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Testing of USA *mcr-1* Colistin-Resistant *E. coli* on MicroScan Panels

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ABSTRACT

Background: In 2015, a report from China first described plasmid-mediated colistin resistance in *Enterobacteriaceae* caused by the *mcr-1* gene. Bacteria with this resistance mechanism have been identified in many countries around the world including the United States and are chiefly in *E. coli*. We tested two *mcr-1* containing colistin-resistant *E. coli* isolated in the United States on MicroScan panels to assess the ability and accuracy of the panels to detect this type of resistance.

Methods: *E. coli* AR 0346 *mcr-1* and *E. coli* AR 0349 *mcr-1* were obtained from the US Centers for Disease Control Antibiotic Resistance bank. Isolates were tested in triplicate on MicroScan NMIC44 panels containing colistin at 2-4ug/ml (not for sale in the United States). Panels were inoculated using either the turbidity or Prompt methods according to the manufacturer's instructions and read visually. Results were compared to expected MIC values given by the AR bank. *E. coli* ATCC 25922 and *P. vulgaris* ATCC 49732 were tested as quality control.

Results: MicroScan panel colistin MICs were 4->4 ug/ml for both *mcr-1* isolates tested and with both inoculation methods. QC was in control.

Conclusion: MicroScan panels accurately detected *mcr-1* mediated colistin resistance in the *E. coli* isolates available from the US Centers for Disease Control Antibiotic Resistance bank.

BACKGROUND

Colistin belongs to an older class of antibacterial agents, the polymyxins, which act on bacterial cells by increasing cell permeability. In 2015, a report from China first described plasmid-mediated colistin resistance caused by the *mcr-1* gene (1). Bacteria with this resistance mechanism have been identified in many countries around the world including the United States and are chiefly in *E. coli* (2). Thus, there is more of an urgency to test colistin. Note that some *Enterobacteriaceae* (e.g. *Proteus, Providencia, Morganella, Serratia* species) are intrinsically resistant to colistin (3,4).

Historically, in vitro evaluation of colistin (polymyxin E) susceptibility has been complicated by its chemical properties. A joint CLSI/EUCAST Polymyxin Breakpoints Working Group was convened to provide recommendations for Minimum Inhibitory Concentrations (MIC) determination of colistin. This group reiterated that broth microdilution is the only validated method (5). Disk diffusion is not recommended for testing bacterial susceptibility or resistance to colistin, due to the poor agar diffusion characteristics (6,7). In July 2016 EUCAST questioned the validity of MICs obtained with gradient diffusion tests (8). The gradient tests "underestimated MIC values by one or more two-fold dilutions, especially for concentrations on or above the breakpoint of 2 mg/L, leading to false susceptible results (Very Major Errors: VME)." Other studies have also urged caution with colistin gradient diffusion testing (9,10). Thus, the only established method for testing colistin is broth microdilution.

MATERIALS AND METHODS

Study Design

Organisms: *E. coli* AR 0346 and *E. coli* AR 0349 with known colistin values were obtained from the US Centers for Disease Control Antibiotic Resistance bank.

Quality Control: *E. coli* ATCC 25922 (expected negative) and *P. vulgaris* ATCC 49132 (expected positive) were tested singly as quality control**.**

Panel Inoculation: All isolates were subcultured twice prior to testing onto trypticase soy agar (TSA) with 5% sheep blood and incubated 18-24 hours at 35°C ± 2°C. The inoculum suspensions for each strain were prepared using either the direct standardization (turbidity standard) method or the Prompt inoculation method according to manufacturer's instructions.

Panels: Isolates were tested in triplicate on NMIC44 panels (For Export only, not available in the United States) and read visually after overnight incubation at 35°C ± 2°C. MicroScan panels contain colistin at 2 and 4ug/ml in cation-adjusted Mueller-Hinton Broth. There is a contraindication for testing colistin with *Acinetobacter* spp. and results are not reported for this organism group.

Data Analysis: Results were compared to expected MIC values given by the AR bank.

RESULTS

- *E. coli* AR 0346, with an expected colistin MIC of 4ug/ml, gave triplicate >4ug/ml MIC results on MicroScan panels, with both turbidity and Prompt panel inoculation methods.
- *E. coli* AR 0349, with an expected colistin MIC range of 2-4ug/ml, gave triplicate >4ug/ml MIC results on MicroScan panels with turbidity panel inoculation method and 4->4ug/ml MIC results with the Prompt inoculation method.
- All quality control results were in specification.

CONCLUSIONS

MicroScan panels accurately detected *mcr-1* mediated colistin resistance in the *E. coli* isolates available from the US Centers for Disease Control Antibiotic Resistance bank.

Colistin not for diagnostic use within the US, available for research use only

RESULTS

Organism	Expected MIC, ug/ml (AR Bank)	Replicate	Colistin MIC, ug/ml MicroScan NMIC44 Panel	
			Turbidity Inoculation	Prompt Inoculation
E. coli AR 0346 *mcr-1*	4	1	>4	>4
		2	>4	>4
		3	>4	>4
E. coli AR 0349 *mcr-1*	2 - 4	1	>4	4
		2	>4	4
		3	>4	>4
E. coli ATCC 25922	- (<=2)	1	- (<=2)	- (<=2)
P. vulgaris ATCC 49732	+ (>4)	1	+ (>4)	+ (>4)

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