

# Molecular mechanisms related to colistin resistance in Enterobacteriaceae

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**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.<sup>1-3</sup> Development of antibiotic resistance is a phenomenon correlated with antibiotic overuse and bacterial evolution.<sup>4</sup> 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.<sup>5</sup> 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 c

hemical modification of old antibiotics. Unfortunately, these antibiotics could not keep pace with the development of antibiotic resistance in bacterial pathogens.<sup>6</sup> Mobile genes conferring resistance to aminoglycosides and broad-spectrum  $\beta$ -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.<sup>7</sup> Emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative bacteria, as well as the lack of

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novel agents against these pathogens, have led to the reintroduction of colistin, an old and valuable antibiotic as a last-resort treatment option.<sup>8</sup>

Colistin, also known as polymyxin E, was isolated in 1947 from the bacterium *Paenibacillus polymyxa* subsp. *colistinus*.<sup>9</sup> This organism also produces colistinase, which inactivates colistin.<sup>10</sup> 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 occur 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 ( $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$ ) from the phosphate groups of membrane lipids.<sup>11,12</sup> Therefore, disruption of LPS may cause increased permeability of the outer membrane and leakage of intracellular contents, ultimately leading to cell death.<sup>13–15</sup> Unfortunately, during the last few decades, the emergence of colistin-resistant isolates has been frequently reported,<sup>10,12</sup> which has increased inappropriate use of this drug, especially as monotherapy could be the cause of this problem.<sup>16–18</sup> In addition, there have been reports of increased infection due to bacteria with intrinsic resistance to colistin, such as *Proteus* spp., *Providencia* spp., *Serratia* spp., and *Morganella* spp.<sup>19–21</sup> 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 *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Salmonella* spp., and *Shigella* spp. It also has activity against common nonfermentative Gram-negative bacteria, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*.<sup>14,22–24</sup> In addition, *Haemophilus influenzae*, *Legionella pneumophila*, *Aeromonas* spp., and *Bordetella pertussis* are naturally susceptible to colistin.<sup>15,22,25,26</sup>

Conversely, among the Enterobacteriaceae, *Proteus* spp. and *Serratia marcescens* have intrinsic resistance to colistin. On the other hand, *Morganella morganii*, *Providencia* spp., *Pseudomonas mallei*, *Burkholderia cepacia*, *Chromobacterium* spp., *Edwardsiella* spp., *Brucella*, *Legionella*, and *Vibrio cholera* are typically resistant to colistin. Colistin is not active against Gram-

negative cocci, such as *Neisseria* spp., gramGram-positive bacteria, anaerobic bacteria, eukaryotic microbes, or mammalian cells.<sup>14,27–31</sup>

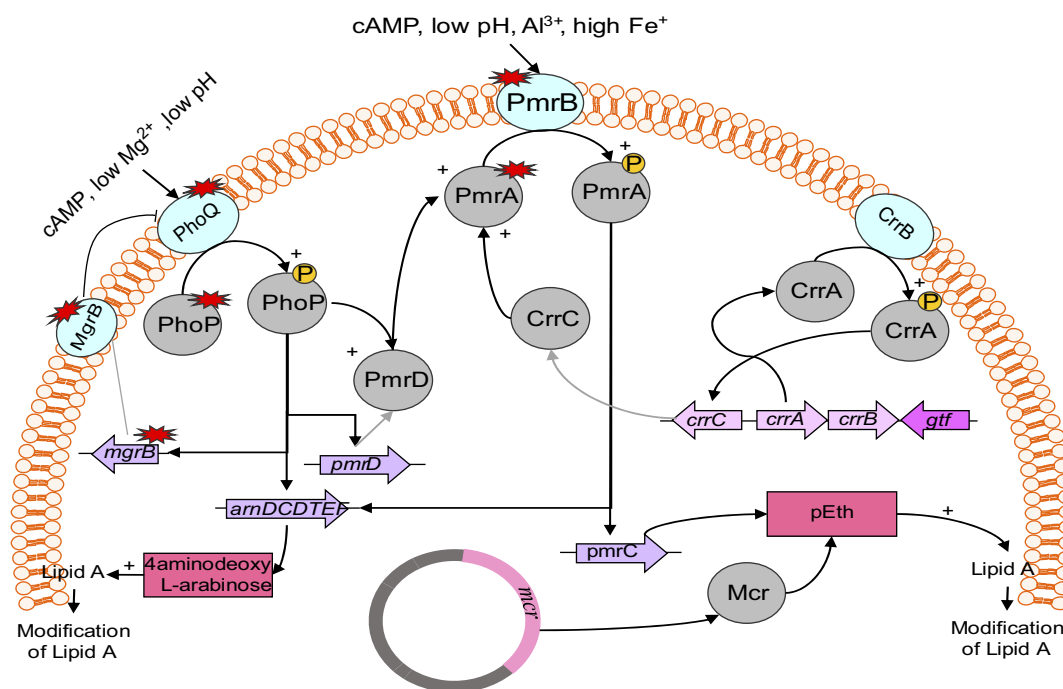
## Mechanisms of colistin resistance in Enterobacteriaceae

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.<sup>15</sup> Resistance to colistin occur 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.<sup>32,33</sup> The other strategy is the overexpression of efflux-pump systems.<sup>34</sup> Another mechanism is overproduction of capsule polysaccharide.<sup>35–37</sup> No enzymatic mechanisms of resistance have been reported, but strains of *P. polymyxa* produce colistinase.<sup>38</sup>

## Intrinsic resistance mechanisms

Resistance to polymyxins occurs naturally in *P. mirabilis* and *S. marcescens* by modification of the LPS via cationic substitution. The mechanism of resistance in these species is linked to expression of the *arnBCADTEF* operon and the *eptB* 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 *P. mirabilis* contains L-Ara4N and the genome of this bacterium contains the *eptC* gene, which is mediated to the modification of LPS with PETN.<sup>39–41</sup> Putative loci in *P. mirabilis* include the *sap* operon encoding a transport protein, ATPase gene, and *O*-acetyltransferase gene, which take part in biosynthesis or transfer of amino arabinose.<sup>42</sup> Also, the existence of *rppA/rppB* TCS has been discovered to play a role in activation of the *arnBCADTEF* operon.<sup>43,44</sup> Similarly, this operon is responsible for intrinsic resistance to colistin in *S. marcescens*, as it has been shown that *arnB* and *arnC* mutants lead to a reduction in susceptibility to colistin (minimum inhibitory concentration [MIC] from 2,048 to 2  $\mu\text{g}/\text{mL}$ ) compared to the wild type.<sup>45</sup>

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.<sup>9,41,43</sup>



**Figure 1** Regulation and plasmid-mediated pathways of lipopolysaccharide modifications in Enterobacteriaceae.

### 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 untransferable via horizontal gene transfer.<sup>46-48</sup> Only one mechanism of resistance has been identified as a transferable mechanism (plasmid-mediated *mcr* gene) so far (Table 1).<sup>9,21</sup>

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 *pmrHFJKLM* operon;<sup>46,49</sup> regulatory two-component systems (TCSs), including PmrAB and PhoPQ, as well as *crrAB*, which regulates the PmrAB system;<sup>50-52</sup> the *mgrB* gene, a negative regulator of TCSs, including PmrAB and PhoPQ;<sup>53</sup> plasmid-mediated *mcr* genes;<sup>54,55</sup> and Cpx and Rcs as regulator of upregulation of capsule biosynthesis and activator of the efflux pump KpnEF regulating the PhoPQ system, respectively.<sup>8</sup>

### *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.<sup>56</sup> The addition of L-arabinoseamine (L-Ara4N) to the 1-phosphate or 4-phosphate group leads to colistin resistance.<sup>46</sup> Generally, L-Ara4N is connected to 4-phosphate and modifies it while PETN is connected to 1-phosphate.<sup>57,58</sup> The *pmrHFJKLM* operon (also named *arnBCDADTEF* or *pbgPE*) and PmrE synthesize L-Ara4N from uridine diphosphate glucuronic acid and fix it to lipid A.<sup>59,60</sup> The biosynthesis of L-Ara4N depends on the *pmr* (*arn*) operon.<sup>61</sup> 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.<sup>56,62</sup> On the other hand, low concentration of  $Mg^{2+}$  or  $Ca^{2+}$  leads to activation of phoQ.<sup>63,64</sup> PmrB activates PmrA by phosphorylation, and PmrA in turns activates regulation of the *pmrABC* and *pmrHFJKLM* operons and the *pmrE* gene. Subsequently, these operons and genes lead to LPS modification by adding PETN and L-Ara4N to lipid A.<sup>56</sup> 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	<i>E. coli</i>	<i>Kpneumoniae</i>	<i>Enterobacter</i> spp.	<i>Salmonella</i> spp.	<i>C. freundii</i>	<i>Protrus mirabilis</i>	<i>Serratia marcescens</i>	References
<i>pmrA/pmrB</i>	Modification of lipid A by <i>armBCADTEF</i> operon, <i>pmrC</i> and <i>pmrE</i> genes	+	+	+	+	-	-	-	65,68,69,129,130
<i>phoP/phoQ</i>	Modification of lipid A by activation of the <i>pmrHFJKLM</i> operon/activation of <i>pmrAB</i> by <i>pmrD</i>	+	+	+	+	-	-	-	50,78,131,132
<i>armBCADTEF</i>	Modification of lipid A by pEtN and L-4AraN	+	+	+	+	-	+	+	9,45,69,129-131
<i>mgrB</i>	Overexpression of <i>phoPQ</i> and activation of <i>pmrHFJKLM</i>	+	+	-	-	-	-	-	51,79
mutation									
<i>ramA</i>	Modulates lipid A biosynthesis	-	+	-	-	-	-	-	110
<i>crbB</i>	Modification of lipid A by upregulation of <i>pmrAB</i> /activation of the glycosyltransferase	-	+	-	-	-	-	-	52
mutation									
<i>mcr1</i>	Phosphoethanolamine transferase	+	+	+	+	+	-	-	85,94,101,133,134
<i>mcr2</i>	Phosphoethanolamine transferase	+	-	-	+	-	-	-	55,135
<i>mcr3</i>	Phosphoethanolamine transferase	+	-	-	+	-	-	-	102,136
<i>mcr4</i>	Phosphoethanolamine transferase	+	-	+	+	-	-	-	103,137,138
<i>mcr5</i>	Phosphoethanolamine transferase	+	-	+	+	-	-	-	104,139
<i>mcr6</i>	Phosphoethanolamine transferase	-	-	-	-	-	-	-	105
<i>mcr7</i>	Phosphoethanolamine transferase	-	+	-	-	-	-	-	106
<i>mcr8</i>	Phosphoethanolamine transferase	+	+	-	-	-	-	-	107
<i>acrB</i>	Phosphoethanolamine transferase	+	+	-	-	-	-	-	36
mutation	Efflux pump								
KpnEF	Efflux pump	-	+	-	-	-	-	-	117
mutation									
<i>sapABCDF</i>	Efflux pump	-	-	-	-	-	+	-	42
mutation									

*pmrABC* and *pmrFHJKLM* 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).<sup>65–69</sup>

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.<sup>21,63,64</sup> 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 *pmrFHJKLM* operon, followed by modification of lipid A;<sup>70,71</sup> and PhoP indirectly activates *pmrA* by bypassing the PmrD connector protein, subsequently activates the transcription of the *pmrHFJKLM* operon and synthesizes PETN, which transfers it to lipid A.<sup>72,73</sup>

Various of PETN-coding genes, such as *eptA* (*pmrC*), *eptB* (*pagC*), and *eptC* (*cptA*), are able to add PETN to different sites of LPS.<sup>74,75</sup> Mutation of the *phoP/Q* genes has been identified in *K. pneumoniae* and *E. coli* that led to acquired colistin resistance.<sup>65,67,76–78</sup>

The *mgrB* gene encodes a small transmembrane protein of 47 amino acids that exerts negative feedback on the PhoPQ TCS.<sup>79</sup> 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 *pmrHFJKLM* operon. Finally, production of L-Ara4N leads to modification of lipid A and colistin resistance.<sup>51</sup>

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*.<sup>67,80–82</sup> 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*.<sup>53,65,80</sup> 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.<sup>53,77</sup> Goulian et al showed that deletion of the *mgrB* gene led to upregulation of the PhoP-regulated gene in *E. coli*.<sup>79</sup>

### CrrAB two-component system

The *crrAB* operon encodes two proteins: CrrA as a regulatory protein and CrrB as a sensor kinase protein.

Wright et al described that mutation of *crrB* leads to colistin resistance in *K. pneumoniae*.<sup>83</sup> The mutated CrrB protein regulates a *crrAB*-adjacent gene that encodes a glycosyltransferase-like protein, which in turn leads to modification of lipid A.<sup>83</sup> 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).<sup>52</sup> However, mutation/inactivation of the *crrB* gene led to activation of the *pmrHFJKLM* 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.<sup>83</sup> 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.<sup>52</sup>

### 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.<sup>54</sup> 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.<sup>54</sup> 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.<sup>9</sup> It is worth noting that the production of *mcr1* leads to resistance to lysozymes.<sup>84</sup>

Following initial findings, *mcr1*-mediating transferable colistin resistance has been reported in several regions, including Europe, Asia, the Americas, and Africa.<sup>85–98</sup> 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.<sup>54,99</sup> 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.<sup>9</sup> The *mcr* gene has also been identified in Enterobacteriaceae isolates, which carry such carbapenemase genes as *bla*<sub>NDM1</sub>, *bla*<sub>NDM5</sub>, *bla*<sub>NDM9</sub>, *bla*<sub>OXA48</sub>, *bla*<sub>KPC2</sub>, and *bla*<sub>VIM1</sub>.<sup>97,100,101</sup>

Recently, Xavier et al reported a novel plasmid-mediated colistin resistance gene, known as *mcr2*, in *E. coli*.<sup>55</sup> Thereafter, *mcr3* and *mcr4* genes were discovered.<sup>102,103</sup> 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.<sup>104</sup>

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*.<sup>105</sup> 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.<sup>106</sup> They suggested that *mcr7*, like *mcr-3*, originated from *Aeromonas* spp.,<sup>102</sup> 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 *bla*<sub>NDM</sub>, which is a great concern.<sup>107</sup> It is notable that *mcr8* has existed for some time and disseminated among *K. pneumoniae*.<sup>107</sup> *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,<sup>55,102,103,108</sup> whereas the origin of *mcr5* remains unknown.<sup>104</sup> 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.<sup>109</sup>

### 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.<sup>110</sup> 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*.<sup>111</sup> 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.<sup>110</sup>

### Role of capsule in colistin resistance

The role of capsular polysaccharide (CPS) has been demonstrated to be protective against cationic antimicrobial peptides, including colistin.<sup>35</sup> *K. pneumoniae* is able to release CPS from its surface.<sup>112</sup> 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.<sup>8,113</sup> 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.<sup>35</sup> 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.<sup>46</sup> 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.<sup>114,115</sup>

### 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.<sup>116,117</sup> 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.<sup>8</sup> In *K. pneumoniae*, this pump is mediated by colistin resistance and other antibiotics, including ceftriaxone, erythromycin, and rifampicin.<sup>117</sup> 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.<sup>117</sup> On the other hand, AcrAB is a part of the AcrAB–TolC complex, which plays a role in colistin resistance. The AcrAB-mutant *E. coli* displays a eightfold increase in colistin susceptibility. It has

been remarked that expression of this pump's proteins is dependent on the PhoPQ TCS.<sup>118</sup> Finally, the *SapABCDF* operon encodes Sap proteins that are constitute of five proteins.<sup>118</sup> In the mutant of *P. mirabilis*, susceptibility to colistin is increased by mutation of the *SapABCDE* operon.<sup>42</sup> 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.<sup>119</sup>

## 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.<sup>120</sup> 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.<sup>121</sup> In addition, rates of cure, 14-day survival, and microbiological eradication are lower in monotherapy compared to combination therapy.<sup>121</sup> 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 prevent the development of colistin-resistant strains, which may improve clinical and microbiological outcomes.<sup>121–126</sup> The colistin–sulbactam combination was recommended against imipenem-resistant *A. baumannii*, particularly in colistin-resistant strains, due to its high in vitro synergistic activity,<sup>121,127</sup> which may be a more favorable combination. Colistin-based combinations with tigecycline, aminoglycosides, and rifampicin have shown synergistic activity against XDR strains,<sup>122,125,128</sup> but tigesycline is disadvantageous in bacteremic patients, because of its low plasma concentrations.<sup>128</sup> In addition, colistin–carbapenem combinations may be preferable in the treatment of *A. baumannii* infections to prevent resistance selection and to decrease the prevalence of *A. baumannii*.<sup>121</sup>

## 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.

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The authors report no conflicts of interest in this work.

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