Polymyxins: Wisdom Does Not Always Come With Age

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We currently face a lack of new antimicrobial therapies in an era of increasingly common multidrug-resistant (MDR) bacteria. The polymyxins have become last-line treatments for patients with MDR bacterial infections. An increasing body of published literature has attempted to answer questions about dosing, pharmacology, and susceptibility testing of these drugs, yet each takes for granted purity and potency of the 2 available polymyxin products. In the case of polymyxin B, true potency may vary by as much as 40% from the content reported in prescribing information. This poor accuracy is related to quality assurance assays established in the 1940s and currently in use, which have been shown to be significantly flawed in recent investigations. This review discusses the limitations of pharmacological knowledge about polymyxin antimicrobials, the clinical impact of these limitations, and suggestions for further study of these drugs in order to optimize their use clinically.

Keywords. polymyxins/pharmacology; polymyxins/therapeutic use; microbial sensitivity tests.

No scientific falsehood is more difficult to expunge than the textbook dogma endlessly repeated in tabular epitome without the original data [1].


With the dramatic increase in multidrug-resistant (MDR) gram-negative infections and the paucity of effective antibiotics to treat these infections, we have been forced to consider alternate treatment strategies including combination antimicrobial therapies and previously retired antibiotics. The polymyxins, originally discovered in the early 1950s and widely used clinically until the 1970s, when less toxic agents became available, are a classic example [2]. Recent years have led to a resurgence in interest and use of systemic polymyxin therapy, with many clinicians adopting polymyxins for use in critically ill patients with the underlying assumption of solid information regarding their potency and quality assurance [2]. Herein, we discuss assumptions about polymyxin antibiotics that have persisted over time and re-raise pharmacological questions pertinent to their clinical use that have been asked in the past but disremembered in the present [3–5]. Table 1 summarizes the concerns raised in this review and their potential clinical impacts.

COMMERCIAL FORMULATIONS

As fermentation products of the bacteria Bacillus polymyxa, 5 different polymyxins were originally isolated: A, B, C, D, and E [6]. Due to their favorable efficacy and toxicity profiles relative to the others, only B and E were ultimately used clinically [6]. Polymyxin B is the sulfate salt of up to 30 related polypeptides; 2 components, and polymyxin B1 and B2 are the most abundant in this clinical product [7]. Polymyxin B is quantified in terms of international units; the package insert for the commercial product states that 1 mg of pure polymyxin B base contains 10 000 international units [8]. Polymyxin E, originally formulated as colistin sulfate, is now predominately available as a prodrug, colistimethate sodium (CMS). Like polymyxin B, colistin is comprised of multiple related polypeptides with 2, polymyxin
Table 1. Summary of Pharmacological Concerns and Potential Clinical Impact

<table>
<thead>
<tr>
<th>Pharmacological Concern</th>
<th>Summary</th>
<th>Clinical Impact</th>
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<tbody>
<tr>
<td>Unclear and varied product potency and purity</td>
<td>Varied chemical composition due to the manufacturing process leads to heterogeneous formulations of both polymyxin B and colistin that may not be consistent across lots and manufacturers</td>
<td>Variation in exposure to active drug may alter patient response with respect to both efficacy and toxicity</td>
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<tr>
<td>Inconsistency in dosing units</td>
<td>A flawed quality assurance assay is used to standardize the quantity of polymyxin antibiotics in terms of international units</td>
<td>Dosing strategies are limited by an inability to accurately know how much drug to which the patient is exposed</td>
</tr>
<tr>
<td>Use of the inactive prodrug CMS</td>
<td>Bioavailability of colistin, and therefore active drug exposure, varies depending on formulation of the prodrug CMS, as well as the patient’s ability to eliminate CMS.</td>
<td>Use of CMS limits the antibacterial potency of colistin, especially in patients without renal impairment</td>
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<tr>
<td>Widespread use of an incorrect susceptibility testing assay</td>
<td>Due to physical properties of polymyxin antibiotics, bacterial MICs, as determined by broth microdilution, have been over-estimated</td>
<td>Concerns and strategies for achieving sufficient drug concentrations to overcome bacterial infection (including loading doses) may be unfounded</td>
</tr>
<tr>
<td>Limited PK/PD data</td>
<td>Inconsistencies in bioanalytical strategies to quantify polymyxin B and colistin as well as the inability to measure free drug concentrations, make comparison between PK/PD studies difficult. Overestimated MICs may alter proposed PK/PD targets</td>
<td>Dosing strategies based on PK/PD analysis may be premature based on the limitations in measuring serum antibiotic concentrations as well as determining bacterial MICs</td>
</tr>
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Abbreviations: CMS, colistimethate sodium; MIC, minimum inhibitory concentration; PK/PD, pharmacokinetic/pharmacodynamic.

E1 and E2, more commonly known as colistin A and colistin B, respectively, being the most abundant [9]. Coly-Mycin M and Colomycin are the 2 branded CMS products currently available in the United States and Europe, respectively [10, 11]. In the United States, CMS is labeled and dosed according to milligrams of colistin base activity (CBA) [11]. CBA appears to be calculated on the basis of international units; however, despite contacting the manufacturer of Coly-Mycin M, these authors were unable to confirm this information (JHP Pharmaceuticals, Medical Affairs, personal communication, 2 February 2012). In Europe, CMS is labeled and dosed according to international units of CMS [10]. The use of both milligrams and units to describe dosages of CMS has led to confusion [12]. Clinicians utilizing clinical studies to inform their dosing strategies must be cognizant that in Europe, CMS is dosed according to the quantity of the prodrug CMS, whereas in the United States, CMS is dosed according to the quantity of the active drug, colistin [10, 11]. Although some studies calculate and publish this conversion for ease of use and global application of data, others, such as a recent study examining high-dose extended-infusion CMS, do not [13, 14].

The unit potency of polymyxin B (10 000 units/mg) and colistin base (30 000 units/mg) serves as the foundation for converting quantities of these drugs from units to milligrams for dosing [12, 15]. However, polymyxin B and colistin are heterogeneous fermentation products that are not composed solely of pure base [7, 9, 16]. This variability raises concerns for underdosing.

### POTENCY AND QUALITY ASSURANCE

Polymyxins and other antibiotics manufactured using fermentation methods have more impurities than do chemically synthesized drugs, which are made in a more controlled environment [17]. Due to chemical heterogeneity, a standard method to define potency must be employed to describe the quantity of active drug. In 1947, Stansly and Schlosser designed an assay for this purpose based on the bioassay used to quantify penicillin [18]. Polymyxin potency was characterized by the antimicrobial activity exhibited in an agar-based diffusion assay [18]. The quantity of drug was measured in units, defined as the concentration of a standard preparation of the specific polymyxin required to inhibit a growth of the test organism. Potency was determined by comparing concentration of the drug in units per milliliter to the zone of inhibition (Figure 1) [18]. The authors recognized a key limitation: Rapid growth of the test organism, coupled with slow diffusion of polymyxins, led to very small zones of inhibition. By altering the temperature and duration of incubation, pH, and adding a solubilizing agent, Stansly and Schlosser created a method that approximated drug potency, although they reported greater assay variability for polymyxins compared to penicillin [18].

This assay was utilized by the World Health Organization (WHO) Expert Committee for Biological Standardization to create an international reference standard in the 1970s [19–21]. International reference laboratories validated the potency of
proposed references for each polymyxin B, colistin sulfate, and CMS and developed the resultant values: 8403, 20 500, and 12 700 IU/mg, respectively [19–21]. These standards remain in practice today.

Two different international standards were established for polymyxin B—the first in 1955, and the second in 1973, after the stock of the original reference was depleted [19]. During development of the second international standard, the committee identified contamination of the proposed standard and significant heterogeneity of results from the reference laboratories and questioned the validity of the data [19]. As reported in their 1973 Bulletin, the data had been determined by collaborative assay and "since it was unlikely that a more accurate estimate of potency could be made and since there was an urgent need for the unit to be defined, this should be done on the basis of the available results" [19]. Although doubts were raised by WHO and in subsequent publications corroborating these findings, the availability of other treatment options in this time period led to declining interest in the polymyxins, and a new assay to define potency was not pursued [5, 19]. The lapse of polymyxin use until recently has been seemingly associated with a collective amnesia regarding previously identified issues of potency and purity.

Current polymyxin B and CMS manufacturers in the United States follow quality assurance protocols put forth by the United States Pharmacopoeia (USP). The USP recommends a derivative of the agar-based bioassay described by Stansly and Schlosser and used by WHO [22]. USP criteria dictate that the clinical product should contain "an amount of polymyxin B sulfate equivalent to not less than 90.0 percent and not more than 120.0 percent of the labeled amount of polymyxin B." [22] According to the polymyxin B package insert, 1 mg can contain as little as 6000 or as many as 10 000 polymyxin B units [8]. Using the polymyxin B USP reference standard, which is different than the WHO reference standard (8131 polymyxin B units/mg), a range of 6000–10 000 units/mg represents potency variation as low as 74% of the reference standard [23]. The USP states that each milligram of colistin sulfate and CMS, respectively, should have a potency of at least 500 µg and 390 µg of colistin. Additional variation of the product is not discussed in the package insert [11, 23].

Bioassay limitations seen with polymyxin B and colistin are not seen with other antibiotics that have used this method of quantification, including penicillin. This is likely related to physical properties of polymyxins, which are large, positively charged, peptide antibiotics [24]. Their size limits their rate of diffusion through solid media [19, 25]. Additionally, positively charged amino acids of the peptide antibiotic structure interact with negatively charged sulfate groups of the agar, further slowing diffusion [18, 24]. In 2001, Gales et al evaluated susceptibility testing methods for colistin and polymyxin B and demonstrated significant variation using agar diffusion [3]. The poor reliability of agar-based diffusion methods to describe polymyxin susceptibility is generally accepted within clinical microbiology circles; however, this knowledge has not transferred to quality assurance standards in the United States [3, 24–26]. Potency variation of polymyxin B, colistin sulfate, and colistimethate sodium raises concerns for consistency in efficacy, toxicity, and susceptibility testing.

**DOSING**

Some practitioners dose polymyxins using international units, whereas others rely upon a conversion to milligrams based on predefined unit per milligram potency standards [15]. Daily doses of Coly-Mycin M (2.5–5.0 mg of CBA) are higher than those of Colomycin normalized to a 70-kg patient (1.5–2.9 mg of CBA) (Table 2). Differing dosing schemes between the branded CMS formulations suggest potency differences between the 2 products.

In 1964, Barnett and colleagues demonstrated that varying the degree of sulfomethylation of colistin alters pharmacologic properties of CMS [4]. Sulphomethylation modifies basic pharmacologic compounds to minimize toxicity [4]. In the case of

**Figure 1.** Polymyxin potency standard curve. International units of activity for polymyxin B, colistimethate sodium, and colistin are defined by a bioassay that defines the unit according to a quantity of drug that inhibits the growth of a test bacterial organism in an agar diffusion assay. Image reproduced with permission from Stansly and Schlosser [18].
colistin, the sulfomethyl derivative, CMS, reduces injection site irritation when colistin is given subcutaneously or intramuscularly [4]. Because colistin contains 5 positively charged amino groups per molecule and each has the potential to become sulfomethylated, the process of creating CMS leads to varying chemical products substituted with sulfomethyl groups at 1 or multiple sites (Figure 2). Greater sulfomethyl substitution of polymyxins, while contributing to decreased infusion-related toxicity, also leads to decreased antibacterial activity, in part related to the greater renal clearance of the prodrug, CMS [4, 13].

In 2010, He et al compared the serum colistin concentrations achieved in rats after dosing each of the 4 commercially available formulations of CMS [27]. They demonstrated inconsistent colistin bioavailability, and therefore active drug exposure, between clinically available formulations [27]. Data from He et al and Barnett et al suggest that, in addition to the potency variation of CMS introduced by the assay determining units of activity, variation in antibacterial activity may also be related to the extent of sulfomethylation [4, 27].

### SUSCEPTIBILITY

Currently, there are discordant recommendations regarding the in vitro determinations and interpretations of minimum inhibitory concentration (MIC) values for the polymyxins from the Clinical and Laboratory Standards Institute (CLSI), the British Society for Antimicrobial Chemotherapy (BSAC), and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The CLSI initially established guidelines for antimicrobial susceptibility testing and clinical breakpoints for these compounds in the late 1970s (example: first supplement to M02-A2-1981), and then withdrew them in the 1980s [3, 28]. The deletion by the CLSI of polymyxin agents was based upon the rarity of clinical use and poor performance of the standardized methods (disk diffusion and MICs), with established interpretative errors. As of 2013, the CLSI has interpretive category breakpoints only listed for *Pseudomonas aeruginosa* (susceptible at ≤2 µg/mL), *Acinetobacter* species (≤2 µg/mL; MIC tests only), and other non-Enterobacteriaceae (≤2 µg/mL; MIC tests only) [25]. Additionally, for each polymyxin B and

<table>
<thead>
<tr>
<th>Product</th>
<th>Conversion Factor&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Units</th>
<th>Milligrams</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymyxin B</td>
<td>10 000 units/mg</td>
<td>15 000–25 000 units/kg/d</td>
<td>1.5–2.5 mg/kg/d</td>
</tr>
<tr>
<td>Coly-Mycin M</td>
<td>30 000 units colistin/mg</td>
<td>75 000–150 000 units CBA/kg/d</td>
<td>2.5–5.0 mg CBA/kg/d</td>
</tr>
<tr>
<td>Colomycin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>30 000 units colistin/mg</td>
<td>12 500 units CMS/mg</td>
<td>1.5–2.9 mg CMS/kg/d</td>
</tr>
</tbody>
</table>

Abbreviations: CBA, colistin base activity; CMS, colistimethate sodium.
<sup>a</sup> A factor of 2.4 (30 000:12 500 units) is used to convert between CMS and colistin base [12].
<sup>b</sup> Doses normalized to a 70-kg patient, the Colomycin package insert recommends 1–2 million units given 3 times daily to patients >60 kg.

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**Figure 2.** Sites available for sulfomethylating colistin. The prodrug of colistin, colistimethate sodium (CMS), is created by sulfomethylating the primary amines located on the molecule (arrows). Formulations of CMS contain a diverse group of colistin molecules that are sulfomethylated on 1 or more of the 5 sites. The sulfomethylated formulations of colistin do not exhibit antibacterial activity. Image adapted from Enzo Life Sciences, [http://www.enzolife sciences.com/ALX-380-272/colistin.-sulfate/](http://www.enzolife sciences.com/ALX-380-272/colistin.-sulfate/). Reproduced with permission from Enzo Life Sciences.
colistin, the CLSI continues to publish breakpoints for disk diffusion when tested against *P. aeruginosa* despite high rates of false-susceptible errors [3, 25]. Etest methods, used by many clinical laboratories, are also associated with recognized technical limitations. The irregular and narrowed ellipse of inhibition around the strip leads to inaccuracy in reading the MIC, which may vary 2-fold or more from a MIC determined using the current reference broth microdilution method [29]. A phenomenon of skipped wells and trailing end points has been seen in susceptibility analyses that use broth microdilution; these make it difficult to ascertain the true MIC [26] Although diffusion- and microdilution-based methods are each associated with limitations, a modified approach to broth microdilution testing significantly improves accuracy of quantitating polymyxin potency [3, 26, 29, 30].

Polymyxin antimicrobials are large, amphipathic molecules, composed of a hydrophilic peptide ring attached to a fatty acid tail [7, 9, 24, 31]. This structure contributes to their detergent-like mode of action against bacterial cell membranes, but also leads to their propensity to “stick” to the surfaces of plastic panels used for broth microdilution testing [2, 30]. Recently, Sader et al demonstrated that the surfactant polysorbate 80 (P-80), when used as a supplement in broth microdilution testing, significantly increases the potency of polymyxins in the assay [30]. On average, MIC results of organisms tested against colistin or polymyxin B in the presence of 0.002% P-80 were 4- to 8-fold lower than organism MICs tested without P-80 (Figure 3) [30]. Although the addition of P-80 overall did not contribute strongly to the recategorizing of isolates as susceptible or not, the greater sensitivity of the assay for organisms positioned in the susceptible MIC range (≤2 µg/mL) is relevant for the use in pharmacokinetic (PK) and pharmacodynamic (PD) analyses and determination of quantitative targets to design rational polymyxin dosing regimens.

Greater guidance is necessary from breakpoint organizations (CLSI, BSAC, EUCAST) for accurate and reproducible testing of polymyxin B and colistin, with associated recommendations for supplements (P-80 or others), species to be tested, relevant quality control ranges, and directions for development of new compounds with polymyxin-like physical/chemical features.

**PHARMACOKINETICS AND PHARMACODYNAMICS**

Significant work has been done and active investigations are ongoing to describe the PK/PD of polymyxins [13, 14, 32]. The PK/PD optimization parameter for polymyxins is considered to be ratio of the area under the curve of unbound, or freely available, drug (fAUC), a value that accounts for total exposure of the drug, to the MIC of the antibiotic required to inhibit a bacterial pathogen, fAUC:MIC [33]. Accurate serum and/or target site antibiotic concentrations and MICs of the bacterial pathogens are required to develop a reliable quantitative value for the PK/PD target, fAUC:MIC.

The availability of colistin as an inactive prodrug, CMS, complicates clinical and PK/PD study [27, 31, 34]. Although CMS readily converts to colistin, especially at higher temperatures (37°C), only about 7% of CMS is converted to colistin systemically [31, 34]. Temperature control can introduce variation to kinetic analyses that do not quickly cool samples prior to quantification [31]. Differences in clearance between CMS, which is predominately cleared renally, and colistin, which predominately undergoes nonrenal
clearance, further complicate dosing regimens [13]. In patients with unimpaired renal function, rapid clearance of CMS may reduce systemic bioavailability of colistin to levels that are not sufficient to overcome a bacterial infection [13]. Average steady-state colistin serum concentrations, not accounting for protein binding of the drug, measure between 1 and 4 µg/mL [13, 35–37]. On the basis of these data, concerns have been raised for achieving effective drug concentrations to optimize bacterial killing, especially for cases in which bacterial MICs exceed 1 µg/mL [13, 35–37]. As current susceptibility testing methods may overestimate bacterial MICs by a 4- to 8-fold difference, the concern for achieving effective serum concentrations of colistin may be unfounded [30].

Given the many issues identified using the prodrug CMS, there is value in developing a formulation of colistin sulfate that can be administered directly [13, 27, 31, 34]. In 2010, Huang et al published a retrospective analysis of 15 critically ill patients with MDR gram-negative bacterial infections who were treated with intravenous colistin sulfate [38]. Eleven of the 15 patients (73.3%) met criteria for clinical improvement or cure; nephrotoxicity independently related to colistin was not identified. Infusion-related toxicity (the original rationale for developing CMS) was not discussed. This study, although small and retrospective, provides proof of concept that may lead to the future abandonment of CMS in favor of colistin sulfate [38].

To conduct pharmacokinetic analyses of the heterogeneous, multicomponent polymyxins, investigators must select which components to quantify. With respect to colistin, 2 components, colistin A and B, comprise 90% of the total clinical product, and all available pharmacokinetic data determine colistin concentrations by summing these 2 components [5, 13, 35–37]. For polymyxin B, however, the 2 most abundant components, B1 and B2, comprise between 70% and 80% of the total formulation; investigators have speculated on their relative contribution to the overall antimicrobial activity of polymyxin B compared to the other components present [5, 16, 39]. In a recent analysis, Abdelraouf et al demonstrated no differences in the pharmacokinetics of 4 separate components of polymyxin B [32]. Additionally, the same group showed no clinically relevant differences in potency based on MICs. On the basis of these data, they suggested polymyxin B1 as a surrogate measure for the disposition and activity of polymyxin B [32]. While this solution would improve ease of use of polymyxin B, the limitations of MIC testing as discussed warrant reevaluation of the potency of the individual polymyxin B components [30, 39]. To accurately interpret pharmacokinetic studies of polymyxin B, more information is needed about how each component contributes to the overall activity and toxicity of the drug.

Two clinical pharmacokinetic studies have investigated polymyxin B in a total of 17 subjects with infections due to MDR, gram-negative bacilli [40, 41]. Kwa et al [40] quantified only concentrations of polymyxin B1, whereas Zavascki et al [41] summed concentrations of polymyxin B1 and B2. Without consensus regarding which component(s) to measure in pharmacokinetic analyses, it is difficult to compare results across studies whose methodologies vary.

**THE FUTURE OF POLYMYXINS**

In the current state of increasingly drug-resistant bacteria and a decreasing supply of new antibiotics, optimizing the use of our limited arsenal is imperative. Although immediate solutions such as higher daily doses and loading doses have led to improved outcomes, long-term strategies for optimizing use of polymyxins include implementing more accurate antimicrobial susceptibility testing, quantifying doses in terms of milligrams of active drug, and abandoning the suboptimal prodrug formulation CMS [14]. Understanding the efficacy, toxicity, and PK profiles of the individual components that comprise polymyxins will allow for dosing in terms of milligrams of the active component(s). Presently very few centers are capable of performing a quantitative assay that measures the individual components of each drug. Therefore, change must be affected globally. Organizations including the USP, European Pharmacopoeia, and WHO should be part of the process to redefine and standardize polymyxins. In the same vein, CLSI, BSAC, and EUCAST need to revise inaccurate susceptibility testing procedures. Until these issues are addressed, management of patients with life-threatening infections treated with polymyxin antibiotics may be significantly compromised.

**Notes**

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14. Dal