



# Chromosomally encoded and plasmid-mediated polymyxins resistance in *Acinetobacter baumannii*: a huge public health threat

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## Abstract

*Acinetobacter baumannii* is an opportunistic pathogen associated with nosocomial and community infections of great clinical relevance. Its ability to rapidly develop resistance to antimicrobials, especially carbapenems, has re-boosted the prescription and use of polymyxins. However, the emergence of strains resistant to these antimicrobials is becoming a critical issue in several regions of the world because very few of currently available antibiotics are effective in these cases. This review summarizes the most up-to-date knowledge about chromosomally encoded and plasmid-mediated polymyxins resistance in *A. baumannii*. Different mechanisms are employed by *A. baumannii* to overcome the antibacterial effects of polymyxins. Modification of the outer membrane through phosphoethanolamine addition, loss of lipopolysaccharide, symmetric rupture, metabolic changes affecting osmoprotective amino acids, and overexpression of efflux pumps are involved in this process. Several genetic elements modulate these mechanisms, but only three of them have been described so far in *A. baumannii* clinical isolates such as mutations in *pmrCAB*, *lpxACD*, and *lpsB*. Elucidation of genotypic profiles and resistance mechanisms are necessary for control and fight against resistance to polymyxins in *A. baumannii*, thereby protecting this class for future treatment.

**Keywords** Outer membrane · Lipid A · Phosphoethanolamine transferase · Efflux pumps · Osmoprotective amino acids · *mcr*

## Introduction

*Acinetobacter baumannii* is a Gram-negative, non-glucose-fermenting, oxidase-negative coccobacillus, most commonly related to healthcare-associated infections [1–3]. It is an opportunistic microorganism that causes several clinical complications in immunocompromised individuals such as pneumonia, bacteremia, meningitis, endocarditis, cellulitis, and uri-

nary tract and soft-tissue infections [4–6]. In hospital settings, *A. baumannii* mainly affects patients in mechanical ventilation of intensive care units (ICUs). It is noteworthy that this microorganism has been responsible for one in five cases of ventilator-associated pneumonia in Europe [7] and 20% of ICUs infections in the USA [8].

The rapid capacity to develop antimicrobial resistance through various intrinsic and acquired mechanisms is notable in *A. baumannii* [2, 9, 10]. In British and American ICUs, more than 25 and 30% of *A. baumannii* isolates, respectively, are resistant to at least three classes of antimicrobials, being considered multidrug resistant (MDR) [11, 12]. Furthermore, in Asia and Eastern Europe countries, higher rates of resistance are observed, with 48 to 85% being MDR [13, 14]. In fact, *A. baumannii* is commonly associated with resistance to ureidopenicillins, cephalosporins (including extended-spectrum drugs), fluoroquinolones, aminoglycosides, and carbapenems [15, 16]. Carbapenems antibiotics are used as the primary option to treat severe MDR Gram-negative bacterial infections [15, 17]. However, carbapenemase-producing *A. baumannii* has increased rapidly on a global scale and are considered to be significant health threats. Thus, confronting

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the problem associated with carbapenem-resistant *A. baumannii*, the biomedical community has revived the use of polymyxins [9, 18, 19].

Isolated in 1947 as secondary metabolites of *Paenibacillus polymyxa* (anteriorly *Bacillus polymyxa*), the polymyxins showed a potent bactericidal effect against several Gram-negative species [20, 21]. They are a cyclic decapeptide consisting of a heptapeptide ring and a tripeptide side chain that attaches to a fatty acid. At physiological pH (7.2), five amino acid residues of the decapeptide portion are positively charged. Thus, the polymyxins can bind to the negative surface of lipopolysaccharide (LPS) that form the outer membrane (OM) of Gram-negative bacteria [21, 22]. After the ionic interaction, the fatty acid chain is inserted inside the OM, disrupting the bacterial membranes and resulting in cellular death [23]. In clinical settings, polymyxin B and colistin (polymyxin E) are frequently used; they have similar biological activities but differ structurally, with D-phenylalanine in polymyxin B replaced by D-leucine in colistin [24]. Until the 1960s, polymyxins had been the most popular therapeutic option for severe infections with Gram-negative bacteria, but their use has been restricted since 1970 because of the significant nephrotoxicity and neurotoxicity associated with prolonged treatment [25].

The re-boosted of polymyxin use increases the selective pressure that favors the resistant strains. Currently has already been reported low sensitivity to colistin and polymyxin B between *Acinetobacter* spp. [19], *Pseudomonas* spp., and Enterobacteriaceae species [26, 27] from several regions of the world. Also, has increased the frequency of infections caused by microorganisms intrinsically resistant to this class, such as *Proteus* spp. and *Serratia* spp. [28]. Moreover, the emergence of a plasmid-carried gene (*mcr*), associated with moderate colistin resistance, makes this scenario even more adverse [29]. Accordingly, the present review summarizes the most up-to-date knowledge about chromosomally encoded and plasmid-mediated polymyxins resistance in *A. baumannii* as well as characterizes the biochemical mechanisms that underlie this phenomenon.

## Chromosomally encoded resistance to polymyxins in *A. baumannii*

### Outer membrane changes

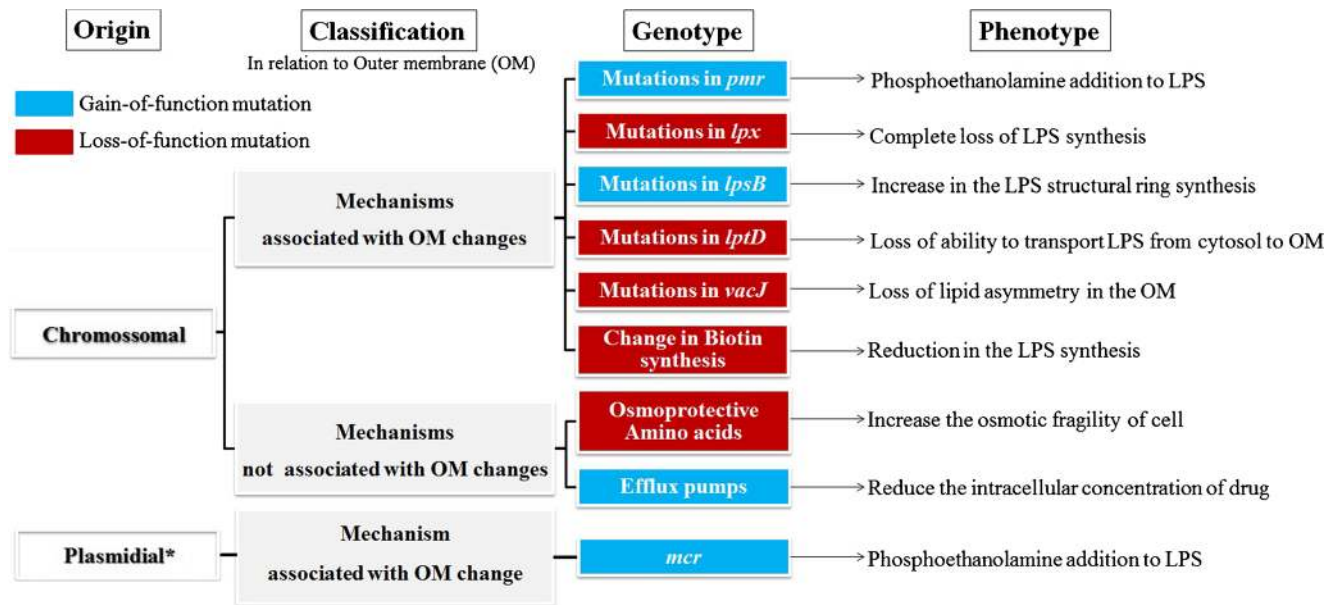
Gram-negative outer membrane (OM) is the molecular target of polymyxins action. Thus, alterations in this component can compromise the bactericidal effect and reduce the microorganism susceptibility to the drug in question [29–34]. The mechanisms underlying polymyxin resistance are complex, but in *A. baumannii*, the mutations that affect the OM stand out [35, 36]. Four different polymyxins resistance

mechanisms have been previously reported to this species, being (i) modification of lipid A structure by the addition of phosphoethanolamine, (ii) complete loss of LPS via mutations in the genes that synthesize lipid A, (iii) reduction in the expression of cofactors involved in LPS synthesis, and (iv) downregulation of proteins that participate in the export and/or stabilization of OM precursors (Fig. 1).

The first mechanism is associated with mutations in *pmrA* and *pmrB* genes [31, 37–40]. They encode a two-component regulatory system that controls the expression of the *pmrC* gene, which encodes lipid A phosphoethanolamine transferase [38, 41], and of the *pmrF* operon, responsible for the expression of the enzymes involved in 4-deoxy-aminoarabinose biosynthesis (Ara4N) [15]. Modification of lipid A, a component of LPS, by the addition of Ara4N or/and phosphoethanolamine, protects the OM from binding and action of polymyxins [42]. The lipid A phosphates are esterified by these metabolites decreasing so the repulsion between adjacent LPS molecules into Gram-negatives OM. Hence, a more compact and lower negatively charged LPS layer is formed, which shows reduced sensitivity to the positively charged polymyxins [43]. However, it should be highlighted that Ara4N biosynthesis is not present in *A. baumannii* [24] and *N. meningitidis* [15] since the operon *pmrF* is absent in these species. Of note, *N. meningitidis* is intrinsically resistant to colistin, suggesting that *pmrC* modulation may be associated with polymyxin B and colistin resistance in *A. baumannii* [44].

Function gain mutations in *pmrA* and/or *pmrB* are associated with high rates of colistin resistance in *P. aeruginosa* [45] and *Salmonella* spp. [46]. Adams et al. (2009) showed the first evidence that the PmrAB two-component system is involved in polymyxins resistance in *A. baumannii*. Initially, they found that the partial deletion of *pmrB* in a colistin-resistant (CoR) *A. baumannii* results in reversion to a colistin-sensitive (CoS) phenotype [31]. Arroyo et al. (2011) [38] demonstrated that partial removal of *pmrC* results in an increase in the sensitivity in CoR *A. baumannii*, with a decrease in polymyxin B minimum inhibitory concentration (MIC) from 4 to 0.25  $\mu\text{g mL}^{-1}$ . The positive regulation of *pmrC* induced by mutations in *pmrAB* is so efficient that specific genetic alterations affecting one or both genes generate a 26- to 292-fold increase in the level of *pmrC* expression.

Mass spectrometric analyses have confirmed that isolates with mutations in *pmrAB* showed the phosphoethanolamine addition in a hepta-acylated lipid A. However, according to Beceiro et al. (2011) [37] even with increased expression of *pmrA* (12.4-fold increase) and *pmrB* (6.8-fold increase) in CoR *A. baumannii*, the amount of the *pmrC* transcription may remains unchanged, suggesting that PmrAB-independent systems are associated with regulation of *pmrC* in *A. baumannii*. Also, little is known about the regulation of PmrAB two-component system in this species. In other Gram-negative bacilli, such as *Salmonella enterica*, *Pseudomonas*



**Fig. 1** Representation summarizing the mechanisms underlying the resistance of *Acinetobacter baumannii* to polymyxin B and colistin.\*The plasmidial mechanism (*mcr* gene) is still not identified in *A. baumannii*

*aeruginosa*, and *Klebsiella pneumoniae*, the PmrAB two-component system is directly or indirectly modulated by the PhoPQ system [47]. However, the absence of the PhoPQ component in *A. baumannii* points to another regulatory factor [15]. Thus, more studies in the direction of elucidating the mechanism(s)/factor(s) that modulate the expression of the *pmrA* and *pmrB* genes, as well as those targeted to describing the regulation *pmrAB*-independent of *pmrC* gene, are important for understanding the dynamics of resistance to polymyxins in *A. baumannii*.

Resistance due to lipid A modifications by a mutation in PmrAB two-component system has a relatively low biologic cost, especially concerning *A. baumannii* virulence [48–50]. Additionally, the sensitivity patterns of amikacin, piperacillin-tazobactam, ciprofloxacin, azithromycin, cefepime, gentamicin, minocycline, tigecycline, ampicillin, and teicoplanin is similar between CoR-*pmrB* mutant and wild-type *A. baumannii* [48, 49]. CoR-*pmrB* mutant strains also develop cross-resistance to antimicrobial that constitutes the innate immune response of host cells, such as LL-37 and lysozyme [50]. These studies suggest that there is a low fitness burden associated with the development of polymyxins resistance via *pmrAB* mutation (Table 1) [19, 58].

The clinical importance of mutations in the *pmrCAB* operon has been demonstrated in several studies (Table 2) [18, 19, 37, 38, 40, 55, 56, 59–62]. Most clinical isolates have shown mutations in the *pmrB*, but mutations in *pmrA* and *pmrC* have also been observed. Substitutions represent the most frequent mutation type for all three proteins, with only two deletions ( $\Delta 32$ –35 and  $\Delta 160$ ) [38] and one insertion (A163) [19] identified, both involving PmrB. The most frequent substitutions

in PmrB were found to be P233S, P360Q, A226V, L208F, and A138T. However, one study revealed that CoS *A. baumannii* also carries substitutions in PmrB, such as A138T and A226V, suggesting that only these mutations may be unrelated to colistin resistance in this species [18]. In PmrA, a highly conserved mutation, with the substitution of proline by histidine at position 102, was observed in 14 isolates of CoR *A. baumannii* [55] and in one bacterium with resistance induced in vitro [31].

Moffatt et al. (2010) confirmed the resistance to polymyxins in *A. baumannii* attributed to the interference with OM synthesis [32]. They have found that a full inactivation of the lipid A biosynthetic genes—*lpxA*, *lpxC*, or *lpxD*—results in a complete loss of surface LPS in *A. baumannii*. Thus, the loss of LPS prevents the essential interaction between it and polymyxins, giving rise to very high colistin MICs. For example, a loss of LPS after the deletion at position 90 of LpxA protein showed a 130-fold increase in colistin MIC. In this context, the transformation of the *A. baumannii* mutant with a wild-type *lpxA* restored the CoS phenotype, reducing the MIC to  $1 \mu\text{g mL}^{-1}$  [32]. Insertion sequences (IS) affecting the Lpx system have also been identified [51, 52]. The insertion of IS element Aba11 into *lpxC* and *lpxA* genes is associated with high resistance to colistin ( $\text{MIC} > 128 \mu\text{g mL}^{-1}$ ) [51] and polymyxin B ( $\text{MIC} > 32 \mu\text{g mL}^{-1}$ ) [53]. In addition, the presence of the ISAbal25 element within *lpxA* in a strain with resistance induced by contact with polymyxin B in vitro was demonstrated in a recent study [52].

Contrasting with the mutations that affected the PmrAB two-component regulatory system, the LPS loss showed a high biologic cost, which limited its spread in clinical

**Table 1** Main characteristics of mechanisms of resistance associated with loss/reduction of susceptibility to polymyxins in *Acinetobacter baumannii*

Gene(s)	Phenotype	Colistin/polymyxin B resistance (MIC range)	Virulence	Susceptibility to other antibiotics	Clinical isolated with the genotype	References
<b>Mechanisms related to outer membrane</b>						
<i>pmrCAB</i>	Addition of phosphoethanolamine to outer membrane	Low to high: 4–128 $\mu\text{g mL}^{-1}$	Maintained	Lower or unchanged <sup>1</sup>	Most frequent	[31, 37–40]
<i>lpxACD</i>	Loss of lipopolysaccharide (LPS)	Moderate to high: 16–> 128 $\mu\text{g mL}^{-1}$	Substantially reduced	Bigger <sup>2</sup>	Less frequent	[32, 48, 49, 51–54]
<i>lpsB</i>	Upregulation of the glycosyltransferase that synthesizes the structural ring of LPS	High: 128 $\mu\text{g mL}^{-1}$	Probably increased	NE	Rare	[30, 55, 56]
<i>lptD</i>	Loss of LPS translocase	Low: 4 $\mu\text{g mL}^{-1}$	Substantially reduced	Bigger <sup>3</sup>	NI	[57]
<i>vacJ</i>	Loss of outer membrane asymmetry	High: > 256 $\mu\text{g mL}^{-1}$	Probably reduced	NE	NI	[33]
Locus AIS_0807	Reduction in biotin synthesis	Low	NE	NE	NI	[30]
<b>Mechanisms not related to outer membrane</b>						
Several Locus <sup>&amp;</sup>	Reduction in the biosynthesis of osmoprotective amino acids	Low	NE	NE	NI	[30]
<i>EmrA-like</i> (Locus AIS_1773) and <i>EmrB-like</i> (Locus AIS_1772)	Expression of EmrAB efflux pump	Low	NE	NE	NI	[34]
<i>tig2C</i>	Expression of Tig2C efflux pump	High: > 256 $\mu\text{g mL}^{-1}$	NE	NE	NI	[33]

NI yet none identified, NE not evaluate

<sup>1</sup> For amikacin, piperacillin-tazobactam, ciprofloxacin, azithromycin, ceftazidime, gentamicin, minocycline, tigecycline, ampicillin, and teicoplanin

<sup>2</sup> For tetracycline, ceftazidime, azithromycin, amikacin, gentamicin, piperacillin-tazobactam, meropenem, ciprofloxacin, rifampicin and vancomycin

<sup>3</sup> For fusidic acid, novobiocin, azithromycin, rifampicin and ciprofloxacin

<sup>&</sup> Locus AIS\_3185, AIS\_1142, AIS\_1143, AIS\_2793, AIS\_2454, AIS\_2023, and AIS\_2024, AIS\_3025

**Table 2** Mutations identified in clinical isolates of polymyxin-resistant *Acinetobacter baumannii* in different countries of the world

Country	Period	Clinical specimen	Polymyxins MIC ( $\mu\text{g mL}^{-1}$ )	Mutations	References
Spain	2001–2008, SENTRY	Bronchoalveolar lavage	64 <sup>a</sup>	PmrB: D64V, L208F	[38]
	2001–2008, SENTRY	Ventricular catheter	64 <sup>a</sup>	PmrC: F90L PmrA: S119T PmrB: P233S; P360Q	[38]
USA	2009	NI/NS	32 <sup>a</sup>	PmrB: F387Y, S403F	[37]
	2001–2008, SENTRY	Blood	1*: 32 <sup>a</sup> 2*: 32 <sup>a</sup> 3*: 16 <sup>a</sup>	1*: PmrB: A226V 2*: PmrB: L208F 3*: PmrB: N256I	[38]
	2001–2008, SENTRY	Pleural fluid	16 <sup>a</sup>	PmrB: R263C, P377L	[38]
Israel	2001–2008, SENTRY	Bronchoalveolar lavage	16 <sup>a</sup>	PmrB: P170Q	[38]
	2001–2008, SENTRY	Bronchoalveolar lavage	8 <sup>a</sup>	PmrC: H499R PmrB: $\Delta$ 32–35, P360Q	[38]
Brazil	2001–2008, SENTRY	Wound	32 <sup>a</sup>	PmrC: T7I PmrB: A211V, $\Delta$ 160	[38]
	2007	Bronchoalveolar lavage	16 <sup>b</sup>	LpxC: N8S, D45N, K130R, S147R, D287N. LpxD: Y6F, E47D, H49Y, D51H, V55A, Y61F, A66T, I98L, S99T, T101K, T118A, V138I, N148D, R170G, I175V, and S178N.	[59]
France	2001–2008, SENTRY	Blood	128 <sup>a</sup>	PmrB: A80V, P170L	[38]
UK	2010	Tracheal lavage	NI/NS	PmrB: E8D	[40, 60]
	2008	Wound	4 <sup>a</sup>	PmrB: M145K	[37]
Saudi Arabia	2009	Bronchoalveolar lavage	32 <sup>a</sup>	PmrB: L87F	[37]
	2006	Blood	128 <sup>a</sup>	PmrB: S14L	[37]
Greece	2009	Blood	16 <sup>a</sup>	PmrB: P233S	[37]
Greece	2011–2013	Several	NI/NS	1*: PmrB: P360Q 2*: PmrB: A138T; A226V 3*: PmrB: Q129L; A138T; A226V	[61]
	2012–2014	Several	16–64 <sup>a,c</sup>	PmrA: G54E PmrC: R109H	[18]
	2008	Wound	128 <sup>a</sup>	PmrB: P233S <i>lpsB</i> : Stop codon to 241K	[55, 61] [55, 61]
Algeria	2009	Bronchoalveolar lavage	32 <sup>a</sup>	PmrB: P170L	[19]
Algeria	2014	Bronchoalveolar lavage	16 <sup>a</sup>	<i>pmrB</i> : Insertion of A163	[19]
Malaysia	2011	NI/NS	4–128 <sup>b,d</sup>	PmrA: P102H <sup>c</sup> . LpxC: K141R and S158R. LpxD: S102T; V141I; R173G; T104K, I178V; T121A; N151D; G169S; E50D; T15A; G186S; S181N. LpsB: H181Y.	[55]
South Korea	2011–2012	1* Bronchoalveolar lavage	1–3. 32– $\geq$ 64 <sup>a</sup>	1–3. <i>lpxA</i> : $\Delta$ 776	[9]
		2* Sputum			
		3* Cerebrospinal fluid			
Italy	2010	4* Wound Bronchoalveolar lavage	4. $\geq$ 64 <sup>b</sup> 128 (two isolates)	4. <i>lpxA</i> : $\Delta$ 776 and insertion 732 PmrB: P233S (two isolates)	[56]

NI/NS not identified or not showed, MIC minimum inhibitory concentration

\*Each number represented one *A. baumannii* clinical isolate differ

<sup>a</sup> MIC for colistin

<sup>b</sup> MIC for polymyxin B

<sup>c</sup> The study evaluates 86 clinical isolates of colistin-resistant *A. baumannii*

<sup>d</sup> The study evaluates four clinical isolates of colistin-resistant *A. baumannii*

<sup>e</sup> All 14 isolates evaluated showed the mutation

environments. The growth rate ( $\mu$ ) during the exponential phase, for example, was found to be significantly lower in  $\Delta$ *lpxA* ( $\mu = 0.85 \pm 0.09$ ),  $\Delta$ *lpxD* ( $\mu = 1.03 \pm 0.09$ ), and  $\Delta$ *lpxC* ( $\mu = 0.49 \pm 0.03$ ) *A. baumannii* in relation to the wild-type ( $\mu = 1.56 \pm 0.27$ ) [54]. Similarly, strains with induced resistance to colistin yield less biomass at the end of

16 h of growth in Mueller-Hinton broth [49] and the capacity for adhesion and formation of biofilms is also compromised under static and dynamic conditions [56]. The virulence of *A. baumannii* *lpxACD* mutant is widely affected such shown by the viability of human lung alveolus cells (A546), which is significantly lower after exposure to strain wild-type. Similar



results were obtained in in vivo infection models [48, 54]. Survival rates of mice [54] and invertebrates *Caenorhabditis elegans* [54] and *Galleria mellonella* [48] were found to be considerably lower after infection with wild-type or *pmrAB* mutants when compared with strains without LPS. Additionally, the loss of LPS alters the host's immune response [63]. Exposure of RAW264.7 macrophages to LPS-deficient *A. baumannii* causes smaller activation of NF- $\kappa$ B and TNF- $\alpha$  [63], as well as increases the sensibility to organic antibacterial components, such as LL-37 [63] and lysozyme [64]. Indeed, it has been observed that MICs for teicoplanin, cefepime, azithromycin, amikacin, gentamicin, piperacillin/tazobactam, meropenem, ciprofloxacin, rifampicin, and vancomycin are considerably lowered after LPS loss in *A. baumannii* (Table 1) [32, 48, 64].

In this direction, few resistant isolates to polymyxins with genetic alterations involving *lpx* have been recovered (Table 2). In clinical strains, different molecular events, such as deletions, point mutations, or insertions, can inactivate any of the first three genes (*lpxA*, *lpxC*, and *lpxD*) in the lipid A biosynthetic pathway of *A. baumannii*. Substitutions are the most frequent genetic events in *LpxC* and *LpxD* [47, 55]. However, concerning *lpxA*, insertions (position 732) and deletions (position 776) related to low sensitivity to colistin and polymyxin B stand out. These mutations alter the size of primary sequences of proteins involved in LPS pathway, making them enzymatically inactive and unable to synthesize lipid A [9].

Recently, four more genes (*lpsB*, *lptD*, *vacJ*, and Locus of biotin synthesis) were shown to have a role to polymyxins resistance in *A. baumannii* (Table 1). These novel resistance mechanisms are poorly known, but some studies are trying to elicit their role in colistin and polymyxin B resistance in this Gram-negative bacillus. The *lpsB* gene contributes to the protection of *A. baumannii* from cationic antimicrobial peptides because it encodes the glycosyltransferase responsible for LPS structural ring synthesis [65, 66], which is directly associated with lower fluidity and higher osmotic resistance of the OM [67]. Time-kill curve study indicates that  $\Delta$ *lpsB* -*A. baumannii* have higher sensitivity to LL-37 and colistin than the wild-type strain [30]. Also, the survival rate is lower after the pulmonary infection of animals with  $\Delta$ *lpsB* strains [30]. These data suggest that mutations associated with overexpression of this gene may contribute to the resistance and virulence of *A. baumannii*. In clinical isolates, polymyxin resistance induced by mutations in *LpsB* has been described (Table 2) [55, 56]. Substitution of a histidine for a tyrosine at position 181 of *LpsB* was found in eight clinical isolates from Malaysia [55]. Another study revealed that the premature add of a stop codon in *lpsB* is also associated with high resistance to colistin (MIC 128  $\mu$ g mL<sup>-1</sup>) but with a reduced ability to form biofilms [56].

The Lpt system component LptD, which is responsible for the insertion of LPS into the OM [57, 68], is also involved with polymyxins resistance in *A. baumannii*. Experimental removal of LptD results in moderate polymyxin B resistance, low virulence, and an increase of sensitivity to antibiotics non-polymyxins [57, 67]. The growth kinetics of  $\Delta$ *lptD* -*A. baumannii* is characterized by an extensive log phase, a slower proliferation rate, a decrease in the exponential phase, and low cell density in the stationary phase [57]. Additionally, the sensitivity to fusidic acid, novobiocin, azithromycin, rifampicin, and ciprofloxacin were found to be higher in  $\Delta$ *lptD* strains than in  $\Delta$ *lpxC* [67]. The accumulation of LPS components into a bacterial cell in  $\Delta$ *lptD* strains impairs the membrane stability and consequently reduces the fitness of *A. baumannii*. Consequently, the pharmacological inhibition of *lpxC* with the compound CHIR-090 as well as antagonism of enzymes  $\beta$ -ketoacyl-ACP synthases I and II by cerulenin, which is essential for the LPS biosynthesis, partially or completely recovered the fitness in *lptD*-mutant *A. baumannii* [57, 69].

A recent study has revealed that the loss of OM asymmetry is also involved in reducing the colistin susceptibility of *A. baumannii*. In several Gram-negatives species, the Vps/VacJ ATP-binding cassette (ABC) transporter system is proposed to function in maintaining the lipid asymmetry of OM, which ensures that the LPS remains on the outer face and phospholipids on the inner face [70]. Nhu et al. (2016) have shown that *A. baumannii* with a single mutation in *VacJ* (R166N) shows a highly colistin-resistant phenotype (MIC > 256  $\mu$ g mL<sup>-1</sup>) [33]. However, several studies have shown that *VacJ* is essential for the virulence of Gram-negative pathogens such as *Shigella flexneri* [71, 72], *Campylobacter lari* [73], *P. aeruginosa* [74], *Actinobacillus pleuropneumoniae* [75], and *Haemophilus parasuis* [76]. Additionally, resistance to phenol in *Pseudomonas putida* [77], to paraquat in *Campylobacter jejuni* [78], to ceftriaxone in *S. enterica* serovar *Typhimurium* [79], and to tetracycline, chloramphenicol, and ciprofloxacin in *P. aeruginosa* [74] are mediated by *vacJ*. Nonetheless, due to the importance of virulence and resistance factor in several Gram-negative bacteria, *VacJ* may be necessary for the fitness of *A. baumannii*, but this remains to be elucidated.

The levels of biotin are also an essential factor related to the susceptibility to polymyxins in *A. baumannii*. It is an important co-factor of lipid metabolism, being that the acetyl-CoA carboxylase complex, which catalyzes the conversion of acetyl-CoA into malonyl-CoA, a rate-limiting step in fatty acid synthesis, only is active when binding to biotin [66]. Higher biotin levels cause increased production of lipid A and increase sensitivity to colistin. Thus, the removal of genes that synthesize this co-factor is related to a reduction in the sensitivity of *A. baumannii* to colistin [30]. Herein, it has been shown that the removal of the A1S\_0807 locus, which

contains genes responsible for the synthesis of biotin, significantly reduces the sensitivity of *A. baumannii* to colistin [30].

### Changes in osmoprotective amino acid metabolism

Some amino acids such as proline, glycine, and aspartate are essential for the balance of solutes in prokaryotic cells. They participate as organic osmolytes and are biosynthesized to a higher degree after exposure of the bacterial cell to conditions of osmotic stress [80, 81]. Therefore, it is expected that quantitative modifications of these amino acids influence microbial sensitivity to compounds that induce osmotic fragility, such as polymyxins (Fig. 1).

According to Hood et al. (2013) [30], after colistin resistance induced in medium supplemented with NaCl (150 mM), *A. baumannii* shows a considerable reduction in the biosynthesis of osmoprotective amino acids. Possibly, this result is due to the negative regulation of genes involved in the production of proline from glutamate and in the metabolism of aspartate. On the other hand, mutations that compromise expression of the enzymes associated with aspartate catabolism, e.g., diaminobutyrate-2-oxoglutarate transaminase, may raise rates of resistance to colistin [30]. Additionally, it has been reported that alterations in genes that are related to biosynthetic feeder pathways of these amino acids, such as those involved in the maintenance of the tricarboxylic acid cycle, also contribute to colistin resistance [30]. Thus, an increase in the synthesis or a reduction in the catabolism of osmoprotective amino acids makes bacterial cells less susceptible to polymyxin-induced lysis (Table 1).

### Efflux pumps

In *A. baumannii*, four categories of efflux pumps are related to antimicrobial resistance, including resistance-nodulation-division (RND), major facilitator (MF), multidrug-toxic compound extrusion (MATE), and small multidrug resistance (SMR) families [82–84]. The ErmAB protein, an efflux pump belonging to the MF family, has been attributed to MDR in several Enterobacteriaceae [85–87]. Some strains of *E. coli* that harbor plasmids containing genes *emrA* and *emrB* show high resistance to antimicrobial detergents [86, 87]. Since polymyxins have amphipathic characteristics and behave similarly to other biological detergents, there is a possibility that this system may be implicated in resistance to the antimicrobials in question (Fig. 1).

To clarify the relationship between the efflux system and the polymyxin resistance in *A. baumannii*, a study has conducted a genomic analysis and revealed the presence of four pairs of genes named *emrA*-like/*emrB*-like in this species. It is noteworthy that the removal of sequence *emrB*-like results in increased sensitivity to colistin in *A. baumannii*, with a reduction in the MIC in a dilution. Time-kill curve studies have also

revealed that the loss of *emrAB* is associated with worse survival of *A. baumannii* after 4 h in plates containing  $1 \mu\text{g mL}^{-1}$  of polymyxin B [34]. In support to the evidence for the role of the EmrAB efflux system in resistance to polymyxins in *A. baumannii*, a strain with laboratory-induced resistance showed a 1.6-fold increase in the expression level of *emrB*-like [34]. These data validate the involvement of EmrAB-like efflux pumps in the decrease of sensitivity to polymyxins in *A. baumannii*; however, the clinical importance of this mechanism remains to be elucidated.

In some microorganisms such as *Pseudomonas putida*, the *tgt2C* gene encodes an efflux pump involved in tolerance to toluene [88]. A recent work showed that in *A. baumannii* with colistin resistance induced in vitro, the substitution of asparagine by methionine at position 104 in Tgt2C is associated with high resistance ( $\text{MIC} > 256 \mu\text{g mL}^{-1}$ ) [33]. This result suggests that *tgt2C* may encode other transporters and promotes efflux of polymyxins, but further research is needed to test whether these antibiotics are in fact substrates of this efflux system.

### Plasmid-mediated resistance to polymyxins in *A. baumannii*

Resistance to colistin in *A. baumannii* was originally chromosomal, which limits its rapid distribution and dissemination [30–32]. However, a plasmid-borne gene, called *mcr-1*, was identified in *Escherichia coli* of animal, human, and environmental origin from China in 2015 [29]. Subsequently *mcr-1.2*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5* variants were also identified [89–93].

The *mcr* genes encode a phosphoethanolamine transferase that leads low to moderate polymyxin resistance ( $\text{MIC}$  from 4 to  $16 \mu\text{g mL}^{-1}$ ) [15]. Structurally, the *N*-terminal region of the enzyme encoded by *mcr* is inserted into the inner membrane, while its *C*-terminal domain continues into the periplasmic space. The latter process allows the addition of phosphoethanolamine resulting from the cleavage of phosphatidylethanolamine in the 3-deoxy-d-manno-octulosonic acid residue of LPS [94, 95].

The diversity of plasmids harboring *mcr* described in Enterobacteriaceae on different continents shows high potential for dissemination of this gene [30]. By the time of this review, *mcr* has been identified in *E. coli*, *K. pneumoniae*, *Salmonella* spp., *Shigella sonnei*, *Klebsiella* (anteriorly *Enterobacter*) *aerogenes*, *Enterobacter cloacae*, *Cronobacter sakazakii*, *Kluyvera ascorbata*, *Citrobacter freundii*, and *Moraxella* spp. [96–99], but in vitro studies also revealed the possibility of gene acquisition from *K. pneumoniae* to *P. aeruginosa* by transformation [100]. In *A. baumannii*, there is still no report of *mcr*-positive isolates but, the rapid dissemination of this gene as well as the real possibility of non-glucose-fermenting Gram-negative bacilli

(e.g., *P. aeruginosa*) to acquire *mcr* from Enterobacteriaceae suggest that this is only a matter of time [33]. Thus, the intensification of surveillance studies is imperative for control of the dissemination of *mcr* and for protection of the class of polymyxins, which are still an important therapeutic option for the treatment of *A. baumannii* extremely-drug-resistant (XDR) infections [15].

## Conclusion remarks

Currently, polymyxin-resistant *A. baumannii* represents less than 1% of clinical isolates, but they pose a significant challenge to public health authorities [101]. Polymyxins are the last pharmacological resource available to treat infections caused by XDR *A. baumannii*. Unfortunately, lineages with low sensitivity to polymyxins have increased in many parts of the world, especially in Europe, Asia, and South America [2, 19]. This fact suggests that the loss of polymyxins to drug resistance seems to be inevitable in the future. Although chromosomal mutations mediate the polymyxin resistance in *A. baumannii*, the emergence of plasmid-mediated *mcr*, which may be transferable between bacterial species and increasing rates of polymyxin resistance in carbapenem-resistant bacteria, is of great concern. Although our understanding of the mechanisms and occurrence of polymyxin resistance has increased in recent years, we know very little about the impact of the different mechanism in the clinic. Thus, reinforcing the detection of polymyxin-resistant isolates must be encouraged so that we can better understand the impacts of each mechanism and outline more effective control measures in each case.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

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