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New update on molecular determinants of colistin resistance in bacteria

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ABSTRACT

Colistin relates to the polymyxin group of antibiotics. This antibiotic is still used to destroy gram-negative bacteria as a last resort. However, resistance to this antibiotic has been reported and is appearing day by day. Not much information is available on the exact mechanisms of resistance to this antibiotic. Also, not enough information about pharmacokinetics and pharmacodynamics is available, so the optimal dose should be determined to use these antibiotics to prevent the toxic effects of this antibiotic. In current study, additionally to their pharmacokinetics and pharmacodynamics, we have presented current knowledge about the genes and two-component systems that may cause such resistance to polymyxin and colistin.

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Abbreviations

CMS, Colistin Methane Sulfonate; FDA, Food and Drug Administration; LPS, Lipopolysaccharides; NADH, Nicotinamide Adenine Dinucleotide + Hydrogen; PK, Pharmacokinetics; PD, Pharmacodynamics; MIC, Minimum Inhibitory Concentration; AUC, Area Under the Curve; PMB, Polymyxin B; EMA, European Medicines Agency; ICU, Intensive Care Units; L-Ara4N, 4-amino-4-deoxy-l-arabinose; pEtN, Phosphoethanolamine; TLC, Thin Layer Chromatography; IS, Insertion Sequence; ESBL, Extended Spectrum Beta-Lactamase; MCR, Mobilized Colistin Resistance; CPS, Capsular Polysaccharide

Introduction

Antimicrobial peptides with a net positive charge are found in leukocyte granules, neutrophils, as well as epithelial secretory cells, protect the body against a variety of microbes. The presence of such antimicrobial peptides is very important in traditional medicine, because they are virus-neutralizing agents that kill bacteria and regulate cytokines in the secretions of urine, blood, skin, milk and saliva (1, 2). Microbial peptides are secreted by gram-positive and -negative bacteria, respectively. Biological and medicinal properties are also found in these bacterial products.

Synthetic lipopeptides have also been the subject of research due to their antibiotic and clinical properties (3).

Two mechanisms have been proposed for the activity of antimicrobial peptides: the main one is based on the interaction with the cell membrane and pore formation, and the other one, which is less frequent, is based on penetrating the cytoplasm and interacting with intracellular substances (4). The first mechanism leads to cell death as a result of increased membrane permeability, cell membrane lysis and release of intracellular contents.

The second mechanism causes cell death in various ways, including inhibiting the synthesis of cell macromolecules, inhibiting the correct folding of proteins, inhibiting the function of enzymes and cell wall synthesis, etc (5).

Antimicrobial peptides are complex in classification and no standard system has been defined for them, however they are usually classified according to their origin, type of expression and chemical composition. Chemists divided them into groups and subgroups according to their chemical structure and antibacterial activity (6). The size of amino acid compounds is another type of classification of such antimicrobial peptides (7, 8). The biologists have classified these antibiotics based on ribosomal and non-ribosomal centers, which polymyxin, vancomycin and actinomycin are in the non-ribosomal group of antibiotics (9, 10).

Structure of polymyxin and colistin

Polymyxins are cationic and branched peptides that penetrate a cellular membrane of gram-negative bacteria and are therefore defined as antibiotics. Due to their toxic effects on the human body, such antibiotics are used only in the form of drugs (11). Polymyxins, like defensins, have gramicidin-like chemical structures that serve as its first line of protection from bacterial attacks on eukaryotic cells. These polypeptides have a tripeptide side chain with a heptapeptide ring, and the terminal fatty acids acetylate the N-terminus (12).

Polymyxins are divided into five groups: -A, -B, -C, -D, and -E, with just polymyxin B and -E (Colistin) being used clinically. Colistin and polymyxin B differ only in one peptide ring and amino acid. Polymyxin B contains phenylalanine, while colistin contains leucine (13). Polymyxin E comes in two forms: negatively charged colistin sulfate or colymycin S (for topical and oral use) as well as negatively charged colistimethate sodium (colistin salt), which are used in injectable forms under the names colistin sodium methanesulfonate (CMS), colimycin M or methacolimycin (Figure 2).

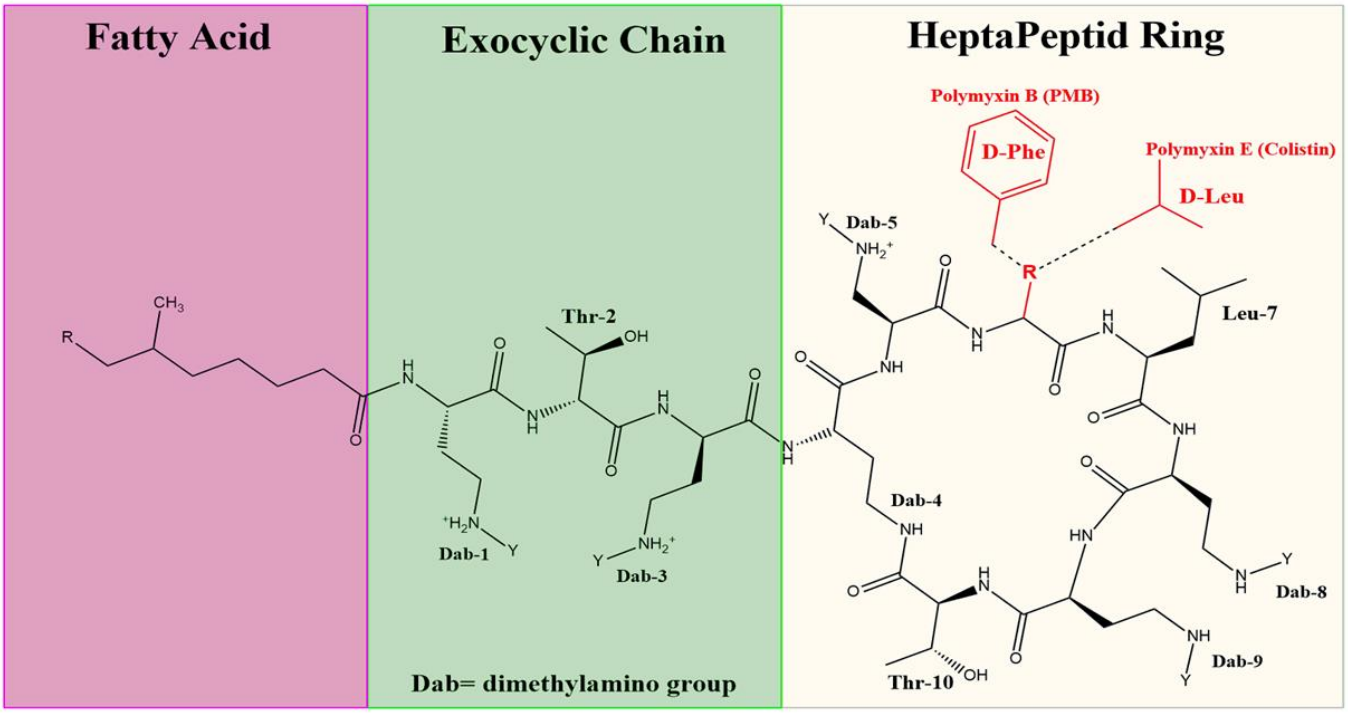

Figure 1. Structure of different polymyxins; Polymyxins are a group of cyclic non-ribosomal polypeptides characterized by the presence of five positively charged amine residues, from di-amino butyric acid (Dab), and a lipophilic tail.

Because of their structural differences, the phrases colistin and colistimethate cannot be used interchangeably in clinical settings (12). Sodium colistin is a less toxic anionic stimulant than colistin sulfate since it is inactive (14). Colistimethate is formed when colistin is combined with formaldehyde and sodium bisulfate. This stimulant must be converted to colistin and other inactive methane sulphate compounds in aqueous media and biological fluids (15, 16). Polymyxin B is an active antibiotic that is administered directly and is one of the most effective permeable components of the cell. Colistin, on the other hand, is an inactive stimulant (17).

Two of the most common injectable formulations of colistin stimulants are CMS and colomycin (18). Colistin sulfate is used orally (such as tablets and syrups) to disinfect the gastrointestinal tract and topically to treat bacterial infections of the skin. CMS is less commonly used intravenously and intramuscularly and may also be given intrauterine or intraventricularly. Intramuscular injection is less commonly used in clinical repetitions due to its variable and painful absorption. Sodium colistimethate is converted to colistin in aqueous solutions (19).

Mechanism of activity

Colistin was first discovered in 1947 by a soil bacterium named Paenibacillus polymyxa (20), and it was first used in Europe and Asia in the 1950s. The FDA approved CMS, a passive stimulant of colistin, as an alternative to injectable colistin in 1959 (21). The use of this antibiotic was banned in the 1970s and 1980s due to neurotoxic and nephrotoxic problems, and was replaced by other alternative antibiotics such as aminoglycosides, quinolones, and beta-lactams, which were less toxic (13). The hydrophobicity of the N-terminal fatty acid sections contributes to colistin's intrinsic toxicity. The sites 6 and 7 are particularly significant due to their antimicrobial activity, side chains 1, 3, 5, and 8 interact with other drugs (Figure 1) (22). The electrostatic interaction of the positive charges residue Dab with the membrane's lipid A phosphate groups allows Mg+2 and Ca+2 (divalent cations) to be displaced from the membrane phosphate groups' negative charges (23). Despite biophysical studies to accurately identify the target mechanism of polymyxins, its exact function has not yet been identified and therefore lipid A is considered the primary target.

There are two hypotheses for the function of polymyxins. Hypothesis 1: Polymyxins are detergents that attack extra-membrane gram-negative bacteria and damage membrane integrity. This disorder causes structural changes. The hydrophilic part is provided by peptides and the hydrophobic part is provided by the end of the chain and fatty acids. LPS destabilizes the bacterial membrane and increases its permeability. As a result, cell death occurs when cell contents leak out (24, 25).

The ability of antibiotics to bind to LPS and release endotoxins is the second hypothesis for polymyxin action. Polymyxins bind to and neutralize LPS lipid-A through fatty acids at the end of the tripeptide side chain, effectively neutralizing LPS's inhibitory effect on mononuclear cell transcription. As a consequence, pro-inflammatory cytokines are less released. These antibiotics also facilitate the excretion of mast cells by secreting histamine, resulting in cellular apoptosis. Endotoxin of gram-negative pathogens is compatible with LPS lipid A, and polymyxins bind to them to neutralize LPS during cell lysis (26, 27). In addition, polymyxins inhibit the NADH Quinone oxidoreductase type 2 enzyme (NDH-2) in the inner membrane of bacteria (28).

Antibacterial activity's scope of polymyxins are usually Gram-negative bacteria, such as those belonging to the Enterobacteriaceae family (E. coli, Enterobacter spp., Klebsiella spp., Citrobacter spp., Salmonella spp., Shigella spp., and Shigella spp.). Polymyxins have also been shown to be effective against Acinetobacter, Pseudomonas, and Stenotrophomonas maltophilia. Proteus, Morganella morganii, Providencia, Serratia marcescens, Pseudomonas mallei, Burkholderia cepacia, Brucella, Legionella, Campylobacter, and Vibrio cholera are among the bacteria that are naturally immune to polymyxins. Polymyxins also lack the function against Gram-negative cocci including Neisseria, Gram-positive bacteria, and active anaerobic bacteria (19).

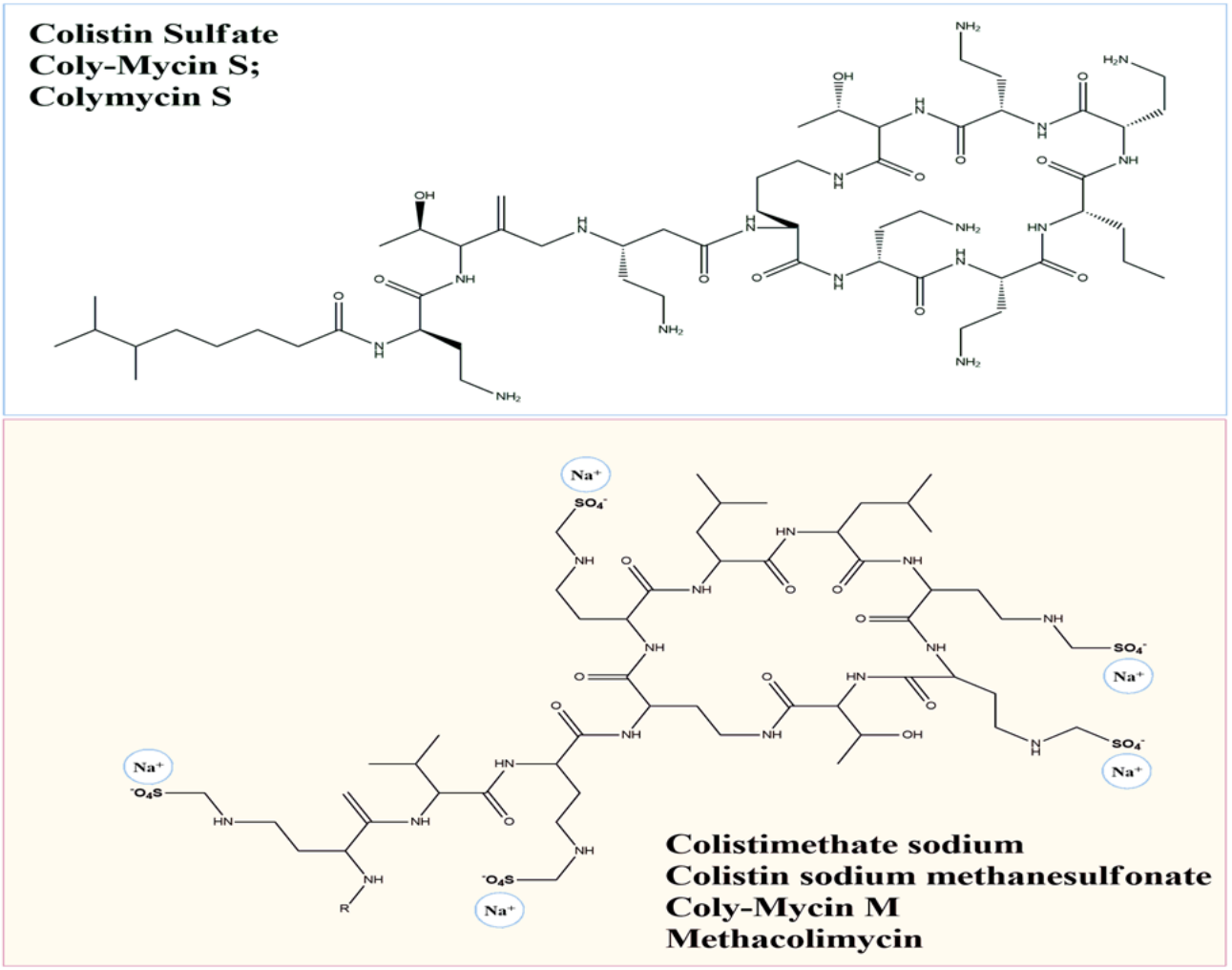

Figure 2. The structure of colistin; Polymyxin E comes in two forms: negatively charged colistin sulfate or colymycin S and negatively charged colistimethate sodium or colistin sodium methanesulfonate (CMS), colimycin M or methacolimycin.

Pharmacodynamics

The pharmacokinetic and pharmacodynamic index (PK-PD) well predicts antibacterial activity to the strains of Acinetobacter and Pseudomonas aeruginosa relative to the time-concentration curve for free drugs, 0-24 h per MIC (fAUC0-24h / MIC). Given that the index is the MIC/Cmax ratio, it shows that exposure to colistin at a moderate interval is more crucial than achieving the highest peak concentration (29).

Quasi-steady colistin concentrations are presented for strains by a MIC of less than 1 µg/ml, averaging 2 µg/ml. In this case, antimicrobial activity is maximized and the risk of kidney toxicity is reduced (30). The antibacterial effect of colistin is very fast and it happens in 5 minutes. If the AUC (Area under the curve)/MIC value is less than required, treatment failure will occur (31). On the other hand, polymyxin B binds to the target faster and it is clinically suitable and superior for blood stream infections (BSI).

The effects of using colistin antibiotic against Klebsiella, Pseudomonas, and Acinetobacter caused resistance increase in these strains (32).

However, the effects that occur after the use of antibiotics in clinical concentrations are as minimal as possible. Hetero colistin resistance is a phenomenon associated with the emergence of a subset of resistance to colistin that can grow at concentrations greater than 4 µg/ml of colistin. These resistances have also been observed in susceptible populations with MICs less than 2 µg/ml for Acinetobacter, Klebsiella and Pseudomonas species.

Pharmacokinetics

The discovery and introduction of polymyxins for clinical use dates back to about 50 years ago. For this reason, they were not as closely monitored for the new drug approval process as they are today. On the other hand, accurate data from PK and PD for the use of polymyxins were not available until recently. In fact, one of the obstacles to progress in this area was the lack of appropriate segregation techniques for CMS plasma concentrations and colostin formation. Advances in chromatographic techniques, for example, have led to quantitative evaluations for each compound separately and the prescribing of colistin and colistimethate (33).

Colistin has been shown to have antimicrobial activity by giving CMS to the body. After CMS is inserted into the body, it is usually removed by the kidneys via filtration and tube secretion (16). CMS is the drug's renal excretion, and some of it is transformed to colistin in the urinary system (13). In a healthy individual, less than 20-25% of the dose of CMS is hydrolyzed to colistin involved in vivo and most of it is absorbed by the kidneys (17). As a result, the concentrations of colistin in the body are due to the initial administration of CMS. Colistin is removed from the non-renal pathway, so there is a large reabsorption in the renal pathway.

If over-administration of CMS and high renal secretion, colistin may be found in the urinary tract. Colistin and polymyxin B despite similar structural form, are different in terms of clinical uses. Polymyxin B, unlike colistin, is administered directly as antibacterial agent. It is also prone to reabsorption of the renal pathway and is mainly eliminated by the non-renal modification mechanism (17).

Dosage schedule:

1. Patients with normal kidney function

CMS should be prescribed carefully as the number of colistin-resistant bacteria increases and re-use of this antibiotic. However, maximum antibacterial activity, reduction of resistance progression, and minimization of side effects should be considered carefully during administration (31). The lowest plasma total C max value after administration of 174 to 250 mg (2 to 3 µg in plasma) from 8 or 12 hours with stable levels in two studies was reported 1.5 to 5.14 µg/ml or 0.68 to 4.65 µg/ml, respectively (34).

Reduced colistin concentrations below the break point of MIC (2 µg/ml) have been shown to be a major barrier to delaying antibiotic treatment, which has been linked to increased mortality, especially in ICU patients, since low colistin concentrations may potentiate resistant populations (35, 36).

Higher CMS is prescribed to patients with normal renal function as a result of low plasma concentrations and high efficacy. The concentration of colistin in the steady state is very variable. Experiments and research have indicated that loading doses and doses higher than MIC be used to achieve sufficient colistin concentrations that result in a better treatment response (37). For patients with normal kidney function, the required dose is a loading dose of 4.5 million units (MU) from the CMS accompanied by a stabilizing dose of 4.5 MU on both days (38). The European Medicines Agency (EMA) recommends 9 MU daily in 2 or 3 intravenous injections for adolescents, 9 MU loading doses of CMS for serious patients, and one dose reduction for patients with renal failure due to creatinine elimination. Since colistin's efficacy and toxicity are proportional to the dosage used, it's clear that it should be used to its full potential to optimize antimicrobial activity while minimizing side effects and progress of resistance (39).

2. Patients with renal insufficiency

Colistin rates are much higher in patients with renal failure, which may be due to reduced elimination of the produced antibiotic or further conversion of CMS to colistin (40). As a result, nephrotoxicity develops in these patients. A loading dose of 9 MU and a stabilizing dose of 4.5 MU per 24 hours is recommended for patients with creatinine removal of 20-25 ml/min. A loading dose of 9 MU and stabilizing doses of 4.5 MU per 48 hours are recommended for patients with creatine depletion less than 20 ml/min (41).

Toxicity

Following intravenous infusion of CMS, today we have a lower rate of toxicity. The low toxicity may be due to the reduction of chemical impurities in the CMS, as well as improved ICU monitoring and the concomitant use of other nephrotoxic drugs. Neurotoxicity similar to nephrotoxicity is dose-dependent and reversible and causes disorders (41). The risk of colistin-induced nephrotoxicity increases with concentrations higher than 2.5-3.3 µg/ml. Other risk factors include the use of nephrotoxic drugs, as well as factors related to the patient (age, sex and severity of the disease, etc.) (42).

Mechanisms of intrinsic resistance to polymyxins

Resistance to polymyxins is naturally associated with the expression of the arnBCADTEF operon and/or eptB gene in P. mirabilis and S. marcescens, resulting in the addition of phosphoethanolamine (PEtN) and the cationic groups of 4-amino-4-deoxy-arabinose (L-Ara4N) to LPS. This change increases the charge on LPS, which is the primary target of polymyxins, and therefore reduces the binding of polymyxins, resulting in inherent resistance in these species (43). The position of the resistance in P. mirabilis contains the SAP operon (a transport protein). This operon is involved in the biosynthesis or transition of amino arabinose and transcribes ATPase and O-acetyltransferase. The RppA/RppB two component system has also been found to be involved in the activation of the arnBCADTEF operon. This operon is also responsible for S. marcescens' intrinsic resistance to colistin, as arnB and arnC mutations have been shown to reduce colistin resistance as compared to wild-type (44).

Acquired resistance mechanisms to polymyxins in Enterobacteriaceae

Colistin resistance in both animals and humans significantly decreased once the drug's usage in animals was outlawed (45). However, polymyxins resistance has been found in many Enterobacteriaceae genera, including Klebsiella pneumonia, E. coli, Enterobacter, and Salmonella. Some bacterial species have unknown mechanisms of colistin resistance, but several molecular mechanisms have been described. The much more common method is to change LPS through cation exchange, which is close to how bacteria those are naturally resistant to polymyxin do it. The addition of cationic groups (L-Ara4N and PEtN) to LPS was found to be responsible for colistin acquired resistance in Enterobacteriaceae strains that were naturally immune to the antibiotic.

Large fragments of genes and operons are involved in the acquired resistance of bacteria to polymyxins. The genes and operons that are directly involved in LPS changes, such as genes responsible for encoding cationic groups and adding them to LPS, including pmrC, pmrE, and pmrHFIJKLM operon; regulatory genes, such as the encoding genes of two-component systems PmrAB and PhoPQ and their regulators (such as mgrB gene, which is a negative regulator of the PhoPQ system) and two-component regulatory system crrAB; plasmid-mediated mcr genes; Cpx and Rcs as upstream regulators of capsule biosynthesis and activator of KpnEF efflux pump are participated (Figure 3) (46). As yet, only one transmissible resistance mechanism (mcr plasmid gene) has been established.

Genes that encode LPS-modifying enzymes

1. The pmrC gene

The PmrCAB operon encodes three proteins, including a phosphoethanolamine (PEtN) phosphotransferase (PmrC), PmrA as a response regulator (also known as BasR), and PmrB as a protein kinase (called BasS). The phosphoethanolamine phosphotransferase PmrC modifies LPS by adding a PEtN group (47) (Figure 3).

2. The pmrHFIJKLM operon and the pmrE gene

Seven proteins are encoded by the pmrHFIJKLM operon, also known as amBCADTEF operon or pbgPE. The L-amino arabinose group (L-Ara4N) is synthesized and fixed to lipid A by pmrHFIJKLM operon and pmrE gene (48) (Figure 3).

Yan et al. (2007), using replacement of kanamycin cassette formation by restriction enzymes and plasmid vectors, were created chromosomal deletions of pmrL and pmrM (genes in the pmrHFIJFKLM operon involved in the lipid A biosynthetic pathway) in polymyxin-resistant Salmonella typhimurium and E. coli. After removal of pmrM-pmrL, a suspension of resistant strains was obtained. The evaluation of lipid-A structure in primary and mutant strains using ESI/MS and thin layer chromatography (TLC) revealed that more than 95 percent of pmrL-pmrM by deletion mutation was free of modified lipid-A with Ara4N was present in resistant early strains. They found that the pmrHFIJKLME operon is involved in lipid A biosynthesis, which leads to polymyxin resistance (48).

3. The pmrA and pmrB genes

The activation of pmrB by periplasmic domains is mediated by environmental stimuli such as macrophage phagosomes, ferric iron (Fe3), aluminum (Al3+), and low pH (e.g., pH 5.5). When bacteria are phagocytosed in macrophages, the two-component systems PmrAB and PhoPQ are activated, allowing bacteria to survive. PmrB is a tyrosine kinase protein that phosphorylates PmrA to activate it. The pmrCAB, the pmrHFIJKLM and the pmrE genes which involved in LPS modification are all activated by PmrA (47) (Figure 3).

Specific mutations have been reported in the pmrA and pmrB genes, which are responsible for the acquired resistance of colistin in Klebsiella, Enterobacter aerogenes, and Salmonella enterica. These mutations activate the PmrAB two-component system, causing rearrangement of the pmrCAB operon with the pmrHFIJKLM operon and the pmrE gene, resulting in the development of PEtN and L-Ara4N and their transition to lipid A. Some polymorphisms in the pmrAB genes of colistin-resistant E. coli have been reported, but the involvement of these mutations in the colistin-resistant phenotype has not been formally demonstrated, since no complementation or mutagenesis has been directly directed.

4. The phoP and phoQ genes that encode the two-component PhoPQ system

Two proteins are encoded by the phoPQ operon, PhoP regulatory protein and phoQ protein kinase. Environmental factors such as macrophage phagosomes, low Mg2+, and low pH (e.g., pH 5.5) activate PhoQ through its periplasmic domain (47). The two-component PhoPQ system expresses genes encoded for magnesium transport, enzymes that modify LPS to develop resistance to cationic antimicrobial peptides, and enzymes that reduce cellular pressure due to acidic pH (49, 50). As a result, the two-component PhoPQ system allows bacteria to survive in low magnesium and acidic pH environments, as well as in the presence of cationic antimicrobial peptides.

PhoQ is a tyrosine kinase protein that phosphorylates PhoP and activates it. PhoP also allows the pmrHFIJKLM operon to be transcribed, which adds L-Ara4N to LPS (Figure 3). PhoP may also cause PEtN to be added to LPS by activating the PmrA protein directly or indirectly via the PmrD binding protein (49, 50).

Several genetic changes in the phoP and phoQ genes, which are responsible for acquired polymyxin resistance in K. pneumoniae and colistin resistance in E. coli, are involved. These mutations are responsible for activating the two-component PhoPQ system and lead to rearrangement of the pmrHFIJKLM operon, resulting in the synthesis of L-Ara4N and its transfer to lipid A (51, 52) (Figure 3).

The modulators of the two component PmrAB and PhoPQ systems

1. The mgrB gene

The mgrB gene is one of the most frequent causes of colistin antibiotic resistance (53, 54). MgrB, also known as YobG, is a small transmembrane protein with 47 amino acids. The mgrB gene is rearranged as a result of PhoP activation, and the MgrB protein suppresses the expression of the PhoQ encoding gene, resulting in negative regulation of the two-component system. (Figure 3). Overexpression of the phoPQ operon, which then activates the pmrHFIJKLM operon, results in the development of L-Ara4N when the mgrB gene (negative regulator of the two-component PhoPQ system) is deactivated (55). Several malignant mutations cause amino acid substitutions and nonsense mutations in the MgrB protein, shortening it and possibly contributing to K. pneumoniae's acquired resistance to colistin. Other changes have been identified, including small nucleotide sequences addition or deletion in the mgrB gene, as well as full deletions from the mgrB locus.

The acquired resistance to colistin in K. pneumoniae and Klebsiella oxytoca is often caused by inactivation of the gene by additive mutation caused by various insertion sequences (IS) belonging to many families at different sites inside the mgrB gene (52, 56, 57). The disruption of the mgrB chromosomal gene also as cause of colistin resistance has recently been identified as a result of the transfer of broad-spectrum beta-lactamase-encoding genes (ESBLs) or carbapenemases. Beta-lactams selective pressure causes the obtaining of beta-lactamase genes, which may be responsible for the co-selection of colistin resistance. Despite the high homology between the mgrB gene sequences of Enterobacteriaceae, a disorder in this gene responsible for acquired resistance to colistin has not been reported in non-Klebsiella and E. coli species (55).

2. The crrAB operon

The CrrAB operon (regulator of colistin resistance) encodes the CrrA regulatory protein and the CrrB protein kinase. The CrrAB operon's physiological function is unclear. Mutations in crrB cause colistin resistance in K. pneumoniae. The CrrB mutant protein controls a gene near crrAB that transcribes a glycosyltransferase-like protein, which contributes to lipid A change. Cheng et al. substituted 6 amino acids in the CrrB protein, resulting in increased resistance to colistin. However, overexpression of the pmrAB operon resulting from mutations or inhibition of the CrrB gene resulted in activation of the pmrHFIJKLM operon as well as the pmrC and pmrE genes. Furthermore, the synthesis and binding of L-Ara4N and PETN to lipid A results in the development of colistin resistance. The CrrC serves as a connector between the CrrAB and PmrAB systems. CrrC overexpression was contributed to the rise of mutations in the crrB gene. Six amino acid substitutions in the CrrB protein, on the other hand, contributed to high phosphorylation of such protein and were thus reported as the cause of K. pneumoniae's acquired resistance to Polymyxins (54, 58).

3. Intrinsic regulator of ramA gene

This gene was discovered in strain K. pneumoniae and is involved in the overall response to antibacterial agents. RamA controls the expression of genes related to permeability barriers. As a result, they can help to reduce antibiotic susceptibility. It has previously been reported that increasing the amount of this regulator induces changes in LPS, decreasing polymyxin sensitivity.

There are three genes in the ramA locus: ramA, romA and ramR. The ramR gene is involved in suppressing the ramA and romA genes. Klebsiella, Citrobacter, Enterobacter, and Salmonella are among the Enterobacteriaceae that have a ramA regulatory gene. This regulatory gene in K. Pneumoniae regulates lipid A biosynthesis, which is linked to permeable barriers. Changes in ramA lead to a decrease in colistin sensitivity. Recently, researchers have shown that increased RamA levels lead to altered LPS and high resistance to colistin. RamA alters bacterial surface, allowing Klebsiella to survive in the presence of colistin.

LpxA, lpxC, lpxD, lpxB, lpxK, lpxL, lpxM, and lpxO are some of the genes responsible for the biosynthesis of lipid A. RamA binds directly to the lpxC, lpxO, and lpxL genes and activates, leading to changes within the lipid A portion of K. pneumoniae. Thus, Klebsiella can survive in antibiotic contests such as colistin (59).

Plasmid resistance to colistin

Although colistin has been known as the last line of defense against pathogenic infections for the past few years, today, in addition to acquired resistance with chromosomal mechanisms, plasmid-mediated resistance with mobile colistin resistance (mcr) genes are prevalent. Figure 4 illustrated the distribution of mcr family genes in the world. The reason for the extent of plasmid resistance is the horizontal transfer of genes and has caused various bacteria to develop resistance to colistin. By encoding phosphoethanolamine transferase, the mcr genes reduce the negative charge on the outer membrane of bacteria and also reduce the affinity for colistin, resulting in antibiotic resistance (60).

It was reported that isolates carrying the mcr-1 gene display resistance to colistin without other resistance mechanisms. The path of circulation and expansion of mcr-1 gene as a first discovered mcr genes is still unclear. The high use of colistin in veterinary medicine, especially in cattle and pigs, has caused the spread of this plasmid gene in animals and humans (25).

According to the results of the phylogenetic tree, the mcr family can be divided into several sub-branches (61). Table 1 illustrates the bacteria in which the mcr gene was first identified, their human or animal origin, and the location of each one. Mcr-1, -2, -6 and mcr-3, -4, -7, -8, -9 and mcr-5, has formed a separate sub-branch alone. Previous studies have shown that mcr-1, -2, -6 is derived from similar samples of Moraxella spp. (60, 62-64), and mcr-3, -7 is derived from Aeromonas spp. (65), and these phylogenetic results are consistent with them.

According to the identification of amino acid sequences, mcr-3, -4, -7, -9 is related in terms of structure based on mcr modeling (65, 66). Most mcr positive isolates are in the various species of Enterobacteriaceae such as K. pneumoniae, S. enterica, E. coli, and Enterobacter spp. Mcr-3 is more prevalent in Aeromonas, which is known as its source (67-69).

The genetic distance of mcr-3, -7 is short, and it seems that they are originated from Aeromonas spp. Mcr-10 has the highest nucleotide compatibility with mcr-9, and its proteins are derived from a common ancestor.

This mcr, when cloned with the colistin-sensitive Enterobacter roggenkmpii strain, multiplies the MIC of colistin (61).

The mcr-6 gene is like a chromosome (63), but other mcr genes are in the form of conjugation plasmids or, ColE-Type mobilized plasmids by helper plasmids (75-77).

With the discovery of the mcr-9 gene, it was revealed that strains carrying the mcr gene do not show the phenotype of colistin resistance due to decreased expression levels (62). Although there is only one mcr determinant in bacterial isolates, recent studies have shown the simultaneous presence of different types of mcr in the same bacterial strain (78).

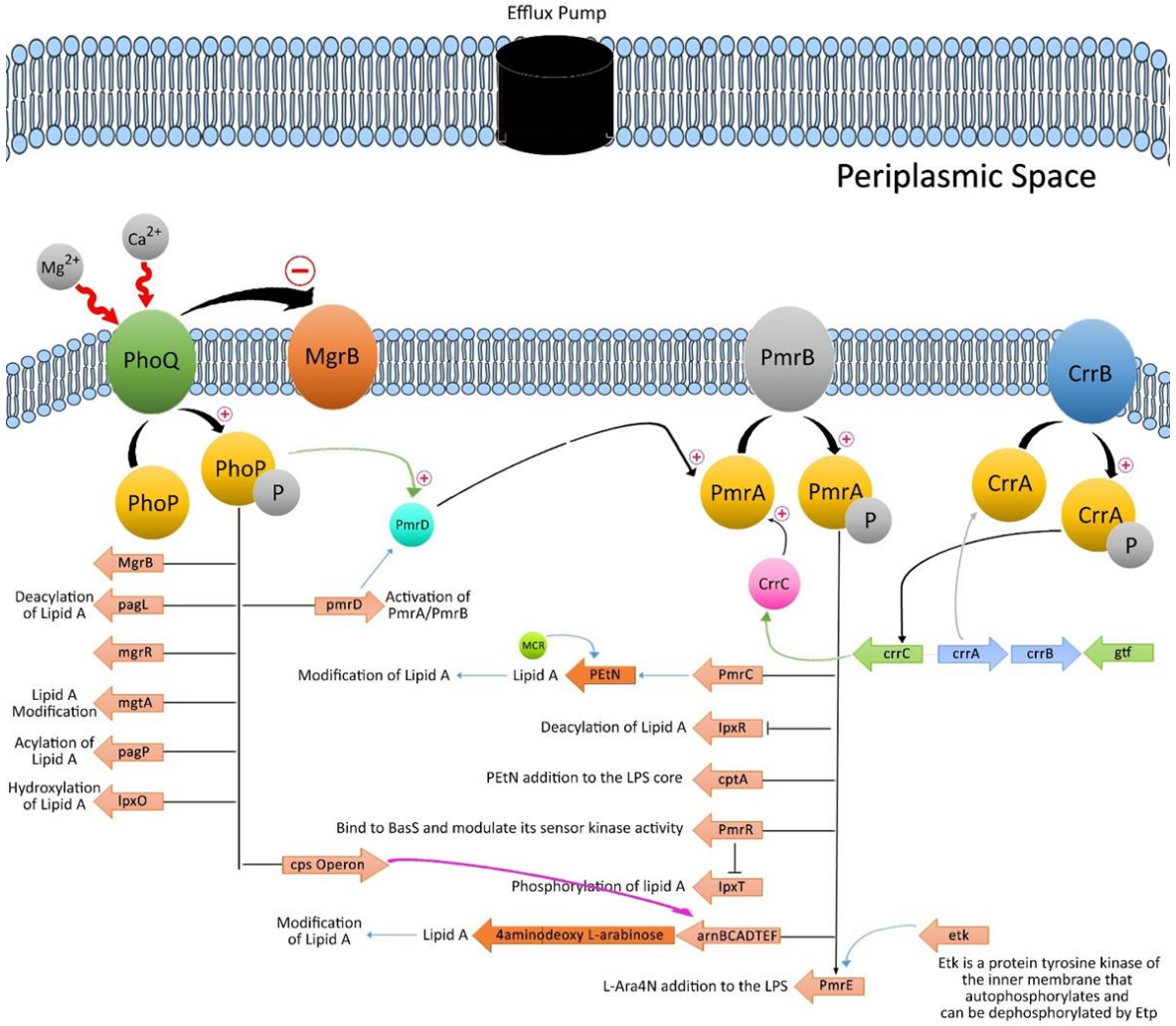

Figure 3. The regulatory pathway of lipopolysaccharide in Enterobacteriaceae. The cell membrane is composed of an inner and outer double layer kept separate by a periplasm. LPS is found in the outer layer of the outer membrane, which is attached to it by Lipid A. Phospholipids and various membrane proteins are found in the inner layer of the outer membrane as well as the entire inner membrane. The PhoPQ two-component system is activated by cAMP, low Mg2+, low MgCl2, low pH, low CaCl2, extracellular DNA, and mutations. This two-component system controls many of the genes required, such as pmrD, pagL, mgrR, pagP, lpxO, and the cps operon, to modify lipopolysaccharide and change the surface area of a cell. PhoPQ and PmrAB two-component systems require ydel genes to create resistance. This gene interacts with an outer membrane purine called OmpD/NmpC. The cps gene is involved in capsular polysaccharide synthesis and phosphorylation of the ugd gene. The activation of PmrA requires the presence of PmrD, high Fe3 +, low Zn2+, low pH, Al3+, low MgCl2 +, low CaCl2, extracellular DNA and CAMP. PmrA-P transcribes genes such as pmrC, cptA, pmrR, and pmrE but controls lpxR gene transcription. Although its effect has not yet been determined, lpxR may be involved in colistin resistance. The creation of mutation by crrC in pmrA, pmrB, crrC genes leads to PmrA activation resulting in pmrC and arnBCADTEF rearrangement.

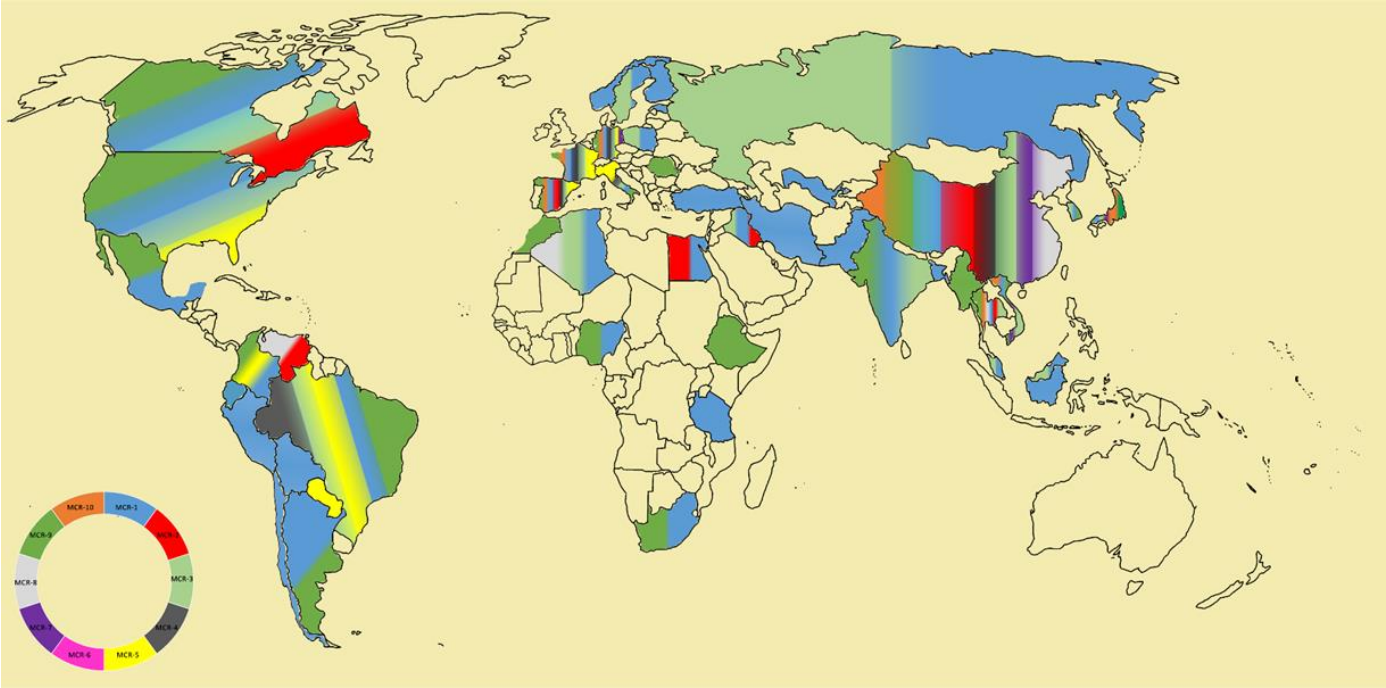

Figure 4: Distribution of the mcr family genes in the world.

Table 1- Origin of differnrt genes in mcr family.

Gene	Bactria	Origin	Place	Reference
mcr-1	*E. coli*	Animal	China	(60)
mcr-2	*E. coli*	Animal	Belgium	(70)
mcr-3	*E. coli*	Animal	Malaysia, China	(71)
mcr-4	*Salmonella, E. coli*	Animal	Italy, Spain, Belgium	(72)
mcr-5	*Salmonella paratyphi B*	Animal	Germany	(73)
mcr-6	*Moraxella species*	Animal	Great Britain	(63)
mcr-7	*Klebsiella pneumonia*	Animal	China	(65)
mcr-8	*Klebsiella pneumonia*	Human	China	(74)
mcr-9	*Salmonella enterica*	Human	Washington State	(62)
mcr-10	*Enterobacter roggenkmpii*	Human	China	(61)

Other mechanisms of polymyxins resistance in Enterobacteriaceae

1. Overproduction of CPS (capsular polysaccharide)

One study has shown that capsular polysaccharide (CPS) act as a protective barrier against polymyxins in K. pneumoniae (79). Rearrangement of capsule biosynthesis genes actually reduces the interaction of polymyxins with the bacterial surface and leads to resistance to polymyxins. Klebsiella strain is able to distribute anionic CPS from its surface. This release leads to entrapment by cationic antimicrobial peptides, such as polymyxins, thus reducing the amount of antibiotics present on the surface of the bacteria.

CPS binds to the bacterial surface through ion interaction with LPS, and this interaction is stabilized by divalent cations. Consequently, the release of CPS in the presence of polymyxins is most likely due to cation-dependent binding perturbations between LPS molecules (80).

2. The role of purines

In S. enterica, the two-component PhoPQ and PmrAB systems have been shown to associate well with OmpD purine and raise bacterial resistance to polymyxins by regulating the Ydel protein (a periplasmic protein) (81).

3. The role of efflux pumps

Although the function of efflux pumps in colistin resistance is not well known, several experiments have shown that they are involved. In reality, variations in the kpnEF and acrAB genes result in a 2-fold reduction in MIC colistin and a rise in the presence of bacteria in low polymyxin concentrations. Adding a small amount of Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) to the test medium with the efflux pump inhibitor reduces the MIC (128 to 512-fold reduction) for resistant strains and partially or completely prevents the regrowth of other resistant strains (82, 83).

Mechanisms of polymyxin resistance in K. pneumoniae, P. aeruginosa and A. baumannii:

1. K. pneumoniae

K. pneumoniae is a gram-negative, rod-shaped bacterium that belongs to the Enterobacteriaceae family and is one of the most common infections in hospitals. Resistance to beta-lactam antibiotics was first reported in this pathogen in 1983 (84). In another report, Klebsiella leads to resistance and failure of treatment against various other antibiotics, including quinolones (85). The emergence of Klebsiella resistance to carbapenems was also reported in 1993 (86). Colistin resistance in K. pneumoniae has been identified in various corners of the world, and it has become a major threat to human health due to restricted treatment options (87, 88). According to studies, the rate of colistin resistance in K. pneumoniae was higher than that of A. baumannii and P. aeruginosa (89-91).

The identification of hetero-resistant isolates is a warning for the rapid growth of colistin resistance and therapeutic failure, as it has happened with other antibiotics and strains (92). According to Arduino et al. (2012), horizontal gene transfer factors such as transposons play a role in the growth of colistin resistance (93). In Enterobacteriaceae, plasmid-mediated mcr, which is responsible for the horizontal transmission of colistin resistance, has been identified (60). As a result, horizontal transfer as much as the selective pressure is one of the most important reasons for the development of colistin resistance.

Recent genomic analyzes have shown that inactivation of mgrB gene, reset of PhoPQ signaling system (94), activation and regulation of pmrHFIJKLM and pmrA operons (95), and presence of arnB gene (96) lead to LPS changes related to colistin resistance in several pathogens factors, including K. pneumoniae.

2. P. aeruginosa

Colistin resistance is mediated by five two-component systems that control LPS changes in P. aeruginosa. Changes in PmrAB and PhoPQ, including those found in Enterobacteriaceae, have been linked to colistin resistance. Genetic changes in these two-component systems result in constructive improvements, such as activation of the pmrHFIJKLM operon transcription, and subsequent addition of L-Ara4N, which converts to LPS and confers colistin resistance. A remarkable point in this direction, unlike what in K. Pneumoniae is observed that colistin resistance is not dependent on the two-component PmrAB system due to PhoPQ changes (97, 98).

The other two-component systems in P. aeruginosa means ColRS, ParRS, and CprRS help with colistin resistance. The ParRS two-component system is involved in acquired resistance to polymyxin (97, 99). Mutations in this scheme allow the pmrHFIJKLM operon construct to be expressed, resulting in the addition of L-Ara4N to LPS. Furthermore, since mutations in the phoQ gene and mutations in the colS gene are linked to high CprRS resistance to colistin. Mutations in the two-component regulatory system ColRS and CprRS can contribute to polymyxin resistance (100). The activation of the phoQ gene or other genes yet to be identified could be responsible for the ColRS and CprRS systems' function. The first enzymatic mechanism by which polymyxin can be modified is Gcn5-related N-acetyltransferase (GNAT) in P. aeruginosa. In recent years, studies on inhibitors of this acetyltransferase have been done (101).

3. A. baumannii

The main mechanism of resistance to colistin in A. baumannii is by adding cationic groups to LPS (qualitative changes of LPS). Acquired colistin resistance, on the other hand, may be the result of a complete loss of LPS activity (slight changes in LPS). In addition, cationic groups in A. baumannii are mediated by mutations in pmrAB.

Mutations in the pmrA and pmrB genes indicate that colistin resistance leads to the synthesis of PEtN by rearrangement of the pmrCAB operon, but does not synthesize L-Ara4N (unlike Enterobacteriaceae) (97, 102, 103). The second mechanism of colistin resistance in A. baumannii is due to changes in lipid A biosynthesis genes (lpxA, lpxC and lpxD), which cause complete loss of LPS.

Mutations identified in these genes are due to substitution mutations, truncation mutation, template-change mutations, or the addition of the ISAba11 sequence (104). Table 2 shows the different mechanisms of colistin resistance in the known bacteria.

Table 2: The different mechanisms of colistin resistance in the known Gram-negative bacteria

Genes/determinants	Resistance mechanisms	Examples of bacteria with this resistance mechanism	References
***acrB* mutation**	Efflux pump	*E. coli*, *K. pneumoniae*	(105, 106)
***KpnEF* mutation**	Efflux pump	*K. pneumoniae*	(107)
***sapABCDF* mutation**	Efflux pump	*P. mirabilis*	(19, 107)
***mgrB* mutation**	Overexpression of *phoPQ* and activation of *pmrHFIJKLM*	*E. coli*, *K. pneumoniae*, *K. oxytoca*	(45, 49-51, 107)
***crrB* mutation**	Modification of lipid A by upregulation of *pmrAB*/ activation of the glycosyltransferase	*K. pneumoniae*	(45, 53)
ramA	Modulates lipid A biosynthesis	*K. pneumoniae*	(55)
pmrA*/*pmrB	Modification of lipid A by *arnBCADTEF* operon, *pmrC* and *pmrE* genes	*E. coli*, *K. pneumoniae*, *S. enterica*, *Enterobacter* sp., *A. baumannii*, *P. aeruginosa*	(45, 47, 101, 105, 106, 108)
phoP*/*phoQ	Modification of lipid A by activation of the *pmrHFIJKLM* operon/activation of *pmrAB* by *pmrD*	*E. coli*, *K. pneumoniae*, *S. enterica*, *Enterobacter* sp., *P. aeruginosa*	(45, 101, 105, 106, 108)
arnBCADTEF	Modification of lipid A by pEtN and L-4AraN	*E. coli*, *K. pneumoniae*, *S. enterica*,	(19, 105, 106)

		Enterobacter sp., *P. mirabilis*, *S. marcescens*	
mcr1-mcr10	Phosphoethanolamine teransferase	*E. coli* (*mcr*-1, 2, 3, 4, 5, 9), *K. pneumoniae* (*mcr*-1, 7, 8), *S. enterica* (*mcr*-1, 2, 3, 4, 5, 9), *S. paratyphi* B (*mcr*-5), *Enterobacter* sp. (1, 4, 5, 10), *Citrobacter freundii* (*mcr*-1, 3), *Aeromonas* sp. (*mcr*-1, 3, 5, 7), *A. baumannii* (*mcr*-1, 2, 3, 4), *P. aeruginosa* (*mcr*-1, 2), *P. mirabilis* (*mcr*-3), *Moraxella* sp., (*mcr*-1, 2, 6)	(61-65, 67, 70, 72, 73, 101, 105, 106, 109, 110)
lpxA, lpxC, lpxD	Inactivation of lipid A biosynthesis abolishing LPS synthesis	*A. baumannii*	(105, 106, 111)
colR/colS, ***cprRS***	LPS additions in response to high Zn2+ modifications of the LPS moiety	*P. aeruginosa*	(100, 111)

Conclusion

The rampant growth of antibiotic resistance makes it difficult to fully depend on the discovery of new antibiotics, antibiotic combinations and inhibitors, so a rational plan to use older antibiotics such as colistin seems reasonable. However, one should be aware of colistin resistance, especially among multidrug-resistant bacteria, and its development through mutations or acquired and intrinsic mechanisms. In this study, several mechanisms of bacterial resistance to the antibiotic colistin, which are mainly based on the change of the LPS structure of the outer membrane, have been presented. Undoubtedly, further research to identify the exact role of each mechanism of colistin resistance will help to develop more effective antibiotics to control microbial infections.

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Conflicts of interest

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Authors' Contributions

EMK conceived the design of the study. MS reviewed literature and participated in data collection. EMK and MS prepared the manuscript. All authors contributed toward drafting and critically revising the paper and agree to be accountable for all aspects of the work.

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