

Influence of Surfactant (Polysorbate-80) on Susceptibility Testing Results for the Polymyxins Using Broth Microdilution Methods

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ABSTRACT

Background: Polysorbate-80 (P-80; also referred as Tween-80) is a surfactant widely employed as a dispersing agent in the preparation of broth microdilution (BMD) panels and/or bacterial inocula used in susceptibility testing. We evaluated the influence of P-80 on the MIC results of colistin (COL) and polymyxin B (PB) generated by reference (CLSI) BMD methods.

Methods: A total of 247 clinical strains of Gram-negative bacilli, including *E. coli* (63), *K. pneumoniae* (61), *Acinetobacter* spp. (ASP; 60) and *P. aeruginosa* (63) were tested for susceptibility (S) against COL and PB by BMD methods according to CLSI standards. The collection was enriched with COL/PB non-susceptible strains. Reference frozen-form BMD panels were prepared at 2x drug concentration with MHB containing 0.004% P-80. An inoculum equal to 0.5 McFarland standard was prepared without P-80 for a final test concentration of 5×10^5 CFU/ml. Final P-80 concentration in the well was 0.002%. Quality control was assured by concurrent testing *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 with all results within ranges published by the CLSI.

Results: MIC results for COL and PB were generally 4- to 8-fold (2 to 3 doubling dilutions) lower when P-80 was added in comparison to the results generated without surfactant (Figure 1). The decrease in the MIC values was greatest for isolates with COL and PB MIC values of ≤ 2 $\mu\text{g/ml}$ (S), compared with isolates with MIC values of ≥ 4 $\mu\text{g/ml}$ (non-S). The P-80 effect was more apparent when testing ASP, and slightly greater with PB compared to colistin.

Conclusions: Significant effects of a surfactant (P-80) on the MIC results for COL and PB were detected for Gram-negative pathogens. We have consistently obtained lower and very reproducible MIC results when a modest concentration (0.002%) of P-80 was added to the MHB used to prepare the MIC panels.

INTRODUCTION

The polymyxins are polypeptides with a basic structure that consist of a fatty acid side chain attached to a polycationic peptide ring composed of 8 to 10 aminoacids. The polymyxins have activity against a wide variety of Gram-negative bacilli, including Enterobacteriaceae and non-fermentative species. The emergence of multidrug-resistant *Pseudomonas aeruginosa*, *Acinetobacter* spp. and *Klebsiella pneumoniae* has required the expanded systemic use of these antimicrobial agents. As polymyxin agents (colistin and polymyxin B) usage increases, the development of polymyxin resistance becomes a clinical concern. Thus, there is a need for standardization of susceptibility testing methods for these compounds.

Polysorbate-80 (also known as Tween-80) is a surfactant widely employed as a dispersing agent in the preparation of broth microdilution panels and/or bacterial inocula used in susceptibility testing; however, there is virtually no data published in the medical literature regarding the influence of this agent on the susceptibility testing results. We evaluate the effect of the addition of polysorbate-80 to Mueller-Hinton broth (MHB) when testing polymyxin B and colistin by the broth microdilution method.

MATERIALS AND METHODS

Organisms: A total of 247 organisms were tested as follows: *Escherichia coli* (63; including 12 polymyxin B-non-susceptible strains [MIC, ≥ 4 $\mu\text{g/ml}$]); *K. pneumoniae* (61; including 11 polymyxin B-non-susceptible strains); *Acinetobacter* spp. (60; including 10 polymyxin B-non-susceptible strains); and *P. aeruginosa* (63; including nine polymyxin B-non-susceptible strains).

Antimicrobials: Polymyxin B was tested with and without polysorbate-80 at a dilution schedule of 64 – 0.03 $\mu\text{g/ml}$. Also, colistin was tested with and without polysorbate-80 at a dilution schedule of 16 – 0.12 $\mu\text{g/ml}$.

Susceptibility testing: Reference frozen-form broth microdilution panels were prepared at 2x drug concentration with MHB containing 0.004% P-80 according to CLSI M07-A9 (2012). An inoculum equal to 0.5 McFarland standard in water was prepared without P-80 for a final test concentration of 5×10^5 CFU/ml. Final P-80 concentration in the well was 0.002%. Quality control was assured by concurrent testing *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 with all results within ranges published by the CLSI (M100-S22, 2012).

RESULTS

- MIC results for polymyxin B and colistin were generally four- to eight-fold (two to three doubling dilutions) lower when 0.002% polysorbate-80 was added to the MHB media in comparison to the results generated without surfactant (Figures 1 to 4).
- The decrease in the MIC values was greatest for isolates having colistin and polymyxin B MIC values of ≤ 2 $\mu\text{g/ml}$, compared with isolates with MIC values of ≥ 4 $\mu\text{g/ml}$ (Figures 3 to 5).
- The polysorbate-80 effect was more apparent with *Acinetobacter* spp. strains compared to other organism species (Figures 6 and 7).
- The polysorbate-80 effect was slightly greater with polymyxin B compared to colistin (Figure 1).

Figure 1. Log₂ variation in the colistin and polymyxin B MIC results when polysorbate-80 (P-80) was added to the media (all species; n=247).

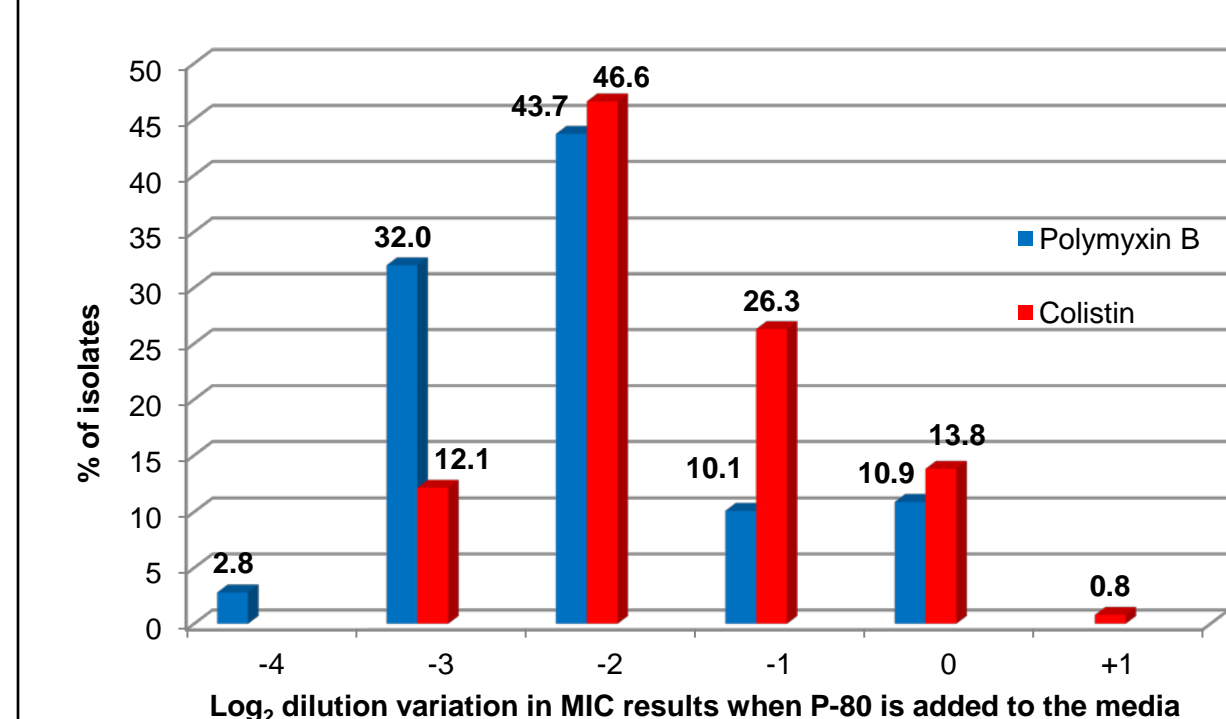


Figure 2. Polymyxin B MIC results with and without polysorbate-80 (P-80) for all species combined (247 isolates).

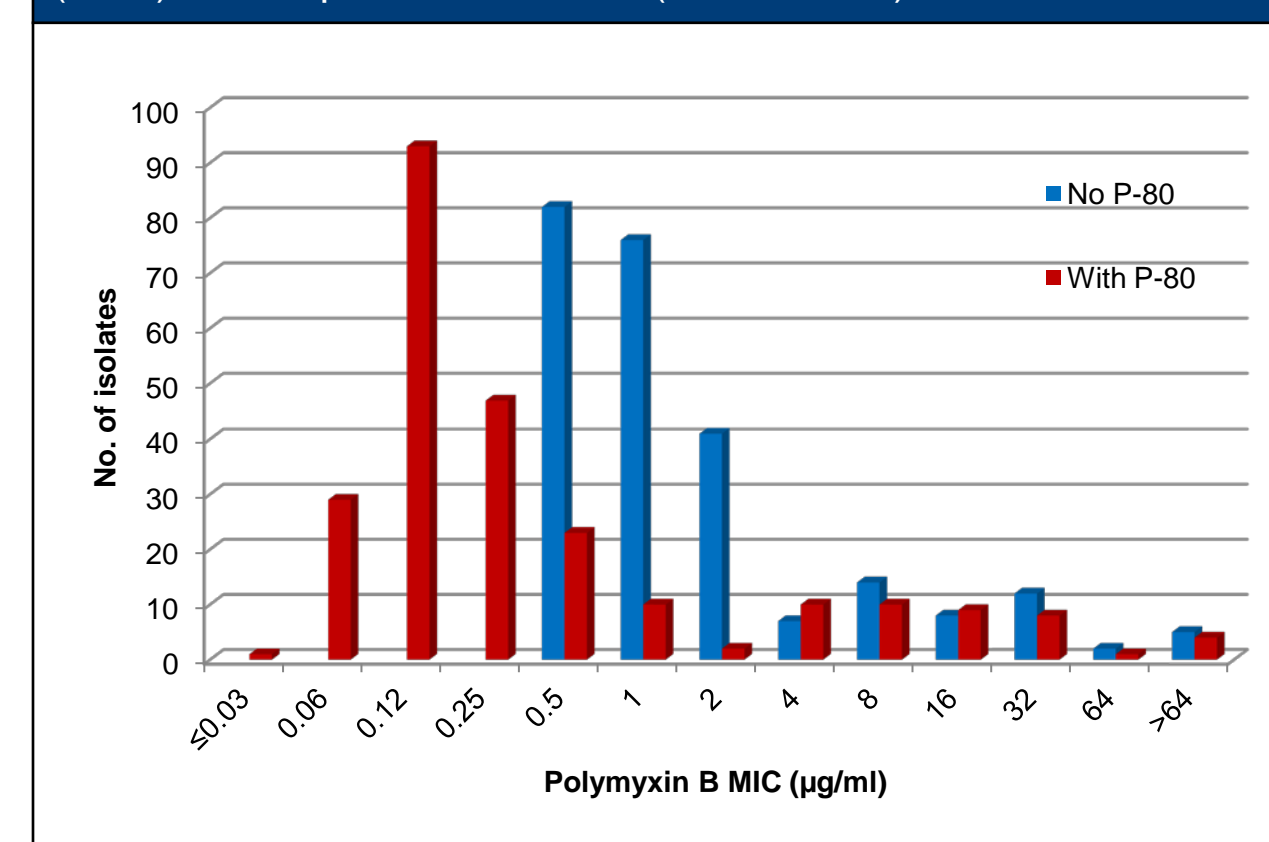


Figure 3. Scattergram showing polymyxin B MIC results obtained with vs. without the addition of polysorbate-80 (P-80) to the MHB (all species; n=247). Black horizontal and vertical lines separate susceptible from non-susceptible strains (MIC, ≥ 4 $\mu\text{g/ml}$).

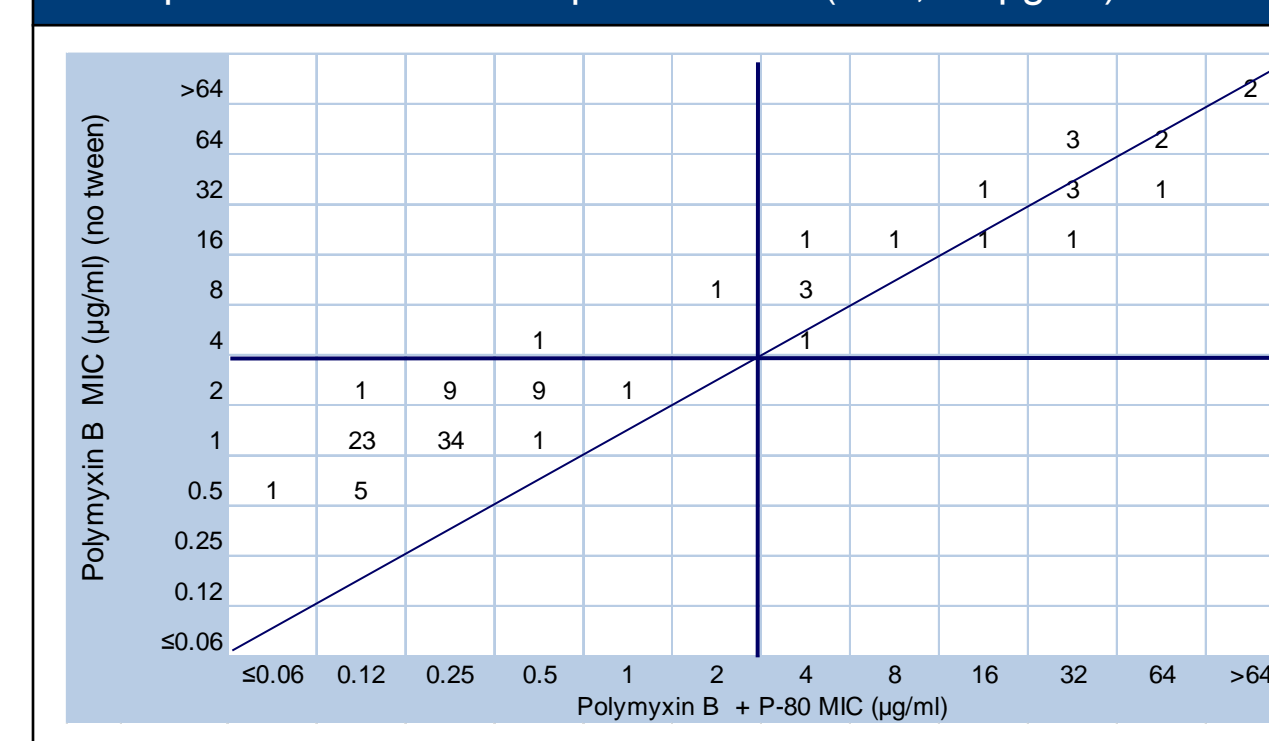


Figure 4. Scattergram showing colistin MIC results obtained with vs. without the addition of polysorbate-80 (P-80) to the MHB (all species; n=247). Black horizontal and vertical lines separate susceptible from non-susceptible strains (MIC, ≥ 4 $\mu\text{g/ml}$).

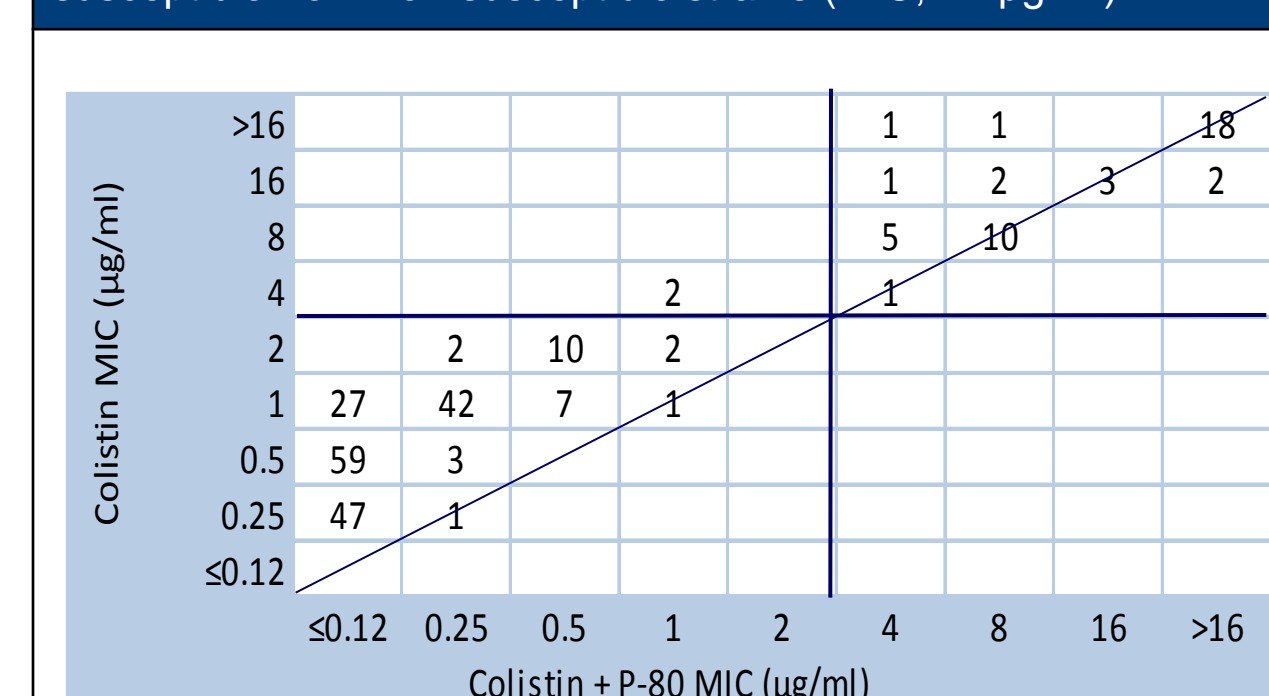


Figure 5. Log₂ variation in the polymyxin B MIC results when polysorbate-80 (P-80) was added to the media stratified by susceptibility to polymyxin B (all species; n=247).

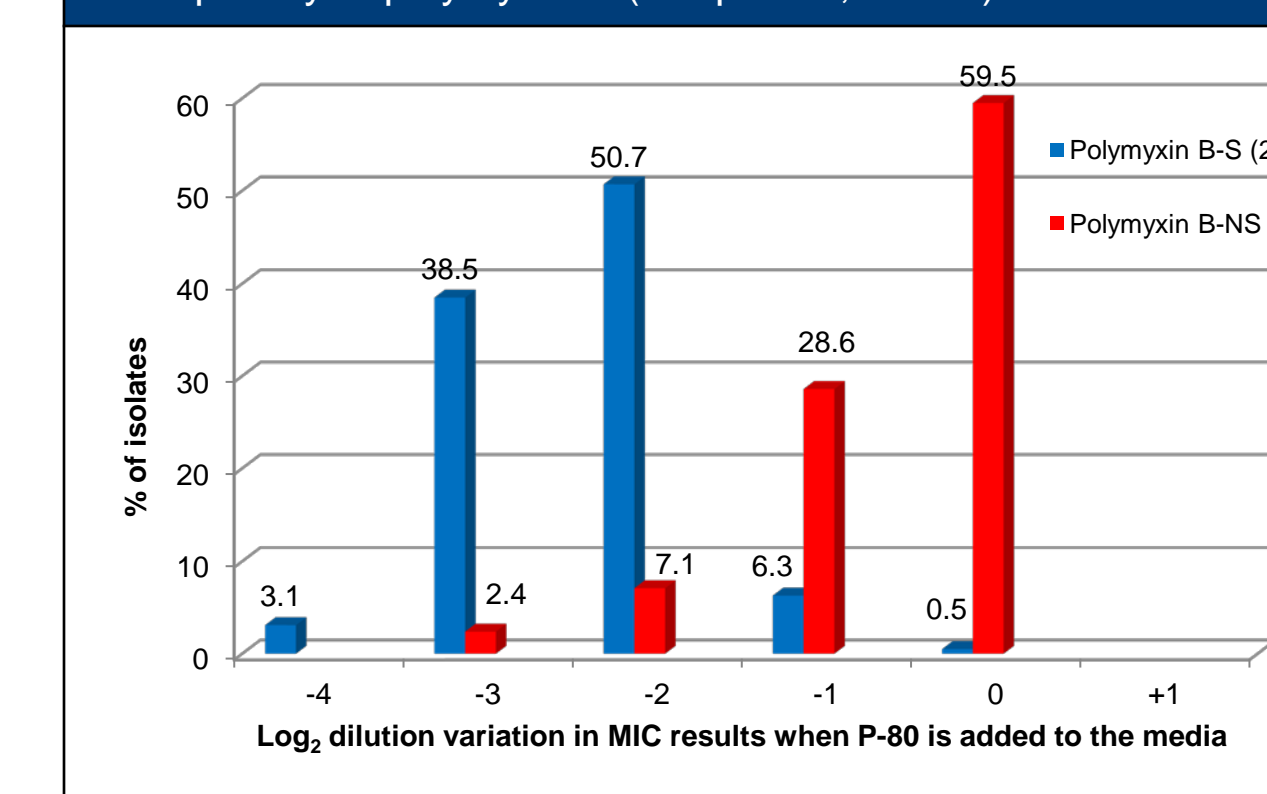


Figure 6. Log₂ variation in the colistin MIC results when polysorbate-80 (P-80) was added to the media stratified by bacterial species/genus.

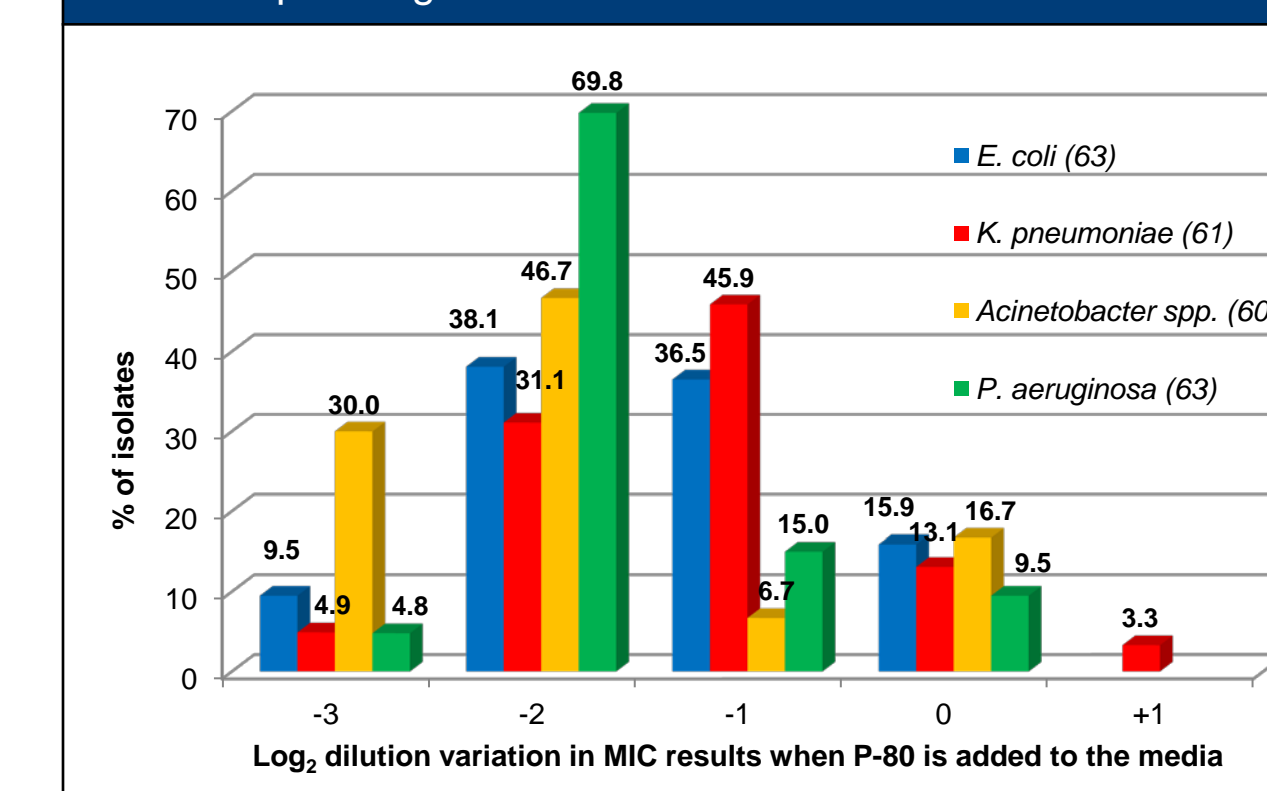
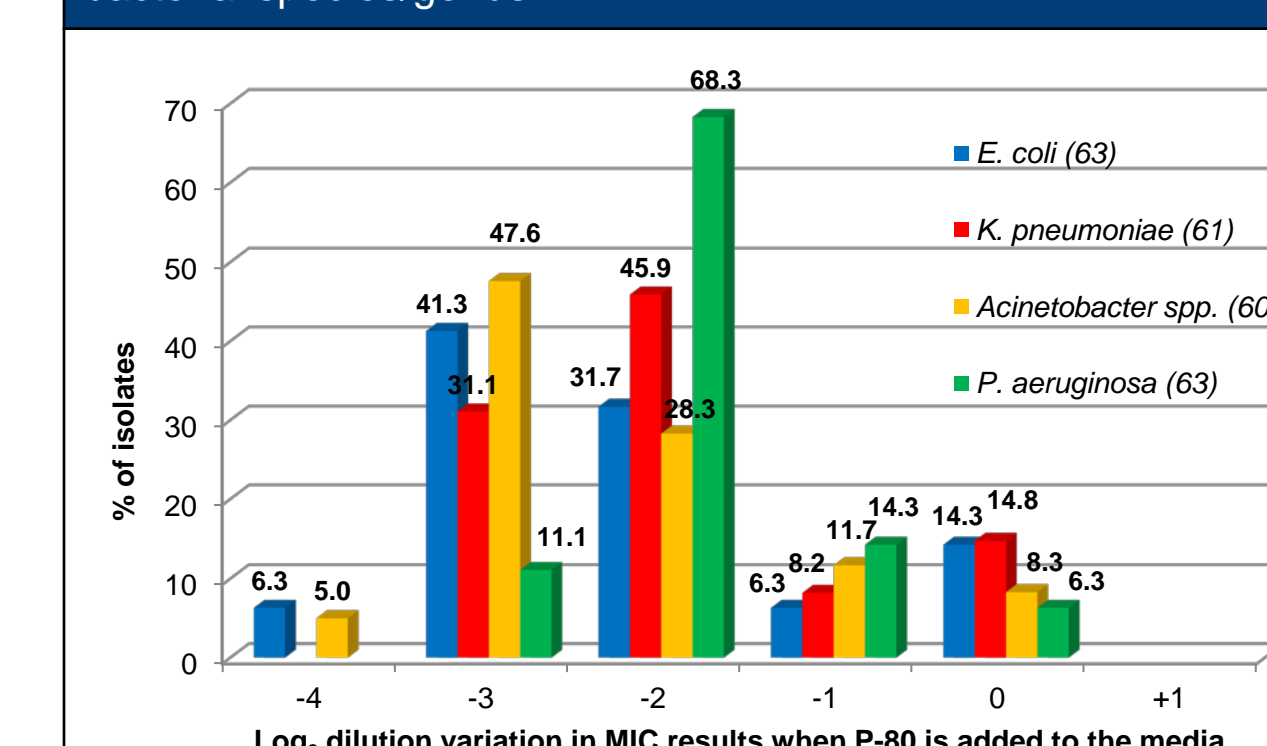


Figure 7. Log₂ variation in the polymyxin B MIC results when polysorbate-80 (P-80) was added to the media stratified by bacterial species/genus.



CONCLUSIONS

- Significant effects of a surfactant (polysorbate-80) on the MIC results for colistin and polymyxin B were detected.
- We have consistently obtained lower and very reproducible MIC results when a modest concentration (0.002%) of polysorbate-80 was added to the MHB used to prepare the BMD MIC panels.
- Although a significant change in the MIC results was noticed when polysorbate-80 was added to the test media, no significant susceptibility category change was observed when the current CLSI breakpoints were applied to the polymyxin agents.
- A new multi-laboratory QC study should be performed using MHB supplemented with 0.002% polysorbate-80 in order to re-establish proper QC ranges for these compounds, and accurately determines polymyxin potencies.

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