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# Assessment of the Antimicrobial Activity of Polymyxin B against Contemporary Gram-negative Bacilli Isolated in Latin America: Results of SENTRY Antimicrobial Surveillance Program (2001)

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## AMENDED ABSTRACT

**Background.** Polymyxins have been used as the drugs of last resort against infections caused by multidrug resistant Gram-negative bacilli (GNB), especially *P. aeruginosa* (PSA) and *Acinetobacter* spp. (ASP). The main objective of this study was to evaluate the antimicrobial activity of polymyxin B against contemporary GNB isolates recently collected through the SENTRY Program.

**Methods**. A total of 2,183 GNB isolated from diverse body sites of infection were studied. The clinical specimens were collected from 10 Latin American medical centers between January and December 2001. Susceptibility (S) to polymyxin B was evaluated by broth microdilution method as recommended by the NCCLS (2000). The S interpretative criteria for polymyxin B was based on former NCCLS documents (ASM-2, 1981). Isolates exhibiting polymyxin MICs ≥ 4 µg/ml were categorized as resistant (R). Quality control was performed using ATCC strains.

**Results.** Although polymyxin B had showed excellent potency against PSA ($MIC_{90}$, ≤ 1 µg/ml) and ASP ($MIC_{90}$, ≤1 µg/ml), 3.7% of the PSA and 5.5% of the ASP were categorized as R to polymyxin B. All of the *E. cloacae*, *M. morganii, Proteus* spp, *S. marcescens*, and *B. cepacia* isolates were R to polymyxin B at concentrations greater than 8 µg/ml, an expected result. In contrast, most of the *E. coli* (98%) and *Klebsiella* spp. (95%) remained susceptible to polymyxin B.

**Conclusions:** Emergence of rare polymyxin-R PSA and ASP isolates were detected by this study. Although future epidemiological studies are necessary to establish the molecular relatedness and risk factors for emergence of such isolates, it is important that the clinical microbiology laboratories be able to detect this possibility. In this manner, the correlation between the microbiology findings and the clinical response for this valuable drug can be assessed.

## INTRODUCTION

Polymyxins are a group of polycationic peptides synthesized naturally by *Bacillus polymyxa*, a non-actinomycete bacteria. Members of this class act primarily on the Gram-negative bacterial cell wall, leading to rapid permeability changes in the cytoplasmic membrane and ultimately cell death. Of the five recognized polymyxins (A-E), only polymyxins B and E (colistin) were advanced to therapeutic use.

Recently, the emergence of multidrug-resistant *P. aeruginosa* and *Acinetobacter* spp. isolates causing life-threatening infections has restored the potential therapeutic indication for the parenteral use of polymyxins. Consequently, clinical microbiology laboratories should be able to perform reliable susceptibility testing for drugs in this class. The National Committee for Clinical Laboratory Standards (NCCLS) does not provide guidance for the testing of polymyxins. In fact, resistance breakpoint criteria for polymyxins were last available in the 1981 NCCLS Approved Standard M2-A2-S2; however, with the very restricted use of polymyxins, the published information was withdrawn from later documents.

The main objective of this study was to evaluate the antimicrobial activity of polymyxin B against contemporary Gram-negative bacilli isolates recently collected through the SENTRY Antimicrobial Surveillance Program in Latin America.

## MATERIALS AND METHODS

Bacterial strains. A total of 2,183 Gram-negative bacilli were collected from 10 Latin American medical centers between January and December 2001. The isolates were collected from diverse body sites, especially blood culture (1,065) and lower respiratory tract (487). The isolates were identified to the species level by the participant medical center and sent to the coordinating laboratory (Iowa, USA) for identification confirmation and reference susceptibility testing. The distribution of species was as follows (number of isolates tested): *Acinetobacter* spp. (163), *Aeromonas* spp. (8), *Burkholderia cepacia* (8), *Citrobacter* spp. (22), *Enterobacter* spp. (189), *Escherichia coli* (453), *Haemophilus influenzae* (48), *Klebsiella pneumoniae* (283), *Klebsiella oxytoca* (26), *Morganella morganii* (8), *Pantoea agglomerans* (10), *Proteus* spp. (32), *Pseudomonas* spp. (7), *Pseudomonas aeruginosa* (406), *Salmonella* spp. (151), *Shigella* spp. (202), *Serratia* spp. (73), *Stenotrophomonas maltophilia* (71) and others (23). Just one isolate per patient was evaluated.

Medical centers. The participant medical centers were distributed throughout five countries (nine cities) including São Paulo, Brasilia, Florianopolis and Porto Alegre in Brazil; Buenos Aires and San Isidro in Argentina; Santiago in Chile (two sites); Mexico City in Mexico; and Caracas in Venezuela (Figure 1).

Susceptibility testing. Antimicrobial susceptibility testing was performed using the reference broth microdilution method as described by the NCCLS [2000]. Quality control was performed by testing *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853. The susceptibility interpretative criteria for polymyxin B was based on former NCCLS documents (ASM-2, 1981). Isolates exhibiting polymyxin MICs ≥ 4 µg/ml were categorized as resistant.

## RESULTS

Polymyxin B was very active against *P. aeruginosa* ($MIC_{90}$, 2 µg/ml) and *Acinetobacter* spp. ($MIC_{90}$, 2 µg/ml). In contrast, it showed variable activity against some other non-fermentative bacilli, such as *B. cepacia* ($MIC_{90}$, ≥ 8 µg/ml) and *Pseudomonas* spp. ($MIC_{90}$, ≥ 8 µg/ml). Nearly 59.5% of the *S. maltophilia* isolates were inhibited at 2 µg/ml of polymyxin.

- Polymyxin B demonstrated excellent in vitro activity against *K. pneumoniae* ($MIC_{90}$, ≤1 µg/ml, 97.8% susceptible) and *E. coli* ($MIC_{90}$, ≤1 µg/ml, 99.4% susceptible) isolates, including those exhibiting the ESBL- phenotype. However, against AmpC-ß -lactamase producing species, the activity of polymyxin B varied. More than 95% of *Citrobacter* spp. ($MIC_{90}$, ≤ 1µg/mL) isolates were susceptible to polymyxin B, while all *Serratia* spp. and *M. morganii* isolates were resistant to this compound with MICs >8 µg/ml. Among *Enterobacter* spp., 21.2% of isolates were resistant.

- Twelve strains of *P. aeruginosa* isolated from the respiratory tract of hospitalized patients with pneumonia exhibited polymyxin B MICs at 4 µg/ml. Among these strains, 7 were isolated from Brazilian medical centers and showed a very similar co-resistance phenotype pattern (? epidemic strain).

- Six isolates of *Acinetobacter baumannii* (3.6%) showed MICs ≥ 4 µg/ml for polymyxin B. In fact, three strains exhibited MICs at > 8 µg/ml, and were isolated from three different medical centers in Brazil. All *A. baumannii* isolates categorized as resistant to polymyxin B were collected from hospitalized patients with bloodstream infections. Only one of those isolates was resistant to carbapenems.

**Figure 1.** Geographic distribution of the Latin American medical centers and the number of isolates referred to the monitoring laboratory.

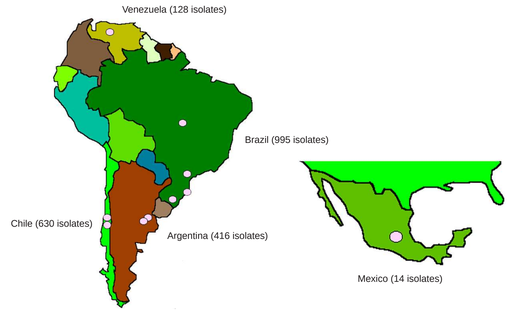


**Table 1.** Polymyxin B MIC frequency distribution according to the organism tested.

| Organism (no. tested) | no. inhibited at MIC in µg/ml (%): | | | | |
|---|---|---|---|---|---|
| | ≤1 | 2 | 4 | 8 | > 8 |
| *Acinetobacter* spp. (163) | 110 (67.5%) | 47 (28.9%) | 3 (1.8%) | 0 (0.0%) | 3 (1.8%) |
| *Aeromonas* spp. (8) | 2 (25.0%) | 2 (25.0%) | 3 (37.5%) | 0 (0.0%) | 1 (12.5%) |
| *B. cepacia* (8) | 2 (25.0%) | 0 (0.0%) | 1 (12.5%) | 0 (0.0%) | 5 (62.5%) |
| *Citrobacter* spp. (22) | 20 (91.0%) | 1 (4.5%) | 0 (0.0%) | 0 (0.0%) | 1 (4.5%) |
| *Enterobacter* spp. (189) | 148 (78.3%) | 1 (0.5%) | 2 (1.1%) | 0 (0.0%) | 38 (20.1%) |
| *E. coli* (453) | 442 (97.6%) | 8 (1.8%) | 3 (0.6%) | 0 (0.0%) | 0 (0.0%) |
| *H. influenzae* (48) | 47 (97.9%) | 1 (2.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| *K. pneumoniae* (283) | 271 (95.7%) | 6 (2.1%) | 3 (1.1%) | 1 (0.4%) | 2 (0.7%) |
| *K. oxytoca* (26) | 25 (96.2%) | 1 (3.8%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| *M. morganii* (8) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 8 (100%) |
| *P. agglomerans* (10) | 9 (90.0%) | 1 (10.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) |
| *Proteus* spp. (32) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 32 (100.0%) |
| *P. aeruginosa* (406) | 141 (34.7%) | 253 (62.3%) | 12 (3.0%) | 0 (0.0%) | 0 (0.0%) |
| *Pseudomonas* spp. (7) | 2 (28.6%) | 2 (28.6%) | 0 (0.0%) | 0 (0.0%) | 3 (42.8%) |
| *Salmonella* spp. (151) | 67 (44.4%) | 25 (16.6%) | 44 (29.1%) | 13 (8.6%) | 2 (1.3%) |
| *Shigella* spp. (202) | 197 (97.5%) | 2 (1.0%) | 2 (1.0%) | 0 (0.0%) | 1 (0.5%) |
| *Serratia* spp. (73) | 0 (0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 73 (100.0%) |
| *S. maltophilia* (71) | 31 (43.7%) | 11 (15.5%) | 17 (23.9%) | 8 (11.3%) | 4 (5.6%) |
| Others (23) | 5 (21.8%) | 3 (13.0%) | 3 (13.0%) | 0 (0.0%) | 12 (52.2%) |
| All strains (2183) | 1519 (69.6%) | 364 (16.7%) | 93 (4.3%) | 22 (1.0%) | 185 (8.4%) |

**Table 2.** *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates resistant to polymyxin B.

| Species | Bank Number | Nation | Medical Center | Body Site | Polymyxin MIC | Cross-resistance[a] |
|---|---|---|---|---|---|---|
| *P. aeruginosa* | 386 | Chile | 42 | LRT[b] | 4 | - |
| *P. aeruginosa* | 4293 | Brazil | 48 | LRT[b] | 4 | C,CAZ,CP,I,M |
| *P. aeruginosa* | 4294 | Brazil | 48 | LRT[b] | 4 | C,CAZ,CP,I,M |
| *P. aeruginosa* | 4302 | Brazil | 48 | LRT[b] | 4 | C,A,CAZ,CP,I,M |
| *P. aeruginosa* | 4303 | Brazil | 48 | LRT[b] | 4 | C,A,CAZ,CP,I,M |
| *P. aeruginosa* | 4311 | Brazil | 48 | LRT[b] | 4 | C,A,CAZ,CP,I,M |
| *P. aeruginosa* | 4381 | Brazil | 46 | LRT[b] | 4 | - |
| *P. aeruginosa* | 4597 | Chile | 43 | LRT[b] | 4 | C |
| *P. aeruginosa* | 4613 | Chile | 43 | LRT[b] | 4 | C |
| *P. aeruginosa* | 4639 | Brazil | 46 | LRT[b] | 4 | C,A,CAZ,CP,I,M |
| *P. aeruginosa* | 7457 | Brazil | 48 | Blood | 4 | CAZ |
| *P. aeruginosa* | 9025 | Brazil | 101 | Blood | 4 | C,A,CAZ,CP,I,M |
| *Acinetobacter* spp. | 501 | Brazil | 48 | Blood | > 8 | CAZ,CP,I,M |
| *Acinetobacter* spp. | 1241 | Brazil | 57 | Blood | > 8 | C,CAZ |
| *Acinetobacter* spp. | 5202 | Brazil | 48 | Blood | 4 | - |
| *Acinetobacter* spp. | 7292 | Brazil | 57 | Blood | 4 | - |
| *Acinetobacter* spp. | 9035 | Brazil | 101 | Blood | 4 | - |
| *Acinetobacter* spp. | 9254 | Brazil | 101 | Blood | > 8 | - |

a. Criteria for resistance according to NCCLS [2002]. Abbreviations: C, ciprofloxacin; A, amikacin; CAZ, ceftazidime; CP, cefepime; I, imipenem; M, meropenem.
b. Lower respiratory tract

## CONCLUSIONS

- Low levels of polymyxin B resistance have been related to the overexpression of OprH caused by genetic mutation. In addition, variation on the cation concentration in the medium may cause increased MICs and, consequently, lead to false-resistant results. Thus, it may be prudent to retest isolates with a polymyxin B MIC of 4 µg/mL with an alternative quantitative methodology.

- Although polymyxin B had been very active against contemporary clinical isolates of *P. aeruginosa* and *Acinetobacter* spp. in Latin America, including those resistant to carbapenems, resistance to this compound appears to be emerging. The clinical significance of the reduced susceptibility to polymyxins observed among these isolates is currently under investigation. If the reduced susceptibility to polymyxins correlates with a poor clinical response, the situation will be disastrous in our region, leaving no efficacious drug for the treatment of serious infections caused by multi-drug resistant *P. aeruginosa* and *Acinetobacter* spp. strains.

- Continued surveillance support by the SENTRY Program will be critical to the monitoring of this resistance pattern in our participating medical centers.

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