

# Methods for colistin testing

## What works and what does not?

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# Antimicrobial susceptibility testing of colistin

- Broth microdilution (BMD)
  - International reference method (ISO 20776-1)
- Agar dilution?
- Gradient tests?
  - Etest, bioMérieux
  - MIC Test Strip (MTS), Liofilchem
- Disk diffusion??

# AST of colistin – dilution methods

- BMD (reference)

- Sulphate salts

- Standard polystyrene trays

- No additives or pre-treatment of plates

- In-house prepared or commercial plates

- Agar dilution

- To be evaluated

See EUCAST Guidance Documents  
[www.eucast.org/guidance\\_documents/](http://www.eucast.org/guidance_documents/)

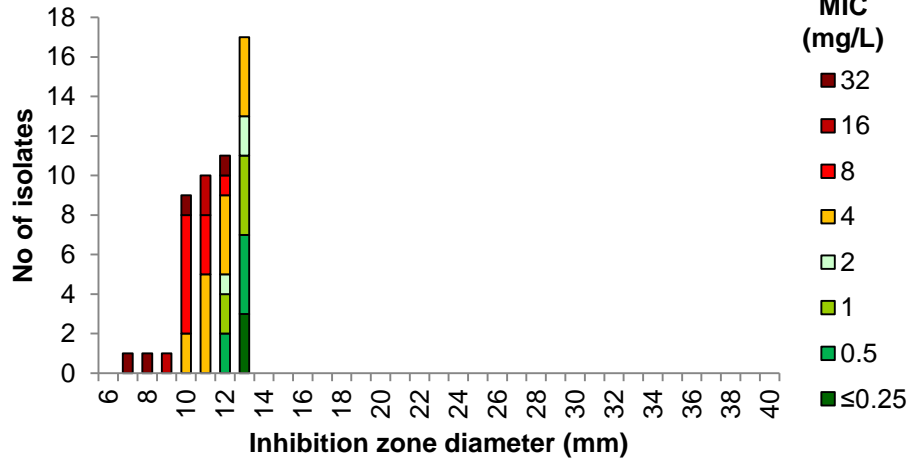
# AST of colistin – diffusion methods

- Gradient tests?
  - Poor correlation with reference BMD
  - Warning on [www.eucast.org](http://www.eucast.org)
- Disk diffusion?
  - Poor separation between resistant and susceptible isolates
- Problems probably related to poor diffusion of colistin in agar

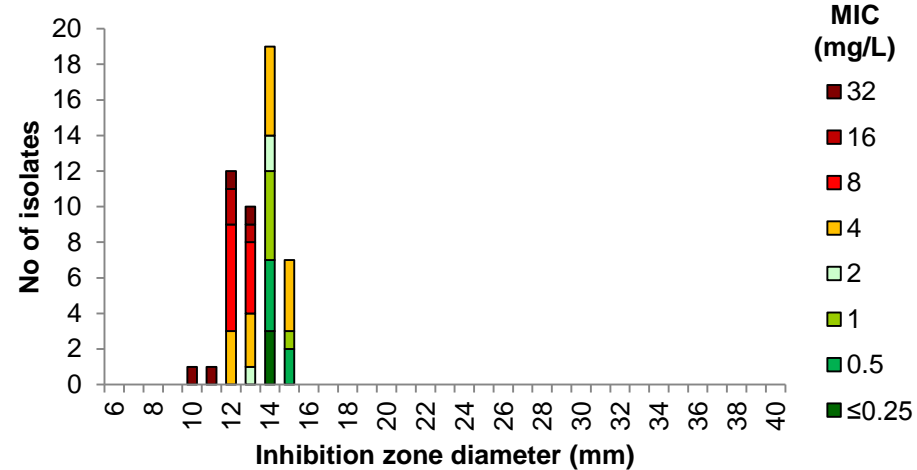
# Results colistin disk diffusion

## Enterobacteriaceae, 50 isolates

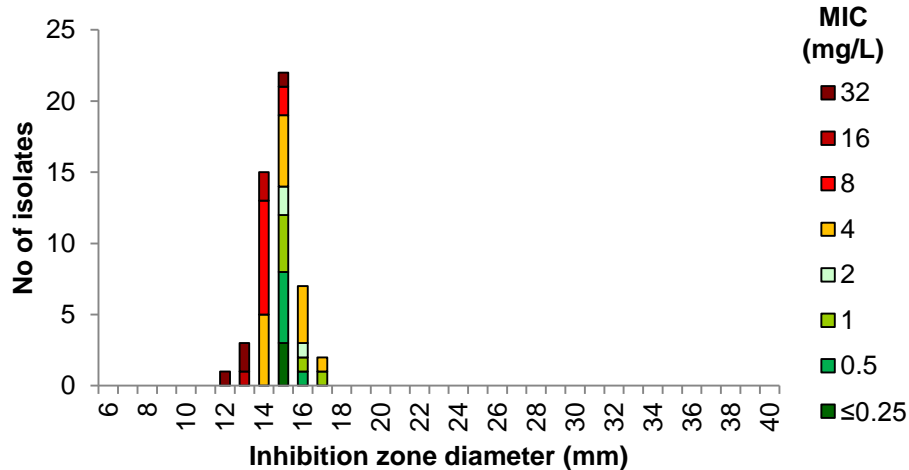
### Colistin 10 µg



### Colistin 25 µg



### Colistin 50 µg



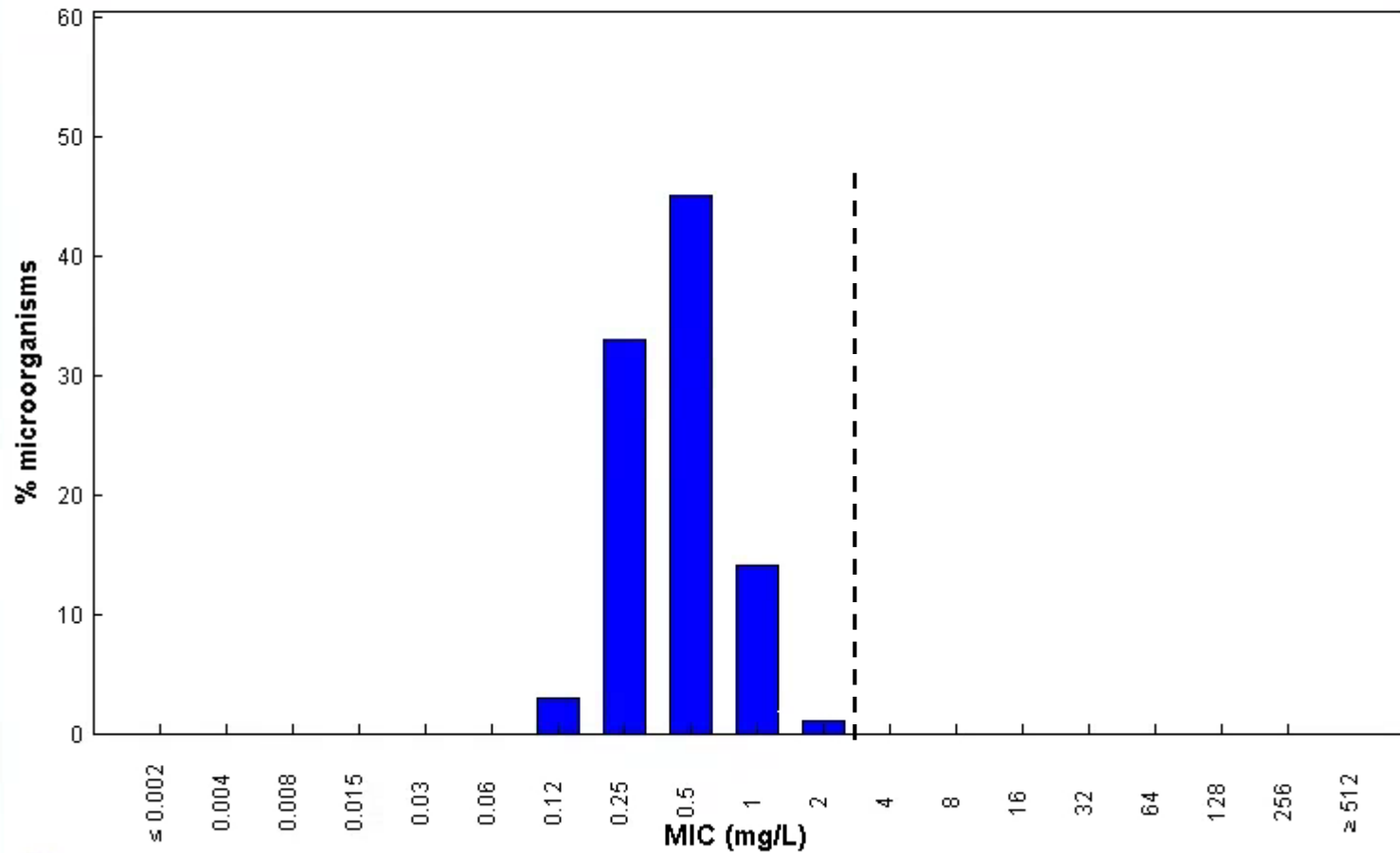
# Colistin MIC distributions

[http://www.eucast.org/mic\\_distributions\\_and\\_ecoffs/](http://www.eucast.org/mic_distributions_and_ecoffs/)

- All distributions are recently reviewed
  - Old distributions with P-80 (tween) removed
  - Only non-truncated distributions included
- ECOFFs established for *E. coli*, *K. pneumoniae* and *P. aeruginosa*
  - More data are needed for Acinetobacter and Salmonella
- Clinical breakpoints:  $S \leq 2$ ,  $R > 2$  mg/L
  - Enterobacteriaceae, *Pseudomonas* spp. and *Acinetobacter* spp.

**Colistin / Escherichia coli**  
**International MIC Distribution - Reference Database 2017-04-03**

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



MIC

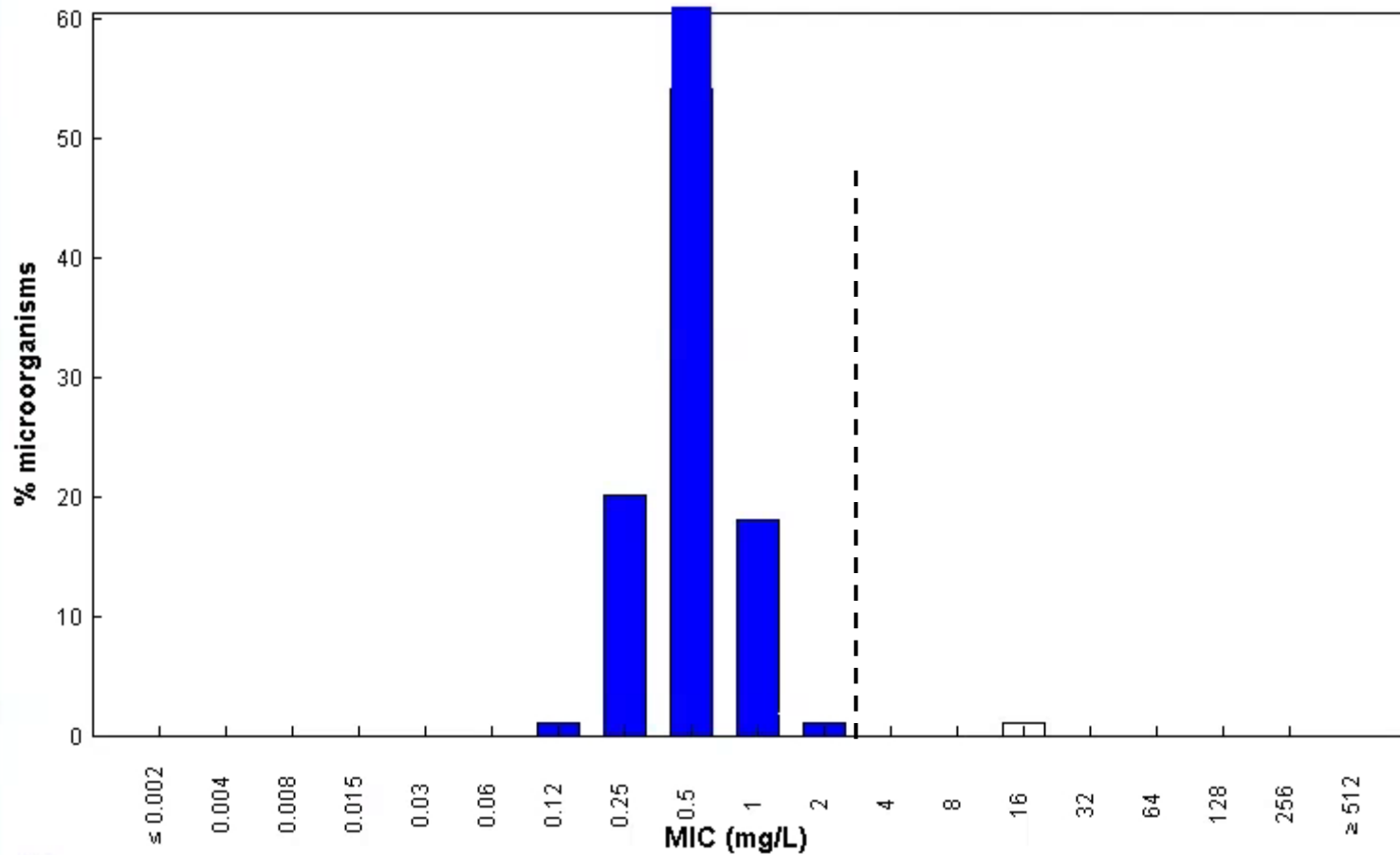
Epidemiological cut-off (ECOFF): 2 mg/L

Wildtype (WT) organisms: ≤ 2 mg/L

6090 observations (15 data sources)

**Colistin / *Klebsiella pneumoniae***  
**International MIC Distribution - Reference Database 2017-04-03**

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

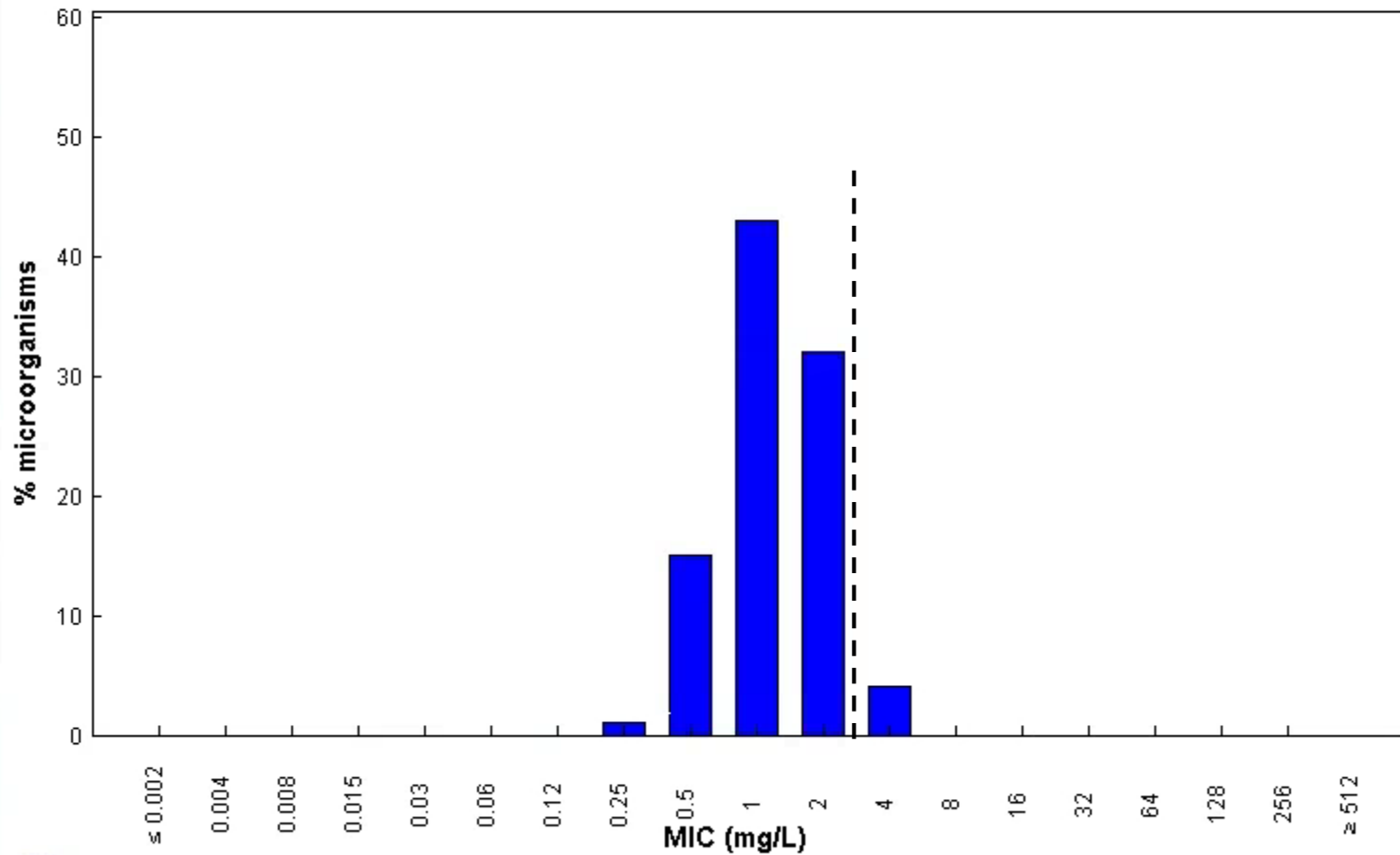
Wildtype (WT) organisms:  $\leq 2$  mg/L

2237 observations (10 data sources)



**Colistin / *Pseudomonas aeruginosa***  
**International MIC Distribution - Reference Database 2017-04-03**

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



MIC

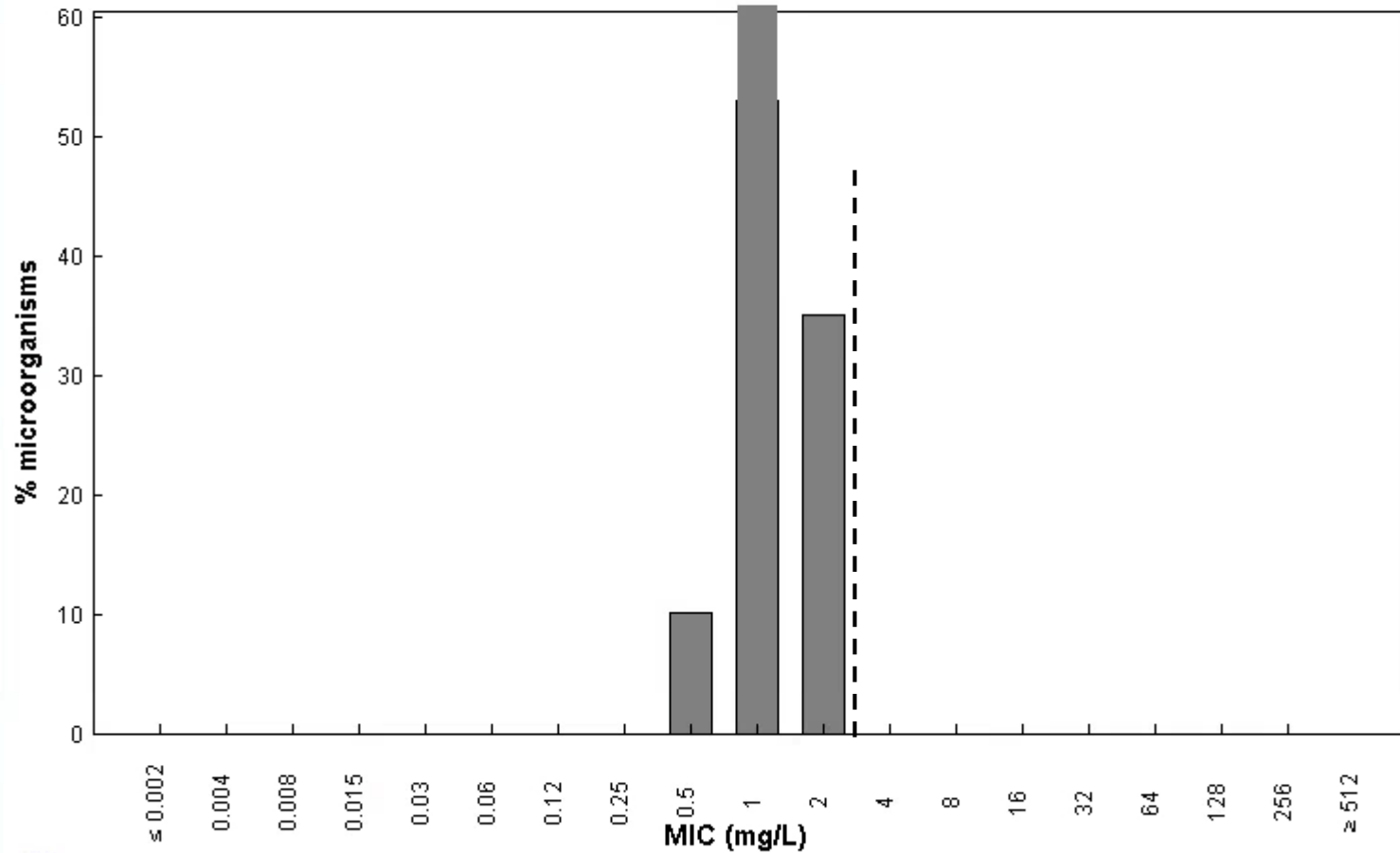
Epidemiological cut-off (ECOFF): 4 mg/L

Wildtype (WT) organisms:  $\leq 4$  mg/L

6579 observations (19 data sources)

**Colistin / *Acinetobacter baumannii***  
**International MIC Distribution - Reference Database 2017-04-03**

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



MIC

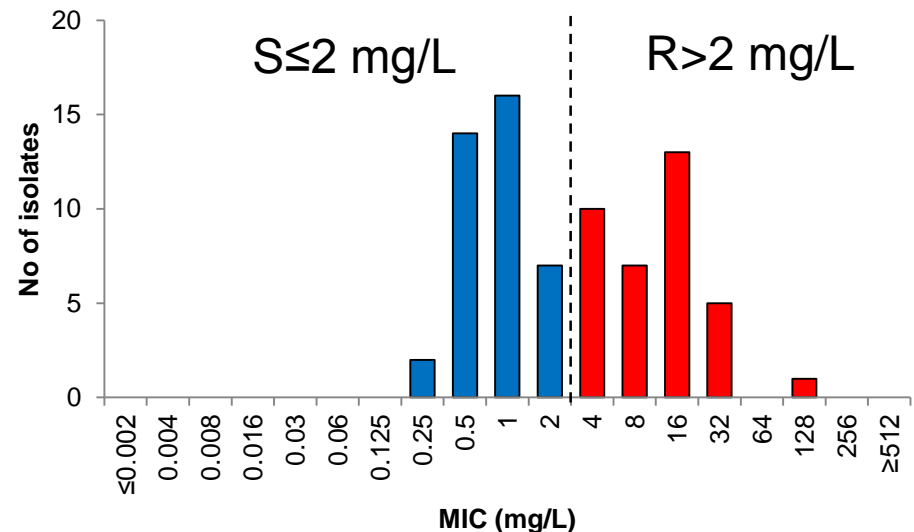
Epidemiological cut-off (ECOFF): -

Wildtype (WT) organisms:

306 observations (2 data sources)

# EUCAST evaluation of colistin MIC methods

- 75 Gram-negative bacteria with varying colistin MICs (0.25-128 mg/L)
  - 14 *E. coli*
    - of which 10 *mcr-1* positive
  - 18 *K. pneumoniae*
    - of which 1 *mcr-1* positive
  - 21 *P. aeruginosa*
  - 22 *Acinetobacter* spp.



# EUCAST evaluation of colistin MIC methods

- BMD
  - According to ISO 20776-1 and EUCAST/CLSI recommendations
  - Frozen panels as reference
  - Commercial freeze-dried panels
    - Sensititre, MICRONAUT-S, MICRONAUT MIC Strip
- Gradient tests
  - Etest
    - Mueller-Hinton (MH) agar: Oxoid, BBL and MHE
  - MIC Test Strip (MTS)
    - Mueller-Hinton (MH) agar: Oxoid and BBL



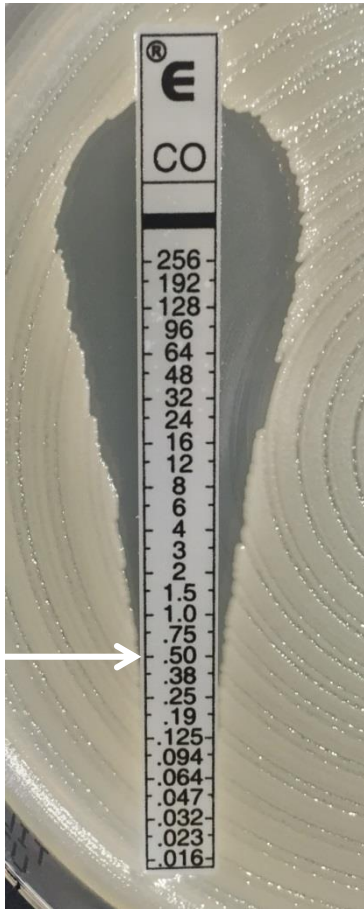
Manual reading  
of MICs

96 well microtiter  
plate



MICRONAUT  
MIC Strip

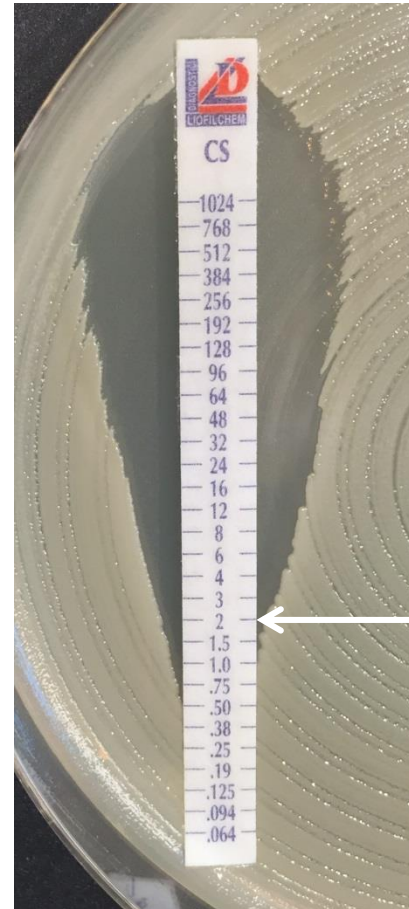
# Typical gradient test result



## 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 were read at the higher value according to the manufacturer's instructions

# Results

Essential agreement for colistin MIC tests (no of MICs being within  $\pm 1$  dilution of reference MIC).

	Organism	<i>E. coli</i> and <i>K. pneumoniae</i> (n=32)	<i>P. aeruginosa</i> (n=21)	<i>Acinetobacter</i> spp. (n=22)	All isolates (n=75)
	Colistin MIC range	0.25-32	0.25-128	0.5-32	0.25-128
Essential agreement (EA)	Sensititre custom plate	27	19	20	66 (96%)
	MICRONAUT-S	31	21	20	72 (96%)
	MICRONAUT MIC-Strip	31	21	22	74 (99%)
	Etest, Oxoid MH	27	13	13	53 (71%)
	Etest, BBL MH	20	11	1	32 (43%)
	Etest, MHE	24	9	2	35 (47%)
	MTS, Oxoid MH	19	12	9	40 (53%)
	MTS, BBL MH	24	12	13	49 (65%)

\* The total number of tests for calculation of EA was 28 for *E. coli*/*K. pneumoniae* and 19 for *P. aeruginosa* due to truncation at  $\leq 0.25$  and  $> 32$  mg/L.

## Essential agreement

BMD: 96-99%

Gradient tests: 43-71% (depending on MH agar used)

# Results

## Categorical agreement for colistin MIC tests

	Organism	<i>E. coli</i> and <i>K. pneumoniae</i> (n=32)	<i>P. aeruginosa</i> (n=21)	<i>Acinetobacter</i> spp. (n=22)	All isolates (n=75)
	Colistin MIC range (mg/L)	0.25-32	0.25-128	0.5-32	0.25-128
Major Errors (ME)	Sensitre custom plate	1	1	2	4
	MICRONAUT-S	2	1	3	6
	MICRONAUT MIC-Strip	2	0	3	5
	Etest, Oxoid MH	2	0	0	2
	Etest, BBL MH	1	0	0	1
	Etest, MHE	2	0	0	2
	MTS, Oxoid MH	0	0	0	0
	MTS, BBL MH	0	0	0	0
Very Major Errors (VME)	Sensitre custom plate	0	0	0	0
	MICRONAUT-S	0	2	0	2
	MICRONAUT MIC-Strip	0	2	0	2
	Etest, Oxoid MH	0	6	6	12
	Etest, BBL MH	1	7	7	15
	Etest, MHE	0	5	4	9
	MTS, Oxoid MH	6	6	4	16
MTS, BBL MH	5	6	7	18	



# Results

## Quality Control (QC) strains

Colistin MIC method	Colistin MIC (mg/L)												
	<i>Escherichia coli</i> ATCC 25922					<i>Pseudomonas aeruginosa</i> ATCC 27853					<i>Escherichia coli</i> NCTC 13846**		
	0.125	0.25	0.5	1	2	0.25	0.5	1	2	4	2	4	8
Reference frozen panel			7	1				8			1	7	
Sensititre custom plate		4*	4				1	7				8	
MICRONAUT-S		5	3					4	4			7	1
MICRONAUT MIC-Strip	1	6	1					8			2	6	
Etest, Oxoid MH		2	5					7				8	
Etest, BBL MH	12						4	8				8	
Etest, MHE	7					3	4				5	3	
MTS, Oxoid MH				6	1			2	5			8	
MTS, BBL MH			1	6				4	3			8	

\* ≤0.25 mg/L

\*\* *mcr-1* positive

QC target value

Upper/lower limit

Red text = MIC out of range

**EUCAST QC table v 7.0:** "For *E. coli* NCTC 13846, the colistin MIC target value is 4 mg/L and should only on occasion be 2 or 8 mg/L."







# Conclusions

- Correlation with reference MICs was good for all BMD methods.
- Gradient tests generally underestimated colistin MICs resulting in very major errors (false susceptibility).
- The poor performance of gradient tests could not be detected with QC strains.

# Warning on [www.eucast.org](http://www.eucast.org)

- Disk diffusion cannot be used for AST of colistin.
- Currently available gradient tests underestimate colistin MIC values (and resistance) and should be avoided.
- Use BMD for AST of colistin, but perform extended quality control.

# EUCAST recommendations for colistin QC

- Use both a susceptible strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) AND the colistin resistant *E. coli* NCTC 13846 (*mcr-1* positive).
  - For *E. coli* NCTC 13846, the colistin MIC target value is 4 mg/L and should only on occasion be 2 or 8 mg/L.


# Further work

- Additional BMD methods will be evaluated
  - Colistin SensiTest (Liofilchem)
  - UMIC (Biocentric)



# Read more!

- Poster 161, ECCMID 2017 (available soon)
  - [www.eccmid.org](http://www.eccmid.org)
  - [http://www.eucast.org/presentations\\_and\\_statistics/eucast\\_at\\_eccmid/](http://www.eucast.org/presentations_and_statistics/eucast_at_eccmid/)
- <http://www.eucast.org/warnings/>

- Organization
- EUCAST News
- Clinical breakpoints
- Expert rules and intrinsic resistance
- Resistance mechanisms
- Guidance documents
- Consultations
- MIC distributions and ECOFFs
- Zone distributions and ECOFFs
- AST of bacteria
- AST of mycobacteria
- AST of fungi
- AST of veterinary pathogens
- Frequently Asked Questions (FAQ)
- Meetings
- Presentations and statistics
-  Warnings!
- Documents
- Videos from EUCAST
- Translations
- Information for industry
- Links
- Contacts



10 May 2016

## The European Committee on Antimicrobial Susceptibility Testing - EUCAST

EUCAST is a standing committee jointly organized by ESCMID, ECDC and European national breakpoint committees. EUCAST was formed in 1997. It has been chaired by Ian Phillips (1997 - 2001), Gunnar Kahlmeter (2001 - 2012), Rafael Canton 2012 - 2016) and Christian Giske (2016 - ). Its scientific secretary is Derek Brown (1997 - 2016) and John Turnidge (2016 - ). Its webmaster is Gunnar Kahlmeter (2001 - ). From 2016, Rafael Canton is the Clinical Data Co-ordinator and Gunnar Kahlmeter the Technical Data Co-ordinator.

EUCAST deals with breakpoints and technical aspects of phenotypic in vitro antimicrobial susceptibility testing and functions as the breakpoint committee of EMA and ECDC. EUCAST does not deal with antibiotic policies, surveillance or containment of resistance or infection control. The Steering Committee is the decision making body. It is supported by a General Committee with representatives from European and other countries, FESCI and ISC. The Steering Committee also consults on EUCAST proposals with experts within the fields of infectious diseases and microbiology, pharmaceutical companies and susceptibility testing device manufacturers.

EUCAST has several subcommittees - [see page Subcommittees](#).  
Most antimicrobial MIC breakpoints in Europe have been harmonised by EUCAST. Breakpoints for new agents are set as part of the licensing process for new agents through EMA. EUCAST breakpoints are available in devices for automated susceptibility testing but with some limitations, depending on the system. A disk diffusion susceptibility test method [calibrated](#) to EUCAST MIC breakpoints is also available.

EUCAST invites anyone with an interest in antimicrobial agents in general and antimicrobial breakpoints in particular to contact EUCAST, ESCMID or one of the

### EUCAST News

27 Mar 2017  
**EUCAST instruction videos in Czech**

15 Mar 2017  
**China joins EUCAST**

13 Mar 2017  
**Breakpoint table v 7.1 published**

13 Mar 2017  
**Coagulase negative staphylococci - MIC vs. zone diameters uploaded**

09 Mar 2017  
**Consultation (9 March - 14 May) on MIC distributions and ECOFFs**

[About Newsfeeds](#)





# EUCAST

EUROPEAN COMMITTEE  
ON ANTIMICROBIAL  
SUSCEPTIBILITY TESTING

European Society of Clinical Microbiology and Infectious Diseases

- Check the EUCAST website regularly for updates on methodology, QC ranges and breakpoints.

[www.eucast.org](http://www.eucast.org)

- For questions and comments, please contact [erika.matuschek@escmid.org](mailto:erika.matuschek@escmid.org) or the EUCAST secretariat (see website).