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Assessment of Colistin and Polymyxin B Antimicrobial Susceptibility Testing Methods Against Non-Fermentative Gram-Negative Bacilli (NFGNB)

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AMENDED ABSTRACT

Background: The emergence of multidrug-resistant (MDR) *P. aeruginosa* (PSA) and *Acinetobacter* spp. (ASP) isolates has restored the potential therapeutic indication for the parenteral use of the polymyxins. We (CLSI Working Group) evaluated the correlation between standardized disk diffusion (DD) zones of inhibition and broth microdilution (BMD) MIC values when testing two polymyxins against contemporary strains of NFGNB.
Methods: A total of 523 clinical NFGNB strains, including 122 ASP, 305 PSA and 96 *S. maltophilia/B. cepacia* (SM/BC) were tested for susceptibility (S) against colistin (COL) and polymyxin B (PB) by BMD and DD methods according to CLSI standards. Five laboratories participated in the study. The MIC portion of the study utilized a common lot of frozen-form, reference BMD panels prepared by TREK Diagnostics; while antimicrobial disks were manufactured by BD. Each participant laboratory tested *E. coli* ATCC 25922 and PSA ATCC 27853 on five occasions as quality control organisms. The BMD and DD results for each drug, as well as COL MIC versus PB MIC, and COL DD versus PB DD results, were compared by regression analysis.
Results: 104 BMD MIC results and 110 DD results were obtained for the QC strains with 96.2% of MIC results and 98.2% of DD results within the CLSI ranges published in 2005 for the polymyxins and 6 comparator agents. At a BMD resistance (R) breakpoint of ≥ 4 µg/ml for both agents, R rates were 5, 4 and 75% for COL and 5, 2 and 57% for PB when testing ASP, PSA and SM/BC; respectively. Poor correlations between BMD and DD results were observed for both COL and PB (*r* = 0.14 to 0.53), with unacceptable false-S (very major) errors for all 3 organism groups evaluated. Excellent correlation was achieved between COL and PB MIC results for ASP and PSA (*r* = 0.85 to 0.90 and >98% of the results were ±1 $\log_2$ dilution). However, the SM/BC group showed COL MIC results 1 or 2 $\log_2$ dilutions higher than those for PB, overall.
Conclusions: The DD method failed to detect NFGNB strains resistant to COL or PB by CLSI BMD tests and should not be routinely used. COL MIC results can be used to predict PB MICs, and vice-versa, when testing ASP and PSA; but against SM/BC these compounds should be tested and reported separately.

INTRODUCTION

The polymyxins are amphipathic polypeptide antimicrobial agents. Their basic structure consists of a fatty acid side chain attached to a polycationic peptide ring composed of 8 to 10 aminoacids. The polymyxins possess a unique mechanism of action, targeting the bacterial cell membrane. The polycationic peptide ring of the polymyxins interacts with the anionic lipopolyssacharide (LPS) molecules in the outer membrane of Gram-negative bacteria, displacing the calcium and magnesium that stabilize the LPS molecules.

Polymyxins B and E (colistin) were introduced into clinical practice in the 1950's for the treatment of Gram-negative infections. However, the parenteral use of these compounds was abandoned in the 1970's when better-tolerated anti-pseudomonal agents became available. The emergence of multidrug-resistant (MDR) *Pseudomonas aeruginosa* and *Acinetobacter* spp. has required the expanded systemic use of these polymyxins. As polymyxins usage increases, the emergence of polymyxin resistance may become a concern.

Thus, there is an urgent need for reliable susceptibility testing methods to predict the clinical response. In 2005, the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) established MIC breakpoints for testing *Acinetobacter* spp. against colistin and polymyxin B. In the present study the CLSI Acinetobacter Working Group evaluated the correlation between standardized disk diffusion zones of inhibition and broth microdilution MIC values when testing these two polymyxins against contemporary strains of non-fermentative Gram-negative bacilli.

MATERIALS AND METHODS

Study Design: Each of five participant laboratories was requested to test non-fermentative Gram-negative bacilli by reference broth microdilution and disk diffusion methods according to a common protocol.

Participating Laboratories:
Center for Disease Control and Prevention, Atlanta, GA;
Duke University, Durham, NC;
JMI Laboratories, North Liberty, IA;
Loyola University Chicago, Chicago, IL;
Massachusetts General Hospital, Boston, MA.

Bacteria Isolates: A total of 523 clinical strains, including 122 *Acinetobacter* spp., 305 *P. aeruginosa*, 68 *S. maltophilia* and 28 *B. cepacia* were tested.

Susceptibility Testing: The isolates were tested for susceptibility against colistin and polymyxin B by broth microdilution and disk diffusion methods according to CLSI standards. The MIC portion of the study utilized a common lot of frozen-form, reference panels prepared by TREK Diagnostics (Cleveland, OH); while antimicrobial disks were manufactured by BD Diagnostics (Sparks, MD). Each participant laboratory tested *Escherichia coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 on five occasions as quality control organisms. MIC and inhibition zone QC ranges for the polymyxins were obtained from the CLSI document M100-S15 (2005).

Breakpoints: Colistin and polymyxin B MIC breakpoints established for *Acinetobacter* spp. by the CLSI (≤ 2 µg/ml for susceptible and ≥ 4 µg/ml for resistance) and the disk diffusion breakpoints from the NCCLS document M2-A2 S2 [1981] (susceptible at ≥ 11 and ≥ 12 mm and resistant at ≤ 8 and ≤ 9 mm for colistin and polymyxin B, respectively) were applied for all pathogens evaluated in the present study. The broth microdilution and disk diffusion results for each drug, as well as colistin MIC versus polymyxin B MIC, and colistin disk diffusion versus polymyxin disk diffusion results, were compared by regression and error-rate bounding analyses.

RESULTS

- A total of 104 MIC results and 110 disk diffusion results were obtained for the ATCC strains with 96.2% of MIC results and 98.2% of disk results within the CLSI polymyxin ranges published in 2005 for the polymyxins.
- Poor correlations between MIC and inhibition zone results were observed for colistin and polymyxin tested against *Acinetobacter* spp. (*r* = 0.31 and 0.11 respectively). In addition, the disk diffusion method was not reliably able to detect resistance (MIC ≥ 4 µg/ml) to these compounds (Figures 1a and b).
- Correlations between MIC and inhibition zone results were higher for both compounds when testing *P. aeruginosa* (*r* = 0.83 and 0.66 for colistin and polymyxin B, respectively; Figures 2a and b). However, isolates with MIC values of 4 µg/ml had inhibition zones in the susceptible category (very major errors).
- The very major error rates (false-susceptible) for *P. aeruginosa* were 3.9% for colistin and 1.3% for polymyxin B, with no major errors. With an intermediate MIC at 4 µg/ml and resistant breakpoint at ≥ 8 µg/ml (which is the resistant breakpoint established by the British Society for Antimicrobial Chemotherapy) the error rates would be acceptable (0.0-0.3% of very major and major errors, and 1.3-4.3% of minor errors).
- Poor correlations between MIC and inhibition zone results and between colistin and polymyxin MIC results were observed for *S. maltophilia* with unacceptable rates of error (Figures 3a and b).
- Excellent correlation was achieved between colistin and polymyxin MIC results for *Acinetobacter* spp. and *P. aeruginosa* (Figures 4 and 5; *r* = 0.85 to 0.90 and >98% of the results were ±1 $\log_2$ dilution). However, *S. maltophilia* showed colistin MIC results one or two $\log_2$ dilutions higher than those for polymyxin B, overall (Figure 6).
- The majority of *B. cepacia* strain showed very high MIC results (>64 µg/ml) for both colisitin and polymyxin B (data not shown).

Figure 1: Scattergram showing the correlation between MIC and disk diffusion results for *Acinetobacter* spp. tested against colistin (1a) and polymyxin B (1b). Solid line indicates current CLSI breakpoint, dashed MIC line indicates suggested resistant breakpoint and dashed zone diameter lines indicates breakpoints from the M2-A2 S2 NCCLS document (1981).

Figure 1a.

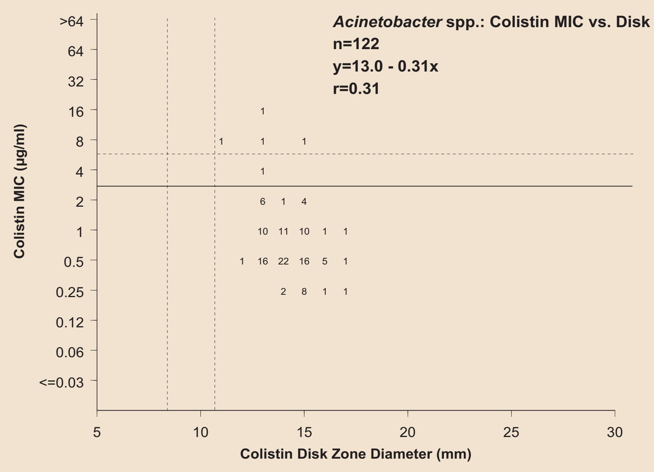


Figure 1b.

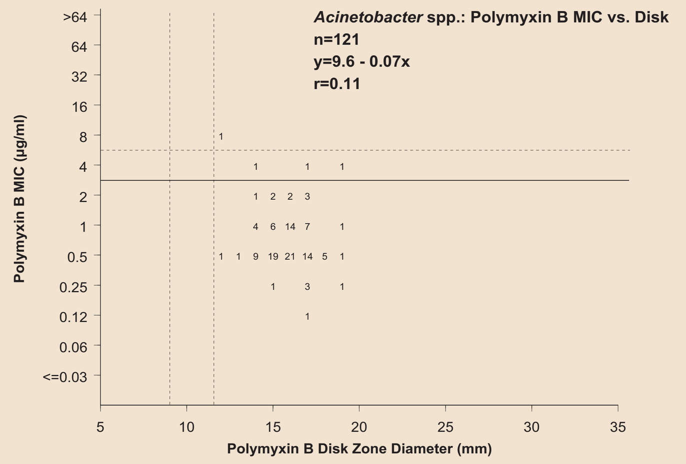


Figure 2: Scattergram showing the correlation between MIC and disk diffusion results for *P. aeruginosa* tested against colistin (2a) and polymyxin B (2b). Solid line indicates current CLSI breakpoint, dashed MIC line indicates suggested resistant breakpoint and dashed zone diameter lines indicates breakpoints from the M2-A2 S2 NCCLS document (1981).

Figure 2a.

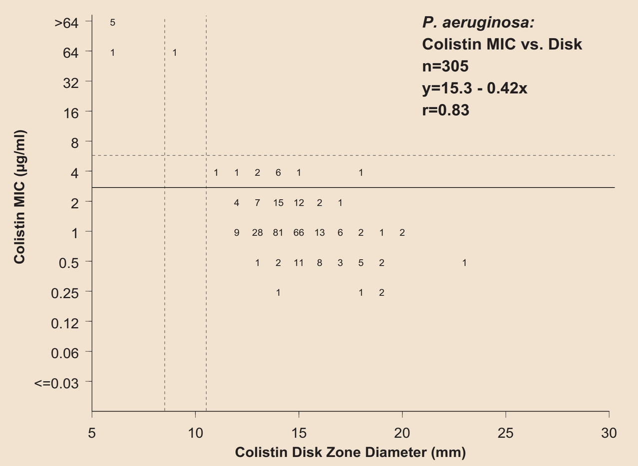


Figure 2b.

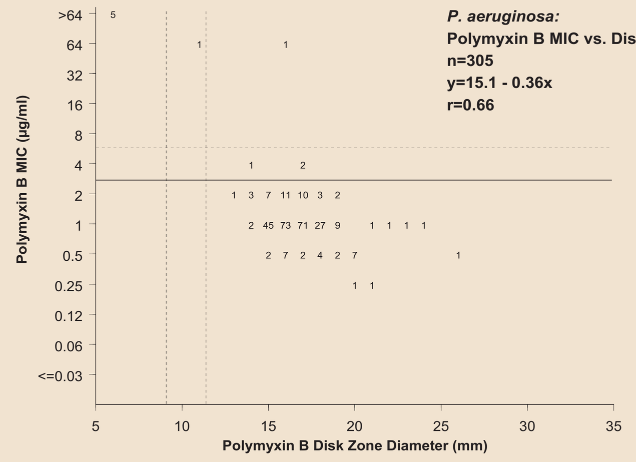


Figure 3: Scattergram showing the correlation between MIC and disk diffusion results for *S. maltophilia* tested against colistin (3a) and polymyxin B (3b). Solid line indicates current CLSI breakpoint, dashed MIC line indicates suggested resistant breakpoint and dashed zone diameter lines indicates breakpoints from the M2-A2 S2 NCCLS document (1981).

Figure 3a.

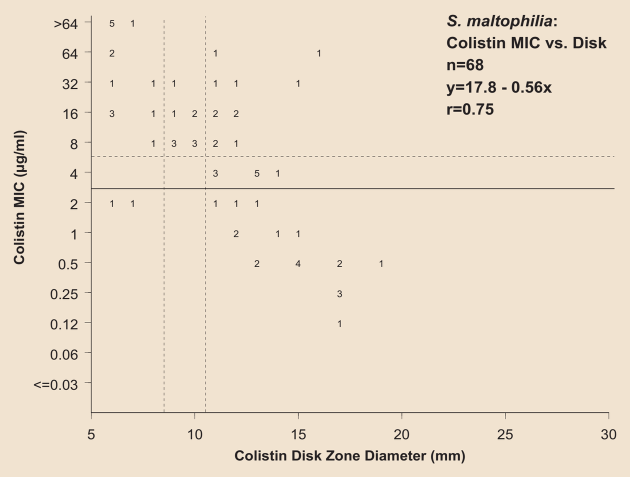


Figure 3b.

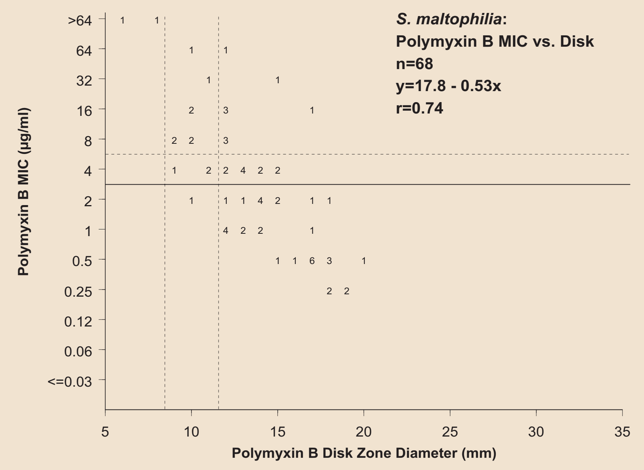


Figure 4: Colistin (COL) and polymyxin B (PB) MIC distibutions for *Acinetobacter* spp.

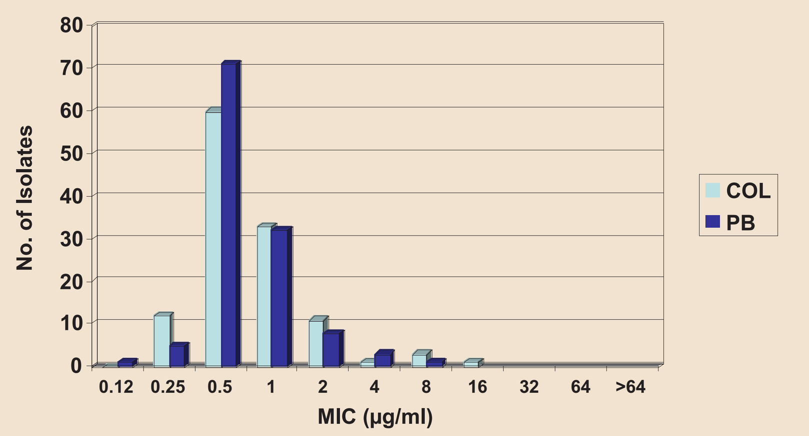


Figure 5: Colistin (COL) and polymyxin B (PB) MIC distibutions for *P. aeruginosa*.

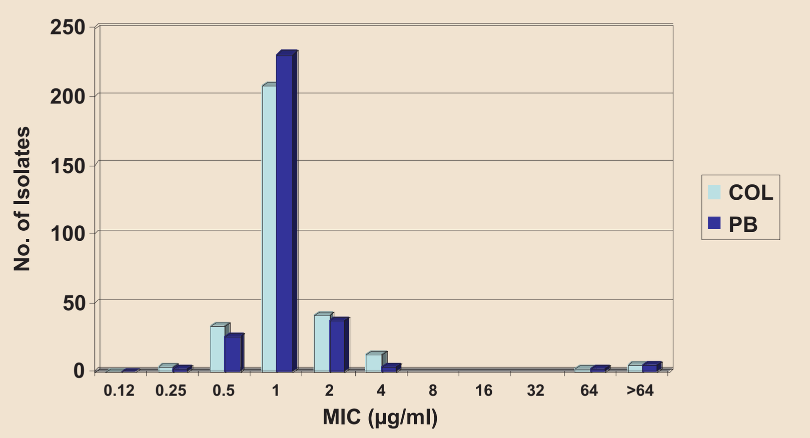
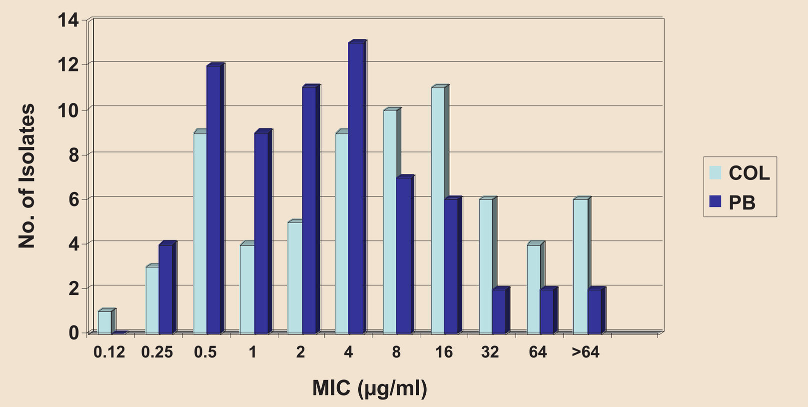


Figure 6: Colistin (COL) and polymyxin B (PB) MIC distibutions for *S. maltophilia*.



CONCLUSIONS

- An acceptable correlation between MIC results for colistin and polymyxin B was obtained with *Acinetobacter* spp. and *P. aeruginosa*, indicating that the laboratory can test only one agent and extrapolate the result to the other.
- The disk diffusion method showed promising results for testing *P. aeruginosa*, especially if the resistant MIC breakpoint was defined as ≥ 8 µg/ml (intermediate at 4 µg/ml). Analysis of clinical and PK/PD data will be necessary to fully evaluate current CLSI MIC breakpoints for these compounds.
- Polymyxin disk diffusion methods showed limited value for testing *Acinetobacter* spp.
- Further studies are necessary to establish MIC and disk diffusion breakpoints for *S. maltophilia*, a population generally having a wide range of MIC values to the polymyxins.

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