



PLASMID OVERFØRBART COLISTINRESISTENS HOS ENTEROBACTERIACEAE

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- Colistin is an increasingly important antimicrobial for human medicine due to the increase of carbapenem resistant organisms

Antibiotika	KPC	MBL	OXA-48
Meropenem	+/-	+/-	+/-
Temocillin	+/-	-	-
Ceftolozan-tazobactam	-	-	-
Ceftazidim-avibactam	++	-	++
Colistin	+	+	+

- ❖ Has been used for > 50 years in veterinary medicine
 - Pigs, poultry and cattle
 - Colibacillosis
 - 99% is oral formulations used in feed or water
 - In Europe use for prophylaxis is not allowed – however metaphylaxis is.....



VETERINARY USAGE OF COLISTIN

Worldwide estimated usage

- ~12.000 tons (2015)
- ~16.500 tons (2021)

Europa

- ~460 tons

Danmark

- 0,7 tons

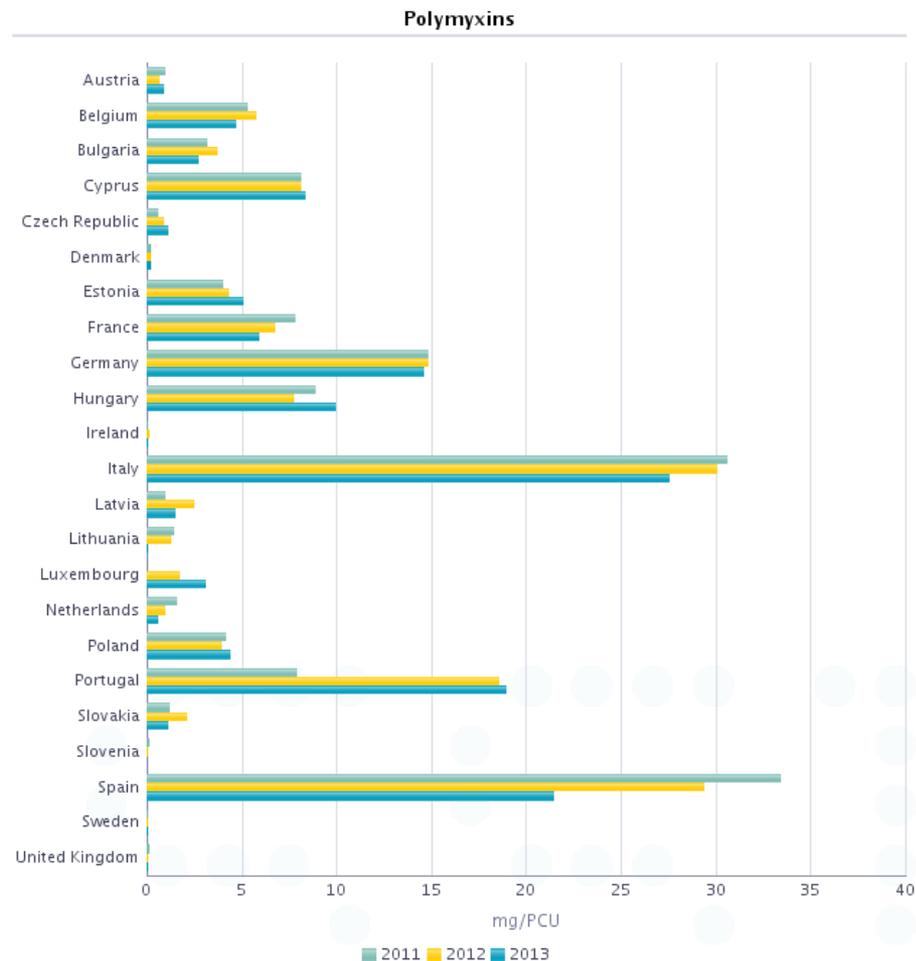


Table 5.3. Consumption of antimicrobial agents for systemic use in somatic hospitals (DDD/100 occupied bed-days), Denmark

ATC group ^(a)	Therapeutic group	Year									
		2005	2006	2007	2008	2009	2010	2011	2012	2013	2014
J01XB	Polymyxins	0.12	0.12	0.05	0.05	0.07	0.10	0.09	0.09	0.16	0.27

- ❖ Colistin is a positively charged antimicrobial peptide
- ❖ Colistin binds to lipid A creating an electrostatic interaction with divalent cations of the outer bacterial membrane, causing a disruption of the cell membrane
- ❖ Resistance (up to recently) has been chromosomally mediated
 - Mutations in the two-component regulatory system PhoPQ-PmrAB
 - leads to an altered charge in the lipopolysaccharide (LPS) and therefore lower binding of the positively charged polymyxins



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



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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^{-3} 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.

Lancet Infect Dis 2015

Published Online
November 18, 2015
[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/S1473-3099\(15\)00463-6](http://dx.doi.org/10.1016/S1473-3099(15)00463-6)

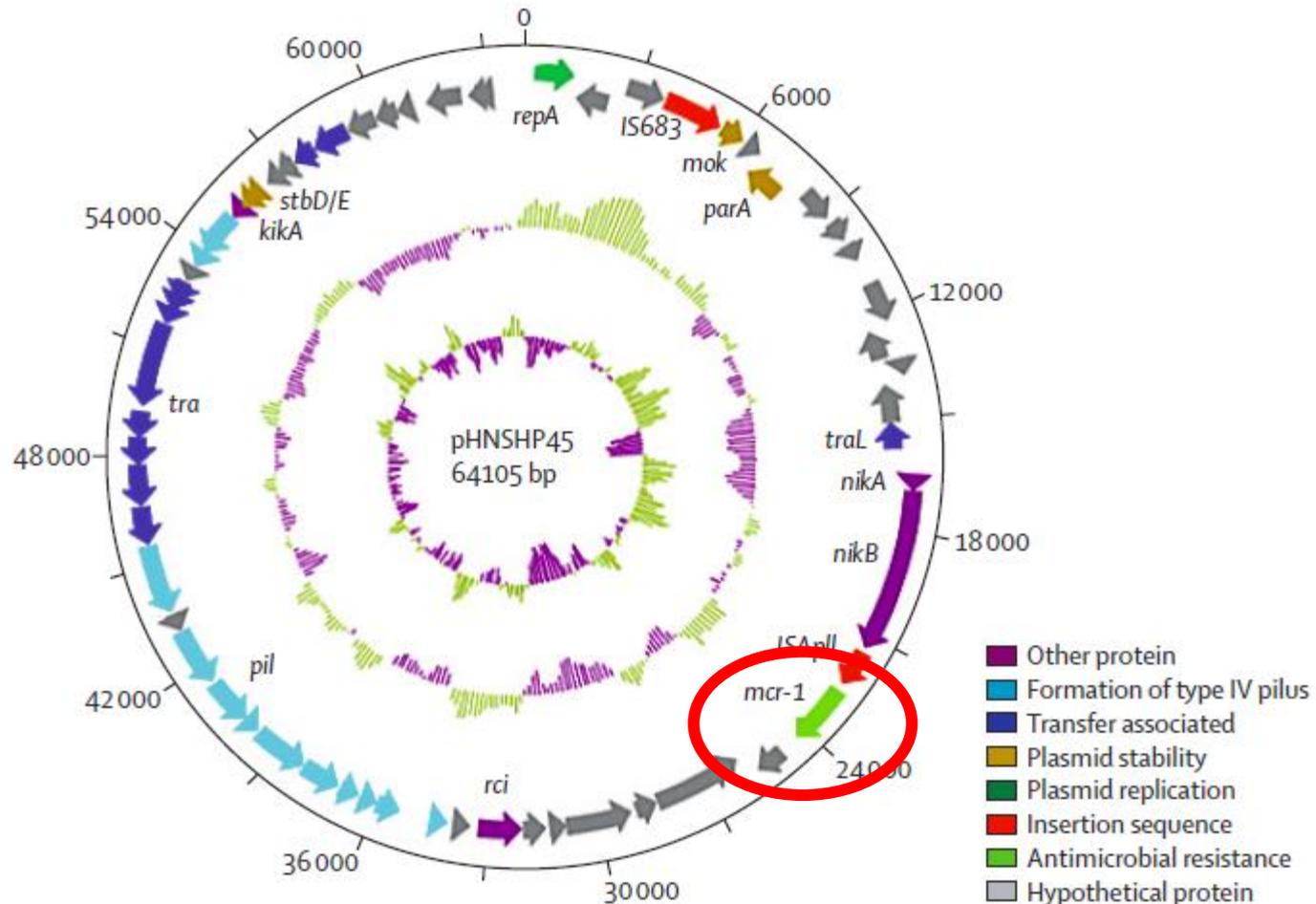
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	Year	Positive isolates (%)/number of isolates
<i>Escherichia coli</i>		
Pigs at slaughter	All	166 (20.6%)/804
Pigs at slaughter	2012	31 (14.4%)/216
Pigs at slaughter	2013	68 (25.4%)/268
Pigs at slaughter	2014	67 (20.9%)/320
Retail meat	All	78 (14.9%)/523
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
<i>Klebsiella pneumoniae</i>		
Inpatient	2014	3 (0.7%)/420

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

INC12 PLASMID



Transferrate: 10^{-1} – 10^{-3} TK/recipients (High!)

- ❖ Not fully elucidated
- ❖ Phosphoethanolamin transferase
 - Similarities to genes from *Neisseria*, *Paenibacillus* and *campylobacter*
- ❖ Phosphoethanolamin is added to lipid A and thereby increases the positive charge which reduces the binding of colistin
- ❖ MIC is typically only slightly increased
 - Susceptible isolates have been reported

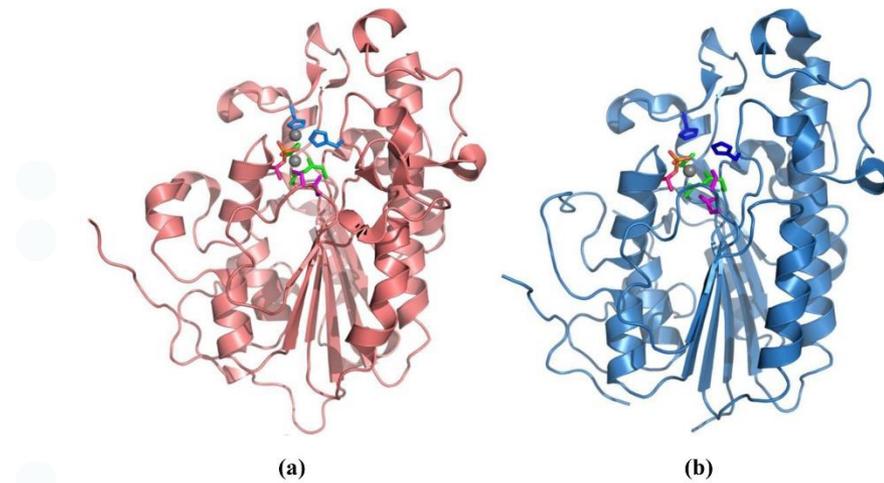


Figure S5. The MCR-1 sequence resembles those of two phosphoethanolamine transferases, **a**) LptA from *Neisseria meningitidis* (pdb ids 4KAY) and **b**) EptC from *Campylobacter jejuni* (pdb ids 4KAY and 4TNO).

EFFECT OF COLISTIN IN MICE THIGH MODEL

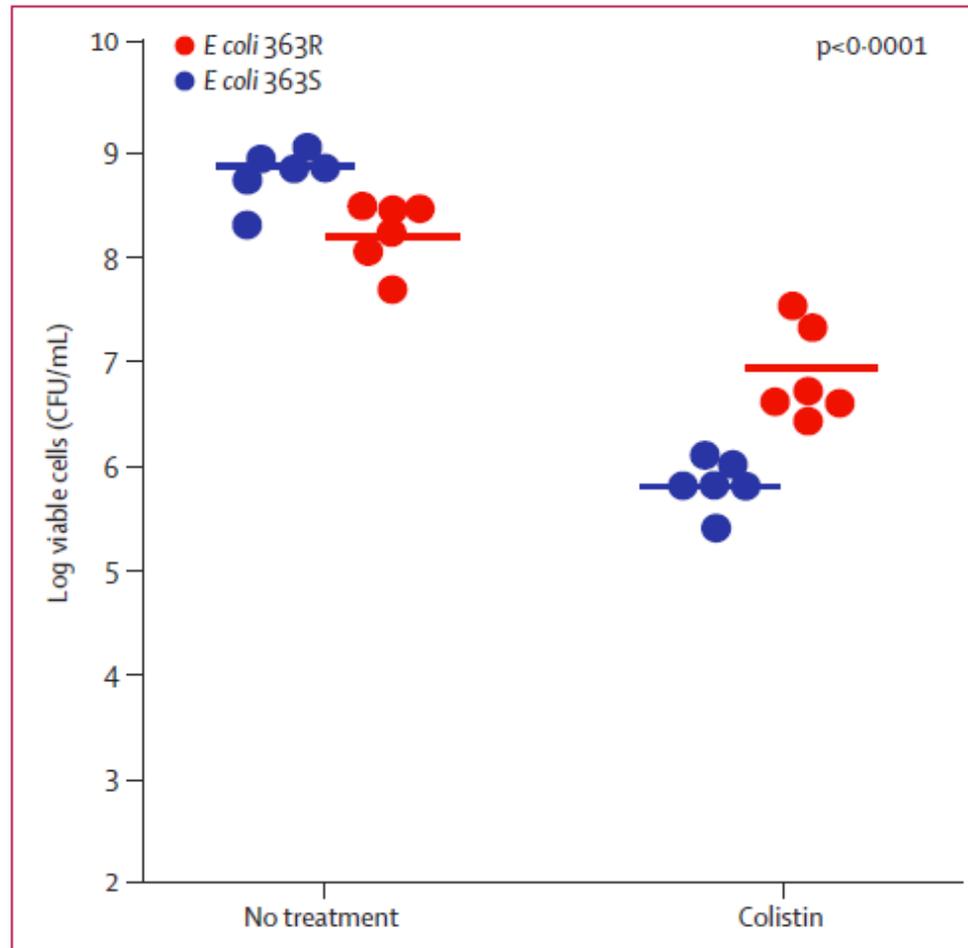
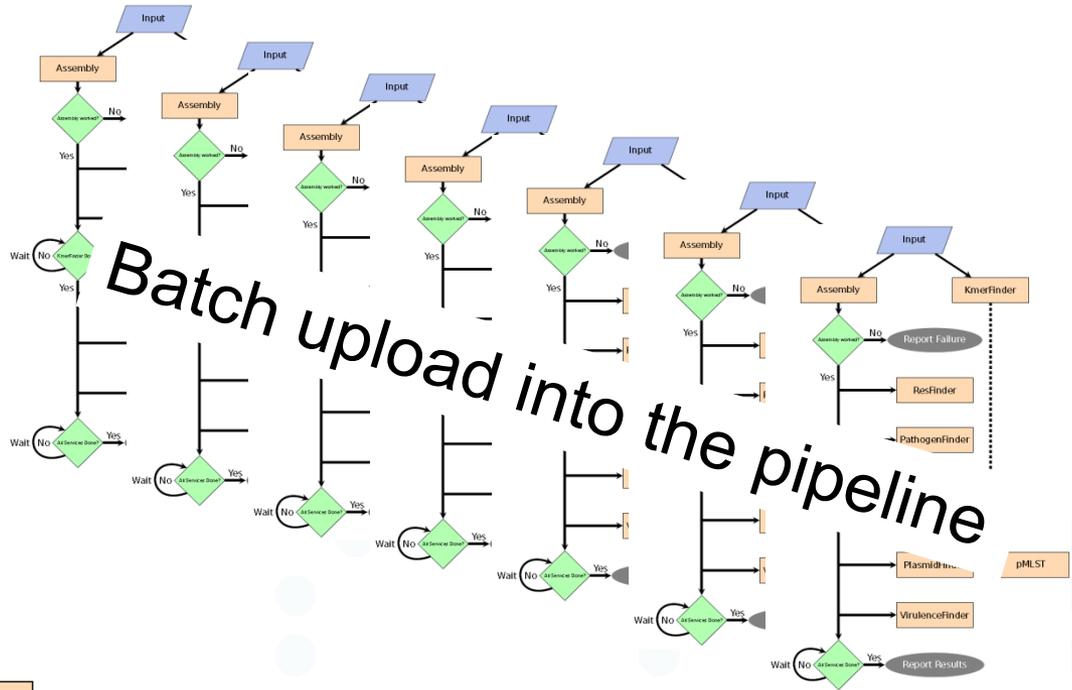
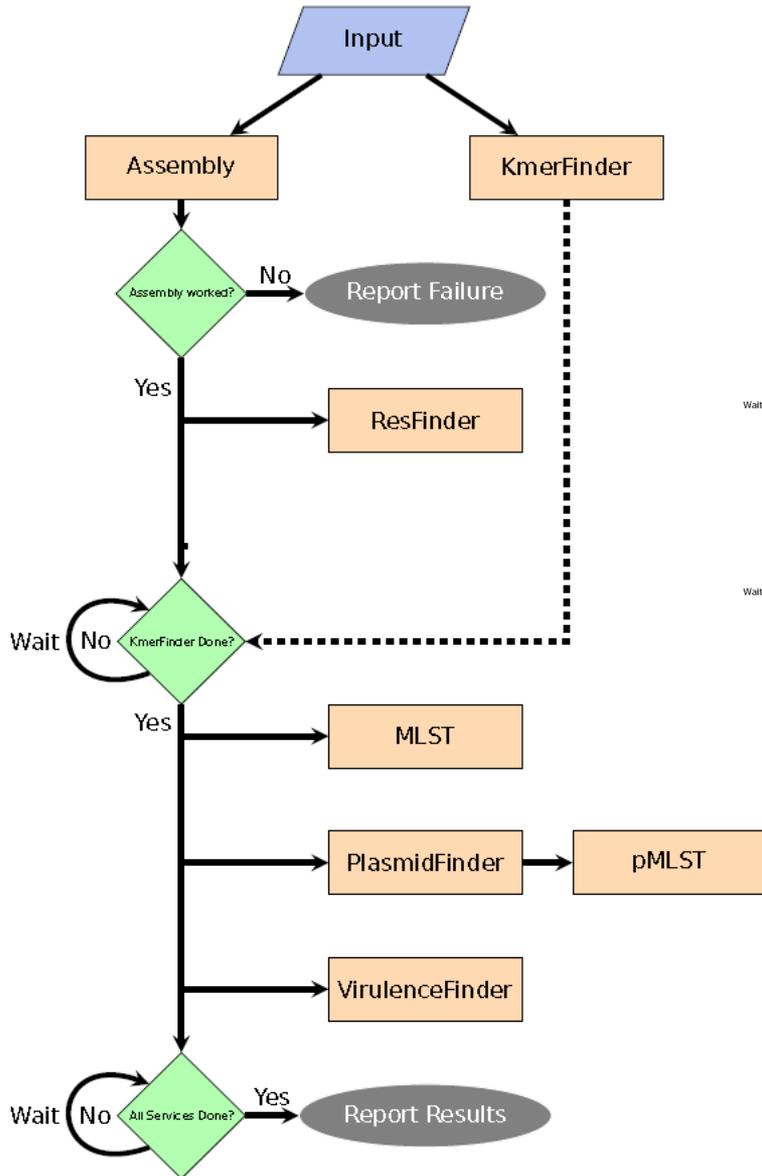


Figure 4: In-vivo effects of colistin treatment (7.5 mg/kg of colistin sulfate per 12 h) in a murine thigh model showing 10^6 CFU infection with *Escherichia coli* with *mcr-1* (363R, red circles) and without *mcr-1* (363S, blue circles)

The CGE Pipeline



RAPID COMMUNICATIONS

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

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Citation style for this article:

Hasman H, Hammerum A, Hansen F, Hendriksen R, Olesen B, Agersø Y, Zankari E, Leekitcharoenphon P, Stegger M, Kaas R, Cavaco L, Hansen D, Aarestrup F, Skov R. Detection of *mcr-1* encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat, Denmark 2015. *Euro Surveill.* 2015;20(49):pii=30085. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085>

Article submitted on 04 December 2015 / accepted on 10 December 2015 / published on 10 December 2015



MCR-1 POSITIVE ISOLATES

TABLE 2

Genotypic characterisation of *mcr-1*-positive *E. coli* isolates, Denmark, November 2015 (n=6)

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

MLST: multilocus sequence typing; ND: not detected.

Furthermore one *E. coli* isolate from i 2011

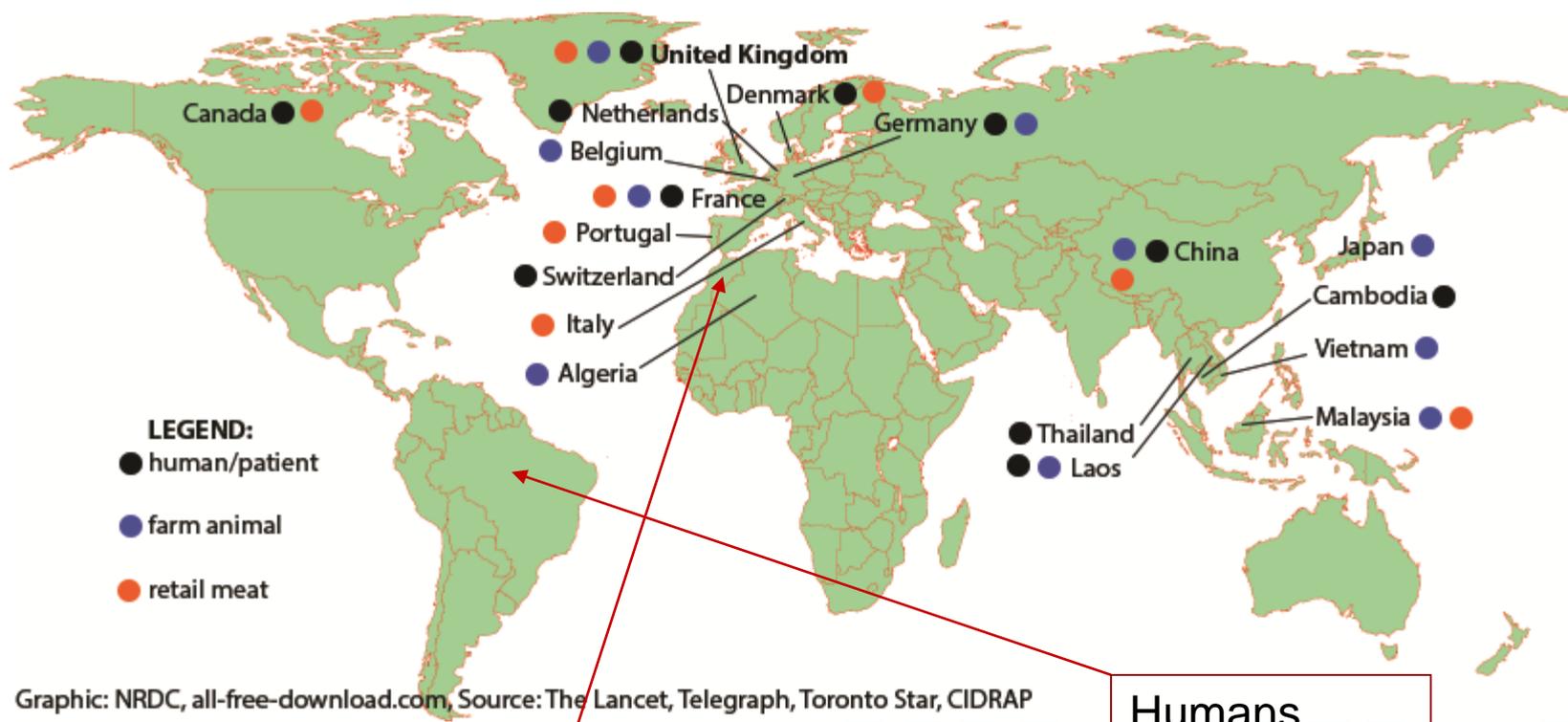
- ❖ >8000 isolates screened since 2008
- ❖ Four isolates positive for *mcr-1*
 - MIC = 8 eller 16 µg/mL
 - Two cases from 2014 (O:4, 5, 12; H:i: -)
 - Two from 2015 (O:4,12; H:i: -)
 - Identical by WGS



EDITORIAL

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

RL Skov ¹, DL Monnet ²



Farm animals

Humans
Farm animals

- ❖ Very few unselected strain collections have been investigated
- ❖ Found widespread in the world

- ❖ *mcr-1* positive isolate found back in the 1980s in China
- ❖ Oldest isolate in Europe
 - Animals: 2005 veal calves, France
 - Humans: 2011, Denmark and Netherlands
- ❖ Isolates are often MDR (selection bias!)
 - Found in non MDR isolates too
- ❖ Found in 6% of French turkeys – unselected!
 - Otherwise low frequencies (reported...)
- ❖ Found in up to 40% of strains from animals | Asia
- ❖ Seemingly increasing prevalence in China!
 - Found among newborn in China

- ❖ Susceptibility testing of colistin is performed by testing colistin sulphate
- ❖ Breakpoints
 - S ≤ 2 mg/L
 - R > 2 mg/L

- ❖ Presently only broth dilution is recommended
 - **NO** disk diffusion / gradient test / agar dilution
 - EUCAST Development laboratory is evaluating these methods

- ❖ Use:
 - uncoated polystyrene microtiter plates;
 - cation adjusted Mueller-Hinton broth without any other additives
 - in particular no polysorbate 80 or other surfactants

- ❖ colistin 3.5 µg/ml. Since some *Streptococcus* and
- ❖ 52 *Staphylococcus* strains may still grow on EBM medium, daptomycin (Novartis, Horsham,
- ❖ 53 UK) was added at a concentration of 10 µg/ml. Noteworthy, daptomycin and not vancomycin
- ❖ 54 was added since vancomycin potentiates the activity of colistin against several gram negatives
- ❖ 55 (6, 7). Amphotericin B (Bristol-Myers-Squibb, Rueil-Malmaison, France) was also added as
- ❖ 56 anti-fungi molecule at a concentration of 5 µg/ml.

❖ Agar: Eosine-Methylene-Blue (EMB) medium

- colistin 4 mg/l
- daptomycin 10 mg/l
- Amphotericin 5 µg/ml.

❖ 88 isolates

- 52 colistin R
 - 45 acquired
 - 7 intrinsic
- 26 Colistin S

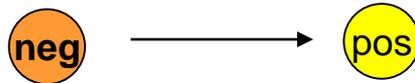
❖ 100 % sensitive and specific

Patrice Nordmann^{a,b*} Aurélie Jayol,^a and Laurent Poirel^a

JCM Accepted Manuscript Posted Online 16 March 2016

❖ Test principle :

Glucose metabolism by alive bacteria involve acidification of the media, which make turned the phenol red from orange to yellow.

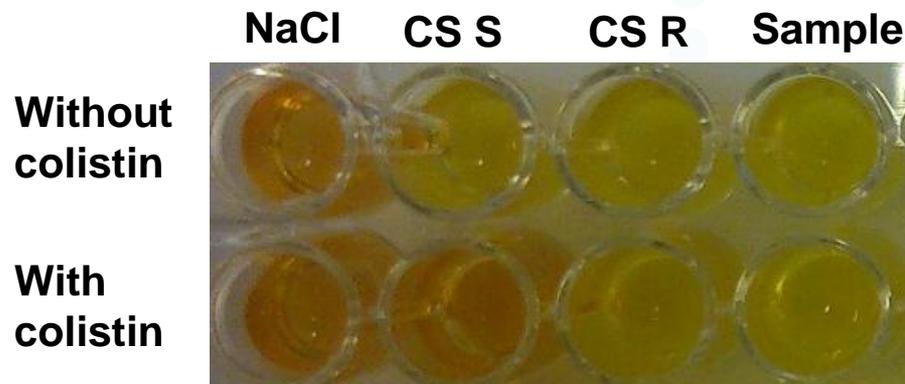


In presence of colistin :

- Strains susceptible to colistin are dead : test 
- Strains resistant to colistin are alive : test 

Nordmann P, Jayol, Poirel L. Rapid detection of polymyxin resistance in ENterobacteriaceae. Emerg Infect Dis. 2016. In press.

- ❖ 3 to 3.5 McFarland optical density (ca. 10^9 CFU/ml)
- ❖ Broth containing (final concentrations)
 - 2.5 % of MHB-CA powder,
 - 0.005 % of phenol red indicator,
 - 1 % of D (+)-glucose
 - colistin of 5 $\mu\text{g/ml}$
- ❖ Incubation
 - 37 C, oxygen present!
- ❖ Reading
 - 15 min , 1,2,3 and 4 hours



- ❖ The recently discovered plasmid borne *mcr-1* has been around for quite some time
 - Significance?

- ❖ BUT we are increasingly using colistin in human medicine and thereby increase the selection pressure

- ❖ Risk management
 - Prudent use of colistin
 - Restriction of use in veterinary medicine
 - EMA have activated the AMEG ad hoc group to produce a revised recommendation for use of colistin



THANK YOU FOR YOUR ATTENTION

Acknowledgements

Christian Giske

Laurent Poirel



