Polymyxins Revisited:
Carbapenem-Resistant Gram-negative Bacteria

Warunee Srisupha-olarn, Pharm.D, BCPS

Master Candidate and Infectious Diseases fellow
Pharmacotherapy Division, College of Pharmacy
The University of Texas at Austin
and
Pharmacotherapy Education and Research Center
The University of Texas Health Science Center San Antonio

Pharmacotherapy Conference
April 30, 2010

Outline:

- To demonstrate characteristics of polymyxin B and polymyxin E.
- To introduce carbapenem-resistant gram-negative bacteria and their resistance mechanisms.
- To evaluate the efficacy of polymyxins against carbapenem-resistant gram-negative bacteria.
1. Introduction
   A. Polymyxins
      i. Polymyxins are lipopeptide antibiotics discovered in the 1950s.\textsuperscript{[1, 2]}
      ii. Polymyxin B and polymyxin E (colistin) are clinically useful; while polymyxin A, C and D are not used because of toxicity.\textsuperscript{[3]}
      iii. Parenteral forms include polymyxin B sulfate (PMB) and colistimethate sodium (CMS).
      iv. Polymyxins were extracted from \textit{Bacillus} spp.
         - Polymyxin B – \textit{B. polymyxa}
         - Polymyxin E (colistin) – \textit{B. colistinus}
      v. Polymyxins are active against gram-negative bacteria.
         - Primarily used in the treatment of multidrug-resistant (MDR) \textit{P. aeruginosa}, \textit{A. baumannii} and \textit{K. pneumoniae} infections.
      vi. Polymyxins structures are shown in Figure 1.\textsuperscript{[4]}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{polymyxin Structures.jpg}
\caption{Structures of polymyxin B sulfate (PMB) and colistimethate sodium (CMS)\textsuperscript{[4]}}
\end{figure}

v. Mechanism of action\textsuperscript{[5, 6]}
   - Polymyxins act as a cation surfactant agent against the outer membrane of gram-negative bacteria.
   - They displace magnesium ions from the phosphate groups of the outer membrane structure and self-penetrate the cell wall to the underlying cytoplasmic membrane.
• Insertion of polymyxins molecules between the phospholipid and protein components of cell membranes results in loss of membrane integrity, leakage of cytoplasmic constituents and cell death.

B. Clinical uses (MDR gram-negative bacteria)
   i. Pneumonia
   ii. Bacteremia
   iii. Urinary tract infection
   iv. Postoperative wound infections
   v. Meningitis

C. Dosing administration[3, 7, 8]
   i. Dosage and dosing units vary by manufacturers.[3, 9]
   ii. Two dosing units are used (milligram or unit).[10]
   iii. Intramuscular route is not recommended because of pain at injection site.[3, 11]

Table 1 Dose of administration

<table>
<thead>
<tr>
<th>Countries</th>
<th>Trade names</th>
<th>Polymyxins</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>U.S.</td>
<td>Coly-mycin M®</td>
<td>Colistin 2.5-5 mg/kg/day,</td>
<td>12500IU/1mg colistimethate</td>
</tr>
<tr>
<td></td>
<td>150mg colistin base or</td>
<td>divided into 2-4 doses</td>
<td>30000IU/1mg colistin</td>
</tr>
<tr>
<td></td>
<td>400mg colistimethate/vial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>U.K.</td>
<td>Colomycin®</td>
<td>≤60kg: CMS 50,000-75,000 IU/kg/d,</td>
<td>12500IU/1mg colistimethate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>divided into 3 doses</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;60kg: CMS 1-2 MIU 3 times a day</td>
<td></td>
</tr>
<tr>
<td>U.S.</td>
<td>Aerosporin®</td>
<td>PMB 1.5-2.5 mg/kg/day,</td>
<td>10000IU/1mg polymyxin B</td>
</tr>
<tr>
<td></td>
<td>500,000IU polymyxin B /vial</td>
<td>divided into 2 doses</td>
<td></td>
</tr>
</tbody>
</table>

D. Renal impairment[3]
   i. Polymyxin B and colistimethate sodium are excreted via renal route.[11, 12]
   ii. Colistimethate sodium is a prodrug of colistin.[12]
   iii. In renal impairment, the excretion of colistimethate sodium by the kidney is decreased therefore amount of colistin base in human is increased.[13]
iv. Dosage adjustment in renal impairment is required for both polymyxins but varies by manufacturers.\[3, 9\]

v. There are limited pharmacokinetic studies on dosage adjustment in renal impairment.\[12, 14\]

E. Adverse drug events (ADEs)\[10\]

i. Since the early 1980s, polymyxins were infrequently used because of nephrotoxicity and neurotoxicity.\[10, 11\]

ii. Both toxicities occur less often now than observed in the past and are reversible upon discontinuation.\[10\]

iii. Nephrotoxicity\[10\]

- In the past, there was no standardized definition of nephrotoxicity and the reported incidences were as high as 58%.
- Recent studies have demonstrated a lower nephrotoxicity (11%).
- Clinical manifestations: increase in serum creatinine, hematuria, proteinuria, oliguria, and acute tubular necrosis
- Appropriate management of electrolyte and fluid imbalance is required.\[10\]

iv. Neurotoxicity \[10\]

- In the past, incidence of paresthesias were 27% with colistimethate sodium; however, recent studies (1995-2005) found only 1.2%.\[10\]
- No neuromuscular blockade has been reported recently.\[15\]
- Clinical manifestations: dizziness, muscle weakness, facial and peripheral paresthesias, visual disturbances, vertigo, confusion, hallucinations, seizures, ataxia, and neuromuscular blockade.\[10\]

v. Other side-effects

- Hypersensitivity reactions, fever, ototoxicity, mild gastrointestinal disorders.\[10\]

2. Revisited in treatment of Gram-negative bacteria

A. Why is an old antibiotic still needed?

i. Prevalence of multidrug-resistant bacteria is increasing.\[16\]

ii. No new antimicrobial agents for MDR gram-negative bacteria are anticipated over the next 10 years.\[17\]
B. Beta-lactams resistance in Gram-negative bacteria
   i. Beta-lactams are the most commonly used worldwide.\textsuperscript{(18)}
   ii. Resistance mechanisms to beta-lactams\textsuperscript{(18, 19)} (Figure 2)
      - Nonenzymatic mechanisms
        a. Modification at PBPs target
        b. Impaired penetration of drug to PBPs target
        c. The pressure of an efflux pump
      - Inactivation of antibiotic by beta-lactamases

![Figure 2 Potential mechanisms of antimicrobial resistance against beta-lactam antibiotics\textsuperscript{(20)}](image)

C. Beta-lactamases
   i. Beta-lactamase production is the most common mechanism of resistance.
   ii. There are several different beta-lactamases\textsuperscript{(16, 20)}
      - Penicillinases
      - Cephalosporinases
      - Oxacillinases
      - AmpC cephalosporinases
      - Extended-spectrum beta-lactamases (ESBL)
      - Carbapenemases
   iii. Carbapenem percent susceptible has been decreasing. (Figure 3)
D. Non-fermenting bacteria

i. Carbapenem resistance in *P. aeruginosa*

- Prevalence of metallo-carbapenemases (MBL), one of carbapenemases, are increasing worldwide.\(^{[22]}\)

- Resistance trends of *P. aeruginosa* to imipenem and ceftazidime from 1986-2003\(^{[23]}\)

a. Cochran-Armitage Chi-square tests for imipenem resistance were statistically significant (p < 0.001) between year 1986 and year 2003 as shown in Figure 4.

Figure 4 Resistance trends of *P. aeruginosa* to imipenem and ceftazidime from 1986-2003.\(^{[23]}\)
Table 2 Drug susceptibilities for imipenem-nonsusceptible *P. aeruginosa* (N=207)\[24\]

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>% susceptible</th>
<th>% resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doripenem</td>
<td>55.6</td>
<td>NA</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0</td>
<td>67.6</td>
</tr>
<tr>
<td>Meropenem</td>
<td>42.5</td>
<td>40.6</td>
</tr>
<tr>
<td>Cefepime</td>
<td>72.5</td>
<td>10.6</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>76.3</td>
<td>18.4</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>72.9</td>
<td>27.1</td>
</tr>
</tbody>
</table>

**ii. Carbapenem resistance in *A. baumannii*\[5\]**

- *A. baumannii* has developed resistance to every antibiotic class.
- Nowadays, carbapenems are used as the most common antibiotic against *A. baumannii.*\[25\]
- The incidence of carbapenem-resistance is 10-15%.\[26\]
- Carbapenem-resistance *A. baumannii* required at least 2 mechanisms; production of carbapenemases and impermeability of outer membrane.\[19\]
- Drug susceptibilities for 492 carbapenem-nonsusceptible *A. baumannii* worldwide is shown in Table 3.\[27\]

Table 3 Drug susceptibilities for carbapenem-nonsusceptible *A. baumannii* (N=92)\[27\]

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>% susceptible</th>
</tr>
</thead>
<tbody>
<tr>
<td>tigecycline</td>
<td>83</td>
</tr>
<tr>
<td>minocycline</td>
<td>83</td>
</tr>
<tr>
<td>amikacin</td>
<td>36</td>
</tr>
<tr>
<td>levofloxacin</td>
<td>6</td>
</tr>
<tr>
<td>piperacillin/tazobactam</td>
<td>4</td>
</tr>
<tr>
<td>cefepime</td>
<td>4</td>
</tr>
<tr>
<td>ceftazidime</td>
<td>2</td>
</tr>
<tr>
<td>ceftriazone</td>
<td>0</td>
</tr>
<tr>
<td>amoxicillin/clavulonic acid</td>
<td>0</td>
</tr>
<tr>
<td>meropenem</td>
<td>0</td>
</tr>
<tr>
<td>imipenem</td>
<td>0</td>
</tr>
</tbody>
</table>
E. Enterobacteriaceae

i. *K. pneumoniae*

- Additional mechanisms of resistance: low-affinity PBP target, porin deficiency, and efflux
- Mainly *Klebsiella pneumoniae*.
- Resistant to many antibacterial groups except tigecycline, amikacin and polymyxins

ii. Carbapenem resistance in *K. pneumoniae*

- Major carbapenemases in the U.S is *K. pneumoniae* carbapenemases (KPC).\(^{[28]}\)
- KPC was firstly reported in North Carolina in 1996 and now dispersed worldwide.\(^{[29, 30]}\)
- Incidence of carbapenem resistance *K. pneumoniae* was 22% in 2006.\(^{[21]}\)
- Percent susceptibilities of carbapenem-resistant *K. pneumoniae* in the U.S and worldwide surveillance are shown in Table 4.

### Table 4 Drug susceptibilities for carbapenem-resistant *K. pneumoniae*

<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Abbreviations New York city(^{[31]}) (N=96)</th>
<th>Abbreviations SENTRY(^{[32]}) (N=60)</th>
</tr>
</thead>
<tbody>
<tr>
<td>tigecycline</td>
<td>TIG 100</td>
<td>100</td>
</tr>
<tr>
<td>amikacin</td>
<td>AMK 97</td>
<td>53.3</td>
</tr>
<tr>
<td>polymyxin B</td>
<td>PMB 91</td>
<td>93.3</td>
</tr>
<tr>
<td>cefotetan</td>
<td>CTT 77</td>
<td>NA</td>
</tr>
<tr>
<td>doxycycline</td>
<td>DOX 76</td>
<td>NA</td>
</tr>
<tr>
<td>cefepime</td>
<td>FEP 70</td>
<td>18.8</td>
</tr>
<tr>
<td>gentamycin</td>
<td>GEN 67</td>
<td>58.3</td>
</tr>
<tr>
<td>tobramycin</td>
<td>TOB 6</td>
<td>NA</td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>CIP 2</td>
<td>14.6</td>
</tr>
<tr>
<td>ceftazidime</td>
<td>CAZ 2</td>
<td>NA</td>
</tr>
<tr>
<td>piperacillin/tazobactam</td>
<td>TZP 1</td>
<td>0</td>
</tr>
<tr>
<td>meropenem</td>
<td>MEM 1</td>
<td>20</td>
</tr>
<tr>
<td>imipenem</td>
<td>IPM 1</td>
<td>21.7</td>
</tr>
<tr>
<td>ertapenem</td>
<td>ETP 0</td>
<td>5</td>
</tr>
</tbody>
</table>
3. Clinical activity of polymyxins
   
   A. There are no randomized, comparison study between polymyxin B and colistimethate sodium in human.\textsuperscript{[11]}

   B. Clinical trials of polymyxins against multidrug-resistant gram-negatives.
      
         
         - Baseline characteristics for colistimethate sodium and polymyxin B were similar except site of infections.
         
         - All isolates had MICs determined via automated system (data not shown).
         
         - Both polymyxins used therapeutic recommended doses
         
         - Median doses were CMS 1-9 MIU/day and PMB 0.4-1.5 MIU/day.
         
         - The major site of infection was bacteremia in both arms.
         
         - All outcomes were comparable (Table 5)

Table 5 Outcomes and safety of colistimethate sodium and polymyxin B

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>CMS n=41 (%)</th>
<th>PMB n=41 (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical success rate</td>
<td>16(39)</td>
<td>16(39)</td>
<td>0.48</td>
</tr>
<tr>
<td>Clinical failure</td>
<td>17(41)</td>
<td>13(32)</td>
<td></td>
</tr>
<tr>
<td>Clinical indeterminate</td>
<td>8(20)</td>
<td>12(29)</td>
<td></td>
</tr>
<tr>
<td>Death during treatment</td>
<td>19(46)</td>
<td>22(54)</td>
<td>0.51</td>
</tr>
<tr>
<td>30-day mortality rate</td>
<td>56%</td>
<td>61%</td>
<td>0.66</td>
</tr>
</tbody>
</table>

- Comment
  
  a. A retrospective study conducted in Brazil with no statistically different
  
  b. Sample size may be too small.
  
  c. The findings are derived from carbapenem-resistant \textit{A. baumannii} infection; the application for other organisms should be carefully considered.
Table 6 Polymyxin B: a new strategy for multidrug-resistant gram-negative organisms

<table>
<thead>
<tr>
<th>Study I</th>
<th>Yuan Z, Tam CH. Expert Opin Drugs.2008;17:661-8.¹⁵</th>
</tr>
</thead>
</table>
| **Objective** | • To review what is known about polymyxin B  
• To identify missing information or gaps for future investigations |
| **Design** | • Pertinent information was reviewed from published literature in English |
| **Results** | This article contains other review information regarding to:  
• Assessment of susceptibility and testing methods  
• Pharmacokinetics and pharmacodynamics  
• Clinical use  
• Mechanism of action and resistance  
• Antiendotoxin activity  
• Toxicity  
  Review literature part:  
• Nine studies were included.  
• No randomized, controlled clinical trials of polymyxin B  
• Intravenous polymyxin B has been used for the treatment of critically ill patients with various nosocomial infections  
• Previous studies demonstrated lower efficacy of the polymyxins for pneumonia, likely due to their large structures and poor penetration into the pleura |
| **Author’s conclusion** | • More understanding is needed on standardized susceptibility testing, serum, and tissue concentrations achieved, antibacterial activity when polymyxin B is combined with other agents, and mechanisms of resistance.  
• A more precise characterization of the relationship between drug concentration and toxicity is required. |
| **Comment** | • There were 6 studies evaluating intravenous polymyxin B  
  o Dose ranged from 15 mg q 12 hrs to 2.5 mg/kg/d  
  o Clinical cure rates ranged from 47% in pneumonia to 100% in bacteremia  
  o Mortality rate ranged from 0% in bacteremia to 51% in pneumonia.  
• The authors did not make conclusion on efficacy of polymyxin B.  
• All studies were observational.  
• There is no dosing unit conversion. |
Table 7  The use of intravenous and aerosolized polymyxins for the treatment of infections in critically ill patients: a review of the recent literature

<table>
<thead>
<tr>
<th>Study II</th>
<th>Falagas ME, Kasiokou SK, Tsiodras S, Michalopoulos A. Clin Med Res2006:4;138-46.[34]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective</td>
<td>To review recent literature regarding to intravenous and aerosolized polymyxins.</td>
</tr>
<tr>
<td>Design</td>
<td>The author did not describe and definite period of “recent”.</td>
</tr>
<tr>
<td>Results</td>
<td>For intravenous polymyxins in critically ill patients</td>
</tr>
<tr>
<td></td>
<td>• No well-designed, randomized control trials conducted to evaluate efficacy and safety of polymyxins for the gram-negative bacterial infections.</td>
</tr>
<tr>
<td></td>
<td>• IV polymyxins were used for pneumonia, bacteremia, urinary tract infections, surgical site infections, abdominal infections, skin and central nervous system infections.</td>
</tr>
<tr>
<td></td>
<td>• All studies included severely ill patients with Acute Physiology and Chronic Health Evaluation II scores ranging from 13-26.</td>
</tr>
<tr>
<td></td>
<td>• The major limitation is that combinations of colistimethate sodium and polymyxin B with other antimicrobial agents were used.</td>
</tr>
<tr>
<td>Author’s conclusion</td>
<td>• Intravenous and aerosolized polymyxins should be considered for the treatment of critically ill patients with multidrug-resistant gram-negative bacterial infections.</td>
</tr>
<tr>
<td></td>
<td>• Strict use of polymyxins is required to prevent the rapid development and dissemination of pandrug-resistant gram-negative bacteria.</td>
</tr>
<tr>
<td>Comment</td>
<td>• Ten studies were included (9 used colistimethate sodium).</td>
</tr>
<tr>
<td></td>
<td>• All literature was published during 1999-2005.</td>
</tr>
<tr>
<td></td>
<td>• Clinical cure rate ranged from 25-100%; the worse result showed in pneumonia group.</td>
</tr>
<tr>
<td></td>
<td>• The authors did not make conclusion on efficacy of polymyxins.</td>
</tr>
<tr>
<td></td>
<td>• The success rate was reported by site of infections.</td>
</tr>
<tr>
<td></td>
<td>• All studies were observational.</td>
</tr>
<tr>
<td></td>
<td>• There is no dosing unit conversion.</td>
</tr>
<tr>
<td></td>
<td>• Only one study evaluate polymyxin B.</td>
</tr>
</tbody>
</table>

C. If we include more recent studies and re analyzed, the clinical cure of polymyxins against MDR gram negative bacteria was about 63%. [35-38]
4. Polymyxin-resistances
   A. Polymyxin B\[^{39}\]
      
      i. Regrowth was observed in both 2.5 mg/kg/d and 20 mg/kg/d regimens against *P. aeruginosa*. (Figure 5A, 5B)
      
      ii. MIC to polymyxin B was 1 mg/L.

   B. Colistin sulfate
      
      i. An in vitro study of colistin sulfate against carbapenem-resistant *A. baumannii*\[^{40}\]
      
      ii. Colistin sulfate showed rapidly killing against *A. baumannii* but regrowth was observed at 24 hours (Figure 6).

---

Figure 5 A Colony count of polymyxin B 2.5 mg/kg/d and B 20 mg/kg/day against *P. aeruginosa* over 4 days.

Figure 6 Colony count of colistin sulfate against *A. baumannii* over 24 hours.
C. Clinical reports of polymyxin-resistant

i. The first polymyxin-resistant *P. aeruginosa* was reported in 2000\cite{41}; now other polymyxin-resistant gram-negative bacteria has been reported worldwide.\cite{42,43,44,45}

ii. Lee et al (2009)\cite{45} reported polymyxin-resistant *K. pneumoniae* after monotherapy of polymyxin B

- Patients were admitted between 2004-2006 in a hospital, NY.
- All 12 patients infected with bacteremia carbapenem-resistant *K. pneumoniae* and received therapeutic dose of polymyxin B.
- 3 of 12 developed polymyxin resistance during treatment.
- MICs to polymyxin B increased ≥21 fold

iii. Prevalence of polymyxins-resistant gram-negative bacteria

- In the United States, up to 6% showed of polymyxin B resistant gram-negative bacteria.\cite{21}
- In South America, 46% and 10% of polymyxin-resistant *A. baumannii* and *P. aeruginosa* were reported.\cite{46,47}
- In Asia: 18% and 28% *A. baumannii* resistant to polymyxin B and colistin.\cite{44}

D. Complete cross-resistance to polymyxin B and colistin.\cite{11,48}

i. Possible resistance mechanisms: alteration of the outer membrane of the bacteria cell, reduction in cell envelope Mg\(^{2+}\) and Ca\(^{2+}\) contents, efflux pumps or enzyme(s).\cite{7,11}

Conclusion

- Polymyxin B and colistimethate sodium are one of the last options to treat carbapenem-resistant gram-negative bacteria.
- Both nephrotoxicity and neurotoxicity are acceptable.
- Monotherapy polymyxins showed suboptimal effectiveness and may lead to resistance.
Reference:


