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Untying the anchor for the lipopolysaccharide: lipid A structural modification systems offer diagnostic and therapeutic options to tackle polymyxin resistance

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Polymyxin antibiotics are the last resort for treating patients in intensive care units infected with multiple-resistant Gram-negative bacteria. Due to their polycationic structure, their mode of action is based on an ionic interaction with the negatively charged lipid A portion of the lipopolysaccharide (LPS). The most prevalent polymyxin resistance mechanisms involve covalent modifications of lipid A: addition of the cationic sugar 4-amino-L-arabinose (L-Ara4N) and/or phosphoethanolamine (pEtN). The modified structure of lipid A has a lower net negative charge, leading to the repulsion of polymyxins and bacterial resistance to membrane disruption. Genes encoding the enzymatic systems involved in these modifications can be transferred either through chromosomes or mobile genetic elements. Therefore, new approaches to resistance diagnostics have been developed. On another note, interfering with these enzymatic systems might offer new therapeutic targets for drug discovery. This literature review focuses on diagnostic approaches based on structural changes in lipid A and on the therapeutic potential of molecules interfering with these changes.

KEY WORDS: antimicrobial resistance; adjuvants; Gram-negative bacteria; MALDI-TOF-MS; MCR-1

Polymyxin B and polymyxin E (colistin) are cationic cyclic lipodecapeptide antibiotics that disrupt the outer membrane of Gram-negative bacteria by displacing divalent cations Ca^{2+} and Mg^{2+} and enabling their interaction with the negatively charged phosphate moieties of lipid A (1), the so-called anchor of the lipopolysaccharide (LPS). This interaction destabilises the LPS, increases the permeability of the bacterial membrane, leads to the leakage of the cytoplasmic content, and eventually kills the bacteria (2). Polymyxins were discovered in 1947 (3) as secondary metabolites isolated from the soil bacterium *Bacillus polymyxa*. A decade later, polymyxins were abandoned in clinical practice due to high nephrotoxicity and neurotoxicity and replaced by the emerging less toxic antibiotics (1, 4).

Today, polymyxins are used as last-resort agents in human medicine to treat infections which are caused by Gram-negative bacteria resistant to all other available antibiotics (5). Therefore, the emerging resistance to this last-resort treatment is one of the gravest public health concerns, as it leaves no reliable treatment options for patients in intensive care units.

The most prevalent form of polymyxin resistance is through structural modifications of lipid A, a polyanionic segment of the LPS. The two most common modifications include the addition of the cationic sugar 4-amino-L-arabinose (L-Ara4N) and/or phosphoethanolamine (pEtN), as presented in Figure 1. The modified structure of lipid A has a decreased net negative charge, which leads to the repulsion of colistin and protects bacteria from membrane disruption (6, 7). These lipid A structural changes follow distinct mechanisms for plasmid- and chromosome-encoded resistance. Moreover, lipid A structures are species-specific. Polymyxin resistance is intrinsic for some bacterial species such as *Proteus* spp., *Burkholderia* spp., *Serratia* spp. (8), and *Moraxella* spp. (9), because their lipid A structure has a lower net negative charge anyway. Other Gram-negative bacteria acquire polymyxin resistance either through plasmid- or chromosome-encoded genes.

Chromosome-encoded resistance is based on the mutation of genes coding for lipid A. The genes which are involved in lipid A biosynthesis are upregulated by the PmrA/PmrB, PhoP/PhoQ, and CrrA/CrrB two-component systems (TCS) or mutations of the *mgrB* gene (7). Genes under TCS control induce *eptA* and *arn* operon members (e.g. *arnT*) required for lipid A modifications (10–12). ArnT transfers L-Ara4N, and EptA adds pEtN moieties to the phosphate groups of lipid A (7, 13–15).

Plasmid-encoded resistance enables horizontal transfer of *mcr* genes, which code for enzymes that transfer pEtN onto the phosphate groups of the lipid A. So far, 10 classes of *mcr* genes have been identified (*mcr*-1 to *mcr*-10) (6, 16–24).

Other mechanisms which contribute to polymyxin resistance in synergy with lipid A modification systems include the expression of efflux pumps, capsule formation, and overexpression of the OprH, an outer membrane protein (25, 26).

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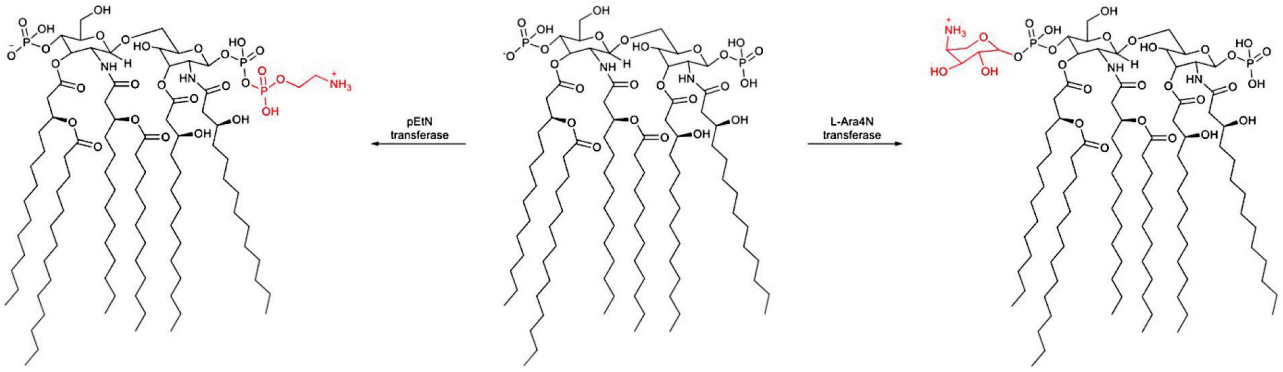


Figure 1 The two most common types of lipid A modifications that lead to colistin resistance

Regardless of the mechanism, polymyxin resistance raises the greatest global concern in all aspects of the One Health paradigm (an interdisciplinary approach that balances the health of people, animals, and the environment): polymyxin-resistant bacteria have been found in environment-, veterinary medicine-, and human medicine-related bacterial strains (27). Although methods for the detection of lipid A structural modifications have mainly been developed for use in human medicine clinical diagnostics, these principles apply for the detection of environmental and veterinary medicine polymyxin-resistant strains. In addition, drugs which may arise from future research could also be utilised in veterinary medicine. The One Health approach to combat polymyxin resistance will be crucial, as one influences the other. For instance, the first *mcr-1* positive bacterial strain has been found in 2016 in China in pigs and later in other food animals (6). Therefore, although it started from veterinary medicine, now *mcr*-positive strains can be found in both human medicine and environmental bacteria.

Although current polymyxin resistance is less frequent than resistance of other classes of antibiotics, we expect an increase in both human and veterinary medicine in the years to come. In this review article we will focus on current potential use of lipid A for clinical diagnostics of resistance and adjuvant therapy.

Lipid A structural analysis in clinical diagnostics

Currently, the gold standard recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for clinical diagnostics of polymyxin resistance used in clinical microbiology laboratories is minimal inhibitory concentration (MIC) (28). Although these diagnostic methods are the most reliable at the moment, they do not inform about the resistance mechanism involved. In clinical settings, this mechanism is generally interpreted through polymerase chain reaction (PCR) after phenotypic resistance has been established (7). Studies have shown that some bacterial strains, referred to as polymyxin heteroresistant, do not show resistance in MIC susceptibility testing but do acquire lipid A modifications along with the genes for polymyxin resistance (29–35). These strains raise concern over their potential to spread polymyxin resistance in clinics, as they usually are not tested for genetic polymyxin resistance. Yet, information about the exact polymyxin resistance mechanism that a bacterium has developed can help us decide the course of hospital treatment. For instance, a patient infected with a bacterium that has plasmid-encoded polymyxin resistance may require quarantine and more cautious behaviour of clinical staff. This is because plasmids can be transferred horizontally from one bacterial species to another. Bacteria that have colonised a hospital area and clinical staff might receive genes for polymyxin resistance by being in close contact with a patient infected by a bacterium carrying plasmid-encoded polymyxin resistance.

Researchers have been working on developing rapid clinical microbiology diagnostic assays which rely on the mass spectrometry (MS) of lipid A structural modifications. Prior to analysis, these bacterial samples require specific treatment to cleave a ketosidic bond in the LPS to release lipid A.

Clinical microbiology laboratories have increasingly been utilising matrix-assisted laser desorption/ionisation – time of flight – mass spectrometry (MALDI-TOF-MS) to identify microbes based on protein profiling. This includes profiling for lipid A structures to develop assays that rapidly determine mechanisms underlying polymyxin resistance in clinical settings (36–44). Molecules analysed by MALDI-TOF-MS need to be ionised prior to separation. Negative ionisation mode is optimal for lipid A because its phosphate groups can easily be ionised into anionic form. In view of the fact that lipid A structures are species-specific, the findings of MS analysis need to be interpreted carefully and individually. To illustrate the rationale of lipid A analysis for clinical diagnostics, we will show examples of lipid A structural modifications for some bacterial species which can be visualised by MALDI-TOF-MS.

Klebsiella pneumoniae has at least four native lipid A structures, which have the MS spectrum m/z values of 1824, 1840, 2063, and 2078 (37, 41, 45). Depending on the mechanism underlying polymyxin resistance, there can be an addition of L-Ara4N or/and pEtN, in which case the m/z values increase by +131 or +123, respectively. The same rationale in structural modifications has been observed in other Gram-negative bacteria. Therefore, chromosome-

encoded resistance can be viewed as lipid A m/z values of 1971 and 2209, plasmid-encoded resistance can be viewed as lipid A m/z values of 1963 and 2201, and, if both mechanisms are involved, as lipid A m/z values of 1963, 2201, and 2209 (37, 41). Table 1 shows the proposed structures of native and modified lipid A for *K. pneumoniae*.

As for *Pseudomonas aeruginosa*, there is no evidence of pEtN addition to lipid A. What we know for now is that the acquired resistance arises from the addition of one or two L-Ara4N moieties to position 1 and/or 4' phosphate in the native lipid A (39). The most common native lipid A structures can be viewed as m/z values of 1446 and 1462. Therefore, chromosome-encoded resistance can be viewed as lipid A m/z values of 1577, 1593, 1708, and 1724 (39). Table 2 shows the proposed structures of native and modified lipid A for *P. aeruginosa*.

Native lipid A structures of *Acinetobacter baumannii* have m/z values of 1728 and 1910. Chromosome-encoded resistance is based on a 4' pEtN addition to lipid A and a +123 shift (38). Table 3 shows the proposed structures (38, 41). Some minor native lipid A peaks include tetraacylated and pentaacylated lipid A with m/z values of 1404 and 1530, respectively (these structures are not included in this review). Plasmid-encoded resistance has not been detected in *A. baumannii* yet.

The most common *Escherichia coli* native lipid A structure has the m/z value of 1796. Chromosome-encoded resistance is identified at m/z of 1927 (L-Ara4N), plasmid-encoded resistance at m/z of 1919, and both mechanisms at respective m/z values of 1919 and 1927 (40, 46). Table 4 shows the proposed structures (40, 41, 46).

Salmonella enterica has at least five native lipid A structures, which correspond to m/z values of 1796, 1812, 1876, 2034, and 2050 (Table 5). Chromosome-encoded resistance is identified as lipid A m/z values of 1927 and 2165 (corresponding to the L-Ara4N addition), and plasmid-encoded resistance as lipid A m/z values of 1919, 1935, 2157, and 2173 (corresponding to the pEtN addition) (36, 41).

Based on the findings from the Larrouy-Maumus research group at Imperial College London, the MALDIxin test, commercialised by Bruker Corporation under the name MBT Lipid Xtract™ kit (47), shows great promise as indicator of polymyxin resistance to be used in clinical diagnostics.

Promising targets in lipid A structural modification systems for adjuvant polymyxin treatment

Currently, polymyxins are the most promising agents for treating infections with extensively drug-resistant (XDR) Gram-negative bacteria. Therefore, we need to foresightedly work on polymyxin adjuvant therapeutics. Adjuvant compounds normally lack inherent antimicrobial activity as single agents, but in combination with a specific antibiotic they potentiate its effects. The idea of polymyxin adjuvant therapy is to reverse polymyxin resistance by inhibiting one of the resistance-driving mechanisms (mechanisms involved in lipid A modifications). Some of the most promising targets include enzymes involved in lipid A modification systems responsible for resistance [e.g. MCR-1, ArnT and diacylglycerol kinase A (DgkA)]. The interactions of colistin with lipid model membranes have been described thoroughly by structural biology studies (48), which have shown how these interactions change when pEtN is added to the phosphate groups of lipid A (49, 50) and how changes in LPS charge renders polymyxins ineffective. Therefore, in theory, for polymyxins to regain their efficacy these changes need to be prevented. In this context, we see another potential application of MALDI-TOF-based polymyxin resistance screening. For instance, analysing lipid A structure would indicate which inhibitor class would be useful for each patient.

MCR-1 is a pEtN transferase encoded by plasmid-borne *mcr-1* genes. Xu et al. (51) have evidenced that MCR-1 [expressed, purified, and incubated with a natural substrate mimetic (nitrobenzodiazole-labelled glycerol-3-pEtN] transfers pEtN to phosphate groups of lipid A in Gram-negative bacteria, while nitrobenzodiazole-glycerol is released. The structure of MCR-1 soluble C-terminal domain has been elucidated through X-ray crystallography (52–56). Its active site is located in the soluble domain, coordinated by an essential Zn^{2+} ion, and has a highly conserved (through the pEtN family) T285 aminoacid residue that acts as a catalytic nucleophile in substrate modifications (52, 57, 58). These findings provide valuable starting point for computer-aided drug design (CADD) in the quest for novel MCR-1 inhibitors (59, 60). Since the discovery of MCR-1, some research groups have been screening for potential MCR-1 inhibitors from natural products (61–66) or developing *de novo* synthesised MCR-1 inhibitors (67). Interestingly, an FDA-approved drug, auranofin (used in the treatment of rheumatoid arthritis), has also shown MCR-1 inhibition (68). So far, this is the most studied lipid A-related target for potential adjuvants. As MCR-1 has been studied quite extensively, there is hope that these findings can be applied to other members of the MCR family. It would be ideal that a candidate drug inhibits all members of the MCR protein family.

As for chromosome-encoded polymyxin resistance, small molecules that downregulate TCS have been found to reverse resistance (69, 70). Another promising target related to chromosome-encoded resistance is ArnT. Ghirga et al. (71) performed a virtual screening for ArnT inhibitors to discover a potent colistin adjuvant BBN149, which can be used as a lead molecule for further development. Quaglio et al. (72) have shown that ent-Beyerane diterpenes inhibit ArnT thanks to their resemblance to L-Ara4N. This means that the structure of these compounds most likely has a similar binding mode as L-Ara4N. Another target which might be of interest is DgkA. As mentioned above, EptA is transferring pEtN to the phosphate groups of lipid A. EptA uses phosphatidylethanoloamine (PE) as its pEtN donor, which results in the formation of diacylglycerol (DAG) as a by-product (10). DAG is quickly phosphorylated by DgkA into phosphatidic acid, a precursor for PE synthesis (73), and DAG accumulation has been

Table 1 *K. pneumoniae* native and modified lipid A structures and their corresponding m/z values (35, 39)

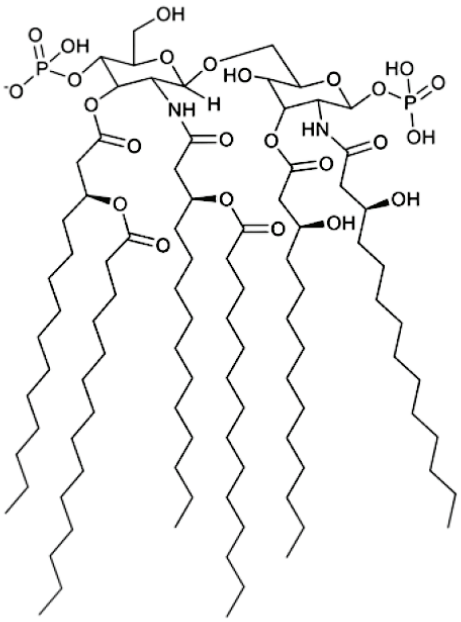
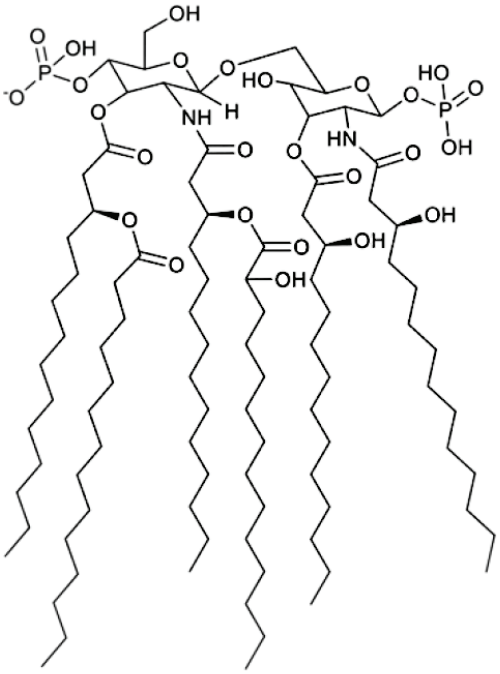
K. pneumoniae		
Type	**m/z**	**Proposed structures**
Native lipid A	1824	
	1840	

Table 1 continued

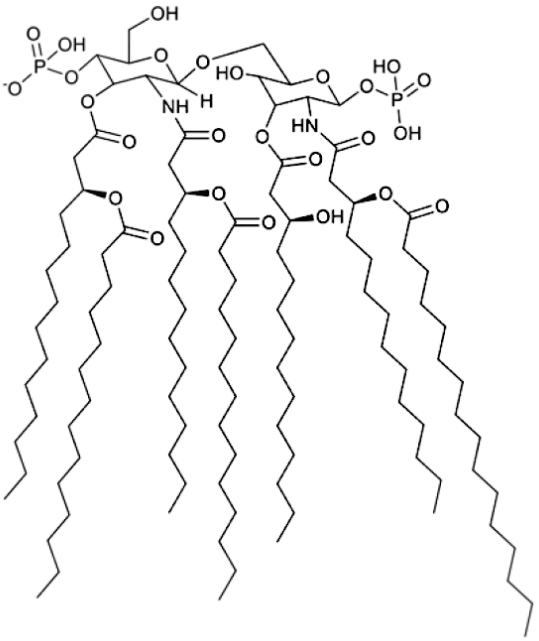
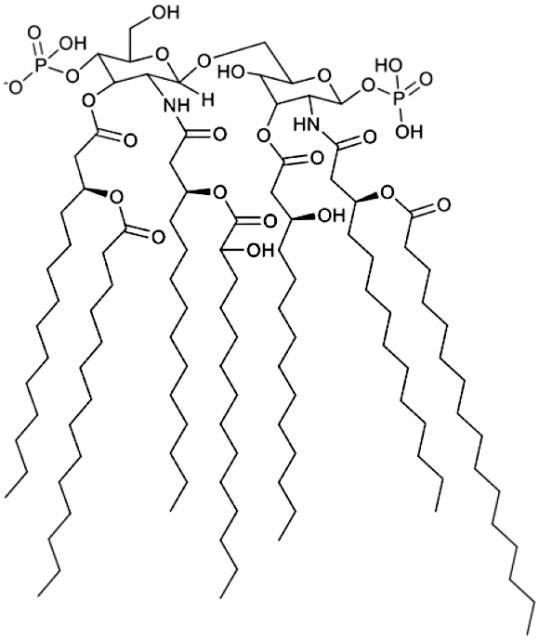
K. pneumoniae		
Type	**m/z**	**Proposed structures**
Native lipid A	2062	
	2078	

Table 1 continued

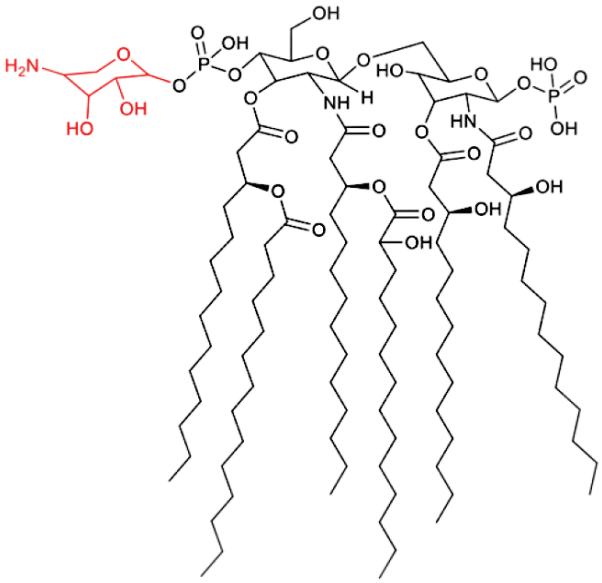
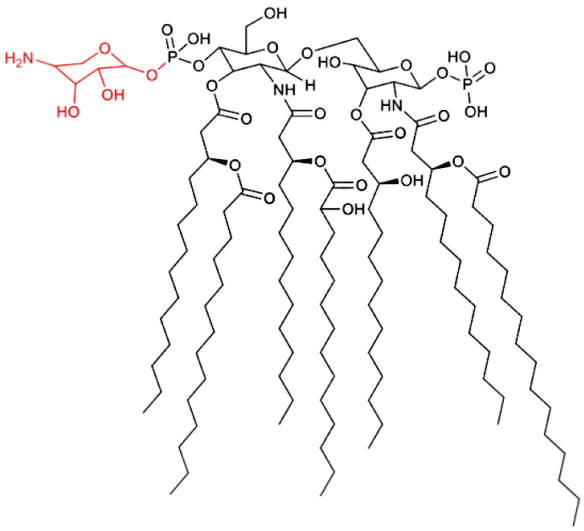
K. pneumoniae		
Type	**m/z**	**Proposed structures**
Chromosome-encoded resistance – L-Ara4N addition	1971 (1840 + 131)	
	2209 (2078 + 131)	

Table 1 continued

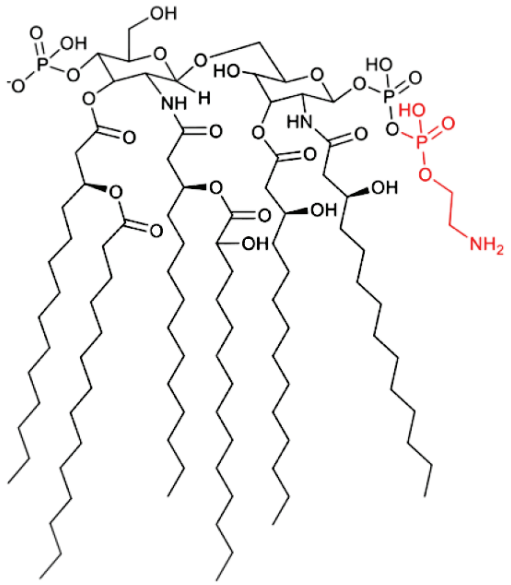
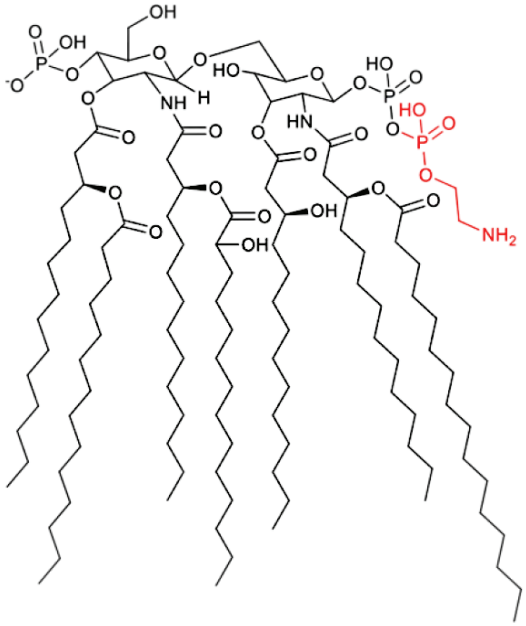
K. pneumoniae		
Type	**m/z**	**Proposed structures**
	1963 (1840 + 123)	
Plasmid-encoded resistance – pEtN addition	2201 (2078 + 123)	

Table 2 *P. aeruginosa* native and modified lipid A structures and their corresponding m/z values (37)

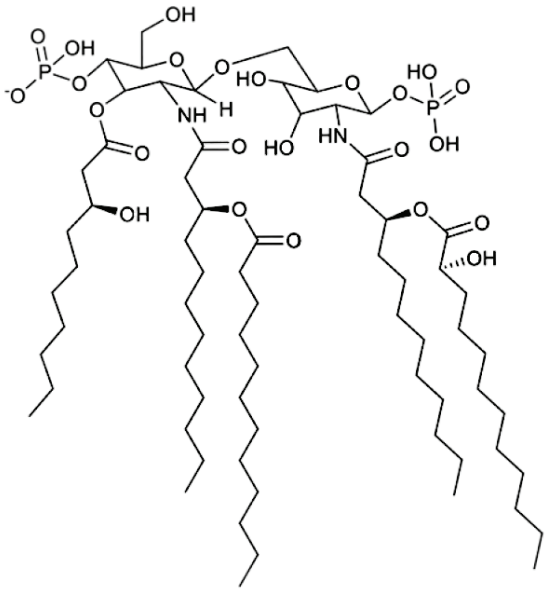
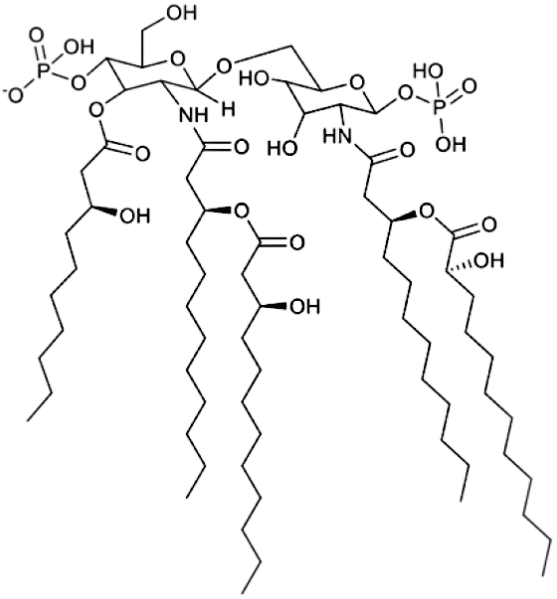
P. aeruginosa		
Type	**m/z**	**Proposed structure**
Native lipid A	1446	
	1462	

Table 2 continued

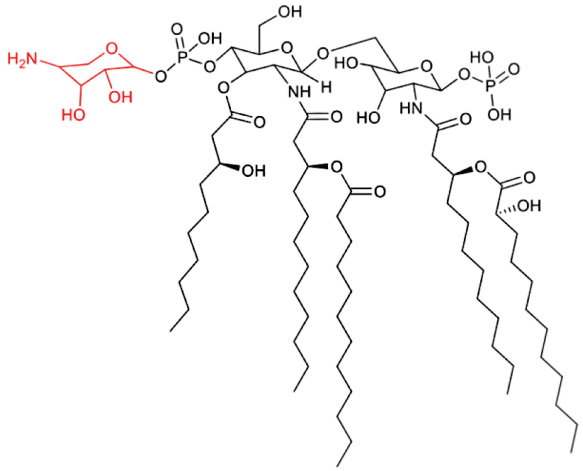
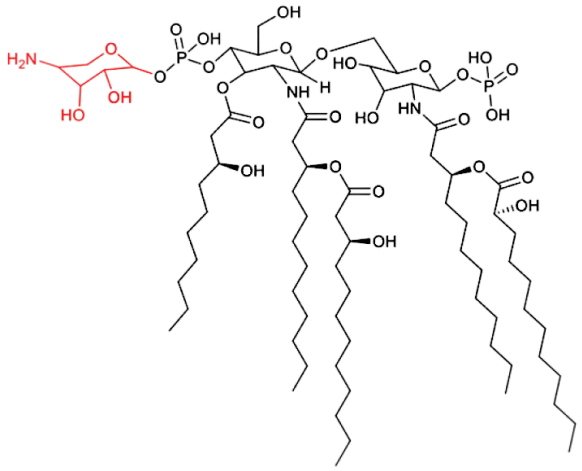
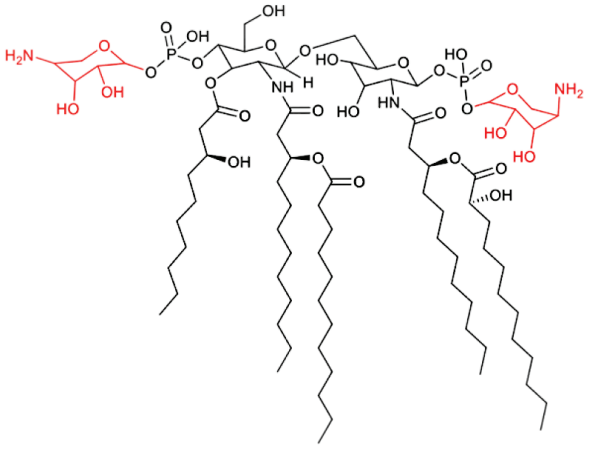
P. aeruginosa		
Type	**m/z**	**Proposed structure**
Chromosome-encoded resistance – L-Ara4N addition	1577 (1446 + 131)	
	1593 (1462 + 131)	
	1708 (1446 + 262)	

Table 2 continued

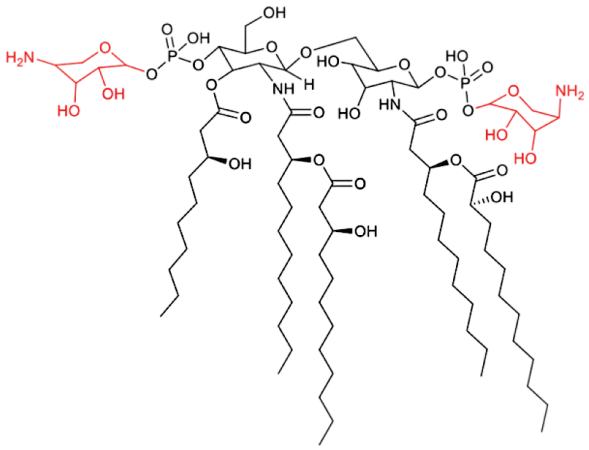
P. aeruginosa		
Type	**m/z**	**Proposed structure**
Chromosome-encoded resistance – L-Ara4N addition	1724 (1462 + 262)	

Table 3 *A. baumannii* native and modified lipid A structures and their corresponding m/z values (36, 39)

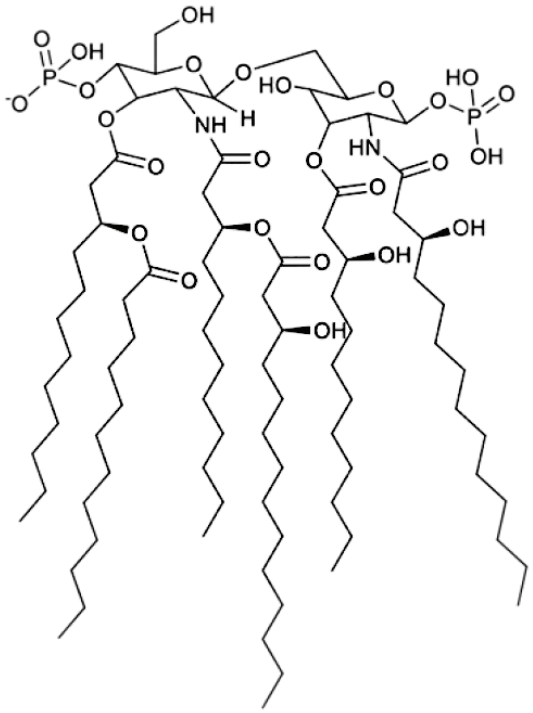
A. baumannii		
Type	**m/z**	**Proposed structure**
Native lipid A	1728	

Table 3 continued

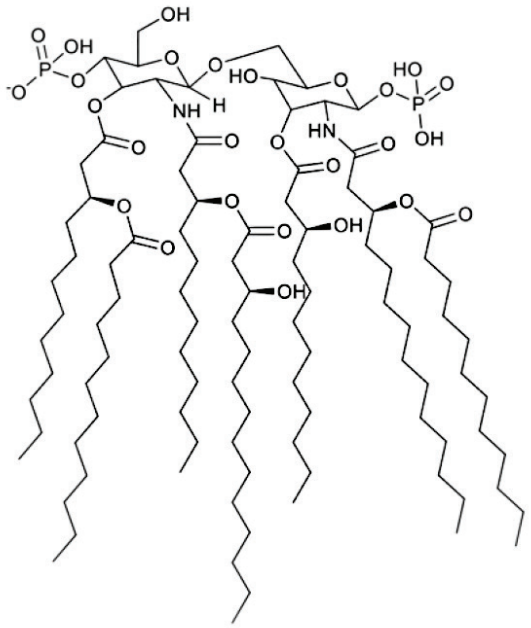
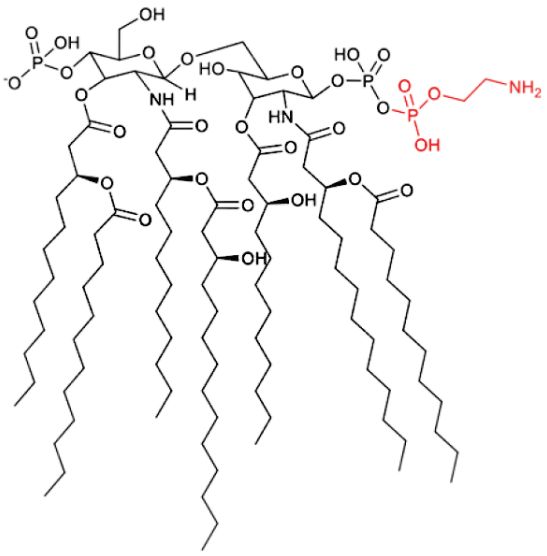
A. baumannii		
Type	**m/z**	**Proposed structure**
Native lipid A	1910	
Chromosome-encoded resistance – pEtN addition	2033 (1910 + 123)	

Table 4 *E. coli* native and modified lipid A structures and their corresponding m/z value (38, 39, 44)

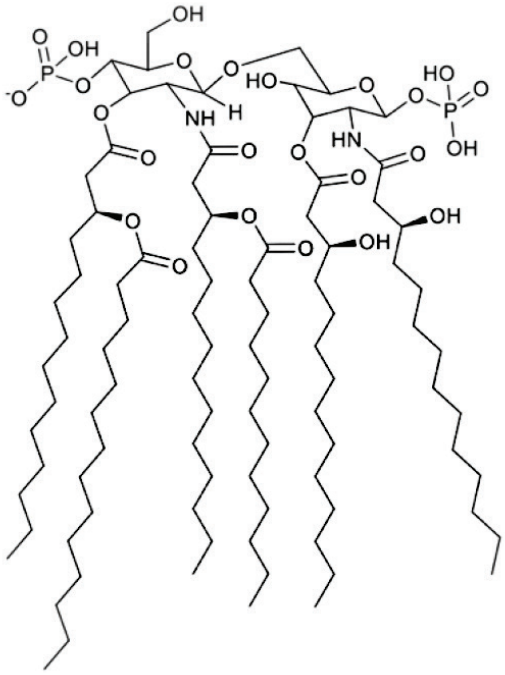
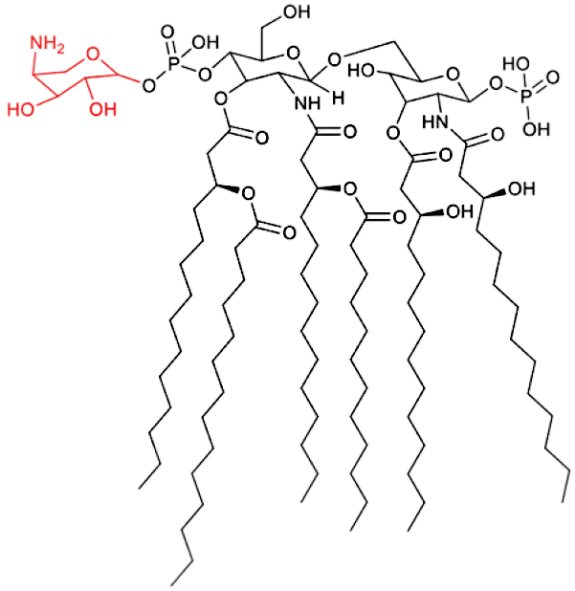
E. coli		
Type	**m/z**	**Proposed structure**
Native lipid A	1796	
Chromosome-encoded resistance – L-Ara4N addition	1927 (1796 + 131)	

Table 4 continued

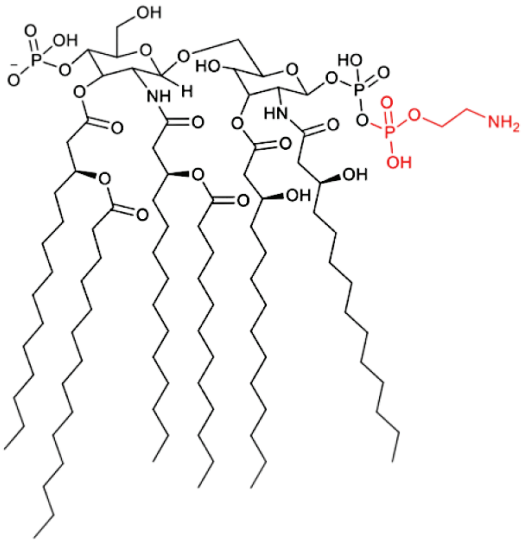
E. coli		
Type	**m/z**	**Proposed structure**
Both plasmid- and chromosome-encoded resistance	1919 (1796 + 123)	

Table 5 *S. enterica* native and modified lipid A structures and their corresponding m/z values (34, 39)

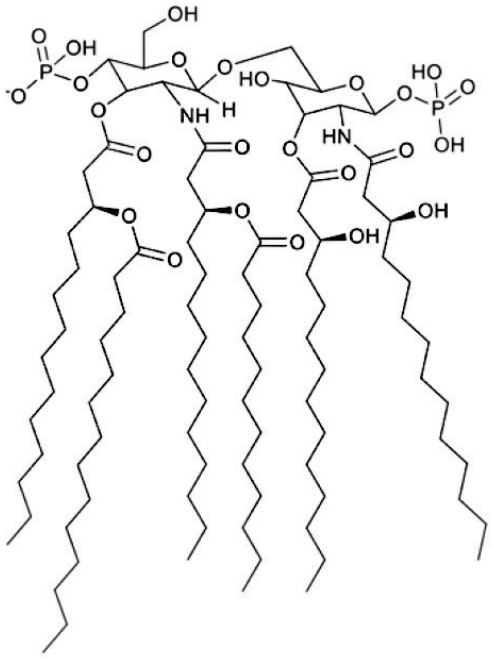
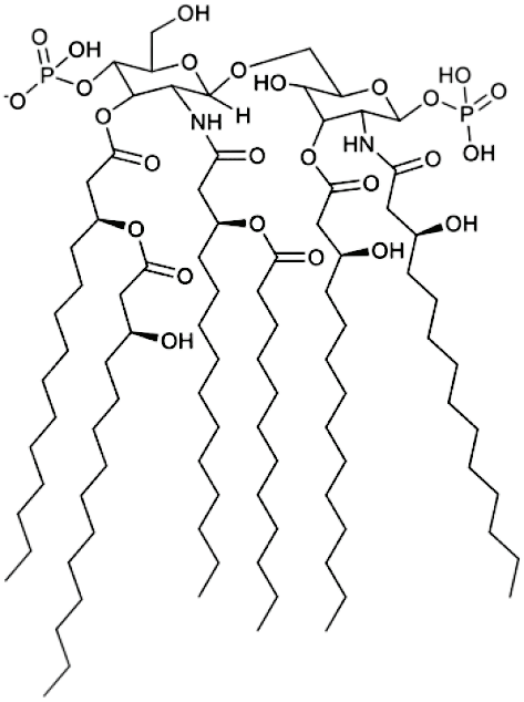
S. enterica		
Type	**m/z**	**Proposed structure**
Native lipid A	1796	
	1812	

Table 5 continued

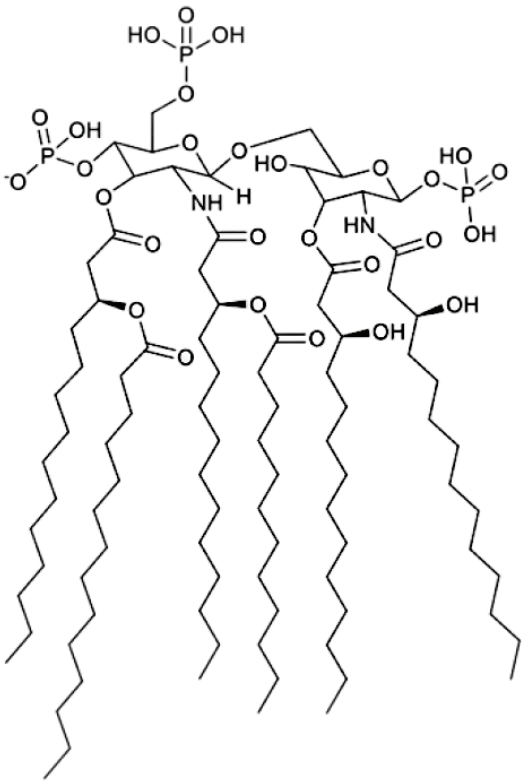
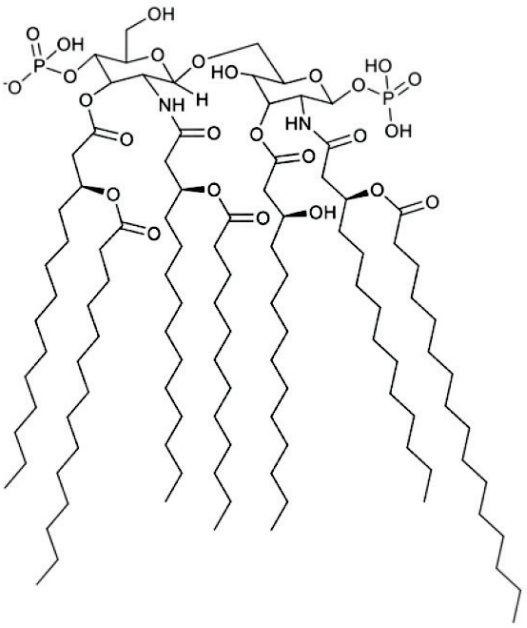
S. enterica		
Type	**m/z**	**Proposed structure**
Native lipid A	1876	
	2034	

Table 5 continued

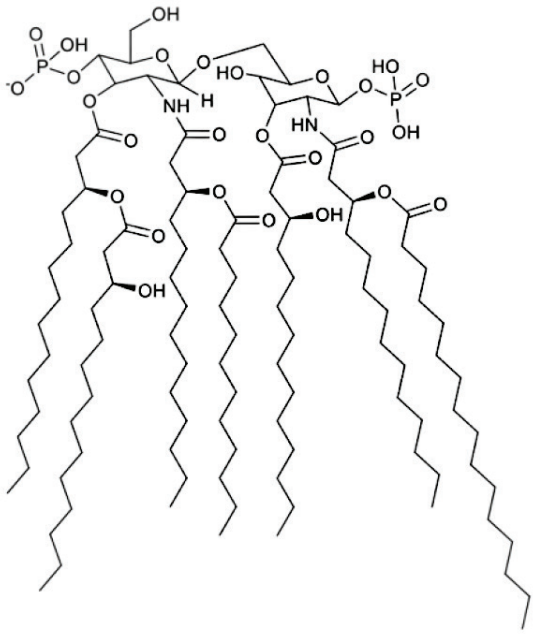
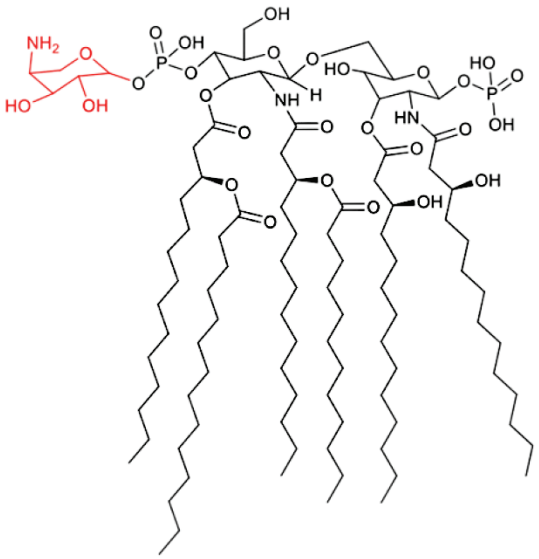
S. enterica		
Type	**m/z**	**Proposed structure**
Native lipid A	2050	
Chromosome-encoded resistance – L-Ara4N addition	1927 (1796 + 131)	

Table 5 continued

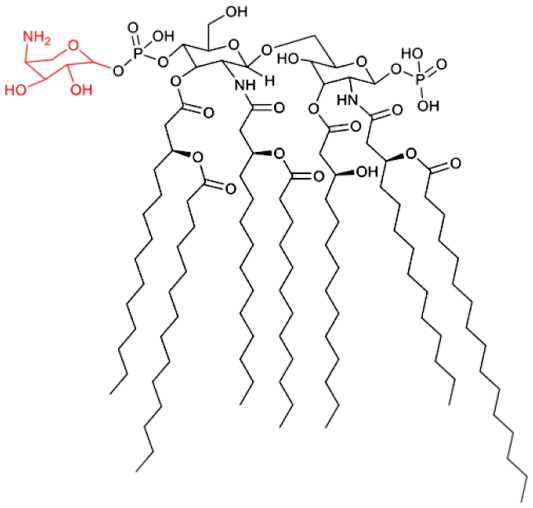
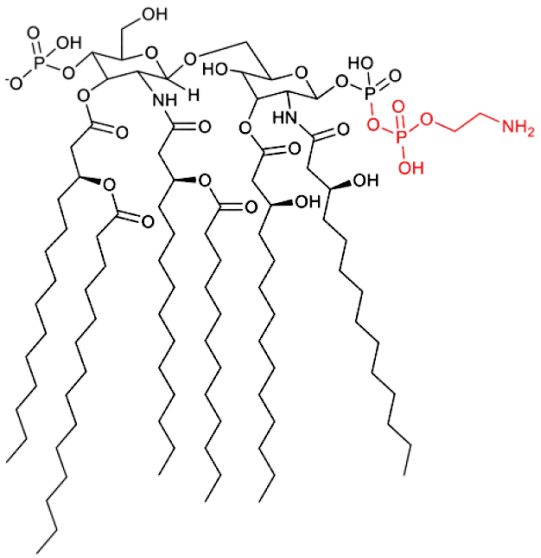
S. enterica		
Type	**m/z**	**Proposed structure**
Chromosome-encoded resistance – L-Ara4N addition	2165 (2034 + 131)	
Plasmid-encoded resistance – pEtN addition	1919 (1796 + 123)	

Table 5 continued

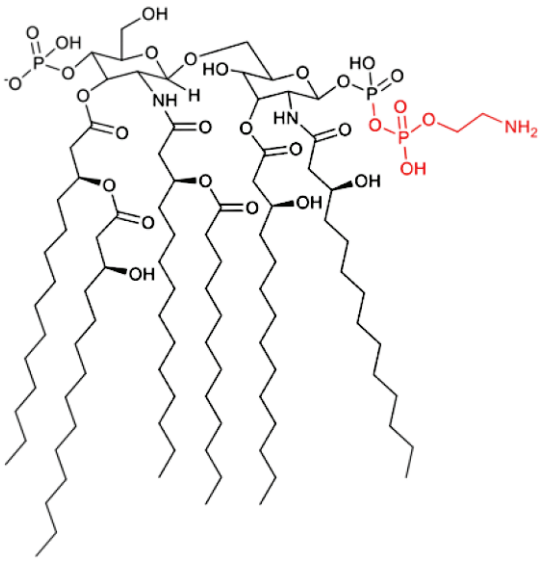
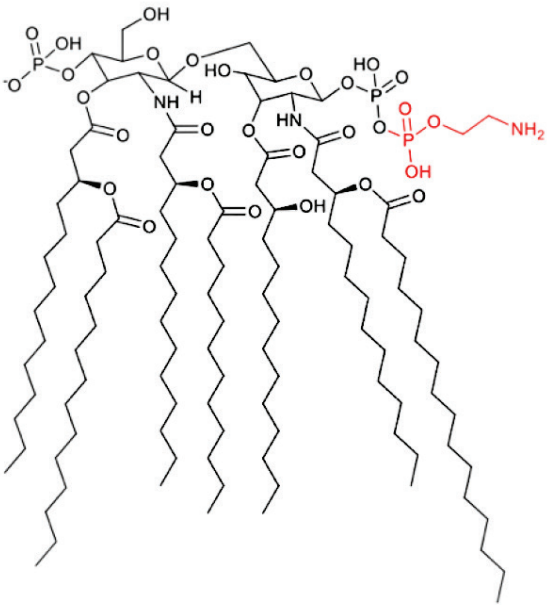
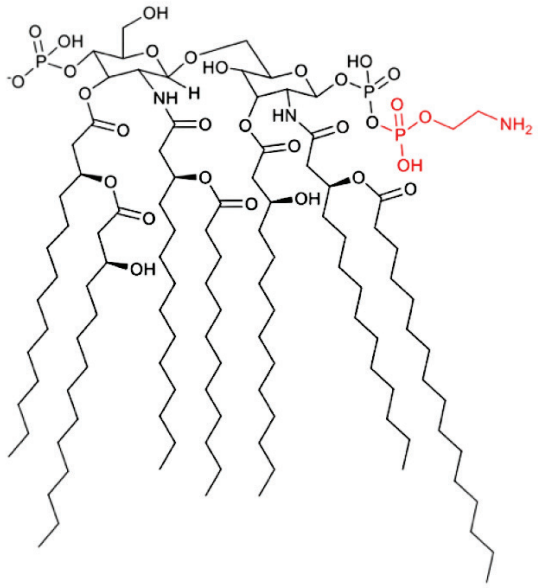
S. enterica		
Type	**m/z**	**Proposed structure**
	1935 (1812 + 123)	
Plasmid-encoded resistance – pEtN addition	2157 (2034 + 123)	

Table 5 continued

S. enterica		
Type	**m/z**	**Proposed structure**
Plasmid-encoded resistance – pEtN addition	2173 (2050 + 123)	

shown to inhibit EptA activity (15, 74). All this points to DgkA as a prominent target of polymyxin resistance adjuvant therapy.

Another potential option are eukaryotic kinase inhibitors, which have been found to re-sensitise bacteria resistant to colistin. Although the mechanism is not clear yet, they have been reported to lower the rate of lipid A modifications by L-Ara4N and pEtN (75). Sertraline, an FDA-approved selective serotonin reuptake inhibitor (SSRI) used for the treatment of depression, has been found to revert polymyxin resistance, as it seems to interfere with the *eptA* lipid A modification pathway (76).

All things considered, there is immense potential for the development of polymyxin adjuvants by targeting lipid A modification systems.

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REFERENCES

1. Velkov T, Thompson PE, Nation RL, Li J. Structure-activity relationships of polymyxin antibiotics. J Med Chem 2010;53:1898–916. doi: 10.1021/jm900999h
2. Li J, Nation RL, Milne RW, Turnidge JD, Coulthard K. Evaluation of colistin as an agent against multi-resistant Gram-negative bacteria. Int J Antimicrob Agents 2005;25:11–25. doi: 10.1016/j.ijantimicag.2004.10.001
3. Ainsworth GC, Brown AM, Brownlee G. 'Aerosporin', an antibiotic produced by *Bacillus aerosporus* Greer. Nature 1947;160(4060):263. doi: 10.1038/160263a0
4. Vaara M. Polymyxins and their potential next generation as therapeutic antibiotics. Front Microbiol 2019;10:1689. doi: 10.3389/fmicb.2019.01689
5. Satlin MJ, Lewis JS, Weinstein MP, Patel J, Humphries RM, Kahlmeter G, Giske CG, Turnidge J. Clinical and Laboratory Standards Institute and European Committee on Antimicrobial Susceptibility Testing position statements on Polymyxin B and colistin clinical breakpoints. Clin Infect Dis 2020;71(9):e523–9. doi: 10.1093/cid/ciaa121
6. Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016;16:161–8. doi: 10.1016/S1473-3099(15)00424-7
7. Hamel M, Rolain JM, Baron SA. The history of colistin resistance mechanisms in bacteria: progress and challenges. Microorganisms 2021;9(2):442. doi: 10.3390/microorganisms9020442
8. Gogry FA, Siddiqui MT, Sultan I, Rizwanul Haq QM. Current update on intrinsic and acquired colistin resistance mechanisms in bacteria. Front Med (Lausanne) 2021;8:677720. doi: 10.3389/fmed.2021.677720
9. Wei W, Srinivas S, Lin J, Tang Z, Wang S, Ullah S, Kota VG, Feng Y. Defining ICR-Mo, an intrinsic colistin resistance determinant from *Moraxella osloensis*. PLOS Genet 2018;14(5):e1007389. doi: 10.1371/journal.pgen.1007389
10. Herrera CM, Hankins JV, Trent MS. Activation of PmrA inhibits LpxT-dependent phosphorylation of lipid A promoting resistance to antimicrobial peptides: Phosphorylation of lipid A inhibits pEtN

addition. Mol Microbiol 2010;76:1444–60. doi: 10.1111/j.1365-2958.2010.07150.x
11. Needham BD, Trent MS. Fortifying the barrier: the impact of lipid A remodelling on bacterial pathogenesis. Nat Rev Microbiol 2013;11:467–81. doi: 10. 1038/nrmicro3047
12. Huang J, Li C, Song J, Velkov T, Wang L, Zhu Y, Li Y. Regulating polymyxin resistance in Gram-negative bacteria: roles of two-component systems PhoPQ and PmrAB. Future Microbiol 2020;15:445–59. doi: 10.2217/fmb-2019-0322
13. Beceiro A, Llobet E, Aranda J, Bengoechea JA, Doumith M, Hornsey M, Dhanji H, Chart H, Bou G, Livermore DM, Woodford N. Phosphoethanolamine modification of lipid A in colistin-resistant variants of *Acinetobacter baumannii* mediated by the *pmrAB* two-component regulatory system. Antimicrob Agents Chemother 2011;55:3370–9. doi: 10.1128/AAC.00079-11
14. Osei Sekyere J, Govinden U, Bester LA, Essack SY. Colistin and tigecycline resistance in carbapenemase-producing Gram-negative bacteria: emerging resistance mechanisms and detection methods. J Appl Microbiol 2016;121:601–17. doi: 10.1111/jam.13169
15. Purcell AB, Voss BJ, Trent MS. Diacylglycerol kinase A is essential for polymyxin resistance provided by EptA, MCR-1, and other lipid A phosphoethanolamine transferases. J Bacteriol 2022;204(2):e00498–21. doi: 10.1128/jb.00498-21
16. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro Surveill 2016;21(27). doi: 10.2807/1560-7917.ES.2016.21.27.30280
17. AbuOun M, Stubberfield EJ, Duggett NA, Kirchner M, Dormer L, Nunez-Garcia J, Randall LP, Lemma F, Crook DW, Teale C, Smith RP, Anjum MF. *mcr*-1 and *mcr-2* (*mcr-6.1*) variant genes identified in *Moraxella* species isolated from pigs in Great Britain from 2014 to 2015. J Antimicrob Chemother 2017;72:2745–9. doi: 10.1093/jac/dkx286
18. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. mBio 2017;8(3):e00543–17. doi: 10.1128/mBio.00543-17
19. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in *d*-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. J Antimicrob Chemother 2017;72:3317–24. doi: 10.1093/jac/dkx327
20. Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, Pezzotti G, Magistrali CF. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill 2017;22(31):30589. doi: 10.2807/1560-7917.ES.2017.22.31.30589
21. Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, Zhang S, Shen J, Shen Z, Wang Y. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. Emerg Microbes Infect 2018;7(1):122. doi: 10.1038/s41426-018-0124-z
22. Wang C, Feng Y, Liu L, Wei L, Kang M, Zong Z. Identification of novel mobile colistin resistance gene *mcr-10*. Emerg Microbes Infect 2020;9:508–16. doi: 10.1080/22221751.2020.1732231
23. Yang YQ, Li YX, Lei CW, Zhang AY, Wang HN. Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. J Antimicrob Chemother 2018;73:1791–5. doi: 10.1093/jac/dky111
24. Carroll LM, Gaballa A, Guldimann C, Sullivan G, Henderson LO, Wiedmann M. Identification of novel mobilized colistin resistance gene *mcr-9* in a multidrug-resistant, colistin-susceptible *Salmonella enterica* serotype Typhimurium isolate. mBio 2019;10(3):e00853–19. doi: 10.1128/mBio.00853-19
25. Campos MA, Vargas MA, Regueiro V, Llompart CM, Albertí S, Bengoechea JA. Capsule polysaccharide mediates bacterial resistance to antimicrobial peptides. Infect Immun 2004;72:7107–14. doi: 10.1128/IAI.72.12.7107-7114.2004
26. Padilla E, Llobet E, Doménech-Sánchez A, Martínez-Martínez L, Bengoechea JA, Albertí S. *Klebsiella pneumoniae* AcrAB efflux pump contributes to antimicrobial resistance and virulence. Antimicrob Agents Chemother 2010;54:177–83. doi: 10.1128/AAC.00715-09
27. Mackenzie JS, Jeggo M. The One Health approach – Why is it so important? Trop Med Infect Dis 2019;4(2):88. doi: 10.3390/tropicalmed4020088
28. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clinical breakpoints – breakpoints and guidance [displayed 31 July 2023]. Available at https://www.eucast.org/clinical_breakpoints.
29. Liao W, Lin J, Jia H, Zhou C, Zhang Y, Lin Y, Ye J, Cao J, Zhou T. Resistance and heteroresistance to colistin in *Escherichia coli* isolates from Wenzhou, China. Infect Drug Resist 2020;13:3551–61. doi: 10.2147/IDR.S273784
30. Lin J, Xu C, Fang R, Cao J, Zhang X, Zhao Y, Dong G, Sun Y, Zhou T. Resistance and heteroresistance to colistin in *Pseudomonas aeruginosa* isolates from Wenzhou, China. Antimicrob Agents Chemother 2019;63(10):e00556–19. doi: 10.1128/AAC.00556-19
31. Chen L, Lin J, Lu H, Zhang X, Wang C, Liu H, Zhang X, Li J, Cao J, Zhou T. Deciphering colistin heteroresistance in *Acinetobacter baumannii* clinical isolates from Wenzhou, China. J Antibiot (Tokyo) 2020;73:463–70. doi: 10.1038/s41429-020-0289-2
32. Band VI, Crispell EK, Napier BA, Herrera CM, Tharp GK, Vavikolanu K, Pohl J, Read TD, Bosinger SE, Trent MS, Burd EM, Weiss DS. Antibiotic failure mediated by a resistant subpopulation in *Enterobacter cloacae*. Nat Microbiol 2016;1(6):16053. doi: 10.1038/nmicrobiol.2016.53
33. Hjort K, Nicoloff H, Andersson DI. Unstable tandem gene amplification generates heteroresistance (variation in resistance within a population) to colistin in *Salmonella enterica*. Mol Microbiol 2016;102:274–89. doi: 10.1111/mmi.13459
34. Charretier Y, Diene SM, Baud D, Chatellier S, Santiago-Allexant E, van Belkum A, Guigon G, Schrenzel J. Colistin heteroresistance and involvement of the PmrAB regulatory system in *Acinetobacter baumannii*. Antimicrob Agents Chemother 2018;62(9):e00788–18. doi: 10.1128/AAC.00788-18
35. El-Halfawy OM, Valvano MA. Antimicrobial heteroresistance: an emerging field in need of clarity. Clin Microbiol Rev 2015;28:191–207. doi: 10. 1128/CMR. 00058-14
36. Dortet L, Bonnin RA, Le Hello S, Fabre L, Bonnet R, Kostrzewa M, Filloux A, Larrouy-Maumus G. Detection of colistin resistance in *Salmonella enterica* using MALDIxin test on the routine MALDI biotyper sirius mass spectrometer. Front Microbiol 2020;11:1141. doi: 10.3389/fmicb.2020.01141
37. Dortet L, Broda A, Bernabeu S, Glupczynski Y, Bogaerts P, Bonnin R, Naas T, Filloux A, Larrouy-Maumus G. Optimization of the MALDIxin test for the rapid identification of colistin resistance in *Klebsiella pneumoniae* using MALDI-TOF MS. J Antimicrob Chemother 2020;75:110–6. doi: 10.1093/jac/dkz405

38. Dortet L, Potron A, Bonnin RA, Plesiat P, Naas T, Filloux A, Larrouy-Maumus G. Rapid detection of colistin resistance in *Acinetobacter baumannii* using MALDI-TOF-based lipidomics on intact bacteria. Sci Rep 2018;8(1):16910. doi: 10. 1038/s41598-018-35041-y
39. Jeannot K, Hagart K, Dortet L, Kostrzewa M, Filloux A, Plesiat P, Larrouy-Maumus G. Detection of colistin resistance in *Pseudomonas aeruginosa* using the MALDIxin test on the routine MALDI biotyper sirius mass spectrometer. Front Microbiol 2021;12:725383. doi: 10.3389/fmicb.2021.725383
40. Furniss RCD, Dortet L, Bolland W, Drews O, Sparbier K, Bonnin RA, Filloux A, Kostrzewa M, Mavridou DAI, Larrouy-Maumus G. Detection of colistin resistance in *Escherichia coli* by use of the MALDI biotyper sirius mass spectrometry system. J Clin Microbiol 2019;57(12):e01427–19. doi: 10.1128/JCM.01427-19
41. Furniss RCD, Kostrzewa M, Mavridou DAI, Larrouy-Maumus G. The clue is in the lipid A: rapid detection of colistin resistance. PLOS Pathog 2020;16(4):e1008331. doi: 10.1371/journal.ppat.1008331
42. Sorensen M, Chandler CE, Gardner FM, Ramadan S, Khot PD, Leung LM, Farrance CE, Goodlett DR, Ernst RK, Nilsson E. Rapid microbial identification and colistin resistance detection via MALDI-TOF MS using a novel on-target extraction of membrane lipids. Sci Rep 2020;10(1):21536. doi: 10.1038/s41598-020-78401-3
43. Smith RD, McElheny CL, Izac JR, Gardner FM, Chandler CE, Goodlett DR, Doi Y, Johnson JK, Ernst RK. A novel lipid-based MALDI-TOF assay for the rapid detection of colistin-resistant *Enterobacter* species. Microbiol Spectr 2022;10(1):e01445–21. doi: 10.1128/spectrum.01445-21
44. Yang H, Smith RD, Chandler CE, Johnson JK, Jackson SN, Woods AS, Scott AJ, Goodlett DR, Ernst RK. Lipid A structural determination from a single colony. Anal Chem 2022;94:7460–5. doi: 10.1021/acs.analchem.1c05394
45. Leung LM, Cooper VS, Rasko DA, Guo Q, Pacey MP, McElheny CL, Mettus RT, Yoon SH, Goodlett DR, Ernst RK, Doi Y. Structural modification of LPS in colistin-resistant, KPC-producing *Klebsiella pneumoniae*. J Antimicrob Chemother 2017;72:3035–42. doi: 10.1093/jac/dkx234
46. Dortet L, Bonnin RA, Pennisi I, Gauthier L, Jousset AB, Dabos L, Furniss RCD, Mavridou DAI, Bogaerts P, Glupczynski Y, Potron A, Plesiat P, Beyrouthy R, Robin F, Bonnet R, Naas T, Filloux A, Larrouy-Maumus G. Rapid detection and discrimination of chromosome- and MCR-plasmid-mediated resistance to polymyxins by MALDI-TOF MS in *Escherichia coli*: the MALDIxin test. J Antimicrob Chemother 2018;73:3359–67. doi: 10.1093/jac/dky330
47. Bruker. MBT Lipid Xtract™ Kit [displayed 15 Jan 2023]. Available at https://www.bruker.com/en/products-and-solutions/microbiology-and-diagnostics/microbial-identification/mbt-lipid-xtract-kit.html
48. Dupuy FG, Pagano I, Andenoro K, Peralta MF, Elhady Y, Heinrich F, Tristram-Nagle S. Selective interaction of colistin with lipid model membranes. Biophys J 2018;114:919–28. doi: 10.1016/j.bpj.2017.12.027
49. Gogry FA, Siddiqui MT, Sultan I, Husain FM, Al-Kheraif AA, Ali A, Haq QMR. Colistin interaction and surface changes associated with *mcr*-1 conferred plasmid mediated resistance in *E. coli* and *A. veronii* strains. Pharmaceutics 2022;14(2):295. doi: 10.3390/pharmaceutics14020295
50. Ma W, Jiang X, Dou Y, Zhang Z, Li J, Yuan B, Yang K. Biophysical impact of lipid A modification caused by mobile colistin resistance gene on bacterial outer membranes. J Phys Chem Lett 2021;12:11629–35. doi: 10.1021/acs.jpclett.1c03295
51. Xu Y, Lin J, Cui T, Srinivas S, Feng Y. Mechanistic insights into transferable polymyxin resistance among gut bacteria. J Biol Chem 2018;293:4350–65. doi: 10.1074/jbc.RA117. 000924
52. Hinchliffe P, Yang QE, Portal E, Young T, Li H, Tooke CL, Carvalho MJ, Paterson NG, Brem J, Niumsup PR, Tansawai U, Lei L, Li M, Shen Z, Wang Y, Schofield CJ, Mulholland AJ, Shen J, Fey N, Walsh TR, Spencer J. Insights into the mechanistic basis of plasmid-mediated colistin resistance from crystal structures of the catalytic domain of MCR-1. Sci Rep 2017;7:39392. doi: 10.1038/srep39392
53. Hu M, Guo J, Cheng Q, Yang Z, Chan EWC, Chen S, Hao Q. Crystal structure of *Escherichia coli* originated MCR-1, a phosphoethanolamine transferase for colistin resistance. Sci Rep 2016;6:38793. doi: 10.1038/srep38793
54. Ma G, Zhu Y, Yu Z, Ahmad A, Zhang H. High resolution crystal structure of the catalytic domain of MCR-1. Sci Rep 2016;6:39540. doi: 10.1038/srep39540
55. Stojanoski V, Sankaran B, Prasad BVV, Poirel L, Nordmann P, Palzkill T. Structure of the catalytic domain of the colistin resistance enzyme MCR-1. BMC Biol 2016;14(1):81. doi: 10.1186/s12915-016-0303-0
56. Liu ZX, Han Z, Yu XL, Wen G, Zeng C. Crystal structure of the catalytic domain of MCR-1 (cMCR-1) in complex with d-xylose. Crystals 2018;8(4):172. doi: 10.3390/cryst8040172
57. Lythell E, Suardíaz R, Hinchliffe P, Hanpaibool C, Visitsatthawong S, Oliveira ASF, Lang EJM, Surawatanawong P, Lee VS, Rungrotmongkol T, Fey N, Spencer J, Mulholland AJ. Resistance to the "last resort" antibiotic colistin: a single-zinc mechanism for phosphointermediate formation in MCR enzymes. Chem Commun 2020;56:6874–7. doi: 10.1039/D0CC02520H
58. Wei P, Song G, Shi M, Zhou Y, Liu Y, Lei J, Chen P, Yin L. Substrate analog interaction with MCR-1 offers insight into the rising threat of the plasmid-mediated transferable colistin resistance. FASEB J 2018;32:1085–98. doi: 10.1096/fj. 201700705R
59. Kai J, Wang S. Recent progress on elucidating the molecular mechanism of plasmid-mediated colistin resistance and drug design. Int Microbiol 2020;23:355–66. doi: 10.1007/s10123-019-00112-1
60. Son SJ, Huang R, Squire CJ, Leung IKH. MCR-1: a promising target for structure-based design of inhibitors to tackle polymyxin resistance. Drug Discov Today 2019;24:206–16. doi: 10.1016/j.drudis.2018.07.004
61. Zhou Y, Wang J, Guo Y, Liu X, Liu S, Niu X, Wang Y, Deng X. Discovery of a potential MCR-1 inhibitor that reverses polymyxin activity against clinical *mcr-1*-positive *Enterobacteriaceae*. J Infect 2019;78:364–72. doi: 10.1016/j.jinf.2019.03.004
62. Zhou Y, Wang T, Guo Y, Liu S, Wang J, Shen Y, Tang S, Wang Y, Deng X. *In vitro*/*vivo* activity of potential MCR-1 inhibitor in combination with colistin againsts *mcr-1*-positive *Klebsiella pneumonia*. Front Microbiol 2018;9:1615. doi: 10.3389/fmicb.2018.01615
63. Zhou Y, Liu S, Wang T, Li H, Tang S, Wang J, Wang Y, Deng X. Pterostilbene, a potential MCR-1 inhibitor that enhances the efficacy of polymyxin B. Antimicrob Agents Chemother 2018;62(4):e02146–17. doi: 10.1128/AAC.02146-17
64. Liu X, Sun X, Deng X, Lv X, Wang J. Calycosin enhances the bactericidal efficacy of polymyxin B by inhibiting MCR-1 *in vitro*. J Appl Microbiol 2020;129:532–40. doi: 10.1111/jam.14635
65. Wang Y, Liu X, Sun X, Wen Z, Wang D, Peng L. A potential inhibitor of MCR-1: an attempt to enhance the efficacy of polymyxin against multidrug-resistant bacteria. Curr Microbiol 2020;77:3256–63. doi: 10.1007/s00284-020-02096-y

66. Xie S, Li L, Zhan B, Shen X, Deng X, Tan W, Fang T. Pogostone enhances the antibacterial activity of colistin against MCR-1-positive bacteria by inhibiting the biological function of MCR-1. Molecules 2022;27(9):2819. doi: 10.3390/molecules27092819
67. Lan XJ, Yan HT, Lin F, Hou S, Li CC, Wang GS, Sun W, Xiao JH, Li S. Design, synthesis and biological evaluation of 1-phenyl-2-(phenylamino) ethanone derivatives as novel MCR-1 inhibitors. Molecules 2019;24(15):2719. doi: 10.3390/molecules24152719
68. Sun H, Zhang Q, Wang R, Wang H, Wong YT, Wang M, Hao Q, Yan A, Kao RY, Ho PL, Li H. Resensitizing carbapenem- and colistin-resistant bacteria to antibiotics using auranofin. Nat Commun 2020;11(1):5263. doi: 10.1038/s41467-020-18939-y
69. Harris TL, Worthington RJ, Hittle LE, Zurawski DV, Ernst RK, Melander C. Small molecule downregulation of PmrAB reverses lipid A modification and breaks colistin resistance. ACS Chem Biol 2014;9:122–7. doi: 10.1021/cb400490k
70. Brackett CM, Furlani RE, Anderson RG, Krishnamurthy A, Melander RJ, Moskowitz SM, Ernst RK, Melander C. Second generation modifiers of colistin resistance show enhanced activity and lower inherent toxicity. Tetrahedron 2016;72:3549–53. doi: 10.1016/j.tet.2015.09.019
71. Ghirga F, Stefanelli R, Cavinato L, Lo Sciuto A, Corradi S, Quaglio D, Calcaterra A, Casciaro B, Loffredo MR, Cappiello F, Morelli P, Antonelli A, Rossolini GM, Mangoni M, Mancone C, Botta B, Mori M, Ascenzioni F, Imperi F. A novel colistin adjuvant identified by virtual screening for ArnT inhibitors. J Antimicrob Chemother 2020;75:2564–72. doi: 10.1093/jac/dkaa200
72. Quaglio D, Mangoni ML, Stefanelli R, Corradi S, Casciaro B, Vergine V, Lucantoni F, Cavinato L, Cammarone S, Loffredo MR, Cappiello F, Calcaterra A, Erazo S, Ghirga F, Mori M, Imperi F, Ascenzioni F, Botta B. *ent*-beyerane diterpenes as a key platform for the development of ArnT-mediated colistin resistance inhibitors. J Org Chem 2020;85:10891–901. doi: 10.1021/acs.joc.0c01459
73. Yao J, Rock CO. Phosphatidic acid synthesis in bacteria. Biochim Biophys Acta 2013;1831:495–502. doi: 10.1016/j.bbalip.2012.08.018
74. May KL. Drown them in their own garbage: a new strategy to reverse polymyxin resistance? J Bacteriol 2022;204(2):e00574–21. doi: 10.1128/jb.00574-21
75. Barker WT, Nemeth AM, Brackett SM, Basak AK, Chandler CE, Jania LA, Zuercher WJ, Melander RJ, Koller BH, Ernst RK, Melander C. Repurposing eukaryotic kinase inhibitors as colistin adjuvants in Gram-negative bacteria. ACS Infect Dis 2019;5:1764–71. doi: 10.1021/acsinfecdis.9b00212
76. Hussein M, Schneider-Futschik EK, Paulin OKA, Allobawi R, Crawford S, Zhou QT, Hanif A, Baker M, Zhu Y, Li J, Velkov T. Effective strategy targeting polymyxin-resistant Gram-negative pathogens: polymyxin B in combination with the selective serotonin reuptake inhibitor sertraline. ACS Infect Dis 2020;6:1436–50. doi: 10.1021/acsinfecdis.0c00108

Odvezivanje sidra lipopolisaharida: sustavi za strukturne modifikacije lipida A nude dijagnostičke i terapijske opcije za borbu protiv polimiksinske rezistencije

Polimiksinski antibiotici zadnja su linija liječenja pacijenata u intenzivnim jedinicama liječenja koji su inficirani multiplorezistentnim gram-negativnim bakterijama. S obzirom na njihovu polikationsku strukturu, njihov mehanizam djelovanja temelji se na ionskoj interakciji s negativno nabijenim dijelom lipopolisaharida koji se naziva lipid A. Najčešći mehanizmi rezistencije na polimiksine uključuju kovalentne modifikacije lipida A: adiciju kationskog šećera 4-amino-L-arabinoze (L-Ara4N) i/ili fosfoetanoloamina (pEtN). Modificirana struktura lipida A sadrži niži neto negativni naboj, što prouzročuje odbojne sile između polimiksina i lipida A. To dovodi do bakterijske rezistencije na urušavanje integriteta stanične membrane. Geni koji kodiraju za enzimske sustave koji sudjeluju u navedenim modifikacijama mogu se prenositi kromosomima ili mobilnim genskim elementima. Stoga su i razvijeni novi pristupi dijagnostici rezistencije. Osim toga, u navedenim enzimskim sustavima postoje i moguće nove terapijske mete za razvoj lijekova. Ovaj pregledni rad usredotočuje se na dijagnostičke metode koje se temelje na strukturnim promjenama u lipidu A i na terapijskom potencijalu molekula koje interferiraju s navedenim strukturnim modifikacijama.

KLJUČNE RIJEČI: antimikrobna rezistencija; adjuvansi; gram-negativne bakterije; MALDI-TOF-MS; MCR-1