

Colistin Testing Methods!

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Colistin Testing ad hoc WG

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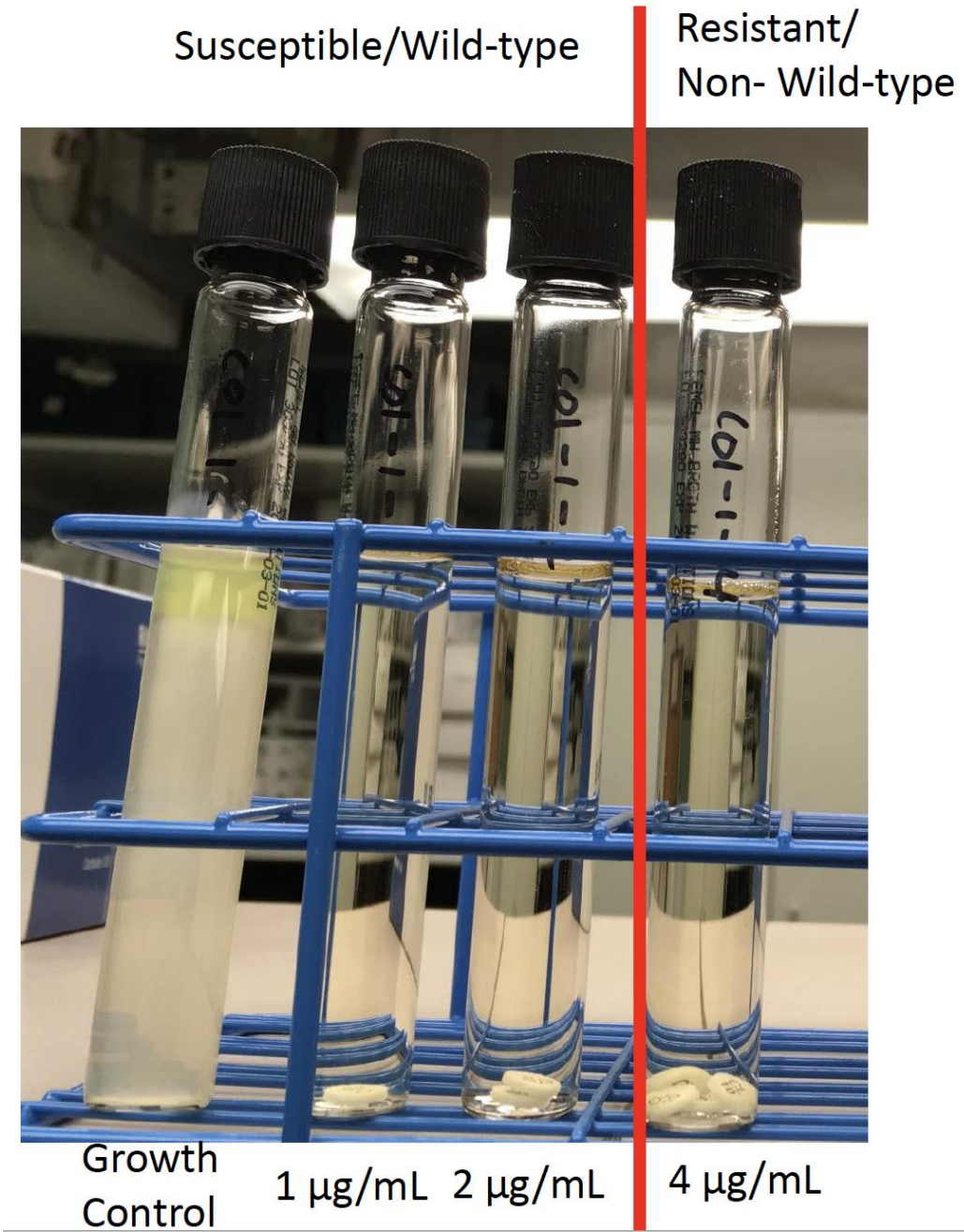
Study Design

- Two methods evaluated for colistin testing:
 - Colistin disk broth elution (CBDE)
 - Colistin Agar Test (CAT, using a 1 uL and 10 uL calibrated loop for inoculum)
- Compared to broth microdilution, performed using 3 lots (brands) of CA-MHB
- Testing was performed at 3 sites: Columbia, Johns Hopkins and Mayo Clinic
- Isolates included:
 - “stock” from laboratory collection, tested at one site
 - “challenge” compiled from all sites + CDC/UCLA/AxDx to ensure enough resistance – tested at each site (i.e., 3 results per isolate)

CBDE Method

- Disks: BD, 10 ug colistin disk
- CA-MHB: Remel (10 mL or 25 mL)
 - 0 disk = growth control
 - 1 disk to 25 mL = 0.4 ug/mL*
 - 1 disk to 10 mL = 1 ug/mL
 - 2 disks to 10 mL = 2ug/mL
 - 4 disks to 10 mL = 4 ug/mL
- Incubate tubes @ RT 20 min
- Add 50 uL 0.5 McFarland organism
- Vortex
- Incubate at 35 C 18-20 h
- Read MIC

* chose to put 1 disk in 25 mL as this is commercially available,
Vs cutting disk in half or aliquoting media



A.



GC 1 µg/ml 2 µg/ml 4 µg/ml

(A) Tubes for a non-carbapenemase-producing carbapenem-resistant *Klebsiella pneumoniae* isolate with a colistin MIC of ≤ 1 µg/ml.

B.



GC 1 µg/ml 2 µg/ml 4 µg/ml

(B) Tubes for an *mcr-1*-producing *Escherichia coli* isolate (CDC AR Bank accession number 493) with a colistin MIC of 4 µg/ml.

CAT Method

- 0, 0.5, 1, 2 and 4 ug/mL colistin MHA plates made (Hardy)
- Used 3 MHA: BD, Remel, Hardy (rotated through study)
- Inoculated with 1 uL or 10 uL loopful of a 1:10 dilution of a 0.5 McFarland suspension
- Incubated 16-20 h, read visually for any growth as +

Study Design (2)

- For each isolate, the same inoculum was used to perform the BMD, CBDE, agar screen methods in parallel
- QC:
 - “routine” *P. aeruginosa* 27583
 - “supplemental” CDC 349 *E. coli* with *mcr-1* (anticipated on-scale results)
- If QC out, 1+ result outside categorical agreement, or skipped wells observed, testing was repeated by all methods 1x

Data analysis

1. For repeats:

1. If repeat resolved issue, took repeat result (presumed random error)
2. If repeat didn't resolve issue, took original MICs
3. If results flipped, excluded result
4. If skips repeated, excluded (n=2 for CBDE, 1 x 1 uL screen, 1x 10 uL screen)

2. Looked @ BMD in Sigma and BD CA-MHB as reference

1. Excluded Oxoid BMD as QC out (multiple lots of BMD attempted)
2. If BD/Sigma BMD not in CA, excluded
 - (n=17 *Acinetobacter*, 5 *Klebsiella* spp, 1 *P. aeruginosa*)
3. If one skipped, excluded (n=3 *Klebsiella* spp.)
4. If in CA, but different MIC: took average (round down)

Data analysis

- Accuracy:
 - Included both challenge & stock isolates in evaluation
 - EA, CA calculated per M23
- Precision:
 - Looked at just challenge isolates

Isolates tested, colistin MIC distributions

Enterobacteriaceae, n= 356 (270 unique isolates)

	Colistin MIC, ug/mL								Excluded*	Total	#Challenge	N Results
	≤0.25	0.5	1	2	4	8	16	>16				
<i>Enterobacter aerogenes</i>	3	2								5		
<i>Enterobacter cloacae</i>	15						1	22		38	6	18
<i>Escherichia coli</i>	45	12	2		19	11		3		92	10	30
<i>Citrobacter spp.</i>	7	3								10	1	3
<i>Klebsiella pneumoniae</i>	23	9	2			2	2	86	5	129	26	78
<i>Klebsiella spp.</i>	18	3	3		1	1	2	41	3	72		
<i>Enterobacter spp.</i>	4	1			1					6		
<i>Providencia stuartii</i>								1		1		
<i>Serratia spp.</i>								1		1		
<i>Morganella morgannii</i>								2		2		

* Excluded due to no BMD agreement; only occurred for stock isolates

Isolates tested, colistin MIC distributions

Non-fermenters, n= 298 (228 unique isolates)

	Colistin MIC, ug/mL								Excluded	Total	Challenge	
	≤0.25	0.5	1	2	4	8	16	>16			# isolates	# results
<i>P. aeruginosa</i>	15	63	54	4	3	1	1	8	1	150	14	42
<i>Acinetobacter</i> spp.	15	25	18	2	1	7	8	55	17	148	21	63

* Excluded due to no BMD agreement; only occurred for stock isolates

BMD vs BMD

Enterics	Sigma	BD MHB								
		<=0.25	0.5	1	2	4	8	16	>16	
	<=0.25	79	7	1			1			
	0.5	34	6		1		1			
	1	12	6	1					1	
	2	1	2	2			1			
	4				1	13	2		1	
	8					5	7	3		
	16						1		4	
	>16					3	1		157	

Acinetobacter sp	Sigma	BD MHB								
		<=0.25	0.5	1	2	4	8	16	>16	
	<=0.25	11		1						
	0.5	6	8	3	1			1	1	
	1	4	8	6	3	1			2	
	2		5	2	1			1	7	
	4							2	3	
	8								9	
	16								2	
	>16			1					55	

Pseudomonas	Sigma	BD MHB								
		<=0.25	0.5	1	2	4	8	16	>16	
	<=0.25	8	1							
	0.5	8	27	5						
	1		30	49	2					
	2	1		3	2		2			
	4					1				
	8					1				
	16								1	
	>16					1			8	

Organism Group	VME	ME
<i>Acinetobacter</i>	16.1%	1.6%
<i>Pseudomonas</i>	14.3%	0%
Enterobacterales	1.9%	0.6%

CBDE results

CBDE, Enterics

Enterics, All sites		348							
	<=0.4	1	2	4	>4		N	%	
<=0.25	97	11	7			EA	328	94.25	
0.5	26	3	1			CA	343	98.56	
1	3	3	1			VME	5	2.55	
2						ME	0	0.00	
4			4	14	3				
8			1	6	7	VME out of EA	1		
16					5				
>16					156				

CBDE, *P. aeruginosa*

Pseudomonas aeruginosa, All sites				148
	<=0.4	1	2	4 >4
<=0.25	13	2		
0.5	57	6		
1	39	9	5	1
2	2	1	1	
4				2
8				1
16				1
>16				8

	N	%
EA	143	96.62
CA	147	99.32
VME	0	0
ME	1	0.74

CBDE, *Acinetobacter* spp.

Acinetobacter spp, 131							N	%
	<=0.4	1	2	4	>4			
						EA	122	93.13
<=0.25	13	2				CA	125	95.42
0.5	18	4	2		1	VME	4	5.63
1	9	6	3			ME	2	3.33
2	1			1				
4				1		VME out of EA	4	
8	1	1	1		4	ME out of EA	1	
16	1			1	6			
>16					55			

CAT

CAT, 1uL Enterobacteriales

Enterics, All sites					347
	<=0.5	1	2	4	>4
<=0.25	113	1			
0.5	30				
1	6	1			
2	1	1			
4				5	14
8			1	1	12
16					5
>16	1			2	153

	N	%
EA	341	98.27
CA	345	99.42
VME	2	1.03
ME	0	0.00

CAT, 1uL *Acinetobacter* spp

Acinetobacter spp, <i>A</i>		130			
		<=0.5	1	2	4 >4
<=0.25		15			
0.5		24	1		
1		16	2		
2		2			
4			1		
8		2	2		2
16		2	4		2
>16			2	2	5 46

	N	%
EA	108	83.08
CA	115	88.46
VME	15	21.43
ME	0	0.00

CAT, 1uL *P. aeruginosa*

Pseudomonas aeruginosa, All sites				149		N	%	
						EA	146	99.32
	<=0.5	1	2	4	>4	CA	146	98.65
<=0.25	15					VME	1	8.33
0.5	41	22				ME	0	0.00
1	23	23	8					
2	1	1	2					
4	1				1			
8				1				
16					1			
>16				2	6			

VME for 1 uL CAT

Site	Study ID	Organism ID	Challenge or Stock	BD BMD	Sigma BMD	BD Sigma mode	1uL	10 uL
Mayo	9	Acinetobacter sp.	Stock	8	>16	16	1	skip
Mayo	62	Acinetobacter sp.	Stock	4	8	4	1	4
Mayo	63	Acinetobacter sp.	Stock	4	>16	8	1	1
Mayo	177	Acinetobacter sp.	Stock	8	>16	16	1	2
JHU	851	Acinetobacter sp.	stock	8	>16	16	≤0.5	≤0.5
JHU	884	Acinetobacter sp.	stock	8	>16	16	≤0.5	1
JHU	1572	Acinetobacter sp.	stock	4	>16	8	≤0.5	2
JHU	A. baumannii #2 JHMI	Acinetobacter sp.	Challenge	>16	>16	>16	2	>4
Columbia	A. baumannii #2 JHMI	Acinetobacter sp.	Challenge	>16	>16	>16	1	>4
JHU	A. baumannii complex #24 Mayo	Acinetobacter sp.	Challenge	16	>16	16	1	1
Columbia	A. baumannii complex #24 Mayo	Acinetobacter sp.	Challenge	8	>16	16	1	1
Mayo	A. baumannii complex #24 Mayo	Acinetobacter sp.	Challenge	>16	>16	>16	1	>4
CUMC	NR5528	Acinetobacter sp.	Stock	8	16	8	1	2
CUMC	NR5817	Acinetobacter sp.	Stock	8	16	8	≤0.5	1
JHU	UCLA-17-08-022-2	Acinetobacter sp.	Challenge	>16	>16	>16	2	>4

VME for 1 uL CAT, cont

Site	Study ID	Organism ID	Challenge or Stock	BD BMD	Sigma BMD	BD Sigma mode	1 uL	10 uL
JHU	CRE 23	Enterobacter cloacae complex	stock	>16	>16	>16	≤0.5	>4
Mayo	91	Enterobacter spp.	Stock	8	4	4	≤0.5	2
JHU	421	Escherichia coli	stock	8	8	8	2	4
Columbia	NR3422	Klebsiella spp.	Stock	4	4	4	1	4
JHU	7150 CUMC	Pseudomonas aeruginosa	Challenge	4	4	4	≤0.5	>4

10 uL CAT, Enterobacteriales

Enterics, All sites					348
	<=0.5	1	2	4	>4
<=0.25	113				
0.5	30				
1	5	2			
2					
4			1	5	15
8				2	12
16					5
>16				1	155

	N	%
EA	347	99.71
CA	347	99.71
VME	1	0.51
ME	0	0.00

10 uL CAT, *Acinetobacter* spp.

Acinetobacter spp, n		130				
		<=0.5	1	2	4	>4
<=0.25		12	2	1		
0.5		23	2			
1		8	10			
2		2				
4					1	
8			3	2	2	
16		1	3	1		2
>16					2	53

	N	%
EA	115	88.46
CA	120	92.31
VME	10	14.29
ME	0	0.00

10 uL CAT, *P. aeruginosa*

Pseudomonas aeruginosa, All sites				148	
	<=0.5	1	2	4	>4
<=0.25	12	3			
0.5	35	26	2		
1	19	23	12		
2	1	1	2		
4					2
8				1	
16					1
>16				1	7

	N	%
EA	143	99.32
CA	148	100.00
VME	0	0.00
ME	0	0.00

10 uL CAT VMEs

Site	Study ID	Organism ID	Challenge or Stock	BD BMD	Sigma BMD	BD Sigma mode	CBDE	AD 1	AD 10
Mayo	63	Acinetobacter sp.	Stock	4	>16	8	>4	1	1
JHU	851	Acinetobacter sp.	stock	8	>16	16	≤0.4	≤0.5	≤0.5
JHU	884	Acinetobacter sp.	stock	8	>16	16	>4	≤0.5	1
JHU	1572	Acinetobacter sp.	stock	4	>16	8	>4	≤0.5	2
Mayo	17.1 rpt	Acinetobacter sp.	Stock	8	>16	16	>4	1	2
CUMC	A. baumannii complex #24 Mayo	Acinetobacter sp.	Challenge	8	>16	16	4	1	1
JHU	A. baumannii complex #24-2	Acinetobacter sp.	Challenge	16	>16	16	>4	1	1
CUMC	NR5528	Acinetobacter sp.	Stock	8	16	8	1	1	2
CUMC	NR5817	Acinetobacter sp.	Stock	8	16	8	≤0.4	≤0.5	1
Mayo	91	Enterobacter spp.	Stock	8	4	4	4	≤0.5	2

Precision Summary (CA only evaluated)

Organism Group	CBDE	CAT 1 uL	CAT 10 uL
Enterobacteriaceae	96% agreement	100%	100%
<i>P. aeruginosa</i>	100%	97.6%	100%
<i>Acinetobacter</i> spp.	100%	90.4%	96.8%

1 laboratory performed agar screen x 3 media lots (A-C) for all isolates in parallel (n=190 tests). This data is for 10uL

	Same MIC	EA	CA
A vs B	92.60%	99.50%	99.50%
A vs C	93.10%	100%	100%
B vs C	93.60%	100%	100%

Vote: 6 Yes, 0 No, 1 abstain

Summary of Testing Methods

Enterobacteriaceae

Method	CA	VME	ME	Notes	Recommendation
CBDE	98.6%	2.6%	0%	VME but in EA for 4/20 <i>mcr-1</i> tests	Approve Method
1 uL CAT	99.4%	1.0%	0%		Don't approve (easier to read 10 uL)
10 uL CAT	99.7%	0.5%	0%	Felt 10 uL loop easier to read	Approve Method

P. aeruginosa

Method	CA	VME	ME	Notes	Recommendation
CBDE	98.6%	0%	0.7%		Approve Method
1 uL CAT	98.6%	8.3%	0%	1 VME only, no growth on repeat, 10 uL ok	Don't approve (easier to read 10 uL)
10 uL CAT	100%	0%	0%		Approve Method

Summary of Methods (2)

Acinetobacter spp.

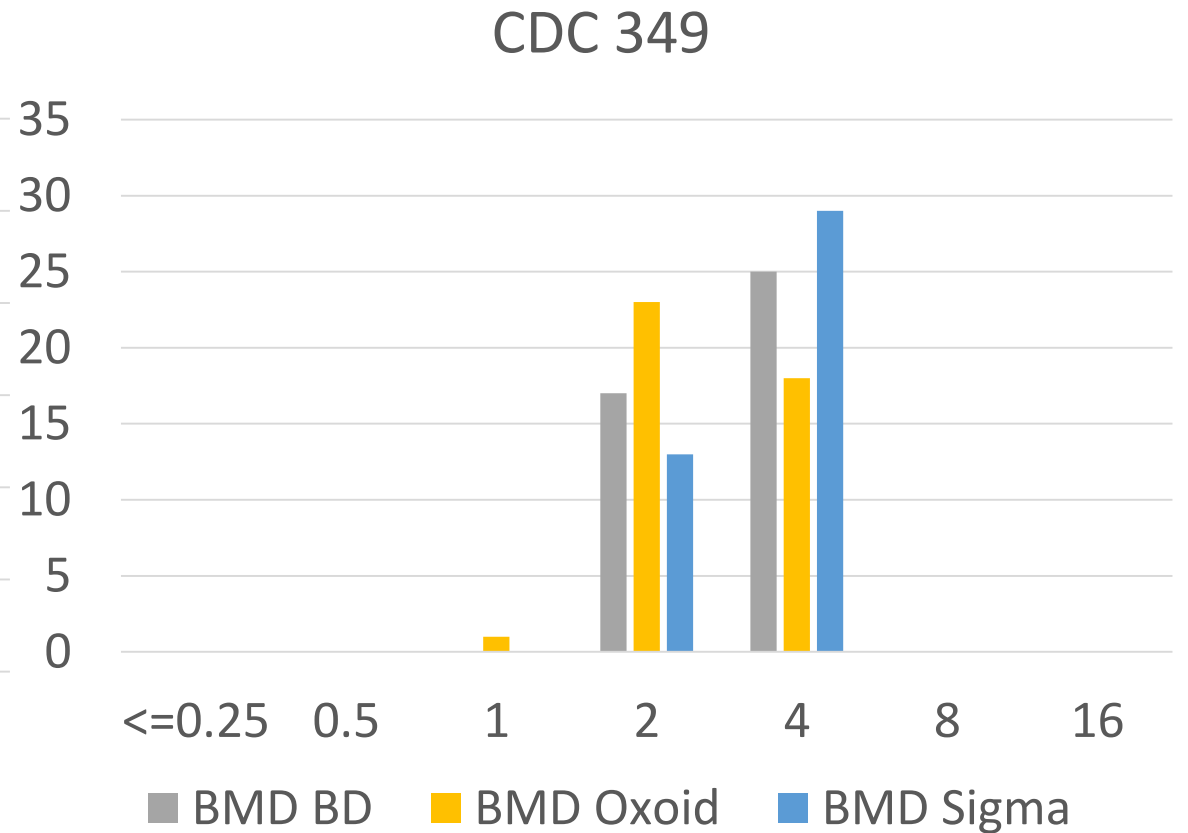
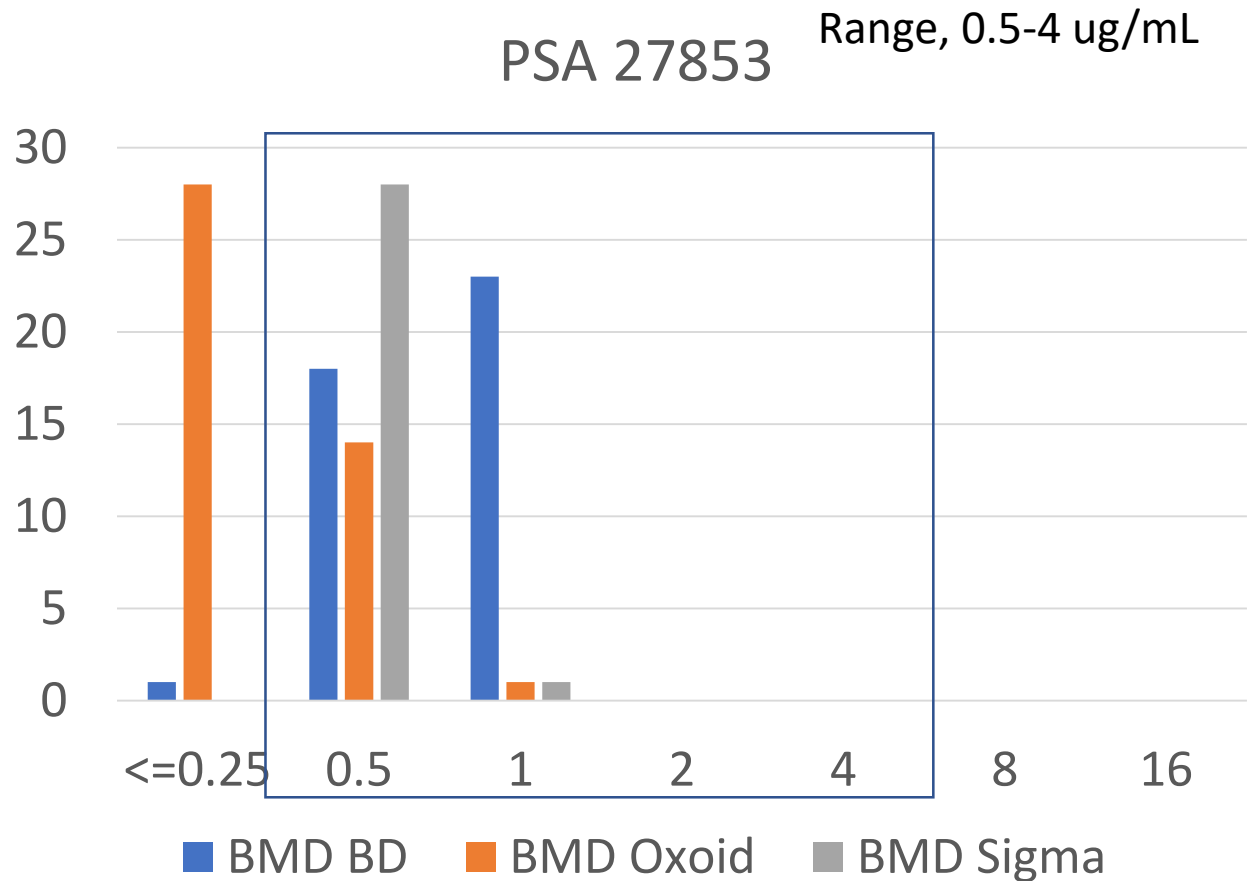
Method	CA	VME	ME	Recommendation
CBDE	95.4%	5.7%	3.3%	Do not approve
1 uL CAT	88.5%	21.4%	0%	Do not approve
10 uL CAT	92.3%	14.3%	0%	Do not approve

No methods were particularly good for *Acinetobacter spp.*
Even BMD vs. BMD was not very good...

QC Testing

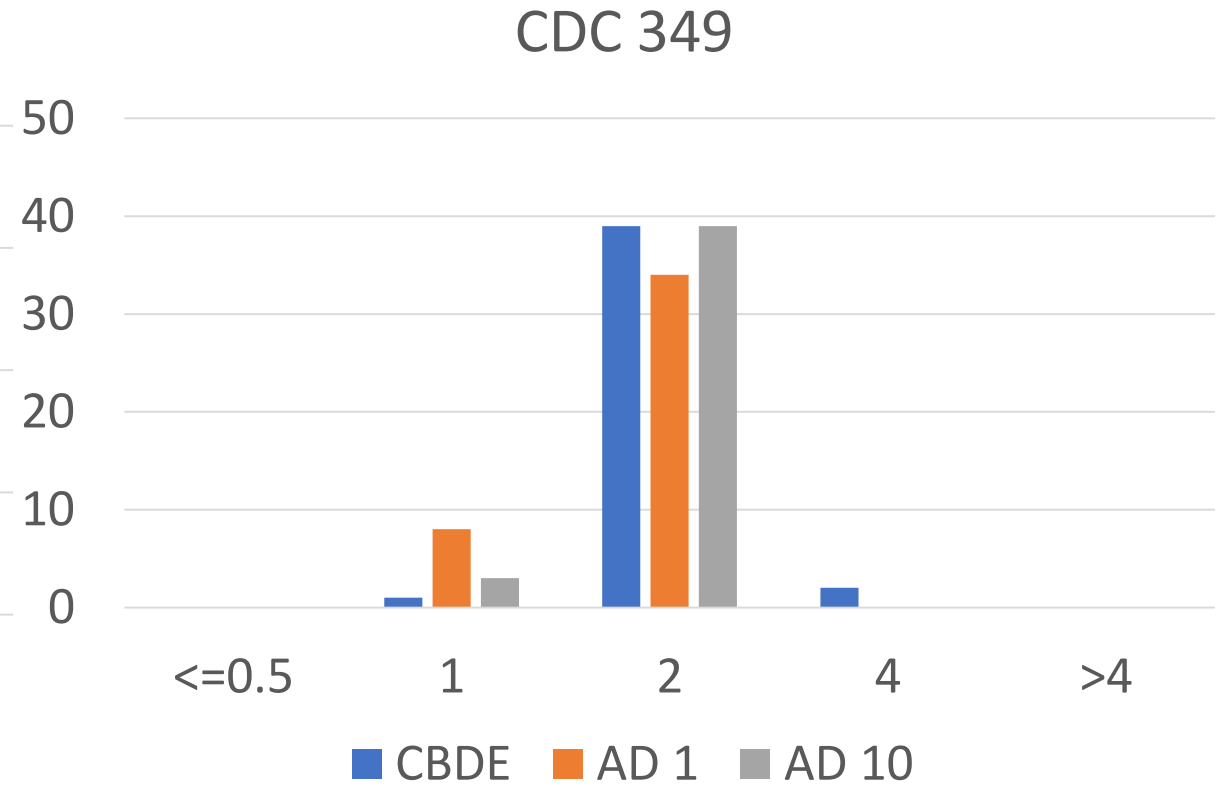
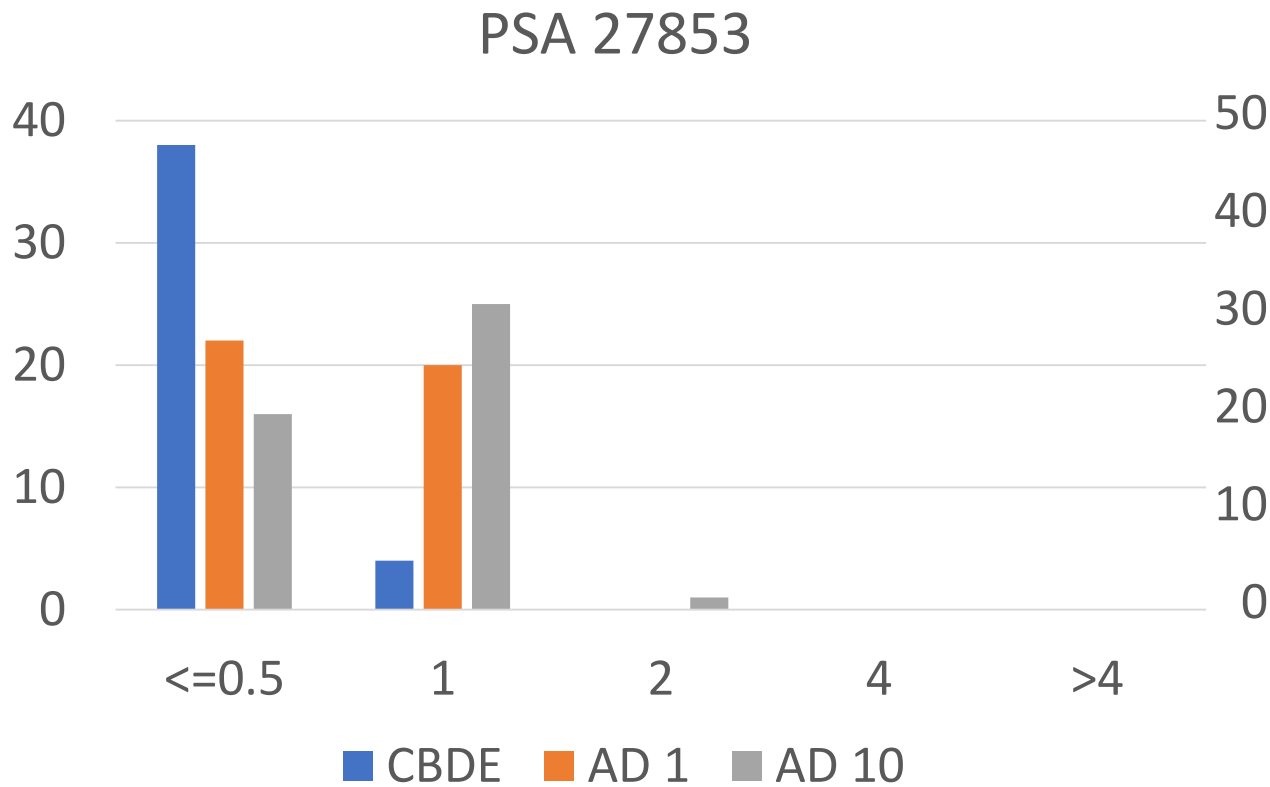
- Daily QC performed by each testing laboratory
- 13-15 results per isolate per laboratory

QC: BMD



Note: Oxoid BMD results were not included in the study

QC: Alternate Methods



Acceptable range, 0.5 – 4 (DOES NOT QC)