Comparison of Vitek 2®, E-test, and MICRONAUT Preprepared Plates With Broth Microdilution for Colistin Testing of *Enterobacteriaceae*

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Background: In the advent of carbapenemase-producing, multiresistant *Enterobacteriaceae* colistin has become more important as a salvage agent. Susceptibility testing of this drug is notoriously problematic because it can bind to plastic material and its diffusion properties preclude the use of disk diffusion testing. Manufacturers of gradient tests and of semiautomated systems have included colistin into their products; however, EUCAST has recently (March 2016) issued a warning against the use of gradient tests, especially if used with the Mueller Hinton agars of some manufacturers. Since broth microdilution is not always available, simple methods are needed. We therefore compared susceptibility results of a collection of 962 strains of enterobacteria when tested with E-Test, Vitek 2 (both bioMérieux) MICRONAUT (Merlin diagnostics) preprepared plates and broth micro dilution (BMD) prepared according to EUCAST recommendations.

Materials and Methods: 963 carbapenemase-positive enterobacterial strains (*K. pneumoniae*, 520, *E. coli*, 191, *E. cloacae*, 117, *C. freundii,* 68, *K. oxytoca*, 46, other, 21) were tested for colistin susceptibility by E-test, performed on MH-E agar, Vitek 2, MICRONAUT preprepared plates and compared to broth microdilution (BMD). Results were assessed according to FDA guidelines 2009.

Results: Essential agreement between BMD and E-test, Vitek 2, and MICRONAUT were 81.7%, 94.9%, and 87%, respectively. However, very major errors (VME) occurred in 8.5%, 29.2% and 4% for E-test, Vitek 2 and MICRONAUT, respectively. For all systems the VME rate was lower when only *K. pneumoniae* was considered (2.5%, 16%, and 4% for E-test, Vitek 2 and MICRONAUT).

Etest Misses Resistant Strains

BMD vs. E-Test All tested species

BMD \ E-test	0.125	0.25	0.5	1	2	4	8	16	32	64	128	512	N
0.125	42	6	1	0	0	0	0	0	0	0	0	0	**857**
0.25	406	144	49	0	0	0	1	1	0	0	0	0	
0.5	129	51	10	1	0	0	0	0	0	0	0	0	
1	3	3	2	1	0	0	0	0	0	0	0	0	
2	4	0	1	2	0	0	0	0	0	0	0	0	
4	3	0	1	0	1	3	0	0	0	0	0	0	**106**
8	1	1	0	0	0	4	3	1	0	0	0	0	
16	0	0	0	0	0	5	11	6	0	0	0	0	
32	2	1	0	0	0	4	15	15	17	6	2	4	

(BMD)

Same result
Essential Agreement
+ one dilution off
VME

Very Major Error rate was 8.4% for Etest and *Enterobacteriaceae*.
These analyses were done for the other systems and for the species *K. pneumoniae* and *E.coli* and results are presented in a a tabulated form.

Error Rates for E-test Are Lower for *K. pneumoniae*

E-Test

All species

BMD		S	R	EA	ME	VME
S	857	855	2	81.7	0.2	8.4
R	106	9	97			
		864	99			

***K. pneumoniae* only**

S	439	438	1	79.6	0.2	2.5
R	81	2	79			
		440	80			

***E. coli* only**

S	186	186		86.4		20
R	5	1	4			
		187	4			

Error Rates for Vitek 2 Are Unacceptable

Vitek 2

All species

BMD		S	R	EA	ME	VME
S	856	852	4	95	0.5	29.2
R	106	31	75			
		883	79			

***K. pneumoniae* only**

S	438	435	3	96.1	0.7	16
R	81	13	68			
		448	71			

***E. coli* only**

S	186	186	0	97.9		60
R	5	3	2			
		189	2			

MICRONAUT (Merlin) Shows Lower Error Rates

MICRONAUT

All species

BMD		S	R	EA	ME	VME
S	219	218	1	87.6	0.5	4.7
R	106	5	101			
		223	102			

***K. pneumoniae* only**

S	126	125	1	90.3	0.8	3.7
R	81	3	78			
		128	79			

***E. coli* only**

S	48	48	0	79.2		20
R	5	1	4			
		49	4			

Conclusions

E-test showed the lowest VME rates if only *K. pneumoniae* were considered. For all enterobacterial species the commercial BMD plates showed the lowest VME rate. For Vitek 2 the errors were always unacceptably high. When colistin susceptibility of *Enterobacteriaceae* is tested, an BMD system should be preferred, for *K. pneumoniae* E-test on MH-E agar may be an acceptable alternative.

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