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Molecular mechanisms of polymyxin resistance: knowns and unknowns

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ABSTRACT

Colistin, also referred to as polymyxin E, is an effective antibiotic against most multidrug-resistant Gram-negative bacteria and is currently used as a last-line drug for treating severe bacterial infections. Colistin resistance has increased gradually for the last few years, and knowledge of its multifaceted mechanisms is expanding. This includes the newly discovered plasmid-mediated colistin resistance gene *mcr-1*, which has been detected in over 20 countries within 3 months of its first report. We previously reported all of the known mechanisms of polymyxin resistance in our first review in 2014, but an update seems necessary in 2016, considering the significant recent discoveries that have been made in this domain. This review provides an update about what is already known, what is new, and some unresolved questions with respect to colistin resistance.

1. Introduction

The increase in antibiotic resistance is a major worldwide health problem. Specifically, severe infections caused by multidrug-resistant bacteria, especially carbapenem-resistant bacteria, as well as the lack of new antibiotics against Gram-negative pathogens have led to a reconsideration of old antibiotics. It is in this context that the use of colistin re-emerged, mainly for infections caused by multidrug-resistant Gram-negative bacteria [1]. Colistin belongs to the polymyxin family and is a polycationic antimicrobial peptide. Unfortunately, the increased and inappropriate use of colistin has led inexorably to the worldwide emergence of colistin-resistant bacteria [2–4].

In 2014, we reviewed all of the reported mechanisms described for polymyxin resistance in clinically important Gram-negative pathogens [5]. However, since then other novel mechanisms have been described, which further unravel the riddle of polymyxin (polymyxin B and colistin) resistance. This has necessitated an update on this subject. This mini-review provides an update about what is known and what is new in colistin resistance, and provides an insight into what is still unknown. It is hoped that this will further provide useful insights regarding which path(s) could be followed with respect to research in polymyxin resistance.

2. Overview of colistin resistance

Studies of the mechanisms of resistance showed that various chromosomal mutations were frequently responsible for acquired colistin resistance in *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and, recently, *Klebsiella pneumoniae* [6–8]. Most mechanisms conferring resistance to colistin are directed against modifications of the lipid A moiety of lipopolysaccharide (LPS), which is the primary target of colistin. Mechanisms that are associated with colistin resistance across different bacterial genera may be different, but more often than not the majority always follow the common pathway of lipid A modifications with 4-amino-4-deoxy-L-arabinose (L-ara4N) and/or phosphoethanolamine (PEtN).

3. Update on what is known about colistin resistance

3.1. Modification of lipid A or 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo) with L-ara4N

Addition of L-ara4N to lipid A is one of the main mechanisms leading to colistin resistance. The most frequent target of L-ara4N is the 4′-phosphate group of lipid A, but it can also be added to the 1-phosphate group or Kdo. The *arnBCADTEF* (also known as *pmrHFIJKLM*) operon synthesises L-ara4N from uridine diphosphate (UDP) glucuronic acid and subsequently transfers it to lipid A [5]. The operon is present in *Salmonella* spp., *K. pneumoniae*, *Escherichia coli* and *P. aeruginosa* but is absent in *A. baumannii* [5].

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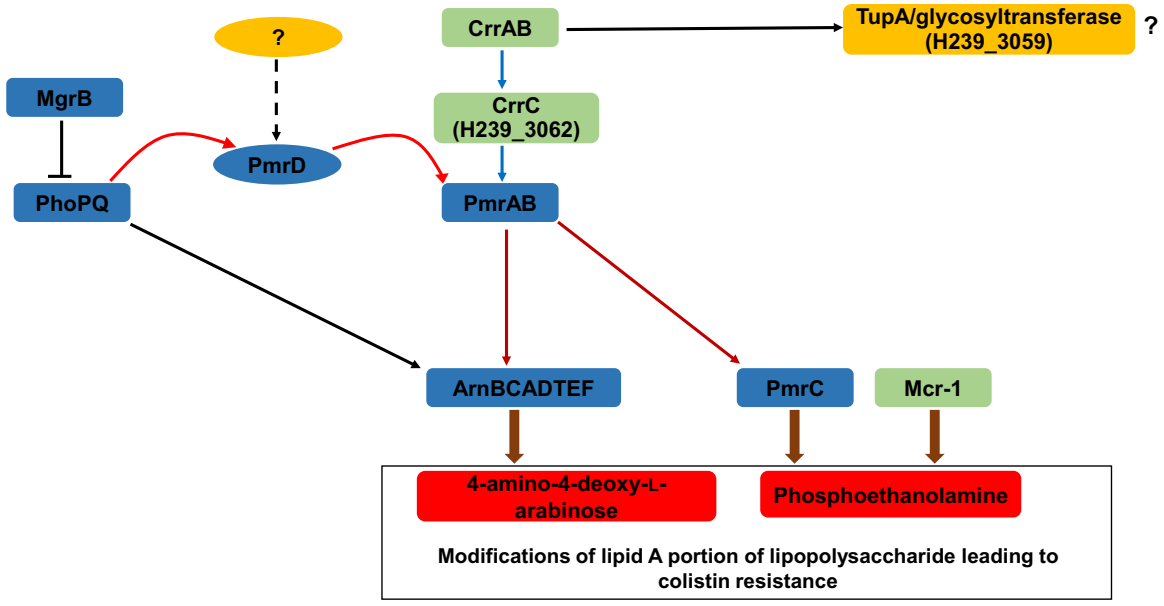

Fig. 1. Model for activation of the two-component systems (TCSs) for colistin resistance in bacteria. Activation of the TCSs, which are mostly, but not exclusively, mutations, stimulates the transcription of lipopolysaccharide (LPS) modification loci *arnBCADTEF* (*pmrHFIJKLM*) and *pmrC*, leading to the synthesis of 4-amino-4-deoxy-L-arabinose and phosphoethanolamine (PEtN), respectively. The recently discovered TCS CrrAB activates PmrAB but not PhoPQ via H239_3062 (now referred to as CrrC) with subsequent upregulation of *arnBCADTEF* and *pmrC*. In addition, a novel PEtN-encoding gene *mcr-1*, which modifies the lipid A of LPS, was also recently identified. All novel protein-encoding genes are shown in green. The missing links (genes) or pathways not yet fully understood are shown in yellow and dashed lines, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Mutations in the two-component systems (TCSs), mainly PhoPQ and PmrAB (Fig. 1), activate this operon in these bacteria with additional TCSs: ParRS, ColRS and CprRS in *P. aeruginosa*. Mutations in PmrAB have been found in *Salmonella* spp. [9,10], *P. aeruginosa* [11] and *A. baumannii* [12] and principally occur in *pmrB*. At the same time, mutations, including deletions in PhoPQ, have been shown to lead to colistin resistance/heteroresistance in *K. pneumoniae* [13–15] as well as resistance in *P. aeruginosa* [16–19] and *Salmonella* spp. [20], whereas this TCS is absent in *A. baumannii*.

Inactivation through numerous mutations (nonsense, missense, deletion, insertional inactivation) of *mgrB*, the negative feedback regulator of PhoPQ, is known to result in constitutive activation of PhoPQ, which thereafter directly activates upregulation of the *arnBCADTEF* operon by bypassing PmrD and PmrAB (Fig. 1) [2,8,21,22]. *mgrB* inactivation appears to be the most frequent colistin resistance mechanism found in *K. pneumoniae* and *Klebsiella oxytoca*, according to published data, with a prevalence between 25.5% and 86.4% based on different studies [2,8,23–27]. Of the 192 strains of *K. pneumoniae* and *K. oxytoca* that have been reported in the literature from 2013 to 2015 (December) with *mgrB* anomalies [2,8,23–33], 77 (40.1%) had an insertion sequence (IS) truncation, 56 (29.2%) had nucleotide deletion(s), 42 (21.9%) had missense or nonsense mutations, and 6 (3.1%) had lost the *mgrB* gene. Among insertions by IS elements, the IS*5* family is the most frequent element found to inactivate *mgrB* (45/77), followed by the IS*1* family (16/77). These ISs can insert in either the promoter or the coding region of *mgrB*, with most insertions occurring between nucleotides 74 and 75 [2,8,24,27,33] within *mgrB*. Stability assays showed that insertional inactivation by an IS was a stable phenomenon [33]. This has been demonstrated for IS*5*-like, IS*3*-like, IS*Kpn14* and IS*102* in different clones that are frequently encountered in clinical samples [33].

3.2. Modification of lipid A or Kdo with PEtN

Lipid A can also be modified by *ept* genes, which add PEtN to lipid A or Kdo. There are different variants of PEtN-encoding genes, such as *eptA* (*pmrC*), *eptB* (*pagC*) and *eptC* (*cptA*), and each is capable of adding PEtN to different parts of LPS [5,21,34]. *pmrC* is regulated by the PmrAB TCS, which itself can be upregulated by PhoPQ. Mutation in *pmrAB* is the main resistance mechanism found in *A. baumannii* [21,34,35], leading to *pmrC* activation and PEtN addition.

3.3. Other lipopolysaccharide modifications

Other genes involved in the LPS biosynthesis pathway have been described as potentially involved in polymyxin resistance. In *Proteus mirabilis*, a mutation in an O-antigen acetyltransferase-encoding gene prevents aminoarabinose fixation, leading to a decrease in colistin resistance. Loss of O-antigen by mutation of genes involved in O-antigen biosynthesis such as *rfaH* in *Yersinia enterocolitica* [36] or *naxD* in *Francisella* spp. [37] decreases polymyxin B resistance, as well as in the *K. pneumoniae* mutant lacking O-antigen [38].

Other regulators recently described that modulate lipid A biosynthesis include *ramA*, a global regulator that can modify the expression of over 68 genes including *lpxC*, *lpxL* and *lpxO*. This regulator is present in some Enterobacteriaceae such as *K. pneumoniae*, *Citrobacter* spp., *Enterobacter* spp. and *Salmonella* spp. The *ramA* locus consists of three genes (*ramA*, *romA* and *ramR*). The latter is a repressor of *ramA* and *romA*. Survival assays in *K. pneumoniae* showed that *ramA* alterations lead to a decrease of colistin susceptibility, suggesting that *ramA*-dependent regulation possesses an alternative pathway for increased resistance to polymyxins [39]. As previously mentioned [5], in addition to modification of lipid A with PEtN, concurrent glycosylation of the phosphate group of lipid A with galactosamine was reported both in laboratory-generated colistin-resistant *A. baumannii* as well as in clinical strains [36].

3.4. Complete loss of lipopolysaccharide

Complete loss of LPS is one of the two resistance mechanisms described in *Acinetobacter* sp. [40]. A mutation occurring in one of the first three genes involved in the lipid A biosynthesis pathway