Molecular mechanisms of polymyxin resistance and detection of \textit{mcr} genes

Patrik Mlynarcik, Milan Kolar

Antibiotic resistance is an ever-increasing global problem. Major commercial antibiotics often fail to fight common bacteria, and some pathogens have become multi-resistant. Polymyxins are potent bactericidal antibiotics against gram-negative bacteria. Known resistance to polymyxin includes intrinsic, mutational and adaptive mechanisms, with the recently described horizontally acquired resistance mechanisms. In this review, we present several strategies for bacteria to develop enhanced resistance to polymyxins, focusing on changes in the outer membrane, efflux and other resistance determinants. Better understanding of the genes involved in polymyxin resistance may pave the way for the development of new and effective antimicrobial agents. We also report novel in silico tested primers for PCR assay that may be able distinguish colistin-resistant isolates carrying the plasmid-encoded \textit{mcr} genes and will assist in combating the spread of colistin resistance in bacteria.

Key words: polymyxin, colistin, resistance, LPS

INTRODUCTION

Constantly increasing antibiotic resistance is a global health problem. In particular, serious infections caused by multi-resistant bacteria, especially carbapenem-resistant bacteria, as well as the lack of new antibiotics against gram-negative pathogens, have led to the revival of older antibiotics. In this context, the use of colistin has been reintroduced, especially in infections caused by multidrug-resistant gram-negative bacteria. Unfortunately, increased and disproportionate use of colistin has led to the emergence of colistin-resistant bacteria worldwide. However, the presence of resistant bacteria to colistin may also occur without any prior exposure to colistin\(^1\). Polymyxins represent a family of antimicrobial cyclic oligopeptides produced by the gram-positive organism \textit{Bacillus polymyxa}. However, only polymyxin B and polymyxin E (colistin) are suitable for clinical use. They act both on the outer and on the cytoplasmic membrane, resulting in loss of integrity in the membrane. Recently, alternative and less characterized mechanisms of action of polymyxins, as well as some bacterial resistance to these antibiotics have been described\(^2\). The reviewed literature shows a number of published studies on polymyxin resistance. In this review, we cover current knowledge on polymyxin resistance mechanisms in bacteria with regard to changes in the outer membrane and efflux. However, we also focus on other polymyxin resistance determinants with unclear and unknown function which may prove to be important components of resistance. Further, we tried to design primers for the detection of all previously described colistin-resistance genes (\textit{mcr}-1 to -7) and their variants.

Overview of polymyxin resistance

Known resistance mechanisms include intrinsic, mutational and adaptive, but recently horizontally acquired resistance has also been described\(^3,4\). The major polymyxin in resistance mechanisms include (i) alteration of the lipopolysaccharide (LPS) moiety, resulting in a reduction of the net LPS negative charge; (ii) mutations in genes; (iii) increased drug efflux; (iv) reduced porin pathway, (v) formation of capsules and (vi) enzymatic inactivation of antibiotic (colistin). However, other mechanisms of polymyxin resistance have also been described (e.g. antioxidative defense mechanisms, hyper-vesiculation).

Electrostatic repulsion of polymyxins by modification of cell surface

The most common polymyxin resistance mechanisms in different pathogenic bacteria are associated with modification the phosphate groups of lipid A with amine substituents, such as 4-amino-4-deoxy-L-arabinose (L-Ara4N) and phosphoethanolamine (PEtn), which are regulated by the two-component systems (TCSs) PhoP/PhoQ and PmrA/PmrB regulatory system. The addition of L-Ara4N and PEtn reduces the net negative charge of bacterial surface and limits its interaction with polymyxins, which ultimately results in increased resistance to polymyxins\(^5,6\). It has been reported that cross-talk between the PhoPQ and PmrAB systems exists in \textit{Salmonella enterica} and is mediated by the protein PmrD which is induced by phosphorylated PhoP (ref.\(^8\)). Recently, it was observed that missense mutations in CrrB is linked to colistin resistance in \textit{Klebsiella pneumoniae} isolates. In this context, CrrC was required to activate PmrAB and thereby connecting
This study described different resistance mechanisms to polymyxin B in various bacteria. For degenerate primers, R = A or G; S = G or C; Y = C or T. Table 1. Sequences of primers used for PCR for detection of mcr genes and their variants.

| Primer name | Sequence (5’ to 3’ direction)* | Target | Length (bases) | Amplicon size | Tm (°C) | Ref. |
|-------------|---------------------------------|--------|---------------|---------------|--------|------|
| MCR-1F      | GTCGATACCCGCGAAATACC            | MCR-1/2/-6 | 19          | 559 bp         | 52     | This study |
| MCR-1R      | GTCTTTTGGTTGCAAAGGC             |        | 19          |               |        |      |
| MCR-2F      | TGATGTTTCTCAAYATGGG             | MCR-3/-7 | 21          | 416 bp         | 54     | This study |
| MCR-2R      | C GATGTTTCTCATAGGTGT            |        | 23          |               |        |      |
| MCR-3F      | GATCGGAGCTGTTTCTG              | MCR-4  | 19          | 380 bp         | 52     | This study |
| MCR-3R      | GGGCGACCATGTAATACA             |        | 19          |               |        |      |
| MCR-4F      | GCTGYAAAGCGCCTGTYGA            | MCR-3/-5 | 19          | 382 bp         | 54     | This study |
| MCR-4R      | TGACTGGARACGTASAGCA           |        | 19          |               |        |      |

*For degenerate primers: R = A or G; S = G or C; Y = C or T.

Other mechanisms related to surface structural changes, acylation and deacylation of lipid A, influence resistance to different types of antimicrobial peptides. These changes have been shown to alter the properties of the outer-membrane permeability barrier. It was shown that activation of PhoPQ system in K. pneumoniae with polymyxin B stimulates PagP (crcD in E. coli) involved in acylation of lipid A (ref. 29). In addition, PagP-like gene, rep in Legionella pneumophilia, also conferred resistance to polymyxin B (ref. 29). In Vibrio cholerae, K. pneumoniae, Escherichia coli and Salmonella Typhimurium, the acylation of lipid A has been shown to be regulated by lpxM (formally msbB or waaA). Inactivation of these genes resulted in a lack of L-Ara4N modification and in a significant decrease in polymyxin resistance 22-25. Furthermore, other genes such as Bmul_2133 and Bmul_2134 have been shown to contribute to polymyxin B resistance in Burkholderia multivorans through alterations in the OM permeability 26. It has been proposed that Bmul proteins appear to confer polymyxin B resistance by the mechanism of hopanoid (analogues of eukaryotic sterols) biosynthesis involved in maintaining membrane fluidity and permeability 27. Moreover, it was observed that genes involved in staphyloxanthin (virulence factor) biosynthesis confer resistance to polymyxin B in S. aureus. The resistance to polymyxin B in this case is derived from its sta-
### Table 2. Strategies used by bacteria to achieve resistance to polymyxins.

| Resistance mechanisms                                         | Genes/determinants involved                                      | Bacteria                                                                 | Ref. |
|---------------------------------------------------------------|------------------------------------------------------------------|-------------------------------------------------------------------------|------|
| **Electrostatic repulsion**                                   | L-Ara4N and PEtn modification of lipid A                        | pmrAB, pmrD, phoPQ, parRS, mcr                                           | 4-8, 10-12 |
| **Activation of LPS-modifying operon by mutations in two-component system** | crrB (crrC)                                                    | Klebsiella pneumoniae                                                    | 9    |
| **D-Ala modification of lipoteichoic acid**                   | greXSR, dra/dlt operon, ltaSR, ciaR                             | Staphylococcus aureus, Bordetella pertussis, Streptococcus gordoni, Listeria monocytogenes, Group B Streptococcus | 13-18 |
| **Unclear**                                                    | outer membrane protein (Omb)                                     | Salmonella enterica                                                     | 19   |
| **Membrane fluidity/permeability**                            | Acylation of lipid A                                            | pagP, lpxM, rec                                                         | 20-25|
| **Putative hopanoid and staphyloxanthin biosynthesis**        | Bmul_2133/Bmul_2134, genes involved in staphyloxanthin biosynthesis | Pseudomonas aeruginosa, Burkholderia multivorans, Staphylococcus aureus | 26, 28|
| **Surface and membrane remodelling**                          | Capsule production                                              | siaD, cps operon, ompA, kpnEF, phoPQ, rcs                               | 20, 29-32, 34 |
| **Alterations in membrane composition**                       | virB, subB<sub>ac</sub>, bvrRS, eps-C-N, cgh, vacJ, waalA, rfbA, ompW, micF, pilMNOPQ operon, parRS, rmaA, brea, ydeI (omdA), ompD (npmc), ygiW (visP), ompF, rcs | Neisseria meningitidis, Klebsiella pneumoniae, Salmonella enterica | 10, 12, 33, 34, 37-47, 49, 50, 52-54 |
| **Alterations of membrane integrity**                         | cas9, tracrRNA, scaRNA, Lol, TolQRA                             | Franciscella novicida, Acinetobacter baumannii                          | 55, 56 |
| **LOS and LPS modifications**                                 | sgpM, pgm, hldA, hldD, opfH, cj1136, waalF, lgtF, gafT, cstH, galU, lic1, lic2A, lpsA, lgtF, opsX, firA, lpxO | Stenotrophomonas maltophilia, Vibrio fischeri, Burkholderia cenocepacia, Escherichia coli, Pseudomonas aeruginosa, Salmonella Typhimurium, Campylobacter jejuni, Haemophilus influenzae | 57-65, 68, 69, 71 |
| **Loss of LPS**                                                | lpxACD, lptD                                                    | Acinetobacter baumannii                                                 | 72, 73 |
| **Efflux and transport**                                      | Mex pumps, AdeABC, HlyD                                         | Pseudomonas aeruginosa, Acinetobacter baumannii                        | 74, 55 |
| **Transport**                                                 | trkA                                                            | Vibrio vulnificus                                                       | 75   |
| **Other polymyxin resistance determinants with known, unclear and unknown function** | bacA, sip, pbs, type IV pilI, w genes, yieM, sodC, sodB, katA, pilB, yidB/pmrc/pagB/pmrf, PA1199, PA2583, PA5548, PA2928, whpZ, PA4041, PA1938, pgm, surA, tolB, gnd, PA0401, pyrB, pdsB, sucC, tepA, arroB, pyrd, mfl, hypothetical protein (rmlD homolog), ampR, iptC, amgS, galU, lptC, wapR, ssg, cgt, kdaA/kdaB, fopC, pta/C/S, ebsA, topA, fshA, gdpP, fabT, sfnH, agaS, manL/N, clpX, deoB, hpt, tls, gmk, nanH, guaA, nupP, ptsI, luxR, fba, ugtL, virK, mirg-14, pgtE, asmA, pbgP, mglB, glqQ-like, feoC, hflc, pitA, rpoE, RCAL2831, mscD, lytB, hpaJ, genes encoding putrescine, yceI, yqjABEF operon, spa, craP, cpxP, micA, rhyB, pgmA, grxD, low pH, extracellular DNA, lack of magnesium/phosphate/ion ions, JSG945, JSG946, JSG948, YPTB0331-0332-0333 and other genes | Brucella ovis, Thermus thermophilus, Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa, Burkholderia cenocepacia, Salmonella enterica, Group B Streptococcus, Brucella suis, Helicobacter pylori, Shevannella oneidensis, Franciscella novicida, Streptococcus pyogenes, Yersinia pseudotuberculosis, Yersinia pestis, Proteus mirabilis, Yersinia enterocolitica, | 3, 26, 28, 38, 43, 49, 61, 77-87, 89, 90, 92, 93, 95, 96, 98-104, 106-111, 113-117 |
**Fig. 1. Proposed polymyxin resistance mechanisms in bacteria.** PhoP-PhoQ, PmrA-PmrB, ColR-ColS, CprS-CprR, ParS-ParR and CbrA-CbrB two-component systems confer bacterial resistance to polymyxin. Diagram of gram-negative cellular envelope, which shows typical inner and outer double layer separated by a periplasm (OM, outer membrane; PS, periplasmic space; CM, cytoplasmic membrane). The outer layer of the outer membrane contains a lipopolysaccharide (LPS), which is anchored to the membrane by the LPS lipid A. The inner layer of the outer membrane and also the entire inner membrane are composed only of phospholipids and the two bilayers can contain a variety of different types of membrane proteins. The figure shows different stimuli that induce various membrane and cytoplasmic proteins which then positively or negatively regulate other proteins. PhoPQ two-component system is activated (green arrow) by cationic antimicrobial peptides (CAMP), low Mg\(^2+\), low pH, low MgCl\(_2^+\), low CaCl\(_2^+\), extracellular DNA and mutations (denoted by red-colored star symbols) in \(\text{phoP} / \text{phoQ}\). PhoPQ controls many genes required for LPS modification and alteration of the cell surface. These include \(\text{pmrD}\), \(\text{pagL}\), \(\text{mgrR}\), \(\text{mgtA}\), \(\text{ugtL}\), \(\text{virK}\), \(\text{mig-14}\), \(\text{pgtE}\), \(\text{ydeI}\), \(\text{pagP}\), \(\text{lpxO}\) and \(\text{cps}\) operon. The activation of this two-component system mediates acylation, deacylation and hydroxylation of lipid A regulated by genes encoding the enzymes Pag, PagL and LpxO, respectively. The expression of EptB (phosphoethanolamine transferase) is suppressed (red line) with MgrR, while EptB is associated with the phosphoethanolamine addition to 3-deoxy-D-manno-oct-2ulosonic acid (KDO). The lipid A is further modified by \(\text{mgtA}\). Transcription of the genes \(\text{ugtL}\), \(\text{virK}\), \(\text{mig-14}\) and \(\text{pgtE}\) participate in the mediation of polymyxin resistance and have functions associated with the alteration of the bacterial envelope. PhoPQ, as well as PmrAB, are required for the induction of \(\text{ydeI}\) which contributes to polymyxin resistance through its interaction with an outer membrane porin OmpD/NmpC. Additional \(\text{cps}\) genes are required for synthesis of polysaccharide capsule and be involved in the phosphorylation of Ugd. By contrast, MgrB, BirR and MicA apply negative feedback to the PhoPQ regulatory system. With the help of PmrD, CbrA and in the presence of CAMP, Zn\(^{2+}\), high Fe\(^{3+}\), low pH, Al\(^{3+}\), vanadate, low MgCl\(_2^+\), low CaCl\(_2^+\) and extracellular DNA, transcription of PmrA-activated gene is induced by PmrAB system. PmrA-P activates transcription of LPS modification loci (i.e., \(\text{wzz}\), \(\text{naxD}\), \(\text{cpta}\), \(\text{pmrg}\), \(\text{ydeI}\), \(\text{pmrc}\) and \(\text{arnBCADTEF-ugd}\)), except for \(\text{lpxR}\), which is downregulated. Synthesis of the O-antigen is controlled by the \(\text{wzz}\) gene products. The \(\text{NaxD}\) and \(\text{LpxR}\) proteins are responsible for the deacylation of lipid A. However, MicF causes downregulation of LpxR synthesis, which is associated with deacylation of lipid A. The initiation of transcription of \(\text{lpxR}\) may play a role in resistance to polymyxin, but its effect on the resistance has not yet been demonstrated. Further, the Cpta protein and PmrG regulate the phosphorylation modification of heptose-I and heptose-II residues on the LPS core, respectively. Likewise, PmrR inhibits the activity of \(\text{lpxT}\), which phosphorylates lipid A. Additionally, phosphorylated PmrA activates the \(\text{arnBCADTEF-ugd}\) and \(\text{pmrc}\) genes, which modify LPS with 4-amino-4-deoxy-L-arabinose (L-Ara4N) and lipid A with PEtn, respectively. Moreover, also mutations in \(\text{pmrA}\), \(\text{pmrB}\) and \(\text{crrB}\) genes through CrrC result in the activation of PmrA, which subsequently upregulates \(\text{pmrc}\) and \(\text{arnBCADTEF-ugd}\). Alternatively, \(\text{pmrc}\) (eptA) can be activated by the ColRS two-component system, mutations in pmrAB or acidic growth conditions. ParR can be phosphorylated and...
controls its regulatory network in response to polymyxins and indolicidin. The ParRS system controls the expression of the porin (oprD), efflux (mexXY-oprM) and LPS modifying (arnBCADTEF-ugd, pmrAB operon). In addition, the arnBCADTEF-ugd can be activated via mutations in the pmrAB and parRS, etk (required for phosphorylation of Ugd), acidic growth conditions and other two-component systems, such as CprRS and CbrAB. However, expression of arnBCADTEF-ugd is negatively regulated by ColRS. CprRS is able to sense CP-26/-28/-29, polymyxins, Bac2A, CRAMP, indolicidin, HHHC6, IDR-1018 and HH17. Further, Zn²⁺ and nitrogen/carbon sources trigger activation of ColRS and CbrAB, respectively, whereas the CbrA protein is also able to induce the oprH-phoPQ operon. Mutations within genes lpxACD and iplD involved in lipid A biosynthesis and assembly of LPS in the outer leaflet of the outer membrane are associated with LPS loss, respectively. These results show that polycationic antibiotics (colistin and polymyxin B) and other factors are capable of promoting the expression of different two-component system and the mexXY operon and coordinated downregulation of the oprD gene by activation of ParRS, which ultimately leads to multiple resistance. In our model LPS represents the major barrier to binding of polymyxins. Further, siaD, ompA, cps, kpnEF, phoPQ and rsc, participate in capsule production leading to an increase in polymyxin resistance. Catalase, KatA, and other detoxifying enzymes, SodB and SodC, are also associated with resistance to polymyxin through their antioxidant defense mechanisms, whereas the vieM gene can mediate resistance by inducing outer membrane vesiculation. Recently, a mobile phosphoethanolamine transferase gene, mcr, has been associated with colistin resistance. It indicates resistance due to modifications of the phosphate groups of lipid A in LPS.

Surface and membrane remodelling

Further cell wall alterations, particularly capsule production, changes in membrane composition, and LOS and LPS modifications, are related with the development of polymyxin resistance. It has been found that the production of capsular polysaccharide or capsule is responsible for resistance to polymyxin B in Neisseria meningitidis and K. pneumoniae. However, synthesis of polysaccharide capsule was regulated by siaD, OmpA and cps operon (wca) (ref.28). In this context, further study showed that PhoPQ is necessary for polymyxin B-triggered induction of cps operon in K. pneumoniae29. Interestingly, a multidrug efflux pump kpnEF mutant showed a defect in capsular synthesis, indicating the direct involvement of KpnEF in capsule synthesis32. In addition, Rcs system (regulator of capsule synthesis) has been described to contribute to polymyxin B resistance in S. enterica for its role in regulating the expression of gene ydeI (ref.31,34). Furthermore, it has been found that the expression of cps operon and ugd is regulated by the Rcs system34, whereas the strain with expressed RcsA transcriptional regulator is able to synthesize colanic acid35. The effect of the capsules in this case lies in increasing electrostatic interaction between capsule polysaccharides and polymyxins, the binding of the cationic polymyxins to the anionic polysaccharides of the capsule, thereby reducing the amount of peptides reaching the bacterial surface and reducing its bactericidal activity36.

With respect to the changes in membrane composition, virB has been implicated in cationic peptide polymyxin B resistance of Brucella ovis and Brucella melitensis through a mechanism that involves modification of cell surface which is achieved by the down-regulation of Omp25/Omp31 family and regulation of type IV secretion system37,38. Other genes such as subB, bvrRS two-component regulatory system, epsCN, cgh (choloyglycine hydrolase), waaL, rbhA, vacJ and ompW, have been shown to contribute to polymyxin B or colistin resistance in many pathogens through alterations in the OM composition39,40,41. It has been found that various environmental signals such as high temperature, oxidative stress, or salicylate have an effect on the expression of porins through micF regulation42. MicF expression has been connected with downregulation of OM porin OmpF mRNA, which contributed to polymyxin B resistance in S. enterica43. In P. aeruginosa, it was found that the ParRS system affects polymyxin B and colistin resistance through down-regulation of the porin (OprD) (ref.10,12). In this context, it has been demonstrated that oligosaccharide/oligonucleotide binding fold (OB-fold) proteins YdeI (OmdA) and YgiW (VisP), and porins [OmpD (NmpC in E. coli) and OmpF] contribute to polymyxin B resistance in S. enterica by cell wall remodelling (interaction between general porins and OB-fold proteins blocks antibiotic entry) or it is also likely that increase antibiotic export33,50. However, the main feature of general porins, for example OmpF in E. coli, is to create a size-selective defined channel for the diffusion of hydrophilic molecules with a certain priority of molecules with charges which are the opposite of the amino acids that line the channels31. It has been also demonstrated that the pilMNOPQ operon encoding components of the type IV pilin secretion system in N. meningitidis contributes to polymyxin B resistance32. In addition, in P. aeruginosa, the small RNA-binding protein RsmA is associated with polymyxin B and colistin resistance through its involvement in the type three secretion system (TTSS) (ref.25). It is believed that pilin secretion apparatus may be the entry gate for several structurally different antimicrobial agents. Moreover, the type III and IV pilin secretion system is involved in the regulation of the delivery of proteins or DNA through the bacterial cell envelope. Further, a mechanism has been described by which B. melitensis maintains a low level of phosphatidyl-ethanolamine in the cell wall by expression of the BveA phospholipase A1 enzyme34. This property of the cell envelope contributes to polymyxin resistance as well as to persistence in the infected host. It has been suggested that BveA is important because it is capable of preventing the formation of pore-like structure and the permeabilization of the cytoplasmic membrane by polymyxin.

Recently, Cheah et al. have described perturbation of the membrane in polymyxin-treated A. baumannii through over-expression of protein complexes involved in mem-
brane homeostasis, namely Lol lipoprotein transport complex and the ToiQRA transmembrane complex. This supports findings related to the reduced integrity and barrier function of the remodelled OM in A. baumannii treated with polymyxin. Further, the genes cas9, tcrRNA and scaRNA in Francisella novicida promote enhanced envelope integrity through the regulation of bacterial lipoproteins and were necessary for polymyxin B resistance.

In view of LPS and LOS changes, inactivation of the genes (spgm, ppgm, hldA and hldD) showed changes in LPS and this correlated with increased susceptibility to polymyxin B in Stenotrophomonas maltophilia, Vibrio fischeri, Burkholderia cenocepacia, E. coli and Proteus mirabilis, respectively. HldA and HldD gene products have been shown to play a role in the modification of heptose sugars. However, spgm and ppgm (phosphoglucanase) have been demonstrated to play a role in catabolism of galactose and in the promotion of UDP-glucose production in E. coli, and LPS and alginate biosynthesis as a homologue of the algC gene in P. aeruginosa, respectively. Moreover, OM protein OprH has been reported to affect resistance to antimicrobial peptide polymyxin B in P. aeruginosa. Polymyxin B resistance resulted from LPS alteration (interaction of OprH with divalent cation-binding sites of LPSs). Further, expression of the cj1136 gene (putative galactosyltransferase), involved in LOS biosynthesis, is associated with Campylobacter jejuni polymyxin B resistance. In this context, insertion inactivation of genes involved in synthesis and extension of LOS, namely waaF, lgtF, galT, cstII and galU in C. jejuni, resulted in decreased resistance to polymyxin B (>15-fold reduction in MIC) (ref.4). Additionally, it has been described elsewhere that galU (involved in L-Ara4N biosynthesis) also in other species such as P. mirabilis, and Yersinia pestis contributes to resistance to polymyxin B. It was further reported that mutations in the genes of Haemophilus influenzae (lic1, lic2A, lpsA, lgtF, opxX) also involved in LOS biosynthesis lead to increased susceptibility to polymyxin B (ref.4). FirA in E. coli and S. Typhimurium has also been shown to be important for lipid A biosynthesis and resistance to polymyxin B (ref.4). Interestingly, UDP-3-O-3-hydroxymyristoyl glucosamine N-acetyltransferase (LpxD) in higher copy number, a FirA homolog, was found in Pseudomonas putida strain HB3267 and may be responsible for higher resistance to polymyxin B than other strains. Furthermore, in one study, it has been reported that other genes regulating, for example, biofilm formation or LPS and LOS modification (ie, lpxO) (ref.4) correlate with increased resistance towards polymyxins, which are described in more detail elsewhere.

It was also found that complete loss of LPS production by mutations in lpxA4D which are involved in lipid A biosynthesis, exhibited a colistin-resistant phenotype in Acinetobacter baumannii. In addition, a mutation in the OM protein, LptD, which allows the final transfer of the newly synthesized LPS, resulted in a complete loss of LPS and decreased susceptibility to polymyxin in A. baumannii.

Efflux and transport

Several different types of multidrug efflux pumps in different pathogens have been shown to confer tolerance towards polymyxin B. For more informations see. Recently, implication of efflux transporter proteins (AdeABC and HlyD family) in polymyxin resistance in A. baumannii has been reported. The AdeABC is homologous to the AcrABC and MexAB-OprM pumps. Further, relationship between TTSS via the RsmA protein and the expression of multidrug efflux (Mex) pumps has also been described in P. aeruginosa, whereas increased expression of MexCD-OprJ or MexEF-OprN was associated with decreased expression of the TTSS regulon. Furthermore, it has been reported, that the potassium uptake protein in Vibrio vulnificus, TrkA, was responsible for resistance to polymyxin B (ref.7). Besides, it has been shown that the reaccumulation of K+ by proteamine-treated cells results in protease expression of PgtE, which in turn degrades protamine, thus preventing the death of bacteria.

Other polymyxin resistance determinants with known, unclear and unknown function

In A. baumannii, some 35 genes have shown to influence colistin resistance. Identified genes have been shown to play roles in the regulation of OM proteins, chaperones, protein biosynthesis factors, and metabolic enzymes (putative role in loss of biological fitness) (ref.7). Furthermore, it was found that additional 30 genes in A. baumannii were involved in resistance to colistin. These were identified to be involved in amino acid transport, lipid and phosphate metabolism (pathways and systems associated with osmotolerance), protein folding, and cell envelope biosynthesis. In K. pneumoniae, in addition to the new two-component system CrrAB characterized by the regulation of colistin resistance through the activation of PmrAB, other genes were also transcriptionally upregulated [genes of cation transport/membrane integrity/efflux transporters (macAB)] and have been linked with LPS modification, cation transport, maintenance of membrane integrity and unknown functions. In Saccharomyces cerevisiae, pbs2 gene was involved in resistance to polymyxin B when overexpressed. However, Pbs2 was found to encode a predicted protein kinase that plays a role in osmoregulation and thus affects the plasma membrane. In addition, it has been found that several genes in V. cholerae, including vc2731 (gspF), vc2732 (gspE), vc0212 (lpxN), vc0224, vc0239, vc1981, associated with type II secretion system, LPS biosynthesis and modification, and unknown functions, were involved in resistance to polymyxin. Also, it has been previously shown that Sip (silica-induced protein) involved in the increase of robustness of the cell surface of Thermus thermophilus helps to protect against peptide antibiotics like polymyxin B (ref.9). Interestingly, Manning and Kuehn revealed that hyper-vesiculating yieM mutant was able to confer polymyxin B and colistin resistance to E. coli by induction of OM vesiculation. It is also worth mentioning that pilus structural subunit PilB of S. agalactiae has been demonstrated to confer polymyxin B resistance and PilB...
contributed to binding of polymyxin B, thereby preventing its interaction with the cell membrane. In this context, type IV pili of *P. aeruginosa* have been shown to be important for resistance to colistin with regulation of motility and development of mushroom caps. Additionally, some PhoP/PhoQ-regulated genes, namely ugtL, virK, migT4 and pgtE, have been shown to contribute polymyxin B and/or CAMP resistance in *S. enterica*, based on potential inhibition of polymyxin binding. However, UgtL has been also described to be involved in dephosphorylation of lipid A. So far, it is still unknown whether this protein functions as an enzyme or as a regulator of the reaction. In addition, genes for the synthesis of putrescine and YceI, found to act as biochemicals, have been recently discovered to mediate polymyxin B resistance in *B. cenocepacia*, most likely by sequestering the antibiotic. Another study has reported that mutants of the yejABEF operon, genes encoding putative ATP-binding cassette (ABC) transporter, were found to be susceptible to polymyxin B. It has been suggested that the transporter system encoded by the yej operon may be involved in virulence regulation in *Brucella* and may also be involved in antimicrobial peptides neutralization, similar to the transporter system encoded by the *Salmonella* yej operon. Furthermore, it was reported that acidic growth conditions were associated with polymyxin resistance and was mediated by transcriptional activity of genes (*yjbD pmrClpapB* and *pmrF*). In another study, it has been reported that the transfer of bacteria to a mildly acidic environment (pH 5.8) resulted in the decrease of LpxT activity and strong induction of the addition of L-Ara4N and PEtn. It is also worth mentioning that lack of magnesium, phosphate and iron ions have all been reported to lead to resistance to antimicrobial peptide polymyxin B. In this context, it has been previously shown that expression of the etk (required for phosphorylation of Ugd) during cultivation of *E. coli* was stimulated with low pH, low concentrations of magnesium and iron ions. In addition, several genes have been implicated in the resistance towards polymyxin B in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* strains, namely, *YPTB0331–0332–0333*, likely through the ferric uptake regulation. Interestingly, the presence of extracellular DNA also has been shown to contribute to colistin and polymyxin B resistance in *S. Typhimurium* and *P. aeruginosa* by activation of PhoPQ and PmrAB systems. Last but not least, additional determinants associated with polymyxin resistance are described in (ref. 3).

Regarding genes with unclear function, there are some genes that have been differently regulated in colistin-resistant strains of *P. aeruginosa* including PA1199, PA2583, PA5548, PA2928 probably participating in LPS biosynthesis as well as non-LPS-mediated genes including PA1980 (eraR), PA5447 (wbpZ), PA4541 and PA1938 (ref.100). Further, it has been previously reported that *P. aeruginosa* genes [PA0401, pyrB, pdsB, sucC, tpiA, aroB, pyrD, mpl, hypothetical protein (rmlD homolog), ampr, lpc, amgS, galU, lpc, wapR, and ssg] play a role in polymyxin B resistance given their potential regulatory function, role in metabolic pathways, LPS biosynthesis and unknown functions. Moreover, gene *cgt* has been reported to be associated with colistin resistance in *Helicobacter pylori*, suggesting its potential involvement in lipid A modification. Moreover, DNA sequence analysis of the mutantized loci, JSG945 (putative O-acetyltansferase, which is essential for the addition of aminoarabinose to lipid A), JSG946, JSG947 (putative ATP synthase), and JSG948 (putative *sap* loci), revealed the role of these genes in conferring resistance to polymyxin B in *P. mirabilis*. It has also been demonstrated in *B. ovis* that resistance to the polymyxin B affects the bacA gene, probably by altering the structure of the bacterial envelope. Furthermore, it was found that a putative *pgm* gene (BRA 0348) in *Brucella suis* and *suc*, tolB, and *gnd* genes in *S. enterica* were shown to be necessary for polymyxin resistance, probably due to its putative OM modification (LPS structure or from destabilization of the membrane) (ref. 43, 104). In this context, it has been found that periplasmic chaperone SurA in *E. coli* plays an important role in transporting the LptD to the OM assembly site. Gattis et al. have shown that three genes *kdnA/kdnB* and *fopC* contribute to polymyxin resistance in many pathogens because of its potential role in OM integrity. From *E. coli* and *Streptococcus pyogenes* mutant screening, a number of other genes (PstA/C/S, EbsA, TopA, FtsH, GdpP, FabT, YfmH, AgaS, ManL/N, ClpX, DeoB, Hpt, TilS, Gmk, NanH, GuaA, NupP, PtsI, LuxR, Fba) have been identified to be required for resistance to polymyxin. These genes encode proteins with potential role in OM integrity and disruption of ExPortal integrity and thereby contribute to polymyxin resistance. In a previous study, it was shown that *asmA, pgp*, *mglB*, *glpQ*-like, *feoC*, *hflC*, *pitA* genes have been implicated in resistance to polymyxin B in *Y. pestis*. This resistance was linked with putative modification of molecules or molecular composition of the bacterial OM (ref. 110). Further, periplasmic chaperone proteins, Spy, ZraP, CpxP, have been reported to play a role in resistance against polymyxin B in *S. enterica* which could be affected by the envelope stress response regulation, while ZraP has been proposed to act to repress the expression of two-component system ZraSR (ref. 111). Interestingly, *σE*-controlled small non-coding RNAs, MicA and RybB, were activated in *S. enterica* treated cells with polymyxin B and are likely to facilitate the remodelling of the OM by reduction of the expression OM proteins (MicA represses OmpA synthesis, while RybB inhibits both OmpC and OmpW expression) (ref. 112). Moreover, MicA has been shown to be a feedback inhibitor of the *phoPQ* system of *E. coli*. Further, the involvement of sodB (A1S_2343) and sodC genes in colistin resistance have been observed in *A. baumannii*, probably by detoxifying reactive oxygen species. In this context, *katA* (catalase) was found to confer resistance to polymyxin B in *S. aureus*. The resistance to polymyxin B was derived from its antioxidative defense mechanisms.

Furthermore, other genes, such as *rpoE* (σE), *BCAL2381*, *mucD*, *ispH* (*BCAL2720*), *lytB*, *hpnJ*, have been found to be related to polymyxin B resistance in *B. cenocepacia* through unknown mechanisms, with the exception of mechanisms that do not contribute to weak binding of
polymyxin to *B. cenocepacia* cells or to poor permeabilization of the outer membrane. However, the gene *ispH* (isopenoid synthesis) increased OM stability and reduced molecular permeability.

Furthermore, *Y. pestis* resistance to cationic peptide polymyxin B is due to changes in unidentified surface structures mediated by regulation of *pgmA* activity. In addition, *grxD* mutant, a gene with unknown function, showed increased susceptibility to polymyxin B in *P. aeruginosa*.

**CONCLUSION**

Polymyxins are powerful bactericidal antibiotics that are effective against gram-negative bacteria. Despite their possible negative effect on the nerves and the kidneys in humans, they represent the last line of defense against persistent multidrug-resistant infections. Research into polymyxin resistance has led to the elucidation of many mechanisms and pathways that in some way affect resistance itself. In this report, we have tried to approach all the mechanisms of resistance described so far, although there are still many unknown and unresolved mechanisms of resistance. Still, we hope that a deeper understanding of the resistance mechanism will improve the ability to design and develop more efficient and toxic derivatives of polymyxins. It is also worth mentioning the discovery of the plasmid-mediated colistin resistance genes, *mcr*, and therefore we can assume that other plasmid-mediated genes will be described in the near future. The primers described in our study may be used for detection of *mcr* genes and their variants, which could ultimately limit the dissemination of colistin-resistant bacteria.

**ABBREVIATIONS**

ABC, ATP-binding cassette; CAMP, cationic antimicrobial peptides; L-Ara4N, 4-amino-4-deoxy-L-arabinose; LPS, lipopolysaccharide; PEtn, phosphoethanolamine; OM, outer membrane; TCSSs, two-component systems; TTSS, type three secretion system.

**Search strategy and selection criteria**

We searched Google Scholar for articles published in English between 1970 and 2018 December using the keywords “polymyxin resistance”, “polymyxin B”, “colistin”, “bacteria”, “genes”, “colistin-resistant”, “LPS” and “mcr”. Bibliographies of all appropriate studies have been reviewed to identify other eligible studies.

**Acknowledgement:** This publication was supported by the National Sustainability Program (LO1304), by the Palacky University Internal Financial Support, project no. IGA_LF_2018_019 and “Increasing internationalization at the Faculty of Medicine and Dentistry, Palacky University Olomouc” (SPP 210015017).

**Author contributions:** PM: wrote the manuscript and conducted the literature review. MK: provided broad ideas and structure, as well as revisions.

**Conflict of interest statement:** The authors declare no conflict of interest.

**REFERENCES**

1. Olaitan AO, Morand S, Rolain JM. Emergence of colistin-resistant bacteria in humans without colistin usage: a new worry and cause for vigilance. Int J Antimicrob Ag 2016;47(1):1-3.

2. Trimbble MJ, Mlynarcik P, Kolar M, Hancock RE. Polymyxin: Alternative Mechanisms of Action and Resistance. Cold Spring Harb Perspect Med 2016;6(10).

3. Hood MJ, Becker KW, Roux CM, Dunnman PM, Skaar EP genetic determinants of intrinsic colistin tolerance in Acinetobacter baumannii. Infect Immun 2013;81(2):542-51.

4. Fernandez L, Jensen H, Bains M, Wiegand I, Gooderham WJ, Hancock RE. The two-component system CprRS senses cationic peptides and triggers adaptive resistance in Pseudomonas aeruginosa independently of ParRS. Antimicrob Agents Chemother 2012;56(12):6212-22.

5. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian GB, Dong BL, Huang XH, Yu LF, Gu DX, Ren HW, Chen XJ, Lv LC, He DD, Zhou HW, Liang ZS, Liu JH, Shen JZ. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infectious Diseases 2016;16(2):161-8.

6. McPhee JB, Lewenza S, Hancock RE. Cationic antimicrobial peptides activate a two-component regulatory system, PmrA-PmrB, that regulates resistance to polymyxin B and cationic antimicrobial peptides in Pseudomonas aeruginosa. Mol Microbiol 2003;50(1):205-17.

7. Moon K, Gottesman S. A PhoQ/P-regulated small RNA regulates sensitivity of Escherichia coli to antimicrobial peptides. Mol Microbiol 2009;74(6):1314-30.

8. Kato A, Groisman EA. Connecting two-component regulatory systems by a protein that protects a response regulator from dephosphorylation by its cognate sensor. Genes Dev 2004;18(18):2302-13.

9. Cheng YH, Lin TL, Lin YT, Wang JT. Amino Acid Substitutions of CrrB Responsible for Resistance to Colistin through CrrC in Klebsiella pneumoniae. Antimicrob Agents Chemother 2016;60(6):3709-16.

10. Muller C, Plesiats P, Jeannot K. A two-component regulatory system interconnects resistance to polymyxins, aminoglycosides, fluoroquinolones, and beta-lactams in Pseudomonas aeruginosa. Antimicrob Agents Chemother 2011;55(3):1211-21.

11. Gutu AD, Sgambati N, Strasserburger P, Brannon MK, Jacobs MA, Haugan E, Kaul RK, Johansen HK, Holby N, Moskowitz SM. Polymyxin resistance of Pseudomonas aeruginosa phoQ mutants is dependent on additional two-component regulatory systems. Antimicrob Agents Chemother 2013;57(5):2204-15.

12. Fernandez L, Gooderham WJ, Bains M, McPhee JB, Wiegand I, Hancock REW. Adaptive resistance to the "last hope" antibiotics polymyxin B and colistin in Pseudomonas aeruginosa is mediated by the novel two-component regulatory system ParP-ParS. Antimicrob Agents Chemother 2010;54(8):3372-82.

13. Herbert S, Bera A, Nerz C, Kraus D, Peschel A, Goerke C, Meehl M, Cheung A, Gottz F. Molecular basis of resistance to muramidase and cationic antimicrobial peptide activity of lysozyme in staphylococci. PLoS Pathog 2007;3(7):e102.

14. Taneya NK, Ganguly T, Bakalietz LO, Nelson KJ, Dubey P, Poole LB, Deora R. D-alanine modification of a protease-susceptible outer membrane component by the Bordetella pertussis dra locus promotes resistance to antimicrobial peptides and polymorphonuclear leukocyte-mediated killing. J Bacteriol 2013;195(22):5102-11.

15. Abachin E, Poyart C, Pellegrini E, Milohanic E, Fiedler F, Berche P, Trieu-Cuot P. Formation of D-arylalanyl-picolinic acid is required for adhesion and virulence of *Listeria monocytogenes*. Mol Microbiol 2002;43(1):1-14.

16. McCormick NE, Halperin SA, Lee SF. Regulation of D-alamination of lipopolysaccharide in *Streptococcus gordoni*. Microbiology 2011;157(Pt 8):2248-56.

17. Mazda Y, Kawada-Matsuo M, Kanbara K, Oogai Y, Shibata Y, Yamashita Y, Miyawaki S, Komatsuzawa H. Association of CiaRH with resistance of *Streptococcus mutans* to antimicrobial peptides in biofilms. Mol Oral Microbiol 2012;27(2):124-35.

18. Quach D, van Sorge NM, Kristian SA, Bryan JD, Shelver DW, Doran
KS. The CiaR response regulator in group B Streptococcus promotes intracellular survival and resistance to innate immune defenses. J Bacteriol 2009;191(7):2023-32.

34. Mouslim C, Groisman EA. Control of the Salmonella ugd gene by a two-component regulatory system. Mol Microbiol 2011;79(5):1318-29.

35. Pilonieta MC, Erickson KD, Ernst RK, Detweiler CS. A protein imported into the bacterial cell enhances protein translation and promotes intracellular infection. Infect Immun 2001;69(7):4276-86.

36. Spinosa MR, Progida C, Tala A, Cogli L, Alifano P, Bucci C. The Neisseria meningitidis capsule is important for intracellular survival in human epithelial cells. Infect Immun 2002;70(11):5167-74.

37. Murray SR, Ernst RK, Bermudes D, Miller SI, Low KB. pmrA(Con) confers pmrHFIJKL-dependent EGTA and polymyxin resistance on Salmonella enterica serovar Typhimurium. J Bacteriol 2007;189(14):4725-34.

38. Martin-Martín AI, Cloeckaert A, Grillo M-J, Vizcaino N. Quorum-sensing and BvrR/BvrS regulation, the type IV secretion system, cyclic glucans, and BacA in the virulence of Brucella ovis: similarities and differences from smooth Brucella. Infect Immun 2012;80(5):1793-83.

39. Wang X, Chen Z, Qiao F, Zhong Z, Xu J, Wang Z, Du X, Ou Q, Yuan J, Jia L, Song H, Sun Y, Huang L. The type IV secretion system affects the expression of Omp25/Omp31 and the outer membrane properties of Brucella melitensis. FEMS microbiology letters 2010;303(1):92-100.

40. Caro-Hernandez P, Fernandez-Lago L, de Miguel M-J, Martín-Martin AI, Cloeckaert A, Grillo M-J, Vizcaino N. Role of the Omp25/Omp31 family in outer membrane properties and virulence of Brucella ovis. Infect Immun 2007;75(8):4050-61.

41. Guzman-Verri C, Manterola L, Sola-Landa A, Parra A, Cloeckaert A, Garin J, Gorvel JP, Moriyon I, Moreno E, Lopez-Goni I. The two-component system BvrR/BvrS essential for Brucella abortus virulence regulates the expression of outer membrane proteins with counterparts in members of the Rhizobiaceae. Proc Natl Acad Sci U S A 2002;99(19):12375-80.

42. Chopra S, Ramkisson K, Anderson DC. A systematic quantitative proteomic examination of multidrug resistance in Acinetobacter baumannii. J Proteomics 2013;84:17-39.

43. Liautard J, Ouahrahni-Bettache S, Jubier-Maurin V, Lafont V, Kohler L, Liautard J. Identification of brucella suis virulence genes involved in resistance to the human innate immune system. Infect Immun 2007;75(11):5167-74.

44. Rogers-Reyes R, Saldias MS, Aurbert DF, El-Halfawy OM, Valmano VA. The sbgB gene of Burkholderia cenocepacia is required for protein secretion, biofilm formation, motility and polymyxin B resistance. Microbiology (Reading, England) 2012;158(9):2315-24.

45. Sikora AE, Lybarger SR, Sandkist M. Compromised outer membrane integrity in Vibrio cholerae Type II secretion mutants. J Bacteriol 2007;189(23):8484-95.

46. Sola-Landa A, Pizarro-Cerda J, Grillo MJ, Moreno E, Moriyon I, Blasco JM, Gorvel JP, Lopez-Goni I. A two-component regulatory system playing a critical role in plant pathogens and endosymbionts is present in Brucella abortus and controls cell invasion and virulence. Mol Microbiol 1998;29(1):125-38.

47. Sassera D, Comandatore F, Gaibani P, D'Auria G, Mariconti M, Landini MP, Sambiri V, Marone P. Comparative genomics of closely related strains of Klebsiella pneumoniae reveals genes possibly involved in colistin resistance. Ann Microbiol 2014;64(2):887-90.

48. Dupont M, De E, Chollet R, Chevalier J, Pages JM. Enterobacter aerogenes OmpX, a cation-selective channel mar- and oso-regulated. Festschrift 2004;569(1-3):27-30.

49. Papenfort K, Pfeiffer V, Mika F, Luchcin S, Hinton JC, Vogel J. SigE-dependent small RNAs of Salmonella respond to membrane stress by accelerating global omp mRNA decay. Mol Microbiol 2006;62(6):1674-88.

50. Moreira CG, Herrera CM, Needham BD, Parker CT, Libby SJ, Fang FC, Trent MS, Sperandio V. Virulence and stress-related periplasmic protein (VisP) in bacterial/host associations. Proc Natl Acad Sci U S A 2013;110(4):1470-5.

51. Achoouk W, Heulin T, Pages JM. Multiple facets of bacterial porins. Fems Microbiology Letters 2001;199(1):1-7.

52. Zheng YL, Ambrose KD, Zughai S, Zhou X, Miller YK, Shafer WM, Stephens DS. Cationic antimicrobial peptide resistance in Neisseria meningitidis. J Bacteriol 2005;187(15):5387-96.

53. Mulcahy H, O’Callaghan J, O’Grady EP, Adams C, O’Gara F. The post-transcriptional regulator RsmA plays a role in the interaction between Pseudomonas aeruginosa and human airway epithelial cells by positively regulating the type III secretion system. Infect Immun 2006;74(5):3025-31.

54. Kerrinnes T, Young BM, Leon C, Roux CM, Tran L, Atliur VL, Winter MG, Tsolis RM. Phospholipase A1 Modulates the Cell Envelope Phospholipid Content of Brucella melitensis, Contributing to Polymyxin Resistance and Pathogenicity. Antimicrob Agents Chemother 2015;59(11):7171-74.

55. Cheah SE, Johnson MD, Zhu Y, Tsuji BT, Forrest A, Bulitta JB, Boyce MG, Tsolis RM. Phospholipase A1 Modulates the Cell Envelope Phospholipid Content of Brucella melitensis, Contributing to Polymyxin Resistance and Pathogenicity. Antimicrob Agents Chemother 2015;59(11):7171-74.

56. Sampson TR, Napier BA, Schroeder MR, Louren M, Zhao J, Chin Y-C, Ratner HK, Llewellyn AC, Jones CL, Laruie H, Merlin D, Zhou P, Endtz
HP Weiss DS. A CRISPR-Cas system enhances envelope integrity mediating antibiotic resistance and inflammasome evasion. Proc Natl Acad Sci U S A 2014;111(30):11163-8.

57. DeLoney CR, Bartley TM, Visick KL. Role for phosphoglucosumate in Vibrio fischeri Euprymna scolops symbiosis. J Bacteriol 2002;184(18):5121-9.

58. Loutet SA, Flanagan RS, Kooi C, Sokol PA, Valvano MA. A complete lipopolysaccharide inner core oligosaccharide is required for resistance of Burkholderia cenocepacia to antimicrobial peptides and bacterial survival in vivo. J Bacteriol 2006;188(6):2073-80.

59. McKay J, Bagley AL, Macdonald RL. Role of phosphoglucosumate of Stenotrophomonas maltophilia in lipopolysaccharide biosynthesis, virulence, and antibiotic resistance. Infect Immun 2003;71(6):3068-75.

60. Subbashchandrabose S, Smith SN, Spurbeck RR, Kole MM, Mobley HLT. Genome-wide detection of fitness genes in uropathogenic Escherichia coli during systemic infection. PLoS pathogens 2013;9(12):e1003788.

61. Macfarlane EL, Kwasnicka A, Ochs MM, Hancock RE. PhoP-PhoQ homologues in Pseudomonas aeruginosa regulate expression of the outer-membrane protein OprH and polymyxin B resistance. Mol Microbiol 1999;34(2):305-16.

62. Young ML, Bains M, Bell A, Hancock RE. Role of Pseudomonas aeruginosa OprM in polymyxin resistance: isolation of an OprM-deficient mutant by gene replacement techniques. Antimicrob Agents Chemother 1992;36(11):2566-6.

63. Javed MA, Cathrawth SA, Baig A, Li J, McNally A, Oldfield NJ, Newell DG, Manning G. Cj136 is required for lipooligosaccharide biosynthesis, hyperinvasion, and chick colonization by Campylobacter jejuni. Infect Immun 2012;80(7):2361-70.

64. Naito M, Friedrich E, Fields JA, Pryjma M, Li J, Cameron A, Gilbert M, Thompson SA, Gaynor EC. Effects of sequential Campylobacter jejuni. Infect Immun 2012;80(7):2361-70.

65. Lin J, Wang Y, Huang KV. Systematic identification of genetic loci required for polymyxin resistance in Campylobacter jejuni using an efficient in vivo transposon mutagenesis system. Foodborne Pathog Dis 2009;6(2):173-85.

66. Jiang SS, Lin TY, Wang WB, Liu MC, Hsueh PR, Liaw SJ. Characterization of UDP-glc dehydrogenase and UDP-glucose pyrophosphorylase mutants of Proteus mirabilis: defectiveness in polymyxin B resistance, swarming and virulence. Antimicrob Agents Chemother 2010;54(5):2000-9.

67. Klein KA, Fukuto HS, Pelletier M, Romanov N, Grabenstein JP, Palmer LE, Ernst R, Bliska JB. A transposon site hybridization screen identifies antimicrobial peptides. J Bacteriol 2002;184(12):3203-13.

68. Morey P, Viadas C, Euba B, Hood DW, Barberan M, Gil C, Grillo MJ, Bengechea JA, Garmendia J. Relative contributions of lipooligosaccharide inner and outer core modifications to non-typeable Haemophilus influenzae pathogenesis. Infect Immun 2010;78(1):1-9.

69. Henry R, Crane B, St Michael F, Cox AD, Adler B, Nation RL, Li J, Boyce MJ, Bengoechea JA, Garmendia J. Relative contributions of lipooligosaccharide inner core oligosaccharide is required for resistance and systemic virulence. FASEB J 2008;22(6):1715-24.

70. Haagensen JA, Klauser M, Ernst RK, Miller SJ, Folkesson A, Tolker-Nielsen T, Molin S. Differentiation and distribution of colistin- and sodium dodecyl sulfate-tolerant cells in Pseudomonas aeruginosa biofilms. J Bacteriol 2007;189(12):3876-87.

71. Brodsky IE, Ernst RK, Miller SJ, Falkow S. msq-14 is a Salmonella gene that plays a role in bacterial resistance to antimicrobial peptides. J Bacteriol 2002;184(12):3203-13.

72. Guina T, Yi EC, Wang H, Hackett M, Miller SJ. A PhoP-regulated outer membrane protease of Salmonella enterica serovar typhimurium promotes resistance to alpha-helical antimicrobial peptides. J Bacteriol 2000;182(4):1077-85.

73. Navarre WW, Halsey TA, Walthers D, Frye J, McClelland M, Potter JL, Kenney LJ, Gunn JS, Fang FC, Libby SJ. Co-regulation of Salmonella enterica genes required for resistance to antimicrobial peptides by SlyA and PhoP/PhoQ. Mol Microbiol 2005;56(2):492-508.

74. Shi Y, Latfi T, Cromie MJ, Groisman EA. Transcriptional control of the antimicrobial peptide resistance ugtL gene by the Salmonella PhoP and SlyA regulatory proteins. J Biol Chem 2004;279(37):38618-25.

75. El-Hafawy OM, Valvano MA. Chemical communication of antibiotic resistance by a highly resistant subpopulation of bacterial cells. PLoS one 2013;8(7):e68874.

76. Eswarapara SM, Panguluri KK, Hensel M, Chakravortty D. The yjeABEF operon of Salmonella confers resistance to antimicrobial peptides and contributes to its virulence. Microbiology 2008;154(Pt 2):666-78.

77. Wang Z, Bie PF, Cheng J, Lu L, Cui BY, Wu QM. The ABC transporter YjeABEF is required for resistance to antimicrobial peptides and the virulence of Brucella melitensis. Sci Rep UK 20166.

78. Groisman EA, Kayser J, Soncin FC. Regulation of polymyxin resistance and adaptation to low-Mg2+ environments. Journal of bacteriology 1997;179(22):7287-90.

79. Soncin FC, Groisman EA. Two-component regulatory systems can interact to process multiple environmental signals. J Bacteriol 1996;178(23):6796-801.

80. Herrera CM, Crofts AA, Henderson JC, Pinaci SG, Davies BW, Tant W. The Vibrio cholerae YprA-YprB two-component system controls virulence through an unknown mechanism. Mol Microbiol 2014;56(1-2).

81. Stumpe S, Bakker EP. Requirement of a large K+-uptake capacity to mediate antibiotic resistance and inflammasome evasion. Proc Natl Acad Sci U S A 2014;111(30):11632-7.

82. Wright MS, Suzuki Y, Jones MB, Marshall SH, Rudin SD, van Duijn D, Kaye K, Jacobs MR, Bonomo RA, Adams MD. Genomic and transcriptomic analyses of colistin-resistant clinical isolates of Klebsiella pneumoniae reveal multiple pathways of resistance. Antimicrob Agents Chemother 2015;59(1):536-43.
Pseudomonas aeruginosa during growth under phosphate-limiting conditions, but is not involved in antimicrobial peptide susceptibility. FEMS microbiology letters 2011;320(2):95-102.

97. Lacour S, Doublot P, Obadia B, Cozzone AJ, Grangeasse C. A novel role for protein-tyrosine kinase Etk from Escherichia coli K-12 related to polymyxin resistance. Res Microbiol 2006;157(7):637-41.

98. Johnson L, Horman SR, Charron-Mazoned L, Turnbl AL, Mulcahy H, Surette MG, Lewenza S. Extracellular DNA-induced antimicrobial peptide resistance in Salmonella enterica serovar Typhimurium. BMC Microbiol 2013;13:115.

99. Mulcahy H, Charron-Mazoned L, Lewenza S. Extracellular DNA chelates cations and induces antibiotic resistance in Pseudomonas aeruginosa biofilms. PLoS pathogens 2008;4(11):e1000213.

100. Lee JY, Na IV, Park YK, Ko KS. Genomic variations between colistin-susceptible and -resistant Pseudomonas aeruginosa clinical isolates and their effects on colistin resistance. J Antimicrob Chemother 2014;69(5):1248-56.

101. Fernandez L, Alvarez-Ortega C, Wiegand I, Olivares J, Kocincova D, Lam JS, Martinez JL, Hancock REW. Characterization of the polymyxin B resistome of Pseudomonas aeruginosa. Antimicrob Agents Chemother 2013;57(1):110-9.

102. McGee DJ, George AE, Trainor EA, Horton KE, Hildebrandt E, Testerman TL. Cholesterol enhances Helicobacter pylori resistance to antibiotics and LL-37. Antimicrob Agents Chemother 2011;55(6):2897-904.

103. McCoy AJ, Liu H, Falla TJ, Gunn JS. Identification of Proteus mirabilis mutants with increased sensitivity to antimicrobial peptides. Antimicrob Agents Chemother 2001;45(7):2030-7.

104. Tamayo R, Ryan SS, McCoy AJ, Gunn JS. Identification and genetic characterization of PmrA-regulated genes and genes involved in polymyxin B resistance in Pseudomonas aeruginosa. Antimicrob Agents Chemother 2015;69(6):3252-60.

105. Denoncin K, Vertommen D, Paek E, Collet JF. The protein-disulfide isomerase DsbC cooperates with SurA and DsbA in the assembly of the essential beta-barrel protein LptD. J Biol Chem 2010;285(38):29425-33.

106. Gattis SG, Chung HS, Trent MS, Raetz CR. The origin of 8-amino-3,8-dideoxy-O-manno-octulosonic acid (Kdo8N) in the lipopolysaccharide of Shewanella oneidensis. J Biol Chem 2013;288(13):9216-25.

107. Nallaparaju KC, Yu JJ, Rodriguez SA, Zogaj X, Manam S, Guentzel MN, Seshu J, Murthy AK, Chambers JP, Klose KE, Arulananandam BP. Evasion of IFN-gamma signaling by Francisella novicida is dependent upon Francisella outer membrane protein C. PLoS One 2011;6(3):e18201.

108. Lamarche MG, Dozois CM, Daigle F, Caza M, Curtiss R, 3rd, Dubreuil JD, Harel J. Inactivation of the pst system reduces the virulence of an avian pathogenic Escherichia coli O78 strain. Infect Immun 2005;73(7):4138-45.

109. Port GC, Vega LA, Nylander AB, Caparon MG. Streptococcus pyogenes polymyxin B-resistant mutants display enhanced ExPortal integrity. J Bacteriol 2014;196(14):2563-77.

110. Guo J, Nair MKM, Galvan EM, Liu S-L, Schifferli DM. Tn5ARAOut mutagenesis for the identification of Yersinia pestis genes involved in resistance towards cationic antimicrobial peptides. Microbial pathogenesis 2011;51(3):121-32.

111. Appia-Ayme C, Hall A, Patrick E, Rajadurai S, Clarke TA, Rowley G. ZraP is a periplasmic molecular chaperone and a repressor of the zinc-responsive two-component regulator ZraSR. Biochem J 2012;442(1):85-93.

112. Coornaert A, Lu A, Mandin P, Springer M, Gottesman S, Guiller M. Mica sRNA links the PhoP regulon to cell envelope stress. Mol Microbiol 2010;76(2):467-79.

113. Heindorf M, Kadar D, Heider C, Skiebe E, Wilharm G. Impact of Acinetobacter baumannii superoxide dismutase on motility, virulence, oxidative stress resistance and susceptibility to antibiotics. PLoS One 2014;9(7):e101033.

114. Pourmaras S, Poulou A, Dafopoulou K, Chabane YN, Kristo I, Makris D, Hardoun J, Cossette P, Tsakiris A, De E. Growth retardation, reduced invasiveness, and impaired colistin-mediated cell death associated with colistin resistance development in Acinetobacter baumannii. Antimicrob Agents Chemother 2014;58(2):828-32.

115. Loutet SA, Mussen LE, Flannagan RS, Valvano MA. A two-tier model of polymyxin B resistance in Burkholderia cepacia complex. Environ Microbiol Rep 2011;3(2):278-85.

116. Felek S, Muszynski A, Carlson RW, Tsang TM, Hinnebusch BJ, Krukonis ES. Phosphoglcomutase of Yersinia pestis is required for autoaggregation and polymyxin B resistance. Infect Immun 2010;78(3):1163-75.

117. Romsang A, Leesukon P, Duangnkern J, Vattanaviboon P, Mongkolsku S. Mutation of the gene encoding monothiol glutaredoxin (GrxD) in Pseudomonas aeruginosa increases its susceptibility to polymyxins. Int J Antimicrob Agents 2015;45(3):314-8.