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Assessment of the Polymyxin B Antimicrobial Activity Tested against 26,921 Clinical Strains of Gram-Negative Bacilli Collected in Europe: Report from 10 Years of the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Objective: To evaluate the in vitro activity of polymyxin B tested against Gram-negative organisms isolated from patients hospitalized in European medical centers. Emergence of multidrug-resistant (MDR) *P. aeruginosa*, *Acinetobacter* spp. and *K. pneumoniae* isolates causing life-threatening infections has restored the potential therapeutic indication for the parenteral use of the polymyxin class (polymyxin B or colistin).

Methods: A total of 26,921 Gram-negative bacilli isolated from diverse sites of infection were tested for susceptibility (S) against polymyxin B by the reference broth microdilution method with results interpreted according to the S breakpoint established by the CLSI (2007) for *Acinetobacter* spp. and *P. aeruginosa* (≤2 mg/L). The isolates were collected through the SENTRY Antimicrobial Surveillance Program between 2001 and 2006 in 34 medical centers located in 12 European countries, Turkey and Israel. Concurrent quality control was obtained and all results were within CLSI ranges.

Results: Polymyxin B showed excellent potency and spectrum against 4,137 *P. aeruginosa* (MIC_{90}, 2 mg/L; 99.5% S) and 1,191 *Acinetobacter* spp. strains (MIC_{90}, ≤1 mg/L; 97.9% S). Among other non-fermentative Gram-negative bacilli, polymyxin B S rates were 92.9% for other *Pseudomonas* spp., 88.5% for *Aeromonas* spp., 78.8% for *S. maltophilia*, but only 25.0% for *Burkholderia cepacia*. Against Enterobacteriaceae, polymyxin B showed excellent activity overall (MIC_{90}, ≤1 mg/L; >98% S) against *Citrobacter* spp., *E. coli* and *Klebsiella* spp.; but inconsistent activity against *Enterobacter* spp. (MIC_{50}, ≤1 mg/L; 83.3% susceptible) and *Salmonella spp.* (MIC_{50}, 2 mg/L, 65.5% S). Very limited activity (MIC_{50}, >8 mg/L) against *Serratia* spp. (6.2% susceptible), indole-positive Proteae (1.5% susceptible) and *Proteus mirabilis* (0.8% susceptible) was documented for polymyxin B.

Conclusions: Polymyxin B was highly active against *Acinetobacter* spp., *P. aeruginosa* and *Klebsiella* spp., including MDR strains. The emergence of acquired resistance to polymyxin B (also colistin) is of great concern since these agents are typically regarded as agents of last resort when no therapeutic options remain. Prudent use of this class is recommended guided by recently developed in vitro testing guidelines (M7-A7, M100-S17).

INTRODUCTION

The polymyxins were discovered in 1947 and introduced into clinical practice in the 1950's. Among the polymyxins, only polymyxins B and E (colistin) were considered clinically safe and were used extensively for the treatment of Gram-negative infections until the 1970's when the development of better tolerated antipseudomonal agents occurred. The emergence of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. has necessitated a return to the systemic use of polymyxins.

The target of antimicrobial action is the bacterial cell membrane, where an electrostatic interaction between the cationic polypeptide and the anionic lipopolysaccharide (LPS) of the outer membrane is observed. This leads to the displacement of magnesium and calcium with increased permeability of the cell envelope, leakage of cell contents and cell death.

The main objective of this study was to assess the contemporary activity and spectrum of polymyxin B against Gram-negative bacilli collected in European hospitals as part of the SENTRY Antimicrobial Surveillance Program (2001 - 2006).

MATERIALS AND METHODS

Study Design. The SENTRY Program monitored the predominant pathogens and their antimicrobial resistance patterns via a broad network of sentinel hospitals in 4 major world regions: Asia-Pacific, Europe, Latin America, and North America (United States and Canada). Approximately 25 medical centers from Europe participate in the program each year. Guided by common protocols, bacterial isolates from diverse body sites were forwarded to the regional monitor: JMI Laboratories in North Liberty, Iowa for confirmation of organism identification and susceptibility testing. Only a single isolate per patient could be referred to the monitoring center. Common reagents and methodologies were used in the reference laboratory.

Bacterial isolates. A total of 26,921 bacterial isolates were collected between January 2001 and September 2006. The isolates originated from 34 medical centers located in 12 European countries, Turkey and Israel. All isolates were identified at the participating institution by the routine methodology in use at each laboratory. The strains were isolated primarily from bloodstream infections (64.5%), pneumonia (17.0%), skin and soft tissue infections (5.6%) and urinary tract infections (4.5%).

Susceptibility testing. Antimicrobial susceptibility testing was performed and interpreted according to the Clinical and Laboratory Standards Institute guidelines for broth microdilution methods. Microdilution panels and broth for inoculation were purchased from Trek Diagnostics Inc. (Cleveland, Ohio, USA). Testing of quality control strains *Escherichia coli* ATCC 25922 and 35218, and *P. aeruginosa* ATCC 27853 were performed for quality assurance purposes.

RESULTS

- Polymyxin B was highly active against *Acinetobacter* spp. (MIC_{90}, ≤1 mg/L; 97.9% susceptible) and *P. aeruginosa* ($MIC_{50/90}$, ≤1/2 mg/L; 99.5% susceptible); but showed variable activity against other non-fermentative Gram-negative bacilli such as *Pseudomonas* spp. other than *P. aeruginosa* ($MIC_{50/90}$, ≤1/2 mg/L; 92.9% susceptible) and *S. maltophilia* ($MIC_{50/90}$, 1/8 mg/L; 78.8% susceptible (Table 1).

- Against Enterobacteriaceae, polymyxin B exhibited broad activity against *Citrobacter* spp. (MIC_{90}, ≤1 mg/L; 99.3% susceptible), *E. coli* (MIC_{90}, ≤1 mg/L; 99.6% susceptible) and *Klebsiella* spp. (MIC_{90}, ≤1 mg/L; 98.3% susceptible). However, activity was inconsistent against *Enterobacter* spp. ($MIC_{50/90}$, ≤1/4 mg/L; 83.3% susceptible).

- Polymyxin B was generally inactive (MIC_{50}, >8 mg/L) against *B. cepacia*, *Serratia* spp., indole-positive Proteae, *P. mirabilis* and *Salmonella* spp. (Table 1).

- Only polymyxin B demonstrated consistent potency and spectrum against *Acinetobacter* spp. (97.9% susceptible) and *P. aeruginosa* (99.5%). Susceptibility rates for other antimicrobial agents tested varied from 36.4% (ciprofloxacin) to 71.4% (imipenem) for *Acinetobacter* spp. and from 70.2% (ciprofloxacin) to 88.5% (amikacin) for *P. aeruginosa* (Table 2).

- Ceftazidime (94.4% susceptible) and meropenem (94.3%) were the most active antimicrobials tested against *B. cepacia*, while minocycline (99.0% susceptible) and trimethoprim/sulfamethoxazole (97.9%) showed the highest susceptibility rates against *S. maltophilia* (Table 2).

Table 1. Antimicrobial activity of polymyxin B tested against Gram-negative bacilli collected in European hospitals.

	% inhibited at MIC (mg/L) of:		
Organism (no. tested)	≤1	2[a]	4
Non-fermentative Gram-negative bacilli			
Acinetobacter spp. (1,191)	91.6	97.9	99.8
B. cepacia (36)	19.4	25.0	27.8
P. aeruginosa (4,137)	79.5	99.5	99.9
Pseudomonas other than *aeruginosa* (210)	83.8	92.9	94.8
S. maltophilia (485)	67.4	78.8	89.9
Enterobacteriaceae			
Citrobacter spp. (415)	96.1	99.3	99.8
Enterobacter spp. (2,181)	87.1	89.0	89.9
E. coli (9,862)	98.7	99.6	99.7
Klebsiella spp. (3,368)	95.5	98.3	98.8
Indole-positive Proteae (598)	1.2	1.5	1.5
P. mirabilis (973)	0.7	0.8	0.8
Salmonella spp. (1,418)	44.9	65.3	88.6
Serratia spp. (713)	5.3	6.2	7.2
Aeromonas spp. (131)	76.3	88.5	92.4

a. Underlined values indicate susceptibility rates according to CLSI breakpoint for *Acinetobacter* spp. and *P. aeruginosa*.

CONCLUSIONS

- Polymyxin B remains very active against contemporary clinical isolates of *P. aeruginosa* and *Acinetobacter* spp. collected in European medical centers, including multidrug-resistant strains.

- In spite of the increasing use of polymyxin B in recent years, no increase in isolation of polymyxin-resistant *Acinetobacter* spp. or *P. aeruginosa* was observed in the monitored 2001 - 2006 period for the SENTRY Program.

- Continued surveillance is critical to the assessment of polymyxin activity, especially against multidrug-resistant non-fermentative Gram-negative bacilli.

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Table 2. Antimicrobial activity of polymyxin B and comparator agents tested against non-fermentative Gram-negative bacilli and *Aeromonas* spp. from European hospitals.

	MIC (mg/L)			
Organism (no.tested)/antimicrobial agent	50%	90%	% Susceptible[a]	% Resistant[a]
Acinetobacter spp. (1,191)				
Polymyxin B	≤1	≤1	97.9	2.1
Imipenem	1	>8	71.4	24.9
Meropenem	1	>8	67.7	21.2
Ceftazidime	>16	>16	38.4	54.6
Cefepime	16	>16	43.6	38.9
Ampicillin/sulbactam	16	>32	47.4	38.8
Piperacillin/tazobactam	>64	>64	37.7	52.9
Ciprofloxacin	>2	>2	36.4	63.1
Amikacin	16	>32	51.8	46.0
Aeromonas spp. (131)				
Polymyxin B	≤1	4	88.5	7.6
Imipenem	≤0.5	2	95.4	2.3
Meropenem	0.12	1	99.2	0.8
Ceftazidime	≤2	≤2	98.5	1.5
Cefepime	≤0.12	0.25	99.2	0.8
Piperacillin/tazobactam	4	>64	67.2	12.2
Ciprofloxacin	≤0.03	0.25	96.9	1.5
Amikacin	2	8	99.2	0.8
B. cepacia (36)				
Polymyxin B	>8	>8	25.0	72.2
Imipenem	4	8	50.0	8.3
Meropenem	2	4	94.3	5.7
Ceftazidime	2	8	94.4	5.6
Cefepime	4	16	88.9	5.6
Piperacillin/tazobactam	4	16	91.7	2.8
Ciprofloxacin	2	>2	41.7	44.4
Amikacin	>32	>32	25.0	66.7

	MIC (mg/L)			
Organism (no.tested)/antimicrobial agent	50%	90%	% Susceptible[a]	% Resistant[a]
P. aeruginosa (4,137)				
Polymyxin B	≤1	2	99.5	0.1
Imipenem	1	>8	77.5	12.9
Meropenem	0.5	>8	80.9	12.2
Ceftazidime	4	>16	75.1	19.5
Cefepime	4	>16	76.0	11.6
Piperacillin/tazobactam	8	>64	81.3	18.7
Ciprofloxacin	0.25	>2	70.2	26.4
Amikacin	4	32	88.5	7.2
Pseudomonas other than *aeruginosa* (210)				
Polymyxin B	≤1	2	92.9	5.2
Imipenem	1	8	82.9	10.0
Meropenem	1	8	82.1	9.2
Ceftazidime	2	>16	80.0	15.2
Cefepime	4	16	79.5	6.2
Piperacillin/tazobactam	8	>64	74.3	19.0
Ciprofloxacin	0.25	>2	73.8	19.5
Amikacin	≤4	16	90.0	5.2
S. maltophilia (485)				
Polymyxin B	≤1	>4	78.8	21.2
Trimethoprim/sulfamethoxazole	≤0.5	1	97.9	2.1
Ticarcillin/clavulanate	32	128	45.8	7.2
Minocycline[b]	≤1	≤1	99.0	1.0
Ceftazidime	16	>16	45.8	40.6
Levofloxacin	1	4	87.2	6.4
Moxifloxacin[c]	0.5	2	84.9	5.7
Ciprofloxacin	2	>2	30.7	37.5
Amikacin	>32	>32	11.3	77.3

a. According to CLSI (2007) breakpoints. Polymyxin B susceptible breakpoint established by the CLSI for *Acinetobacter* spp. and *P. aeruginosa* (≤2 mg/L) was utilized for all pathogens. Resistant breakpoint established by the CLSI for *P. aeruginosa* (≥8 mg/L) was applied for all organisms except *Acinetobacter* spp. which has a distinct resistant breakpoint (≥4 mg/L).
b. Only isolates collected in 2004 (103 strains) were tested against minocycline.
c. Breakpoints of ≤1 mg/L (susceptible) and ≥4 mg/L (resistant) were used for comparison purposes only.