# AST issues of polymyxins and their implications for the routine laboratory

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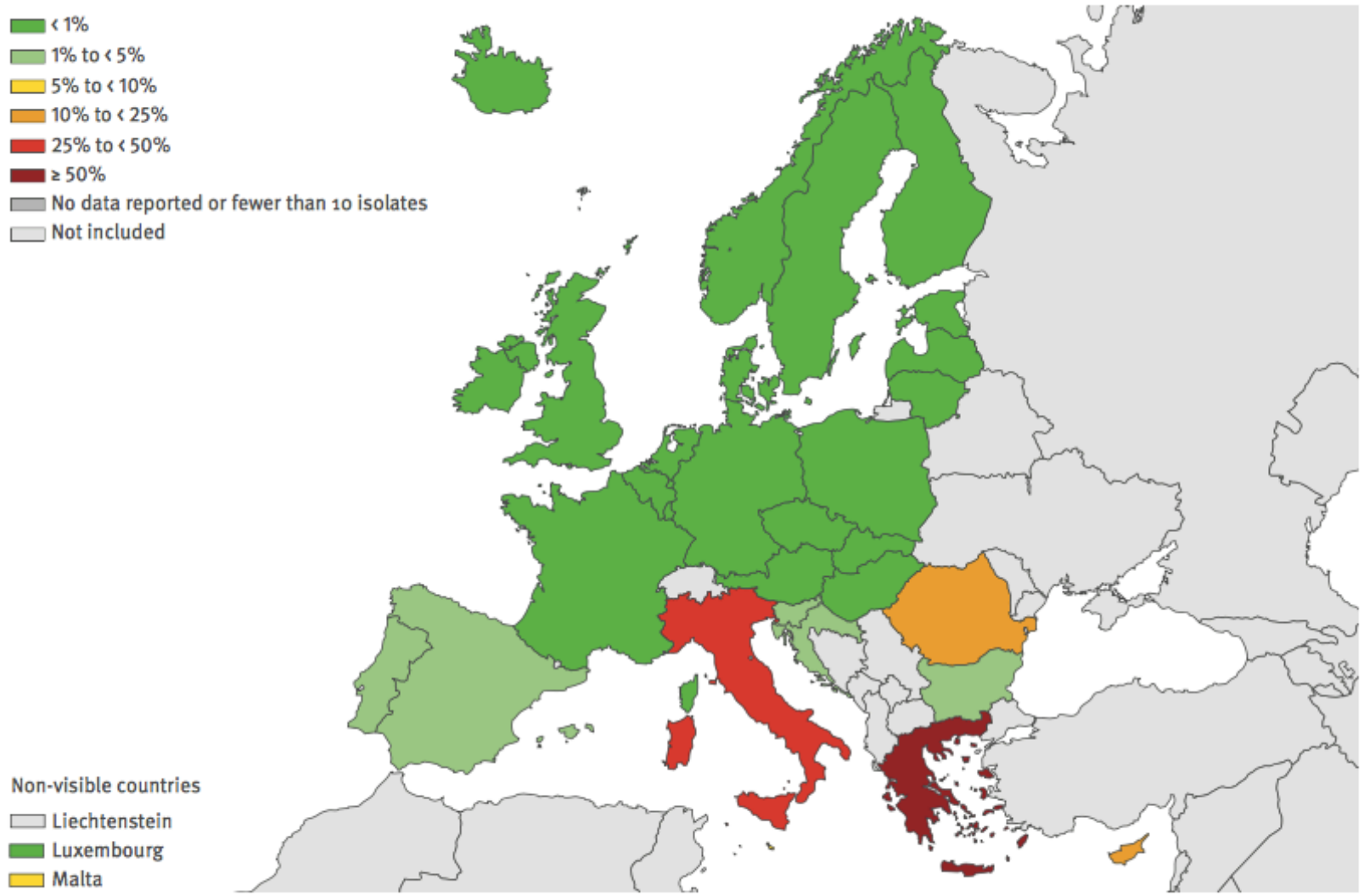
Vienna, 23 March 2017

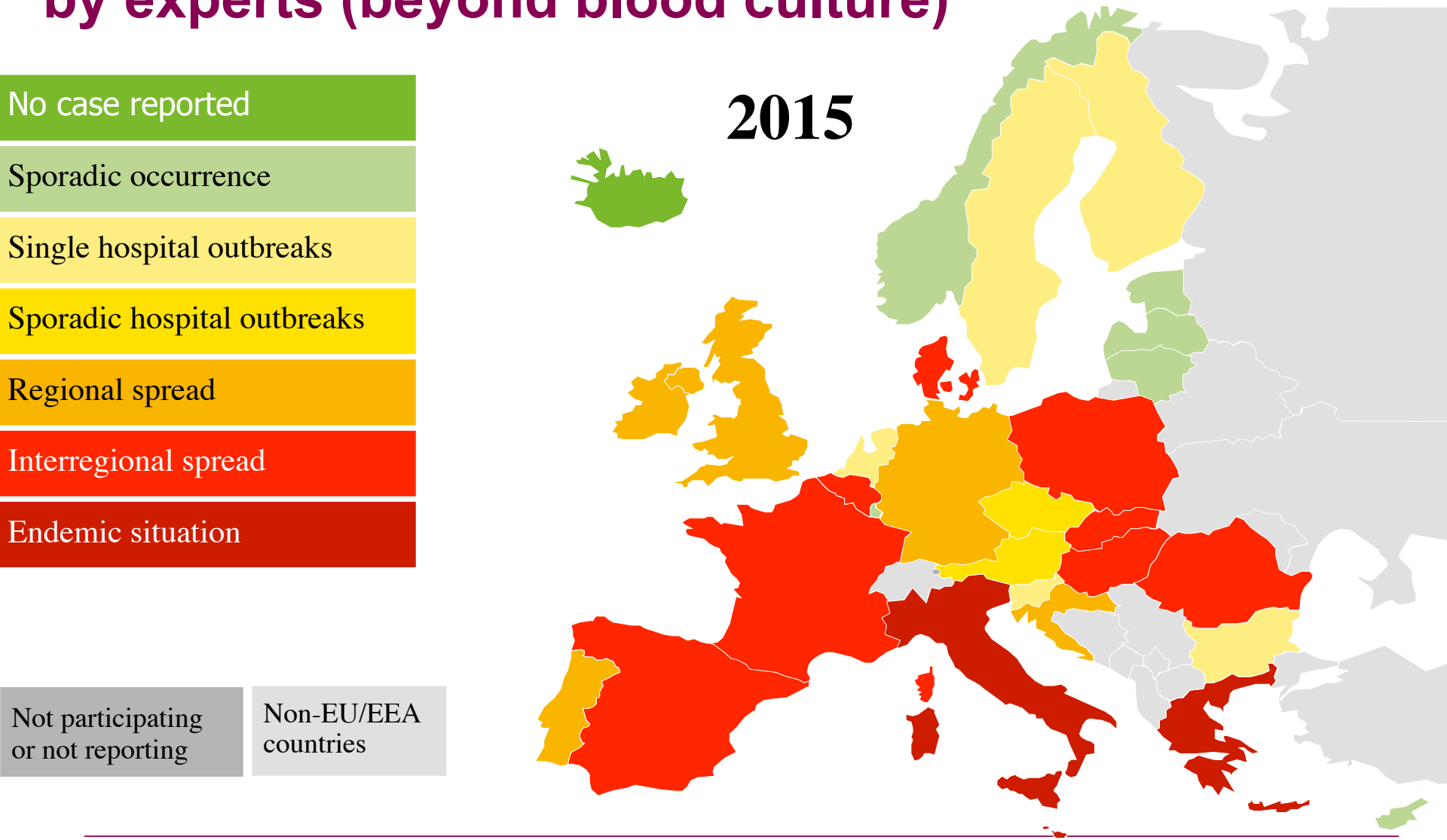
# Significance of colistin as a drug

# Carbapenem resistance in Europe, 2015



# CPE dissemination in Europe assessed by experts (beyond blood culture)

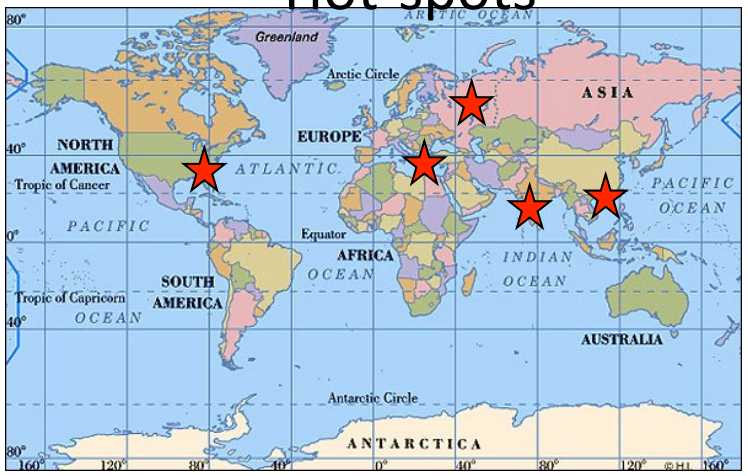
Karolinska Institutet




Source: Glasner C et al., Eurosurveillance 2015 18(28). Albiger B, et al. Eurosurveillance 2015 20(45)

# Global epidemiology

”Hot-spots”




**KPC:**
North-America
Israel
Greece
China

**VIM/IMP:**
Greece
Southeast Asia

**NDM:**
India
Pakistan
Bangladesh
Balkan

**OXA-48:**
Turkey
North-Africa
India

# Which treatment alternatives are available?

| Antibiotika | ESBL | AmpC | KPC | MBL | OXA-48 |
|---|---|---|---|---|---|
| Meropenem | + | + | +- | +- | +- |
| Temocillin | + | + | +- | - | - |
| Ceftolozane -tazobactam | + | - | - | - | - |
| Ceftazidime -avibactam | + | + | + | - | + |
| Colistin | + | + | + | + | + |

# Colistin use

## Veterinary medicine

- Top 5 most commonly used antibiotic in the EU


## Human medicine

- Used mainly for ophthalmic and topical infections
- In cystic fibrosis patients (systemic or nebulised)
- Surgical prophylaxis via SDD
- Treatment of healthcare-associated infections due to MDR Gram-negative bacteria

# 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. |
| Netherland | 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. |
| **EU/EEA** | **0.008** | **0.011** | **0.014** | **0.012** | **0.012** | | **<0.001** | **n.s.** |
| 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. |

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.

- 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

# Colistin resistance: mechanisms

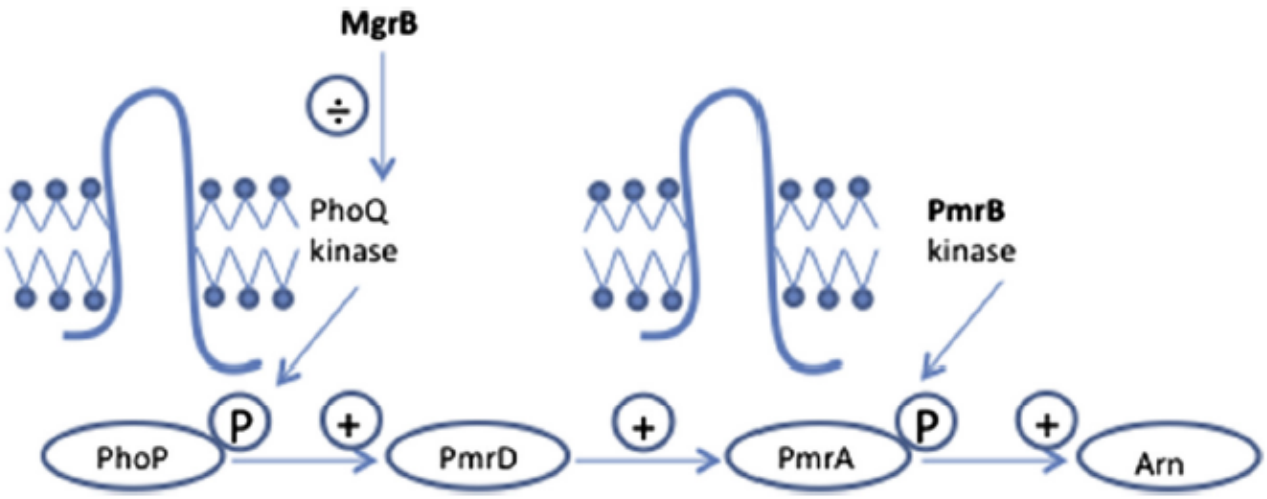
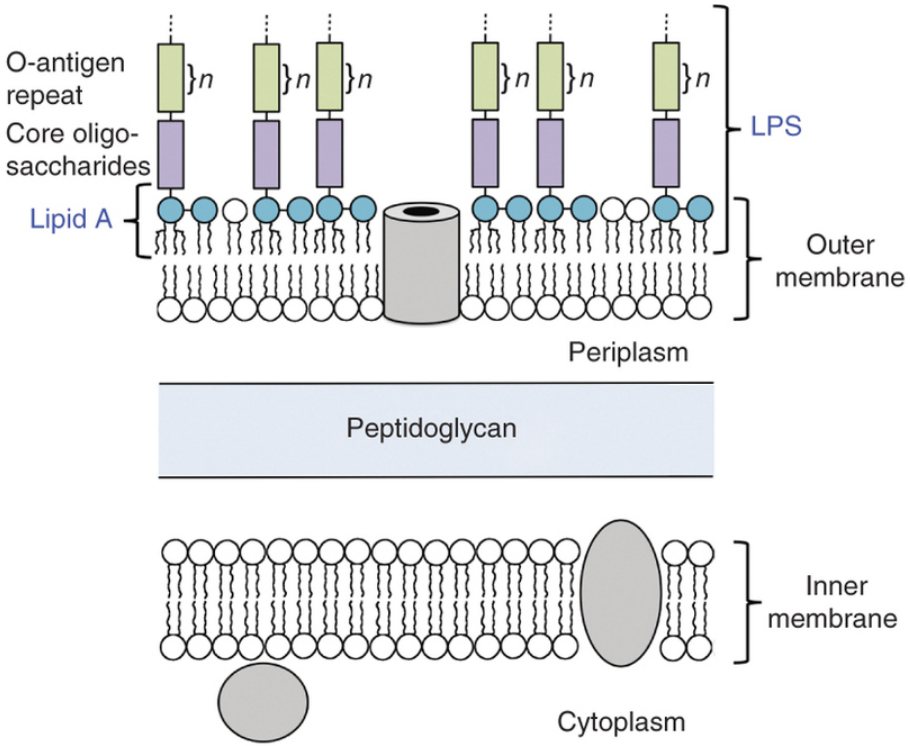
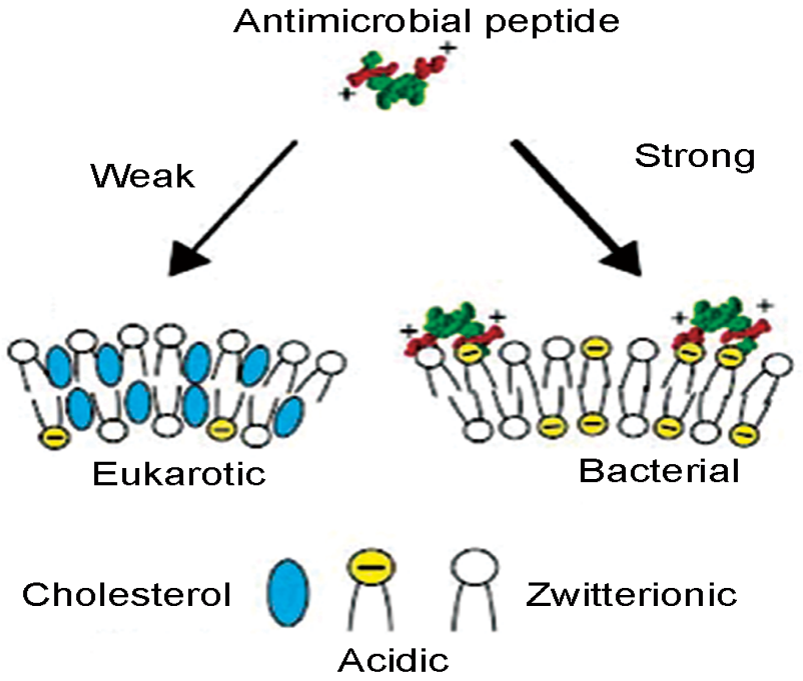



FIG. 1. Two-component regulatory system for modification of charge in LPS of Gram-negative bacilli. Membrane-bound kinases affecting transcription of *arn* complex through intermediate PmrD are shown. Circled P denotes phosphorylation; circled minus sign denotes negative regulation of transcription; 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

# How common is colistin resistance?

**TABLE I. Colistin resistance in Enterobacteriaceae ($n$ = 8341) and *Pseudomonas aeruginosa* ($n$ = 2191) in study of 31 medical centres, 2011–2012**

| Species/category[a] | Colistin resistance (EUCAST) (%) |
|---|---|
| *P. aeruginosa* ($n$ = 2191) | 0.2 |
| MDR *P. aeruginosa* ($n$ = 698) | 0.6 |
| *Escherichia coli* ($n$ = 3843) | 0.5 |
| *E. coli* with ESBL ($n$ = 715) | 0.6 |
| *Klebsiella pneumoniae* ($n$ = 1408) | 5.4 |
| *K. pneumoniae* with ESBL ($n$ = 633) | 9.7 |
| *Klebsiella oxytoca* ($n$ = 304) | 0.7 |
| *Enterobacter* spp. ($n$ = 899) | 10.9 |
| *Citrobacter* spp. ($n$ = 389) | 0.3 |

Data from Sader *et al.* [7].
EUCAST, European Committee on Antimicrobial Susceptibility Testing; ESBL, extended-spectrum β-lactamase; MDR, multidrug resistant.
[a]Species with natural resistance to colistin (*Proteae*, *Serratia*) are not included.

# Polymyxin resistance in 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

# The emergence of mobile 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**

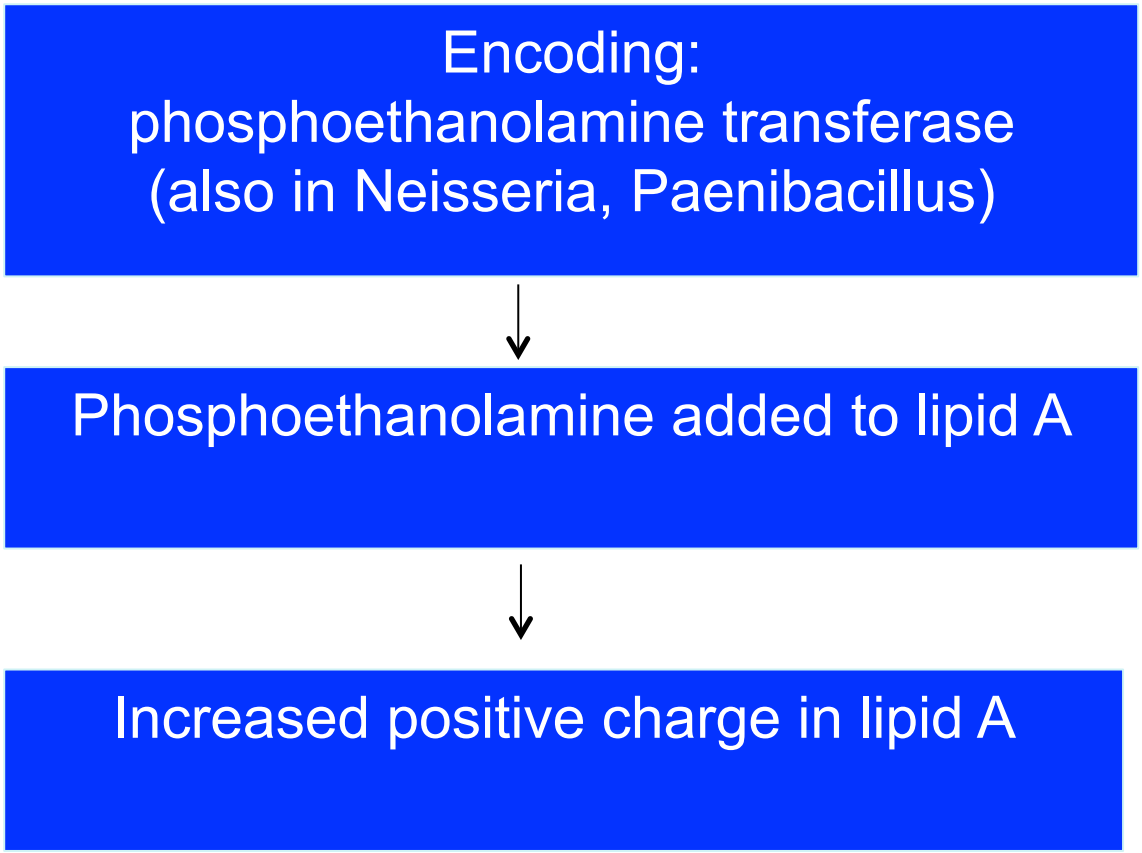
**Background** Until now, polymyxin resistance has involved chromosomal mutations but has never been reported via horizontal gene transfer. During a routine surveillance project on antimicrobial resistance in commensal *Escherichia coli* from food animals in China, a major increase of colistin resistance 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

Published Online November 18, 2015 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?



# Level of resistance conferred by MCR-1

| | Origin | Polymyxin E (colistin) | Polymyxin B |
|---|---|---|---|
| *Escherichia coli* SHP45 (*mcr-1*) | Pig | 8·0 | 4·0 |
| *E coli* C600 | .. | 0·5 | 0·5 |
| *E coli* C600+ pHNSHP45 *mcr-1*) | Transconjugant | 8·0 | 4·0 |
| *E coli* E11 (ST131, KPC-2-producer) | Human | 0·5 | 0·5 |
| *E coli* E11 (ST131, KPC-2-producer) + pHNSHP45 (*mcr-1*) | Transformant | 4·0 | 2·0 |
| *Klebsiella pneumoniae* MPC11 | Human | 0·5 | 0·5 |
| *K pneumoniae* MPC11 + pHNSHP45 (*mcr-1*) | Transformant | 8·0 | 4·0 |
| *K pneumoniae* 1202 (ST11, KPC-2-producer) | Human | 0·5 | 0·5 |
| *K pneumoniae* 1202 (ST11, KPC-2-producer) + pHNSHP45 (*mcr-1*) | Transformant | 4·0 | 4·0 |
| *Pseudomonas aeruginosa* HE26 | Human | 0·5 | 0·5 |
| *P aeruginosa* HE26 + pHNSHP45(*mcr-1*) | Transformant | 8·0 | 4·0 |
| *E coli*W3110+ pUC18 | Laboratory strain | 0·5 | 0·5 |
| *E coli*W3110 + pUC18-*mcr-1* | Transformant | 2·0 | 2·0 |

*Table 1:* **Minimum inhibitory concentration (mg/L) for parental strain, transformants, and transconjugant**

# Susceptibility of wild-type Enterobacteriaceae

Antimicrobial: **Colistin** (Method: **MIC** )

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

| | 0.002 | 0.004 | 0.008 | 0.016 | 0.032 | 0.064 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | ECOFF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acinetobacter baumannii | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 163 | 108 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | ND |
| Citrobacter freundii | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ND |
| Citrobacter koseri | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ND |
| Enterobacter aerogenes | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 55 | 150 | 44 | 10 | 3 | 3 | 3 | 0 | 0 | 4 | 0 | 0 | 2.0 |
| Enterobacter cloacae | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 255 | 398 | 76 | 15 | 6 | 9 | 17 | 23 | 8 | 22 | 2 | 2 | 2.0 |
| Escherichia coli | 0 | 0 | 0 | 0 | 0 | 2 | 231 | 2058 | 2776 | 874 | 74 | 16 | 14 | 5 | 8 | 2 | 30 | 0 | 0 | 2.0 |
| Klebsiella pneumoniae | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 451 | 1220 | 411 | 36 | 17 | 14 | 35 | 14 | 5 | 9 | 0 | 1 | 2.0 |
| Pseudomonas aeruginosa | 0 | 0 | 0 | 0 | 1 | 6 | 24 | 114 | 1051 | 2872 | 2118 | 287 | 30 | 48 | 7 | 3 | 12 | 0 | 6 | 4.0 |
| Raoultella ornithinolytica (Klebs. oxytoca) | 0 | 0 | 0 | 0 | 0 | 16 | 10 | 143 | 406 | 192 | 22 | 6 | 1 | 10 | 2 | 1 | 2 | 0 | 1 | 2.0 |
| Salmonella dublin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 30 | 108 | 65 | 1 | 0 | 0 | 0 | 0 | 0 | ND |
| Salmonella enteritidis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 11 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | ND |

Ac-

# Prevalence of MCR-1 in various isolates

| | Year | Positive isolates (%)/number of isolates |
|---|---|---|
| *Escherichia coli* | | |
| Pigs at slaughter | All | 166 (20·6%)/804 |
| Pigs at slaughter | 2012 | 31 (14·4%)/216 |
| Pigs at slaughter | 2013 | 68 (25·4%)/268 |
| Pigs at slaughter | 2014 | 67 (20·9%)/320 |
| Retail meat | All | 78 (14·9%)/523 |
| Chicken | 2011 | 10 (4·9%)/206 |
| Pork | 2011 | 3 (6·3%)/48 |
| Chicken | 2013 | 4 (25·0%)/16 |
| Pork | 2013 | 11 (22·9%)/48 |
| Chicken | 2014 | 21 (28·0%)/75 |
| Pork | 2014 | 29 (22·3%)/130 |
| Inpatient | 2014 | 13 (1·4%)/902 |
| *Klebsiella pneumoniae* | | |
| Inpatient | 2014 | 3 (0·7%)/420 |

*Table 2:* Prevalence of colistin resistance gene *mcr-1* by origin

# Murine thigh model colistin treatment

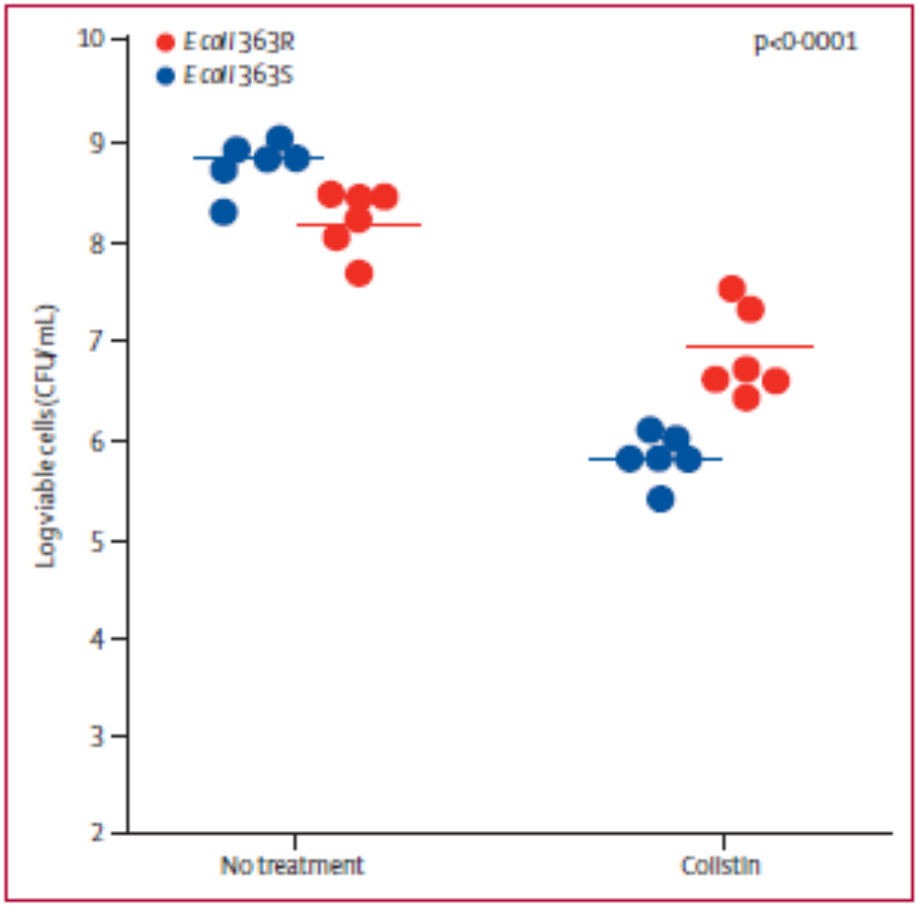



*Figure 4:* In-vivo effects of colistin treatment (7·5 mg/kg of colistin sulfate per 12 h) in a murine thigh model showing $10^8$ CFU infection with *Escherichia coli* with *mcr-1* (363R, red circles) and without *mcr-1* (363S, blue circles)

# Epidemiology in China


*Figure 1:* **Map of China**

# Early situation in Europe

***Numbers of extended spectrum beta-lactamase and AmpC-producing E. coli isolates obtained and analysed by WGS from chicken meat, humans and carbapenemase-producing isolates from humans tested for mcr-1 using ResFinder, Denmark, November 2015 (n=914)***

| Isolate origin | No. of isolates analysed by WGS | No. of sequences positive for *mcr-1* |
|---|---|---|
| ESBL- and AmpC-producing *E. coli* isolates from Danish chicken meat (2012–2014) | 125 | 0 |
| ESBL- and AmpC-producing *E. coli* isolates from imported chicken meat (2012–2014) | 255 | 5 |
| ESBL- and AmpC-producing *E. coli* isolates from human bloodstream infections (January 2014– beginning of November 2015) | 417 | 1 |
| Carbapenemase-producing isolates from humans (January 2014–beginning of November 2015) | 117 | 0 |

ESBL: extended spectrum beta-lactamase; No: number; WGS: whole-genome sequence.

Hasman H. Eurosurveillance 2015

# MCR-1 positive *E. coli*, Denmark

| Isolate name | Origin | Year of detection | MLST | Resistance genes detected by ResFinder besides *mcr-1* | Detection of chromosomal mutations encoding resistance to quinolones |
|---|---|---|---|---|---|
| 0412016126 | Chicken meat | 2012 | ST359 | *aadA1, aadA5, aph(3')-Ic, bla* $_{CMY-2}$, *bla* $_{TEM-1B}$, *dfrA1, strA, strB, sul1, sul2, tet*(B) | GyrA (S83L, D87N) ParC (E62K) |
| 0412044854 | Chicken meat | 2012 | ST48 | *aadA1, bla* $_{CMY-2}$, *bla* $_{TEM-1B}$, *dfrA1, mph(B), strA, strB, sul1, sul2, tet*(A) | GyrA (S83L) |
| 0412049521 | Chicken meat | 2012 | ST131 | *aadA1, bla* $_{SHV-12}$, *bla* $_{CMY-2}$, *strA, strB, sul1, dfrA1, tet*(A) | ND |
| 0413040864 | Chicken meat | 2013 | ST1112 | *aadA1, aadA2, bla* $_{SHV-12}$, *cmlA1, sul3, tet*(A) | ND |
| 14042624 | Chicken meat | 2014 | ST2063 | *aadA1, aadA2, bla* $_{SHV-12}$, *cmlA1, sul3* | ND |
| ESBL20150072 | Human, bloodstream infection | 2015 | ST744 | *aadA5, bla* $_{CMY-2}$, *bla* $_{CTX-M-55}$, *bla* $_{TEM-1B}$, *catA1, dfrA17, floR, fosA, mph*(A), *rmtB, strA, strB, sul1, sul2, tet*(A) | GyrA (S83L, D87N) ParC (E62K) |

Hasman H. Eurosurveillance 2015

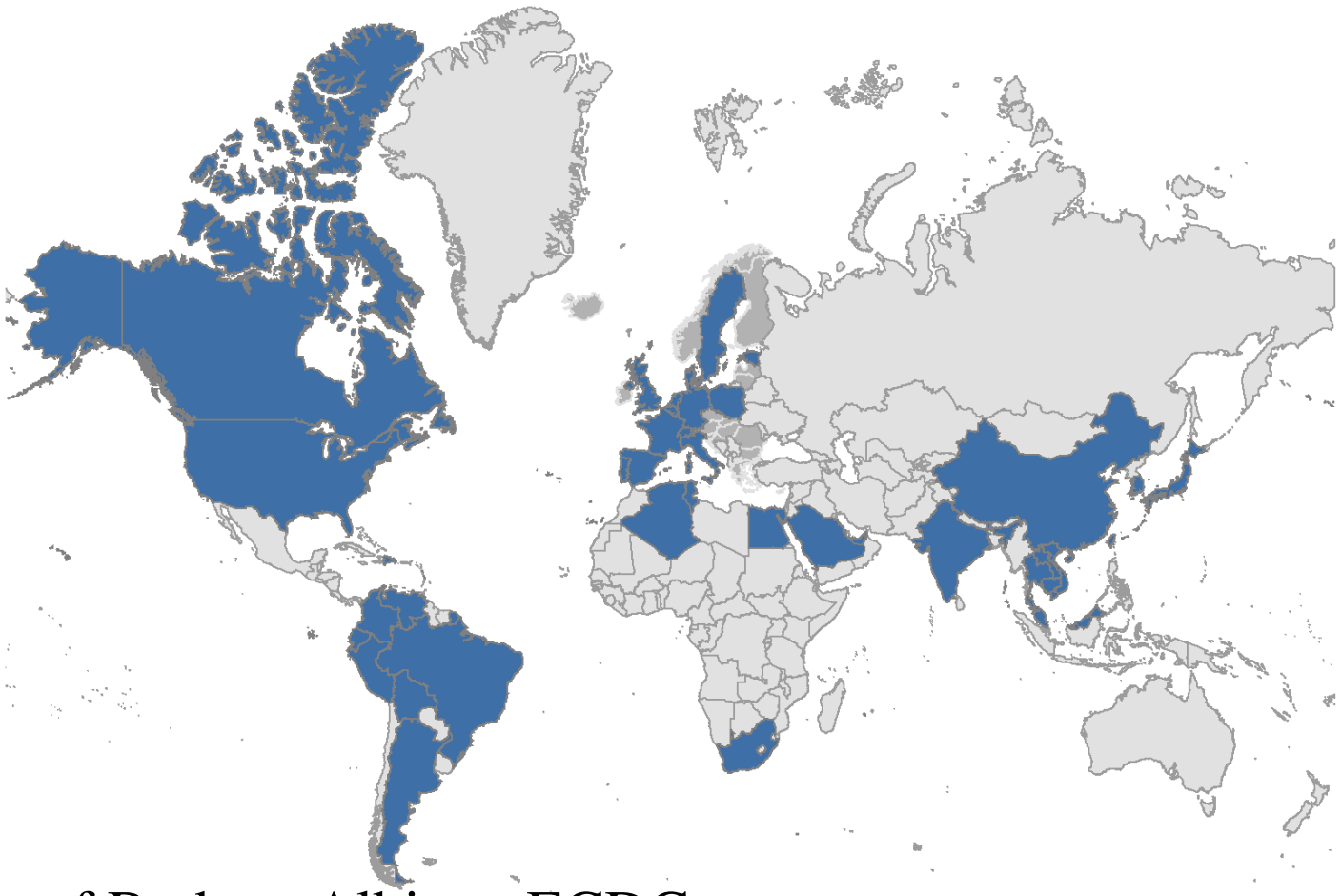
# Intensive hunt for MCR-1

Within 6 months, *mcr-1* gene was isolated:

- → from food animals, the environment and various types of meat and vegetables
- → from patients and asymptomatic human carriers (including international travellers)
- → from various bacterial species
- → found in 27 countries on 5 continents (except Australia/Oceania and Antarctica)
- → carried by several plasmids (e.g., IncI2, IncHI2, IncP, IncFIB and IncX4)

# 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

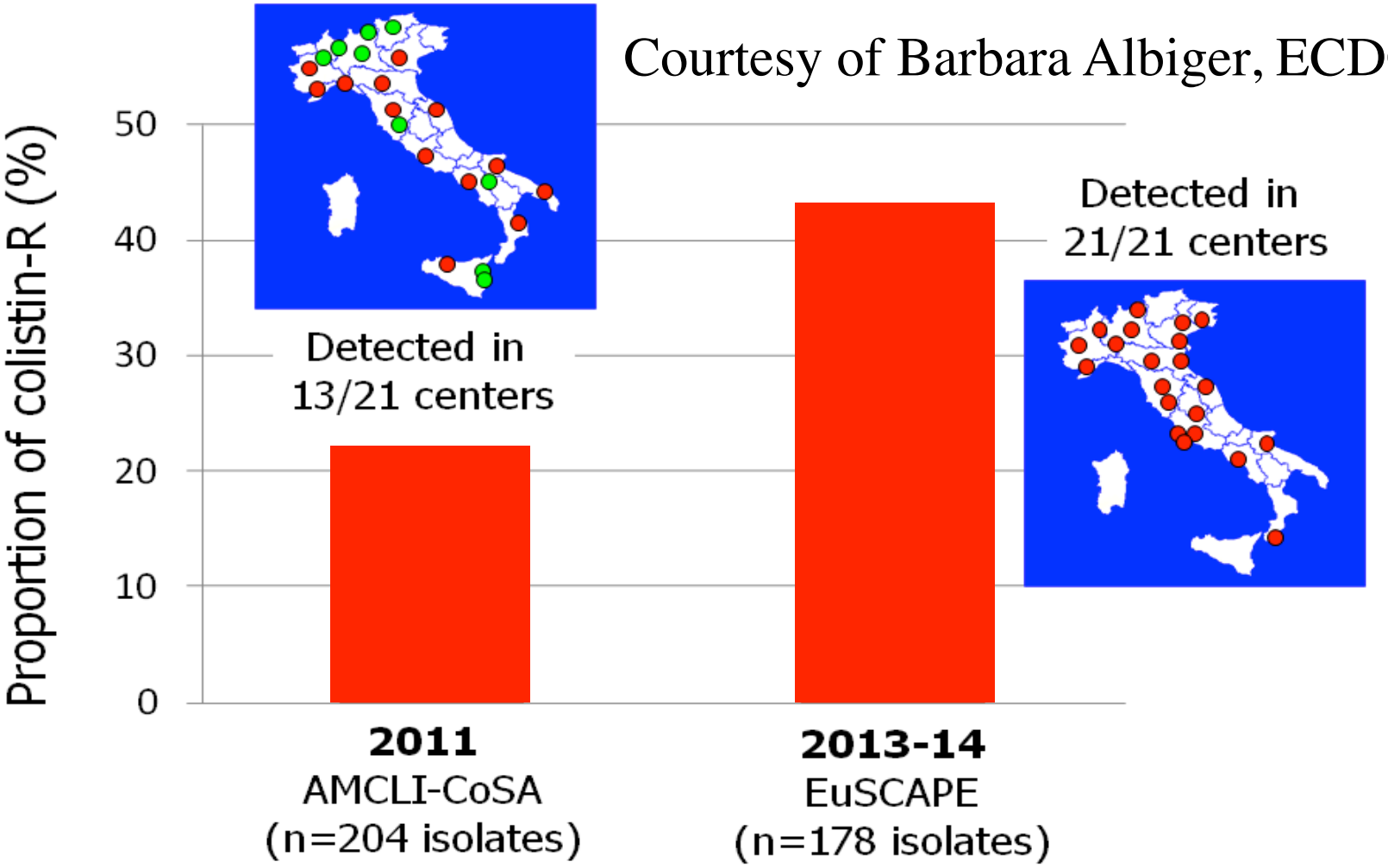
- Colistin-resistant *Escherichia coli* isolated during 2011–12 from passive surveillance of diarrhoea in 52 calves and 53 piglets in Belgium: 13 had MCR-1
- 10 negative strains were subjected to NGS, identifying a plasmid with an MCR-1 homologue in 3 strains (76.8% similarity)
- Found in ST10 (n=2, 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)

# Colistin and carbapenem co-resistance: national surveys (Italy), 2011 and 2013-14

Courtesy of Barbara Albiger, ECDC




Source: Giani T, et al. Eurosurveill 2013; Monaco M, et al. Eurosurveill 2014.

# 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*$_{\text{OXA-48}}$ and *mcr*-1**
- **2014**, Germany: patient with a foot wound infection with *E. coli* harbouring ***bla*$_{\text{KPC-2}}$ and *mcr*-1**
- **2015**, Switzerland: 83-year old male patient with urinary tract infection due to *E. coli* with ***bla*$_{\text{VIM}}$ and *mcr*-1**
- **2014**, Italy: rectal swab of leukemic child with *Klebsiella pneumoniae* ST512 with ***bla*$_{\text{KPC-3}}$ and *mcr*-1.2**
- **2014**, USA: patient with a urinary tract infection with *E. coli* harbouring ***bla*$_{\text{NDM-5}}$ and *mcr*-1**

Source: Ellis C, et al. Diagn Microbiol Infect Dis 2013; Yang J. Toronto Star (5 January 2016); Falgenhauer L, et al. Lancet Infect Dis 2016; Poirel L, et al. Lancet Infect Dis 2016; Di Pilato V, et al. Antimicrob Agents Chemother 2016. Mediaville et al., mbio 2016.

# ECDC: risk of the colistin resistance for clinical and patient management

- An increasing challenge for appropriate patient therapy
- Fewer treatment options if colistin resistance is associated to multiple other resistance genes (= MDR strain becoming XDR/PDR strain)
- Increased risk of fatal outcomes is associated with colistin resistance

Source: ECDC RRA on plasmid-mediated colistin resistance in *Enterobacteriaceae*. 13 June 2016.

# Actions undertaken to prevent spread

- European Medicines Agency (EMA): polymyxins should only be used as a second line treatment in animals and that their sales should be minimised across all EU Member States
  - → Goal: **overall reduction of approximately 65%** for veterinary use at an EU level
  - → Improvement of the conditions of animal husbandry and alternative measures
- ECDC Roadmap: new sentinel genomic-based surveillance module for carbapenem and colistin resistant *Enterobacteriaceae* in the EU/EEA
  - → Goal: identify internationally disseminated epidemic colistin resistant, carbapenem resistant *Enterobacteriaceae* clones that carry *mcr* gene and to monitor their geographic distribution across the EU/EEA

Source: EMA Updated advice on the use of colistin products in animals within the European Union: development of resistance and possible impact on human and animal health. 27 July 2016; ECDC roadmap for integration of molecular and genomic typing into European-level surveillance and epidemic preparedness – Version 2.1, 2016-19. 2016

# Challenges in AST

# Colistin MIC distributions and ECOFFs

Antimicrobial: **Colistin** (Method: **MIC** )

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

| | 0.002 | 0.004 | 0.008 | 0.016 | 0.032 | 0.064 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | ECOFF |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Acinetobacter baumannii | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 33 | 163 | 108 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | ND |
| Citrobacter freundii | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 4 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ND |
| Citrobacter koseri | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 8 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ND |
| Enterobacter aerogenes | 0 | 0 | 0 | 0 | 0 | 4 | 4 | 55 | 150 | 44 | 10 | 3 | 3 | 3 | 0 | 0 | 4 | 0 | 0 | 2.0 |
| Enterobacter cloacae | 0 | 0 | 0 | 0 | 0 | 0 | 16 | 255 | 398 | 76 | 15 | 6 | 9 | 17 | 23 | 8 | 22 | 2 | 2 | 2.0 |
| Escherichia coli | 0 | 0 | 0 | 0 | 0 | 2 | 231 | 2058 | 2776 | 874 | 74 | 16 | 14 | 5 | 8 | 2 | 30 | 0 | 0 | 2.0 |
| Klebsiella pneumoniae | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 451 | 1220 | 411 | 36 | 17 | 14 | 35 | 14 | 5 | 9 | 0 | 1 | 2.0 |
| Pseudomonas aeruginosa | 0 | 0 | 0 | 0 | 1 | 6 | 24 | 114 | 1051 | 2872 | 2118 | 287 | 30 | 48 | 7 | 3 | 12 | 0 | 6 | 4.0 |
| Raoultella ornithinolytica (Klebs. oxytoca) | 0 | 0 | 0 | 0 | 0 | 16 | 10 | 143 | 406 | 192 | 22 | 6 | 1 | 10 | 2 | 1 | 2 | 0 | 1 | 2.0 |
| Salmonella dublin | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 24 | 30 | 108 | 65 | 1 | 0 | 0 | 0 | 0 | 0 | ND |
| Salmonella enteritidis | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 3 | 11 | 15 | 0 | 0 | 0 | 0 | 0 | 0 | ND |

Ac-

# Colistin AST

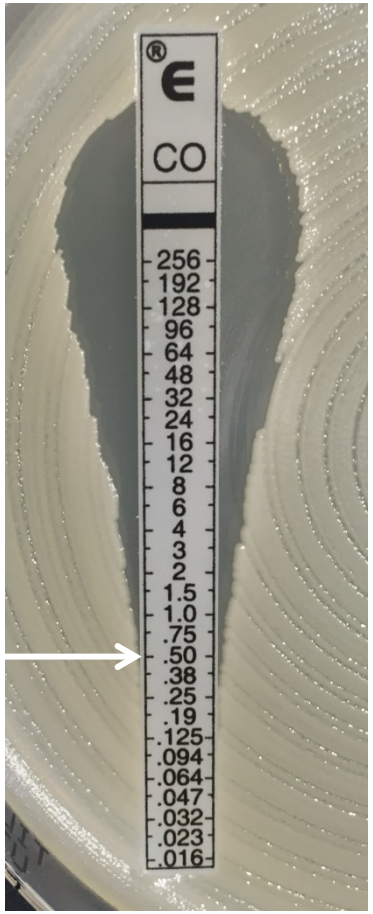
- BMD
  - → Microtiter trays (standard polystyrene, no additives or pretreatment of plates)
  - → Automated BMD?
- Agar dilution?
- Gradient tests (Etest®, MTS®)?
- Disk diffusion?

# EUCAST Development Laboratory Study

- 96 Enterobacteriaceae with previously known colistin MICs
  - →DTU (Copenhagen)
  - →SSI (Copenhagen)
  - →Bochum (Sören Gatermann)

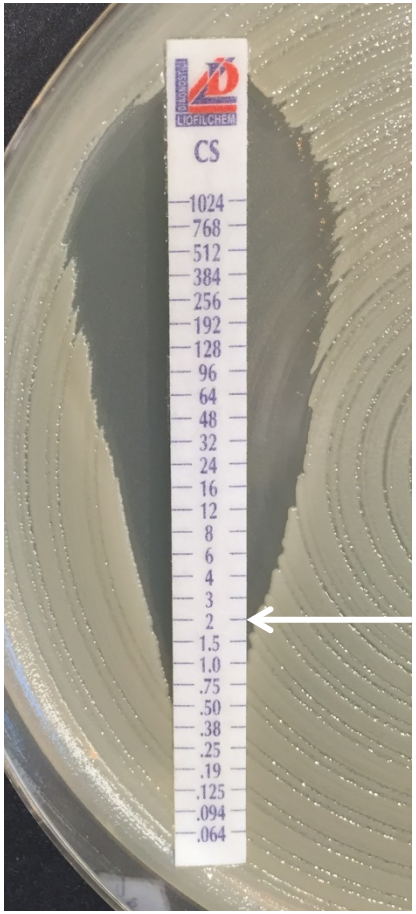
| Organism | No of isolates | | | mcr-1 | | |
|---|---|---|---|---|---|---|
| | Total | S | R | pos | neg | not tested |
| *Salmonella* spp. | 59 | 23 | 36 | 11 | 30 | 18 |
| *Escherichia coli* | 19 | 6 | 13 | 12 | 4 | 3 |
| *Klebsiella pneumoniae* | 14 | 0 | 14 | | 12 | 2 |
| *Enterobacter* spp. | 3 | 0 | 3 | | 3 | |
| *Hafnia alvei* | 1 | 0 | 1 | | 1 | |

# Typical gradient test results




**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

# Materials and methods

- **BMD** according to EUCAST/CLSI (no addition of P-80/Tween)
  → For 50 selected isolates, BMD was repeated once

- **Gradient tests (50 selected isolates)**
  → Etest, bioMérieux
  → MIC Test Strip (MTS), Liofilchem
  → (M.I.C.E, Oxoid/Thermo Fisher not available)

- **Disk diffusion (50 selected isolates)**
  → 10, 25 and 50 µg disks from Oxoid, BD and Mast

- Oxoid and BBL MH used in parallel for gradient tests and disk diffusion
  → In addition, colistin Etest was tested on MH-E (as recommended by bioMerieux)

# Etest/Oxoid MH vs BMD – all species

| | COL BMD | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Colistin Etest Oxoid MH** | **≤0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** |
| **0.125** | | | | | | | | |
| **0.25** | | | | | | | | |
| **0.5** | 2 | | 1 | 1 | 2 | | | |
| **1** | 1 | 6 | 5 | 2 | 2 | | | |
| **2** | | | | | 1 | | | |
| **4** | | | | | 9 | 4 | 1 | 2 |
| **8** | | | | | 1 | 6 | 2 | |
| **16** | | | | | | | | 2 |
| **32** | | | | | | | | |
| **64** | | | | | | | | |

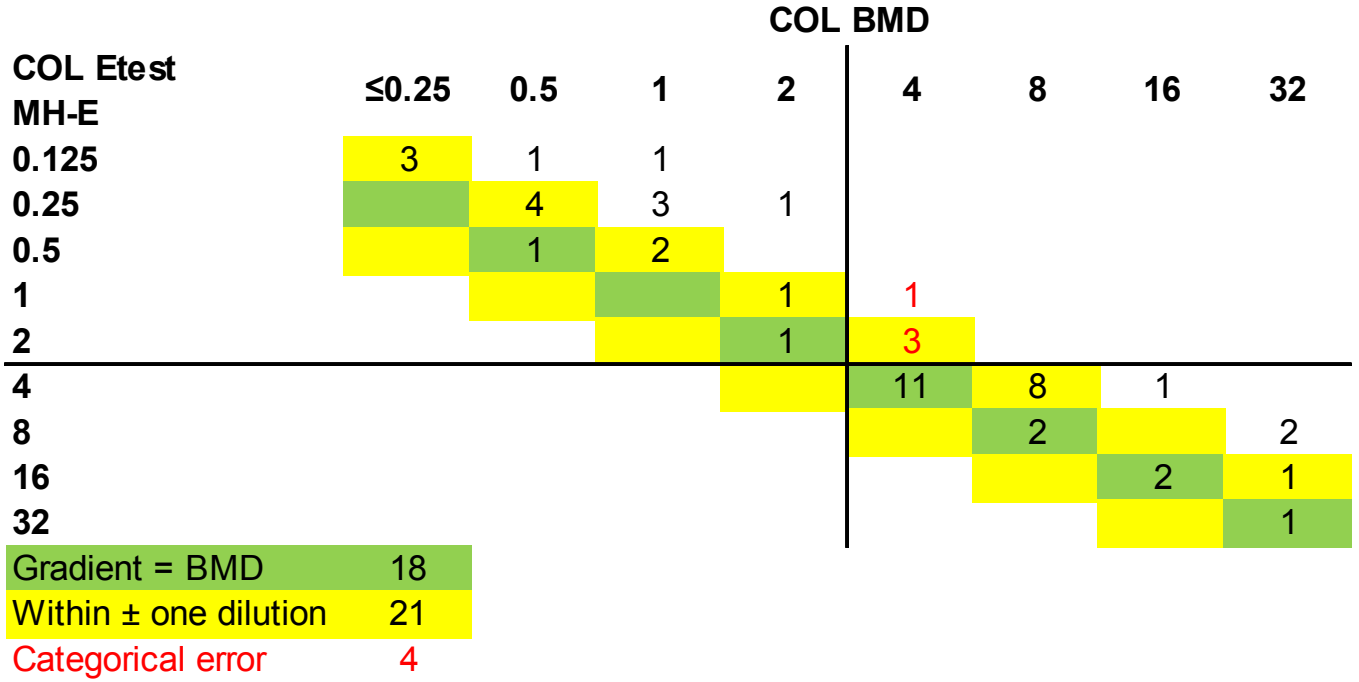
| | |
|---|---|
| Gradient = BMD | 20 |
| Within ± one dilution | 21 |
| Categorical error | 5 |

# Etest/BBL MH vs BMD – all species

| | COL BMD | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Colistin Etest BBL MH** | **≤0.25** | **0.5** | **1** | **2** | **4** | **8** | **16** | **32** |
| **0.125** | 2 | 1 | 1 | | | | | |
| **0.25** | 1 | 5 | 3 | 1 | 2 | | | |
| **0.5** | | | 2 | 2 | 2 | | | |
| **1** | | | | | | | | |
| **2** | | | | | 2 | 1 | | |
| **4** | | | | | 9 | 5 | 1 | 2 |
| **8** | | | | | | 4 | 2 | |
| **16** | | | | | | | | 2 |
| **32** | | | | | | | | |
| **64** | | | | | | | | |

| | |
|---|---|
| Gradient = BMD | 14 |
| Within ± one dilution | 20 |
| Categorical error | 7 |

# Etest/MH-E vs BMD – all species

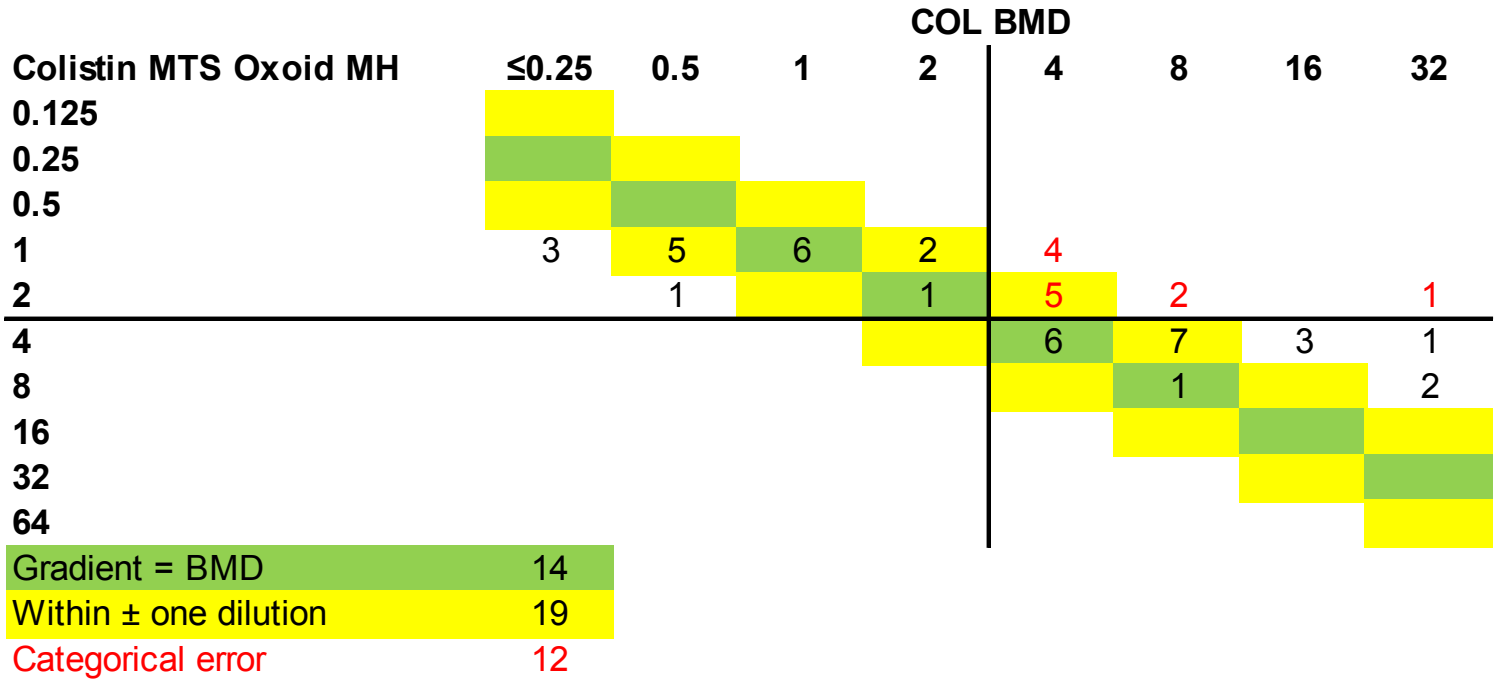


| COL Etest MH-E | COL BMD ≤0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|---|---|
| 0.125 | 3 | 1 | 1 | | | | | |
| 0.25 | | 4 | 3 | 1 | | | | |
| 0.5 | | 1 | 2 | | | | | |
| 1 | | | | 1 | 1 | | | |
| 2 | | | | 1 | 3 | | | |
| 4 | | | | | 11 | 8 | 1 | |
| 8 | | | | | | 2 | | 2 |
| 16 | | | | | | | 2 | 1 |
| 32 | | | | | | | | 1 |

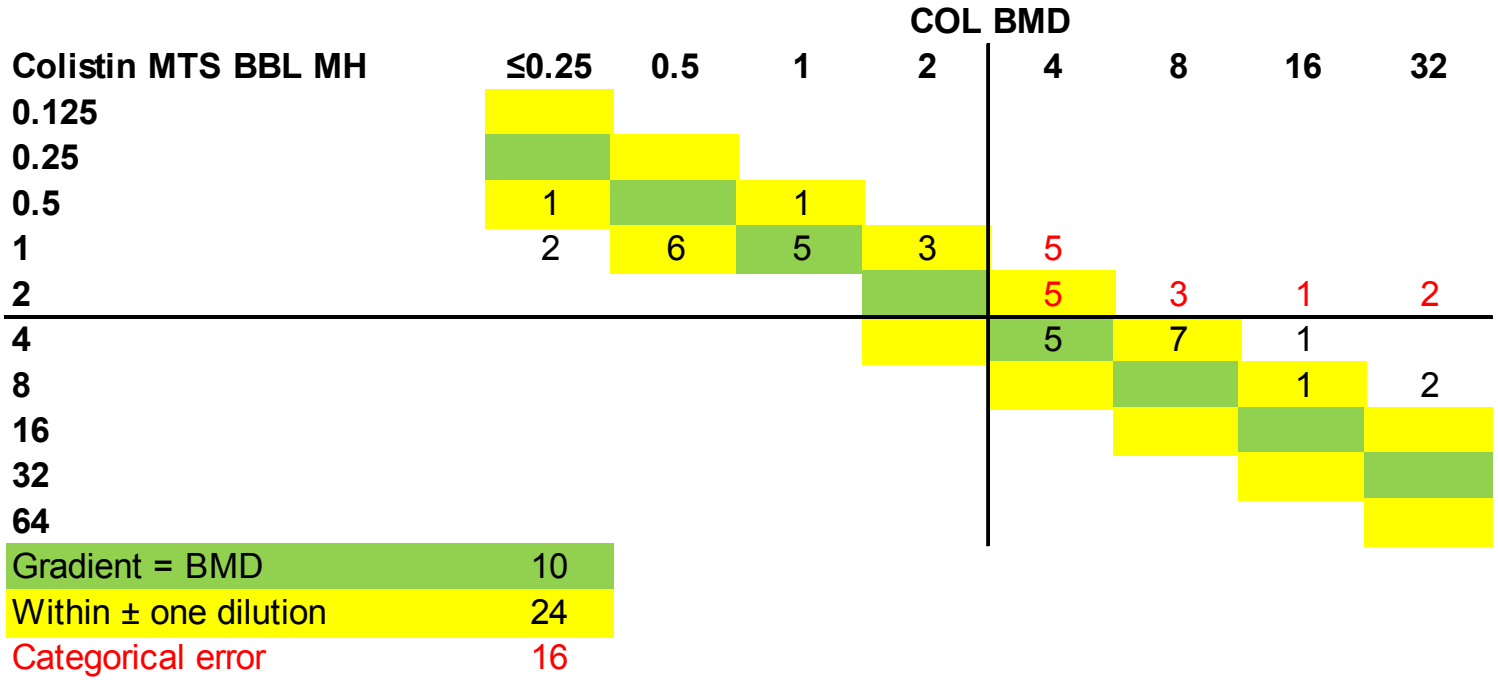
| | |
|---|---|
| Gradient = BMD | 18 |
| Within ± one dilution | 21 |
| Categorical error | 4 |

The four isolates with categorical errors (all *Salmonella* spp.) had colistin BMD MICs at 4 mg/L in both tests (BMD 1st test and BMD 2nd test)

# MTS/Oxoid MH vs BMD – all species



# MTS/BBL MH vs BMD – all species

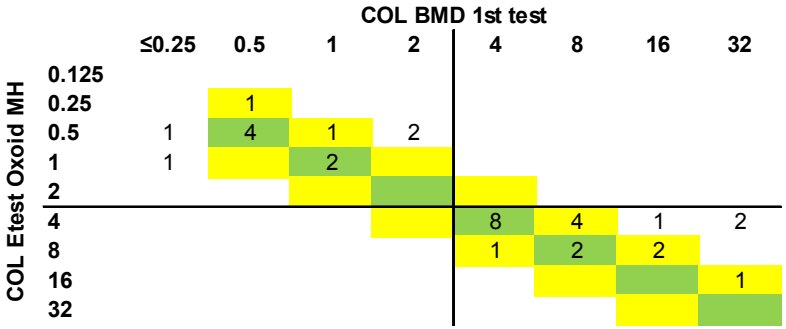


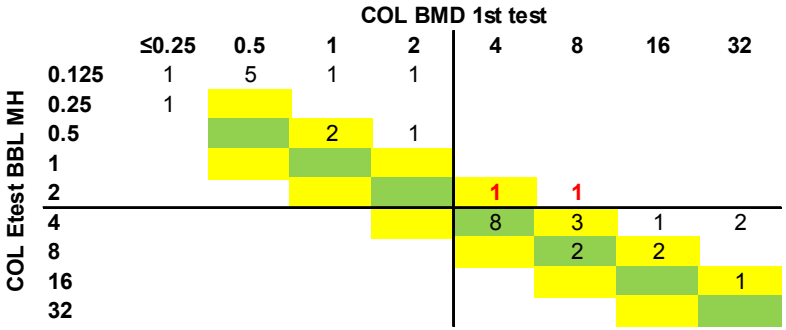
| Colistin MTS BBL MH | COL BMD ≤0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 |
|---|---|---|---|---|---|---|---|---|
| 0.125 | | | | | | | | |
| 0.25 | | | | | | | | |
| 0.5 | 1 | | 1 | | | | | |
| 1 | 2 | 6 | 5 | 3 | 5 | | | |
| 2 | | | | | 5 | 3 | 1 | 2 |
| 4 | | | | | 5 | 7 | 1 | |
| 8 | | | | | | | 1 | 2 |
| 16 | | | | | | | | |
| 32 | | | | | | | | |
| 64 | | | | | | | | |

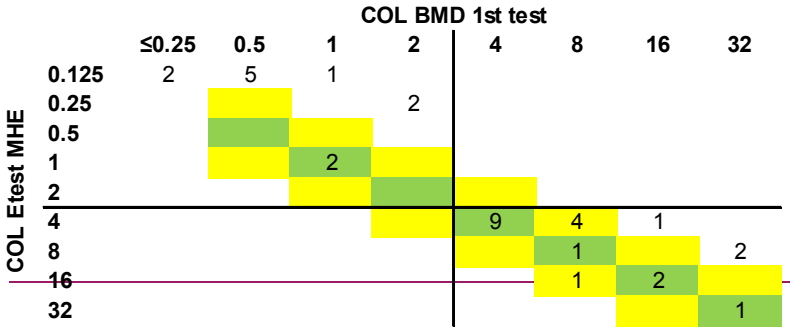
| | |
|---|---|
| Gradient = BMD | 10 |
| Within ± one dilution | 24 |
| Categorical error | 16 |

# *E. coli* (n=15) + *K. pneumoniae* (n=18)

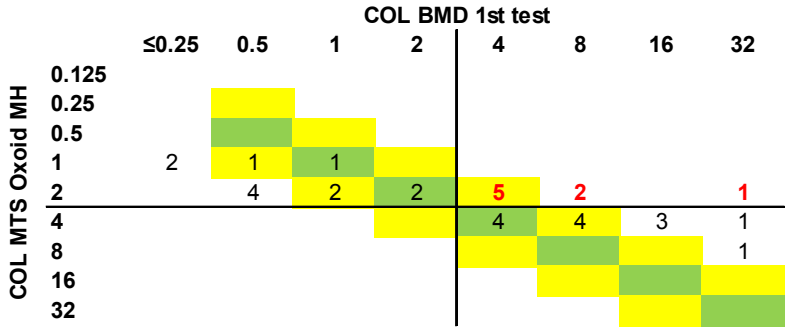
## Etest® (bioMérieux)

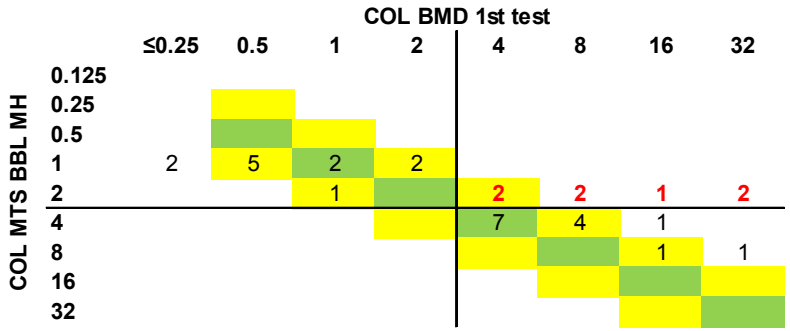


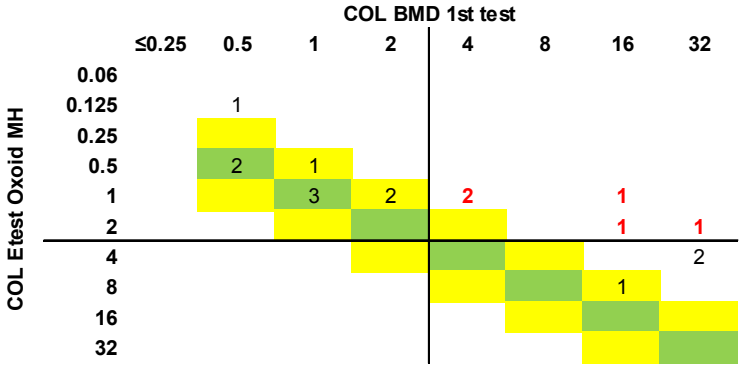

## MTS® (Liofilchem)

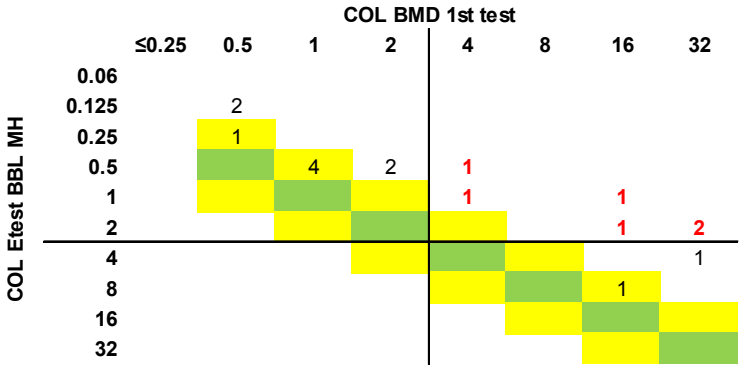


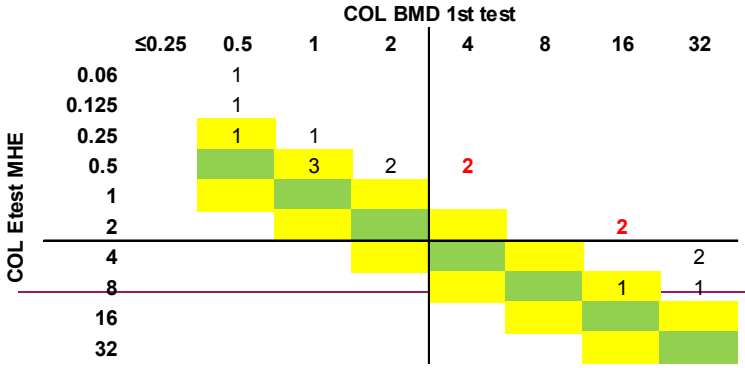



# *P. aeruginosa* (n=17)

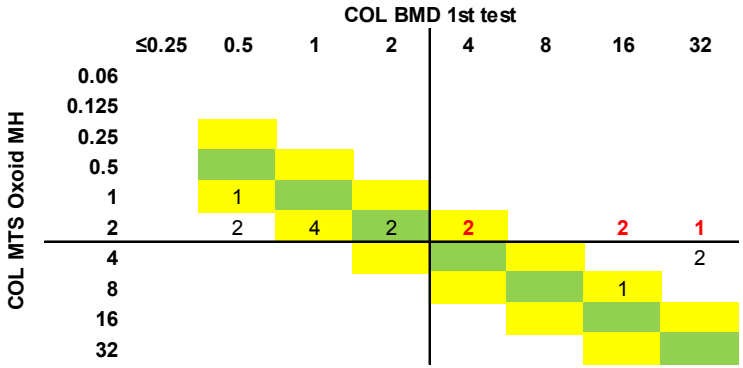
## Etest® (bioMérieux)

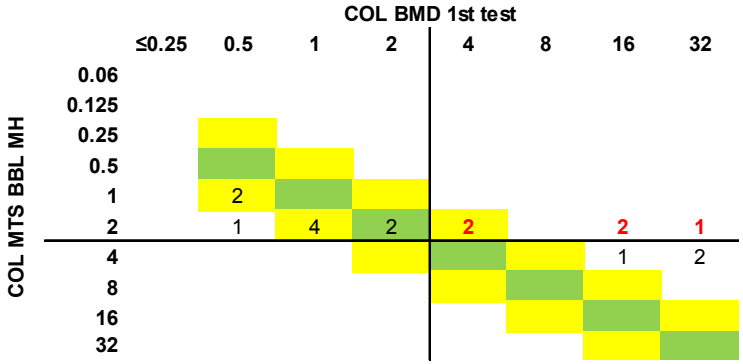


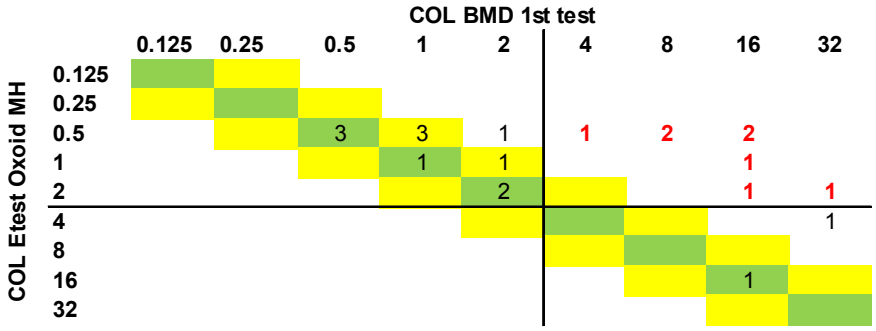

## MTS® (Liofilchem)

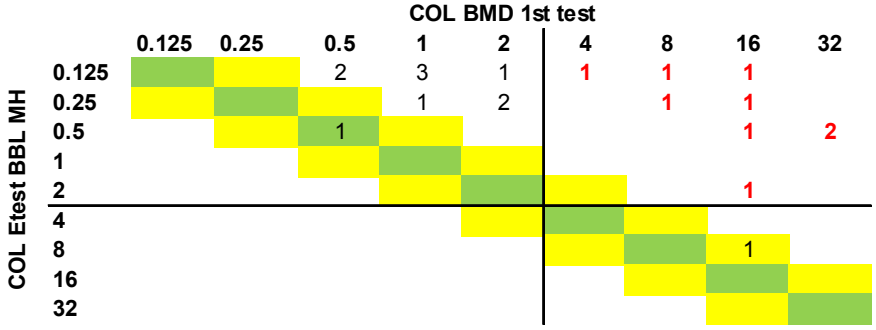


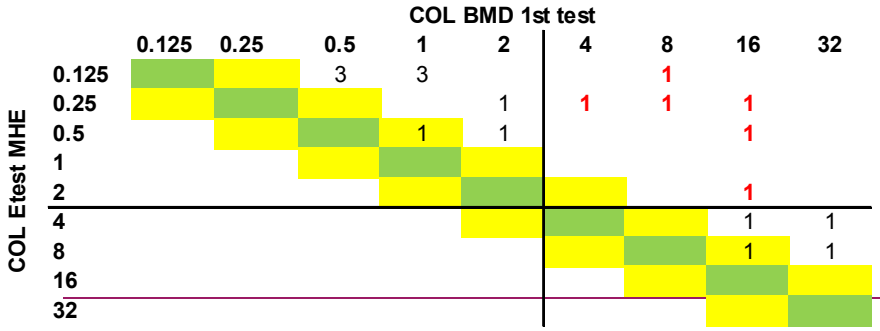



# *Acinetobacter* spp. (n=20)

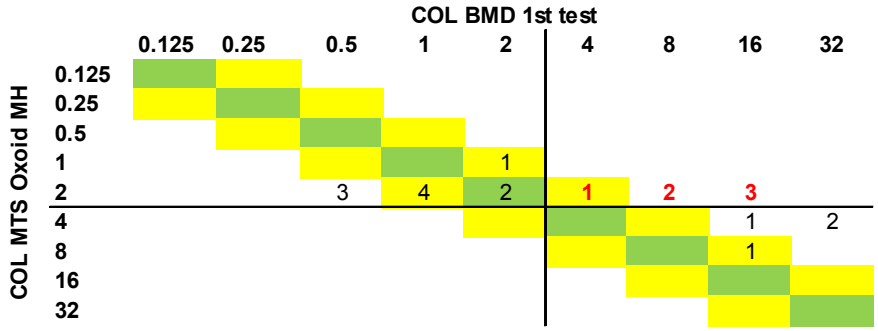
## Etest® (bioMérieux)

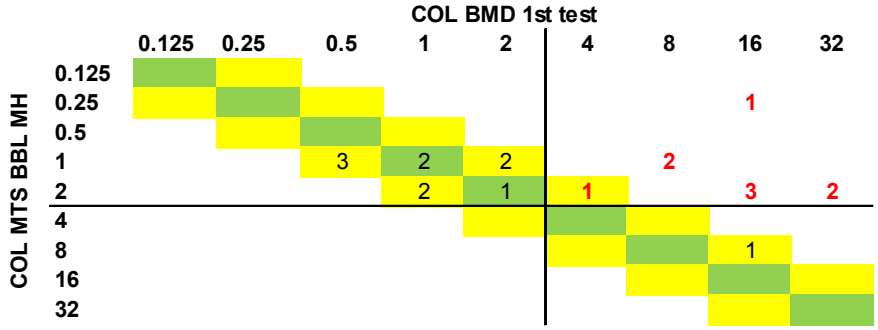


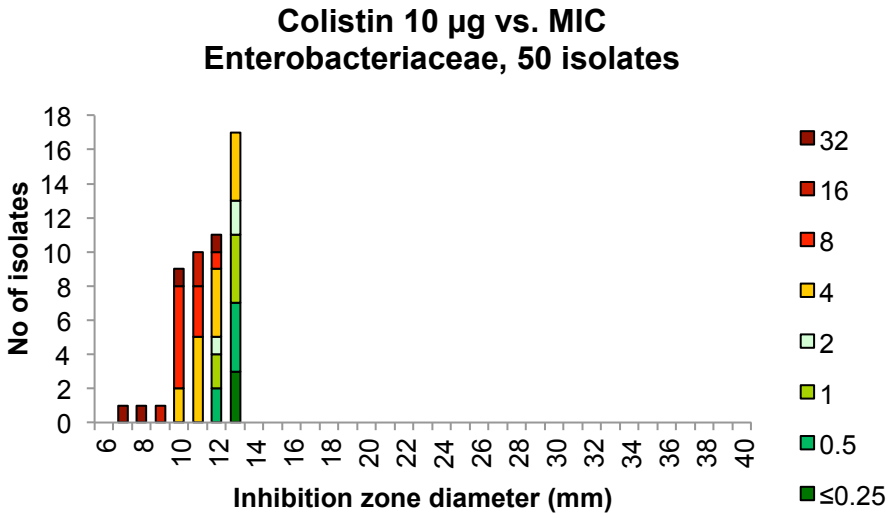



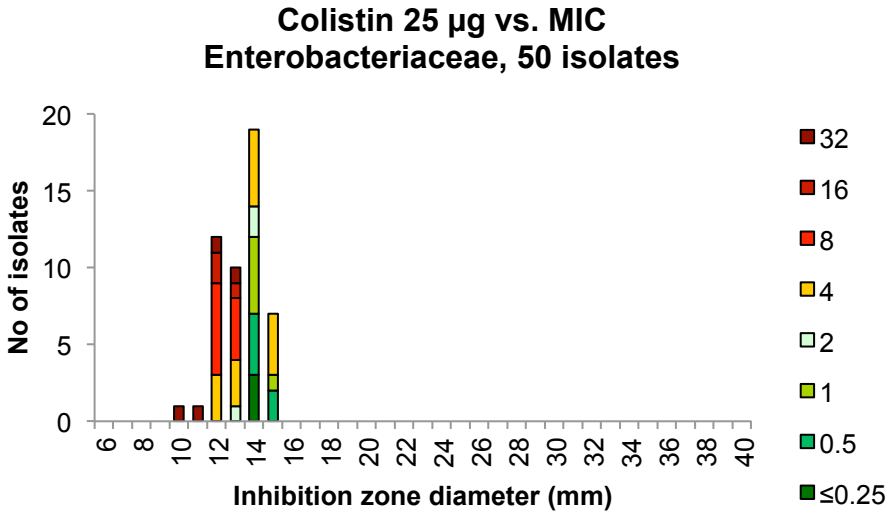

## MTS® (Liofilchem)

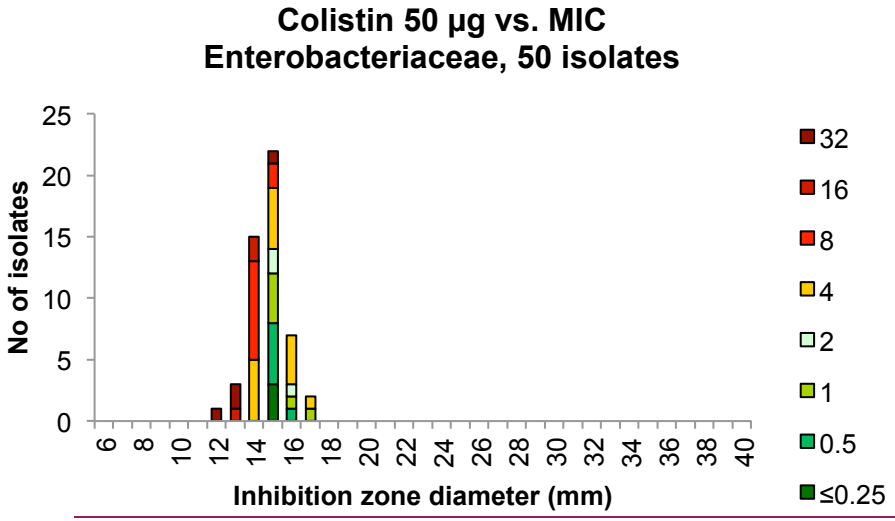


# Colistin disk diffusion




No difference between disks from Oxoid, BD and Mast observed (only data for Oxoid disks shown)

# Summary of results

| | Organism | *E. coli* and *K. pneumoniae* (n=32) | *P. aeruginosa* (n=21) | *Acinetobacter* spp. (n=22) | All isolates (n=75) |
|---|---|---|---|---|---|
| | Colistin MIC range (mg/L) | 0.25-32 | 0.25-128 | 0.5-32 | 0.25-128 |
| EA[1] | SEMPA1[4] | 27 | 19 | 20 | 66 (96%) |
| | MICRONAUT-S | 31 | 21 | 20 | 72 (96%) |
| | 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 | 10 | 13 | 47 (63%) |
| ME[2] | SEMPA1 | 1 | 1 | 2 | 4 |
| | MICRONAUT-S | 2 | 1 | 3 | 6 |
| | MIC-Strip | 2 | 0 | 0 | 2 |
| | 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 |
| VME[3] | SEMPA1 | 0 | 0 | 0 | 0 |
| | MICRONAUT-S | 0 | 2 | 0 | 2 |
| | 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 |

BMD-based

Gradient tests

Matuschek E et al. Poster ECCMID 2017

# Preliminary results automated systems

| Code | Organism | COL BMD 1 | COL BMD 2 | Vitek | Vitek 1 | Vitek 2 | Vitek 3 | Vitek 4 | Vitek 3 | Vitek 1 | Vitek 4 | Vitek 1 | Vitek 3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | Escherichia coli | 8 | 8 | 4 | 2 | 2 | 2 | 4 | 4 | 4 | 8 | 4 | 4 |
| 9 | Escherichia coli | 4 | 4 | 2 | 2 | 2 | 2 | 8 | 8 | 8 | 4 | 4 | 8 |
| 11 | Escherichia coli | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 14 | Escherichia coli | 4 | 4 | 2 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 19 | Escherichia coli | 4 | 4 | 4 | 4 | 8 | 4 | 8 | 8 | 8 | 8 | 4 | 8 |
| 26 | Klebsiella pneumoniae | 8 | 8 | 2 | 2 | 2 | 2 | 8 | 4 | 4 | 4 | 4 | 4 |
| 36 | Salmonella Dublin | 4 | 4 | 1 | ?0.5 | ?0.5 | ?0.5 | ?0.5 | ?0.5 | ?0.5 | 4 | 1 | 4 |
| 37 | Salmonella Dublin | 4 | 4 | ?0.5 | 2 | 1 | ?0.5 | ?0.5 | ?0.5 | ?0.5 | ?0.5 | ?0.5 | ?0.5 |
| 42 | Salmonella Dublin | 4 | 4 | ?0.5 | 2 | 2 | 2 | 1 | 2 | 2 | 1 | 1 | 1 |
| | **Laboratory** | | | **Firenze** | **Malta** | **Malta** | **Malta** | **Malta** | **Malta** | **Malta** | **Malta** | **Malta** | **Malta** |
| | Card | | | AST-N202 | AST-N222 | AST-N222 | AST-N222 | AST-N222 | AST-N222 | AST-N222 | AST-N222 | AST-N222 | AST-N222 |

| Code | Organism | COL BMD 1 | COL BMD 2 | Phoenix | Phoenix | MicroScan |
|---|---|---|---|---|---|---|
| 5 | Escherichia coli | 8 | 8 | 4 | 4 | >4 |
| 9 | Escherichia coli | 4 | 4 | 4 | 4 | >4 |
| 11 | Escherichia coli | 8 | 8 | 4 | 4 | >4 |
| 14 | Escherichia coli | 4 | 4 | 4 | 4 | >4 |
| 19 | Escherichia coli | 4 | 4 | 4 | 4 | >4 |
| 26 | Klebsiella pneumoniae | 8 | 8 | >4 | >4 | >4 |
| 36 | Salmonella Dublin | 4 | 4 | ?1 | ?1 | >4 |
| 37 | Salmonella Dublin | 4 | 4 | ?1 | ?1 | >4 |
| 42 | Salmonella Dublin | 4 | 4 | | ?1 | >4 |
| | **Laboratory** | | | **Leverkusen** | **Rigshosp CPH** | **Madrid** |
| | Card | | | NMIC-ID-402 | NMIC-417 | NC 53 |

? = ≤

# Work ongoing at Karolinska

- Challenge collection with 37 colistin resistant strains, 13 susceptible
- Agar dilution on MH and MHF containing colistin (1-6 mg/L range)
  - →1/50 had a VME (false susceptibility) – *P. aeruginosa* with MIC 1 mg/L with agar dilution and 4 mg/L with broth microdilution (repeat will be done)
  - →3/50 ME (false resistance) – will also be repeated (1 *A. baumannii*, 2 *P. aeruginosa*)
  - →No discrepancies were seen with Enterobacteriaceae
  - →New round of testing will be done with 3 mg/L as a tentative breakpoint agar
- Same collection tested with automated broth microdilution (ARIS, ThermoFisher)

# Warnings on www.eucast.org

- When AST products (disks, media, gradient tests etc.) do not perform to the expected standard
  → EDL informs the manufacturer and publish a warning on www.eucast.org

- EDL does not systematically test all products
  → The lack of a warning does not imply that there is no problem with the product in question

- Contact the EDL for advice if you suspect that problems with AST is related to a particular product

www.eucast.org



# EUCAST warnings concerning antimicrobial susceptibility testing products or procedures.

The EUCAST disk diffusion development laboratories, a network of laboratories coordinated from the EUCAST development laboratory in Växjö, Sweden, from time to time discover products (disks, media batches, gradient tests or procedures) which are not performing to the expected standard. When this is the case we inform the manufacturer and publish a warning on this page.

We do not systematically test all products so the lack of a warning does not imply that there is no problem with the product in question.

Laboratories which experience problems with a susceptibility test method, and suspect that this may be related to a particular product, may contact EUCAST for advice.

1. **Problems with piperacillin-tazobactam gradient tests from two manufacturers** - the warning issued 2015 will be removed when problem is resolved.
2. **Wide variation in disk quality in 16 disks from nine manufacturers** - the warning issued in 2015 and reiterated 2016 will be removed when issue is resolved.
3. **Problems with colistin gradient tests from both bioMérieux and Liofilchem** - the warning issued July 2016 will be removed when issue is resolved.

## 1. Problems with piperacillin-tazobactam gradient tests from two manufacturers.

*This warning, issued 2015, will be removed when problem is solved.*

Following reports of problems related to piperacillin-tazobactam gradient tests, several batches of tests from bioMérieux and Liofilchem (from both single packs and multipacks) were evaluated at the EUCAST Development Laboratory, Växjö, Sweden (January 2016). Enterobacteriaceae and Pseudomonas aeruginosa (type strains and strains with resistance mechanisms) and broth micro dilution were used.

Gradient tests from both manufacturers gave variable and unreliable results. The difference between gradient tests and broth micro dilution was not systematic, some values were two dilutions too high, others too low. There was also considerable variation between batches from the same manufacturer.

We urge manufacturers to seriously consider these problems and users to introduce internal quality control of each procedure. We have detected no reason

# Conclusions

- Colistin resistance is increasing, not the least in carbapenemase-producing Enterobacteriaceae
- Resistance so far mostly related to chromosomal resistance mechanisms
- Plasmid-mediated resistance observed initially in ESBL-producers, later in CPE
  → Likely to continue increasing in prevalence and thus significance
- Major problems in AST with gradient tests
- Uncertainties about performance of automated AST
- Disk diffusion has been reconfirmed to be inappropriate
- Agar MIC and automated broth microdilution under evaluation
  → Might be options for clinical labs
  → Commercial BMD strips are also available
- Reference labs are advised to implement BMD

# Acknowledgments


- EUCAST Development Laboratory (EDL) – Gunnar Kahlmeter and Erika Matuschek
- Jacob Östman and Anni-Maria Örmäla-Odegrip (own research group)
- Barbara Albiger and Dominique Monnet, ECDC