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Resistance to polymyxins: Mechanisms, frequency and treatment options

Matthew E. Falagas[a,b,c,*], Petros I. Rafailidis[a,b], Dimitrios K. Matthaiou[a,d]

[a] Alfa Institute of Biomedical Sciences (AIBS), Athens, Greece
[b] Department of Medicine, Henry Dunant Hospital, Athens, Greece
[c] Department of Medicine, Tufts University School of Medicine, Boston, Massachusetts, USA
[d] Department of Medicine, "G. Gennimatas" General Hospital, Thessaloniki, Greece

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ABSTRACT

Polymyxins act by binding to lipid A moiety of the bacterial lipopolysaccharide and subsequently disintegrating the bacterial membranes. The most important mechanism of resistance includes modifications of the bacterial outer membrane structure, including lipopolysaccharide. Lipopolysaccharide modification is mostly mediated by PmrA/PmrB and PhoP/PhoQ two-component regulatory systems. These mechanisms exist with some differences in many gram-negative bacterial species. Resistance to polymyxins is generally less than 10%. In specific regions, such as the Mediterranean basin, Korea and Singapore, they tend to be higher. Heteroresistance to polymyxins is associated with exposure to polymyxins and especially suboptimal therapeutic dosage. Polymyxin combination regimens, tigecycline and fosfomycin may be useful options for the treatment of polymyxin-resistant gram-negative infections.

1. Introduction

Polymyxins include polymyxin B and colistin (polymyxin E), and are derivatives of the *Bacillus polymyxa* subspecies *colistinus*. They belong to a diverse group of natural antimicrobials found in eucariotic cells called cationic antimicrobial peptides. Structurally, they are decapeptides bound to a fatty acid chain. They consist of a seven-member cyclic ring of aminoacids with a tripeptide side chain. The side chain links to the lipidic part of the molecule. The heptapeptide ring is the same between the two polymyxins with the exception of a single aminoacid, which is phenylalalanine in polymyxin B and leucine in colistin (Kwa et al., 2007).

Polymyxins were discovered in the late 1940's and were widely used until the mid-1980's when they were forsaken due to the reported adverse events, namely nephrotoxicity. They remained in clinical practice for the management of pseudomonal lung infections in patients with cystic fibrosis and in topical solutions with other antimicrobials for the treatment of ear or eye infections. They reappeared as an option for the management of gram-negative infections (administration by the intravenous, and/or nebulized or intrathecal route) for non-cystic fibrosis patients after the emergence of multidrug-resistant pathogens and the subsequent restriction of possible alternatives (Falagas and Kasiakou, 2005). Despite their relatively recent reintegration in clinical practice, resistance to polymyxins constitutes already an issue of significance.

Polymyxins are active against gram-negative pathogens including *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella* spp., *Escherichia coli* and other enterobacteriaceae. However, there are species possessing intrinsic resistance, such as *Providencia* spp., *Neisseria* spp., *Proteus* spp., *Serratia marcescens* and *Burkholderia cepacia*. Polymyxins are not active against gram-positive bacteria nor against anaerobes.

2. Mechanism of action

Lipopolysaccharide (LPS) is a structural component of the bacterial outer membrane consisting of O antigen, a core polysaccharide and lipid A, which anchors in the outer membrane (Raetz and Whitfield, 2002). It bears negative charge and confers to the integrity and stability of the bacterial outer membrane. Polymyxins, having positive charge, displace Mg^{2+} or Ca^{2+} and bind on lipid A component resulting in the destabilization and disruption of the outer and inner membranes (Brown and Tsang, 1978; Clausell et al., 2007; Davis et al., 1971; Newton, 1956; Schindler and Osborn, 1979). The most potent part of the polymyxin molecule is the hydrophobic lipid tail. Polymyxin nonapeptide, which is the acyl part remaining after the removal of the lipidic component, exerts antimicrobial properties though to a lesser extent. It has lesser affinity than the hydrophobic tail, but sensitizes bacteria to the

* Corresponding author at: Alfa Institute of Biomedical Sciences (AIBS), 9 Neapoleos Street, 15 123, Marousi, Greece. Tel.: +30 694 611 0000; fax: +30 210 683 9605.
E-mail address: m.falagas@aibs.gr (M.E. Falagas).

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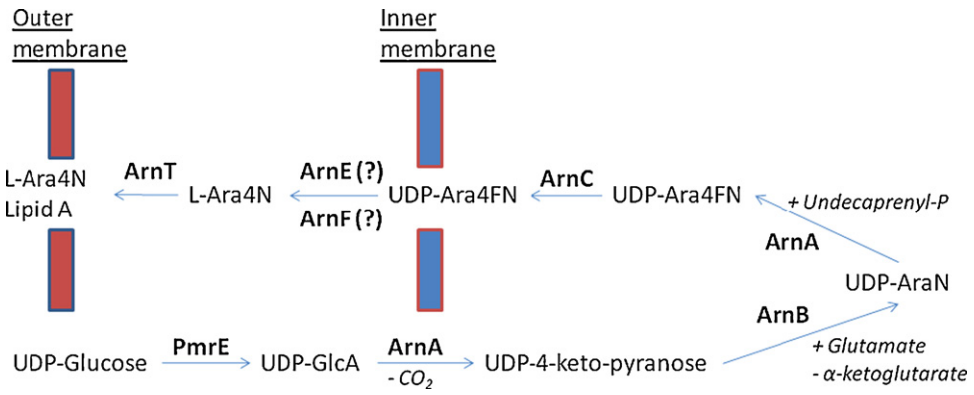

Fig. 1. Biosynthesis of L-Ara4N and modification of lipid A.

effect of other antibiotics and human neutrophils (Rose et al., 2000; Tsubery et al., 2000; Warren et al., 1985).

3. Mechanisms of resistance

Gram-negative bacteria may develop resistance through mechanisms that are common for colistin and polymyxin B. The most important mechanism involves modifications of the bacterial outer membrane, mainly through the alteration of the LPS moiety (Kline et al., 2008; Raetz and Whitfield, 2002). However, further modifications of the bacterial outer membrane may confer to polymyxin resistance (Campos et al., 2004; Moore et al., 1984). Another mechanism includes the development of an efflux pump/potassium system found in *Yersinia* spp. (Bengoechea and Skurnik, 2000). Although no enzymatic mechanisms of resistance has been reported so far, strains of *B. polymyxa* are known to produce colistinase (Ito-Kagawa and Koyama, 1980).

The modification of the LPS occurs with the addition of 4-amino-4-deoxy-L-arabinose (LAra4N) to a phosphate group in lipid A. This addition causes an absolute increase in lipid A charge, thus lowering the affinity of positively charged polymyxins (Kline et al., 2008).

The biosynthesis of LAra4N (Fig. 1) depends on the genes of polymyxin resistance operon, formerly known as *pmr*, which has been renamed as *arn* (Reeves et al., 1996). This operon includes *pmrHFIJKLM* genes. First, UDP-glucose is dehydrogenated to UDP-glucuronic acid (UDP-GlcA) by PmrE dehydrogenase. UDP-GlcA is subsequently decarboxylated and transaminated by ArnA(PmrI) and ArnB(PmrH) to UDP-AraN (Gatzeva-Topalova et al., 2005a, 2004). UDP-Ara4N is formylated by ArnA to UDP-Ara4FN and transferred to the bacterial inner membrane by ArnC(PmrF). UDP-Ara4FN is deformylated and transferred from the inner to the outer bacterial membrane by mechanisms that are not fully understood, although ArnE(PmrM) and ArnF(PmrL) may have a role in the transportation of UDP-Ara4FN across the bacterial inner membrane (Yan et al., 2007). Finally, ArnT(PmrK) tranfers LAra4N to lipid A (Gatzeva-Topalova et al., 2005b; Raetz and Whitfield, 2002). These genes were first studied in *S. enterica serotype Typhimurium* and *E. coli* (Gunn and Miller, 1996). However, similar pathways exist in other gram-negative bacteria, such as *P. aeruginosa*, *B. cepacia*, *Salmonella* spp., and *Yersinia pestis* (Breazeale et al., 2005).

The biosynthesis of LAra4N is mediated by PmrA/PmrB and PhoP/PhoQ two-component regulatory systems (Fig. 2). PmrB is a sensor cytoplasmic membrane-bound kinase with a histidine residue in its cytoplasmic domain. It is activated by high concentrations of Fe^{3+} or by low pH. Upon activation, it phosphorylates the aspartate residue of PmrA, which is a regulator protein of *arn* operon (McPhee et al., 2003). PhoQ is another sensor cytoplasmic membrane-spanning kinase, which is activated by low concentrations of Mg^{2+} or Ca^{2+} (Gunn and Miller, 1996; Moskowitz et al., 2004). Upon activation, it phosphorylates PhoP regulator, which in turn activates *pmrD* transcription. PrmD has a protective role by inhibiting PmrA dephosphorylation, thus conferring in the promotion of *arn* transcription (Fu et al., 2007). PmrA, in turn, exerts a negative feedback effect by repressing *pmrD* transcription under conditions that activate PmrA, such us high Fe^{3+} concentrations (Kato et al., 2003).

These mechanisms, although common in gram-negative bacteria, exhibit interspecies variation. PmrA/PmrB and PhoP/PhoQ systems, as described, are present in *Salmonella* spp. and were well studied in *S. enterica serotype typhimurium* (Gunn and Miller, 1996). In *Y. pestis*, PmrD is not present. Both systems may directly promote the transcription of *arn* operon (Winfield et al., 2005). In *E. coli*, although PhoP/PhoQ exists and promotes the transcription of *pmrD*, it does not activate PmrA/PmrB system (Winfield and Groisman, 2004). In *P. aeruginosa*, Mg^{2+} concentrations directly regulate PmrA/PmrB system (McPhee et al., 2003). Furthermore, *P. aeruginosa* biofilm cells exhibiting high metabolic activity were reported to develop tolerance to colistin, as they upregulated *arn* transcription (Pamp et al., 2008). In *A. baumannii*, resistance mechanisms are not well understood. However, mutations in the PmrA/PmrB encoding genes are linked to polymyxin resistance (Adams et al., 2009).

Further modifications of the bacterial outer membrane include the increased production of capsule polysaccharide (CPS) in *K. pneumoniae*. CPS limits the interaction of polymyxins with their target sites. Thus, upregulation of CPS productions confers to increased polymyxin resistance (Campos et al., 2004). Furthermore, increased levels of the outer membrane protein H1 inhibits the action of

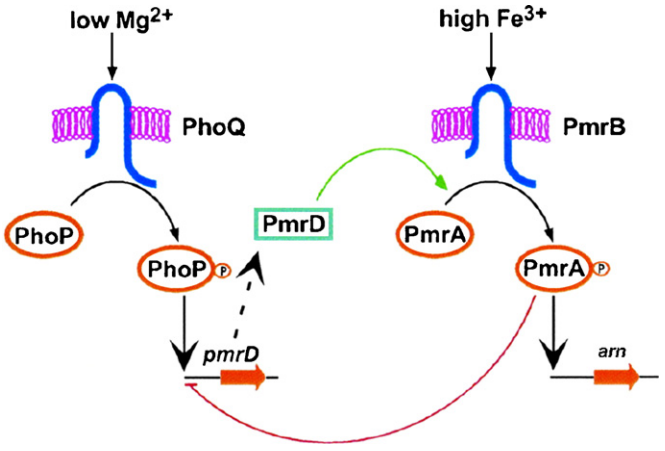

Fig. 2. PmrA/PmrB and PhoP/PhoQ two-component regulatory systems (Figure adapted from Kato A et al. PNAS 2003;100:4706-4711©).