Molecular mechanisms related to colistin resistance in Enterobacteriaceae

Abstract: Colistin is an effective antibiotic for treatment of most multidrug-resistant Gram-negative bacteria. It is used currently as a last-line drug for infections due to severe Gram-negative bacteria followed by an increase in resistance among Gram-negative bacteria. Colistin resistance is considered a serious problem, due to a lack of alternative antibiotics. Some bacteria, including Pseudomonas aeruginosa, Acinetobacter baumannii, Enterobacteriaceae members, such as Escherichia coli, Salmonella spp., and Klebsiella spp. have an acquired resistance against colistin. However, other bacteria, including Serratia spp., Proteus spp. and Burkholderia spp. are naturally resistant to this antibiotic. In addition, clinicians should be alert to the possibility of colistin resistance among multi-drug-resistant bacteria and development through mutation or adaptation mechanisms. Rapidly emerging bacterial resistance has made it harder for us to rely completely on the discovery of new antibiotics; therefore, we need to have logical approaches to use old antibiotics, such as colistin. This review presents current knowledge about the different mechanisms of colistin resistance.

Keywords: colistin, Enterobacteriaceae, two-component system, lipid A, mcr genes

Introduction

Antibiotic resistance, which started in the 1970s among Gram-negative bacteria, is a crucial global problem. Development of antibiotic resistance is a phenomenon correlated with antibiotic overuse and bacterial evolution. Microorganisms can use several mechanisms to adapt against antimicrobial agents and environmental stimulants. Bacteria can use genetic alterations in their genes to form genes with improved performance to overcome antibiotics. Modification in only a few base pairs in DNA causing replacement of one or a few amino acids in an important target, such as cell structure or cell wall and enzymes, leads to new resistance strains. Initially, the problem of bacterial resistance to antibiotics was solved by the invention of the latest categories of antibiotics, including aminoglycosides, glycopeptides, and macrolides, and further by the chemical modification of old antibiotics. Unfortunately, these antibiotics could not keep pace with the development of antibiotic resistance in bacterial pathogens. Mobile genes conferring resistance to aminoglycosides and broad-spectrum β-lactams can transfer between species and are one of the important factors accounting for the progressive erosion of antimicrobial activity in both hospital and community settings. Emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria, as well as the lack of...
novel agents against these pathogens, have led to the reintroduction of colistin, an old and valuable antibiotic as a last-resort treatment option.\textsuperscript{8} Colistin, also known as polymyxin E, was isolated in 1947 from the bacterium \textit{Paenibacillus polymyxa} subsp. \textit{colistinus}.\textsuperscript{9} This organism also produces colistinase, which inactivates colistin.\textsuperscript{10} Colistin is a polycationic antibiotic, and has significant activity against Gram-negative bacteria, such as Enterobacteriaceae. The outer cell membrane of Gram-negative bacteria is the main site of action for colistin. When colistin binds to lipopolysaccharides in the outer membrane, electrostatic interaction occurs between the \(\alpha,\gamma\)-diaminobutyric acid of colistin and the phosphate groups of the lipid A region of lipopolysaccharide (LPS). It competitively displaces divalent cations (\(Ca^{2+}\) and \(Mg^{2+}\)) from the phosphate groups of membrane lipids.\textsuperscript{11,12} Therefore, disruption of LPS may cause increased permeability of the outer membrane and leakage of intracellular contents, ultimately leading to cell death.\textsuperscript{13–15} Unfortunately, during the last few decades, the emergence of colistin-resistant isolates has been frequently reported,\textsuperscript{10,12} which has increased inappropriate use of this drug, especially as monotherapy could be the cause of this problem.\textsuperscript{16–18} In addition, there have been reports of increased infection due to bacteria with intrinsic resistance to colistin, such as \textit{Proteus} spp., \textit{Providencia} spp., \textit{Serratia} spp., and \textit{Morganella} spp.\textsuperscript{19–21} In this article, we assess different mechanisms of colistin resistance in Enterobacteriaceae.

**Activity spectrum of colistin**

Colistin is a narrow-spectrum antimicrobial agent that has significant activity against most members of the Enterobacteriaceae family, including \textit{Escherichia coli}, \textit{Enterobacter} spp., \textit{Klebsiella} spp., \textit{Citrobacter} spp., \textit{Salmonella} spp., and \textit{Shigella} spp. It also has activity against common nonfermentative Gram-negative bacteria, such as \textit{Acinetobacter baumannii}, \textit{Pseudomonas aeruginosa}, and \textit{Stenotrophomonas maltophilia}.\textsuperscript{14,22–24} In addition, \textit{Haemophilus influenzae}, \textit{Legionella pneumophila}, \textit{Aeromonas} spp., and \textit{Bordetella pertussis} are naturally susceptible to colistin.\textsuperscript{15,22,25,26}

Conversely, among the Enterobacteriaceae, \textit{Proteus} spp. and \textit{Serratia marcescens} have intrinsic resistance to colistin. On the other hand, \textit{Morganella morganii}, \textit{Providencia} spp., \textit{Pseudomonas mallei}, \textit{Burkholderia cepacia}, \textit{Chromobacterium} spp., \textit{Edwardsiella} spp., \textit{Brucella}, \textit{Legionella}, and \textit{Vibrio cholerae} are typically resistant to colistin. Colistin is not active against Gram-negative cocci, such as \textit{Neisseria} spp., gram-Gram-positive bacteria, anaerobic bacteria, eukaryotic microbes, or mammalian cells.\textsuperscript{14,27–31}

**Mechanisms of colistin resistance in Enterobacteriaceae**

Although the main mechanism of resistance to colistin is unclear, Gram-negative bacteria employ several mechanisms to protect themselves against colistin toward other polymyxins (Figure 1). According to the literature, most colistin-resistance mechanisms are adaptive mechanisms that occur after in vitro exposure.\textsuperscript{15} Resistance to colistin occurs with LPS modification via different routes. The most common strategies for resistance to colistin are modifications of the bacterial outer membrane through alteration of the LPS and reduction in its negative charge.\textsuperscript{32,33} The other strategy is the overexpression of efflux-pump systems.\textsuperscript{34} Another mechanism is overproduction of capsule polysaccharide.\textsuperscript{35–37} No enzymatic mechanisms of resistance have been reported, but strains of \textit{P. polymyxa} produce colistinase.\textsuperscript{38}

**Intrinsic resistance mechanisms**

Resistance to polymyxins occurs naturally in \textit{P. mirabilis} and \textit{S. marcescens} by modification of the LPS via cationic substitution. The mechanism of resistance in these species is linked to expression of the \textit{arnBCADTEF} operon and the \textit{epdB} gene. In this way, the 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (pEtN) cationic groups are added to the LPS by this operon and gene, respectively. It has been shown that the LPS of \textit{P. mirabilis} contains L-Ara4N and the genome of this bacterium contains the \textit{epdC} gene, which is mediated to the modification of LPS with PETN.\textsuperscript{39–41} Putative loci in \textit{P. mirabilis} include the \textit{sap} operon encoding a transport protein, ATPase gene, and \(O\)-acetyltransferase gene, which take part in biosynthesis or transfer of amino arabinose.\textsuperscript{42} Also, the existence of \textit{rrpA/rrpB} TCS has been discovered to play a role in activation of the \textit{arnBCADTEF} operon.\textsuperscript{43,44} Similarly, this operon is responsible for intrinsic resistance to colistin in \textit{S. marcescens}, as it has been shown that \textit{arnB} and \textit{arnC} mutants lead to a reduction in susceptibility to colistin (minimum inhibitory concentration [MIC] from 2.048 to 2 \(\mu\)g/mL) compared to the wild type.\textsuperscript{45}

This modification of LPS and the increase in its charge give rise to the affinity of colistin decrease for binding to LPS. Therefore, intrinsic resistance has occurred in these species.\textsuperscript{9,41,43}
Acquired resistance mechanisms in Enterobacteriaceae

Acquired colistin-resistance mechanisms have been recognized in some members of Enterobacteriaceae family, such as E. coli, Salmonella spp., Klebsiella spp., and Enterobacter spp., and remain unknown for other bacterial species. Resistance mechanisms are presumed to be linked to chromosomal mutation untransferrable via horizontal gene transfer. Only one mechanism of resistance has been identified as a transferable mechanism (plasmid-mediated mcr gene) so far (Table 1).

Many genes and operons play a role in modification of LPS, which in turn leads to colistin resistance. These include: genes and operons responsible for encoding enzymes that have a direct role in LPS modification, such as the pmrC and pmrE genes and the pmrHFIJKL operon; regulatory two-component systems (TCSs), including PmrAB and PhoPQ, as well as crrAB, which regulates the PmrAB system; the mgrB gene, a negative regulator of TCSs, including PmrAB and PhoPQ; plasmid-mediated mcr genes; and Cpx and Rcs as regulator of upregulation of capsule biosynthesis and activator of the efflux pump KpnEF regulating the PhoPQ system, respectively.

mgrB gene and regulators of PmrAB and PhoPQ two-component systems

Some operons and regulators have a role in the modification of LPS by PmrAB and PhoPQ TCSs. The pmrABC operon encodes PmrA (BasR) as a regulator protein, PmrB (BasS) as a cytoplasmic membrane-bound sensor kinase, and PmrC as a putative membrane protein. The addition of L-arabinoseamine (L-Ara4N) to the 1-phosphate or 4'-phosphate group leads to colistin resistance. Generally, L-Ara4N is connected to 4'-phosphate and modifies it while PETN is connected to 1-phosphate. The pmrHFIJKL operon (also named arnBCDADTEF or pbgPE) and PmrE synthesize L-Ara4N from uridine diphosphate glucuronic acid and fix it to lipid A. The biosynthesis of L-Ara4N depends on the pmr (arn) operon. Moreover, under environmental stimulants, such as macrophage phagosomes, the high concentration of iron (Fe^{3+}) and exposure to aluminum (Al^{3+}), as well as acidic pH, leads to activation of PmrB. On the other hand, low concentration of Mg^{2+} or Ca^{2+} leads to activation of phoQ. PmrB activates PmrA by phosphorylation, and PmrA in turns activates regulation of the pmrABC and pmrHFIJKL operons and the pmrE gene. Subsequently, these operons and genes lead to LPS modification by adding PETN and L-Ara4N to lipid A. Mutation of pmrA/pmrB results in upregulation of the
### Table 1 Acquired and intrinsic strategies employed by Gram-negative bacteria for achieving resistance to colistin

| Genes          | Gene function                                                                 | E. coli | Kpneumoniae Enterobacter spp. | Salmonella spp. | C. freundii Protrus mirabilis | Serratia marcescens | References |
|----------------|-------------------------------------------------------------------------------|---------|-------------------------------|-----------------|-------------------------------|---------------------|------------|
| pmrA/pmrB      | Modification of lipid A by pmrB genes                                          | +       | +                             | +               | −                             | −                   | 65,68,69,129,130 |
| phoP/phoQ      | Modification of lipid A by activation of the pmrHFIJKLM operon/activation of pmrAB by pmrD | +       | +                             | +               | −                             | −                   | 50,78,131,132    |
| pmrC           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 9,45,69,129--131  |
| pmrD           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 51,79        |
| pmrE           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 110          |
| pmrF           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 52          |
| pmrG           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 110          |
| pmrI           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 133,134      |
| pmrJ           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 55,135      |
| pmrK           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 102,137      |
| pmrL           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 103,137      |
| pmrM           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 104,139      |
| pmrN           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 105          |
| pmrO           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 106          |
| pmrP           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 107          |
| pmrQ           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 36           |
| pmrR           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 117          |
| pmrS           | Overexpression of phoPQ and activation of pmrHFIJKLM                            | +       | +                             | −               | −                             | −                   | 42           |
pmrABC and pmrFHIJKLM operons and pmrE gene. Mutation within the pmrA and pmrB genes leading to colistin resistance has been described in Klebsiella pneumoniae and Salmonella enterica (Table 1).\(^{65-69}\)

On the other hand, the phoPQ TCS encodes PhoP as a regulator protein and PhoQ as a sensor kinase. Under conditions of low magnesium or calcium, acidic PH, or cationic antimicrobial peptide, PhoPQ is activated and protects bacteria.\(^{21,63,64}\) Activated PhoPQ leads to modification of lipid A via two routes: PhoQ activates PhoP by its kinase activity via phosphorylation, which activates transcription of the pmrFHIJKLM operon, followed by modification of lipid A;\(^ {70,71}\) and PhoP indirectly activates pmrA by bypassing the PmrD connector protein, subsequently activates the transcription of the pmrHFIJKLM operon and synthesizes PETN, which transfers it to lipid A.\(^ {72,73}\)

Various of PETN-coding genes, such as eptA (pmrC), eptB (pagC), and eptC (cpta), are able to add PETN to different sites of LPS.\(^ {74,75}\) Mutation of the phoPQ genes has been identified in K. pneumoniae and E. coli that led to acquired colistin resistance.\(^ {55,67,76-78}\)

The mgrB gene encodes a small transmembrane protein of 47 amino acids that exerts negative feedback on the PhoPQ TCS.\(^ {79}\) This protein inhibits the kinase activity of PhoQ, which in turn represses expression of the phoQ gene. Nevertheless, mutation/inactivation of the mgrB gene results in upregulation of the phoPQ operon and subsequent activation of the pmrHFIJKLM operon. Finally, production of L-Ara4N leads to modification of lipid A and colistin resistance.\(^ {51}\)

Various mutations or disruptions of the mgrB gene have been reported, such as deletion, nonsense, missense, inactivation, and insertional mutations. According to reports, mgrB inactivation is the most common mechanism for colistin resistance in K. pneumoniae and K. oxytoca.\(^ {67,80-82}\) In addition, it has been described that inactivation of the mgrB gene by diverse insertion sequences at different sites of this gene is the other mgrB mutation that often occurs in K. pneumoniae.\(^ {53,65,80}\) Other alterations that have been reported in the mgrB gene include nonsense and missense mutations, leading to premature termination and amino-acid substitutions in mgrB, respectively.\(^ {53,77}\) Goulian et al showed that deletion of the mgrB gene led to upregulation of the PhoP-regulated gene in E. coli.\(^ {79}\)

**CrrAB two-component system**

The crrAB operon encodes two proteins: CrrA as a regulatory protein and CrrB as a sensor kinase. Wright et al described that mutation of crrB leads to colistin resistance in K. pneumoniae.\(^ {83}\) The mutated CrrB protein regulates a crrAB-adjacent gene that encodes a glycosyltransferase-like protein, which in turn leads to modification of lipid A.\(^ {83}\) In Cheng et al's study, six amino-acid substitutions in the CrrB protein led to high resistance to colistin (MICs of colistin 512–2,048 µg/mL).\(^ {85}\) However, mutation/inactivation of the crrB gene led to activation of the pmrHFIJKLM operon and the pmrC and pmrE genes through overexpression of the pmrAB operon. Furthermore, the production and addition of L-Ara4N and PETN to lipid A lead to acquisition of resistance to colistin.\(^ {83}\) It was demonstrated that CrrC afforded a connection between the CrrAB and pmrAB systems. Mutation of the crrB gene led to increased crrC transcription. On the other hand, it has been suggested amino-acid substitutions of the CrrB protein result in increased autophosphorylation of this protein, consequently leading to colistin resistance.\(^ {52}\)

**Plasmid-mediated resistance to colistin**

Plasmid-mediated colistin is a significant challenge and global concern, because of easy transfer of colistin-resistance genes to susceptible strains.\(^ {54}\) The mcr genes are responsible for horizontal transfer of colistin resistance. These plasmid-mediated genes were first reported in E. coli isolated from pigs and meat in China, November 2015.\(^ {54}\) MCR is a member of the PETN enzyme family, and its expression leads to addition of PETN to lipid A. According to the literature, isolates carrying the mcr1 gene display resistance to colistin without other resistance mechanisms. The existence of mcr1 in isolates is enough for colistin resistance without other resistance mechanisms, as isolates carrying this gene displayed a four- to eightfold increase in colistin MIC.\(^ {9}\) It is worth noting that the production of mcr1 leads to resistance to lysozymes.\(^ {84}\)

Following initial findings, mcr1-mediating transferable colistin resistance has been reported in several regions, including Europe, Asia, the Americas, and Africa.\(^ {85-98}\) There is a hypothesis that mcr1 originated in animals, particularly pigs and cattle, and subsequently spread to humans, though the proportion of mcr1-positive isolates is low in humans compared to animals.\(^ {54,99}\) This transmissible gene has been reported from diverse genera of Enterobacteriaceae, including E. coli, Klebsiella spp., Enterobacter spp., Salmonella spp., Shigella spp., and Cronobacter spp., but mostly from E. coli. Some plasmids containing the mcr1 gene carry other genes that are resistant to other antibiotics, such as β-lactams, aminoglycosides, quinolones,
sulfonamides, tetracyclines, and fosfomycin. The mcr gene has also been identified in Enterobacteriaceae isolates, which carry such carbapenemase genes as blaNDM1, blaNDM5, blaNDM9, blaOXA48, blaKPC2, and blaVIM1.2,5,9,10

Recently, Xavier et al reported a novel plasmid-mediated colistin resistance gene, known as mcr2, in E. coli.63 Thereafter, mcr3 and mcr4 genes were discovered.102,103 Finally, in July 2017, Borowiak et al reported a new gene of the mcr family from Salmonella paratyphi B were carried in transposons instead of plasmids.104

In addition, three mobile colistin-resistance genes (mcr6, mcr7, and mcr8) were discovered in 2018. AbuOun et al discovered a new variant of mcr2 from Moraxella pluranimalium that they renamed mcr6.1.105 They suggested that Moraxella spp. may contain a natural reservoir of mcr, and mcr-harboring Moraxella appeared in pig populations. Yang et al found K. pneumoniae isolates harbored a new mcr variant, mcr7.1, recovered from chickens in China.106

They suggested that mcr7, like mcr-3, originated from Aeromonas spp.,102 and its structure was similar to mcr3. In addition, mcr7 displayed 78% nucleotide identity to the mcr3 gene. Eventually, a new mobile genetic element, mcr-8, was discovered in K. pneumoniae. It was identified as the coexistence of mcr8 and the carbapenemase-encoding gene blanDM, which is a great concern.107 It is notable that mcr8 has existed for some time and disseminated among K. pneumoniae.108 mcr2–8 are similar to mcr1, as PETN leads to the addition of phosphoethanolamine to lipid A, followed by colistin resistance (Figure 1). Both mcr1 and mcr2 genes originated from Moraxella spp. In addition, mcr3 and mcr4 genes line up closely with PETN from Aeromonas spp. and Shewanella frigidimarina, respectively,55,102,103,108 whereas the origin of mcr5 remains unknown.104 Although mcr is a plasmid-mediated gene, recently Zurfluh et al identified the mcr1 gene on chromosomes of E. coli strains. Therefore, there is a hypothesis that this gene can be integrated in the genome of some isolates.109

Role of regulator RamA

The ramA locus has three genes: ramA, romA, and ramR. The ramR gene plays a role as a repressor of the ramA and romA genes. Some Enterobacteriaceae possess a ramA regulator, such as K. pneumoniae, Citrobacter spp., Enterobacter spp., and Salmonella spp. In K. pneumoniae, this regulator modulates lipid A biosynthesis and is related to permeability barriers. It has been shown that ramA alterations lead to reductions in colistin susceptibility. Recently, researchers showed that increased levels of RamA resulted in LPS modification and increased resistance to colistin.110 RamA applied changes to the bacterial surface and Klebsiella survived against colistin. Several genes are associated with lipid A biosynthesis, including lpxA, lpxC, lpxD, lpxB, lpxK, lpxL, lpxM, and lpxO.111 RamA binds directly to and activates the lpxC, lpxO, and lpxL2 genes and leads to alterations within the lipid A moiety in K. pneumoniae. Therefore, Klebsiella can survive in such antibiotic challenges as colistin.110

Role of capsule in colistin resistance

The role of capsular polysaccharide (CPS) has been demonstrated to be protective against cationic antimicrobial peptides, including colistin.55 K. pneumoniae is able to release CPS from its surface.112 The number of capsule layers is related to resistance level. It has been observed that K. pneumoniae with several layers was more resistant to colistin than isolates with few layers.8,113 However, upregulation of a capsular biosynthesis gene led to a reduction in the interaction of colistin with the target site in K. pneumoniae, followed by increased colistin resistance.55 Consequently, there are some regulators of capsule formation, such as Cpx (conjugative pilus expression) and Rcs (regulator of capsule synthesis). Cpx and Rcs also appear to contribute to colistin resistance by activating the efflux pump KpnEF and regulating the PhoPQ TCS, respectively.36 Furthermore, the ugd gene plays a role in CPS and L-Ara4N biosynthesis in that its phosphorylation is related to the synthesis of capsular and colistin resistance.114,115

Role of efflux pumps

A few studies have suggested that efflux-pump systems are involved in colistin resistance. Efflux pumps, such as the KpnEF, AcrAB and Sap proteins, have been reported in Enterobacteriaceae. By activation of these pumps, resistance to colistin is increased.116,117 The efflux pump KpnEF is a member of the Cpx regulon (responsible for capsule synthesis in K. pneumoniae) and belongs to the SMR protein family. In K. pneumoniae, this pump is mediated by colistin resistance and other antibiotics, including ceftriaxone, erythromycin, and rifampicin.117 It has been observed that mutations in KpnEF (as a member of the small MDR efflux-pump family) lead to more susceptibility and a doubled reduction in the MIC of colistin.117 On the other hand, AcrAB is a part of the AcrAB–ToIC complex, which plays a role in colistin resistance. The AcrAB-mutant E. coli displays an eightfold increase in colistin susceptibility. It has
been remarked that expression of this pump's proteins is dependent on the PhoPQ TCS.\textsuperscript{118} Finally, the Sap\textsubscript{ABCDEF} operon encodes Sap proteins that are constitute of five proteins.\textsuperscript{118} In the mutant of \textit{P. mirabilis}, susceptibility to colistin is increased by mutation of the Sap\textsubscript{ABCDEF} operon.\textsuperscript{32} It has been shown that the use of efflux-pump inhibitors in the test medium carbonyl cyanide 3-chlorophenylhydrazone leads to a reduction in MIC for colistin-resistant strains.\textsuperscript{119}

**Logical approaches to use of colistin**

Recent studies have suggested colistin is the foremost therapeutic option of XDR Gram-negative bacteria in recent years, owing to its potent bactericidal efficacy.\textsuperscript{120} Combination therapies of colistin with other antibiotics are superior to colistin monotherapy for XDR strains, due to rapid selection of resistance in some strains, heteroresistance during colistin monotherapy, and lower clinical efficacy during colistin-based combination.\textsuperscript{121} In addition, rates of cure, 14-day survival, and microbiological eradication are lower in monotherapy compared to combination therapy.\textsuperscript{121} Moreover, several combination therapies have been recommended to decrease the development of resistance. The combination of colistin with other drugs, such as carbapenems, sulbactam, tigecycline, aminoglycosides, and rifampicin, has been recommended to the development of colistin-resistant strains, which may improve clinical and microbiological outcomes.\textsuperscript{121–126} The colistin–sulbactam combination was recommended against imipenem-resistant \textit{A. baumannii}, particularly in colistin-resistant strains, due to its high in vitro synergistic activity,\textsuperscript{121,127} which may be a more favorable combination. Colistin-based combinations with tigecycline, aminoglycosides, and rifampicin have shown synergistic activity against XDR strains,\textsuperscript{122,125,128} but tigecycline is disadvantageous in bacteremic patients, because of its low plasma concentrations.\textsuperscript{128} In addition, colistin–carbapenem combinations may be preferable in the treatment of \textit{A. baumannii} infections to prevent resistance selection and to decrease the prevalence of \textit{A. baumannii}.\textsuperscript{121}

**Conclusion**

The main target for colistin is lipid A of the LPS in Gram-negative bacteria, leading to disruption of the bacterial membrane and resulting in cellular death. In recent decades, the increasing use of colistin in clinical settings, mainly in veterinary clinics, has led to the emergence of colistin resistance. Many studies have shown that the prevalence of colistin resistance has increased rapidly among Enterobacteriaceae. Clinicians should be alert to the possibility of colistin resistance among MDR bacteria and the development of colistin resistance through mutation or adaptation mechanisms. Rapidly emerging bacterial resistance has made it harder for us to rely completely on the discovery of new antibiotics; therefore, we need to have logical approaches to use older antibiotics, such as colistin.

**Acknowledgments**

This study received no funding, and was the authors’ own work. We thank the staff of the Drug Applied Research Center for their support.

**Disclosure**

The authors report no conflicts of interest in this work.

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