Resistance to Polymyxins in France

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CHUV Centre hospitalier universitaire vaudois

NARA
Nationales Referenzlaboratorium zur Früherkennung neuer Antibiotikaresistenzen und Resistenzmechanismen

Prof. Patrice Nordmann

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4224–4229 Vol. 55, No. 9
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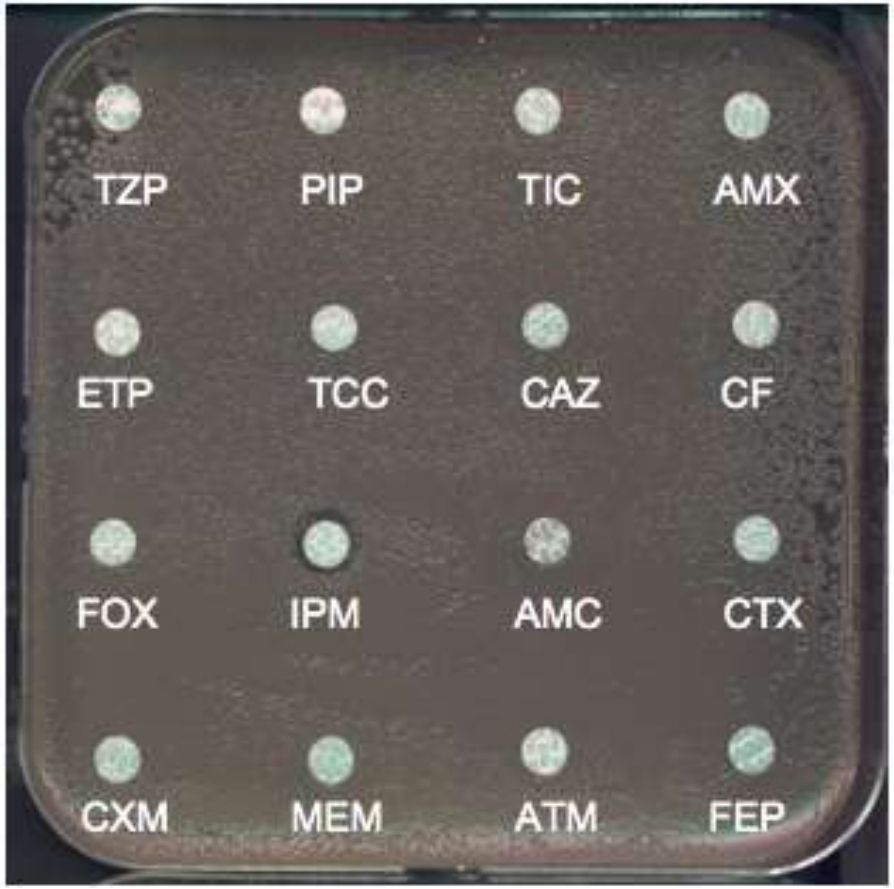
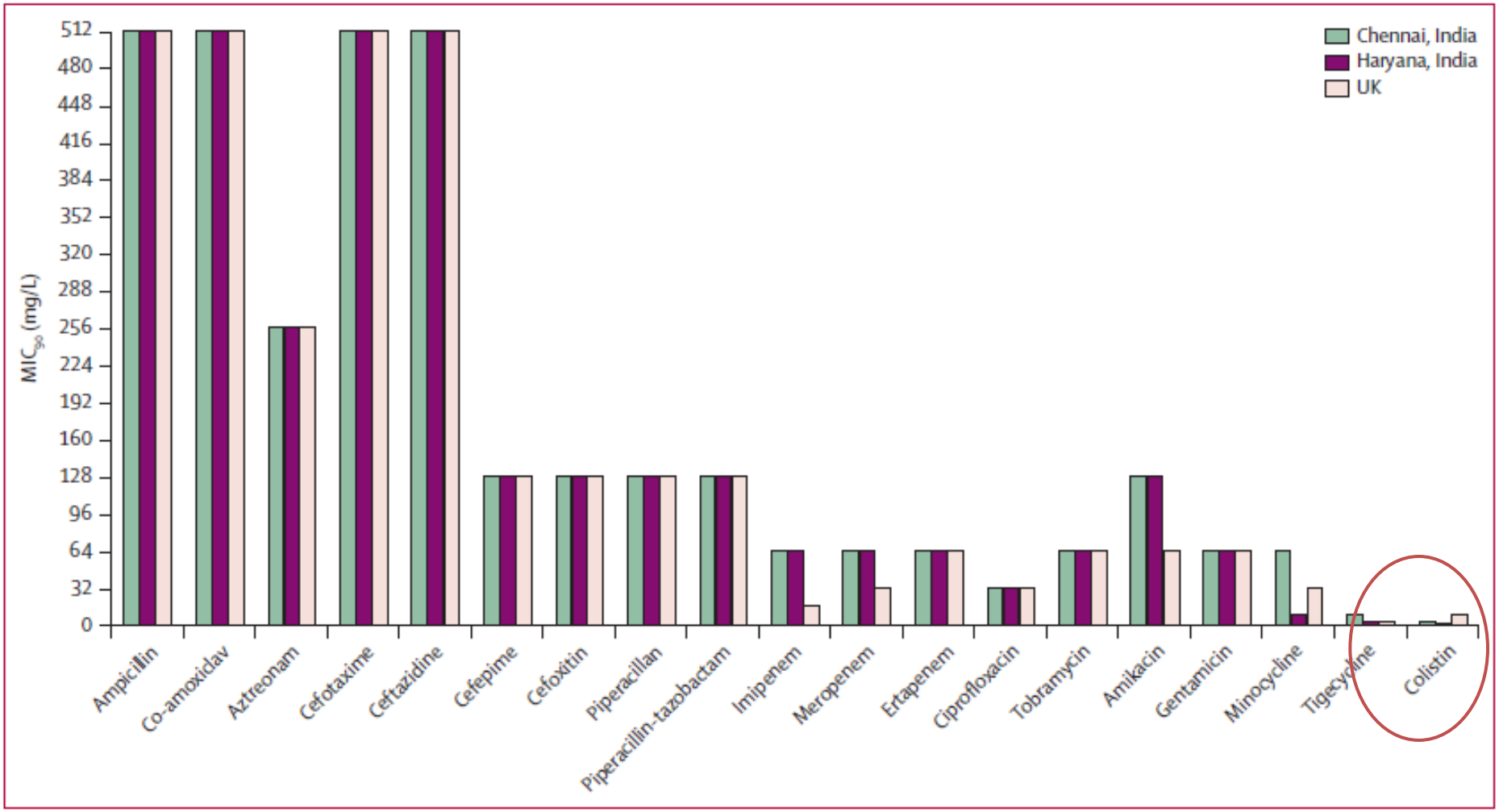
Analysis of the Resistome of a Multidrug-Resistant NDM-1-Producing *Escherichia coli* Strain by High-Throughput Genome Sequencing[▽]

Laurent Poirel, Rémy A. Bonnin, and Patrice Nordmann*

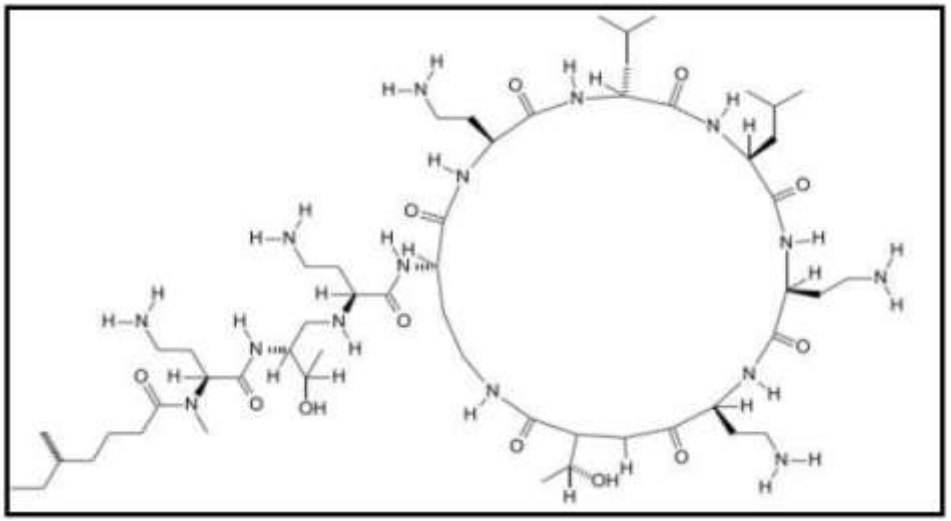


NDM producers in *Enterobacteriaceae*



Lancet Infect Dis 2010; 10: 597–602

The polymyxins; colistin and polymyxin B



Colistin

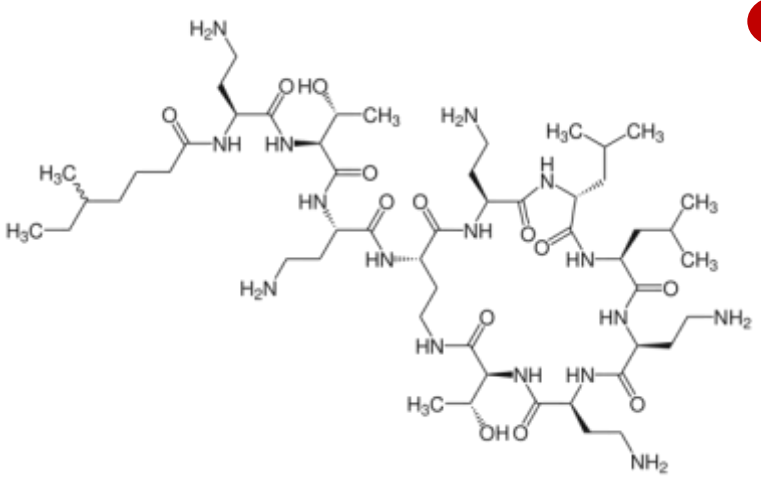
- Synthesis by *Bacillus polymyxa* spp colistinus
- Discovered in the 1940's
- High rates of toxicity (mainly nephrotoxicity)
- Renewed interest in mid-2000's to treat multidrug-resistant Gram-negative bacteria: MDR *Klebsiella*, *Acinetobacter* and *Pseudomonas* sp.

Colistin use, 2018

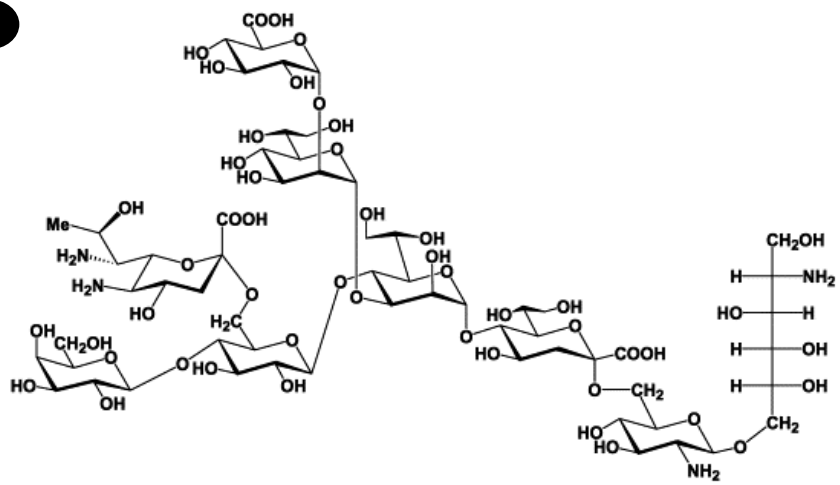
Mostly in veterinary medicine (prophylaxis and metaphylaxis)

Mechanism of action

Colistin



Lipid A

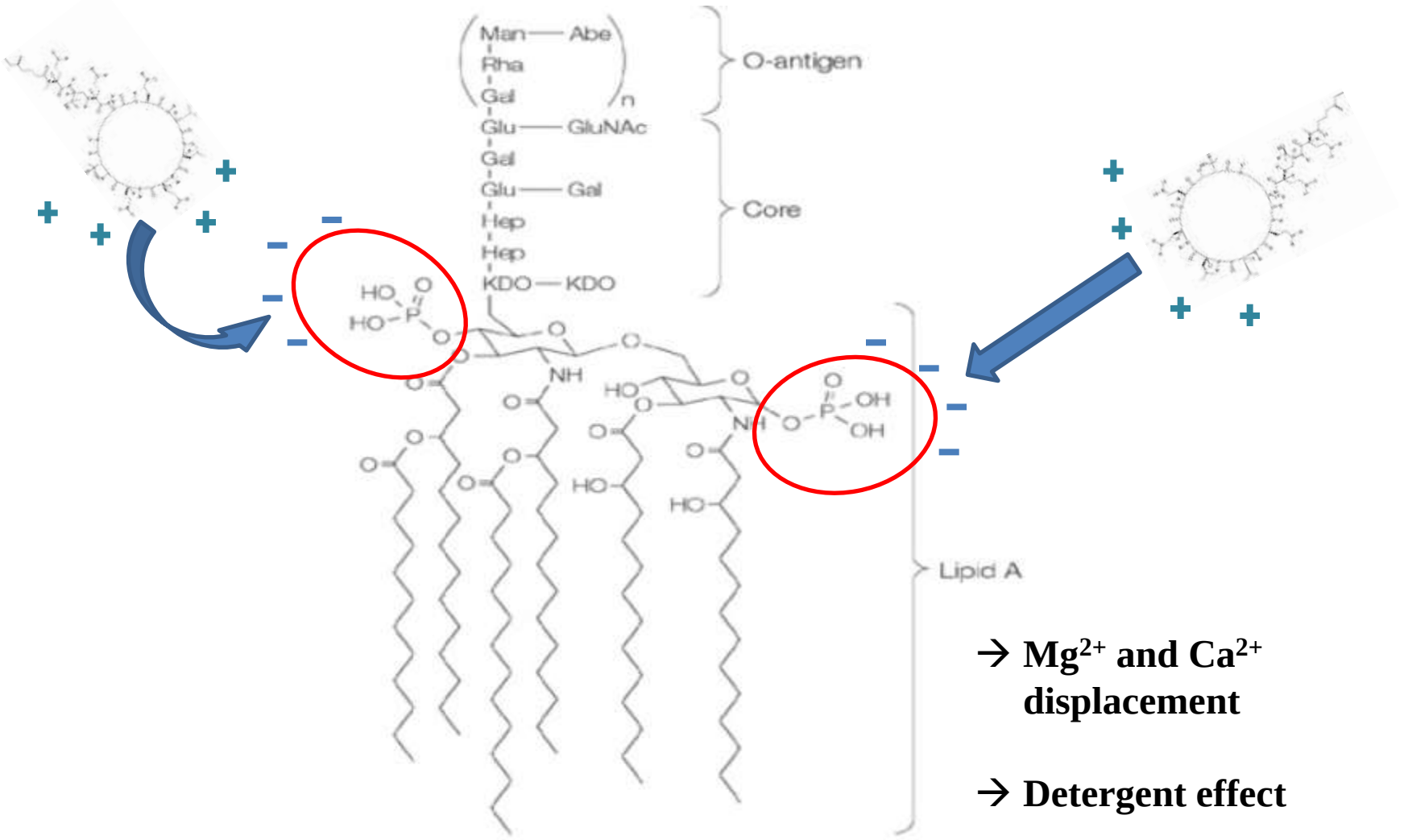


Colistin is a cationic antibiotic that is composed of a cyclic heptapeptide covalently attached to a fatty acyl chain

Lipopolysaccharide (LPS) of Gram-negative bacteria is composed by :

- Lipid A
- Core
- Oligosaccharide O

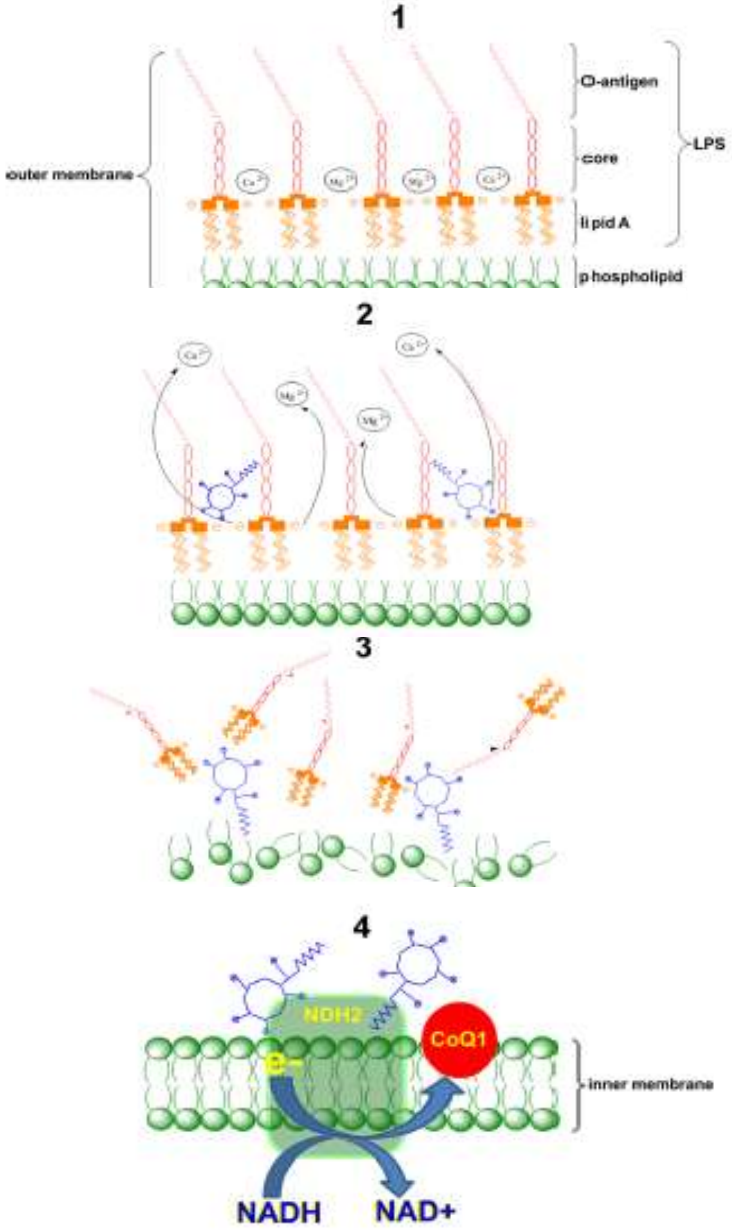
Target of colistin : LPS



→ **Mg^{2+} and Ca^{2+} displacement**

→ **Detergent effect**

Mechanism of action



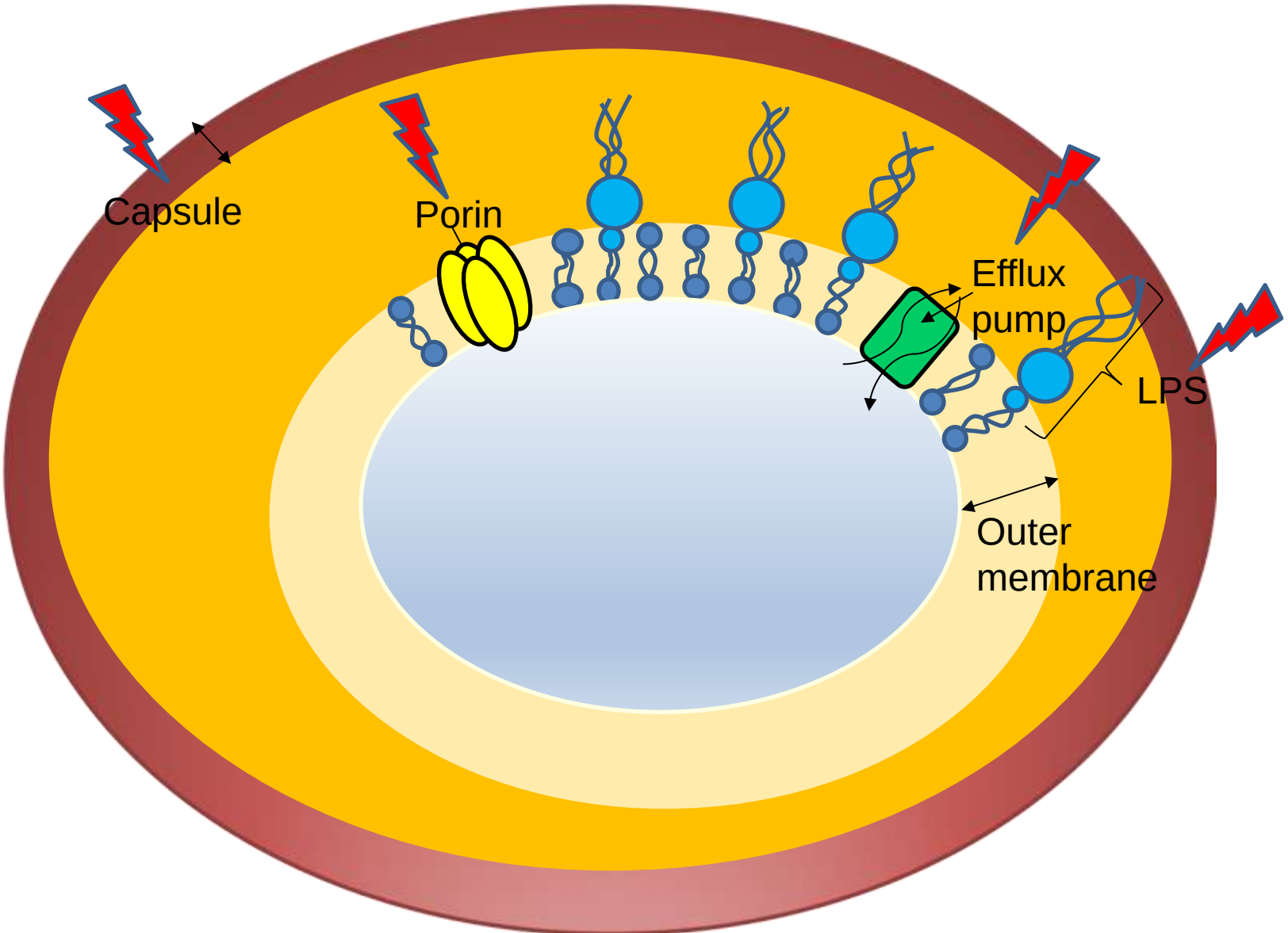
1. Fixation

2. Displacement of divalent cation (Ca^{2+} et Mg^{2+})

3. Destabilisation of the outer membrane

4. Penetration throughout the inner membrane and inhibition of the respiratory enzymes NDH2

Multiple chromosomal mechanisms of colistin resistance



Role of LPS in polymyxin resistance

❖ Loss of LPS :

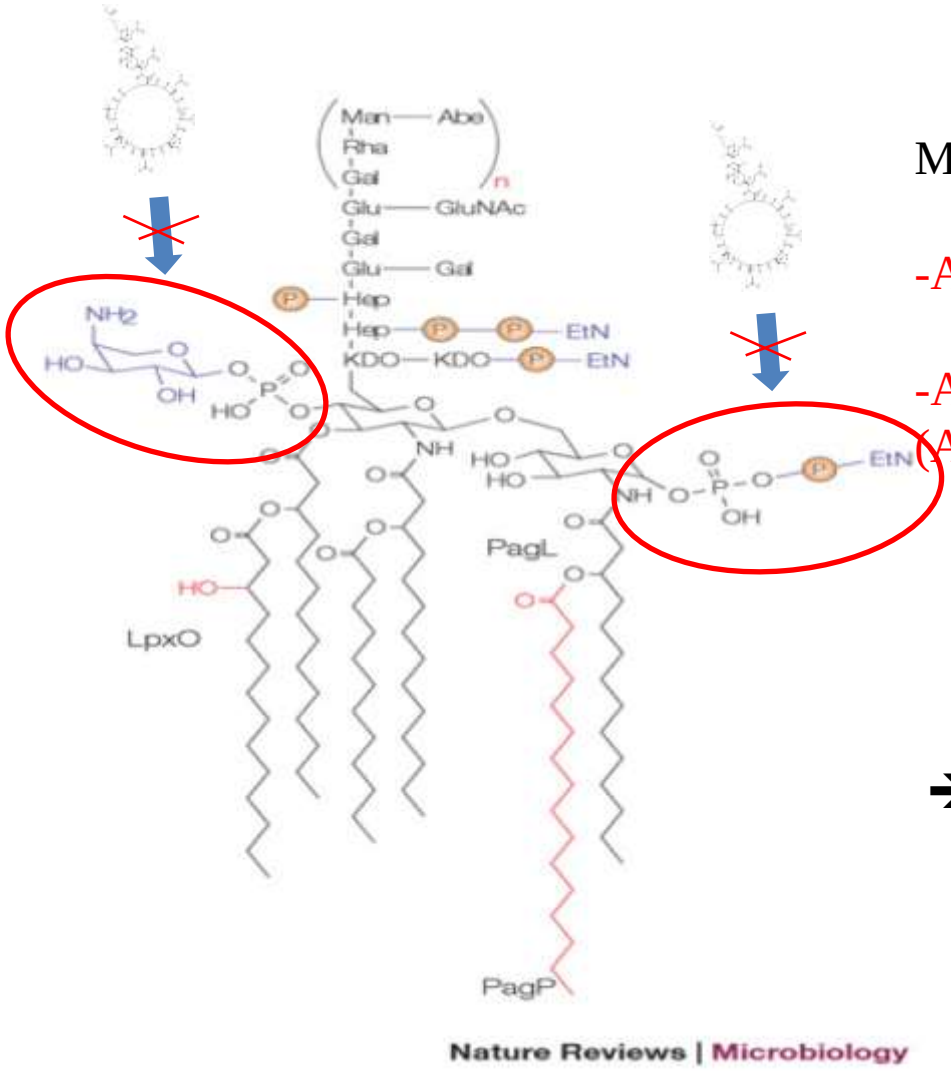
Inactivation of lipid A biosynthesis genes (*lpxA*, *lpxC* and *lpxD*) cause loss of LPS and prevent the interactions of polymyxins with its binding sites on the LPS, described in *A. baumannii*

❖ LPS modifications : the main mechanism of resistance to colistin :

Addition of 4-amino-4-deoxy-L-arabinose (LAra4N) and or phosphoethanolamine (pEtN) to lipid A ➔ Increase of positive charges ➔ decreased affinity for LPS

Synthesis of L-Ara4N and pEtN mediated by PmrA / PmrB, PhoP / PhoQ, and *mgrB* gene

Modification of the chemical structure of the LPS



Modifications :

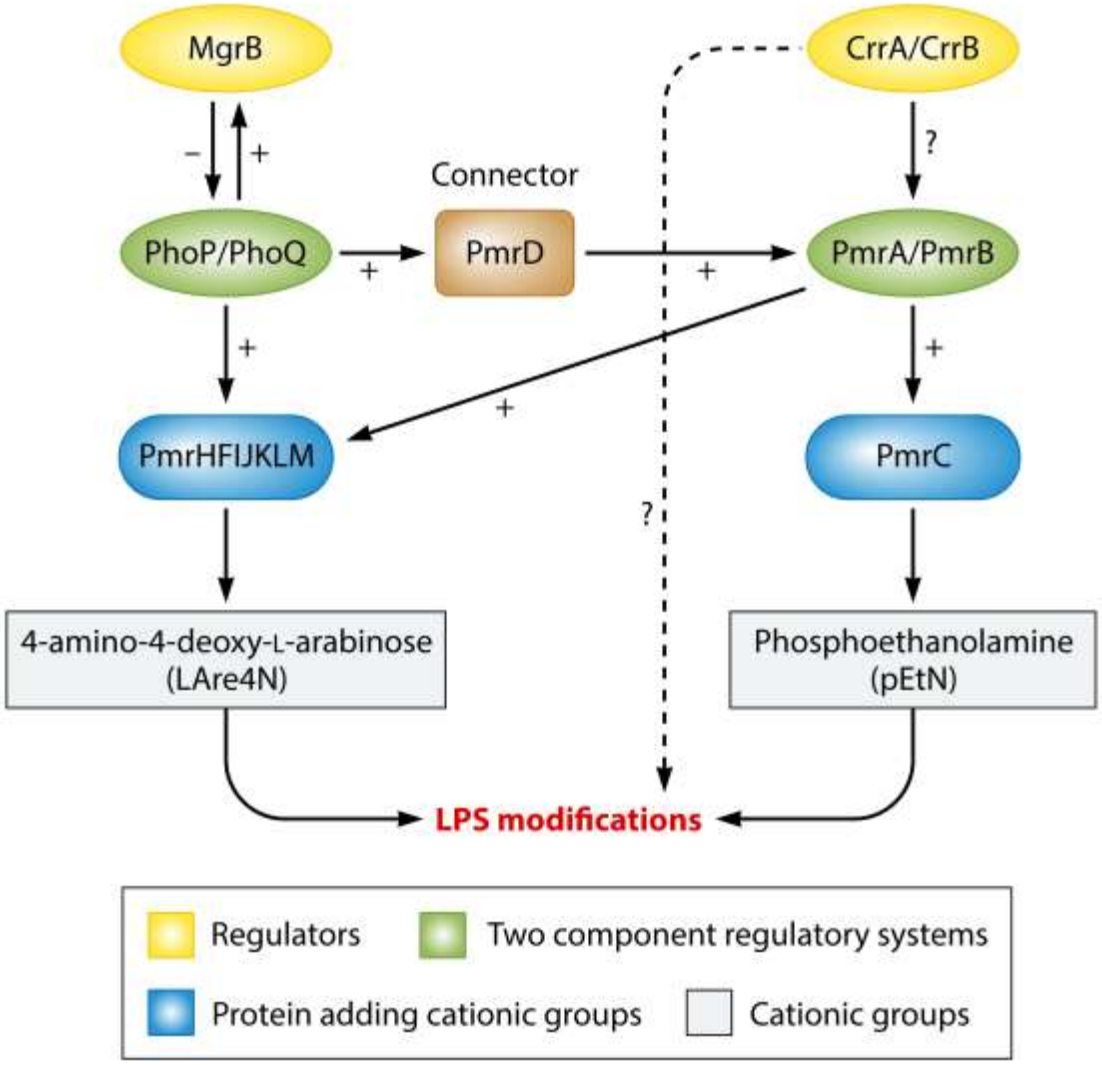
-Addition of phosphoethanolamine (pEtN)

-Addition of 4-amino-4-deoxyl-L-arabinose (Ara4N)

➔Adaptation against hostile environment

➔Lower affinity for cationic molecules such as colistin

Modification of the LPS structure in *K . pneumoniae*



SURVEILLANCE AND OUTBREAK REPORT

National survey of colistin resistance among carbapenemase-producing *Enterobacteriaceae* and outbreak caused by colistin-resistant OXA-48-producing *Klebsiella pneumoniae*, France, 2014

A Jayol [1], L Poirel [1], L Dortet [2 3 4], P Nordmann [1 2 5]

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Citation style for this article:
Jayol A, Poirel L, Dortet L, Nordmann P. National survey of colistin resistance among carbapenemase-producing Enterobacteriaceae and outbreak caused by colistin-resistant OXA-48-producing Klebsiella pneumoniae, France, 2014. Euro Surveill. 2016;21(37):pii=30339. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2016.21.37.30339

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From January 2014 to December 2014, 972 consecutive non-replicate carbapenemase-producing *Enterobacteriaceae* isolates from colonised or infected patients were collected at the Associated French National Reference Centre as part of the French national survey on antimicrobial resistance. It included 577 *Klebsiella* spp. (59%), 236 *Escherichia coli* (24%), 108 *Enterobacter* spp. (11%), 50 *Citrobacter* spp. (5%), and a single *Salmonella* spp. isolate (0.1%). Of 561 *K. pneumoniae* isolates, 35 were found to be resistant to colistin (6.2%). PFGE analysis revealed a clonal outbreak involving 15 *K. pneumoniae* isolates belonging to sequence type ST11, recovered in a single hospital in the Picardie region in northern France. Those clonally related isolates showed variable levels of resistance to colistin, ranging from 4 to 64 mg/L. They harboured the bla_{OXA-48} carbapenemase gene and the $bla_{CTX-M-15}$ extended-spectrum beta-lactamase gene. Among the 91 *Enterobacter cloacae* isolates, seven were resistant to colistin and produced different types of carbapenemases. Surprisingly, none of the *E. coli* and *Citrobacter* spp. isolates showed resistance to colistin. This national survey including carbapenemase-producing isolates recovered in 2014 reported a high rate of colistin resistance in *K. pneumoniae* and *E. cloacae* (6.2% and 7.7%, respectively) in France.

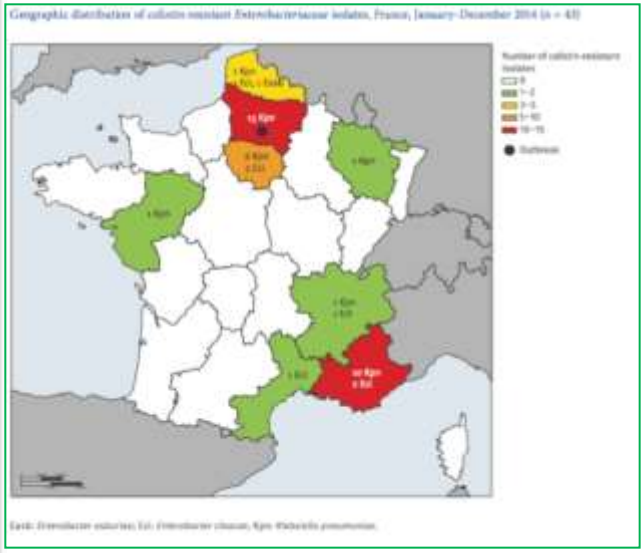
currently almost unknown in most parts of the world. In Italy, an increase in carbapenemase-producing *Enterobacteriaceae* has been noted in the past years, but the situation remains unknown in France [1]. The lack of information about the prevalence of colistin resistance among multidrug-resistant enterobacterial isolates derives from several reasons: (i) so far, there has been limited interest in that field, (ii) methods used for determination of colistin susceptibility are not adequate, and (iii) the lack of well-defined breakpoints does not allow precise determination of prevalence. However, the recent identification of a plasmid-borne polymyxin resistance determinant (MCR-1) raised a very serious concern in that resistance to colistin might widely disseminate [2].

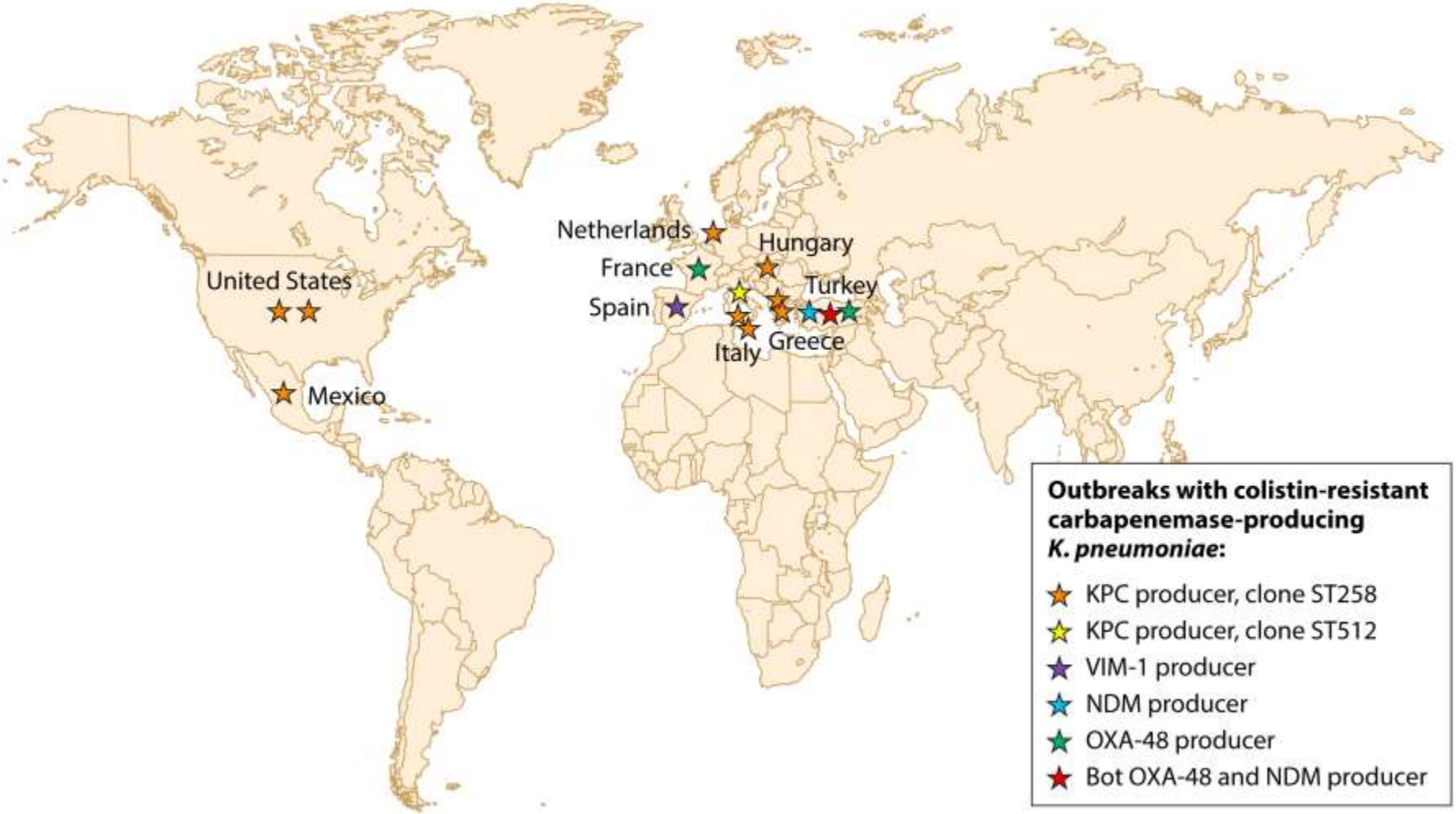
The aim of this study was to evaluate retrospectively the prevalence of colistin resistance among a collection of CPE strains recovered in France during a period of one year and to analyse the phenotypic, genotypic features and clonality of the colistin-resistant isolates.

Methods

Carbapenemase-producing *Enterobacteriaceae* isolates

From January to December 2014, 972 consecutive





Clinical Microbiology Reviews®

2017

Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes

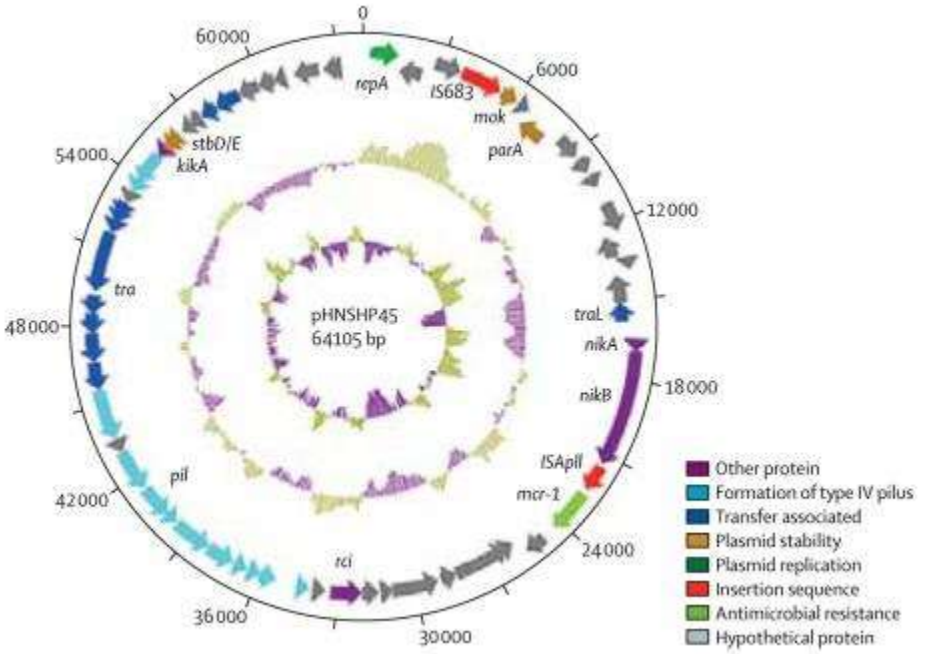
Laurent Poirel[a,b,c] Aurélie Jayol[a,b,c] Patrice Nordmann[a,b,c,d]

Lancet Infect Dis, Nov 28, 2015

Plasmid-mediated resistance to colistin

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

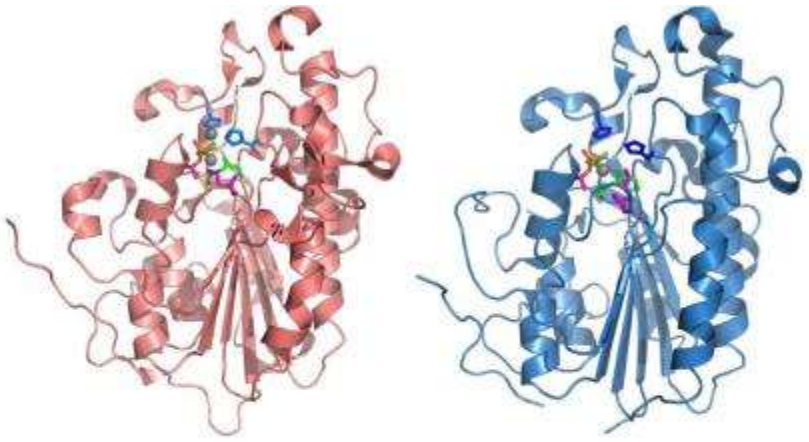
Yi-Yun Liu, Yang Wang*, Timothy R Walsh, Ling-Xian Yi, Rong Zhang, James Spencer, Yohei Doi, Guobao Tian, Baolei Dong, Xianhui Huang, Lin-Feng Yu, Danxia Gu, Hongwei Ren, Xiaojie Chen, Luchao Lv, Dandan He, Hongwei Zhou, Zisen Liang, Jian-Hua Liu, Jianzhong Shen*



	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**

The MCR-1 protein; a phosphoethanolamine transferase



(a) (b)

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).

Clinical Microbiology Reviews®

Polymyxins: Antibacterial Activity, Susceptibility Testing, and Resistance Mechanisms Encoded by Plasmids or Chromosomes

Laurent Poirel[a,b,c] Aurélie Jayol[a,b,c] Patrice Nordmann[a,b,c,d]

- A 16-fold increase in MIC of polymyxins (colistin and polymyxin B)

- From 0.5 µg/ml (recipient *E. coli*) to 8 µg/ml (transconjugant)

Co-occurrence of extended spectrum β lactamase and MCR-1 encoding genes on plasmids

Published Online
January 7, 2016
http://dx.doi.org/10.1016/S1473-3099(16)00007-4

Findings reported by Yi-Yun Liu and colleagues[1] identified the plasmid-borne gene *mcr-1* encoding resistance to colistin with a high prevalence in *Escherichia coli* isolates from animals, foodstuff, and human beings in China. The same gene was then reported in Europe (Denmark) among extended-spectrum β lactamase (ESBL) and AmpC-producing *E coli* isolates from chicken meat and human infections, but at a very low prevalence.[2]

We screened ESBL-positive *E coli* isolates collected in France for colistin resistance. Isolates were collected between 2005 and mid-2014 from faeces of diarrhoeic veal calves at farms, as part of a survey in the context of the French antimicrobial resistance Resapath surveillance network for animal pathogens. We screened these

For the **Resapath network** see http://www.resapath.anses.fr

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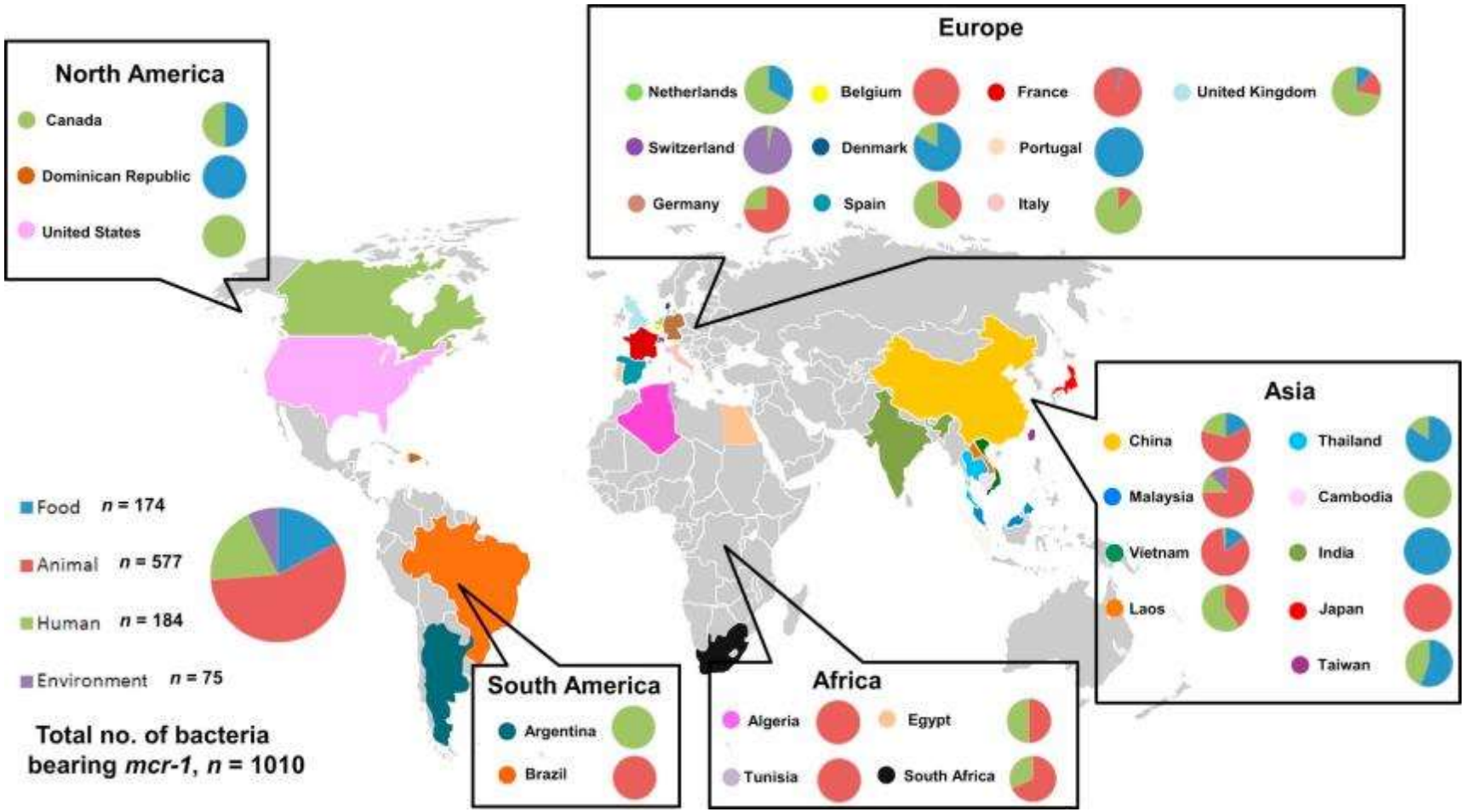
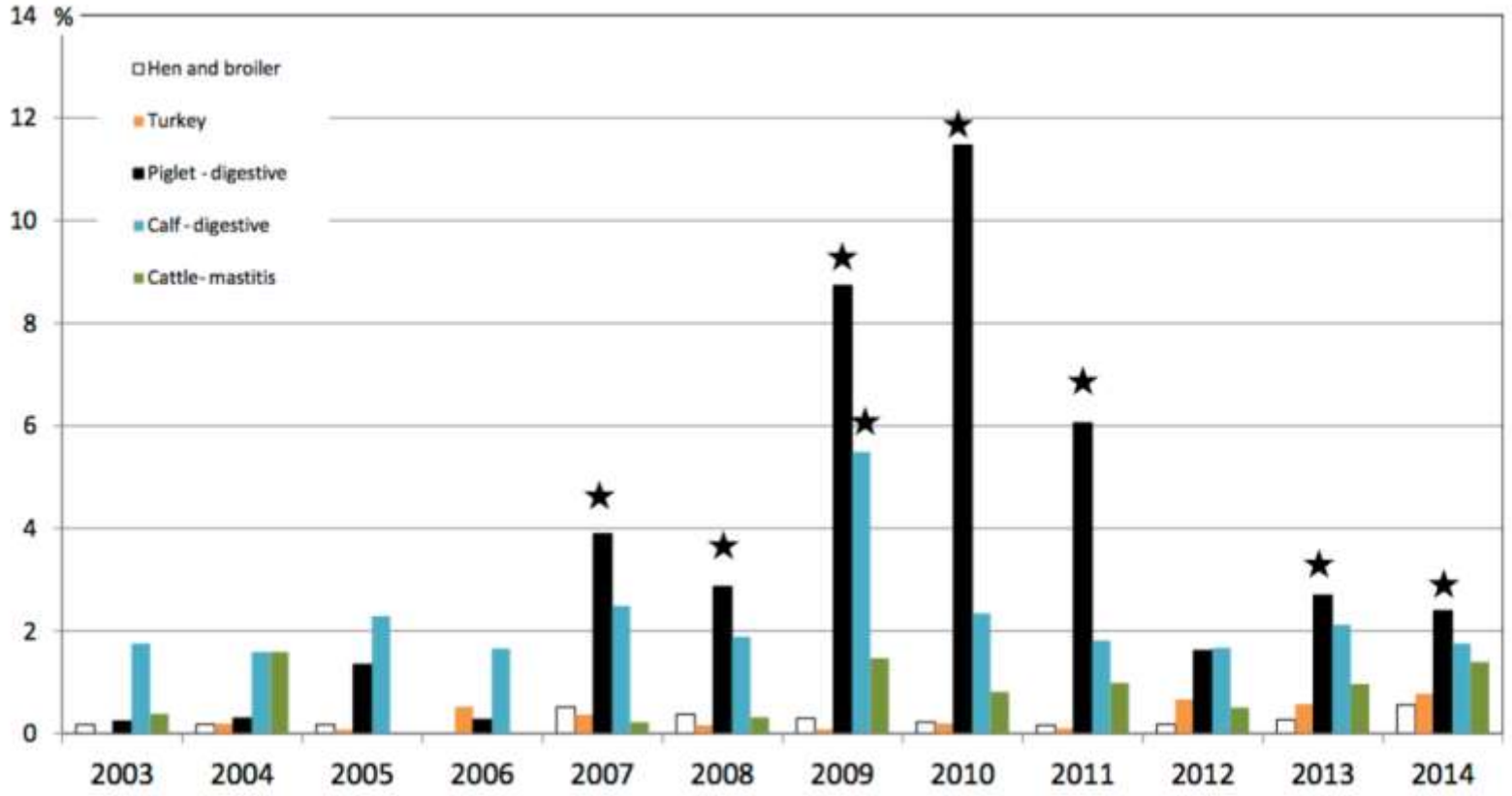
Global distribution of plasmid-mediated *mcr-1* colistin-resistant strains isolated from environments, foods, animals and humans (November 2015 to April 2016).



Baron S et al. Int J Antimicrob Agents 2016;48:583-591

Colistin Resistance- *E. coli*- animals-France



Kempf et al. Int J Antimicrob Agents 2016

Plasmid-mediated colistin resistance: one-health world issue

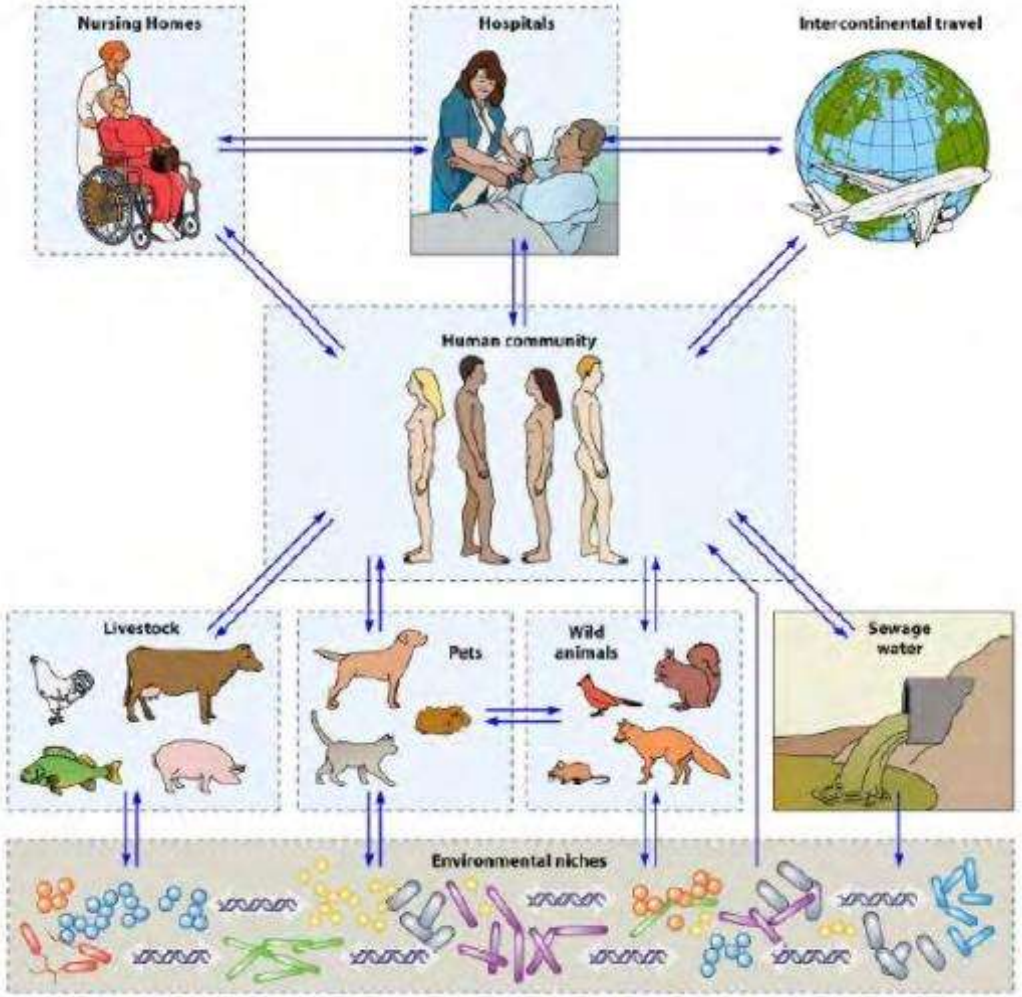


Tableau 2. Protéines de résistance plasmidiques à la colistine.

Protéine	Espèce progénitrice	Taille de la protéine	Pays de découverte
MCR-1	*Moraxella sp.*	541 aa	Chine
MCR-2	*Moraxella pluranimalium*	538 aa	Belgique
MCR-3	*Aeromonas sp.*	541 aa	Chine
MCR-4	*Shewanella frigidimarina*	541 aa	Espagne, Belgique
MCR-5	*Cupriavidus gilardii* ?	547 aa	Allemagne

MCR in *Salmonella* sp.

Dissemination of the *mcr-1* colistin resistance gene

Published Online December 17, 2015 http://dx.doi.org/10.1016/S1473-3099(15)00538-1

In response to the Yi-Yun Liu and colleagues' finding of a mobile genetic element responsible for colistin resistance, *mcr-1*,[1] and the accompanying Comment asking "is plasmid-mediated colistin resistance a purely Chinese phenomenon?"[2], we, and others,[3,4] can now reply no. As part of routine surveillance, we screened 8684 salmonella isolates collected during 2012–13 from the French agricultural food sector for colistin resistance using disk diffusion.[5] Between October and December, 2013, 27 isolates that showed a reduced susceptibility to colistin (ie, zone of inhibition <15 mm) were further assessed using a colistin concentration gradient assay. Five isolates (0·06%) had a distinctly different minimum inhibitory concentration (≥4 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 in silico replicon typing identified various plasmid backbones that were distinct from those reported by Liu and colleagues (table).[1] The isolates harboured a 1626 bp sequence with 100% homology to the recently described *mcr-1*.[1] Colistin resistance, although extraordinarily rare, was reported in epidemiologically, regionally, and serologically unrelated salmonella isolates, and, surprisingly, all were of the O:4 serogroup (serotypes Derby, Schwarzengrund, 1,4,[5],12:i:-, and Paratyphi B). Whether the product of *mcr-1*, MCR-1, confers resistance in a limited number of lipopolysaccharide structures or whether our findings were a remarkable coincidence is unclear. If not coincidental, this finding might offer the prospect of limited dissemination within the salmonella genus and this potentially warrants further investigation in Enterobacteriaceae. Interrogation of the genomes using nucleotide alignment of genes previously associated with colistin resistance in Enterobacteriaceae, specifically *pmrAB*, *phoPQ*, and *mgrB*,[6] showed no mutations that explained the resistant phenotype. The genomes of the four *mcr-1*-positive strains were deposited in GenBank under the accession numbers LNCZ00000000, LKJK00000000, LKJJ00000000, and LKJD00000000.

Broader distribution—in terms of geography and bacterial genera—of plasmid-associated *mcr-1* is evident because it has now been identified outside Asia.[1] Saliently, 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 concentration gradient strips). Furthermore, *mcr-1* has now been associated with several plasmid incompatibility types. If these plasmids do contain the *mcr-1* gene, as suggested by our interrogation of the draft genomes, and are mobile, or at least mobilisable, dissemination of these plasmids harbouring *mcr-1* in salmonella and other bacteria seems possible, if not probable. Interrogation of other horizontally transferable elements will provide broader understanding of the probable distribution of this gene.

Our findings, and those of others,[1] reinforce the need to reconsider the use of in-feed colistin in veterinary

	2013LSAL02374	12CEB43375AL	12CEB2196SAL	2013LSAL04524
Serotype	Derby	Paratyphi B	Paratyphi B	1,4,[5],12:i:-
Year	2013	2012	2012	2013
Sample type	Chipolata sausage	Ready-to-cook guinea fowl pie	Chicken breast with skin	Boot swabs from broiler farm
French department	62	56	85	01
AMP	Non-res	Non-res	Non-res	Res
AMC	Non-res	Non-res	Non-res	Non-res
CAZ	Non-res	Non-res	Non-res	Non-res
CHL	Res	Res	Res	Res
CEF	Non-res	Non-res	Non-res	Non-res
CIP	Non-res	Res	Res	Non-res
CST	Res	Res	Res	Res
CTX	Non-res	Non-res	Non-res	Non-res
GEN	Non-res	Res	Res	Res
KAN	Non-res	Non-res	Non-res	Non-res
NAL	Non-res	Res	Res	Non-res
OFX	Non-res	Non-res	Non-res	Non-res
STR	Res	Res	Res	Res
SSS	Res	Res	Res	Res
SXT	Res	Res	Res	Res
TET	Res	Res	Res	Res
GenBank accession number	LNCZ00000000	LKJK00000000	LKJJ00000000	LKJD00000000
Plasmid replicon harbouring mcr-1	IncP	IncX4	IncX4	IncP

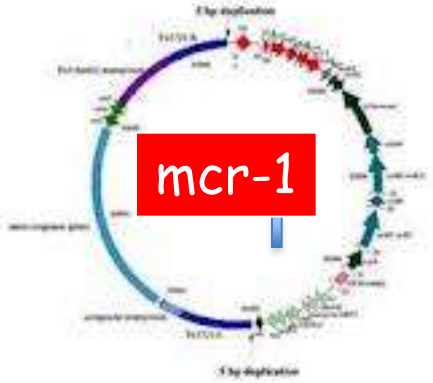
AMP=ampicillin. AMC=amoxicillin-clavulanic acid. CAZ=ceftazidime. CHL=chloramphenicol. CEF=cephalotin. CIP=ciprofloxacin. CST=colistin. CTX=cefotaxime. GEN=gentamicin. KAN=kanamycin. NAL=nalidixic acid. OFX=ofloxacine. STR=streptomycin. SSS=sulfonamides. SXT=trimethoprim-sulfamethoxazole (co-trimoxazole). TET=tetracycline. Res=resistance. Non-res=not resistant.

Table: **Isolate information for *mcr-1* positive isolates selected for whole genome sequencing**

Webb et al. 2016
Lancet Infect Dis

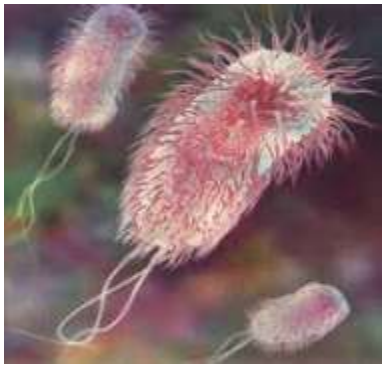
Transfer of plamid-mediated colistin resistance *mcr-1* gene to carbapenamase producers ?

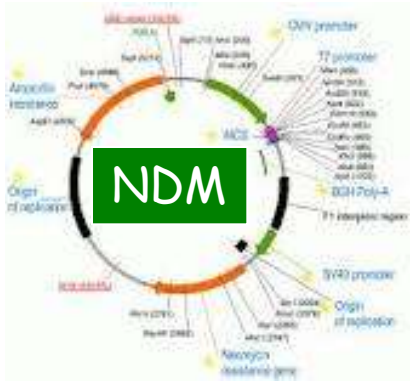
E. coli

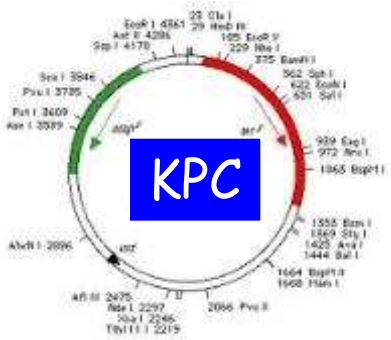


?

K. pneumoniae



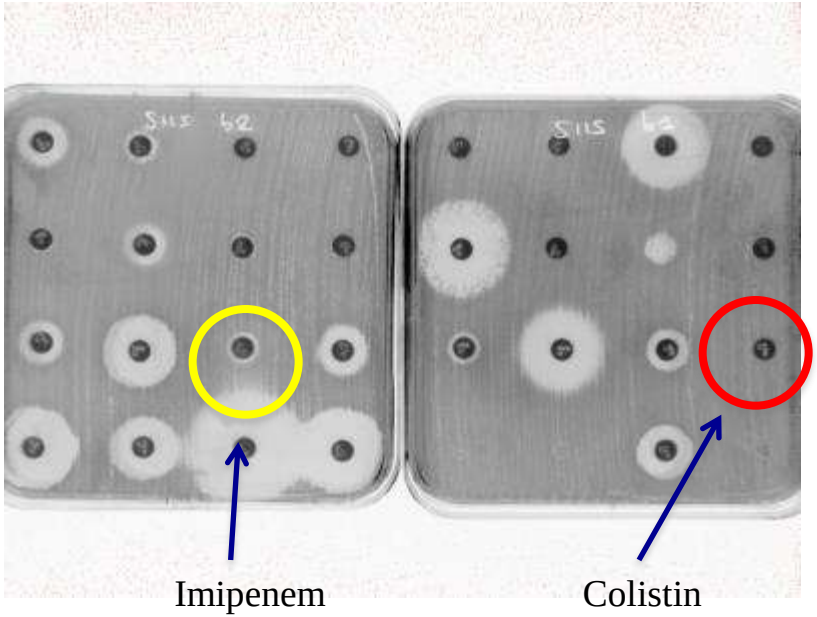




Emergence of plasmid-mediated carbapenem and colistin resistance in *E. coli* in Europe

- Patient from Switzerland, December 2015
- No history of travel abroad
- No colistin-based treatment
- Urinary tract infection: community-acquired

- *E. coli* isolate resistant to carbapenems, fluoroquinolones, aminoglycosides (except amikacin), chloramphenicol, trimethoprim-sulfamethoxazole, and colistin
• Metallo-ß-lactamase VIM-1 +
• Phosphoethanolamine transferase MCR-1



L. Poirel, N.Kieffer, N Liassine, P. Nordmann
Lancet Infect Dis, 2016

.. Then, 2016-2017, MCR-1 also identified with NDM-1, NDM-2, NDM-9, KPC-2 and OXA-48 in *E. coli, K. pneumoniae* and *C. sakazakii*

JOURNAL OF MEDICAL MICROBIOLOGY

SHORT COMMUNICATION

Saly *et al.*, *Journal of Medical Microbiology* 2017;66:842–843

DOI 10.1099/jmm.0.000497

MICROBIOLOGY SOCIETY

Prevalence of faecal carriage of colistin-resistant Gram-negative rods in a university hospital in western France, 2016

Marion Saly,[1,2] Aurelie Jayol,[1,2,3,4,5,*] Laurent Poirel,[3,4,5] Francis Megraud,[1] Patrice Nordmann[3,4,5,6] and Veronique Dubois[1,2]

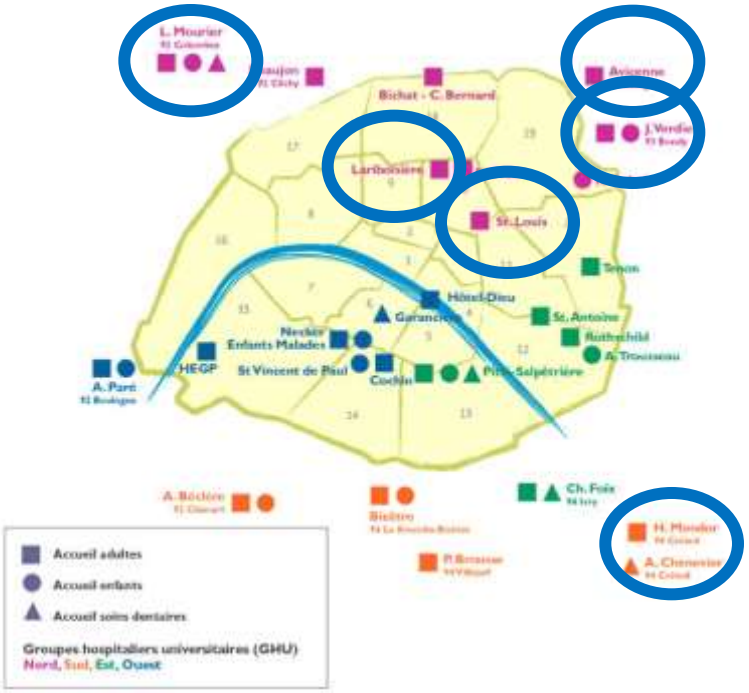
Abstract

Plasmid-mediated and chromosomally-encoded colistin resistance is increasingly being reported worldwide. We aimed to determine the prevalence of faecal carriage of colistin-resistant Gram-negative rod isolates in a university hospital in western France. From February to May 2016, rectal swabs from 653 patients hospitalized in various clinical settings were recovered and subsequently screened for colistin resistance using the SuperPolymyxin medium. Antimicrobial susceptibilities were determined according to EUCAST guidelines. Genetic detection of plasmid-mediated colistin resistance was performed by PCR. The faecal carriage with intrinsic colistin-resistant isolates was high (23 %), while the faecal carriage with Gram-negative rods showing acquired resistance was low (1.4 %). No isolate carried the plasmid-mediated *mcr-1*/*mcr-2* genes. It was noteworthy that none of the patients carrying isolates with acquired colistin resistance had previously received a colistin-based treatment, while these isolates were not multidrug resistant.

The COLI-RED study: population study (Decousser et al.)

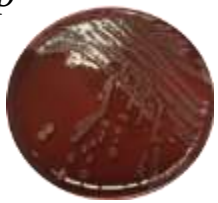
- 6 hospitals in the Paris area
- 3-month period (2016-2017)
- all patients screened systematically upon admission
  - to an intensive care unit
  - anywhere in the hospital if the patient showed risk for carriage of emerging extensively drug-resistant bacteria such as carbapenemase-producing *Enterobacteriaceae* or vancomycin-resistant enterococci (French regulalory action)

  Rectal swab (Eswab®)
- one specimen per patient
- patient information guaranteed

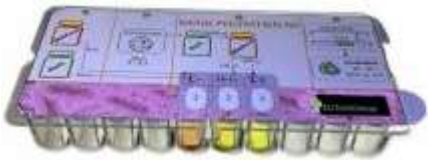


The COLI-RED study: method (1)

- Direct inoculation of one drop of transport medium on **Superpolymyxin®** plate (*ElitechGroup Microbiology, France*) *Nordmann, Jayol, Poirel. J Clin Microbiol 2016*



- **If positive:**
  - Each type of colonies was re-inoculated after standardization of the inoculum on another Superpolymyxin® plate
  - **If positive:**
    - **Identification** using **MALDI-TOF** (discarded if *Proteus sp. / Providencia sp./ Morganella sp./Hafnia alvei*)
    - **Confirmation test = Rapid Polymyxin NP test** ® (*ElitechGroup Microbiology, France*)



Strategy for rapid identification of polymyxin resistance

MCR-1

MCR-1.2

AcrAB, KpnEF overexpression

OmpD defect

PmrB defect

MCR-2

PhoP, PhoQ defect

PmrA defect

Intrinsic Resistance
(Hafnia alvei, Serratia, Proteus..)

CcrB
defect

Mgr B defect

RamA

Capsule overproduction

AcrAB, KpnEF overexpression

MCR-1.2

PmrA defect

RamA

MCR-2

Mgr B defect

MCR-1

OmpD defect

CcrB
defect

Intrinsic Resistance
(Hafnia alvei, Serratia, Proteus..)

PmrB defect

Rapid Culture

Biochemistry, molecular biology

Rapid Polymyxin NP test

RESEARCH

Rapid Detection of Polymyxin Resistance in *Enterobacteriaceae*

Patrice Nordmann, Aurélie Jayol, Laurent Poirel

For identification of polymyxin resistance in *Enterobacteriaceae*, we developed a rapid test that detects glucose metabolization associated with bacterial growth in the presence of a defined concentration of colistin or polymyxin B. Formation of acid metabolites is evidenced by a color change (orange to yellow) of a pH indicator (red phenol). To evaluate the test, we used bacterial colonies of 136 isolates expressing various mechanisms of colistin resistance (chromosomally encoded, and plasmid-mediated MCR-1) and 83 colistin-susceptible isolates. Sensitivity and specificity were 98.9% and 96.4%, respectively, compared with the standard broth microdilution method. This new test is inexpensive, easy to perform, sensitive, specific, and can be completed in <2 hours. It could be useful in countries facing endemic spread of carbapenemase producers and for which polymyxins are last-resort drugs.

Among the most clinically significant multidrug-resistant bacteria are carbapenemase-producing *Enterobacteriaceae*. Because these bacteria usually remain susceptible to polymyxins, an old class of antimicrobial drugs almost abandoned in the 1970s because of their potential toxicity, interest in polymyxins (colistin and polymyxin B) has been renewed worldwide (*1*,*2*). However, the increasing use of colistin explains why acquired colistin resistance may now be added to the carbapenem resistance trait in *Enterobacteriaceae* (*3*).

The standard reference technique for determining susceptibility to polymyxins is broth microdilution, which requires fastidious attention and a long time (24 h) to perform (*4*). Other techniques for determining susceptibility to polymyxins (disk diffusion and Etest) have been proposed and also provide results in 18–24 h. Because of poor diffusion of polymyxin molecules in agar, rates of false susceptibility are high (up to 52%) (*4*,*5*).

Acquired resistance to colistin in *Enterobacteriaceae* results mainly from modification of lipopolysaccharide (*6*). Addition of phosphoethanolamine, 4-amino-4-arabinose, or both to lipopolysaccharide decreases polymyxin binding to the bacterial outer membrane. Addition of these groups may be associated with chromosome-encoded mechanisms (mutations in PmrAB or PhoPQ two-component systems or alterations of the *mgrB* gene) (*6*). A recent report revealed that addition of phosphoethanolamine may also be plasmid mediated through the *mcr-1* gene, which confers the first known plasmid-mediated resistance to colistin in isolates from humans and animals (*7*). More recently, the *mcr-1* gene was identified in several plasmid backbones, mostly in *Escherichia coli* (*8*–*10*). There is therefore a need for a test that enables rapid detection of polymyxin resistance in *Enterobacteriaceae* and that may contribute to its containment.

We developed a test (the rapid polymyxin NP [Nordmann/Poirel] test) that detects bacterial growth in the presence of a defined concentration of a polymyxin. Bacterial growth detection (or absence) is based on carbohydrate metabolism (*11*). Acid formation associated with carbohydrate metabolism in *Enterobacteriaceae* can be observed through the color change of a pH indicator. This test is rapid (<2 h) and easy to perform.

Materials and Methods

Isolate Collection

To evaluate the performance of the rapid polymyxin NP test, we used 208 isolates collected from clinical samples worldwide. This collection included 115 *Enterobacteriaceae* isolates resistant to polymyxin: 5 isolates of intrinsically polymyxin-resistant species (*Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia stuartii*, and *Serratia marcescens*) and 110 isolates of various enterobacterial species (*Klebsiella* spp., *E. coli*, *Enterobacter* spp., and *Hafnia alvei*) with acquired resistance to polymyxins (online Technical Appendix, http://wwwnc.cdc.gov/EID/article/22/6/15-1840-Techapp1.pdf), including a previously reported heteroresistant *Klebsiella pneumoniae* isolate for which MIC for colistin was high (*12*). The other 83 enterobacterial isolates belonged to various species and were susceptible to polymyxins (online Technical Appendix).

MIC Determination

To determine MICs for polymyxins, we used the broth microdilution method in cation-adjusted Mueller-Hinton broth (MHB-CA, reference 69444; Bio-Rad, Marnes-La-Coquette, France) as recommended by Clinical Laboratory

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	NaCl alone	Susceptible strain	Resistant strain
Colistin -			
Colistin +			

1. Results: < 2 h (currently AST, 24 h à 48 h)

2. Useful for antibiotic stewardship , isolation of colonized/infected patients

1.Sensibility 99%, specificity 95-98%

Results (1)

- **1,217** rectal swabs originating from :
  - Patients at risk for carriage of **emerging extensively drug-resistant bacteria**: 13%
  - Patients **admitted to ICU: 87%**
    - and hospitalized since:
      - Less than 48 hours: **80%**
      - More than 48 hours: **20%**
- = **a relevant snapshot of the colistin resistance prevalence mostly from the community setting**
- Rate of *E. coli* growing on SuperPolymyxin®

  With a **positive confirmation test** (Rapid Polymyxin NP test®); n=**168** (12.7 % of patients)

Results (2) Prevalence of *mcr* genes

- **All colistin-resistant *E. coli* isolates (n = 168) tested**

✓ **7 *mcr*-1-positive**

✓ **No other *mcr* gene detected**

✓ **161 *mcr*-negative and colistin-resistant strains**

Results (3): Genotype analysis

- **The 7 *mcr*-1 positive *E. coli* isolates were submitted to whole genome sequencing**
- **The strain backgrounds corresponded to commensal phylogroups (A, B1, E, and Clade I)**
- **The ST types were all different and all but one corresponded to *E. coli* backgrounds always identified from animal sources**
- **The plasmid scaffolds bearing the *mcr-1* gene were diverse, corresponding to the formerly identified *mcr-1*-positive plasmids (IncHI2, IncX3, IncP)**

Origin of this high rate of colistin resistant *E. coli*?

- Antimicrobial selective pressure? Unlikely owing to:
  - The community origin for a large part of the patients (>80%)
  - The low consumption of polymyxin in/out hospital setting
- Co-selection of colistin resistance through another way /mechanism beyond the use of colistin?

Genetic determinant?

- WGS in progress ; Almost all colistin-resistant and non-MCR producing *E. coli* possess a background corresponding to human commensal strains
- Most of those isolates possess mutations in chromosomal genes involved in LPS modification

Take home message

- Chromosome and plasmid-mediated colistin resistance are now identified, in particular in *E. coli* and *K. pneumoniae*
- Plasmid-mediated resistance is mostly in *E. coli* among animal isolates
- Association of plasmid-mediated colistin resistance and carbapenemases has been already reported
- High rate of « isolated » polymyxin resistance in E. coli in humans of unknown signification
- Rapid diagnostic technique for identification of polymyxin resistance is now available