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Themed Issue: Resurrection of old antibiotics

Resistance to polymyxins in Gram-negative organisms

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ABSTRACT

Polymyxins have recently been re-introduced into the therapeutic arsenal to combat infections caused by multidrug-resistant Gram-negative bacteria. However, the emergence of strains resistant to these last-resort drugs is becoming a critical issue in a growing number of countries. Both intrinsic and transferable mechanisms of polymyxin resistance have been characterised. These mechanisms as well as the epidemiological data regarding four relevant bacterial pathogens (*Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*) are considered in this review. A special focus is made on plasmid-mediated resistance and the spread of *mcr* genes.

1. Introduction

The gradual increase in antibiotic resistance that started in the 1970s among Gram-negative bacteria is becoming a critical global issue [1–3]. Multidrug-resistant (MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) strains of *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* are now reported worldwide. Interspecies transfer of mobile genes conferring resistance to broad-spectrum β-lactams and aminoglycosides are one of the factors accounting for the progressive erosion of antimicrobial activity both in the community and hospital settings [4–6]. Because of the current shortage of novel anti-infective drugs to combat infections caused by recalcitrant isolates, the polymyxins (colistin and polymyxin B) have been re-introduced into the therapeutic arsenal as last-resort drugs [7,8]. However, as the use of these polycationic agents is increasing to treat humans and animals, bacterial resistance has emerged in many parts of the world, leaving clinicians unarmed to treat patients.

Polymyxin resistance in Gram-negative bacteria is primarily due to post-translational modification of the lipopolysaccharide (LPS) molecules that form the outer layer of the outer membrane. In most resistant strains, substituents such as 4-amino-4-deoxy-L-arabinose (L-Ara4N), phosphoethanolamine (pEtN) or galactosamine are enzymatically added to the lipid A or the LPS core; alternatively, the LPS part of the outer membrane may be completely lost in some other isolates [9–13]. By decreasing the net negative charge of phosphate residues, these LPS alterations tend to prevent the binding of polymyxin molecules to the bacterial surface and their further penetration into the cell interior where they are supposed to exert their bactericidal activity. Expression of most of the genes of the LPS modification pathway is under the control of a variety of two-component systems (TCSs) such as PhoP–PhoQ (PhoPQ) and PmrA–PmrB (PmrAB). Each of these phosphorelays is composed of a transmembrane sensor histidine kinase (e.g. PhoQ, PmrB), which is subject to self-phosphorylation under specific stress conditions, and a cognate cytoplasmic response regulator (e.g. PhoP, PmrA), which when phosphorylated by the kinase in turn modulates the expression of target genes. Some mutations in the genes encoding these TCSs result in constitutive upregulation of the LPS modification pathway and thus polymyxin resistance because of membrane impermeability. Polymyxin resistance rates are still low in many countries but are increasing steadily in some others such as Greece and Italy [14]. However, the recent identification of a plasmid-borne colistin resistance gene (*mcr-1*) in human, animal and environmental strains of Enterobacteriaceae may potentially worsen this situation at the global scale [15]. Indeed, reports from all continents multiply on the isolation of *mcr-1*-positive strains [16]. The goal of the present review is to provide readers with the most recent mechanistic and epidemiological data on polymyxin resistance in human Gram-negative pathogens.

2. Intrinsic resistance mechanisms to polymyxins in Enterobacteriaceae

2.1. Escherichia coli

The lipid A of *E. coli* contains a β-1′-6-linked glucosamine disaccharide backbone phosphorylated at the 1′ and 4′ positions, which is decorated by six fatty acyl chains. It is now well established that addition of pEtN and L-Ara4N molecules to the LPS phosphate groups by enzymes EptA and ArnT, respectively, strongly increases the

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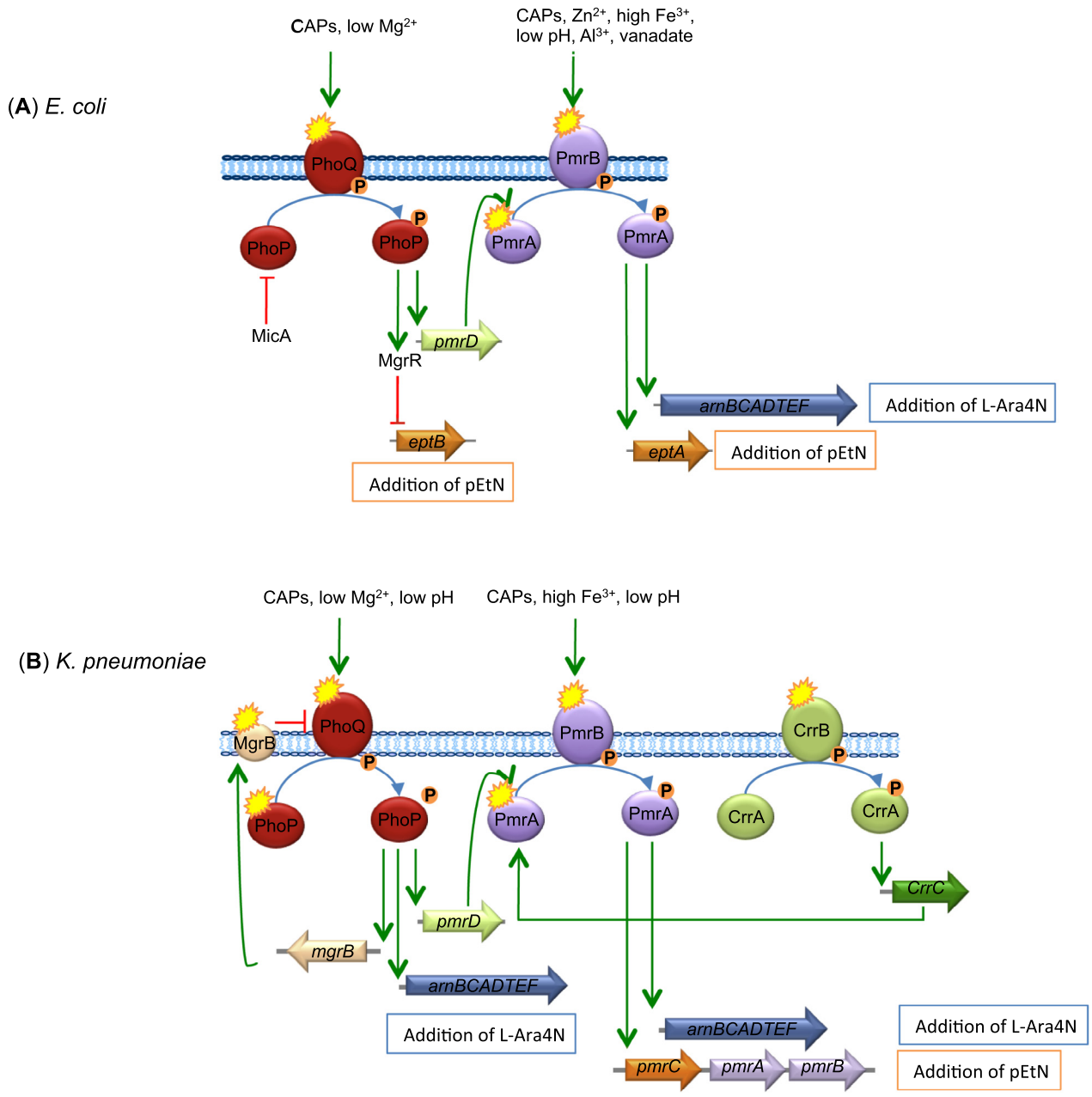

Fig. 1. Schematic representation of regulation of genes involved in polymyxin resistance in clinical isolates of (A) *Escherichia coli* and (B) *Klebsiella pneumoniae*. In both species, resistance to polymyxins is induced by cationic compounds such as colistin, low Mg^{2+} concentrations, acidic pH and high Fe^{3+} concentrations, which activate the two-component systems (TCSs) PhoPQ and/or PmrAB. Subsequent activation of operon *arnBCADTEF* (also called *pmrHFIJKLM*), the *eptA* gene or the *pmrC* gene triggers the synthesis and addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (pEtN) to lipid A, respectively. PmrAB is also activated by PhoPQ via the product of the *pmrD* gene. In *K. pneumoniae* (B), the *arnBCADTEF* operon can be directly activated by PhoP. In *E. coli* only (A), a first small RNA, MgrR, directly represses the expression of *eptB*, a gene required for addition of pEtN to the lipopolysaccharide (LPS) core, whilst a second small RNA, MicA, represses the *phoP* gene. In both *E. coli* and *K. pneumoniae* (A and B) clinical isolates, alterations (represented by yellow asterisks) in histidine kinases PhoQ and PmrB or in the response regulator PmrA lead to constitutive activation of the TCSs PmrAB or PhoPQ. Furthermore, in *K. pneumoniae* (B), inactivation of *mgrB* results in colistin resistance through activation of PhoPQ, whilst mutations in histidine kinase CrrB activate PmrAB through CrrC. CAPs, cationic antimicrobial peptides (including polymyxins). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

resistance of *E. coli* to cationic antimicrobial peptides (CAPs) including colistin [minimum inhibitory concentration (MIC) × 4- to 32-fold) [9]. Various membrane stress conditions are also known to trigger LPS modification through activation of the TCSs PhoPQ and PmrAB. The inner membrane sensor PhoQ is activated when bacteria grow in low Mg^{2+} environments or in the presence of CAPs, whereas PmrB senses molecular signals generated by exposure to CAPs, acidic pH, Zn^{2+}, Al^{3+}, vanadate (VO_3^-) or high Fe^{3+} concentrations (Fig. 1A) [17–21]. As mentioned above, mutations in operons *pmrAB* and *phoPQ* or several loci (*micA*, *mgrR*, *etk*) involved in complex regulatory pathways may potentially result in constitutive activation of the *eptA* and *arnT* genes [22] (Fig. 1A). Analysis of polymyxin-resistant *E. coli* strains of human (urine, stools) or animal (swine) origin revealed the occurrence of mutations in the genes *pmrA* (R81S), *pmrB* (T156K, A159V, G161V, +I92, Δ7–12) and *phoQ* (E375K) [23–25]. More surprisingly, some of these mutations seemingly emerged in the absence of colistin treatment, thus suggesting that factors other than the polymyxin itself might select for such mutants