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# To Add or Not To Add Polysorbate 80: Impact on Colistin MICs for Clinical Strains of *Enterobacteriaceae* and *Pseudomonas aeruginosa* and Quality Controls

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**Colistin adherence to plastics can be diminished by adding a surfactant, i.e., polysorbate 80. Incorporating polysorbate 80 resulted in 3 twofold and 2 twofold modal MIC decreases for *Enterobacteriaceae* and *Pseudomonas aeruginosa*, respectively. The reproducibility of the quality controls (QCs) with and without polysorbate 80 supports the use of *Escherichia coli* strain 25922 and *P. aeruginosa* strain 27853.**

Colistin (CST) readily adheres to plastics, like those used in the manufacturing of microtiter trays. Studies have shown that adding polysorbate 80 (P80) lessens the adhesion of CST to plastics and thus significantly reduces the MICs of Gram-negative bacteria (1). The purpose of this study was to evaluate the impact of P80 on CST MICs and quality control (QC) values. Six U.S. hospitals collected nonduplicate nonurine *Escherichia coli* ($n = 169$), *Klebsiella pneumoniae* ($n = 157$), and *Pseudomonas aeruginosa* ($n = 163$) samples over the period of June 2013 to March 2014. The isolates were transferred to Trypticase soy agar slants for shipping, and once received at the central processing laboratory (at the Center for Anti-Infective Research and Development, Hartford Hospital, Hartford, CT, USA), the isolates were transferred onto Trypticase soy agar plates containing 5% blood for MIC determination. CST and P80 were purchased from Sigma (St. Louis, MO). The CST MIC trays were prepared with and without the addition of P80 using the Biomek 3000 (Beckman Instruments, Inc., Fullerton, CA). P80 was added directly to the cation-adjusted Mueller-Hinton broth (BBL, BD Diagnostic Systems, Sparks, MD) and dispensed into the 96-well U-bottom MIC tray (Sarstedt, Inc., Newton, NC) for a final concentration of 0.002%, prior to storage at −80°C. MIC testing for both the clinical and nonclinical isolates was undertaken using Clinical and Laboratory Standards Institute (CLSI)-defined broth microdilution methods. Colony counts were performed on each isolate to verify the correct inoculum. As recommended by the CLSI, *E. coli* strain 25922 and *P. aeruginosa* strain 27853 were utilized as quality control strains (2).

The results of this experiment showed the modal MICs for *E. coli* and *K. pneumoniae* were 0.5 μg/ml for CST and 0.06 μg/ml for CST with P80. The modal MICs of *P. aeruginosa* were 1 μg/ml for CST and 0.25 μg/ml for CST with P80. The addition of P80 to organisms with CST MICs of ≥2 μg/ml had no impact on MIC values. Consistent with the ranges published by the CLSI, *P. aeruginosa* 27853 had a QC mode of 1 μg/ml, with a range of 0.5 to 2 μg/ml for CST ($n = 70$), and a mode of 0.25 μg/ml, with a range of 0.25 to 0.5 μg/ml for CST with P80 ($n = 36$). Likewise, *E. coli* 25922 had a QC mode of 1 μg/ml, with a range of 0.5 to 2 μg/ml for CST ($n = 32$), and a mode of 0.125 μg/ml, with a range of 0.06 to 0.25 μg/ml for CST with P80 ($n = 30$). In conclusion, the addition of P80 to colistin resulted in 3 twofold decreases in the modal MIC values for the *Enterobacteriaceae* and 2 twofold decreases for *P. aeruginosa*; however, for isolates with colistin MICs of ≥2 μg/ml, the addition of the surfactant did not alter the initial values. As a result of the MIC reproducibility of the QCs with and without P80, our data support the continued use of *E. coli* 25922 and *P. aeruginosa* 27853, as previously published by the CLSI.

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