

Colistin antimicrobial susceptibility testing - evaluating available methods

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**Colistin/Polymyxin B
susceptibility testing
is difficult !**

**...and the literature is difficult to
assess**

Because of issues with "binding of polymyxins to plastic" and "synergy between polymyxins and polysorbate", we have studies from three eras:

- **BEFORE:** regular BMD trays (no surfactants), variable plastic quality
- **DURING:** BMD trays with surfactant (a period of 3 – 5 years 2010 –15)
- **AFTER:** regular BMD polystyrene trays without surfactants
- post-EUCAST/CLSI subcommittee report (-16)

Recommendations for MIC determination of colistin (polymyxin E) As recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group

Colistin (polymyxin E) MIC determination is associated by several methodological issues. The issues have been extensively investigated by the CLSI-EUCAST joint Polymyxin Breakpoints Working Group and the following method for determination of colistin MIC was agreed:

1. Reference testing of Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter* spp. is by the ISO-standard broth microdilution method (20776-1). Note:
 - a. Cation-adjusted Mueller-Hinton Broth is used
 - b. No additives may be included in any part of the testing process (in particular, no polysorbate-80 or other surfactants)
 - c. Trays must be made of plain polystyrene and not treated in any way before use
 - d. Sulphate salts of polymyxins must be used (the methanesulfonate derivative of colistin must not be used - it is an inactive pro-drug that breaks down slowly in solution)

2. Susceptibility testing by other methods, including agar dilution, disk diffusion and gradient diffusion, cannot be recommended until historical data have been reviewed or new study data have been generated. Work on these methods is ongoing.

Colistin breakpoints

EUCAST breakpoints	S \leq	R $>$	ECOFF
<i>E. coli, K. pneumoniae</i>	2	2	2
<i>Pseudomonas aeruginosa</i> *	2	2	4
<i>Acinetobacter baumannii</i> *	2	2	2

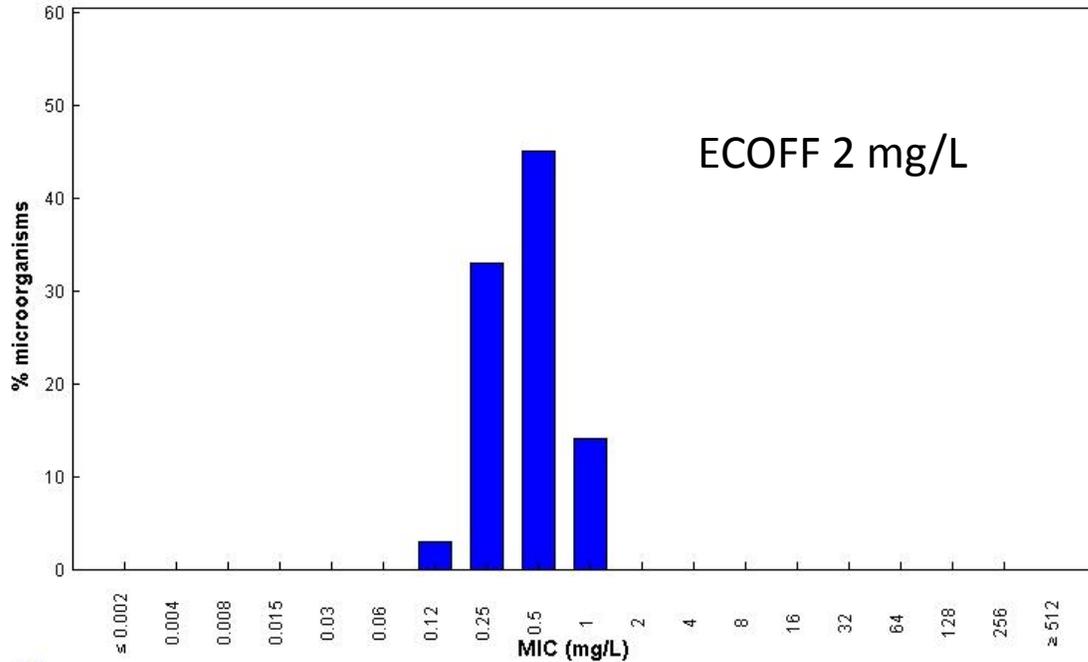
*CLSI breakpoints for *Pseudomonas* and *Acinetobacter*.

There is no INTERMEDIATE category for colistin in either EUCAST or CLSI. Thus all errors will be major or very major.

Colistin Wild Type MIC distributions

Colistin / *Escherichia coli*
International MIC Distribution - Reference Database 2018-03-31

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



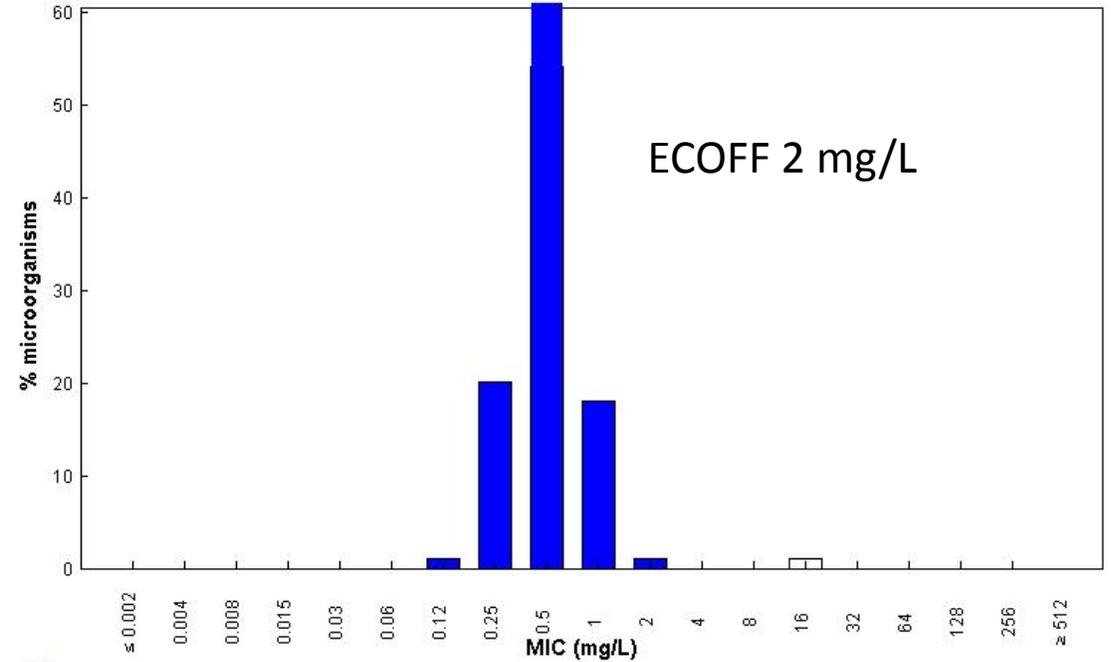
MIC
Epidemiological cut-off (ECOFF): 2 mg/L
Wildtype (WT) organisms: ≤ 2 mg/L

6064 observations (14 data sources)

E. coli; n=6064/14

Colistin / *Klebsiella pneumoniae*
International MIC Distribution - Reference Database 2018-03-31

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): 2 mg/L
Wildtype (WT) organisms: ≤ 2 mg/L

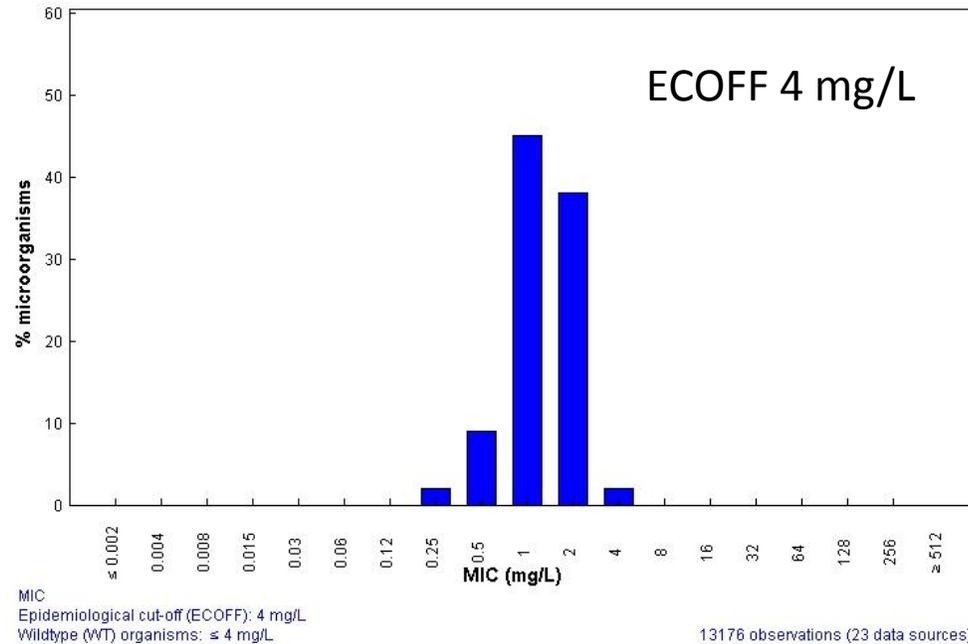
2237 observations (10 data sources)

K. pneumoniae; n=2237/10

Evaluations of Colistin AST methods

Colistin / *Pseudomonas aeruginosa*
International MIC Distribution - Reference Database 2018-03-31

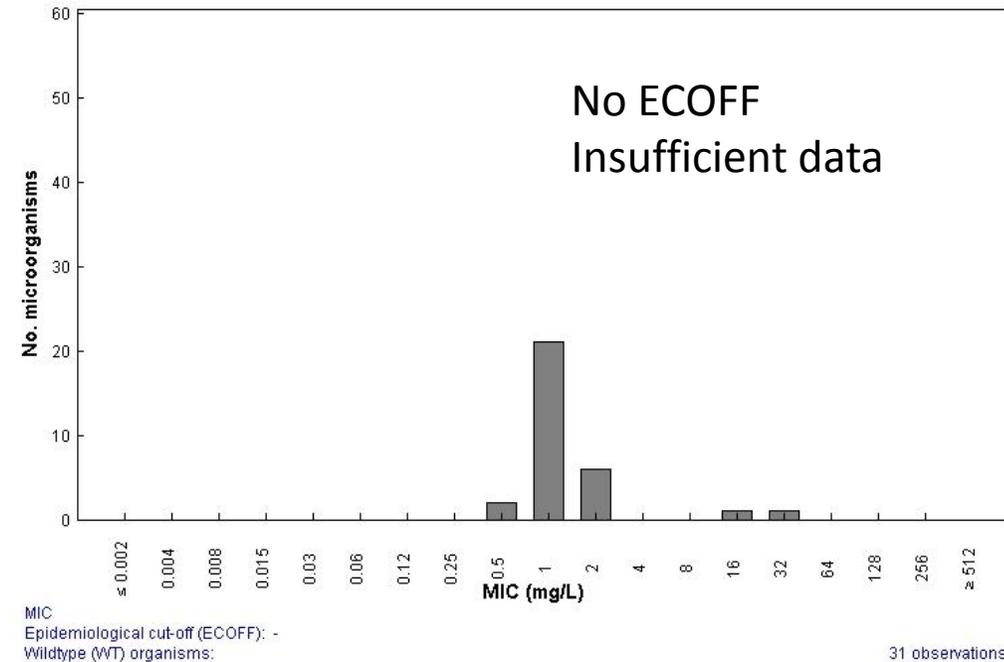
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



Ps. aeruginosa; n=13176/23

Colistin / *Acinetobacter baumannii*
International MIC Distribution - Reference Database 2018-04-20

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



A.baumannii – data lacking (31/1)

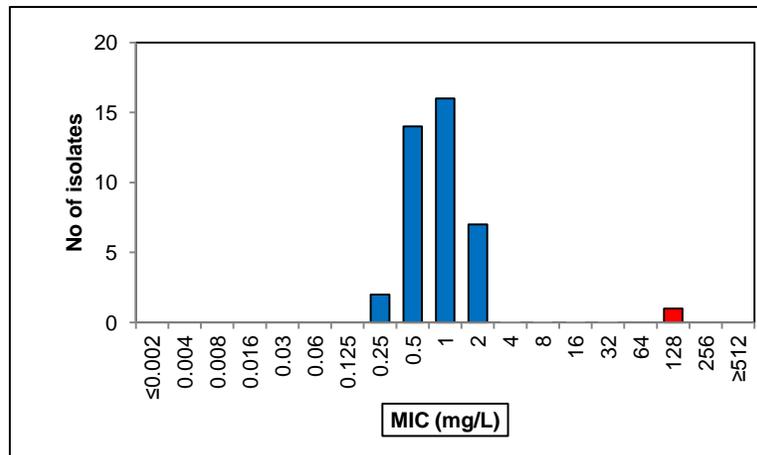
Phenotypic AST

- **broth microdilution**
 - **Reference – frozen panels and cation adjusted MH media**
 - Freeze-dried panels: many **commercially available** tests (but gradual manufacturing changes).
- **agar dilution**
 - For AST but also affects selective screening agars
- **gradient tests** (Etest, MTS).
- **disk diffusion.**
- **semi-automated devices** (Vitek2, Phoenix, MicroScan) with different panels/cards and (often short) concentration ranges;
- the recent **NP tests.**

Evaluations are affected by the choice of isolates

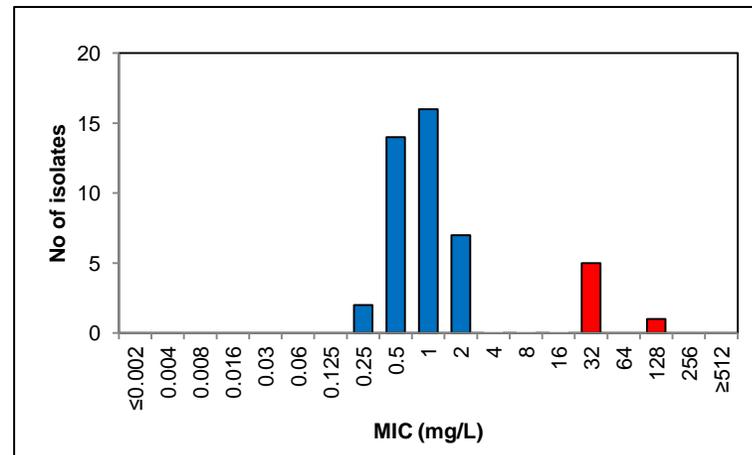
E.coli, *K.pneumoniae*, *Ps.aeruginosa* and *Acinetobacter baumannii* ...

Many wild type, no LLR



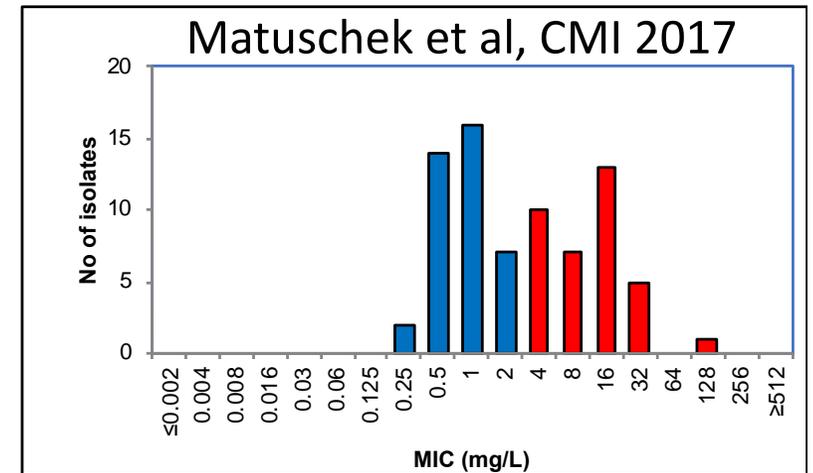
Easy
– no challenge

Many wild type, few R mainly HLR



Medium difficult
– little challenge

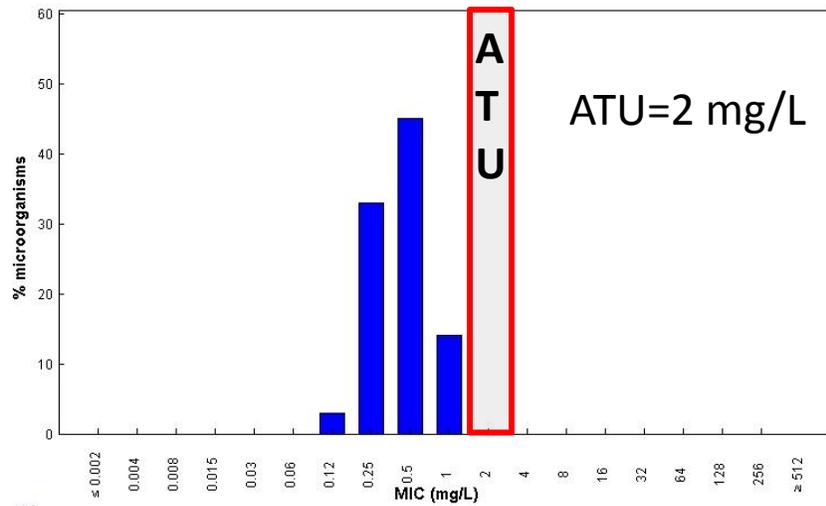
Mixture of WT, LLR & HLR



Difficult
– great challenge

Colistin / Escherichia coli
International MIC Distribution - Reference Database 2018-03-31

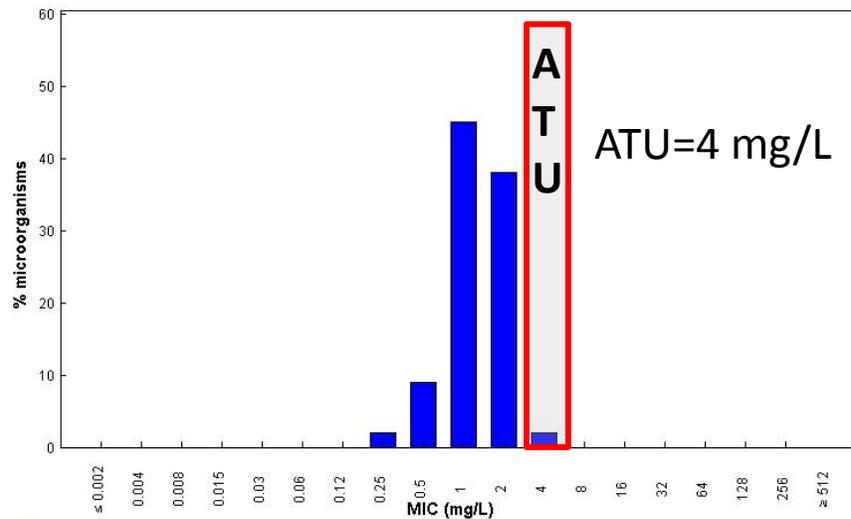
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): 2 mg/L
Wildtype (WT) organisms: ≤ 2 mg/L
6064 observations (14 data sources)

Colistin / Pseudomonas aeruginosa
International MIC Distribution - Reference Database 2018-03-31

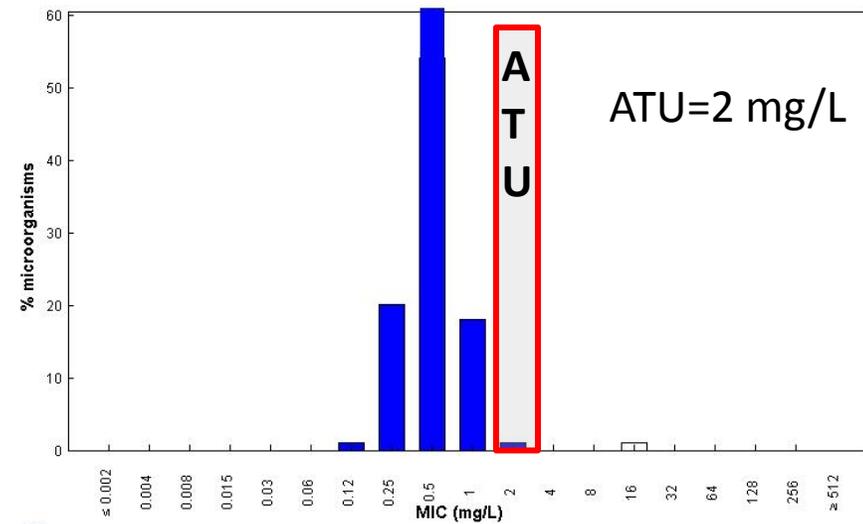
MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): 4 mg/L
Wildtype (WT) organisms: ≤ 4 mg/L
13176 observations (23 data sources)

Colistin / Klebsiella pneumoniae
International MIC Distribution - Reference Database 2018-03-31

MIC distributions include collated data from multiple sources, geographical areas and time periods and can never be used to infer rates of resistance



MIC
Epidemiological cut-off (ECOFF): 2 mg/L
Wildtype (WT) organisms: ≤ 2 mg/L
2237 observations (10 data sources)

The EUCAST solution - pending a final decision 2018

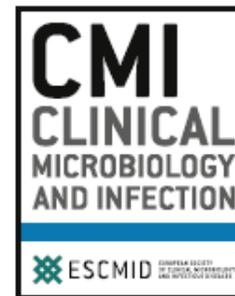


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Original article

Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.

E. Matuschek*, J. Åhman, C. Webster, G. Kahlmeter

EUCAST Development Laboratory, Växjö, Sweden

EUCAST evaluation of colistin MIC methods

- **Reference method (BMD)**
 - According to ISO 20776-1 and EUCAST/CLSI recommendations
 - Reference: Frozen panels, full dilutions, no truncation
- **BMD commercially available tests** - freeze-dried panels from:
 - Commercial Sensititre™ (custom plate, Thermo Fisher Scientific)
 - MICRONAUT-S™, MICRONAUT MIC Strip™ (Merlin)
 - ComASP™ (**previously SensiTest**), Liofilchem
 - UMIC™ (Biocentric)
- **Gradient tests**
 - Etest™ (bioMérieux)
 - Mueller-Hinton (MH) agar: Oxoid, BBL and MHE
 - MIC Test Strip™ (Liofilchem)
 - Mueller-Hinton (MH) agar: Oxoid and BBL
- **Disk diffusion** (several disk strengths)

BMD commercial products

Sensititre™ custom plate
(Thermo Fisher Scientific)



ComASP™ (**previously SensiTest**) (Liofilchem)

- four tests per panel
- broth included in kit



MICRONAUT-S™
(Merlin) –one isolate per row



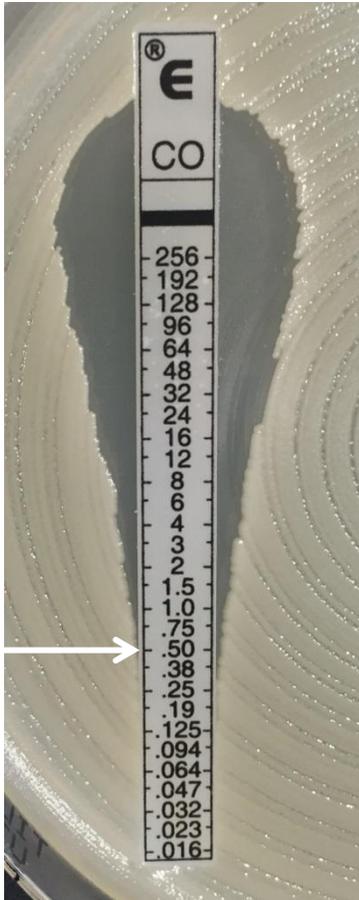
MICRONAUT MIC-Strip™
(Merlin) –single strips



UMIC™
(Biocentric) –single strips



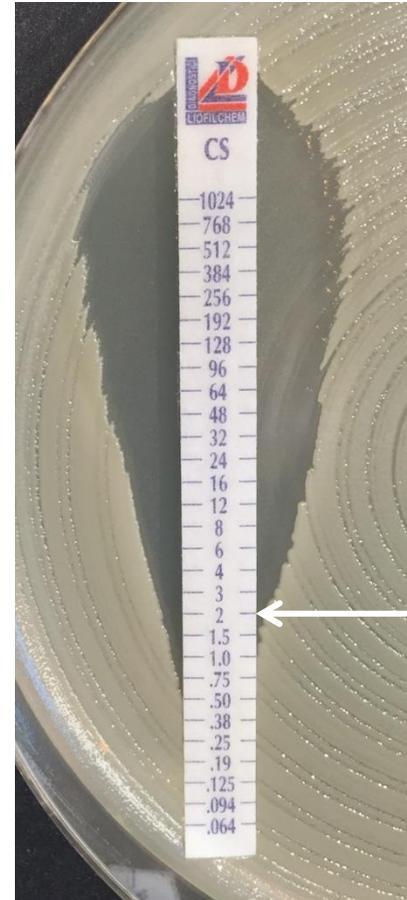
Typical gradient test result



Etest™

Often very narrow ellipse with a dip

MICs were read at the bottom of the ellipse according to the manufacturer's instructions



MTS™

Often 0.5-1 dilution higher on the right side of the strip

MICs should be read at the higher value according to the manufacturer's instructions

Antimicrobial susceptibility testing of colistin - evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.

Matuschek E¹, Åhman J², Webster C², Kahlmeter G².

Table 1

Colistin MIC distributions with reference broth microdilution for 75 Gram-negative bacterial isolates

Organism	Number of isolates	Colistin reference MIC (mg/L)									
		0.25	0.5	1	2	4	8	16	32	64	128
<i>Escherichia coli</i>	14	1	3	1	8		1				
<i>Klebsiella pneumoniae</i>	18		4	2	2		4	4	2		
<i>Pseudomonas aeruginosa</i>	21	1	2	7	2	2	2	3	1		1
<i>Acinetobacter</i> spp.	22		5	6	3			6	2		
Total	75	2	14	16	7	10	7	13	5	0	1

Examples of results for BMD and gradient tests

Commercial BMD (freeze-dried panel)

	Colistin reference MIC (mg/L)										
	0.25	0.5	1	2	4	8	16	32	64	128	
0.125											
0.25	1	1									
0.5	1	10	1								
1		2	7								
2		1	8	1	2						
4				4	5						
8				2	3	5	4				
16					2	6	1				
32						3	2				
64							2			1	
128											

Gradient test

	Colistin reference MIC (mg/L)										
	0.25	0.5	1	2	4	8	16	32	64	128	
0.125	1	12	4				1				
0.25		2	2	2	1		1				
0.5			7	3			1				
1				3							
2					1	2	1				1
4				2	8	3	5				
8						1	3	3			
16						1	1	1			
32							1				
64											
128											

- MIC 1 dilution below reference
- MIC identical to reference
- MIC 1 dilution above reference

Essential agreement (reference \pm 1 dilution) = green + yellow highlights **Categorical errors** = within red boxes

Agar dilution vs. commercial BMD products

MIC method	Manufacturer	Essential agreement (%)	Categorical agreement (%)	Categorical errors (No, of 75)	
				ME ^A	VME ^B
Agar dilution standard inoculum	in-house	73	85	2	9
Agar dilution high inoculum	in-house	80	88	3	6
Sensititre custom plate (BMD)	Thermo Fisher Scientific	96	95	4	0
MICRONAUT-S (BMD)	MERLIN Diagnostika	96	89	6	2
MICRONAUT MIC-Strip (BMD)	MERLIN Diagnostika	99	91	5	2
SensiTest (BMD)	Liofilchem	88	89	7	1
UMIC (BMD)	Biocentric	82	92	3	3

^A ME (Major Error) = False resistant

^B VME (Very Major Error) = False susceptible

Phenotypic AST - conclusion

- Broth microdilution
- Agar dilution
- Disk diffusion
- Gradient tests
- Semiautomated AST devices (MicroScan, Phoenix, Vitek2)

- Broth microdilution
- ~~Agar dilution~~
- ~~Disk diffusion~~
- ~~Gradient tests~~
- ~~Semiautomated AST devices (MicroScan, Phoenix, Vitek2)~~
Many studies report failures but the reports in the literature exhibit scattered results and error rates

New phenotypic methods (microcalorimetry, flowcytometri, masspec, computerassisted microscopy, **NP-tests**, etc)
Genotypic methods – targeting single defined resistance genes or through sequencing.
Accelerate – research only.

Thank you!