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Use of 0.002% Polysorbate-80 (PS-80) for Determination of Colistin MICs Produces Results that are Compatible with Colistin Pharmacokinetics/Pharmacodynamics

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ABSTRACT

Background: Colistin is used for the treatment of multi-drug-resistant gram-negative infections. However, optimal methods for colistin susceptibility testing and interpretation are unclear. Recent studies show that current dosing recommendations lead to serum colistin concentrations lower than the susceptibility breakpoint. Pharmacokinetic/pharmacodynamic (PK/PD) modeling suggests that, based on current MIC methodology and CLSI breakpoints, the probability of target attainment (PTA) for treating gram-negative infections is less than 10%. This contrasts with some studies that suggest colistin therapy is as effective as better-studied agents in some settings. These observations suggest that current MIC methods are over estimating true MICs and that addition of PS-80 may provide more accurate MIC values for modeling. The purpose of this study was to compare the AUC_{24}/MIC using standard colistin dosing and the calculated epidemiological cut-off values (ECOFF) for *Enterobacteriaceae* and *Pseudomonas aeruginosa* with and without PS-80 and to calculate PTA of a range of targets.

Methods: Published Monte-Carlo simulations were used to define the PK profiles of colistin dosed at 5 mg/kg/d. Colistin MICs for 962 *Enterobacteriaceae* and 350 *P. aeruginosa* from Canadian hospitals were determined with and without 0.002% PS-80 using M100-S24 CLSI recommendations. ECOFFs were calculated using standard statistical methods. Various PTA were calculated from both methods.

Results: See table 1.

Conclusions: The current AST method (M100-S25, 2015) provides MICs incompatible with target attainment even with permissive PK/PD targets (e.g. AUC24/MIC >30) and may over-estimate actual MIC. Addition of 0.002% PS-80 provides MICs that appear more compatible with PK/PD targets that predict positive clinical outcomes. However, clinical susceptibility breakpoints would need to be reconsidered if 0.002% PS-80 is used for in vitro susceptibility testing.

BACKGROUND

Colistin (Polymyxin E) is an antimicrobial developed over 50 years ago and abandoned due to concerns of nephrotoxicity (1). However, a resurgence of use has occurred in the context of treating multi-drug resistant infection, particularly for those caused by *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* sp. Prior to their release, no rigorous microbiological or pharmacokinetic studies were performed, and optimal dosing and methods for susceptibility testing remain unclear (1,2). Until recently, dosing recommendations for treatment of severe gram-negative infection are 5 mg/kg/d of colistin base up to 300 mg, which provides a median steady-state serum concentration of approximately 1.8 mg/L colistin in population models (3). This relatively low total serum concentration, combined with protein binding of 59 – 74% (4), is at odds with MIC values of organisms considered clinically susceptible to colistin (i.e. MIC ≤ 2 µg/mL) (5) as well as the observed, albeit often anectotal, clinical response of patients treated with colistin. These observations would suggest that current methodologies over-estimate the true colistin MIC of clinical isolates. This has led to the suggestion that polysorbate-80, a surfactant that reduces binding (and thus inactivation) of colistin to plastics used in susceptibility testing panels, should be added to broths used in colistin MIC determination. Addition of PS-80 results in lower apparent colistin MICs in microbroth dilution panels, however, it remains unclear if the lower MICs are a better approximation of true MIC and thus a more accurate estimation for use in pharmacokinetic/pharmacodynamic modeling or if the lower MICs are the result of synergistic antimicrobial activity demonstrated by some studies between PS-80 and colistin (5).

The purpose of this study was to compare the AUC_{24}/MIC using standard colistin dosing and the calculated epidemiological cut-off values (ECOFF) for *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter* spp. with and without PS-80 and to calculate PTA of a range of targets. We used the AUC_{24}/MIC as the PD parameter of choice because it has been determined to be the most predictive of clinical success with colistin in animal models (2).

MATERIALS & METHODS

Isolates were collected as part of the CANWARD 2014 studies occurring between January 2014 and December 2014. 15 Canadian hospitals in 8 provinces contributed clinically relevant isolates. *Enterobacteriaeceae* (*Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae, K. oxytoca*), *Pseudomonas aeruginosa* and *Acinetobacter* species were included in this study. In total, colistin MICs for 962 *Enterobacteriaceae* and 350 *P. aeruginosa* were determined with and without 0.002% PS-80 using M100-S24 and CLSI M07-A10 CLSI recommendations (6,7). ECOFFs were calculated using standard statistical methods (8).

For the determination of probability of target attainment (PTA), published Monte-Carlo simulations were used to define the PK profiles of colistin dosed at 5 mg/kg/d up to 300 mg/d (see Table 2). PTA was determined for the following pharmacodynamic targets using ECOFFs with and without PS-80: AUC_{24}/ECOFF > 30, AUC_{24}/ECOFF > 60 and AUC_{24}/ECOFF > 120.

RESULTS

TABLE 1: PTA for various AUC_{24}/MIC targets based on ECOFFs calculated for *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp.

Organism	ECOFF (no PS-80)	PTA AUC_{24}/ECOFF>30	PTA AUC_{24}/ECOFF>60	PTA AUC_{24}/ECOFF>120	ECOFF (PS-80)	PTA AUC_{24}/ECOFF>30	PTA AUC_{24}/ECOFF>60	PTA AUC_{24}/ECOFF>120
P. aeruginosa (350)	4 µg/mL	0%	0%	0%	1 µg/mL	88.6%	7.5%	0%
E. coli (624)	1 µg/mL	88.6%	7.5%	0%	0.25 µg/mL	99.9%	99.4%	88.6%
K. pneumoniae (186)	2 µg/mL	7.5%	0%	0%	0.25 µg/mL	99.9%	99.4%	88.6%
E. cloacae (86)	1 µg/mL	88.6%	7.5%	0%	0.25 µg/mL	99.9%	99.4%	88.6%
K. oxytoca (43)	1 µg/mL	88.6%	7.5%	0%	0.25 µg/mL	99.9%	99.4%	88.6%
Enterobacteriaceae (962)	1 µg/mL	88.6%	7.5%	0%	0.25 µg/mL	99.9%	99.4%	88.6%
Acinetobacter spp. (19)	2 µg/mL	7.5%	0%	0%	0.5 µg/mL	99.4%	88.6%	7.5%

TABLE 2: PK parameters derived from Monte-Carlo simulation of 5000 cases used in this study data from Zelenitsky *et al.* (3)

	Weight (kg)	Creatinine clearance (mL/min)	Dose (mg)	Dose (mg/kg)	Concentration at Steady state (mg/L)	AUC_{24}
Minimum	45.2	60.0	225.9	3.2	0.55	13.1
Maximum	94.5	120.0	300.0	5.0	3.98	95.5
Median	70.0	90.6	300.0	4.3	1.77	42.6
Arithmetic Mean	70.0	90.3	295.9	4.3	1.82	43.7
Standard Deviation	9.9	17.3	12.1	0.5	0.47	11.4

MICs using PS-80 were generally 4 – 8 fold lower that those without PS-80.

Assuming 60% protein binding, all ECOFF MICs determined without PS-80 were higher than the expected mean steady-state free serum concentrations of colistin using 5 mg/kg/d dosing.

Animal models suggest Total AUC_{24}/MIC > 60 is associated with 1-2 $\log_{10}$ killing of *Pseudomonas aeruginosa*. This target is not achievable using recommended dosing for isolates with a colistin MIC of 2 µg/mL or greater.

CONCLUSIONS

ECOFF MICs without PS-80 are higher than the expected mean free serum concentrations of colistin using 5 mg/kg/d dosing, strongly suggesting that true MIC values are over-estimated.

Integrating population MIC values for *Enterobacteriaceae* (excluding the genera *Proteus, Providencia* and *Morganella)*, *Pseudomonas aeruginosa* and *Acinetobacter* species with PK/PD models of colistin dosing suggests the addition of PS-80 would provide MIC values more compatible with PK/PD target attainment using recommended colistin dosing while even very permissive PK/PD targets are unattainable using MIC values without PS-80.

Adding PS-80 provides MIC values that generate significantly higher PTAs across a range of targets and PTA remains relatively high with AUC_{24}/MIC target values similar to better studied antimicrobials where AUC_{24}/MIC is the PD parameter that predicts outcomes (e.g. AUC_{24}/MIC > 120 for fluoroquinolones).

If PS-80 were to be routinely added to colistin microbroth panels, susceptibility breakpoints would need to be reconsidered and likely lowered.

Although widely used and not thought of having significant antibacterial activity, reports of synergism between PS-80 and colistin need to be fully investigated prior to changing susceptibility methodology.

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REFERENCES

1. Kassamali Z.,i Jain R., Danziger LH. International Journal of Infectious Diseases 30 (2015) 125–132.
2. Dudhani RV., Turnidge JD, Nation RL and Li J. J Antimicrob Chemother 2010; 65: 1984–1990.
3. Zelenitsky SA, Rubinstein E, Ariano RE, Zhanel GG. J Antimicrob Chemother. 2013; 68 Suppl 1:i67-72.
4. Mohamed AF, Karaiskos I, Plachouras D, Karvanen M, Pontikis K, Jansson B, Papadomichelakis E, Antoniadou A, Giamarellou H, Armaganidis A, Cars O, Friberg LE. Antimicrob Agents Chemother. 2012 ;56(8):4241-9
5. Figura N, Marcolongo R, Cavallo G, Santucci A, Collodel G, Spreafico A, Moretti E.BMC Microbiol. 2012;12:217.
6. CLSI. *Methods for Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Eighth Edition.* CLSI document M07-A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
7. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement.* CLSI document M100-S25. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
8. Turnidge J, Kahlmeter G, Kronvall G. Clin Microbiol Infect. 2006 May;12(5):418-25