Emergence of colistin resistance in Gram-negative bacteria

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Polymyxins

- Lipopeptides
- Amphiphilic molecule (positively charge heptapeptide, tripeptide side, chain and terminal hydrophobic fatty acid chain)
- *Paenibacillus polymyxa* (Japan 1947)
- 5 major compounds (A to E):
  - polymyxin B
  - polymyxin E (colistin)

polymyxin E (colistin)

PM: 1200 D

polycationic Cyclic heptapeptide

Hydrophobic acetylated fatty acid tail

Tripeptide side chain
Colistin in brief

Polypeptidic antibiotic  
Family: Polymyxines

Polymyxine E (=colistine)  
Natural substance  
From *Bacillus polymyxa* var. *colistinus* in 1950

Therapeutic use starts in 1960’s

**Natural resistance**  
- All gram positive  
- *Proteus*  
- *Morganella morganii*  
- *Serratia marcescens*  
- *Yersinia pseudotuberculosis*  
- ...  

**Active against**  
- *E. coli*  
- *Salmonella* spp.  
- *Shigella* spp.  
- *Klebsiella pneumoniae*,  
  *K. oxytoca*  
- *Enterobacter cloacae*,  
  *E. aerogenes*  
- *Citrobacter*
Human health: colistin use

1960’s-1990’s: **Golden time for antimicrobial Therapy**

Colistin rapidly revealed toxic (nephrotoxic, neurotoxic, ...) → not used from 70’s

1990’s: **first patients with MDR Gram-**: colistin use revisited

≈ 2010: acquired-resistance to colistin reported in the literature (K. pneumoniae, E. coli)

2012: WHO classified colistin as critical for human health
Mode of action: alteration of the LPS

- Replacement of divalent cations stabilizing the LPS by large polycationic structures
  - Disorganization of the structure of LPS
  - Destabilization of the outer membrane leaflet
  - Increasing in permeability of the cytoplasmic membrane
  - Leakage of cytoplasmic content
  - Cellular death (bactericidal activity)

-Concentration dependent activity; PK/PD parameters: Cmax/CMI; AUC/CMI
Mechanisms of resistance to colistin

LPS modifications:
- pEtN
- L-Ara4N
- Lipid deacylation
- Lipid acylation
Acquired resistance to colistin

- Modification of LPS by *chromosomal encoded* resistance mechanisms
  - Complex regulation pathways of LPS modification in Gram-negative
  - Several genes/operons involved in modification of LPS (addition of cationic groups)

- Mutations in two-component systems or deletions/insertions in regulators
  - Increased expression of proteins adding cationic groups
  - Diminution of negative charge of LPS leads to decrease affinity of colistin to LPS and to resistance

- Overexpression of efflux pump systems
- Capsular trapping of colistin

⇒ NO STRAIN TO STRAIN TRANSFER
⇒ LOW RISK OF DISSEMINATION

From L. Poirel et al. Clin Microbio Rev 2017

Addition of 4-Amino-4deoxy-L-ARA leads to higher resistance rate than addition of pEtn
Since November 2015, acquired resistance to colistin involved also plasmid mediated resistance.

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

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Lancet Infect Dis

MCR-1
Plasmid encoded phosphoethanolamine transferase

Mcr-1 plasmid transferable between several species (high-frequency)
Plasmid-mediated resistance to colistin

The Lancet Infectious Diseases (Nov. 18th, 2015)

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

Yi-Yin Liu*, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Yong Zheng, James Spencer, Yuehe Dai, Guobing Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchen Lv, Dandan He, Hongwei Zhou, Zixin 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.

Methods The mcr-1 gene in E coli strain SHP45 was identified by whole plasmid sequencing and subcloning. MCR-1 mechanistic studies were done with sequence comparisons, homology modelling, and electrospray ionisation mass spectrometry. The prevalence of mcr-1 was investigated in E coli and Klebsiella pneumoniae strains collected from five provinces between April, 2011, and November, 2014. The ability of MCR-1 to confer polymyxin resistance in vivo was examined in a murine thigh model.

Findings Polymyxin resistance was shown to be singularly due to the plasmid-mediated mcr-1 gene. The plasmid carrying mcr-1 was mobilised to an E coli recipient at a frequency of 10⁻¹ to 10⁻² cells per recipient cell by conjugation, and maintained in K pneumoniae and Pseudomonas aeruginosa. In an in-vivo model, production of MCR-1 negated the efficacy of colistin. MCR-1 is a member of the phosphoethanolamine transferase enzyme family, with expression in E coli resulting in the addition of phosphoethanolamine to lipid A. We observed mcr-1 carriage in E coli isolates collected from 78 (15%) of 523 samples of raw meat and 166 (21%) of 804 animals during 2011–14, and 16 (1%) of 1322 samples from inpatients with infection.

Interpretation The emergence of MCR-1 heralds the breach of the last group of antibiotics, polymyxins, by plasmid-mediated resistance. Although currently confined to China, MCR-1 is likely to emulate other global resistance mechanisms such as NDM-1. Our findings emphasize the urgent need for coordinated global action in the fight against pan-drug-resistant Gram-negative bacteria.

15-20% in animals (Pigs/Chicken) 5-25% in food animal products <1% in hospital patients

Table 2: Prevalence of colistin resistance gene mcr-1 by origin
Specimens:
- Human
- Cattle
- Piglets
- Swines
- Companion animals
- Vegetables
- Animal feed
- Water
- Environment
- Wild birds

Number of isolates:
- 1
- 2-5
- 6-10
- 11-50
- 51-100
- >100

1st August 2016
Dortet et al. J. Antiinfectieux 2016
...and other colistin-resistance mcr-1 resistance gene variants

**mcr-2 in animals (pigs)**
- 76% nucleotide identity with mcr-1
- in Belgian in porcine coli-R *E. coli*
- Located on a transferable plasmid IncX4
- harboured on a mobile element (IS1595)
- likely originating from *Moraxella* spp.

**mcr-1.2 in humans**
- 99% nucleotide identity with mcr-1
- KPC-3 producing *K. pneumoniae* (ST 512)
- Surveillance culture (rectal swab) in a hospitalized patient
- IncX4 plasmid (very similar to mcr-1)
- found in *E. coli* and *K. pneumoniae* in distant geographic areas
Diversity of mcr-1 plasmids among human Enterobacteriaceae isolates in Belgium

Characterization of mcr-1 plasmids from 6 E. coli and 4 Salmonella enterica human strains isolated at 5 belgian laboratories, period 2014-2017

- IncHI2, IncX4 or an IncA/C plasmid background
- Similar mechanisms of mcr-1 acquisition for the different replicon types (remnants of ISApl1 or associated repeat regions found in each mcr-1 operon).

P.-J. Ceyssens et al. ISP, ECCMID 2017
Resistance to colistin

Resistant Status and Evolution Trends of *Klebsiella pneumoniae* Isolates in a University Hospital in Greece: Ineffectiveness of Carbapenems and Increasing Resistance to Colistin

I.K. Neonakis, G. Samonis, H. Messaritakis, S. Baritaki, A. Georgiladakis, S. Maraki, D.A. Spandidos

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>1996 (n = 114)</th>
<th>1997 (n = 105)</th>
<th>1998 (n = 126)</th>
<th>2005 (n = 143)</th>
<th>2006 (n = 194)</th>
<th>2007 (n = 293)</th>
<th>2008 (n = 329)</th>
<th>Period A value¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>10</td>
<td>19</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>IPM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>14</td>
<td>26</td>
<td>29</td>
<td>50</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

RESEARCH NOTE

Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients

CR pathogen *Klebsiella pneumoniae* 30 (20) 1 5 1 7 (23)

Resistance to colistin

Resistence Status and Evolution Trends of *Klebsiella pneumoniae* Isolates in a University Hospital in Greece: Ineffectiveness of Carbapenems and Increasing Resistance to Colistin

... also in Italy

SURVEILLANCE AND OUTBREAK REPORTS

Ongoing spread of colistin-resistant *Klebsiella pneumoniae* in different wards of an acute general hospital, Italy, June to December 2011

C. Mammina (caterina.mammina@unipa.it), C. Bonura, F. Di Bernardo, A. Aleo, T. Fasciana, C. Sodano, M. A. Saporito, M. S. Verde, R. Tetamo, D. M. Palma

1. Department of Sciences for Health Promotion G D' Alessandro, University, Palermo, Italy
2. Laboratory of Clinical Microbiology, ARNAS General Hospital Cívico, di Cristina e Benfratelli, Palermo, Italy
3. II Intensive Care Unit, ARNAS General Hospital Cívico, di Cristina e Benfratelli, Palermo, Italy

Colonization and infection by colistin-resistant Gram-negative bacteria in a cohort of critically ill patients

<table>
<thead>
<tr>
<th>CR pathogen</th>
<th>Colonization, No (%)</th>
<th>Infection</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>30 (20)</td>
<td></td>
</tr>
<tr>
<td>VAP</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>BSI</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>7 (23)</td>
<td></td>
</tr>
</tbody>
</table>


- **COL-R rate higher in KPC KP** (19%) than in OXA-48 KP (8%); \(p<0.001\)
- Global increase of I/R rates in KP from 2014 to 2015 for:
  - **COL-R** (6% to 14%; \(p=0.007\)) especially in **KPC KP** (\(p<0.001\))

Huang et al., AST data of CPE - RICAI Paris, Dec. 2015

Coli R > 2 µg/ml
Plasmid-mediated resistance to colistin in CPE from humans in Belgium

SURVEILLANCE AND OUTBREAK REPORT

Increasing proportion of carbapenemase-producing Enterobacteriaceae and emergence of a MCR-1 producer through a multicentric study among hospital-based and private laboratories in Belgium from September to November 2015

TD Huang¹, P Bogaerts¹, C Berhin¹, M Hoebek¹, C Bauraing¹, Y Glupczynski¹, on behalf of a multicentre study group²
1. National Reference Laboratory for Antibiotic-Resistant in Gram-negative bacilli, CHU UCL Namur, Université Catholique de Louvain (UCL), Belgium
2. The members of the group are listed at the end of the article

First OXA-48 E. coli MCR-1 detected from a wound sample of a 43y old patient hospitalized in a Belgian hospital in September 2015

### MDRO with acquired resistance to colistin in Belgium tested for mcr-1

Retrospective testing of 129 colistin-resistant gram-negative isolates received at the NRC during 2014-2015 (2 OXA-48 positive *E. coli* strain mcr-1 positive)

<table>
<thead>
<tr>
<th>Species</th>
<th>N tested</th>
<th><em>mcr-1</em> POS</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>K. pneumoniae</em></td>
<td>74</td>
<td>0</td>
<td><em>PmrA/PmrB, PhoP/PhoQ mutations</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>mgrB</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(mutation/délétion/IS)</td>
</tr>
<tr>
<td><em>K. oxytoca</em></td>
<td>4</td>
<td>0</td>
<td><em>mgrB</em> WT</td>
</tr>
<tr>
<td><em>E. cloacae</em></td>
<td>8</td>
<td>0</td>
<td><em>mgrB</em> WT</td>
</tr>
<tr>
<td><em>E. aerogenes</em></td>
<td>2</td>
<td>0</td>
<td><em>mgrB</em> WT</td>
</tr>
<tr>
<td><strong>E. coli</strong></td>
<td><strong>25</strong></td>
<td><strong>2</strong></td>
<td><em>PmrA/PmrB, PhoP/PhoQ mutations</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>mgrB</em> WT</td>
</tr>
<tr>
<td><em>C. freundii</em></td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp.</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>12</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

P. Bogaerts et al. Poster P0692
ECCMID 2017
### MCR-1 positive Enterobacteriaceae isolates confirmed at the NRC (2014-Q1/2017)

<table>
<thead>
<tr>
<th>LIS Nr</th>
<th>DATE OF SAMPLE</th>
<th>SPECIMEN ID</th>
<th>LABORATORY</th>
<th>SPECIES</th>
<th>AMPI</th>
<th>AMOX/CLAV</th>
<th>TEMO</th>
<th>PIP/TAZO</th>
<th>CEFUR</th>
<th>CFOX</th>
<th>CTRX</th>
<th>AZTR</th>
<th>CEFEP</th>
<th>AMK</th>
<th>GEN TA</th>
<th>CIPRO</th>
<th>TRI. MS/MX</th>
<th>PCR mcr-1</th>
<th>Resistance mech</th>
</tr>
</thead>
<tbody>
<tr>
<td>1052592401</td>
<td>03/05/14</td>
<td>CNR20140385</td>
<td>A</td>
<td>E. coli</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>I</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>mcr-1 CPE (OXA-48)</td>
</tr>
<tr>
<td>1111989601</td>
<td>15/02/16</td>
<td>COL20160015</td>
<td>B</td>
<td>E. coli</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>mcr-1 CPE (KPC)</td>
</tr>
<tr>
<td>1113836501</td>
<td>03/03/16</td>
<td>COL20160017</td>
<td>C</td>
<td>Salmonella spp.</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>I</td>
<td>R</td>
<td>mcr-1 PENICILLINASE (TEM)</td>
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<tr>
<td>1149142201</td>
<td>29/08/2016</td>
<td>COL20170063</td>
<td>F</td>
<td>E. coli</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
<td>R</td>
<td>mcr-1 PENICILLINASE (TEM)</td>
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<td>06/11/2016</td>
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<td>S</td>
<td>mcr-1 PENICILLINASE (TEM)</td>
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<td>CNR20170093</td>
<td>D</td>
<td>E. coli</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
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<td>S</td>
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<td>CNR20170238</td>
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<tr>
<td>1157142301</td>
<td>16/04/17</td>
<td>CNR20170381</td>
<td>E</td>
<td>E. coli</td>
<td>R</td>
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<td>S</td>
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<td>S</td>
<td>S</td>
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<tr>
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<td>11/04/17</td>
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<td>E. coli</td>
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<td>S</td>
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<td>S</td>
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<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

Data from the NRC for antimicrobial resistant Gram-negative bacilli (May 2017)
Guidelines for AST to colistin

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.

Published on www.eucast.org 22 March 2016
Colistin breakpoints according to CLSI and EUCAST

<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td></td>
<td>MIC</td>
<td>Diameter</td>
<td>MIC</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>≤ 2</td>
<td>&gt;2</td>
<td>—</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>≤ 2</td>
<td>&gt;2</td>
<td>—</td>
</tr>
<tr>
<td>Acinetobacter spp*</td>
<td>≤ 2</td>
<td>&gt;2</td>
<td>—</td>
</tr>
</tbody>
</table>

†No clinical breakpoints but epidemiological cut-off values (ECV): applies only to specific species (E. aerogenes, E. cloacae, E. coli, K. pneumoniae)

No zone diameter breakpoints anymore in 2017 (*Use a MIC method*)

EUCAST recommend a susceptible QC strain (*E. coli* ATCC 25922 or *P. aeruginosa* ATCC 27853) and one resistant QC strain (NCTC13846; *mcr-1* positive) for colistin testing
Available methods for testing colistin resistance

1) **Dilution methods**
   - macrodilution
   - microdilution (commercially available systems, Sensititre plates, MIC strip tests)
     (>95% EA and CA with ISO BMD reference method)

2) **Automated systems**
   - VITEK, Phoenix (+/-95% EA, few studies)
     *(Not FDA validated for testing colistin)*

3) **Phenotypic qualitative methods**
   - **Rapid Polymyxin NP test (2-4 3):** Sensitivity: 99%, Specificity: 95% *(for Enterobacteriaceae)*; direct detection of colistin-resistance also possible from blood culture
   - **SuperPolymyxin selective culture medium:** direct detection of carriage from clinical specimens; sensitivity: 100%, specificity: 100%

4) **Genotypic molecular methods**
   - Commercial molecular methods for detection of plasmid mediated resistance genes *(mcr-1)*
   - In house PCR protocols *(mcr-1, mcr-1.2, mcr-2)*

   **Disk diffusion and MIC gradient strip methods not recommended (CLSI/EUCAST joint working group)**
Comparison of MIC methods for Colistin AST for Gram-negative bacteria

Comparative evaluation of 5 commercial MIC methods (BMD plates/strips, gradient MIC tests)

Table 1. Essential and categorical agreements for colistin MIC tests for 76 Gram-negative bacteria with MICs on frozen broth microdilution panels as reference.

<table>
<thead>
<tr>
<th>Organism</th>
<th>E. coli and K. pneumonia</th>
<th>P. aeruginosa</th>
<th>Acinetobacter</th>
<th>All isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensititre custom plate</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MICRONAUT-T5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MICRONAUT MIC-Strip</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

- The total number of tests for calculation of EA was 28 for E. coli K. pneumonia and 10 for P. aeruginosa due to truncation at 0.25 and 1.0 mg/L.

**Results**

Essential and categorical agreements for the five methods are shown in Table 1. The correlation with reference MICs was good for all BMD methods but poor for gradient tests (Figure 1). Skipped wells occurred occasionally on all BMD panels and resulted in unreliable results unless retested. The BMD methods tended to overcall resistance to a small extent, resulting in a few major errors. Gradient tests generally underestimated MICs, resulting in a significant number of false susceptible results (very major errors). For Elast., very major errors were more abundant for P. aeruginosa and Acinetobacter spp. than for E. coli and K. pneumonia.

For BMD methods, all QC results were within ranges, except for one reading below the range for MICRONAUT MIC-Strip with E. coli ATCC 25922 (Table 2). All MICs for MTS were within range for both QC strains. All Elast MICs were out of range for E. coli ATCC 25922 on BBL and MH agar, and below range or in the lower part of the range for P. aeruginosa ATCC 27853. For E. coli ATCC 13846, all MICs were within 1.1 dilution of the expected 4 mg/L.

Table 2. Quality control results per MIC method and QC strain.

<table>
<thead>
<tr>
<th>QC strain</th>
<th>E. coli ATCC 25922</th>
<th>P. aeruginosa ATCC 27853</th>
<th>P. aeruginosa ATCC 27853</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Expected MIC</strong></td>
<td>4 mg/L</td>
<td>8 mg/L</td>
<td>16 mg/L</td>
</tr>
<tr>
<td><strong>MIC</strong></td>
<td>4 mg/L</td>
<td>8 mg/L</td>
<td>16 mg/L</td>
</tr>
<tr>
<td><strong>Result</strong></td>
<td>4 mg/L</td>
<td>8 mg/L</td>
<td>16 mg/L</td>
</tr>
</tbody>
</table>

**Conclusions**

The commercial BMD methods reliably determined colistin MICs when no skipped wells were present. The correlation between gradient tests and reference MICs was poor, even when QC results were within range. This was probably related to the poor dilution of colistin in agar. Based on the results of this study, EUCAST recommends laboratories to use BMD methods for colistin MIC determination and advice against the use of gradient tests at this point.

For more information, please contact erika.matuschek@ecsmid.org

**Highest level of correlation between reference BMD and commercial BMD plates/strips**

Skip wells observed with BMD -> unreliable results need of retesting (5-10%)

**Poor results with gradient tests** (large number of VME (false-susceptible results)

Matuschek et al., ECCMID 2017
Laboratory detection of colistin resistance in Belgium

**K. pneumoniae CNR20150622: KPC-3**

Belgian National EEQ2017/2 (n=110 labs)

Colistin MIC: 16 µg/ml (RESISTANT)
Non sense mutation in *mgrB* (Y41 STOP)
PCR mcr-1/mcr-2: NEGATIVE

Categorized as Colistin-R by 104/110 respondents (94.5%)

- One third of labs use a non Recommended testing method
- A minority (5%) uses MBD method (Sensititre)

6 labs reporting false-susceptible results (VME):
- disk diffusion (n=5); Automate (Phoenix) (n=1)

With permission from K. Vernelen
Belgian National EQC (ISP-WIV)
Conclusions

- Avoid/Limit polymyxin use in animals
- Optimize clinical use in humans (indications, posology/drug monitoring)
- Avoid dissemination of MCR-producing isolates almost at the hospital level (MDRO):
  - Identify colonized patients (risk factors)
  - Single room
  - Dedicated staff

- Microbiologists challenge
  - Importance of monitoring colistin-R
  - Testing methodologies have to be consolidated
  - Development of accurate rapid tools for the detection of colistin resistant isolates (and also of mcr-producers)