The emergence of $mcr-1$ in *Salmonella* and *E. coli*

Sophie Granier
NRL-AR France
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*
- …
Colistin use and resistance

Before November, 18th, 2015
Microbiologist: Colistin tool

- Comfortable tool to confirm bacterial identification
- Susceptibility test unreliable
  - bad diffusion in agar
  - adhesion to plastic
- What does resistance look like?

Before November, 18th, 2015

Serratia marcescens
Cockade image
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

Before November, 18th, 2015
Colistin resistance mechanisms are chromosomal
Animal health: Colistin use

Before November, 18th, 2015
Animal health: Colistin use

- Sales
- Resistance
- Expert elicitation data

Colibacilloses, salmonellosis

Poultry
++ : chicken, laying hens, digestive disorders
++++ : turkeys, digestive disorders

Pigs
++++++ : post-weaning, digestive disorders + edema
+++ : before weaning & fattening, digestive disorders

Cattle
+++ : associated with penis, gynecology (theriogenology)
++++ : veal calves, digestive disorders

Rabbits
++ : maternity, digestive disorders
++++ : fattening, digestive disorders

Before November, 18th, 2015
Le directeur général

Maisons-Alfort, le 23 septembre 2015

AVIS
de l’Agence nationale de sécurité sanitaire de l’alimentation, de l’environnement et du travail

relatif à la saisine 2015-SA-0118 concernant les antibiotiques critiques pour la santé humaine et animale

The colistin exception
November, 17th, 2015

Préservons l’efficacité des antibiotiques, ensemble !

La colistine, quant à elle, ne doit pas être considérée comme critique, car, à ce jour, les mécanismes de résistances observés vis-à-vis de cet antibiotique ne sont pas portés par des éléments génétiques transférables. De même, le taux de résistance constaté reste raisonnable, « ne mettant pas en péril l’efficacité de cette molécule en médecine humaine ». Pour ces raisons, sa criticité n’a pas été retenue. Cependant, l’Anses estime nécessaire de surveiller l’évolution de la résistance à cet antibiotique.

Agreement on colistin exception

Before November, 18th, 2015
Salmonella Antimicrobial resistance French Surveillance 2012
Resistance ou Artefact?

Hattie E. Webb

TEXAS TECH UNIVERSITY
### French *Salmonella* Network vs. EFSA AMR Surveillance

<table>
<thead>
<tr>
<th>French <em>Salmonella</em> Network</th>
<th>EFSA AMR Surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td>≈3,500 - 4,000 isolates / year</td>
<td>Randomly select 170 isolates from each: - layers  - broilers  - turkeys</td>
</tr>
<tr>
<td>no sample scheme</td>
<td></td>
</tr>
<tr>
<td>Food animal samples</td>
<td>Farm level environmental samples</td>
</tr>
<tr>
<td>Samples are voluntary based from ≈150 public and private labs in France</td>
<td>Regulated at a European level</td>
</tr>
<tr>
<td>Disk-diffusion</td>
<td>Micro-broth dilution</td>
</tr>
</tbody>
</table>

**2012-2013 databases ➔ 27 presumptive colistin-resistant isolates**
We identified five strains that we believe are TRULY resistant to colistin.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Sample type</th>
<th>Description</th>
<th>Sample date</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>4,12:i:- **</td>
<td>animal health surveillance</td>
<td>boot swabs from chicken facility</td>
<td>OCT 28, 2013</td>
<td>Dept. 01</td>
</tr>
<tr>
<td>Derby</td>
<td>food</td>
<td>chipolata sausage</td>
<td>JAN 03, 2013</td>
<td>Dept. 62</td>
</tr>
<tr>
<td>Paratyphi B</td>
<td>food</td>
<td>guinea fowl pie (before cooked)</td>
<td>OCT 16, 2012</td>
<td>Dept. 56</td>
</tr>
<tr>
<td>Paratyphi B</td>
<td>food</td>
<td>chicken breast with skin</td>
<td>JUN 05, 2012</td>
<td>Dept. 85</td>
</tr>
<tr>
<td>Schwarzengrund</td>
<td>animal health surveillance</td>
<td>boot swab from broiler farm</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**confirmed as monophasic variant of Typhimurium by PCR
PAST RESEARCH – COLISTIN RESISTANCE

©Hattie E. Webb, 2013
Salmonella Antimicrobial resistance French Surveillance 2012-2013

5 colistine R Salmonella isolates from 8684 analyses

Which mechanisms?
Strategy: WGS

Hattie E. Webb, PhD candidate

Texas Tech University
Characterization of colistin resistance mechanisms in Salmonella of French origin

Background:
Colistin became available for clinical use in 1959. Human medicine abandoned colistin use shortly after due to initial reports of renal toxicity. Detectable resistance in Europe has continuously used colistin.

Colistin has been rediscovered in human medicine and is of the few effective drugs for treating the prevailing MDR gram-negative infections. Consequently, there is a need to understand the use of colistin in veterinary medicine to preserve its efficacy for human medicine.

Objectives:
- Identify truly colistin-resistant Salmonella strains isolated from food animal products and their environments in France.
- Characterize the regions of the genome mediating colistin resistance.

Methods:
- Microbiological and molecular analysis.
- Genotypic characterization.

Results:
- Colistin resistance is rare in Salmonella.
- No mutations found within the PhoPQ and PmrAB two-component systems of the five colistin-resistant strains.
- Future plans: Identify genetic changes between colistin-resistant and -susceptible strains that may be conferring colistin resistance.

Conclusions:
- Colistin resistance is rare in Salmonella.
- No mutations found within the PhoPQ and PmrAB two-component systems of the five colistin-resistant strains.
- Future plans: Identify genetic changes between colistin-resistant and -susceptible strains that may be conferring colistin resistance.

Hattie E. Webb, PhD candidate
Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study


Summary
Background Until now, polymyxin resistance has involved chromosomal mutations but has never been reported to be due to 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, harbouring a plasmid 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. All isolates were serotyped, andin all cases the plasmid-encoding the mcr-1 gene were found to be conjugative. 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 pig meat at retail and in Chinese poultry and swine farms between April, 2011, and November, 2014. The ability of MCR-1 to confer polymyxin resistance in vivo was studied by examining the susceptibility of mcr-1-positive E. coli to colistin in a murine thigh model.

Findings Polymyxin resistance was shown to be singularly due to the plasmid-mediated mcr-1 gene. The plasmid was mobilised to an E.coli recipient at a frequency of $10^{-3}$ to $10^{-5}$ cells per recipient cell by conjugation and maintained in K pneumoniae and Pseudomonas aeruginosa. In an in-vivo model, production of MCR-1negatively affected the efficacy of colistin. MCR-1 is a member of the phosphoethanolamine transferase enzyme family, with expression of the mcr-1 gene 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 (1.4%) 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 emphasise the urgent need for coordinated global action in the fight against pan-drug-resistant Gram-negative bacteria.

### Escherichia coli

<table>
<thead>
<tr>
<th>Type</th>
<th>Year</th>
<th>Percentage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pigs at slaughter</td>
<td>2012</td>
<td>31 (14.4%)</td>
<td>216</td>
</tr>
<tr>
<td>Pigs at slaughter</td>
<td>2013</td>
<td>68 (25.4%)</td>
<td>268</td>
</tr>
<tr>
<td>Pigs at slaughter</td>
<td>2014</td>
<td>67 (20.9%)</td>
<td>320</td>
</tr>
<tr>
<td>Retail meat</td>
<td>All</td>
<td>78 (14.9%)</td>
<td>523</td>
</tr>
<tr>
<td>Chicken</td>
<td>2011</td>
<td>10 (4.9%)</td>
<td>206</td>
</tr>
<tr>
<td>Pork</td>
<td>2011</td>
<td>3 (6.3%)</td>
<td>48</td>
</tr>
<tr>
<td>Chicken</td>
<td>2013</td>
<td>4 (25.0%)</td>
<td>16</td>
</tr>
<tr>
<td>Pork</td>
<td>2013</td>
<td>11 (22.9%)</td>
<td>48</td>
</tr>
<tr>
<td>Chicken</td>
<td>2014</td>
<td>21 (28.0%)</td>
<td>75</td>
</tr>
<tr>
<td>Pork</td>
<td>2014</td>
<td>29 (22.3%)</td>
<td>130</td>
</tr>
<tr>
<td>Inpatient</td>
<td>2014</td>
<td>13 (1.4%)</td>
<td>902</td>
</tr>
</tbody>
</table>

### Klebsiella pneumoniae

<table>
<thead>
<tr>
<th>Type</th>
<th>Year</th>
<th>Percentage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inpatient</td>
<td>2014</td>
<td>3 (0.7%)</td>
<td>420</td>
</tr>
</tbody>
</table>
Colistin resistance: a major breach in our last line of defence

In hospital practice, clinicians have been hampered by the recent development of new antibiotics active against multiresistant Gram-negative bacilli. However, recently approved vancomycin-like 

collesterol-attachment or colistin-sensitive organisms do not provide activity against all Gram-negative bacilli, with notable gaps in their coverage, including the notorious New Delhi metallo-β-lactamase 5-producing organisms and many strains of carbapenem-resistant Acinetobacter baumannii. For this reason, the polymyxins (colistin and polymyxin B) remain the last line of defence against many Gram-negative bacilli. Colistin-resistant and even pan-drug-resistant Gram-negative bacilli have already been reported. Hypersusceptibility is due to chromosomally mediated modification of the imidazoles A, resulting in induced affinity for the polymyxins. Clones of colistin-resistant organisms have spread in some hospitals, but have not seriously affected the use of polymyxins. These resistance genes are generally not transmissible between bacteria and so have not disseminated widely.

In The Lancet Infectious Diseases, Yi-Yun Liu and colleagues describe plasmid-mediated colistin resistance for the first time. The implications of this finding are enormous. The investigators reported that the plasmid bearing the colistin resistance mechanism was readily passed between Escherichia coli strains, including strains with known epidemic potential, such as STEC. Furthermore, the plasmid could be passed to Deinococcus and pseudomonas aeruginosa strains. The plasmids were quite stable, implying that even in the absence of selective pressure by colistin, the plasmids would be maintained. It therefore seems inevitable that plasmid-mediated transfer of colistin resistance will seriously limit the lifespan of the polymyxins as the backbone of regimens against multiply resistant Gram-negative bacilli.

How did this come about? and is there anything we can do to limit the sale of spread of colistin resistance? Colistin has been used in agriculture since the 1950s. Indeed, in 2010 it was the fifth most sold group of antimicrobials used in agriculture in Europe. Historical data on its use in agriculture in Asia are limited. However, it is clear that its current use is substantial.

Liu and colleagues present data from China showing that E.coli from pigs at slaughter and from wild chickens and porc have high rates of plasmid-mediated colistin resistance. The same mechanism was found in E.coli and K.pneumoniae isolates from Chinese patients in hospital. These findings suggest that the links between agricultural use of colistin, colistin resistance in slaughtered animals, colistin resistance in food, and colistin resistance in human beings are now complete. One of the few solutions to uncoupling these connections is limitation of use of colistin in agriculture. This will require substantial political will and we call upon Chinese leaders to act rapidly and decisively. Failure to do so will create a public health problem of major dimensions.

In plasmid-mediated colistin resistance a purely Chinese phenomenon? A recent report has described colistin resistance in E.coli from a pig and a pens in Laos. The pig and human colistin-resistant E.coli isolates were indistinguishable by pulsed field gel electrophoresis suggesting animal to human transmission. No known chromosomally encoded colistin resistance mechanisms were identified in these isolates, raising the question as to whether they could also have unrecognised plasmid-mediated colistin resistance mechanisms. As noted by Liu and colleagues, E.coli bearing genes very similar to those than they describe causing plasmid-mediated colistin resistance have recently been detected in Malaysia.

Given that substantial use of colistin in agriculture is highly likely throughout southeast Asia, it would hardly be surprising that plasmid-mediated colistin resistance will soon be detected in this region. At least one manufacturer of colistin for agriculture is based in India, raising the spectre of untraceable NDMA-mediated colistin-resistant strains occurring in the Indian subcontinent.

In 2012, WHO reclassified colistin as critically important for human medicine. This classification remains true despite ongoing development of new antibiotics against multiply resistant Gram-negative bacilli. There have been previous calls for curtailing the use of polymyxins in agriculture. We must all reiterate these appeals and take them to the highest levels of government or face increasing numbers of patients for
Is plasmid-mediated colistin resistance a purely Chinese phenomenon?
Colistin resistance

Results

- 4 of 5 \textit{mcr-1}-positive
  - \textit{S. Schwarzengrund \textit{mcr-1}-negative}
- 100\% homology
- Contigs with the \textit{mcr-1} also include:
  - \textit{IncX4} replicon sequence
  - \textit{IncP} replicon sequence
- Little sequence similarity with plasmid described by Liu \textit{et al.}
Dissemination of the mcr-1 colistin resistance gene

In response to the Yi-Yun Liu and colleagues’ finding of a mobile genetic element responsible for colistin resistance, mcr-1 and the accompanying Comment asking “Is planned-mediated colistin resistance a genuinely Chinese phenomenon?”, we, and others,15-17 can now reply no. As part of routine surveillance, we screened ESBL Salmonella isolates collected during 2012–13 from the French agricultural food sector for colistin resistance using disk diffusion. Between October and December, 2012, 27 isolates that showed a reduced susceptibility to colistin (c; zone of inhibition ≤15 mm) were further assessed using a colistin concentration gradient assay. Five isolates (18%) had a distinctively different minimum inhibitory concentration (≥12 mg/ml) and were defined as colistin-resistant. In 2014, whole-genome sequencing of the five isolates was done and resultant sequences were assembled and interrogated for mutations and genetic elements associated with colistin resistance.

We identified mcr-1 in four of five phenotypically colistin-resistant isolates. Furthermore, mcr-1 was associated with plasmid DNA and on silver staining. Typing of some plasmids found to differ from those reported by Liu and colleagues (table). The isolates harboured a 16SrD gene sequence with 100% homology to the recently described mcr-1. Colistin resistance, although extraordinarily rare, was reported in epidemiologically, regionally, and geographically related Salmonella isolates, and, surprisingly, also seen in the O4 serogroup (serotypes Derby, Schwartzengrund, 1,4,[5,12]:X, and Paratyphi B). Whether the product of mcr-1, MCR-1, confers resistance in the limited number of lipopolysaccharide structures in which we have found mcr-1, the mechanism by which MCR-1 is able to confer resistance is unknown. We have now been identified outside Asia. Clearly, we describe its presence in an important foodborne pathogen recovered from food and animal environments and associated with well described phenotypic resistance by disk diffusion, broth microdilution, and combination (gradient) tests. Furthermore, mcr-1 has been associated with several plasmid types and has been found in Enterobacteriaceae, specifically, mcr-1, mcr-2, and mcr-3. This shows that the genetic diversity of the mcr-1 gene, as suggested by our sequence analysis, is widespread among the mcr-1 genotypes and is mobile. At least mcr-1, mcr-2, and mcr-3, all encode resistance to at least moderate strains of these plasmids and other bacteria, as possible, not probable. Interrogation of other horizontally transferred elements will provide a broader understanding of the probable distribution of this gene.
RAPID COMMUNICATIONS

Detection of mcr-1 encoding plasmid-mediated colistin-resistant Escherichia coli isolates from human bloodstream infection and imported chicken meat, Denmark 2015

H Hasman 1, AM Hammerum 1, F Hansen 1, RS Hendriksen 2, B Olesen 3, Y Agersø 4, E Zankari 5, P Leekitcharoenphon 4, M Stegger 1, RS Kaas 1, LM Cavaco 2, DS Hansen 3, FM Aarestrup 2, RL Skov 1
1. Department of Microbiology and Infection Control, Statens Serum Institut, Copenhagen, Denmark
2. National Food Institute, Technical University of Denmark, Lyngby, Denmark
3. Department of Clinical Microbiology, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark
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EDITORIAL

Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds

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2. Office of Chief Scientist, European Centre for Disease Prevention and Control, Stockholm, Sweden

Correspondence: Robert L. Skov (rsk@ssi.dk)

Citation style for this article:

Article submitted on 01 March 2016 / accepted on 03 March 2016 / published on 03 March 2016

Summarizing 28 « Me too! » publications
mcr-1 Geographic distribution (updated 5 April 2016)

→ Highly conserved mcr-1 gene
→ Genetic environment extremely diverse
Rapid communication
PREVALENCE OF mcr-1 IN COMMENSAL ESCHERICHIA COLI FROM FRENCH LIVESTOCK, 2007 TO 2014

A Perrin-Guyomard 1, M Bruneau 1, P Houët 1, K Deleurme 1, P Legrandois 1, C Poirier 1, C Soumet 1, P Sanders 1

+ Author affiliations

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Received: 04 February 2016; Accepted: 11 February 2016

Colistin resistance was investigated in 1,696 isolates collected from 2007 to 2014 within the frame of the French livestock antimicrobial resistance surveillance programme. The mcr-1 gene was detected in all commensal Escherichia coli isolates with a minimum inhibitory concentration to colistin above the 2 mg/L cut-off value (n=23). In poultry, mcr-1 prevalence was 5.9% in turkeys and 1.8% in broilers in 2014. In pigs, investigated in 2013, this prevalence did not exceed 0.6%. These findings support that mcr-1 has spread in French livestock.

<table>
<thead>
<tr>
<th>Year</th>
<th>Animals</th>
<th>E. coli strains tested for MIC N</th>
<th>E. coli strains resistant to colistin N</th>
<th>Proportion of mcr-1 positive (n) among colistin-resistant E. coli strains (N) n/N</th>
<th>Prevalence of mcr-1 positive E. coli strains % (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>Turkeys</td>
<td>239</td>
<td>14</td>
<td>14/14</td>
<td>5.9 (2.9–8.8)</td>
</tr>
<tr>
<td></td>
<td>Broilers</td>
<td>227</td>
<td>4</td>
<td>4/4</td>
<td>1.8 (0.1–3.5)</td>
</tr>
<tr>
<td>2013</td>
<td>Pigs</td>
<td>196</td>
<td>1</td>
<td>1/1</td>
<td>0.5 (0.0–1.5)</td>
</tr>
<tr>
<td></td>
<td>Broiler</td>
<td>193</td>
<td>3</td>
<td>3/3</td>
<td>1.6 (0.0–3.3)</td>
</tr>
<tr>
<td>2012</td>
<td>Pigs</td>
<td>194</td>
<td>0</td>
<td>N.a.</td>
<td>N.a.</td>
</tr>
<tr>
<td></td>
<td>Broiler</td>
<td>201</td>
<td>0</td>
<td>N.a.</td>
<td>N.a.</td>
</tr>
<tr>
<td>2011</td>
<td>Pigs</td>
<td>200</td>
<td>1</td>
<td>1/1</td>
<td>0.5 (0.0–1.5)</td>
</tr>
<tr>
<td>2007</td>
<td>Turkeys</td>
<td>ND*</td>
<td>ND*</td>
<td>0/246</td>
<td>0 (0.0–1.2)</td>
</tr>
<tr>
<td>Total</td>
<td>All</td>
<td>1,450</td>
<td>23</td>
<td>N.a.*</td>
<td>N.a.*</td>
</tr>
</tbody>
</table>

Ci: confidence interval; MIC: minimum inhibitory concentration; N.a.: not applicable; ND: not determined.

* As susceptibility to colistin was not tested in 2007, each isolate obtained in that year was tested for the presence of mcr-1.
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](http://www.eucast.org) 22 March 2016
b. No additives may be included in any part of the testing process (in particular, no polysorbate-80 or other surfactants)

Susceptibility Testing of the Polymyxins: Where Are We Now?

Romney M. Humphries

Antimicrobial susceptibility testing for the polymyxins—colistin and polymyxin B—is fraught with technical challenges. Key among these is the propensity of the polymyxins to adsorb to polystyrene, a material often used for in vitro minimum inhibitory concentration testing devices. This effect may be mitigated by the addition of a surfactant such as polysorbate 80; however, concern exists that polysorbate 80 may act synergistically with the polymyxins and artificially lower minimum inhibitory concentrations. Furthermore, the polymyxins diffuse poorly through agar, compromising the performance of both disk diffusion and Etest methods. Very few peer-reviewed studies have investigated in vitro susceptibility test methods for the polymyxins, and it is clear that an in vitro test that reliably predicts the activity of the polymyxins in vivo has yet to be defined. This review describes the methods available and challenges associated with susceptibility testing of colistin and polymyxin B and discusses the current breakpoints for both agents.

Keywords: polymyxins, susceptibility testing, broth microdilution.

(Pharmacotherapy 2014;***(**):***-***) doi: 10.1002/phar.1505

Surfactants such as P-80 to waters used for BMD is a f both commercial manufac-tories, as it is used with organisms in the test panel. A deat is frequently not ethods sections of published 80 is not mentioned in the rds Organization BMD doc uentes the rationale for form a exact interactions between P-80, the polymyxins, and the gram-negative cell wall.

Regardless, the CLSI subcommittee voted in January 2014 to pursue polymyxin BMD testing without surfactants, including P-80, over concerns for potential synergy between the two agents. The ramifications to this decision will depend largely on the clinical breakpoints established for the polymyxins in the coming years, in the impact of polymyxin resistance in BMD.
Microbiologist: detect *mcr-1*

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.

**PAST RESEARCH – COLISTIN RESISTANCE**

©Hattie E. Webb, 2013
A universal culture medium for screening polymyxin-resistant gram negatives

Patrice Nordmann, Aurélie Jayol, and Laurent Poirel

Table 1. Preparation of the SuperPolymyxin medium

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Stock solution</th>
<th>Quantity or volume to adda</th>
<th>Final concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMB agar powder</td>
<td>-</td>
<td>15 g</td>
<td>3.75 %</td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td>400 ml</td>
<td></td>
</tr>
<tr>
<td>Colistin sulfate</td>
<td>20 mg/ml in water in glass tubes</td>
<td>70 µl</td>
<td>3.5 µg/ml</td>
</tr>
<tr>
<td>Deptomycin</td>
<td>20 mg/ml in water</td>
<td>200 µl</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>20 mg/ml in D(+)glucose 10%</td>
<td>100 µl</td>
<td>5 µg/ml</td>
</tr>
</tbody>
</table>

*aThe volume of 400 ml of SuperPolymyxin medium was for i.e. twenty plates.

Figure 1. Polymyxin-resistant lactose-positive E. coli (A), polymyxin-resistant K. pneumoniae (B), polymyxin-resistant lactose-negative E. coli (C), and mix of a heavy inoculum of P. mirabilis and a low inoculum of polymyxin-resistant K. pneumoniae (C) growing on the SuperPolymyxin medium.
PCR for plasmid-mediated colistin resistance
(protocol optimized at Statens Serum Institut, Copenhagen)

December 2015
Version 1

Lina Cavaco, Rene Hendriksen

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, Yohi Dei, Guобоо Tian, Baolui Dong, Xianhui Huang, Lin-Feng Yu, Dania Gu, Hongwei Ren, Xiaojie Chen, Luchao Lu, Dandan He, Hongwei Zhou, Zixue Liang, Jian-Hua Li, Jianzhong Shen
ResFinder 2.1

ResFinder identifies acquired antimicrobial resistance genes in total or partial sequenced isolates of bacteria.
Fasta file with test sequence: Test_sequence
NOTE: Currently ResFinder focuses on acquired genes and does therefore not find chromosomal mutations (NAL, FUS, high-level CIP, RIF resistance, etc.)

View the version history of this server.

Database Updates

The ResFinder database download site

- 16-March-2016 New variants of aph-genes added to aminoglycoside
- 11-March-2016 Lager cleanup in the databases
- 01-March-2016 blaCTX-M-38 was chanced from AY753197 to AY822595 beta-lactamase.
- 26-Feb-2016 Names of Van genes in the glycopeptide database corrected.
- 03-Feb-2016 blaLEN11 was removed from database beta-lactamase.
- 22-Dec-2015 blaOXA-436 was added in database beta-lactamase.
- 09-Dec-2015 blaFRI-1 was added in database beta-lactamase.
- 25-Nov-2015 blaPAM-1, blaTMB-1 and blaTMB-2 was added in database beta-lactamase.
- 24-Nov-2015 Colistin databases created containing the gene mcr-1 colistin.
Conclusion
Colistin resistance and public health: a change of paradigm?

Human Use
→ Critically important for human health?

Veterinary Use
→ Risk of therapeutic failure?
→ Critically important for animal health?

Microbiologist: Colistin challenge
→ Monitoring colistin R is important!
→ Methodologies still to be consolidated
Dr Hattie E. Webb                 Guy Loneragan

Pôle Antibiorésistance – LNR résistance antimicrobienne
Réseau Salmonella
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