3 History, Chemistry and Antibacterial Spectrum

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Abstract

Polymyxins are naturally occurring cyclic lipopeptides that were discovered more than 60 years ago. They have a narrow antibacterial spectrum, which is mainly against Gram-negative pathogens. The dry antibiotic pipeline, together with the increasing incidence of bacterial resistance in the clinic, has been dubbed 'the perfect storm'. This has forced a re-evaluation of 'old' antibiotics, in particular the polymyxins, which retain activity against many multidrug-resistant (MDR) Gram-negative organisms. As a consequence, polymyxin B and colistin (polymyxin E) are now used as the last therapeutic option for infections caused by 'superbugs' such as *Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Klebsiella pneumoniae*. This chapter covers the history, chemistry and antibacterial spectrum of these very important last-line lipopeptide antibiotics.

Keywords

Discovery of polymyxins · Aerosporin · Chemical structure · Methanesulphonate · Antibacterial spectrum

3.1 History

3.1.1 Discovery

The polymyxins are a family of chemically distinct antibiotics produced by the widely distributed Gram-positive spore-forming soil bacterium *Paenibacillus polymyxa* (previously known as *Bacillus polymyxa*) (Table 3.1). They were first identified in the 1940s simultaneously by three different research groups working independently in the field of antibiotic discovery [1–3]. Initially, Benedict and Langlykke at the Northern Regional Research Laboratories in the United States published a paper in July of 1947 describing the antbacterial properties of crude liquid cultures of *Paenibacillus polymyxa* [1]. Later that month Stansley, Shepherd and White at the Stamford Research Laboratories of the American Cyanamid Company in the United States published a paper

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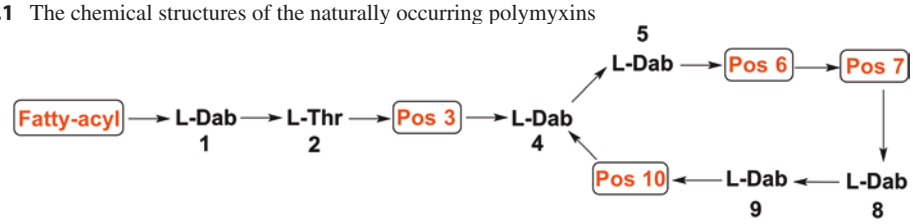
Table 3.1 The chemical structures of the naturally occurring polymyxins



Polymyxin	Fatty-acyl group	Pos 3	Pos 6	Pos 7	Pos 10
A_1	(*S*)-6-methyloctanoyl	D-Dab	D-Leu	L-Thr	L-Thr
A_2	6-methylheptanoyl	D-Dab	D-Leu	L-Thr	L-Thr
B_1	(*S*)-6-methyloctanoyl	L-Dab	D-Phe	L-Leu	L-Thr
B_2	6-methylheptanoyl	L-Dab	D-Phe	L-Leu	L-Thr
B_3	octanoyl	L-Dab	D-Phe	L-Leu	L-Thr
B_4	heptanoyl	L-Dab	D-Phe	L-Leu	L-Thr
B_5	nonanoyl	L-Dab	D-Phe	L-Leu	L-Thr
B_6	3-hydroxy-6-methyloctanoyl[a]	L-Dab	D-Phe	L-Leu	L-Thr
B_1-Ile (Circulin A)	(*S*)-6-methyloctanoyl	L-Dab	D-Phe	L-Ile	L-Thr
B_2-Ile (Circulin A)	6-methylheptanoyl	L-Dab	D-Phe	L-Ile	L-Thr
Dab3-B_1	(*S*)-6-methyloctanoyl	D-Dab	D-Phe	L-Leu	L-Thr
Dab3-B_2	6-methylheptanoyl	D-Dab	D-Phe	L-Leu	L-Thr
C_1[†]	6-methyloctanoyl[b]	L/D-Dab	D-Phe	L-Thr	L-Thr
C_2[†]	6-methylheptanoyl	L/D-Dab	D-Phe	L-Thr	L-Thr
D_1	(*S*)-6-methyloctanoyl	D-Ser	D-Leu	L-Thr	L-Thr
D_2	6-methylheptanoyl	D-Ser	D-Leu	L-Thr	L-Thr
E_1 (Colistin A)	(*S*)-6-methyloctanoyl	L-Dab	D-Leu	L-Leu	L-Thr
E_2 (Colistin B)	6-methylheptanoyl	L-Dab	D-Leu	L-Leu	L-Thr
E_3	octanoyl	L-Dab	D-Leu	L-Leu	L-Thr
E_4	heptanoyl	L-Dab	D-Leu	L-Leu	L-Thr
E_7	7-methyloctanoyl	L-Dab	D-Leu	L-Leu	L-Thr
E_1-Ile	(*S*)-6-methyloctanoyl	L-Dab	D-Leu	L-Ile	L-Thr
E_1-Val	(*S*)-6-methyloctanoyl	L-Dab	D-Leu	L-Val	L-Thr
E_1-Nva	(*S*)-6-methyloctanoyl	L-Dab	D-Leu	L-Nva	L-Thr
E_2-Ile	6-methylheptanoyl	L-Dab	D-Leu	L-Ile	L-Thr
E_2-Val	6-methylheptanoyl	L-Dab	D-Leu	L-Val	L-Thr
E_8-Ile	7-methylnonanoyl	L-Dab	D-Leu	L-Ile	L-Thr
F[†]	6-methyloctanoyl[b]	L/D-Dab	D-Leu/D-Ile	L-Leu/L-Ile/L-Ser	L-Leu/L-Ile/L-Ser
F[†]	6-methylheptanoyl	L/D-Dab	D-Leu/D-Ile	L-Leu/L-Ile/L-Ser	L-Leu/L-Ile/L-Ser
F[†]	octanoyl	L/D-Dab	D-Leu/D-Ile	L-Leu/L-Ile/L-Ser	L-Leu/L-Ile/L-Ser
M_1 (Mattacin)	(*S*)-6-methyloctanoyl	D-Dab	D-Leu	L-Thr	L-Thr
M_2 (Mattacin)	6-methylheptanoyl	D-Dab	D-Leu	L-Thr	L-Thr
P_1	(*S*)-6-methyloctanoyl	D-Dab	D-Phe	L-Thr	L-Thr
P_2	6-methylheptanoyl	D-Dab	D-Phe	L-Thr	L-Thr
S_1	6-methyloctanoyl[b]	D-Ser	D-Phe	L-Thr	L-Thr
T_1	6-methyloctanoyl[b]	L-Dab	D-Phe	L-Leu	L-Leu
T_2	6-methylheptanoyl	L-Dab	D-Phe	L-Leu	L-Leu

L-Dab = L-2,4-diaminobutyric acid, D-Dab = D-2,4-diaminobutyric acid, D-Phe = D-phenylalanine, L-Leu = L-Leucine, L-Ile = L-Isoleucine, L-Val = L-Valine, L-Nva = L-Norvaline, L-Ser = L-Serine, D-Ser = D-Serine, L-Thr = L-Threonine

[a]stereochemistry at C3 and C6 not confirmed, [†] position of amino acid residues is speculative

[b]stereochemistry at C6 not confirmed

describing the isolation and partial purification of an antibiotic substance from *Paenibacillus polymyxa* which they designated 'Polymyxin' [2]. This organism produced on agar a wide zone of inhibition of the Gram-negative pathogen *Salmonella schottmuelleri*. The 'polymyxin' entity was unique in its remarkable specificity for Gram-negative bacteria, which distinguished it from all antibiotics previously reported. In August of 1947 Brownlee and co-workers at the Wellcome Physiological Research laboratory in England published their work on the identification of an antibiotic substance from an organism identified as *Bacillus aerosporus*, isolated from the soil of a market garden in Surry in 1946 [3]. They initially called this antibiotic 'Aerosporin' and like the antibiotic 'Polymyxin', Aerosporin had selective antimicrobial activity against Gram-negative bacteria. Brownlee and Bushby went on to further identify the chemotherapeutic and pharmacological properties of 'Aerosporin' showing that the substance they had isolated was a basic peptide [4]. Subsequently, researchers at both the Stamford and Wellcome labs determined that the three groups were working with different strains of *P. polymyxa* and that the antibiotic called 'Polymyxin' was also a basic peptide that was chemically distinct from 'Aerosporin' yet had a very similar antimicrobial spectrum and biological activity. It was concluded that the two antibiotics belonged to the same family of antibiotic compounds [5–15]. By international agreement the generic name of 'polymyxin' was adopted for all the antibiotics derived from *P. polymyxa* and a nomenclature was developed that described the chemically distinct groups of antibiotics, which comprise the polymyxin family [16, 17]. With this new nomenclature 'Aerosporin' became known as polymyxin A, while 'Polymyxin' became known as polymyxin D. Three other chemically distinct antibiotics isolated from *P. polymyxa* strains by researchers at the Wellcome labs during this period became known as polymyxin B, C and E [11]. Colistin (polymyxin E) was first described in 1950 and obtained from *Bacillus* (*Aerobacillus*) *colistinus*, a new species isolated from a soil sample in Japan [18]. Colistin was originally thought to be distinct from polymyxins, although the striking pharmacological and chemical similarities of colistin to the entire polymyxin group of antibiotics were recognized from the outset [19–21]. It was eventually determined that colistin was structurally identical to polymyxin E and that they were in fact the same compound [22–24]; colistin, however, was the name ultimately adopted in the literature. During this period the exact chemical structures of the polymyxins remained speculative [12–14, 25]. It was known that that they were peptides and possibly cyclic in nature. Individual amino acid residues had been identified and it was also established that they contained a fatty acyl group that had been identified as the *S*-6-methyloctanoyl acyl group. In 1954, Hausmann and Craig made the discovery that polymyxin B was in fact composed of two individual peptide components that differed only in the structures of the fatty-acyl groups they contained [26]. These two peptide components were labelled polymyxin B_1 and B_2 (Table 3.1). It was soon established that the presence of multiple peptide components with variations in their structures, primarily their fatty-acyl component, was a feature common to all of the polymyxin groups. In 1963, Suzuki and co-workers at the Osaka Univeristy in Japan finally determined the absolute chemical structures for polymyxin B_1, polymyxin B_2 and colistin A (polymyxin E_1) followed by colistin B (polymyxin E_2) in 1964 (Table 3.1) [27]. They went on to also confirm the structures of polymyxin D_1 and D_2 (Table 3.1) [28]. These polymyxins were all identified as being cyclic lipopeptides. Since the initial discovery of the polymyxin A, B, C, D and E groups of lipopeptides, five other groups of polymyxins containing multiple unique lipopeptide components have been identified from *P. polymyxa* strains which include the polymyxin F [29], M [30, 31], P [32, 33], S [34, 35] and T [34, 36] groups (Table 3.1). The structures and chemistry of the polymyxins are discussed in more detail in the next section of this chapter.

3.1.2 Adoption into Clinical Practice

Although the polymyxin compounds were recognized to exhibit similar antimicrobial activity, there were striking differences in their potential for eukaryotic cell toxicity [5, 6, 37–39]. For example, Brownlee et al. [37] demonstrated severe though reversible renal toxicity in rats with polymyxin A, C and D, and likewise with polymyxin A in rabbits and dogs (polymyxins C and D not tested); polymyxin B and especially colistin (polymyxin E), which were tested in all species, produced significantly less nephrotoxicity in all cases. Interestingly, in contrast to what is now known about the nephrotoxicity of both polymyxin B and colistin, the authors in that study commented that this "*lends support to the view that it [i.e. colistin] has little nephrotoxic activity*". Early reports such as this indicating substantially reduced renal toxicity from colistin and polymyxin B are likely the reason that of the five polymyxin antibiotic groups initially discovered, only these two were further developed and adopted into clinical practice. Nevertheless, while the prevailing view at the time was that colistin and polymyxin B were generally safe compounds the potential for toxicity, especially renal toxicity, was well recognized [39, 40]. Subsequently, research was undertaken to examine ways to reduce further their toxicity.

3.1.3 Sulphomethyl Derivatives

The reaction of a primary amine with an aldehyde and sodium sulphite to convert a basic substance to labile alkane sulphonic acids was introduced into drug synthesis in the early 1900s in a successful attempt to reduce the toxicity of phenetidine without loss of antipyretic activity [41]. The reaction is equally applicable to basic polypeptides such as the polymyxins (the chemistry of which is discussed later in this chapter), and the treatment of polymyxins with formaldehyde and sodium bisulphite was first reported by Stansly et al. [2]. These investigators showed that a sulphomethyl derivative of 'Polymyxin' (later shown to be polymyxin D) produced less acute toxicity than the parent antibiotic. Subsequent studies demonstrated similar results with the sulphomethylated derivatives of both colistin and polymyxin B [21, 39]. Interestingly, Stansly et al. [2] also reported substantially less painful irritation at subcutaneous or intramuscular injection sites with the sulphomethylated derivative than with the unsubstituted lipopeptide, a common problem with the polymyxins initially considered by some to be more significant than the potential renal toxicities. This is exemplified by Barnett et al. [40] who in 1964 commented that "*In the literature much value has been attached to the reduction in acute intravenous toxicity achieved by the sulphomethylation of the polymyxins, but with these antibiotics this toxicity is of no therapeutic importance because even in the unsubstituted form they have a satisfactory therapeutic index. The use of the polymyxins has, however, been much affected by the pain that develops at the site of intramuscular injection and by an undeserved reputation for nephrotoxicity. The painful reactions are undoubtedly avoided by using the sulphomethylated derivatives*." Indeed, sulphomethylation was applied by Koyama [42] in 1957 specifically to overcome this problem with colistin. As will be discussed below colistin is still administered in the clinic intravenously as its sulphomethylated derivative.

3.1.4 Commercial Preparations

The polymyxins colistin and polymyxin B became available clinically in the late 1950s and early 1960s [43, 44]. Presently, 'colistin' is commercially available in two different forms, namely colistin sulphate [1264-72-8, CAS registry number], hereafter referred to as colistin, and its sulphomethylated derivative, sodium colistin methanesulphonate [8068-28-8] (CMS, also known as colistimethate sodium, sodium colistimethate, penta-sodium colistimethanesulphate and sulphomethyl colistin); polymyxin B is only available as polymyxin B sulphate [1405-20-5] [45]. Colistin, which is poorly absorbed from the gastrointestinal tract and through skin [21, 37, 46], has been formulated as an oral prep-

aration (indicated for bowel decontamination) and topical preparations (indicated for bacterial skin, eye and ear infections), but is not used parenterally due to its high potential to elicit toxicity upon intravenous administration (median lethal dose (LD_{50}) = 5.46 mg/kg in mice) [21]. CMS is poorly absorbed from the adult gastrointestinal tract [47] and its sodium salt, in lyophilized form, is the form of 'colistin' that is administered parenterally, most commonly intravenously [48, 49]. However, it may also be administered intramuscularly, intrathecally, intraventricularly, and via inhalation, the latter a common route of administration for patients with cystic fibrosis. Although CMS can be administered intramuscularly at the same doses as intravenously, intramuscular administration is not commonly used in clinical practice because of variable absorption and severe pain at the injection site [50].

It is important not to use the terms colistin and CMS interchangeably, as the chemistry, antibacterial activity, toxicity and pharmacokinetics of these two entities differ substantially. Unfortunately, despite the urging of Goodwin [51] who as early as 1969 pointed out the potential confusion that may arise when the general term 'colistin' is used in reference to either colistin sulphate or CMS (as was common practice at the time; for examples, see Kunin [52], and Schwartz et al. [21]), authors to this day still occasionally report and discuss 'colistin' in generic terms which makes determination of even the preparation used (colistin sulphate or CMS) difficult. For the purposes of this and all remaining discussions, colistin sulphate will hereafter be referred to as colistin.

3.1.5 Clinical Use

In terms of their clinical use, the only difference between polymyxin B and the two commercially available forms of 'colistin' (colistin sulphate and CMS) is that polymyxin B is not indicated for oral use. Otherwise, polymyxin B sulphate can be administered via intravenous, intramuscular, inhalational, intrathecal or topical routes [45]. With the introduction of polymyxins to clinical practice, colistin was marketed as offering greater or equal antibacterial potency as compared with polymyxin B and, as the methanesulphonate (i.e. CMS), was said to lack serious toxic effect in patients [19, 21, 39, 53–57]. It was demonstrated that larger doses of CMS were required for effectiveness and thus the rate of nephrotoxicity approximated that of polymyxin B [39]; this, together with the noted reduction of pain at injection sites with the sulphomethylated derivatives, may explain why the use of CMS was adopted far more widely than polymyxin B. Interestingly, in 1961 the sodium salt of a sulphomethyl derivative of polymyxin B was administered in large doses intramuscularly and intraventricularly in five children with secondary meningitis due to *Pseudomonas pyocyanea* (now *Pseudomonas aeruginosa*) [43]. This was done in an attempt to reduce the meningeal irritant and nephrotoxic properties of polymyxin B. With all five patients cured and no toxicity observed, the authors recommended this derivative of polymyxin B for future use in the treatment of such infections. However, for reasons, which may never be known, the sulphomethylated derivative of polymyxin B was never adopted into regular clinical practice. At present there is greater worldwide use of colistin compared to polymyxin B. Notably, a survey across 56 different countries revealed formulations of polymyxins used were CMS (48.6%), colistin (sulfate) (14.1%), both (1.4%), polymyxin B (1.4%), and unknown [58]; respondents from 11 countries had no access to polymyxins. Intravenous formulations were used by 84.2% of respondents, aerosolised or nebulised colistin by 44.4%, and oral colistin for selective gut decontamination by 12.7% [58].

Despite the early belief that colistin and polymyxin B were relatively safe drugs, and the use of less toxic CMS as the parenteral form of 'colistin', clinical reports began to emerge which suggested a high incidence of nephrotoxicity and neurotoxicity following intravenous administration in a considerably large number of patients [59–67]. As a consequence, use of polymyxins declined in the 1970s with the arrival of potentially less toxic antimicrobials such as the aminoglycosides, which possessed the same or broader

antibacterial spectra. However, a resurgence in their use began in the late 1980s when colistin (the most commonly used polymyxin) was reintroduced to manage infection or colonisation by *P. aeruginosa* in patients with cystic fibrosis [68]. More recently, with the emergence of multidrug-resistant (MDR) Gram-negative 'superbugs' resistant to almost all other available antibiotics [69–72], and a lack of novel antimicrobial agents in the drug development pipeline for Gram-negative infections [70–76], the place of polymyxins in therapy is presently being re-evaluated. With no new antibiotics to treat these infections to become available in the foreseeable future [71, 74], 'old' polymyxins are often the only available therapeutic options. As a consequence the use of polymyxins, especially CMS, has increased dramatically over the last decade [48, 49, 68, 77–83]. The growing importance of polymyxins as a treatment option for MDR Gram-negative infections is exemplified by the growing problem of New Delhi metallo-β-lactamase (NDM)-producing Enterobacteriaceae. Since the first identification on the Indian subcontinent in December 2009 of NDM-1-producing *Klebsiella pneumoniae* [84], NDM-producing Enterobacteriaceae (mainly *K. pneumoniae* and *E. coli*) have spread rapidly to more than 20 countries in all continents [85–87]. Many of these NDM-producing MDR isolates are only susceptible to polymyxins.

3.2 Chemistry

From a chemical perspective, the polymyxins are non-ribosomal cyclic lipopeptides and the general structure is illustrated in Table 3.1. They are decapeptides containing an intramolecular cyclic heptapeptide amide-linked loop between the amino group of the side chain of the diaminobutyric acid (Dab) residue at position 4 and the carboxyl group of the *C*-terminal threonine residue. They also have several other distinguishing structural features, which include four or five non-proteogenic Dab residues, which are charged at physiological pH. Four of these Dab residues are always found at positions 1, 5, 8 and 9 in the polymyxin scaffold and are always of the L-configuration. Position 2 of the polymyxin scaffold always contains a conserved hydrophilic L-threonine residue. Position 3 sees variation and can contain either a D or L-Dab residue or a D-serine residue. Position 6 always contains a conserved hydrophobic residue that is of the D-configuration and varies between phenyalanine, leucine. Position 7 sees the greatest variation and can either contain one of several hydrophobic residues including leucine, isoleucine, valine, norvaline or the hydrophilic residue threonine. The stereochemistry at position 7 is always of the L-configuration. Position 10 in most cases has an L-threonine residue but in at least one case contains an L-leucine residue. In regards to the *N*-terminal fatty-acyl group, six chemically distinct fatty acyl groups that vary in length from 7 to 9 carbons have been identified to date. These include (*S*)-6-methyloctanoyl, 6-methylheptanoyl, octanoyl, heptanoyl, nonanoyl and 3-hydroxy-6-methyloctanoyl. Like many other antimicrobial peptides, this mixture of lipophilic and hydrophilic groups makes them amphipathic, a chemico-physical property which is essential for their activity [88]. This also allows them to be readily water soluble (*e.g.* logP values for colistin A and colistin B are −3.15 and −3.68, respectively) [89]. The relationship between these structural features and the activity of the polymxyins is discussed in detail in Chap. 20: Discovery of Novel Polymyxin-Like Antibiotics.

Examination of the literature to date reveals that 37 unique polymyxin lipopeptides have been isolated and structurally identified from the *P. polymyxa* species [27–33, 35, 36, 90–96]. The chemical structures of these individual lipopeptides are illustrated in Table 3.1. These have been classified into 10 different groups (A, B, C, D, E, F, M, P, S and T) with each group being structurally defined and loosely classified by the presence of unique amino acid residue(s) or amino acid stereochemistry in their amino acid sequence at positions 3, 6, 7 and 10 (Table 3.1). These distinct groups of polymyxins have each been labelled with a letter. Each group can contain several individual lipopeptide components which differ from one another in the chemical structure

of the fatty-acyl group they present at their *N*-terminus and in some cases the residue presented at position 7. The individual lipopeptide components of each 'polymyxin' group are labelled with a number. This nomenclature is demonstrated in Table 3.1. It is important to note here that the use of this classification system to label newly discovered polymyxins has not always been consistent as evident with the labelling of the individual components of the polymyxin E group (Table 3.1). In the case of the polymyxin C and F lipopeptides, the amino acid residue and fatty acyl composition of the lipopeptides in these two groups have been identified; however, the stereochemistry and exact positions of the amino acids are yet to be unambiguously determined. Therefore, in Table 3.1 the position of the amino acid residues for the individual lipopeptides in these two groups is speculative and based on the structural trends observed in the other polymyxin groups. To date no examples have been reported in the literature of individual polymyxin producing *P. polymyxa* strains producing 'cross mixtures' containing lipopeptides from the different polymyxin groups. Furthermore the polymyxins are always produced as mixtures of the individual lipopeptide components of that group and never as a single lipopeptide component [90, 92–95, 97]. The relative abundance of the individual components produced does vary from strain to strain and in the commercial manufacture of polymyxins from the same strain, batch-to-batch variation can be observed [92, 93, 98, 99]. Of the different 'polymyxin' groups identified to date, only the lipopeptide components of the polymyxin B and E (Colistin) groups have undergone extensive structural analysis [92–95]. This is a reflection of the fact that only 'mixtures' of individual polymyxin B lipopeptides as well as 'mixtures' of individual polymyxin E lipopeptides are used therapeutically in the clinic. The European (Ph. Eur.) and British Pharmacopeias (BP) have established limits on the minimum amount of certain components required in colistin and polymyxin B products [100, 101]. For colistin products, colistin A and B together with three minor components must constitute ≥ 77% of the total content; for polymyxin B products, no less than 80% of total content is to consist of polymyxin B1, B2, and two minor components. Notably, similar composition limits for colistin or polymyxin B are absent from the United States Pharmacopoeia (USP) [102]. The remaining discussion will focus only on the chemical structures of the lipopeptide components of these two groups of polymyxins.

3.2.1 Chemistry of the Polymyxin B Lipopeptides

Structurally, the lipopeptides of the polymyxin B group are generally defined by the presence of a D-phenylalanine residue at position 6, an L-leucine residue at position 7 and an L-Dab residue at position 3. To date, seven individual polymyxin B lipopeptide components have been identified (Table 3.1) [92, 95, 96]. Of these seven lipopeptides, six contain structurally different branched and non-branched *N*-terminal fatty-acyl groups varying in length from 7 to 9 carbons, which have been labelled polymyxin B_1 to B_6. The 6-methyloctanoyl fatty-acyl group of polymyxin B_1 and B_1-Ile has a stereo-centre at C6, which has been identified as being the (*S*)-configuration. Polymyxin B_6 is unique in that its fatty-acyl group contains a hydroxyl group at C3, which is not present in the fatty acyl chains of the other polymyxin B lipopeptides. This unique fatty acyl group also has two stereo-centres at C3 and C6, however the absolute stereochemistry of these two stereo-centres is yet to be reported. Interestingly, polymyxin B_1-Ile, the seventh polymyxin B lipopeptide is almost identical to polymyxin B1 except that it contains an isoleucine residue at position 7, but is still considered part of the polymyxin B group. Although isoleucine is only a structural isomer of leucine it is still a structurally distinct residue. In terms of relative abundance of individual components found in polymyxin B mixtures, polymyxin B_1 and B_2 are always the major lipopeptide components. Notably, the proportion of the different lipopeptide components in polymyxin B can vary between different brands and even between different batches from the same manufacturer [99].

In commercial preparations of polymyxin B, the lipopeptide components are always provided as their corresponding sulfate salts.

3.2.2 Chemistry of the Polymyxin E (Colistin) Lipopeptides

The polymyxin E (colistin) group of lipopeptides is generally defined by the presence of a D-leucine residue at position 6, an L-leucine residue at position 7 and an L-Dab residue at position 3 (Table 3.1). To date, 11 individual polymyxin E lipopeptide components have been identified (Table 3.1) [93, 94]. Like the polymyxin B lipopeptides the individual lipopeptide components of the polymyxin E group (polymyxin E_1, E_2, E_3, E_4, E_7 and E_8) contain structurally distinct branched and non-branched *N*-terminal fatty-acyl groups, varying in length from 7 to 9 carbons. The 6-methyloctanoyl fatty-acyl group of polymyxin E_1, E_1-Val, E_1-Ile and E_1-Nva contains a stereo-centre at C6, which has been identified as being the (*S*)-configuration. The inconsistent nature of the nomenclature used for labelling the polymyxins can be observed here with several polymyxin E lipopeptides (Polymyxin E_1-Val, E_1-Ile, E_1-Nva, E_2-Val, E_2-Ile, E_8-Ile) having structurally different amino acid residues (valine, norvaline and isoleucine) at position 7 (Table 3.1). Furthermore, no polymyxin E_5, E_6 or E_8 has been reported in the literature. In terms of relative abundance of individual components found in polymyxin E mixtures, polymyxin E_1 (colistin A) and E_2 (colistin B) are always the major lipopeptide components. Similar to commercial preparations of polymyxin B, the lipopeptide components of commercial preparations of polymyxin E are always provided as their corresponding sulfate salts.

As mentioned previously, polymyxin E (colistin) is administered intravenously as colistin methanesulphonate (CMS); CMS is an inactive prodrug of colistin [103]. CMS is chemically formed via the reaction of the amino groups of the Dab residues of polymyxin E with formaldehyde and sodium bisulphite to form sulphomethylated derivatives of each of the Dab groups (Fig. 3.1) [40, 57]. This derivatization of the amino groups of the Dab residues neutralizes the positive charge at physiological pH and imparts a negative charge through the sulphonate group, which is fully deprotonated at physiological pH. *In vivo* the sulphomethyl groups are not stable and readily undergo hydrolysis resulting in conversion back to the free amino groups to give the active form of colistin [103–114]. In the preparation of commercial CMS products, this conversion of the Dab residues to their corresponding sulphomethyled derivatives is not a complete process and as a result some of the Dab residues remain unreacted. This potentially means that even for a single polymyxin E (colistin) lipopeptide component (e.g. polymyxin E1 [colistin A]), there can be a large number of unique chemical entities in CMS, depending on the location and number (i.e. which Dab residue) of methanesulphonate groups attached. As a result commercial batches of CMS are provided as complex mixtures of fully and partially sulphomethylated derivatives [113]. Currently, no limits on the minimum or maximum amount of each potential sulphomethylated derivative within a CMS product have been established by the Ph. Eur, BP and USP [100–102].

3.2.3 Future Perspective

As we look towards the future the renewed interest in the use of polymyxins as a therapeutic option for treating MDR Gram-negative infections, alongside the constant improvement in the analytical techniques available for the identification and structural elucidation of natural products, is likely to result in the discovery of new polymyxin groups and new lipopeptide components within existing polymyxin groups. As such a more consistent use of the nomenclature for the structural classification of polymyxins is required. Therefore, the implementation of a new internationally recognised nomenclature system for structurally classifying the polymyxins is required. On a final note, an important question that still remains to be answered: *what is the physiological/biological significance of all of these individual polymyxin lipopeptides*?

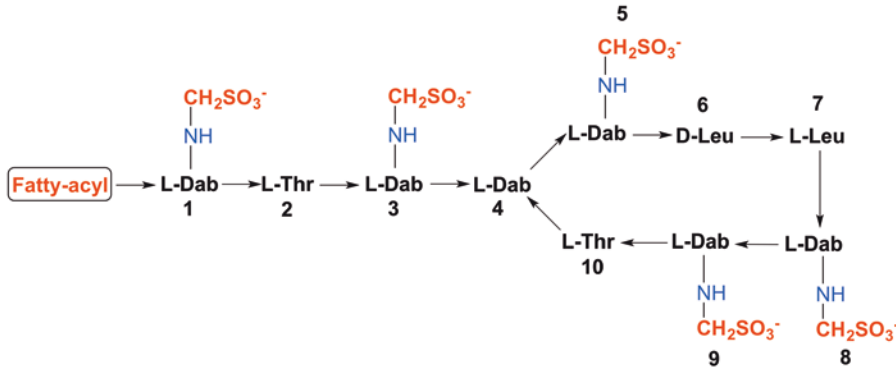


Fig. 3.1 Chemical structure of colistin methanesulphonate (CMS)

3.3 Antibacterial Spectrum

Given the structural similarities between colistin and polymyxin B as outlined above, many aspects of their antimicrobial spectrum of activity, clinical uses, toxicity and mechanism of action and resistance are shared by both [38, 45]. Both have essentially identical *in vitro* potencies (as measured by minimum inhibitory concentration [MIC]) and spectrum of activity against the commonly encountered Gram-negative organisms responsible for MDR nosocomial infections, and display a near-complete degree of cross-resistance [38, 49, 115, 116]. They exhibit a narrow antibacterial spectrum, mostly against common Gram-negative pathogens. They retain excellent bactericidal activity against most common species of Gram-negative bacilli or coccobacilli including *P. aeruginosa* [115, 117–128], *Acinetobacter* spp. [115, 117, 119, 120, 124, 125, 127, 129–131] and Enterobacteriaceae such as *Klebsiella* spp. or *E. coli* [115, 117, 119, 120, 124, 127, 132–134], the organisms against which they are most commonly used clinically. However, resistance in these and other species is increasing in some regions [119, 128, 135–147]. Interestingly, colistin-resistant isolates of several key species have been shown to be more susceptible to other antibiotics than their colistin-susceptible parent strain [128, 148, 149]. Worryingly, colistin heteroresistance (the presence of resistant subpopulations within an isolate that is susceptible based upon its MIC) has been reported in *P. aeruginosa* [150–152], *A. baumannii* [152–157], *K. pneumoniae* [144, 152, 158] and *Enterobacter cloacae* [156].

Either colistin, polymyxin B or both have also been shown to be active against *Enterobacter* spp. [117, 119, 159], *E. coli* [21, 117, 119, 124, 134, 159], *Salmonella* spp. [21, 117, 159], *Shigella* spp. [21, 117, 159], *Citrobacter* spp. [117, 159], *Haemophilus* spp. [160], *Bordetella pertussis* [40], *Legionella* spp. [161] and most *Aeromonas* species except *Ae. jandaei* (*Ae. hydrophila* has inducible resistance) [159, 162]. Polymyxins have also been reported to be potentially active against several mycobacterial species including *Mycobacterium xenopi*, *M. intracellulare, M. tuberculosis, M. fortuitum,* and the rapidly growing, non-pathogenic species *M. phlei and M. smegmatis* [163]. Activity against *Campylobacter* species [164, 165] and *Stenotrophomonas maltophilia* [120, 121, 166, 167] is variable, while activity against *Bartonella* species is borderline [168, 169]. Polymyxins are generally inactive against *Vibrio* spp. [159, 170], *Providentia* spp. [117, 171], *Serratia* spp. [21, 117, 124, 171, 172], *Proteus* spp. [21, 124, 171], *Morganella morganii* [173], *Helicobacter* pylori [159, 174, 175], *Neisseria* spp. (meningococci and gonococci) [21, 159, 176], *Brucella* spp. [21, 159], *Edwardsiella tarda* [177], *Burkholderia cepacia* complex [120, 178], *P. pseudomallei* [179] and *Moraxella catarrhalis* [159, 176]. Polymyxins have no significant activity against most Gram-positive bacteria, anaerobes, parasites or fungi [21, 38, 180–182]. The lack of activity against Gram-positive bacteria is likely

due to the binding selectivity of polymyxins to lipopolysaccharide, the principal component of the outer leaflet of the outer membrane of Gram-negative organisms but absent in Gram-positive organisms [88].

Table 3.2 contains significant large-scale surveillance studies of antimicrobial susceptibility, which have included polymyxins conducted since 2001. As can be seen from these studies polymyxins generally remain highly active against their target Gram-negative pathogens, primarily *P. aeruginosa*, *A. baumannii* and *K. pneumoniae*. However, while the large SENTRY Antimicrobial Surveillance Program conducted between 2006 and 2009 and which contained 40,625 isolates of Gram-negative bacilli showed polymyxin-resistance generally remained stable across the collection period, a greater trend towards resistance in *Klebsiella* spp. from the Asia-Pacific and Latin American regions was noted [127]. Also noteworthy is that in the SENTRY collection, 12% of the imipenem-resistant isolates of *K. pneumoniae* were also resistant to colistin [133].

That sulphomethyl derivatives of polymyxins (including CMS) possessed substantially reduced antibacterial activity *in vitro* (as determined by MIC measurement) [21, 40, 56] and *in vivo* [21, 40] was well known from the earliest times of development. While some had speculated that the activity of sulphomethylated forms of both colistin and polymyxin B derived from unmasking of the five free amino groups present in each of the parent antibiotics, it had not been possible to determine whether any of the components had intrinsic antibacterial activity [40]. Given the sulphomethylated form of polymyxin B was never adopted into clinical practice, the uncertainty surrounding whether CMS possessed antibacterial activity in its own right persisted until recent times. Such uncertainty resulted in MIC measurements for 'colistin' having been performed using colistin [183] or CMS [117], or both [21, 136, 184]. Additionally, confusion surrounded microbiological assays used to measure 'colistin' concentrations in biological fluids. Study of the antibacterial activity of CMS, the parenteral form of colistin, had proven complicated due to the *in vitro* and *in vivo* conversion of CMS to colistin and a lack of analytical methods capable of differentiating between colistin initially present in a sample and colistin subsequently formed from CMS; on this latter point, microbiological assays are incapable of such differentiation. In 2006 Bergen et al. [103] employed previously developed high-performance liquid chromatography (HPLC) assays [185–187] which are capable of separately quantifying the concentrations of colistin and CMS (the CMS concentration determined using this approach representing the concentration of CMS (i.e. the penta-sulphomethylated species) and the numerous partially-sulphomethylated species that are intermediates in the conversion of CMS to colistin) to show that CMS may therefore be regarded as an inactive pro-drug of colistin. Additionally, this study demonstrated that the use of CMS is inappropriate for MIC measurement.

3.4 Conclusions

The polymyxins are a family of chemically distinct cyclic lipopeptide antibiotics with high specificity for Gram-negative bacteria. The chemistry of this diverse group of amphipathic compounds is complex, with each group consisting of mixtures of individual lipopeptides. Two polymyxins, polymyxin B and colistin, have been used clinically for approximately 60 years. Commercially, polymyxin B is available as polymyxin B sulphate whereas colistin is available as colistin sulphate and its sulphomethylated derivative, sodium colistin methanesulphonate (CMS); CMS is the form of 'colistin' that is administered parenterally. As polymyxins are of biological origin, the proportion of the different lipopeptide components in commercial preparations of polymyxin B or colistin vary between different brands and even between different batches from the same manufacturer. Similarly, commercial batches of CMS are provided as complex mixtures of fully and partially sulphomethylated derivatives.

Worldwide, the clinical use of colistin (predominantly as CMS) far exceeds that of poly-

Table 3.2 Summary of large-scale antimicrobial surveillance studies published from 2001–2014

Reference	Year	Polymyxin form	Species (No. of isolates)	MIC breakpoint used (mg/L)		No. of resistant isolates (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
				S	R				
Gales et al. [120]	2001	Colistin	*Acinetobacter* spp. (60)	≤2	≥4	2 (3.3)	≤1	2	≤1–32
			B. cepacia (12)	≤2	≥4	12 (100)	>128	>128	>128
			K. pneumoniae (9)	≤2	≥4	0 (0)	≤1	–	≤1–2
			P. aeruginosa (80)	≤2	≥4	0 (0)	≤1	2	≤1–2
			S. maltophilia (23)	≤2	≥4	17 (26.1)	≤1	8	≤1–64
			Others[a] (16)	≤2	≥4	10 (62.5)	64	>128	≤1–>128
		Polymyxin B	*Acinetobacter* spp. (60)	≤2	≥4	3 (5.0)	≤1	2	≤1–8
			B. cepacia (12)	≤2	≥4	12 (100)	>128	>128	>128
			K. pneumoniae (9)	≤2	≥4	0 (0)	≤1	–	≤1–2
			P. aeruginosa (80)	≤2	≥4	0 (0)	≤1	2	≤1–2
			S. maltophilia (23)	≤2	≥4	17 (26.1)	2	8	≤1–64
			Others[a] (16)	≤2	≥4	10 (62.5)	64	>128	≤1–>128
Fosse et al. [162]	2003	Colistin or CMS not specified	*Aeromonas* spp. (387)	≤2	≥4	83 (21.4)	ND	ND	<0.25–>256
Nicodemo et al. [167]	2004	Colistin	*S. maltophilia* (70)	≤2	≥4	17 (24.3)	2	4	0.12–32
		Polymyxin B	*S. maltophilia* (70)	≤2	≥4	16 (22.7)	2	4	0.25–16
Tan and Ng [119]	2006	Colistin	*P. aeruginosa* (33)	≤2	≥4	11 (33)	2	4	2–16
			Acinetobacter spp. (18)	≤2	≥4	0 (0)	≤1	2	<1–2
			E. coli (13)	≤2	≥4	0 (0)	≤1	≤1	<1–2
			Enterobacter spp. (8)	≤2	≥4	2 (25)	≤1	16	<1–16
			K. pneumoniae (16)	≤2	≥4	1 (6.3)	≤1	≤1	<1–4
			S. maltophilia (17)	≤2	≥4	17 (100)	128	>256	8–>256

(continued)

Table 3.2 (continued)

Reference	Year	Polymyxin form	Species (No. of isolates)	MIC breakpoint used (mg/L)		No. of resistant isolates (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
				S	R				
Gales et al. [159]	2006	Polymyxin B	*Acinetobacter* spp. (2621)	≤2	≥4	55 (2.1)	≤1	2	≤1–>8
			Aeromonas spp. (368)	≤2	≥4	104 (28.3)	≤1	>8	≤1–>8
			Alcaligenes spp. (121)	≤2	≥4	44 (36.4)	2	>8	≤1–>8
			B. cepacia (153)	≤2	≥4	135 (88.2)	>8	>8	0.5–8
			P. aeruginosa (8705)	≤2	≥4	113 (1.3)	≤1	2	≤1–>8
			Pseudomonas spp. (non-*aeruginosa;* 282)	≤2	≥4	33 (11.7)	≤1	4	≤1–>8
			S. maltophilia (1256)	≤2	≥4	347 (27.6)	1	8	≤0.12–>8
			Other non-enteric Gram-negative bacilli (302)	≤2	≥4	168 (55.6)	4	>4	<1–>8
			Citrobacter spp. (895)	≤2	≥4	8 (0.9)	≤1	≤1	≤1–>8
			Enterobacter spp. (4693)	≤2	≥4	784 (16.7)	≤1	>8	≤1–>8
			E. coli (18,325)	≤2	≥4	92 (0.5)	≤1	≤1	≤1–>8
			Klebsiella spp. (8188)	≤2	≥4	147 (1.8)	≤1	≤1	≤1–>8
			Indole-positive *Proteus* spp. (895)	≤2	≥4	883 (98.7)	>8	>8	≤1–>8
			P. mirabilis (1931)	≤2	≥4	1917 (99.3)	>8	>8	≤1–>8
			Salmonella spp. (2909)	≤2	≥4	698 (24.0)	≤1	4	≤1–>8
			Shigella spp. (828)	≤2	≥4	8 (1.0)	≤1	≤1	≤1–>8
			Serratia spp. (1919)	≤2	≥4	1815 (94.6)	>8	>8	0.25–>8
Yau et al. [130]	2009	Colistin	*A. baumannii* (30)	≤2	≥4	1 (3.3)	0.5	1	0.5–128

Walkty et al. [124]	2009	Colistin	*P. aeruginosa* (561)	≤2	≥4	47 (2)	2	2	0.5–>16
			E. coli (1732)	≤2	≥4	11 (0.6)	0.5	1	≤0.06–>16
			K. pneumoniae (515)	≤2	≥4	15 (2.9)	0.5	1	0.12–>16
			E. cloacae (186)	≤2	≥4	30 (16.1)	0.5	>16	0.12–>16
			P. mirabilis (119)	≤2	≥4	119 (100)	>16	>16	≥16
			S. marcescens (108)	≤2	≥4	106 (98.1)	>16	>16	1–>16
			K. oxytoca (108)	≤2	≥4	5 (4.6)	0.5	1	0.25–>16
			S. maltophilia (83)	≤2	≥4	70 (84.3)	8	>16	0.25–>16
			E. aerogenes (37)	≤2	≥4	1 (2.7)	0.5	1	0.25–4
			A. baumannii (31)	≤2	≥4	2 (6.5)	1	2	0.5–>16
Hawser et al. [132]	2010	Colistin	*K. pneumoniae* (303)	≤2	≥4	8 (2.6)	0.25	0.5	≤0.12–>4
Landman et al. [188]	2010	Polymyxin B	*K. pneumoniae* (3050)	≤2	≥4	6 (0.2)	1	1	≤0.12–>4
Lee et al. [128]	2011	Colistin	*P. aeruginosa* (215)	≤2	≥8	16 (7.4)	2	2	≤8
		Polymyxin B	*P. aeruginosa* (215)	≤2	≥8	0 (0.0)	1	2	≤2
Sader et al. [133]	2011	Colistin	*K. pneumoniae* (9774)	≤2	≥4	136 (1.4)	ND	ND	NS
		Polymyxin B	*K. pneumoniae* (9774)	≤2	≥4	156 (1.6)	ND	ND	NS
Gales et al. [115]	2011	Colistin	*Acinetobacter* spp. (4686)	≤2	≥4	42 (0.9)	≤0.5	1	NS
			E. coli (17035)	≤2	>2	34 (0.2)	≤0.5	≤0.5	NS
			Klebsiella spp. (9774)	≤2	>2	147 (1.5)	≤0.5	≤0.5	NS
			P. aeruginosa (9130)	≤2	>4	37 (0.4)	1	1	NS
		Polymyxin B	*Acinetobacter* spp. (4686)	≤2	≥4	37 (0.8)	≤0.5	≤0.5	NS
			E. coli (17035)	≤2	>2	17 (0.1)	≤0.5	≤0.5	NS
			Klebsiella spp. (9774)	≤2	>2	137 (1.4)	≤0.5	≤0.5	NS
			P. aeruginosa (9130)	≤2	>4	9 (0.1)	1	1	NS
Gales et al. [127]	2012	Colistin	*Acinetobacter* spp. (845)	≤2	≥4	10 (1.2)	≤0.5	1	≤0.5–>4
			Enterobacter spp. (451)	≤2	>2	78 (17.3)	≤0.5	>4	≤0.5–>4
			E. coli (1517)	≤2	>2	3 (0.2)	≤0.5	≤0.5	≤0.5–>4
			Klebsiella spp. (1052)	≤2	≥8	32 (3.0)	≤0.5	≤0.5	≤0.5–>4
			P. aeruginosa (1099)	≤2	≥8	1 (0.1)	1	2	≤0.5–>4

(continued)

Table 3.2 (continued)

Reference	Year	Polymyxin form	Species (No. of isolates)	MIC breakpoint used (mg/L)		No. of resistant isolates (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)
				S	R				
Quale et al. [189]	2012	Polymyxin B	*E. coli* (3049)	≤2	≥4	6 (0.2)	1	1	≤0.12–>8
			K. pneumoniae (1155)	≤2	≥4	46 (4)	1	2	0.25–>16
			Enterobacter spp. (199)	≤2	≥4	48 (24)	1	>8	≤0.12–>16
			A. baumannii (407)	≤2	≥4	12 (3)	1	2	≤0.25–>16
			P. aeruginosa (679)	≤2	≥8	3 (0.5)	1	2	0.25–4
Queenan et al. [131]	2012	Colistin	*Acinetobacter* spp. (514)	≤2	≥4	27 (5.3)	1	2	0.12–>32
Jones et al. [126]	2013	Colistin	*P. aeruginosa* (586)	≤2	≥8	0 (0.0)	1	2	≤0.25–4
Zhanel et al. [125]	2013	Colistin	*E. coli* (5451)	–	–	–	0.25	0.5	≤0.06–>16
			ESBL *E. coli* (231)	–	–	–	0.5	1	≤0.06–4
			P. aeruginosa (2183)	≤2	≥8	24 (1.1)	2	2	≤0.06–>16
			K. pneumoniae (1659)	–	–	–	0.5	1	≤0.06–>16
			E. cloacae (637)	–	–	–	0.5	>16	≤0.06–>16
			P. mirabilis (415)	–	–	–	>16	>16	0.5–>16
			K. oxytoca (411)	–	–	–	0.5	1	0.12–>16
			S. marcescens (412)	–	–	–	>16	>16	0.5–>16
			S. maltophilia (378)	–	–	–	8	>16	0.25–>16
			E. aerogenes (163)	–	–	–	0.5	1	0.12–>16
			C. freundii (123)	–	–	–	0.5	0.5	0.12–1
			A. baumannii (104)	≤2	≥4	3 (3.2)	1	2	0.25–>16
Nakamura et al. [134]	2014	Colistin	*E. coli* (174)	≤2	>2	7 (4.0)	0.5	2	NS
			K. pneumoniae (37)	≤2	>2	5 (13.5)	0.5	2	NS

[a]*E. aerogenes* (four strains), *E. cloacae* (one strain), *M. morganii* (two strains), *P. mirabilis* (two strains), *P. rettgeri* (two strains), and *S. marcescens* (five strains)
ND not determined, *NS* not specified

myxin B. Relegated to the 'back shelf' in the 1970s due to toxicity concerns, the emergence of MDR Gram-negative 'superbugs' resistant to almost all other available antibiotics has resulted in their progressive reintroduced into clinical practice over the last two decades. Given they retain excellent bactericidal activity against most common species of Gram-negative bacilli or coccobacilli, they have become increasingly important as salvage therapy for otherwise untreatable infections caused by MDR Gram-negative organisms.

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