



# Colistin resistance - an update

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# Which treatment alternatives are available for XDR Gram-negatives?

Antibiotika	ESBL	AmpC	KPC	MBL	OXA-48
Meropenem	+	+	+-	+-	+-
Temocillin	+	+	+-	-	-
Ceftolozane -tazobactam	+	-	-	-	-
Ceftazidime -avibactam	+	+	+	-	+
<b>Colistin</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>	<b>+</b>

# Colistin use in human medicine

**Table 5.** Trends in consumption of polymyxins in EU/EEA countries, 2010–2014 (expressed in DDD per 1 000 inhabitants and per day)

Country	2010	2011	2012	2013	2014	Trends in consumption of polymyxins, 2010–2014	Average annual change 2010–2014	Statistical significance
Finland (b)	0	0	0	0	0			n.a.
Lithuania (a)			0	0	0			n.a.
Norway	0.0002	0.0004	0.0006	0.0006	0.0006		<0.001	significant
Poland (a)					0.001			n.a.
Latvia	0	0	0.003	0.002	0.001		<0.001	n.s.
Sweden	0.000	0.001	0.001	0.001	0.001		<0.001	n.s.
Netherlands	0.006	0.003	0.002	0.003	0.002		-0.001	n.s.
Bulgaria	0	0	0	0	0.002		<0.001	n.s.
Estonia	<0.001	<0.001	0.002	0	0.002		<0.001	n.s.
Denmark	0.002	0.002	0.002	0.001	0.003		<0.001	n.s.
Luxembourg	0.005	0.005	0.005	0.006	0.003		<0.001	n.s.
Slovenia	0.001	0.002	0.003	0.003	0.005		0.001	n.s.
United Kingdom (a)(d)				0.005	0.006			n.a.
Hungary	0.002	0.004	0.005	0.006	0.007		0.001	significant
France	0.008	0.008	0.008	0.008	0.008		<0.001	n.s.
Malta	0.026	0.004	0.002	0.006	0.011		0.003	n.s.
<b>EU/EEA</b>	<b>0.008</b>	<b>0.011</b>	<b>0.014</b>	<b>0.012</b>	<b>0.012</b>		<b>&lt;0.001</b>	<b>n.s.</b>
Ireland	0.014	0.014	0.015	0.015	0.013		<0.001	n.s.
Portugal (c)	0.013	0.018	0.019	0.020	0.019		0.001	n.s.
Croatia	0.055	0.010	0.029	0.003	0.019		0.008	n.s.
Slovakia (a)			0.020	0.023	0.025			n.a.
Italy	0.012	0.011	0.019	0.023	0.025		0.004	significant
Greece (a)		0.078	0.085	0.084	0.095			n.a.
Belgium	0.008	0.009	0.006	0.008				n.a.

- Still low in the EU/EEA
- 600 times lower than in veterinary medicine
- Nearly doubled between 2010 and 2014
- Significant increasing trends in several EU Member States
- Follows the rise of MDR Gram-negative HAI

The number for EU/EEA refers to the corresponding population-weighted mean consumption, calculated by summing the products of each country's consumption in DDD per 1 000 inhabitants and per day × country population as in Eurostat, and then dividing this sum by the total EU/EEA population.

(a) These countries did not report data for all years during the period 2010–2014.

(b) Finland: data include consumption in remote primary healthcare centres and nursing homes.

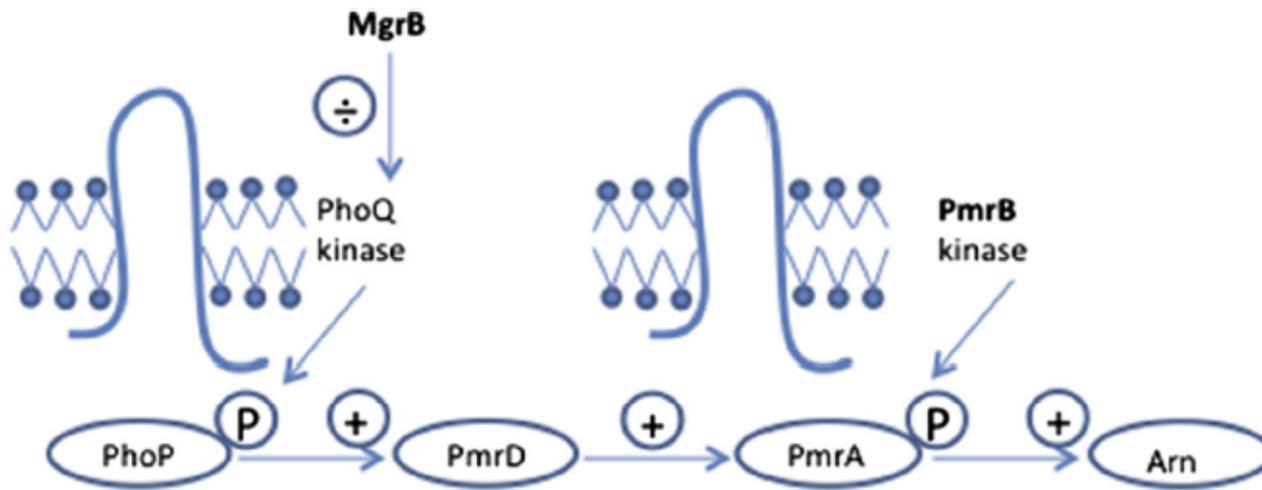
(c) Portugal: data relate to public hospitals only.

(d) United Kingdom: data do not include consumption from UK-Wales (2013) or UK-Northern Ireland (2014).

n.a.: not applicable; linear regression was not applied due to missing data.

n.s.: not significant.

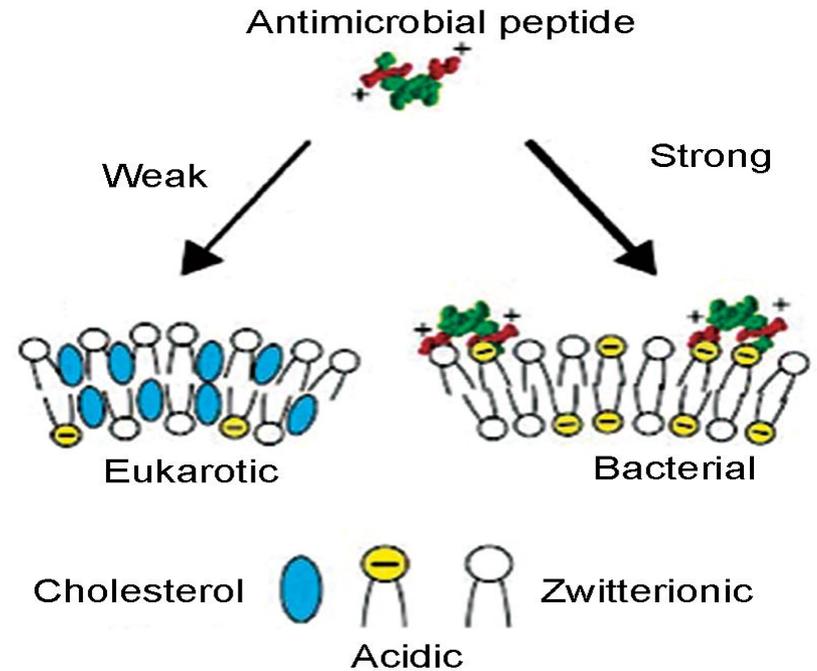
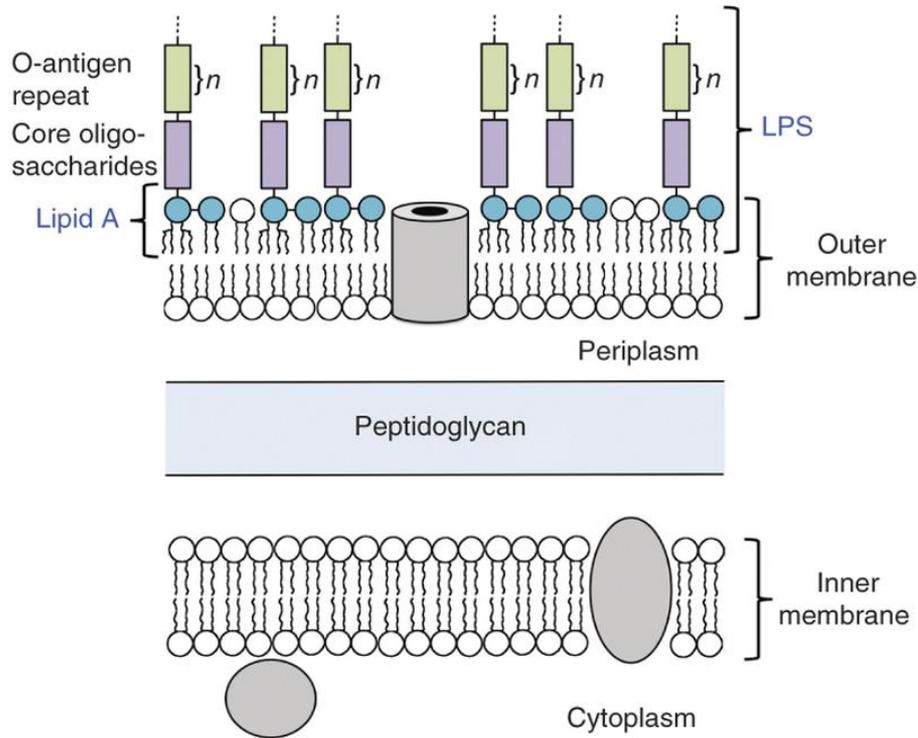
# Colistin resistance: mechanisms



**FIG. 1.** Two-component regulatory system for modification of charge in LPS of Gram-negative bacilli. Membrane-bound kinases affecting transcription of *arn* complex through intermediate PmrD are shown. Circled P denotes phosphorylation; circled minus sign denotes negative regulation of transcription; encircled plus sign denotes positive regulation. The two most common sites of mutation are shown in bold.

Giske CG. CMI 2015

# Consequences of *arn* transcription



Decreased negative charge in lipid A = weaker interaction with polymyxins

# Polymyxin R – EARS-Net 2015

- Twenty-one countries reported AST data for polymyxins for a total of 6 029 isolates (26.3% of all reported *K. pneumoniae* isolates) in 2015
- **Overall: 8.8% resistance in *K. pneumoniae***
- 95% of the isolates with combined carbapenem and polymyxin resistance were reported from Greece and Italy
- **Carbapenem-resistant isolates: 31.9% polymyxin resistant**
- Carbapenem-susceptible isolates: 2.6% polymyxin resistant
- **Majority: likely to be chromosomal resistance**

# The emergence of plasmid-mediated colistin resistance

Articles

## Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study



Yi-Yun Liu\*, Yang Wang\*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen

### Summary

**Background** Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance was observed. When an *E coli* strain, SHP45, possessing colistin resistance that could be transferred to another strain, was isolated from a pig, we conducted further analysis of possible plasmid-mediated polymyxin resistance. Herein, we report the emergence of the first plasmid-mediated polymyxin resistance mechanism, MCR-1, in Enterobacteriaceae.

*Lancet Infect Dis* 2015

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[http://dx.doi.org/10.1016/S1473-3099\(15\)00424-7](http://dx.doi.org/10.1016/S1473-3099(15)00424-7)

See Online/Articles

<http://dx.doi.org/10.1016/>

# How does MCR confer resistance?

Encoding:  
phosphoethanolamine transferase  
(also in *Neisseria*, *Paenibacillus*)



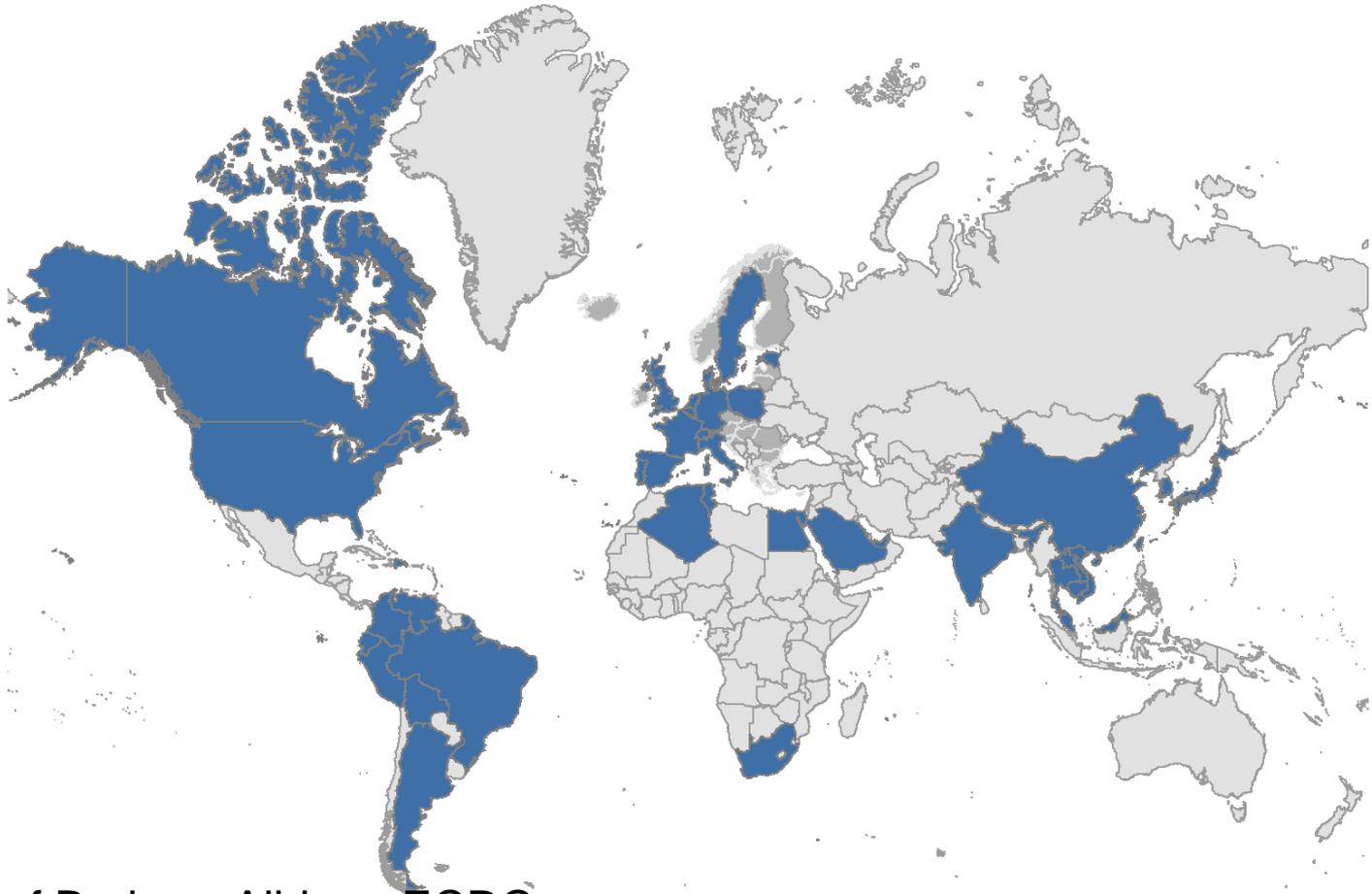
Phosphoethanolamine added to lipid A



Increased positive charge in lipid A

# Current global prevalence of *mcr-1*

Countries (n=41) reporting of *mcr-1* in samples of animals, environmental and human origin



Courtesy of Barbara Albiger, ECDC

Map produced on: 7 Sep 2016. Administrative boundaries: ©EuroGeographics, ©UN-FAO

# MCR-1.2 – novel variant

- Detected in *K. pneumoniae* of ST512 (CC258), epidemic clone producing KPC-3
- From surveillance rectal swab in leukemic child, 2014
- Found on transferable IncX4 plasmid whose structure was very similar to that of *mcr-1*-bearing plasmids
- Susceptible to amikacin and tigecycline
- No chromosomal mutations were detected
- 16-fold increase in MICs in *E. coli* transconjugants
- Di Pilato V et al. AAC 2016; 60: 5612

# MCR-2 – another novel variant

- Colistin-resistant *Escherichia coli* isolated during 2011–12 from passive surveillance of diarrhoea in 52 calves and 53 piglets in Belgium: 13 had MCR-1
- 10 negative strains were subjected to NGS, identifying a plasmid with an MCR-1 homologue in 3 strains (**76.8% similarity**)
- Found in ST10 (n=2, porcine) and ST167 (n=1, bovine)
- All plasmid IncX4 – high transfer frequency
- Xavier BB et al. Eurosurv 2016; 27

# And finally: MCR-1.3

- Detected on an IncP plasmid in *Salmonella* Typhimurim
- Two SNPs compared with MCR-1
- Lu X et al. AAC 2017 (In press)

# CPEs with MCR

- **2013**, Canada: 62-year old female patient with previous healthcare in Egypt and duodenal and sigmoid perforation, and *E. coli* with both ***bla*<sub>OXA-48</sub>** and ***mcr-1***
- **2014**, Germany: patient with a foot wound infection with *E. coli* harbouring ***bla*<sub>KPC-2</sub>** and ***mcr-1***
- **2015**, Switzerland: 83-year old male patient with urinary tract infection due to *E. coli* with ***bla*<sub>VIM</sub>** and ***mcr-1***
- **2014**, Italy: rectal swab of leukemic child with *Klebsiella pneumoniae* ST512 with ***bla*<sub>KPC-3</sub>** and ***mcr-1.2***
- **2014**, USA: patient with a urinary tract infection with *E. coli* harbouring ***bla*<sub>NDM-5</sub>** and ***mcr-1***

# Antimicrobial susceptibility testing of colistin

# Dilution methods

- Broth microdilution (BMD)
  - International reference method (ISO 20776-1)
  - Sulphate salts
  - Standard polystyrene trays
  - No additives or pre-treatment of plates
  - In-house prepared or commercial plates
- Agar dilution
  - To be evaluated

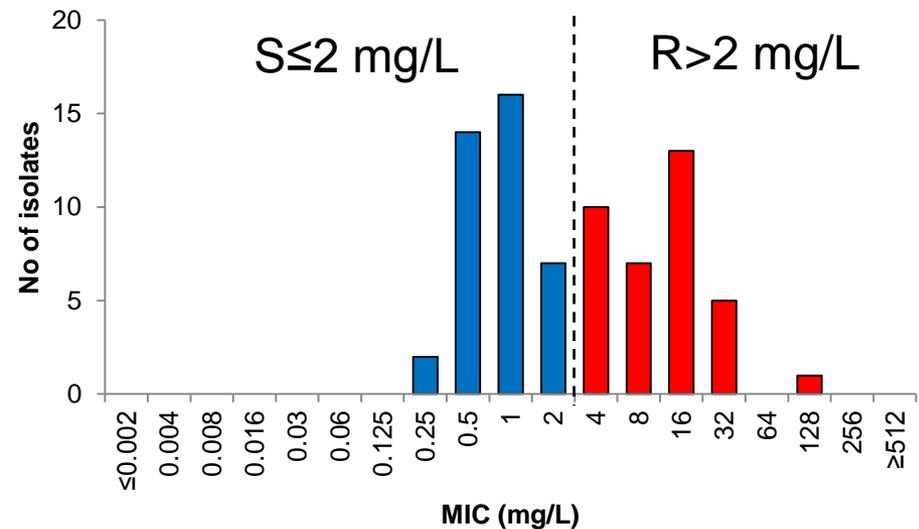
See EUCAST Guidance Documents  
[www.eucast.org/guidance\\_documents/](http://www.eucast.org/guidance_documents/)

# Diffusion methods

- Gradient tests?
  - Poor correlation with reference BMD
  - Warning on [www.eucast.org](http://www.eucast.org)
- Disk diffusion??
  - Poor/no separation between resistant and susceptible isolates.
  - No recommendation to use colistin disk diffusion in standardised methods, but disks can be purchased...
- The poor performance of diffusion tests is probably due to poor diffusion of colistin in agar.

# 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 (custom plate, Thermo Fisher Scientific)
    - MICRONAUT-S, MICRONAUT MIC Strip (MERLIN)
    - 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

# BMD products

**Sensititre** custom plate  
(Thermo Fisher Scientific)

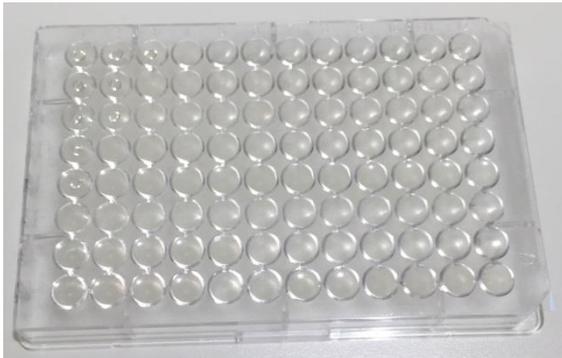


**SensiTest**  
(Liofilchem)

- four tests per panel
- broth included in kit



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



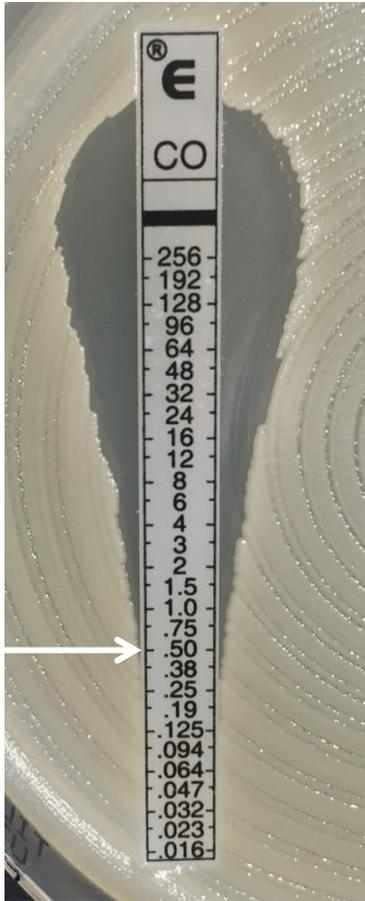
**MICRONAUT MIC-Strip**  
(Merlin) –single strips



**UMIC**  
(Biocentric) –single strips



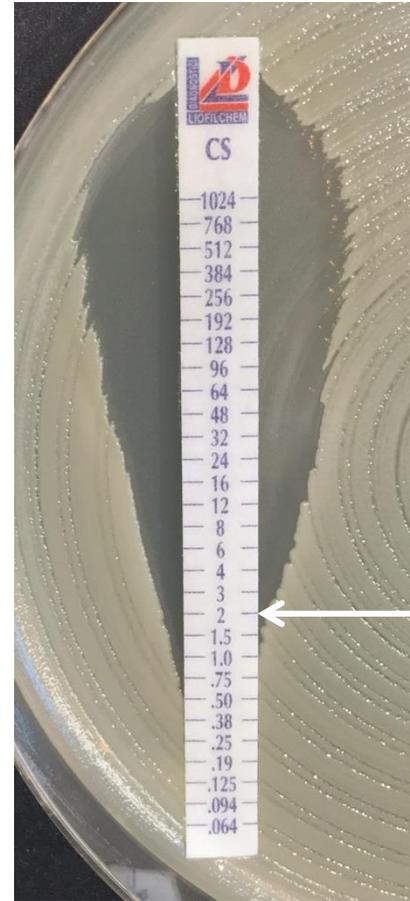
# Typical gradient test result



## 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

# Results

## Quality Control (QC) strains

BMD methods	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	
SensiTest			5	1				6				6	
UMIC	3	3	2					5	1		2	8	

\* ≤0.25 mg/L

Gradient tests	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
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	

 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."

# Essential and categorical agreement

BMD methods	Essential agreement (%)
Sensititre custom plate	96
MICRONAUT-S	96
MICRONAUT MIC-Strip	99
SensiTest	88
UMIC	82

Gradient tests	Essential agreement (%)
Etest, Oxoid MH	71
Etest, BBL MH	43
Etest, MHE	47
MTS, Oxoid MH	40
MTS, BBL MH	49

**Essential agreement** = No of MICs being within  $\pm 1$  dilution of reference MIC

# Essential and categorical agreement

BMD methods	Essential agreement (%)	Categorical agreement (No, of 75)	
		ME	VME
Sensititre custom plate	96	4	0
MICRONAUT-S	96	6	2
MICRONAUT MIC-Strip	99	5	2
SensiTest	88	7	1
UMIC	82	3	3

Gradient tests	Essential agreement (%)	Categorical agreement (No, of 75)	
		ME	VME
Etest, Oxoid MH	71	2	12
Etest, BBL MH	43	1	15
Etest, MHE	47	2	9
MTS, Oxoid MH	40	0	16
MTS, BBL MH	49	0	18

Underestimating resistance!

**Essential agreement** = No of MICs being within  $\pm 1$  dilution of reference MIC

**ME** = Major Error (false resistance)

**VME** = Very Major Error (false susceptibility)







# Conclusions

- Correlation with reference MICs was good for BMD methods.
  - Excellent correlation for Sensititre and MICRONAUT.
- 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.
- Disk diffusion cannot be used for AST of colistin!
- Warning on [www.eucast.org](http://www.eucast.org) on the use of disk diffusion and gradient tests for AST of colistin.

# EUCAST recommendations for colistin QC

- Use both
  - A susceptible strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853)
  - **and** the colistin resistant (*mcr-1* positive) *E. coli* NCTC 13846 (CCUG 70662)
    - 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.

EUCAST QC Tables v 7.0, 2017