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Analysis of the Influence of Surfactant (Tween-80) Concentrations on Susceptibility Testing Results for the Polymyxins by Broth Microdilution Methods

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ABSTRACT

Background: 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. The objective of the study was to evaluate the influence of Tween-80 on the MIC results of polymyxins generated by reference BMD methods (CLSI/NCCLS).

Methods: A total of 257 clinical strains of Gram-negative bacilli were tested for susceptibility against colistin (COL), polymyxin B (PB), gentamicin (GENT) and tetracycline (TET) by BMD methods according to CLSI standards (M7-A7, 2006). An inoculum equal to 0.5 McFarland standard (1 ml) was prepared and diluted into a 29 ml H_20 blank tube with and without 0.02% Tween-80. Then reference frozen-form BMD panels, prepared by TREK Diagnostics (Cleveland, OH), were inoculated using disposable inoculators. 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-S16, 2006).

Results: MIC results for COL and PB were generally 4- to 8-fold lower when Tween-80 was added to the inoculum in comparison to the results generated with an inoculum prepared without surfactant. The decrease in the MIC values was greatest for isolates with COL and PB MIC values of ≤ 2 µg/ml, compared with isolates with MIC values of > 4 µg/ml. For both polymyxins, the MICs were equal for both inocula (± Tween-80) at higher MIC values (resistant levels). For GENT and TET the MIC results obtained with and without surfactant were very similar.

Conclusions: Significant effects of a surfactant (Tween-80) on the MIC results for COL and PB were detected for Gram-negative pathogens having MIC values ≤ 2 µg/ml. We have consistently obtained lower and very reproducible MIC results when a modest concentration (0.02%) of Tween-80 was used in the inoculum preparation.

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 amino acids. The polymyxins have activity against a wide variety of Gram-negative bacilli, including Enterobacteriaceae and non-fermentative isolates. The emergence of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. has required the expanded systemic use of these polymyxins. As polymyxin usage increases, the development of polymyxin resistance becomes a concern. Thus, there is a need for standardization of susceptibility testing methods for these compounds.

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 susceptibility testing results. We evaluated the influence of Tween-80 on the MIC results for the polymyxins generated by reference broth microdilution methods.

MATERIALS AND METHODS

Bacterial Isolates: A total of 257 contemporary clinical strains of Gram-negative bacilli collected worldwide were evaluated in the study, including:

1. *Acinetobacter* spp. — 38 strains
2. *Burkholderia cepacia* — 16 strains
3. *Pseudomonas aeruginosa* — 97 strains
4. *Stenotrophomonas maltophilia* — 19 strains
5. Enterobacteriaceae — 87 strains

Susceptibility Testing: The isolates were tested against colistin and polymyxin B by broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI) standards (M7-A7, 2006). An inoculum equal to 0.5 McFarland standard (1 ml) was prepared and diluted into a 29 ml H_20 blank tube with and without Tween-80 at 0.02%. Then reference frozen-form panels, prepared by TREK Diagnostics (Cleveland, OH), were inoculated using disposable inoculators (final Tween-80 concentration at 0.002%). Gentamicin and tetracycline were tested as controls. Quality control was assured by concurrently testing *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 with all results within ranges published by the CLSI (M100-S16, 2006).

RESULTS

- MIC results for colistin and polymyxin B were 4- to 8-fold lower when Tween-80 was added to the testing media via inoculum compared to the results of testing without surfactant (Table 1 and Figures 1 and 2). This difference was only noted when MIC results were ≤ 2 µg/ml. When the MIC values were ≥ 4 µg/ml (CLSI resistant breakpoint), Tween-80 had virtually no effect on the MIC results for these compounds.
- The effect of Tween-80 on colistin and polymyxin B MIC results was similar for all organism groups tested (Table 1).
- No effect of Tween-80 was noticed when testing gentamicin (Table 1 and Figure 3) or tetracycline (data not shown).

Table 1. Comparison of MIC results for colistin, polymyxin B and gentamicin when tested by broth microdilution with and without Tween-80.

	Cumulative % inhibited at MIC (µg/ml):												
Organism (no. tested)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>
Acinetobacter spp. (38)													
Colistin													
No Tween	0	0	0	4	14	14	4[a]	1	1	0	0	0	0
0.002% Tween	2	4	15	8	5	3	0	0	1	0	0	0	0
Polymyxin B													
No Tween	0	0	0	3	21	9	4	1	0	0	0	0	0
0.002% Tween	5	8	14	6	2	2	0	1	0	0	0	0	0
Gentamicin													
No Tween	0	0	0	2	11	5	1	4	2	0	0	-	13
0.002% Tween	0	0	0	4	7	7	1	4	2	0	1	-	12
B. cepacia (16)													
Colistin													
No Tween	0	0	0	0	0	2	1	0	0	0	0	0	13
0.002% Tween	0	0	0	0	0	1	1	1	0	0	0	0	13
Polymyxin B													
No Tween	0	0	0	0	0	1	2	0	0	0	0	0	13
0.002% Tween	0	0	0	1	0	2	0	0	0	0	0	0	13
Gentamicin													
No Tween	0	0	0	0	0	0	0	0	0	1	3	-	12
0.002% Tween	0	0	0	0	0	0	0	0	0	2	3	-	11
P. aeruginosa (97)													
Colistin													
No Tween	0	0	0	0	5	78	13	1	0	0	0	0	0
0.002% Tween	0	1	19	65	11	1	0	0	0	0	0	0	0
Polymyxin B													
No Tween	0	0	0	0	4	85	8	0	0	0	0	0	0
0.002% Tween	0	3	41	50	3	0	0	0	0	0	0	0	0
Gentamicin													
No Tween	0	1	1	3	9	37	21	4	1	4	5	-	11
0.002% Tween	0	2	0	3	11	35	21	5	0	5	5	-	10
S. maltophilia (19)													
Colistin													
No Tween	0	0	1	1	2	2	1	2	4	3	1	0	2
0.002% Tween	3	0	1	1	1	1	2	1	4	2	1	1	1
Polymyxin B													
No Tween	0	0	0	2	3	5	4	3	1	0	0	0	1
0.002% Tween	2	4	1	1	2	4	2	0	2	1	0	0	1
Gentamicin													
No Tween	0	0	0	0	0	0	0	0	0	3	4	-	12
0.002% Tween	0	0	0	0	0	0	0	1	1	1	5	-	11
Enterics (87)													
Colistin													
No Tween	0	0	0	31	21	3	0	0	1	1	0	0	30
0.002% Tween	18	25	5	7	0	0	0	0	1	1	0	0	30
Polymyxin B													
No Tween	0	0	0	1	46	8	0	1	0	1	0	1	29
0.002% Tween	20	23	11	1	0	0	0	1	0	1	0	1	29
Gentamicin													
No Tween	0	0	6	37	25	6	1	0	3	1	4	-	4
0.002% Tween	0	0	8	31	27	9	0	2	2	1	2	-	5
All isolates (257)													
Colistin													
No Tween	0	0	1	36	42	97	20	5	6	4	1	0	45
0.002% Tween	23	30	40	81	17	6	3	2	6	3	1	1	44
Polymyxin B													
No Tween	0	0	0	6	74	108	18	5	1	1	0	1	43
0.002% Tween	27	38	67	59	6	8	2	2	2	2	0	1	43
Gentamicin													
No Tween	0	1	7	42	45	48	23	8	6	9	16	-	52
0.002% Tween	0	2	8	38	45	51	22	12	5	9	16	-	49

a. Boxes indicate breakpoints for susceptibility by CLSI criteria (2006). Colistin and polymyxin B breakpoints were established for *Acinetobacter* spp. only but applied here for all organisms.

Figure 1. Scattergram showing the correlation between MIC results when testing colistin by broth microdilution methods with and without 0.002% Tween-80. Solid lines indicate CLSI [2006] breakpoints for *Acinetobacter* spp. (≤ 2 µg/ml for susceptible and ≥ 4 µg/ml for resistant); while dashed lines indicate resistant breakpoint established by the British Society for Antimicrobial Chemotherapy.

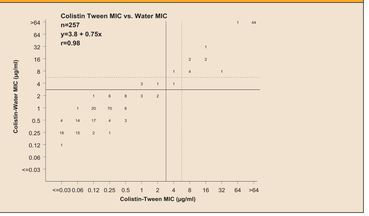


Figure 2. Scattergram showing the correlation between MIC results when testing polymyxin B by broth microdilution methods with and without 0.002% Tween-80. Solid lines indicate CLSI [2006] breakpoints for *Acinetobacter* spp. (≤ 2 µg/ml for susceptible and ≥ 4 µg/ml for resistant); while dashed lines indicate resistant breakpoint established by the British Society for Antimicrobial Chemotherapy for colistin.

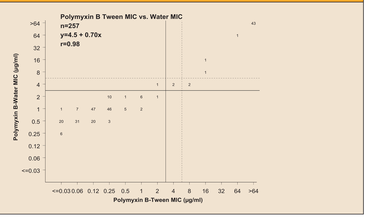
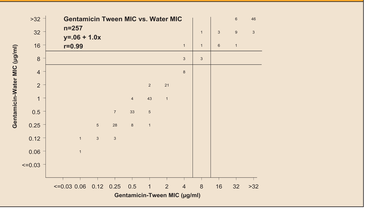


Figure 3. Scattergram showing the correlation between MIC results when testing gentamicin by broth microdilution methods with and without 0.002% Tween-80. Solid lines indicate CLSI [2006] breakpoints.



CONCLUSIONS

- MIC results for colistin and polymyxin B are 2 to 3 log_2 dilutions lower if Tween-80 is added to the test media compared to results obtained by CLSI standard methods (no Tween-80). The effect of the surfactant seems to be greater at lower MIC levels with nearly no effect when the MIC is ≥ 4 µg/ml.
- Although a significant change in the MIC results was noticed when Tween-80 was added to the test media, no significant susceptibility category change was observed when the current *Acinetobacter* spp. CLSI breakpoints were applied to the polymyxin agents.

SELECTED REFERENCES

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