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In Vitro Potency of Various Polymyxin B Components[▽]

In view of the emergence of multidrug resistance among Gram-negative bacteria, polymyxin B has emerged as one of the therapeutic agents of last resort. Despite being available for decades, the pharmacological understanding of polymyxin B remains very limited and is a major hindrance to the optimal use of this agent. Alarmingly, resistance to polymyxin B has been reported (3, 5). Since the commercial formulation of polymyxin B (USP) is an unspecified mixture of chemically related compounds (e.g., polymyxin B1, B2, etc.), there have been concerns as to whether these components have identical pharmacological properties (9, 10). Our earlier work suggested that there was no considerable interbatch variability in the proportions of different components from several manufacturers (2); in this study, we examined the relative *in vitro* potencies of different polymyxin components.

Polymyxin B sulfate (USP) powder was purchased from Sigma (St. Louis, MO). The major components of the polymyxin B were purified by a preparatory-scale liquid chromatography method (Cayman Chemical Company, Inc., Ann Arbor, MI). The components (>80% purity, except for polymyxin B1 at >95%) were identified by their sequence of elution times and mass/charge ratios (7). Polymyxin B1 and isoleucine-polymyxin B1 were further distinguished using amino acid analysis. A stock solution in sterile water was prepared, aliquoted, and stored at −70°C. Prior to each susceptibility testing, an aliquot of the drug was thawed and diluted to the desired concentrations. Three standard wild-type microorganisms, *Pseudomonas aeruginosa* ATCC 27853, *Acinetobacter baumannii* ATCC BAA 747, and *Klebsiella pneumoniae* ATCC 13883 (American Type Culture Collection, Rockville, MD), were used in the study. In addition, 3 clinical multidrug-resistant isolates (*P. aeruginosa* 9019, *A. baumannii* 1261, and *K. pneumoniae* VM9) were examined; their sources and molecular characterization have been reported previously (4, 6, 8). The bacteria were stored at −70°C in Protect (Key Scientific Products, Round Rock, TX) storage vials. Fresh isolates were subcultured twice on 5% blood agar plates (Hardy Diagnostics, Santa Maria, CA) for 24 h at 35°C prior to each experiment. MICs were determined in cation-adjusted Mueller-Hinton II broth (Ca-MHB) (BBL, Sparks, MD), using a broth dilution method as described by the CLSI (1). The studies were conducted in triplicate and repeated at least once on a separate day. The results were normalized to those obtained with polymyxin B (USP) on the same day.

The MICs of polymyxin B (USP) and its various components for the bacterial isolates are shown in Table 1. Overall, there was no considerable difference in *in vitro* potency among the bacterial isolates examined. The differences (if any) among the polymyxin B components were mostly within the interday variability typically accepted for such experimental setups. Apparently, the differences in molecular structure among the polymyxin B components did not interfere with their antimicrobial activity. To our knowledge, this is the first study to evaluate the antimicrobial activity of polymyxin B at the individual component level and provides much-needed information to evaluate the benefit-to-risk ratio of this multicomponent antimicrobial agent. Investigations are ongoing to examine the pharmacokinetics and nephrotoxicity of various polymyxin B components. Collectively, these data would improve our understanding of polymyxin B and may facilitate its optimal use clinically.

TABLE 1. Susceptibilities of various bacteria to polymyxin B

Bacterium	Polymyxin B (USP) MIC (μg/ml), susceptibility[a]	Susceptibility[a] to indicated component				
		Polymyxin B1	Isoleucine-polymyxin B1	Polymyxin B2	Polymyxin B3	Polymyxin B4
P. aeruginosa ATCC 27853	2, 1×	2×	2×	2×	1×	2×
A. baumannii ATCC BAA 747	1, 1×	2×	1×	1×	1×	2×
K. pneumoniae ATCC 13883	1, 1×	2×	2×	2×	1×	4×
P. aeruginosa 9019	4, 1×	1×	1×	2×	1×	1×
A. baumannii 1261	2, 1×	2×	1×	2×	0.5×	2×
K. pneumoniae VM9	2, 1×	1×	1×	2×	0.5×	1×

[a] The polymyxin B (USP) MIC is set as the reference (1×) for comparison.

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Vincent H. Tam*
Department of Clinical Sciences and Administration
University of Houston
College of Pharmacy
1441 Moursund Street
Houston, Texas 77030

Henry Cao
Kimberly R. Ledesma
Department of Clinical Sciences and Administration
University of Houston
College of Pharmacy
Houston, Texas

Ming Hu
Department of Pharmacological and Pharmaceutical Sciences
University of Houston
College of Pharmacy
Houston, Texas

*Phone: (713) 795-8316
Fax: (713) 795-8383
E-mail: vtam@uh.edu

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