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Maximizing Antibacterial Activity of Polymyxin B: Microbiological component analyses alone and in combination

P-0717

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Abstract

Objectives: The individual components that comprise polymyxin B vary in their antimicrobial activity. As a product of fermentation, polymyxin B composition varies between manufacturers and lots. One component, polymyxin B1, comprises two-thirds of the drug yet demonstrates the least *in vitro* antimicrobial activity. Our objective was to evaluate which combination of polymyxin B components demonstrates the greatest antibacterial activity.

Methods: Polymyxin B1, B2, Ile-B1, and B3 were obtained from the commercial manufacturer, Toku-E. All combinations of 2 components were evaluated using checkerboard panels. Broth microdilution was conducted according to CLSI M07-A9 with dilutions ranging from 0.06 – 4 µg/ml for each component. 12 strains of lactose- and non-lactose fermenting Gram-negative bacilli were tested in duplicate. Synergy was defined as ≥ 4-fold lowering of both component MICs when combined. Partial synergy was defined as ≥ 4-fold decrease in MIC of one component and a 2-fold decrease in MIC of the 2nd component. Additive was defined as a 2-fold decrease in MICs of both components. Indifference was defined as no change in component MICs. Antagonism was defined as ≥ 4-fold increase in both component MICs.

Results: One combination met criteria for synergy against the Enterobacteriaceae: B3 + Ile-B1. Against *P. aeruginosa* and *A. baumannii*, B3 + Ile-B1 demonstrated partial synergy. No combinations demonstrated antagonism. Combinations employing B3 and/or Ile-B1 demonstrated the greatest rates of partial synergy. The B1/B2 combination was least likely to show any level of synergy (13%), with 42% occurrences of indifference. This contrasts to 0-8% indifference and 42-67% partial or complete synergy for all non-B1 combinations.

Conclusion: The dominant components of polymyxin B products (B1 and B2) are those associated with the lowest probability of improved activity when combined. The minor components demonstrate the greatest antimicrobial activity. Refining the composition of polymyxin B to increase the abundance of more active minor components could enhance antibacterial properties of this pharmaceutical.

Introduction

- **Polymyxin B is produced by a fermentative process; composition varies between manufacturers and lots.[1-3]**
- **We have demonstrated previously that individual components comprising polymyxin B vary in their antimicrobial activity. The more abundant components demonstrated less *in vitro* antimicrobial activity compared to less abundant components.[4]**
- **The objective of this investigation was to evaluate antimicrobial activity of polymyxin B components in combination.**

Methods

- **Polymyxin B1, B2, Ile-B1, and B3 were obtained from the commercial manufacturer, Toku-E (715 W. Orchard Dr. Ste 3 Bellingham, WA 98225 USA).**
- **Each combination of 2 components was evaluated using checkerboard panels conducted in cation-adjusted Mueller-Hinton broth without 0.002% P-80.**
- **Dilutions ranged from 0.06 – 4 µg/mL for both drug components**
- **Twelve strains of Gram-negative clinical pathogens were tested in duplicate.**
- **Combination activity was defined according to the following categories:**

Synergy	**≥ 4-fold decrease in each component MIC when combined**
Partial synergy	**≥ 4-fold decrease in MIC of one component and a 2-fold decrease in MIC of the other component**
Additive	**2-fold decrease in the MICs of both components**
Indifference	**No change in component MIC values when combined**
Antagonism	**≥ 4-fold increase in both component MIC values when combined**

Results

Table 1: Activity of polymyxin B components (four) tested in combination

Combination	Organism Group	Antagonism	Indifference	Additive	P. Synergy	Synergy
B1/B2	Enterobacteriaceae[1]	0	1	9	2	0
	P. aeruginosa[2]	0	6	0	0	0
	A. baumannii[2]	0	3	2	1	0
B1/Ile-B1	Enterobacteriaceae	0	3	7	2	0
	P. aeruginosa	0	0	5	1	0
	A. baumannii	0	0	2	4	0
B1/B3	Enterobacteriaceae	0	3	4	5	0
	P. aeruginosa	0	4	0	2	0
	A. baumannii	0	0	4	2	0
B2/Ile-B1	Enterobacteriaceae	0	0	6	6	0
	P. aeruginosa	0	0	6	0	0
	A. baumannii	0	0	2	4	0
B2/B3	Enterobacteriaceae	0	1	5	6	0
	P. aeruginosa	0	0	0	6	0
	A. baumannii	0	1	1	4	0
B3/Ile-B1	Enterobacteriaceae	0	1	4	6	1
	P. aeruginosa	0	0	5	1	0
	A. baumannii	0	0	1	5	0

[1]Enterobacteriaceae: 3 strains *E. coli*, 3 strains *K. pneumoniae*
[2]Three strains each of *P. aeruginosa* and *A. baumannii*

Figures 1a – 1d: Component activity in combination by organism

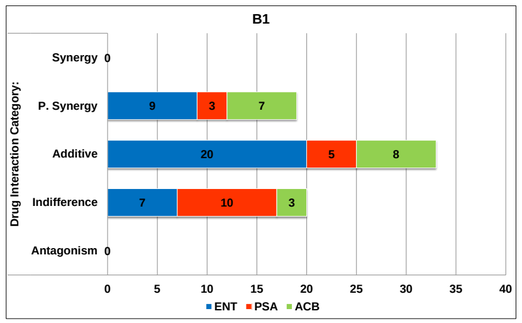

Figure 1a

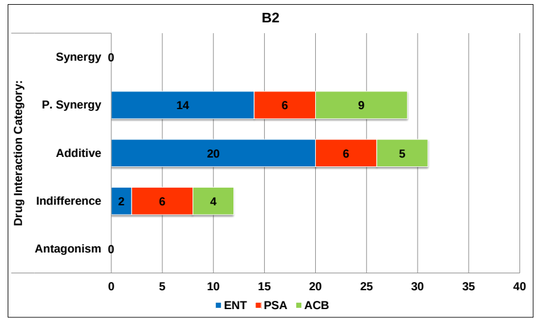

Figure 1b

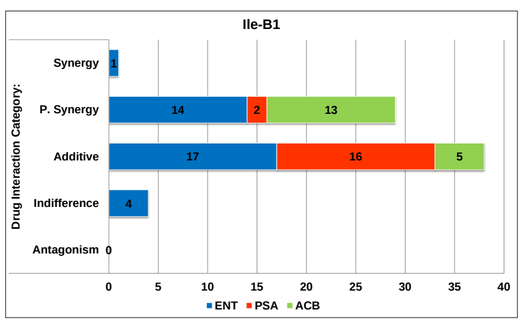

Figure 1c

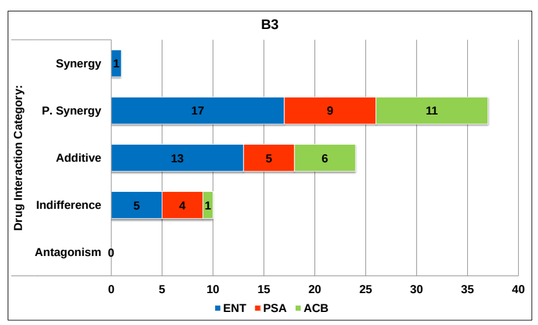

Figure 1d

ENT: Enterobacteriaceae, PSA: *Pseudomonas aeruginosa*, ACB: *Acinetobacter baumannii*

Summary

- **Synergy was seen with one combination, B3/Ile-B1, against the Enterobacteriaceae**
- **Synergy of any combinations against either *P. aeruginosa* or *A. baumannii* was not identified**
- **No instances of antagonism were identified**
- **Combinations including B1 demonstrated the highest rates of indifference**
- **Combinations including B3 demonstrated the highest rates of partial synergy**

Conclusions

- **Our analyses indicate the dominant components of polymyxin B products (B1 and B2) are those associated with the lowest probability of improved antibacterial activity when combined.**
- **While these results should be confirmed with additional investigations including time-kill studies, preliminary data indicate the formulation of polymyxin B could be refined to maximize antimicrobial activity.**

References

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