# Résistances Emergentes aux antibiotiques


UNIVERSITÉ DE FRIBOURG
UNIVERSITÄT FREIBURG

Instituts thématiques Inserm
Institut national de la santé et de la recherche médicale

Paris

Centre hospitalier universitaire vaudois

NARA
Nationales Referenzlaboratorium zur Früherkennung neuer Antibiotikaresistenzen und Resistenzmechanismen

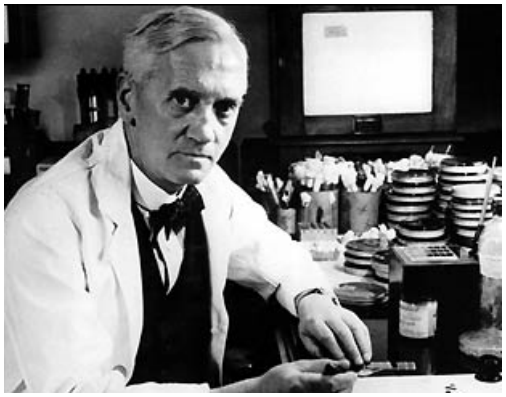
**Prof. Patrice Nordmann**

# Discovery of Penicillin

1932 Country doctor Cecil Paine tried “Fleming’s droplets” on patients. Showed his successful results to Howard Florey who was looking for a new project.

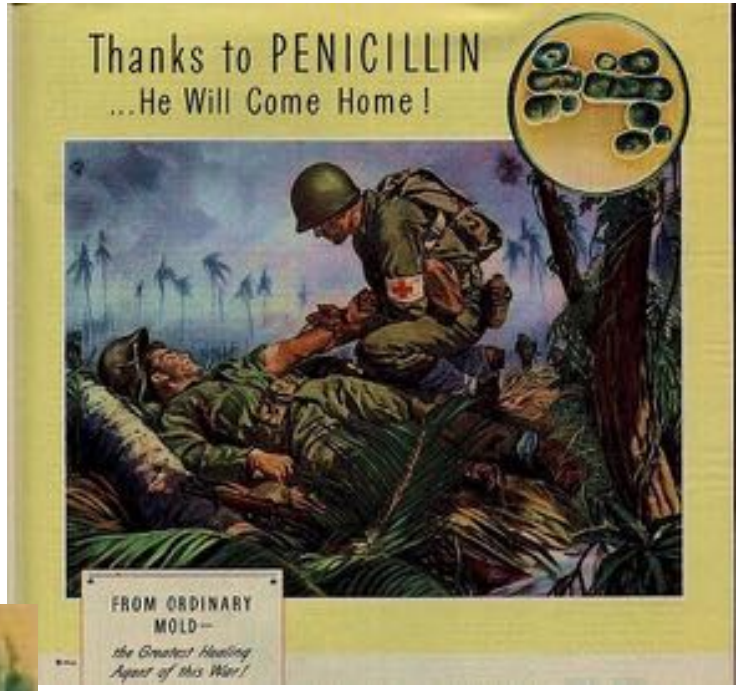
1938-40 Florey & Chain isolated the active principle - penicillin - from the droplets and tested it.

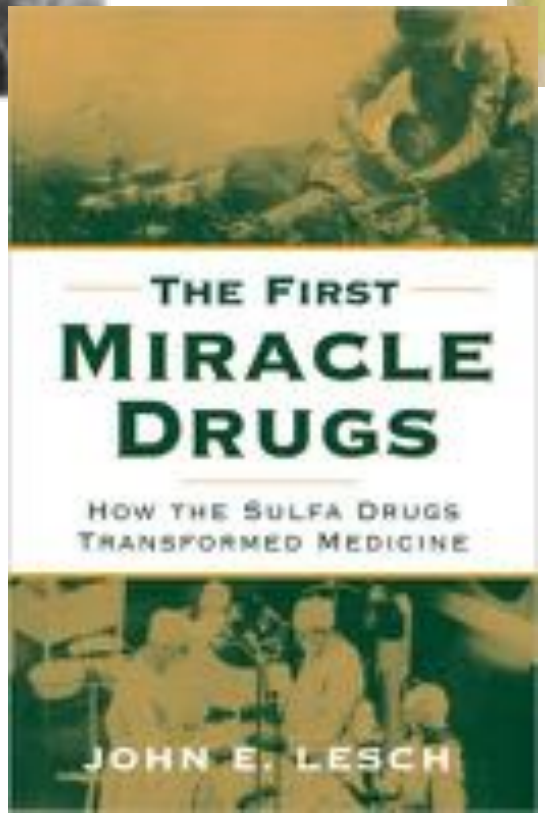
The first patients treated showed improvement but it was not possible to make enough material to complete the cure.

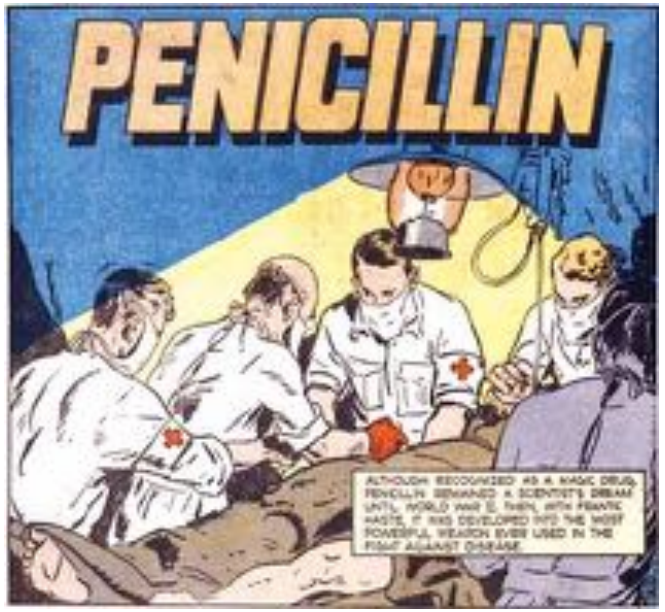


1945 Fleming, Chain and Florey received the Nobel Prize in Medicine.


The new, modern way to protect small injuries—
Sulfa-thiazole Band-Aid!


Thanks to PENICILLIN
...He Will Come Home!
FROM ORDINARY MOLD—
the Greatest Healing Agent of this War!


THE FIRST
MIRACLE DRUGS
HOW THE SULFA DRUGS TRANSFORMED MEDICINE
JOHN E. LESCH


PENICILLIN

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## Global Health Threats of the 21st Century

FINANCE & DEVELOPMENT, December 2014, Vol. 51, No. 4

PDF version

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For Immediate Release

September 18, 2014

## FACT SHEET: Obama Administration Takes Actions to Combat Antibiotic-Resistant Bacteria

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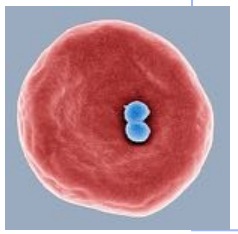
## NATIONAL STRATEGY FOR COMBATING ANTIBIOTIC-RESISTANT BACTERIA

*d States will work domestically and internationally to prevent, detect ness and death related to infections caused by antibiotic- resistant menting measures to mitigate the emergence and spread of antibiotic resistance and ensuring the continued availability of therapeutics for the treatment of bacterial infections.*

September 2014



THE WHITE HOUSE
WASHINGTON

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72 NRP

Antimicrobial Resistance
National Research Programme

A one health approach

Call for proposals

2016

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Antibiorésistance –
un objectif, une stratégie

Maintenir l'efficacité des antibiotiques

Stratégie Antibiorésistance
StAR

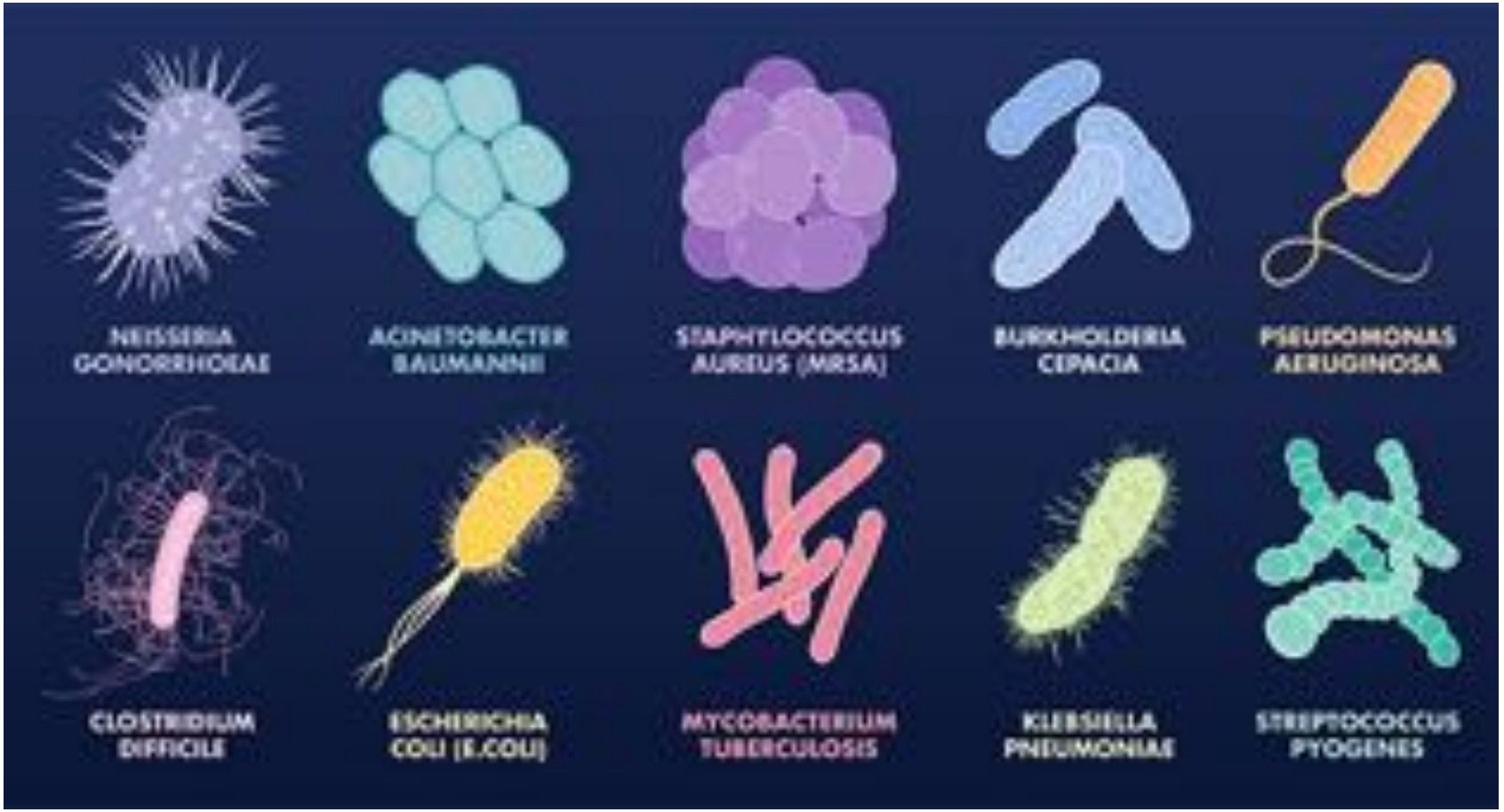
---

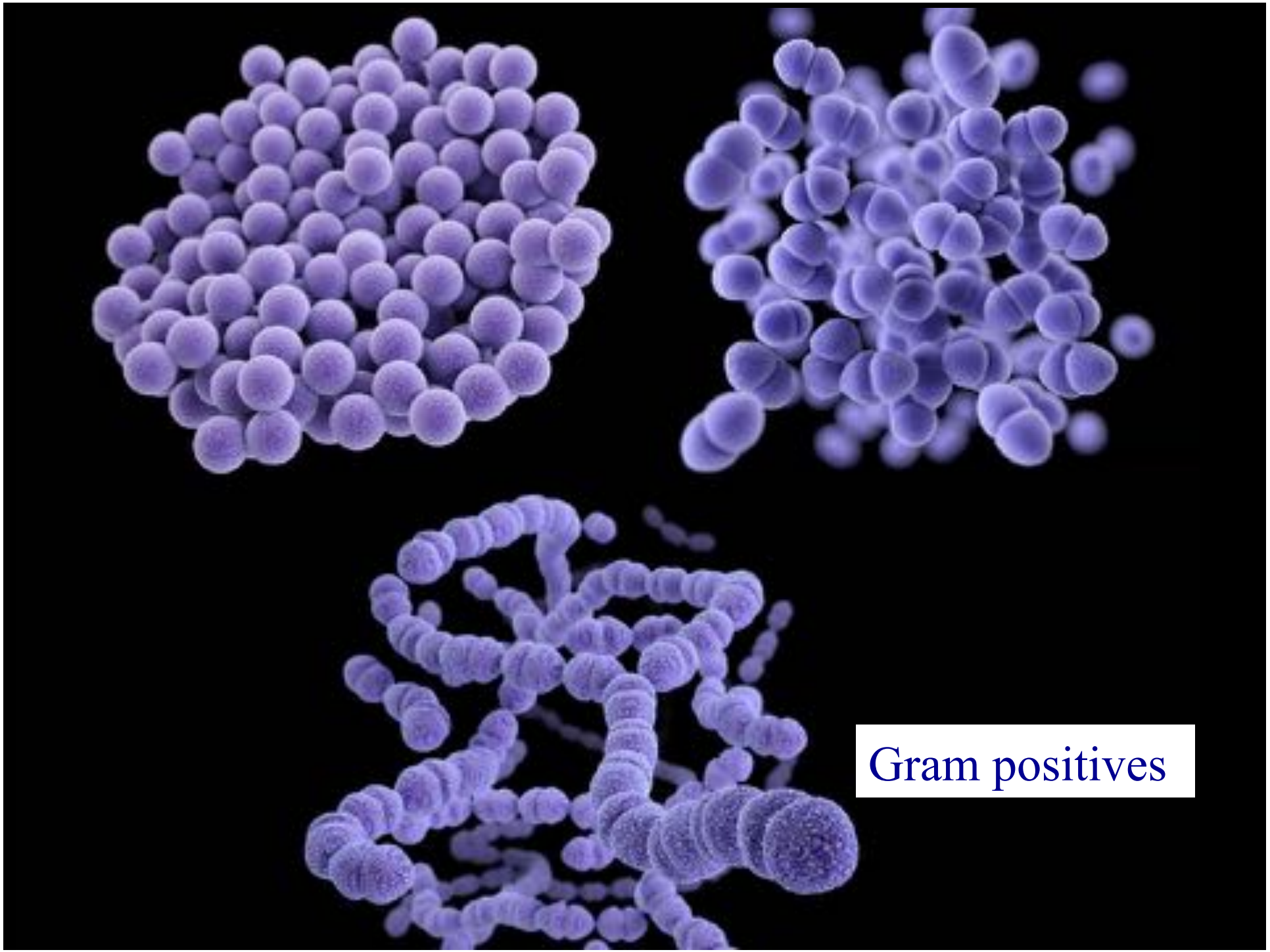
## Huit domaines d'action

Les mesures de la Stratégie Antibiorésistance concernent l'être humain, les animaux, l'agriculture et l'environnement. Elles se répartissent en huit domaines d'action. La stratégie se fonde sur l'approche One Health.

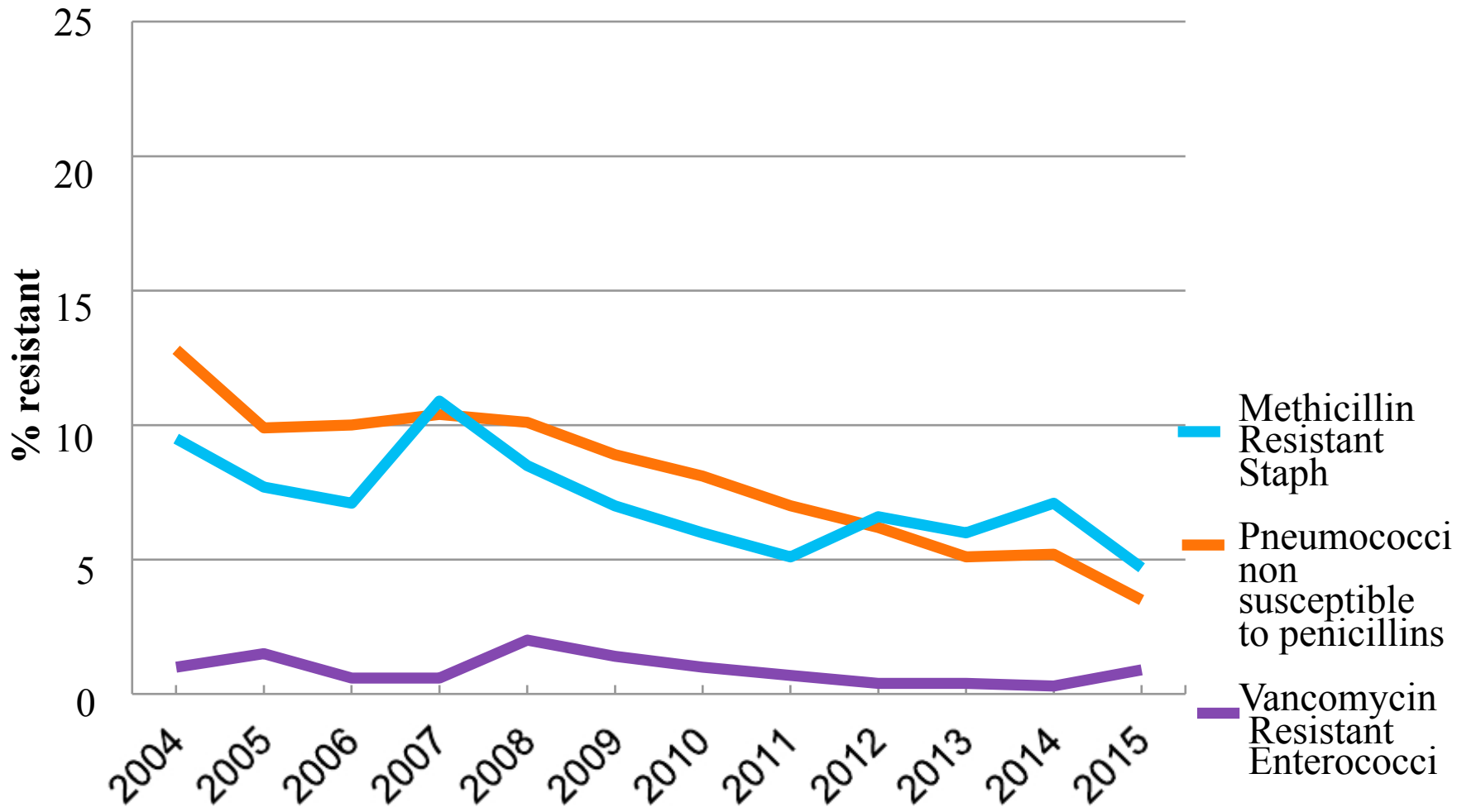

www.bag.admin.ch/fr/star

# Most « dangerous bacteria… »




Gram positives

# Proportion (%) of multidrug resistant pathogens in invasive isolates, 2004-2015




BAG Bulletin 2015, 35:650-1

# Novel antibiotics: ceftaroline/ceftopibrole

- Broad spectrum cephalosporins with anti-MRSA activity
- Spectrum:
  - MRSA, VISA, VRSA
  - Multidrug-resistant *Streptococcus pneumoniae*
- Mechanism of action: cell wall synthesis inhibition
  - Inhibition of transpeptidation (high affinity for PBP2a)
- Bactericidal

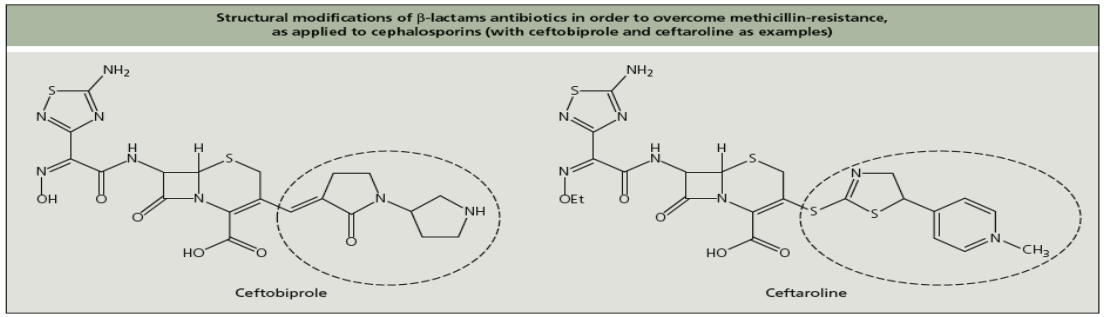
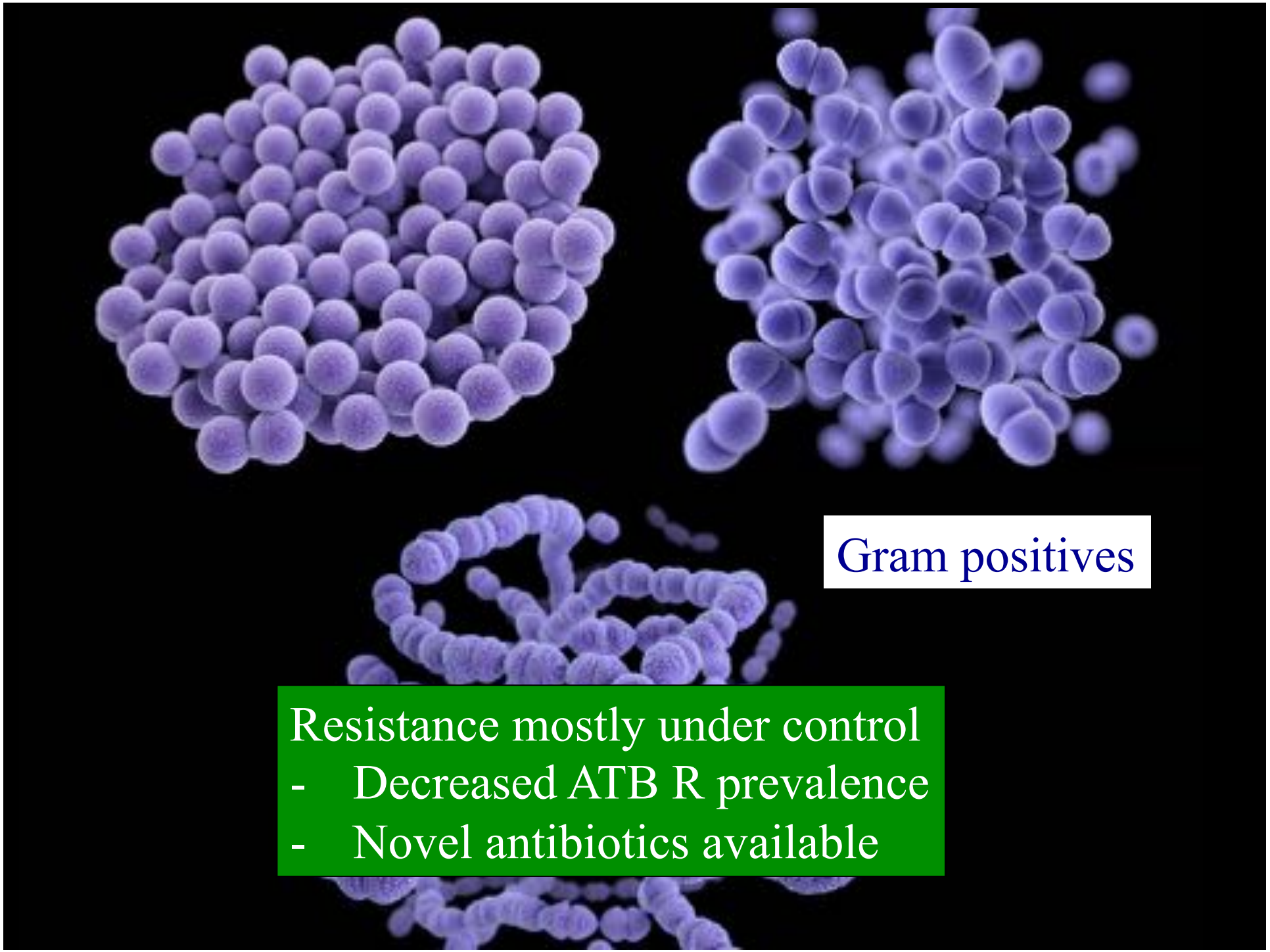


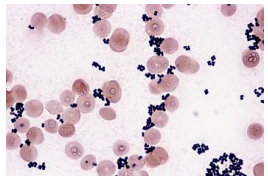

Fig. 130.4 Structural modifications of β-lactam antibiotics in order to overcome methicillin resistance, as applied to cephalosporins (with ceftobiprole and ceftaroline as examples). The bulky hydrophobic moieties (dotted-lined ellipse) added to the molecules forces a conformational change in PBP2a resulting in the opening of the active site and allowing acylation (inactivation) by the antibiotic. Although activity is largely restored towards methicillin-resistant organisms, MICs remain still typically one to four dilutions higher than for susceptible ones. The increase in lipophilicity also makes it necessary to administer the molecules as prodrugs – medocaril for ceftobiprole and fosamyl for ceftaroline (not shown).


Gram positives
Resistance mostly under control
- Decreased ATB R prevalence
- Novel antibiotics available

# Main microbial pathogens in humans

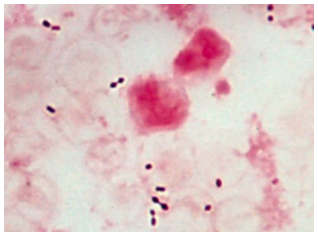
## Gram positives

*Staphylococcus*



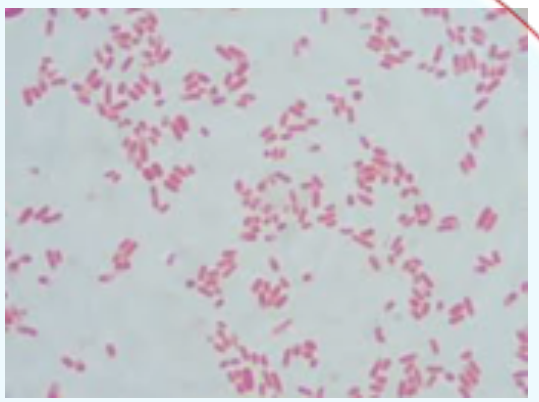
*Enterococcus,*

*Streptococcus*

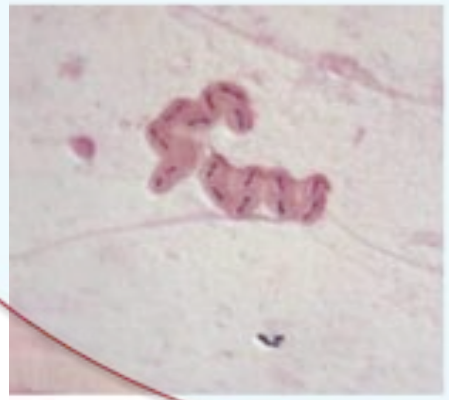


## Gram negatives

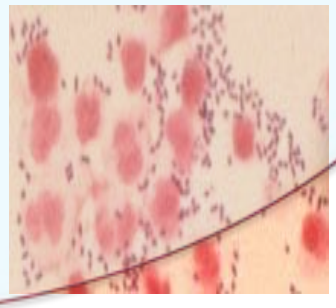
*Enterobacteriaceae (E. coli, K.pneumoniae…)*



*Pseudomonas aeruginosa*



*Acinetobacter baumannii*



# Pouvoir pathogène de *E. coli*

. infections urinaires

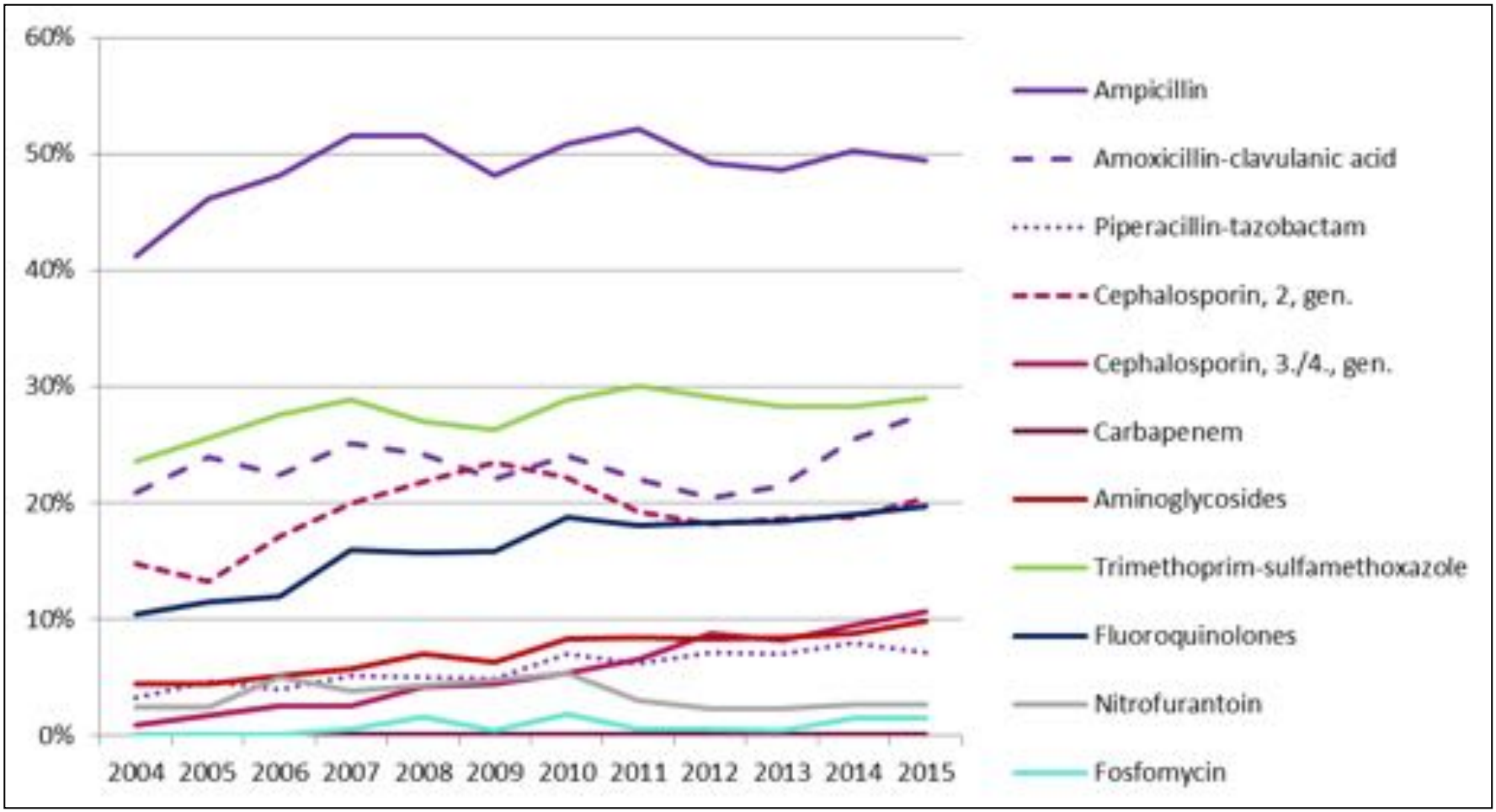
. infections intra-abdominales

. entérites

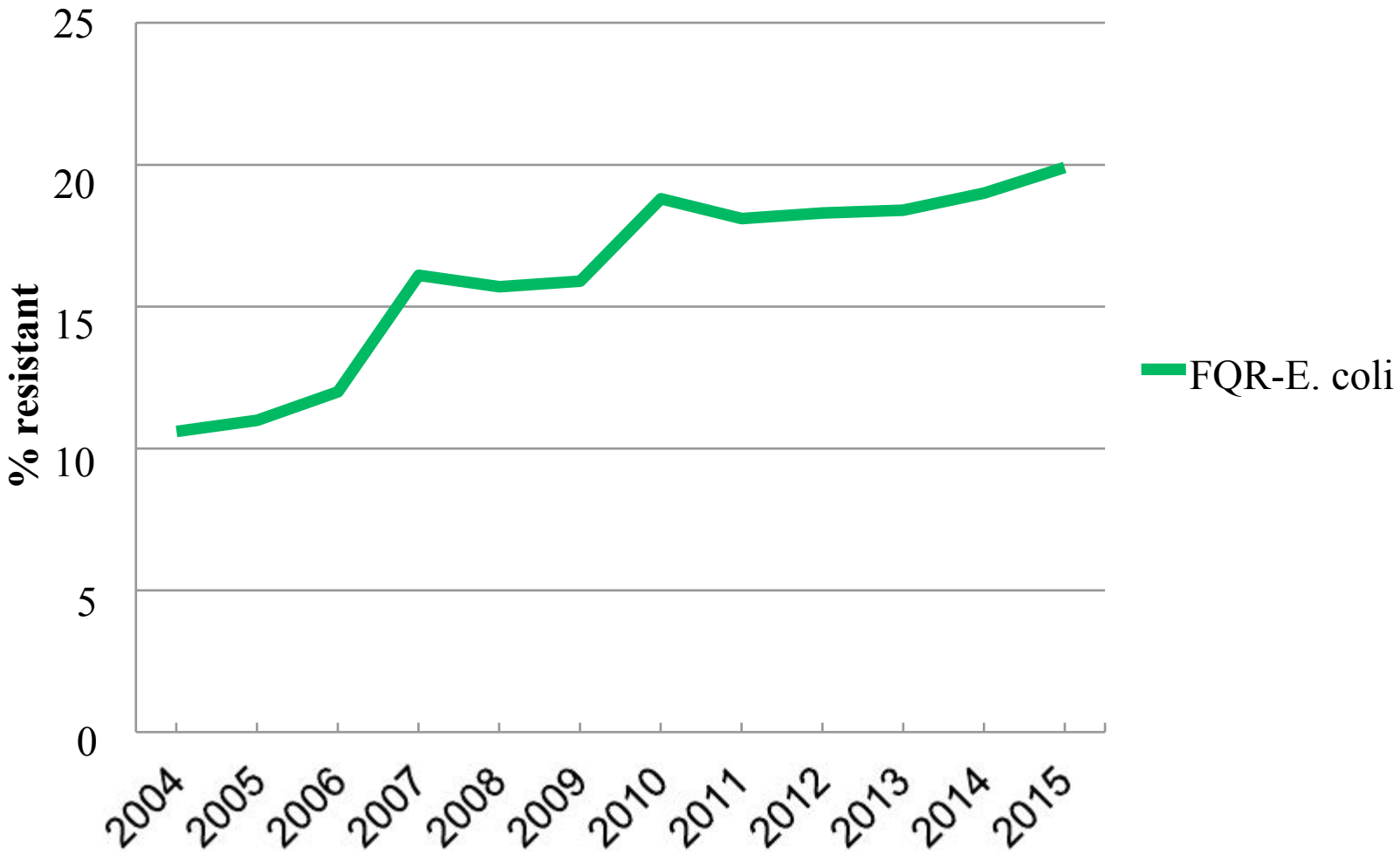
. méningites néonatales

. pneumopathies….

# Résistance aux antibiotiques des souches invasives de *Escherichia coli*, Suisse, 2004-2015

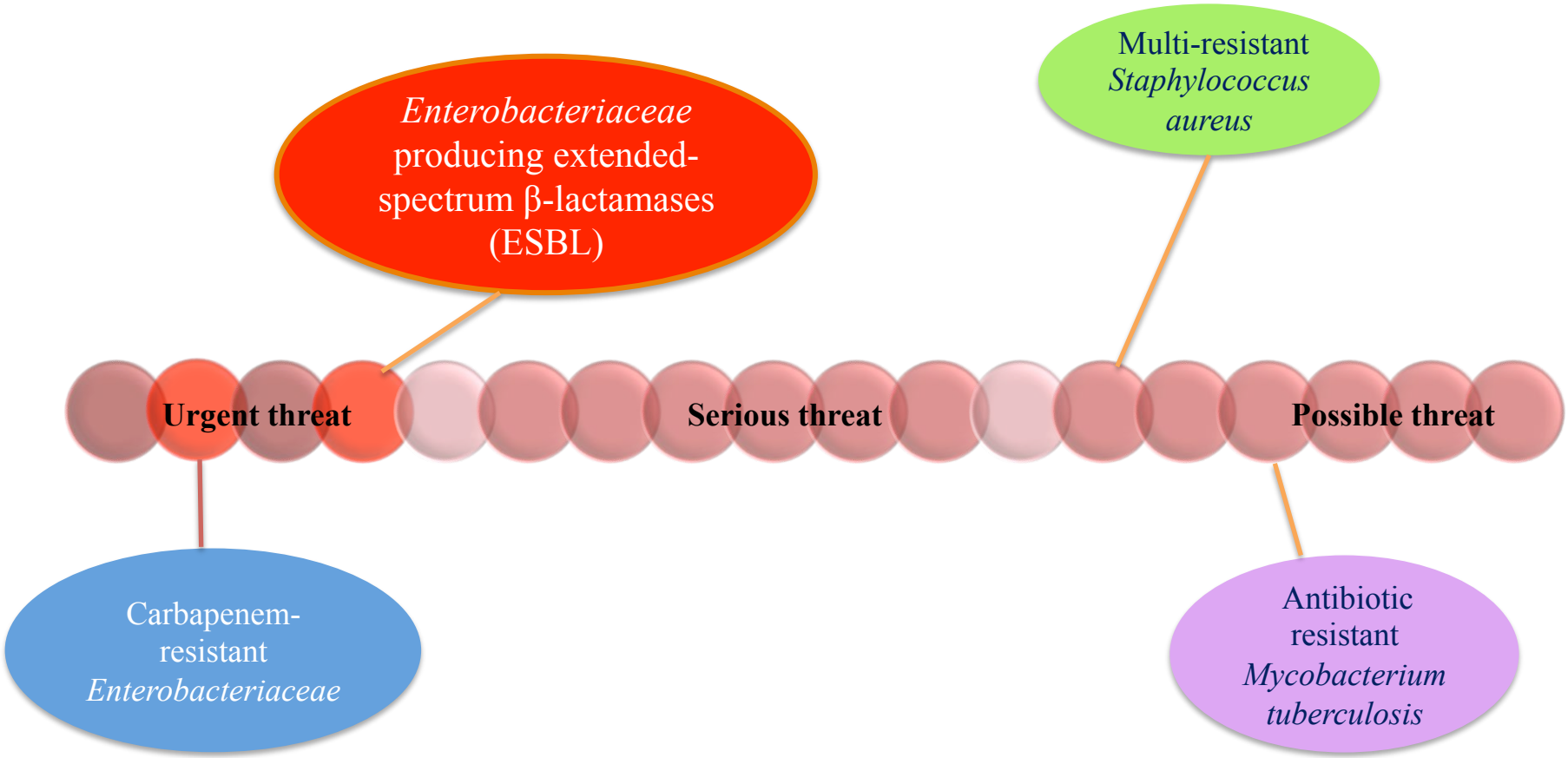


# Proportion (%) de souches invasives de *E. coli* résistantes aux fluoroquinolones Suisse, 2004-2015




BAG Bulletin 2015, 35:650-1

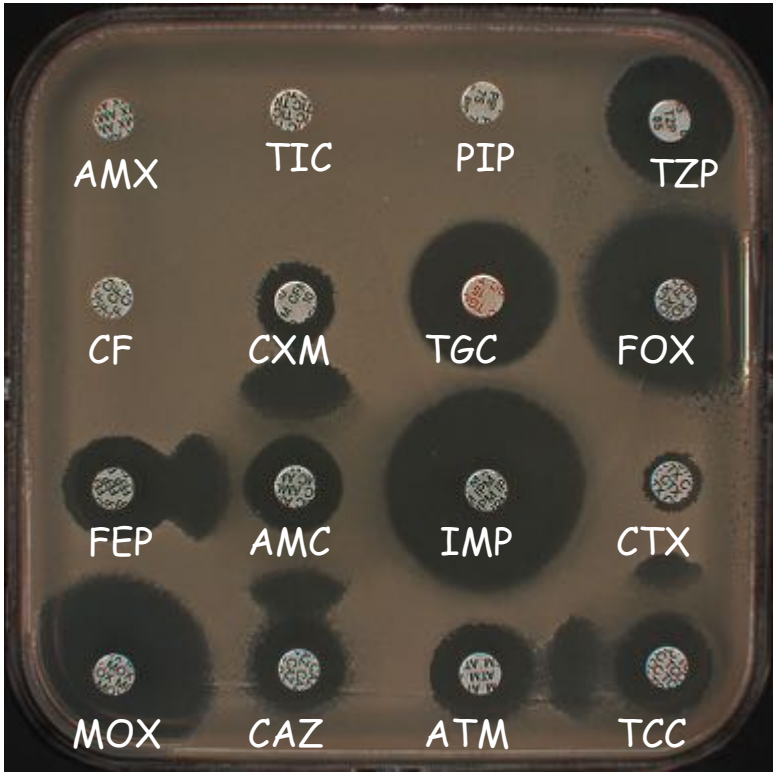
# Emerging Resistance threats, CDC – USA-2015



# Extended-spectrum ß-lactamases (ESBLs)

| Penicillin/Amino-penicillins | Ureido-penicillins | 1/2nd generation cephalosporins | 3 rd generation cephalosporins | 4/5th generation cephalosporins |
|---|---|---|---|---|
| • Penicillin G<br>• Ampicillin<br>• Amoxicillin | •Ticarcillin<br>•Piperacillin | •Cefazolin<br>• Cefuroxime | • Ceftriaxone<br>• Ceftazidime | • Cefepime<br>• Ceftaroline |

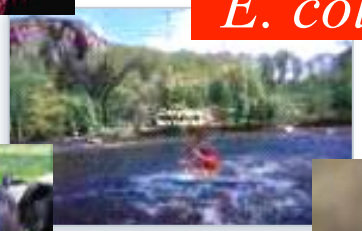
# Multidrug resistance of ESBL-producing *Escherichia coli*

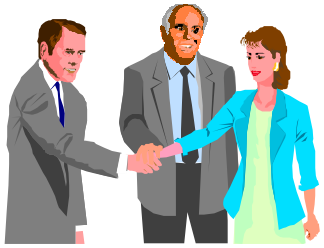



# Plasticity of the ESBL (CTX-M) genes; from the environment to infected and hospitalized patients

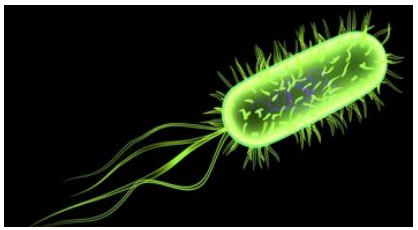
*Kluyvera sp.* (CTX-M)

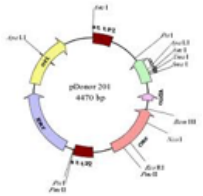
*E. coli* (CTX-M)





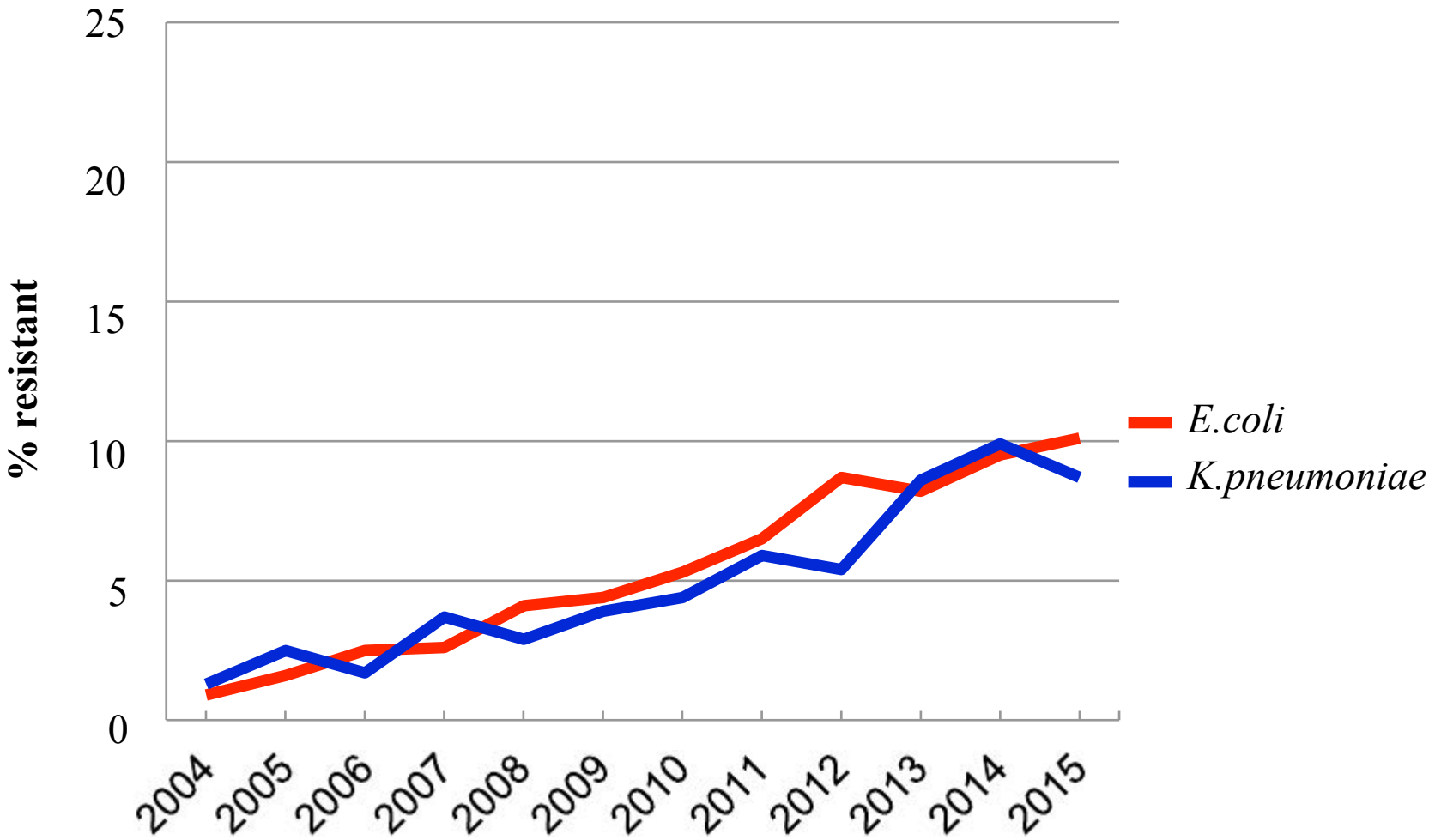
*K. pneumoniae* (CTX-M)





*E. coli* (CTX-M)

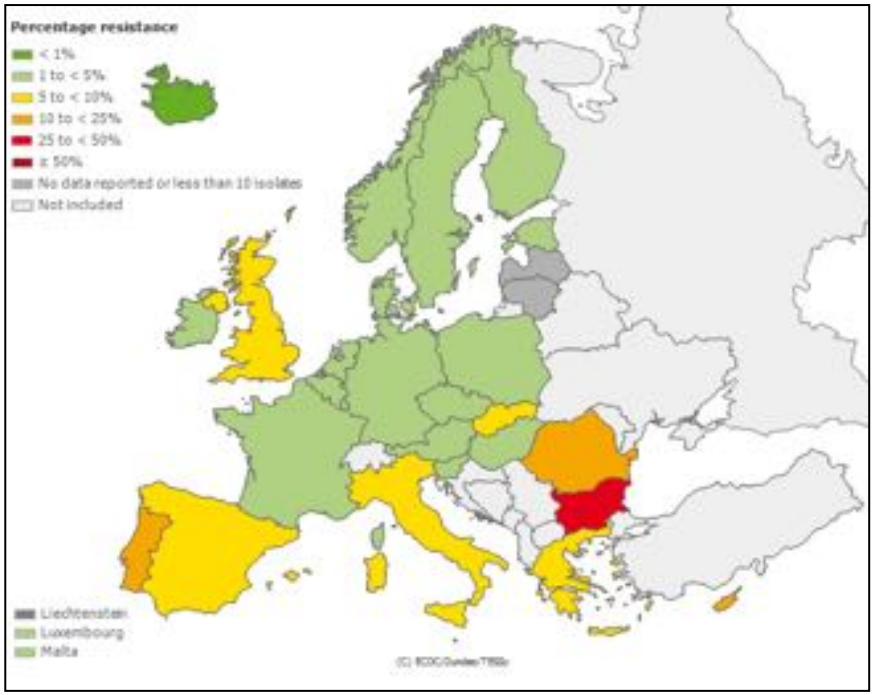
# **Proportion (%) of** extended-spectrum cephalosporin resistant in invasive isolates, 2004-2015 in Switzerland



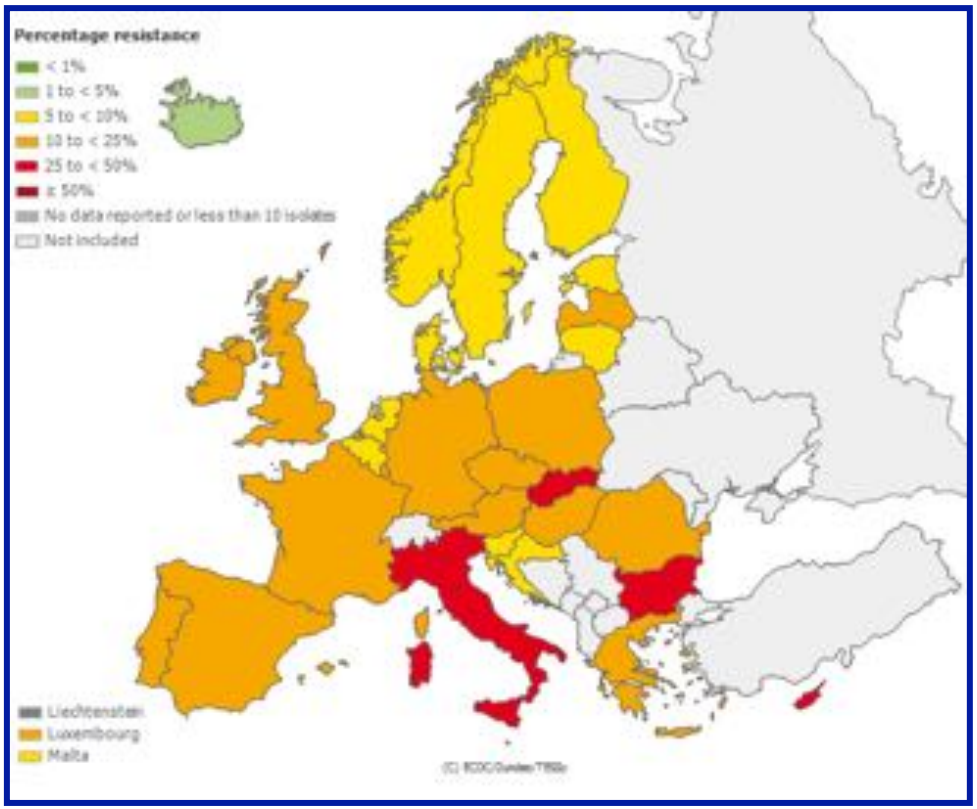

BAG Bulletin 2015, 35:650-1

# Résistance aux céphalosporines à large spectre
# Souches invasives de *E. coli*

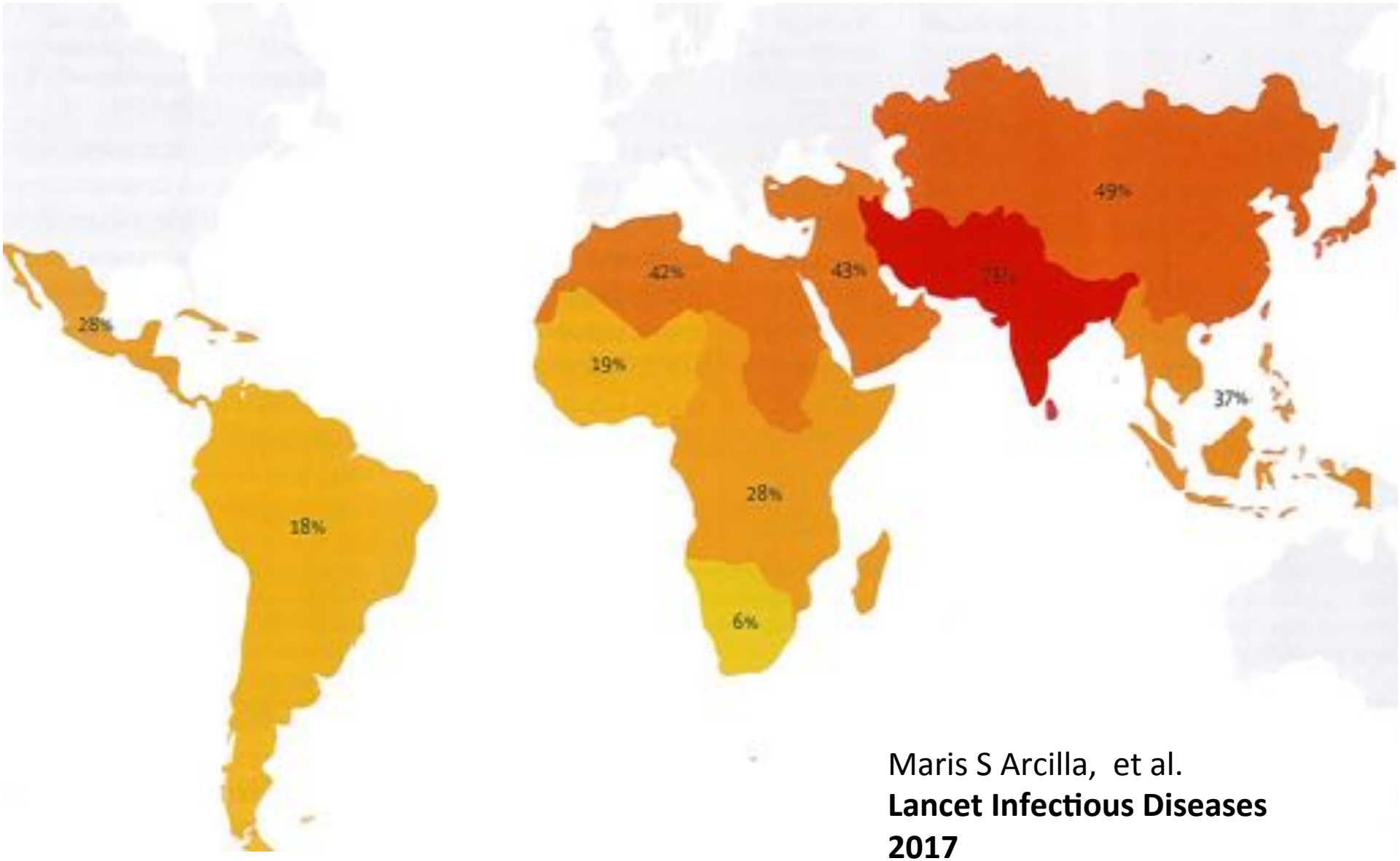
2005




2014

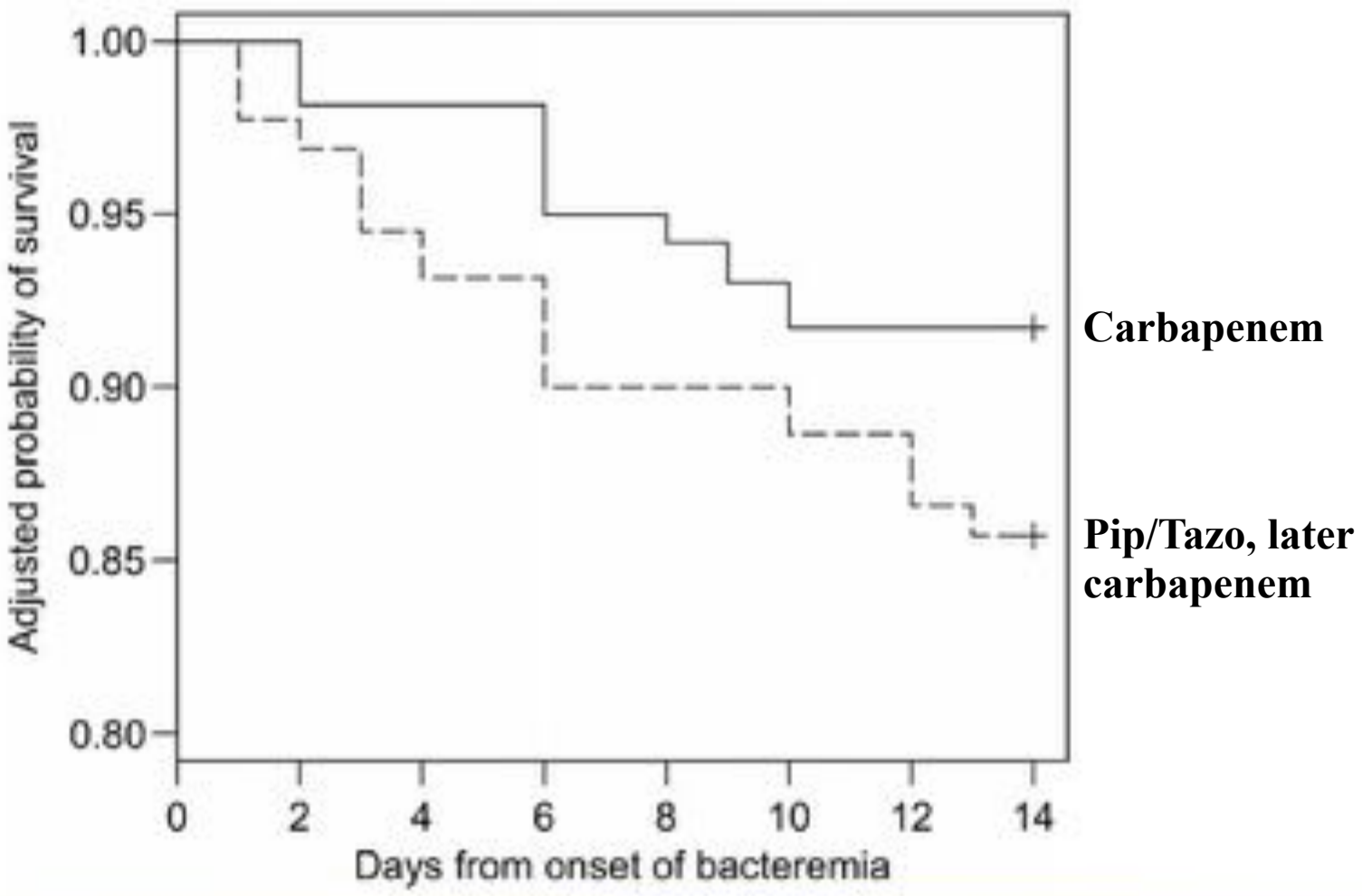


# Percentages of travellers that acquired ß-lactamase-producing *Enterbobacteriaceae*

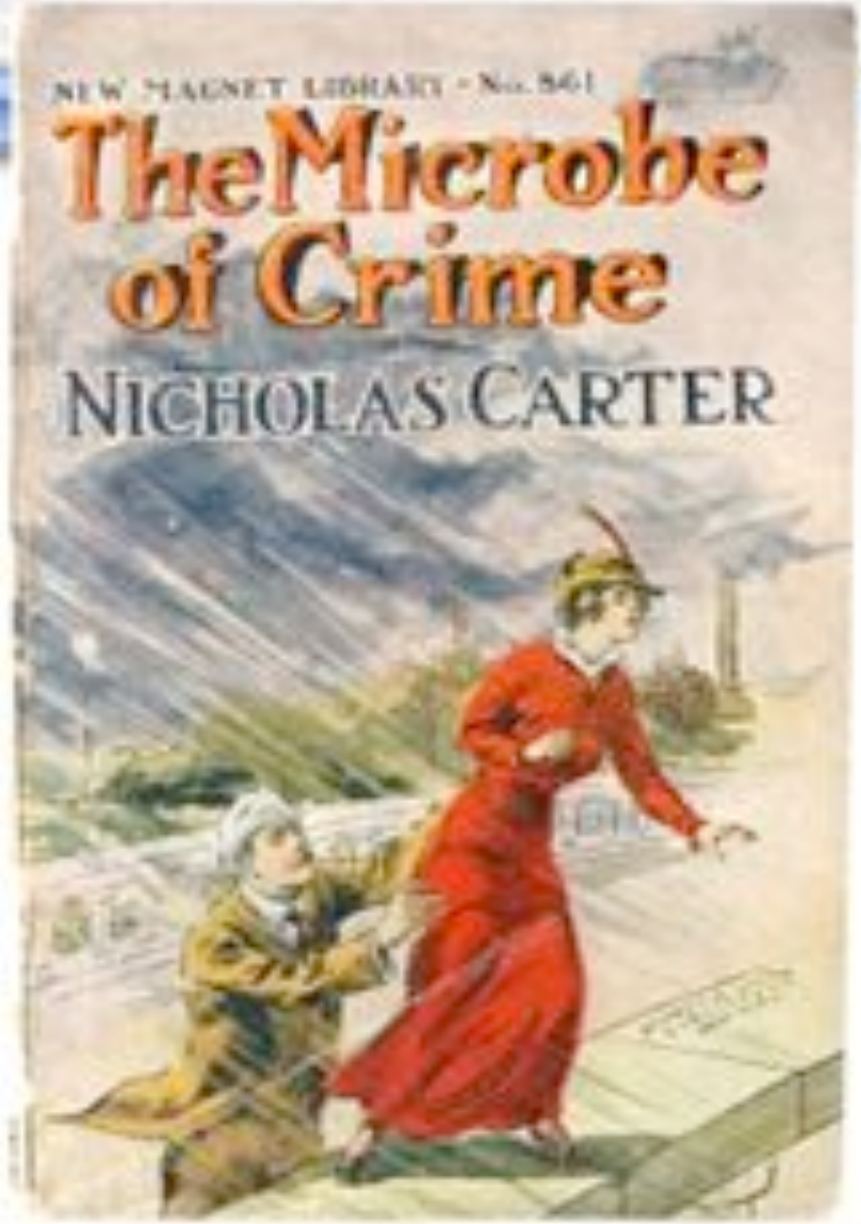



Maris S Arcilla, et al.
**Lancet Infectious Diseases**
**2017**

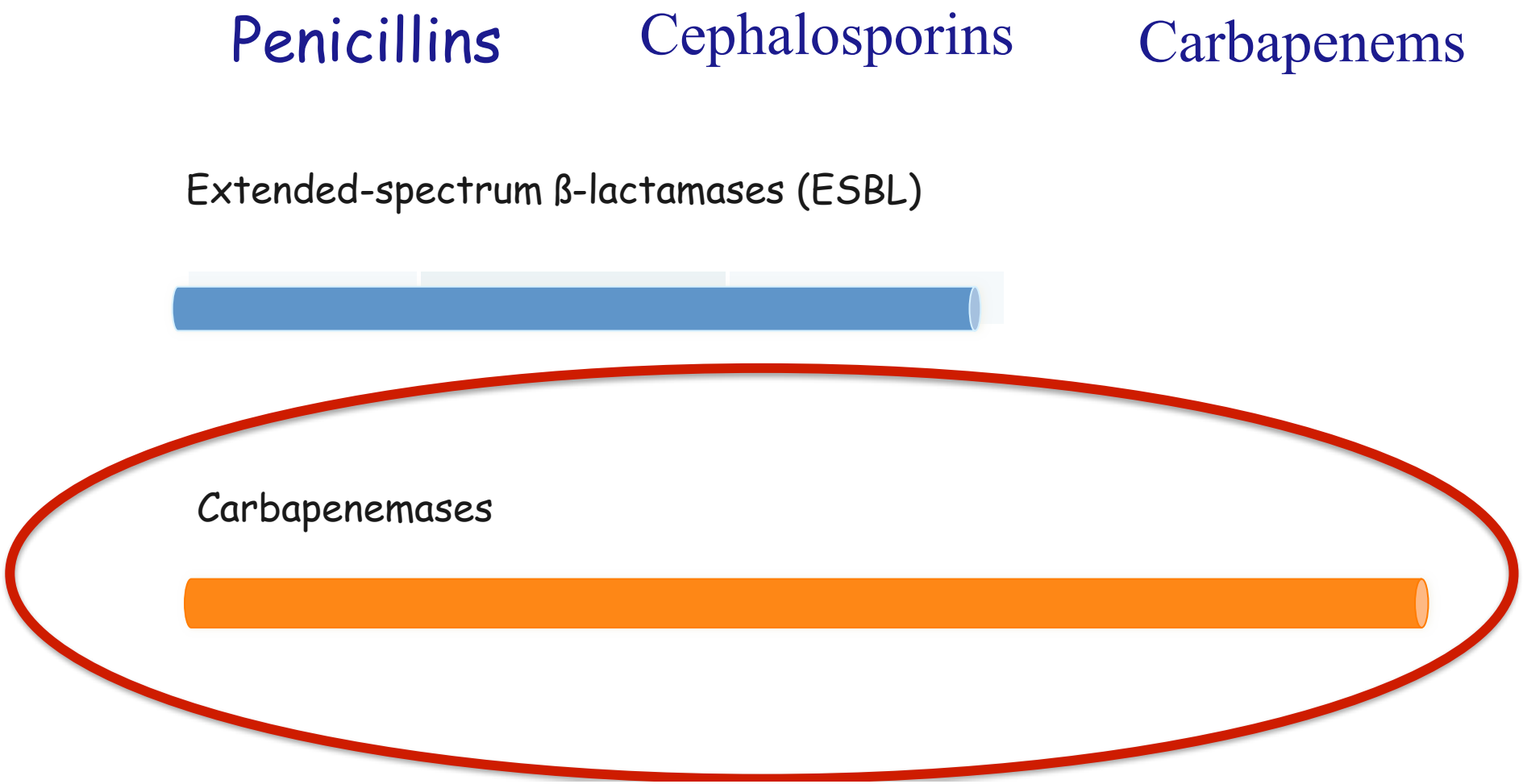
# Probability of survival of patients with ESBL bacteremia




Clin Infect Dis 2015, 60:1319–25


NEW MAGNET LIBRARY - No. 861
The Microbe of Crime
NICHOLAS CARTER

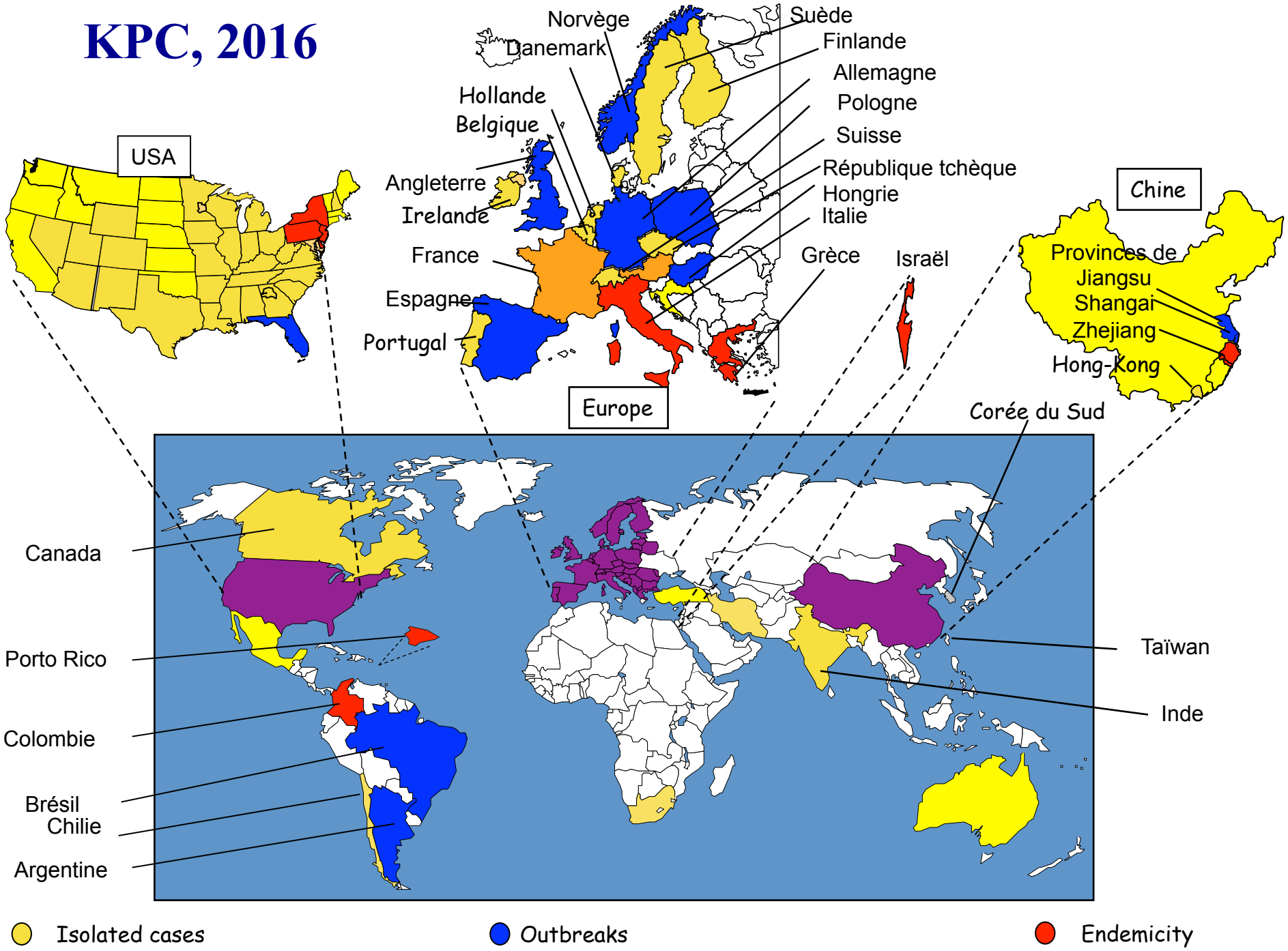
# Broad-spectrum ß-lactamases in gram negatives



# KPCs; Klebsiella Pneumoniae Carbapenemase

Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of *Klebsiella pneumoniae*


# KPC, 2016


USA
Norvège
Suède
Danemark
Finlande
Allemagne
Hollande
Pologne
Belgique
Suisse
République tchèque
Angleterre
Hongrie
Irelande
Italie
France
Grèce
Israël
Espagne
Portugal
Europe
Chine
Provinces de
Jiangsu
Shangai
Zhejiang
Hong-Kong
Corée du Sud
Canada
Taïwan
Porto Rico
Inde
Colombie
Brésil
Chilie
Argentine
Isolated cases
Outbreaks
Endemicity

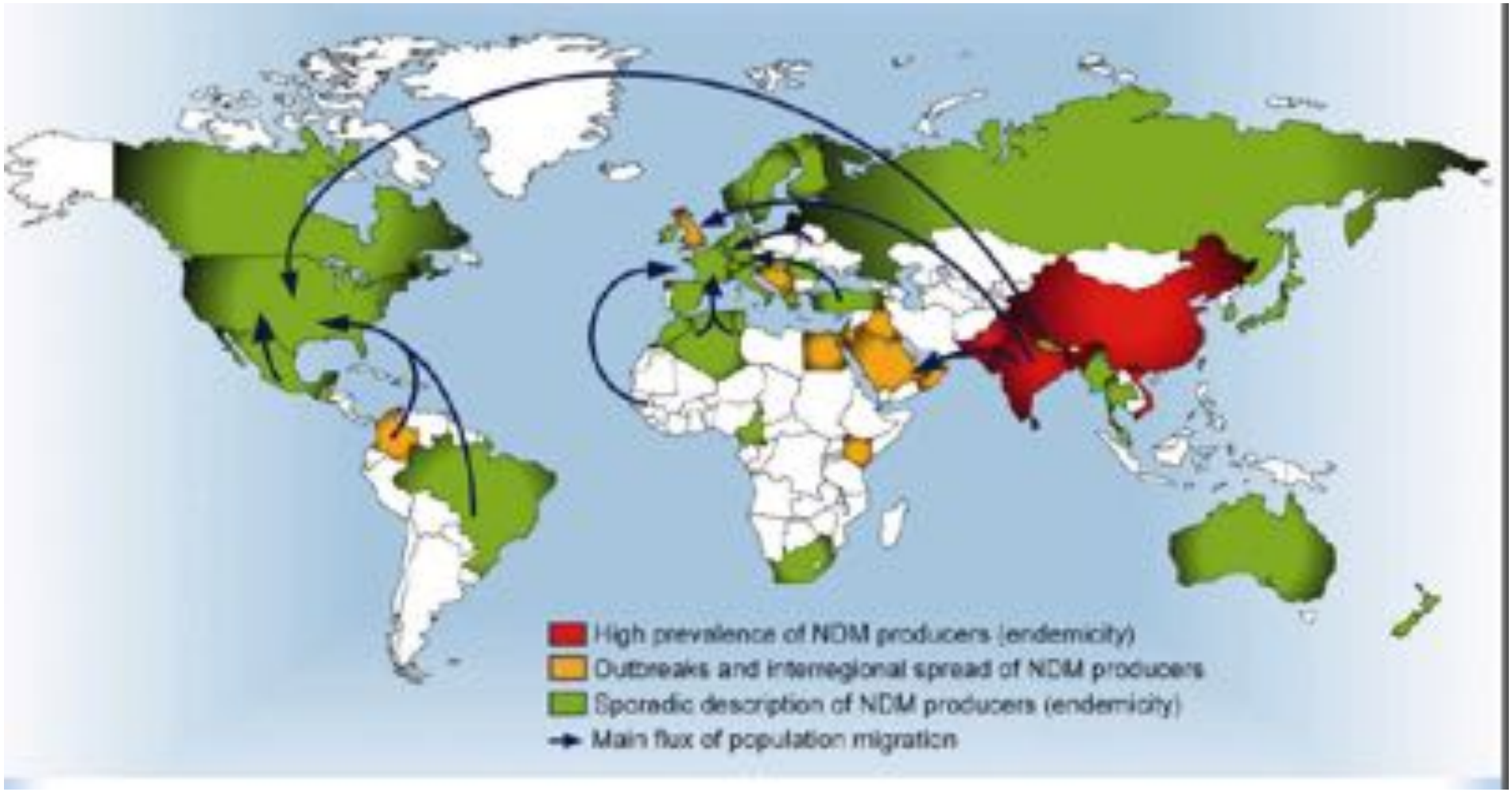
AAC.2009; 53 5046-5054

# Characterization of a New Metallo-β-Lactamase Gene, $bla_{NDM-1}$, and a Novel Erythromycin Esterase Gene Carried on a Unique Genetic Structure in *Klebsiella pneumoniae* Sequence Type 14 from India[▽]

Dongeun Yong,[1,2] Mark A. Toleman,[2] Christian G. Giske,[3] Hyun S. Cho,[4] Kristina Sundman,[5] Kyungwon Lee,[1] and Timothy R. Walsh[2]*

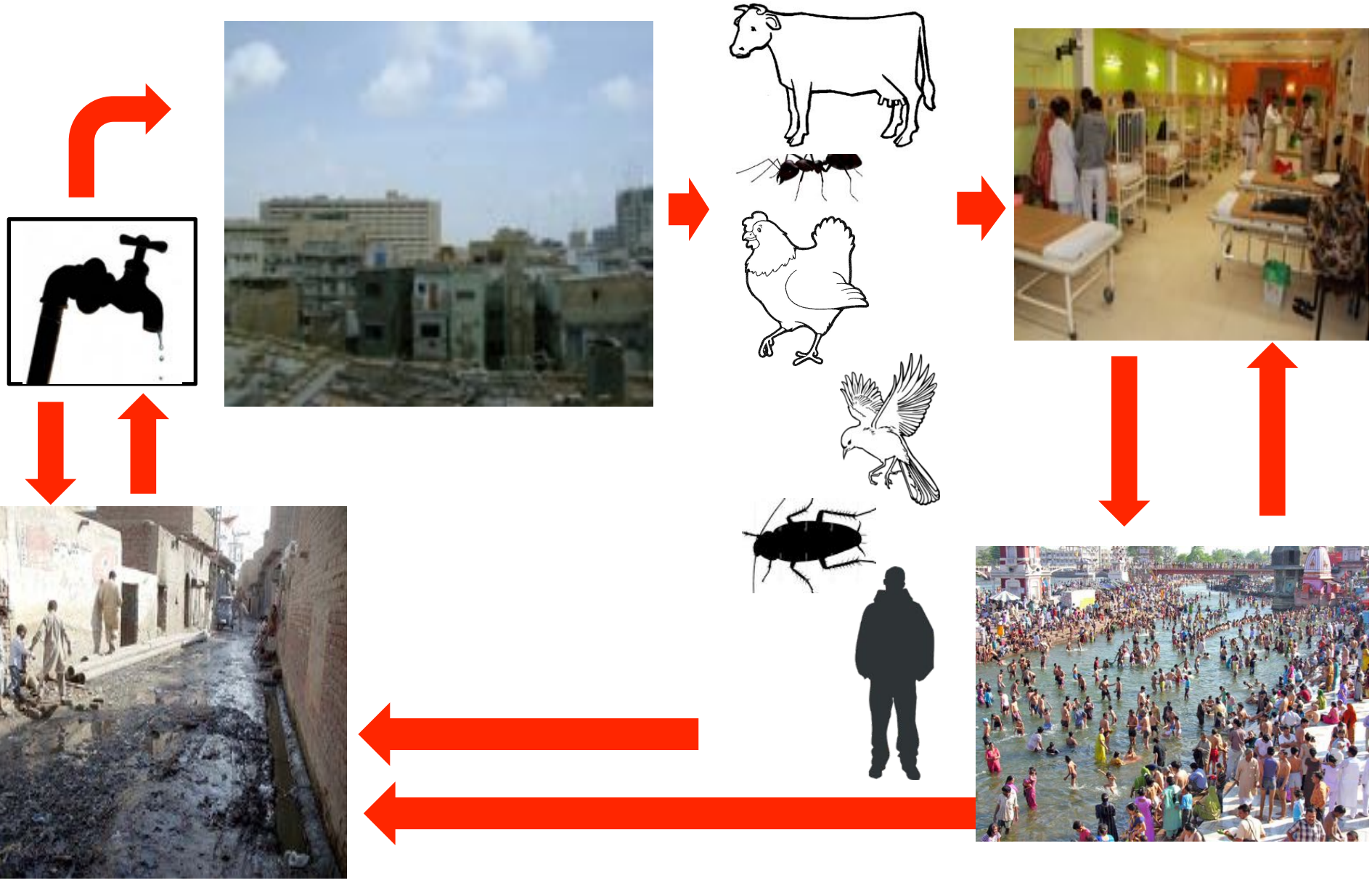
*Yonsei University College of Medicine, Research Institute of Antimicrobial Resistance, Seoul, Republic of Korea[1]; Department of Medical Microbiology, Cardiff University, Cardiff, United Kingdom[2]; Clinical Microbiology, MTC—Karolinska Institutet, Karolinska University Hospital, Stockholm, Sweden[3]; Yonsei University College of Life Science and Biotechnology, Seoul, Republic of Korea[4]; and Department of Clinical Microbiology, Örebro University Hospital, Örebro, Sweden[5]*


# Wordwide spread of NDM carbapenemases in *Enterobacteriaceae*




*Nordmann et al EID 2015*

# Compartmentalization of sectors is not valid anymore




Infection
DOI 10.1007/s15010-017-1007-2

ORIGINAL PAPER

# Environmental pollution with antimicrobial agents from bulk drug manufacturing industries in Hyderabad, South India, is associated with dissemination of extended-spectrum beta-lactamase and carbapenemase-producing pathogens

Christoph Lübbert[1,2] · Christian Baars[3] · Anil Dayakar[4] · Norman Lippmann[2,5] · Arne C. Rodloff[2,5] · Martina Kinzig[6] · Fritz Sörgel[6,7]

Received: 27 February 2017 / Accepted: 8 March 2017

# OXA-48 + CTX-M-15

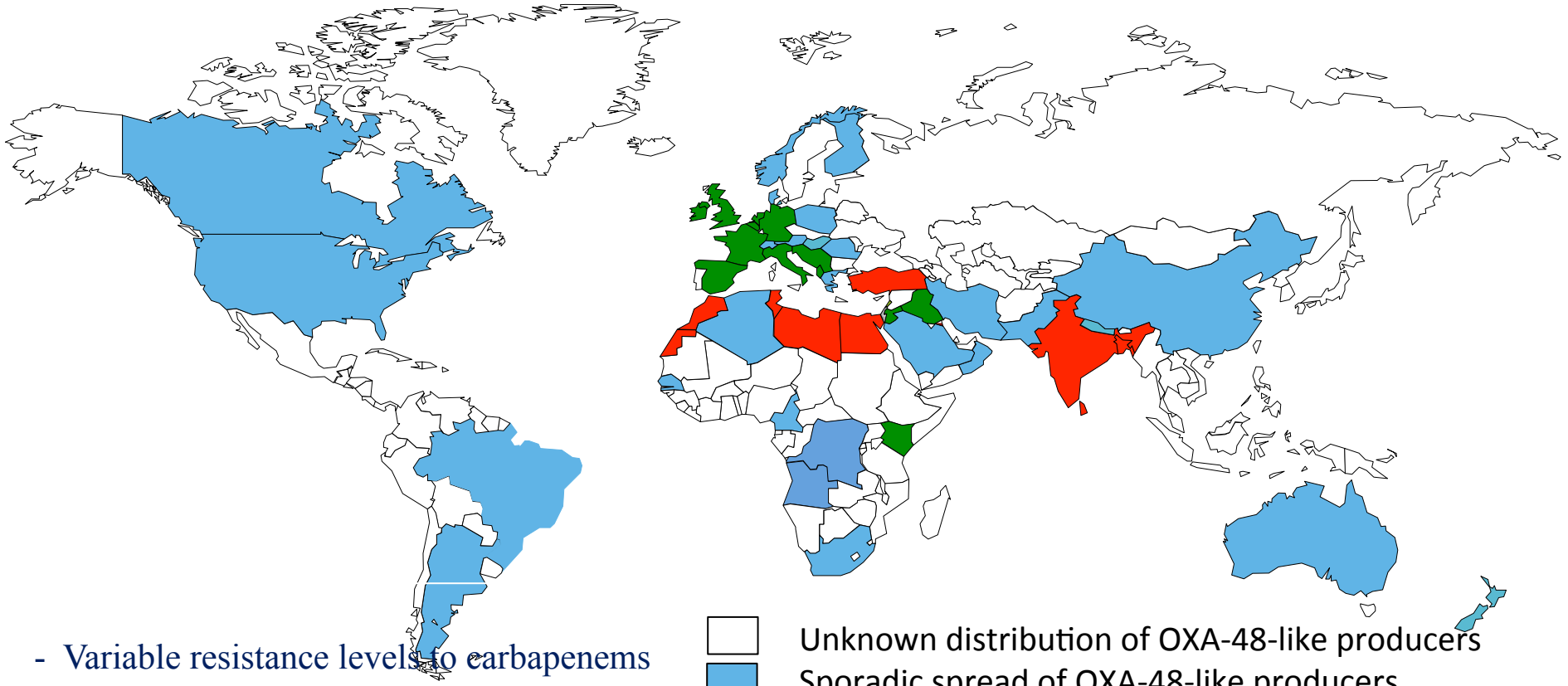



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2004, p. 15–22 Vol. 48, No. 1
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.1.15–22.2004


## Emergence of Oxacillinase-Mediated Resistance to Imipenem in *Klebsiella pneumoniae*

Laurent Poirel,[1] Claire Héritier,[1] Venus Tolün,[2] and Patrice Nordmann[1]*

# OXA-48-like producers- *Enterobacteriaceae*, 2017



- Variable resistance levels to carbapenems
- Main known reservoirs; North Africa, Middle East, Turkey and India
- Community aquisition(+++) High frequency transfer
- *K. pneumoniae, E.cloacae, E.coli (++)*

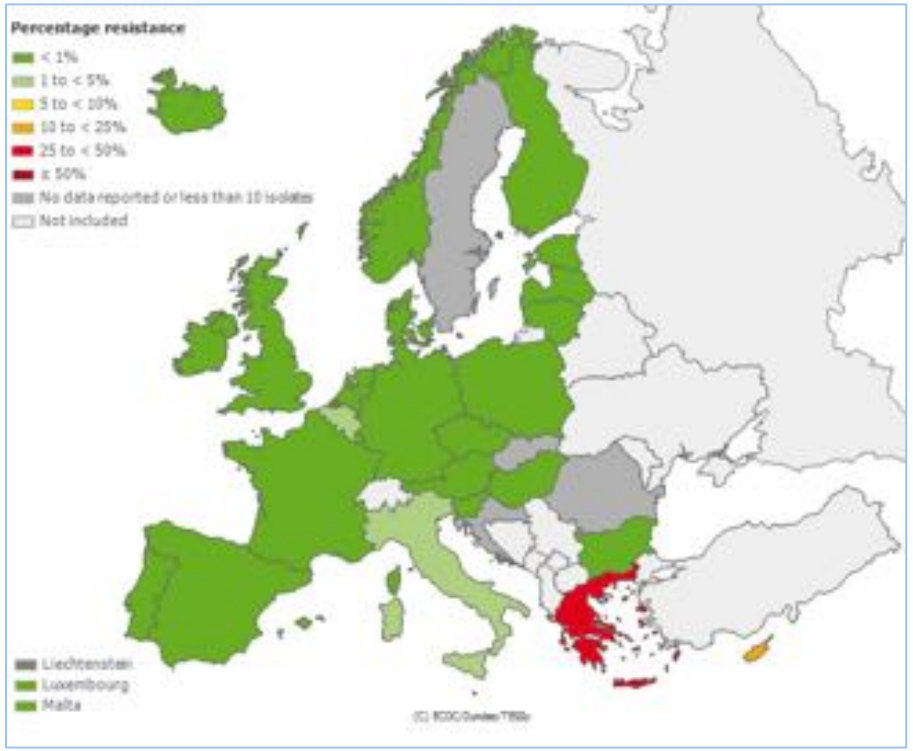
Unknown distribution of OXA-48-like producers
Sporadic spread of OXA-48-like producers
Outbreaks due to OXA-48-like producers
Endemicity of OXA-48-like producers

*Nordmann & Poirel, 2016 Clin Microb Infect,*

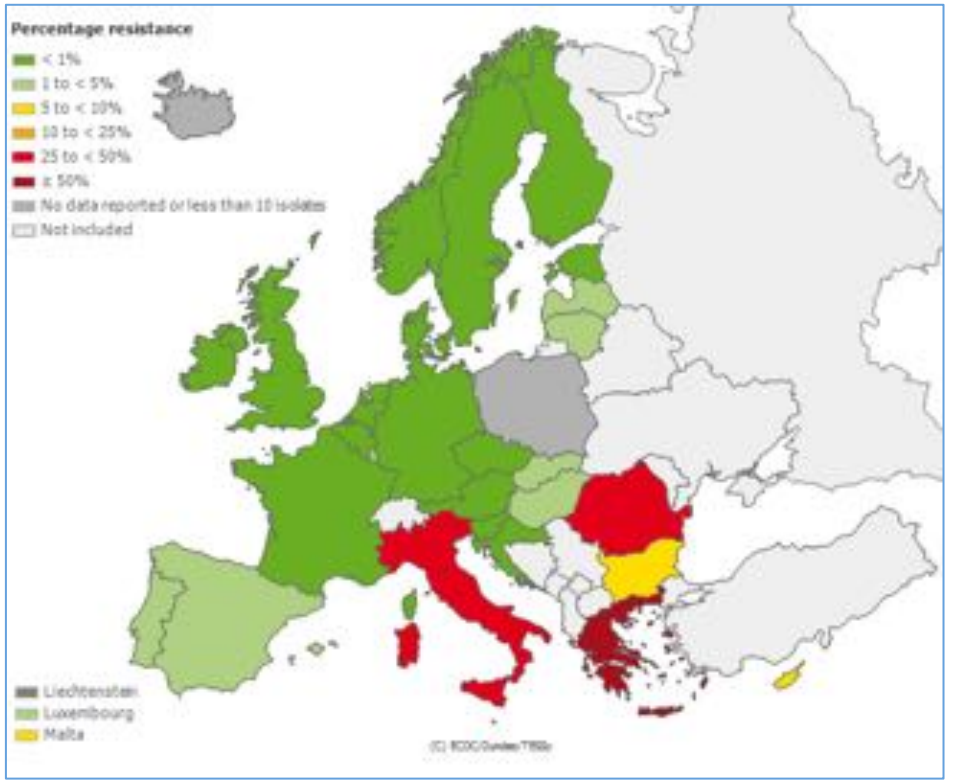
# Carbapenemase producers are spreading now in the community

# Carbapenem-resistant *K. pneumoniae*

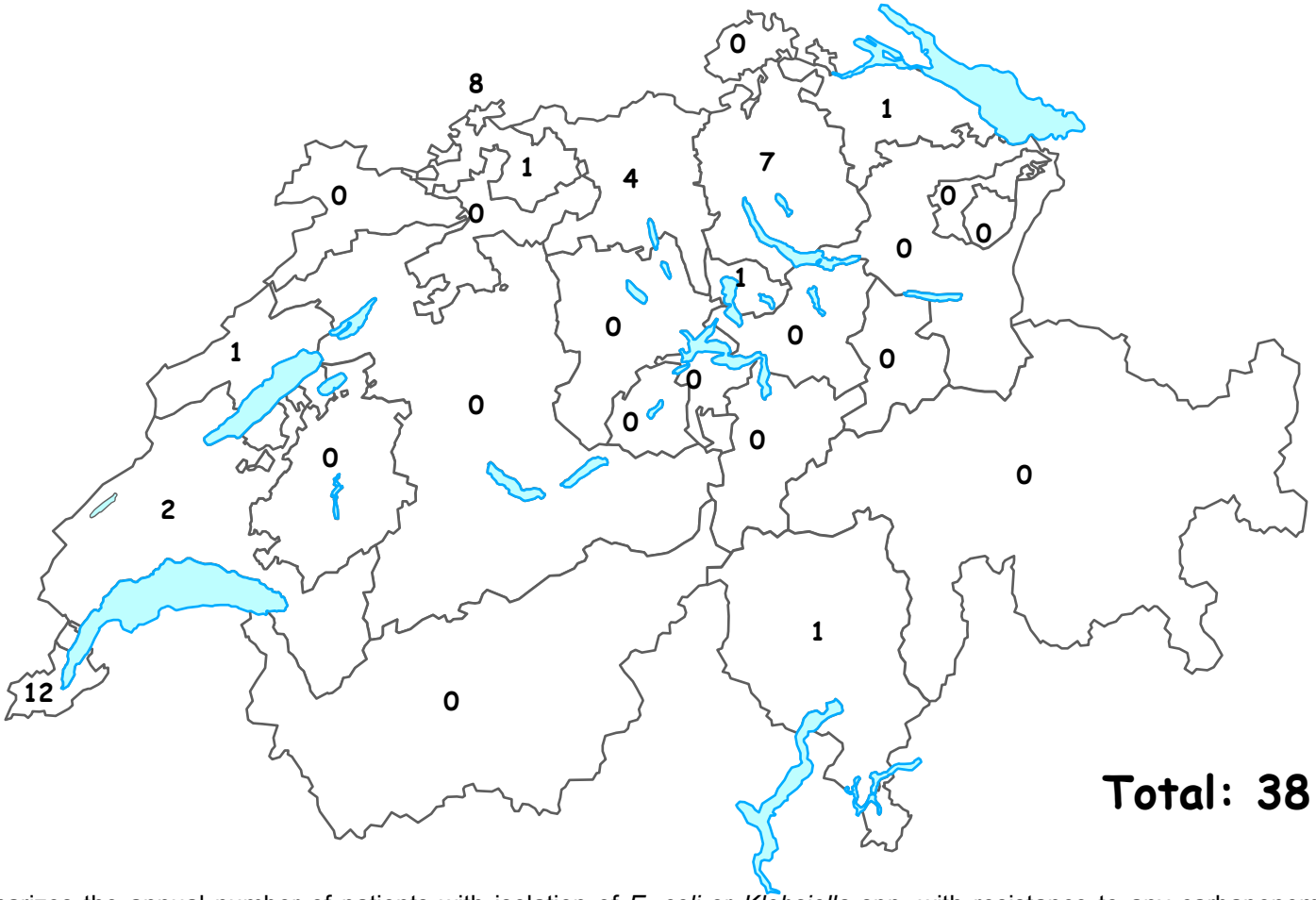
2009




2016

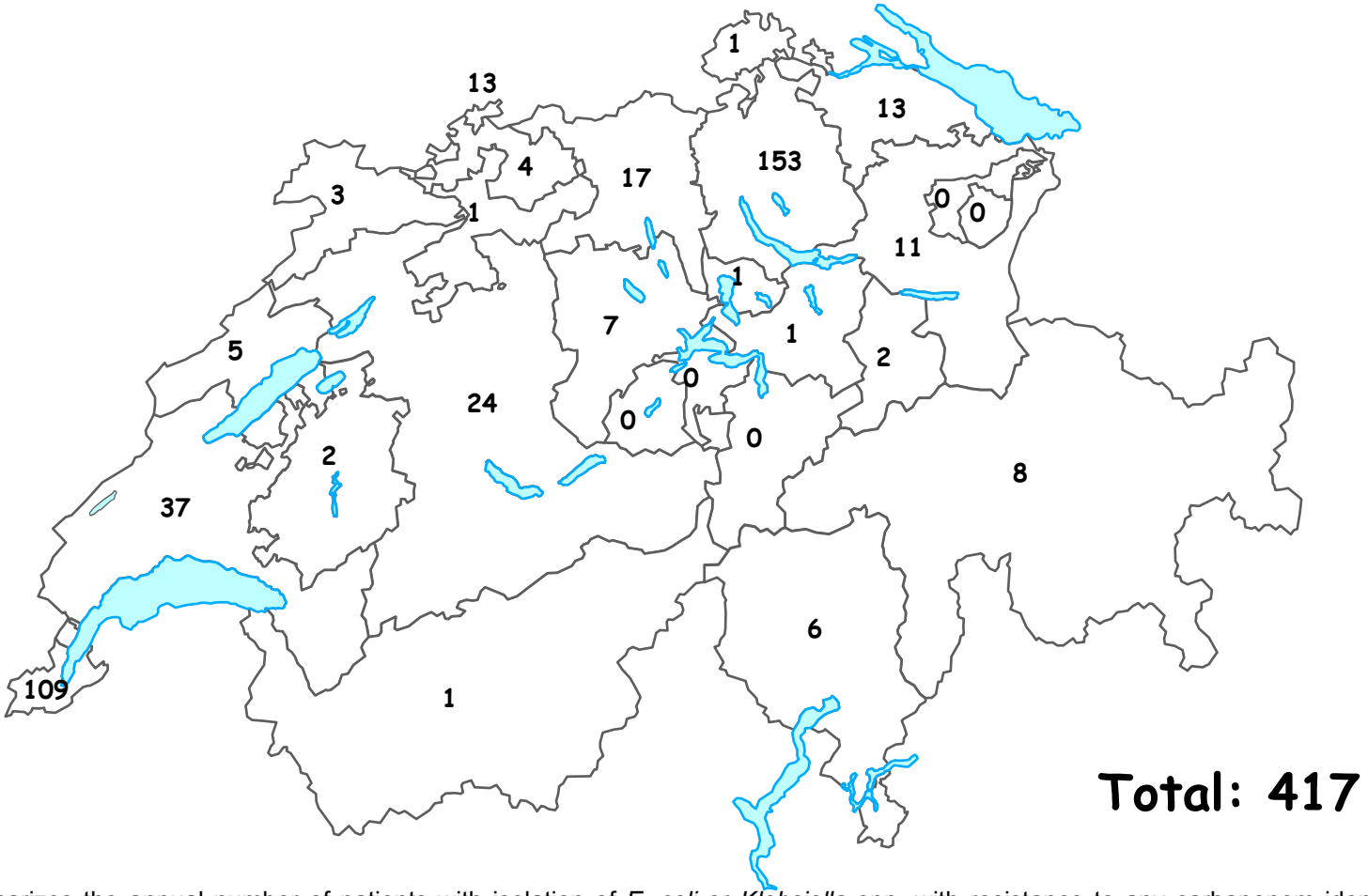


# *E. coli* and *Klebsiella* spp. being resistant to carbapenems, year 2009



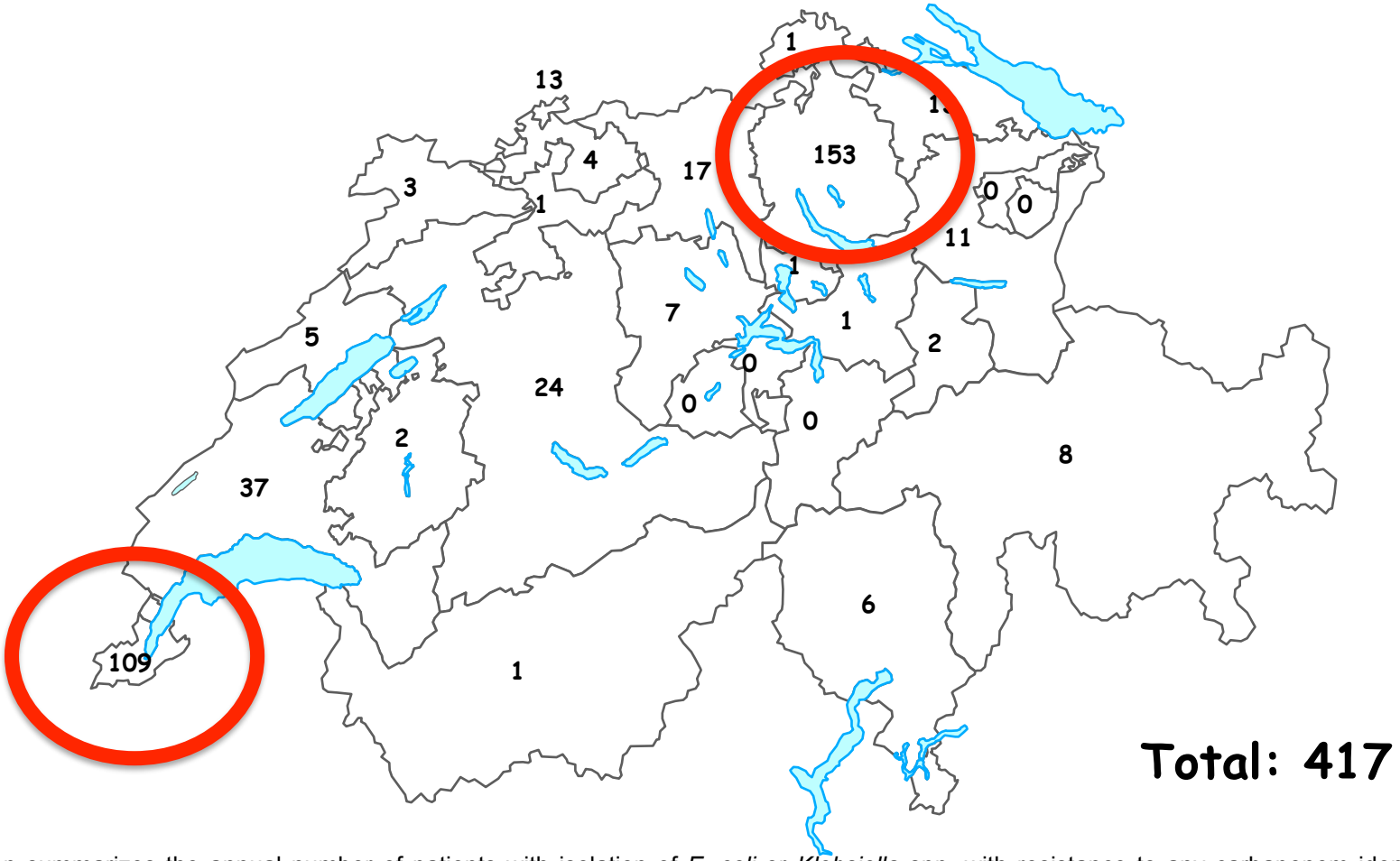

This map summarizes the annual number of patients with isolation of *E. coli* or *Klebsiella* spp. with resistance to any carbapenem identified by Swiss clinical microbiology laboratories. The geographic distribution indicates the location of the microbiological laboratory and, for reasons of data protection, not the patient's residence. The  number represents data collected in the ANRESIS database.

# *E. coli* and *Klebsiella* spp. with resistance to carbapenems, year 2014




This map summarizes the annual number of patients with isolation of *E. coli* or *Klebsiella* spp. with resistance to any carbapenem identified by Swiss clinical microbiology laboratories. The geographic distribution indicates the location of the microbiological laboratory and, for reasons of data protection, not the patient's residence. The number represents data collected in the ANRESIS database.

# *E. coli* and *Klebsiella* spp. with resistance to carbapenems, year 2014




This map summarizes the annual number of patients with isolation of *E. coli* or *Klebsiella* spp. with resistance to any carbapenem identified by Swiss clinical microbiology laboratories. The geographic distribution indicates the location of the microbiological laboratory and, for reasons of data protection, not the patient's residence. The number represents data collected in the ANRESIS database.

# Carbapenemase-producing Enterobacteriaceae in Switzerland from 2013 to 2016

**Ramette A[1], Zbinden R[2], Schrenzel J[3], Nordmann P[4], Kronenberg A[1] and the Swiss Centre for Antibiotic Resistance (ANRESIS)**

*[1]Institute for Infectious Diseases, University of Bern, Bern; [2]Institute for Medical Microbiology, University of Zurich, Zurich*
*[3]Laboratory of Bacteriology, Geneva University Hospitals, Geneva; [4]Molecular and Medical Microbiology, Department of Medicine, University Fribourg*

## Introduction and purpose

Increasing rates of carbapenem-producing Enterobacteriaceae (CPE) in Europe and all over the world are of great concern because of the broad resistance to multiple antibiotics, which reduces considerably therapeutic options. So far no data was available for Switzerland and the aim of this study was to analyse CPE data available for Switzerland from 2013 to 2016.

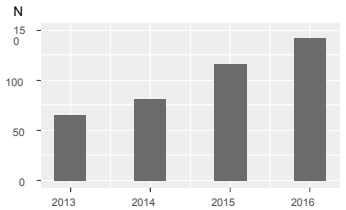



**Fig. 1**. Total number of CPE isolates.

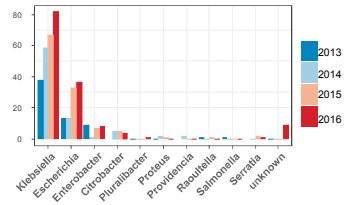



**Fig. 2**. Temporal distribution of the most prevalent CPE genera.

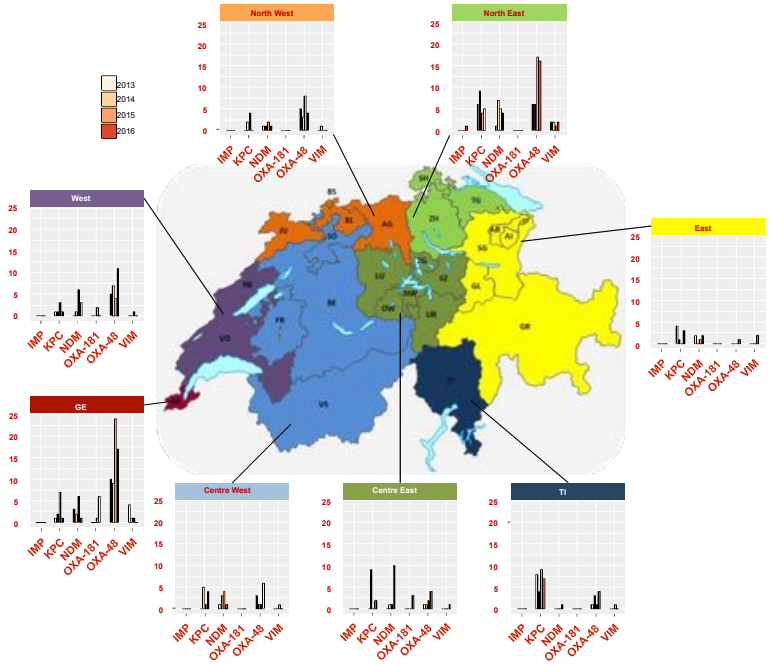



**Fig. 4** Regional and temporal distribution of CPE genotypes in Switzerland from 2013 to 2016.

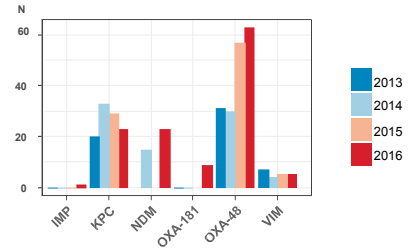



**Fig. 3**. Distribution of CPE genotypes over time.

| | IRR | 95% CI | Pvalue |
|---|---|---|---|
| **Year** | 1.14 | (1.04,1.26) | 0.008 * |
| **Region** | | | |
| Centre | | | |
| West | 0.68 | (0.43,1.06) | 0.087 |
| East | 0.90 | (0.44,1.87) | 0.783 |
| GE | 1.65 | (1.02,2.68) | 0.041 * |
| North East | 1.17 | (0.76,1.80) | 0.482 |
| North West | 0.71 | (0.44,1.16) | 0.174 |
| TI | | | |
| **Sex** (male) | 0.85 | (0.55,1.32) | 0.462 |
| **Type of specimen** | 1.16 | (0.74,1.83) | 0.523 |
| Blood | | | |
| Respiratory tract | 1.45 | (1.16,1.81) | 0.001 * |
| Stool | | | |
| Urine | | | |
| Wound | 2.64 | (1.80,3.88) | 0.000 * |
| | 1.98 | (1.34,2.92) | 0.001 * |
| | 3.70 | (2.60,5.27) | 0.000 * |
| | 2.18 | (1.58,3.00) | 0.000 * |
| | 1.43 | (0.94,2.16) | 0.095 |

**Table 1.** Factors affecting the number of CPE isolates per canton per year. IRR: incident rate ratio; multivariable Poisson regression.

## Methods

In 2013 the Swiss Society for Microbiology defined a network of 8 Swiss expert laboratories, capable of identifying and characterizing CPE according to EUCAST guidelines. All Swiss microbiology laboratories were asked to send all suspected human CPE cases to one of the expert laboratories for characterizing the isolates. Data was then collated by the Swiss Antibiotic Resistance Centre ANRESIS for epidemiological analysis. In 2016 CPE was defined as notifiable disease by the Federal Office of Public Health, and data are from the mandatory reports to the FOPH.

## Results

After exclusion of duplicate entries, a total of 416 isolates originating from 361 patients were characterized, with 69, 89, 121, and 142 isolates reported from 2013, 2014, 2015 and 2016, respectively **(Fig. 1)**. The species most frequently isolated were *Klebsiella pneumoniae* (n=240, 60%), *Escherichia coli* (n=95, 24%), and *Enterobacter spp.* (n=25, 6%) **(Fig. 2)**.

Out of 418 carbapenemase genotypes, the most frequently found were OXA (n=193, 46%, mostly OXA-48 with n=181, 43%), KPC (n=105, 25%) and NDM (n=71, 17%) **(Fig. 3)**.

From 2013 to 2016, the number of KPC-harboring isolates was relatively stable (about 25 per year in total), whereas both numbers of NDM and OXA harboring isolates increased, from 9 to 25, and from 30 to 60 cases, respectively, in this time period. At the regional level, highest number of CPE isolates were identified in the Geneva and North East regions **(Fig. 4; Table 1)**.

## Conclusions and outlook

Molecular data indicate a high diversity of different carbapenemases, with OXA-48, KPC- and NDM-type carbapenemases being the most prevalent in Switzerland. Overall OXA-48 and NDM producers are increasing as observed in other European countries such as in France.

Significant temporal and regional trends were found and the ongoing mandatory reporting scheme will provide further epidemiological data that will help define possible interventions in the future.

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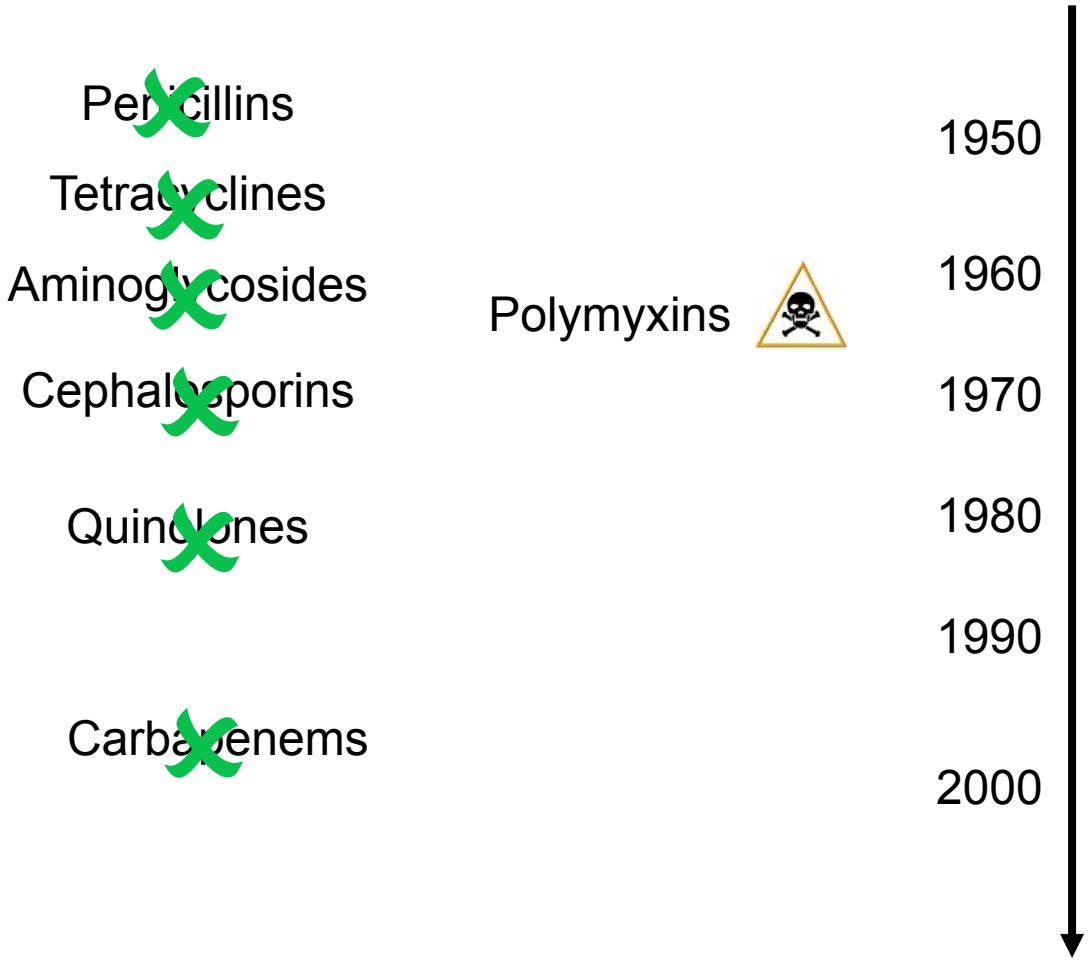
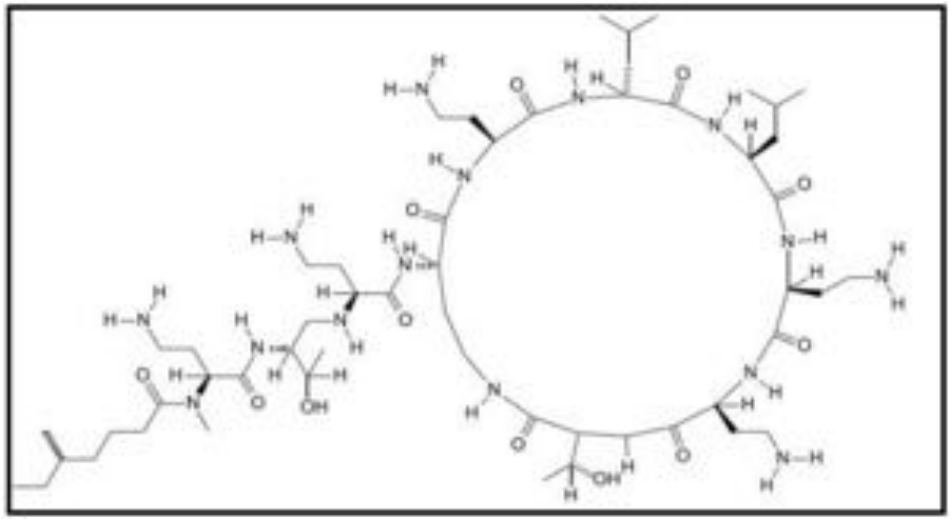
None of the authors has a conflict of interest to disclose.

# Rise of Antimicrobial Resistance in Gram-negative bacteria



# The polymyxins; colistin and polymyxin B



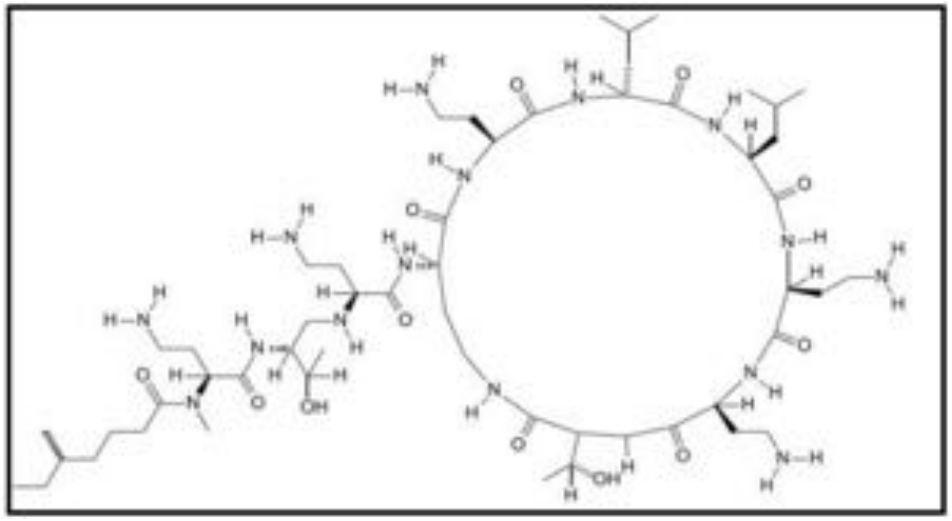
**Colistin**

- Synthesis by *Bacillus polymyxa* spp colistinus
- Discovered in the 1940's
- High rates of toxicity (mainly nephrotoxicity)

# Colistin use, 2017

Mostly in veterinary medicine (prophylaxis and metaphylaxis)


# The polymyxins; colistin and polymyxin B



**Colistin**

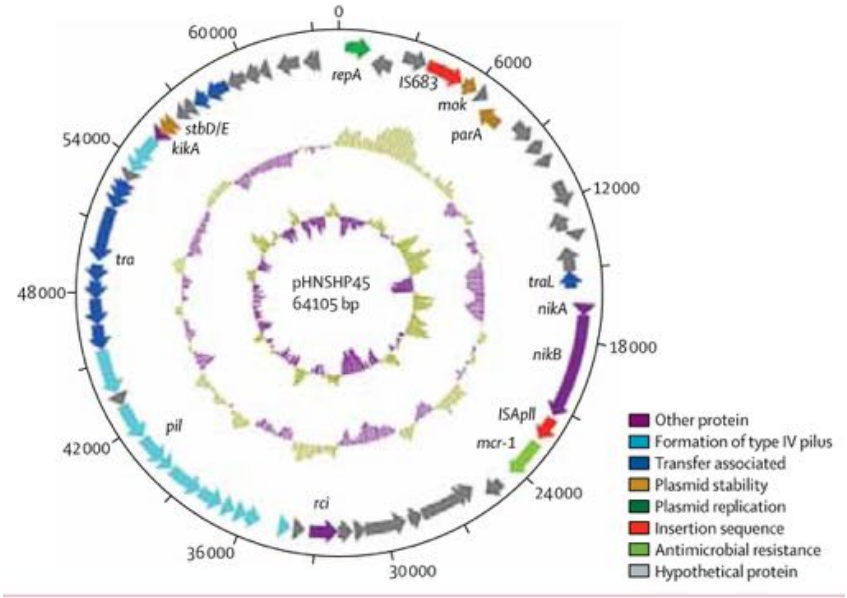
- Synthesis by *Bacillus polymyxa* spp colistinus
- Discovered in the 1940's
- High rates of toxicity (mainly nephrotoxicity)
- **Renewed interest in mid-2000's to treat multidrug-resistant Gram-negative bacteria: MDR *Klebsiella, Acinetobacter* and *Pseudomonas* sp.**

Lancet Infect Dis, Nov 28, 2015

# Plasmid-mediated resistance to colistin

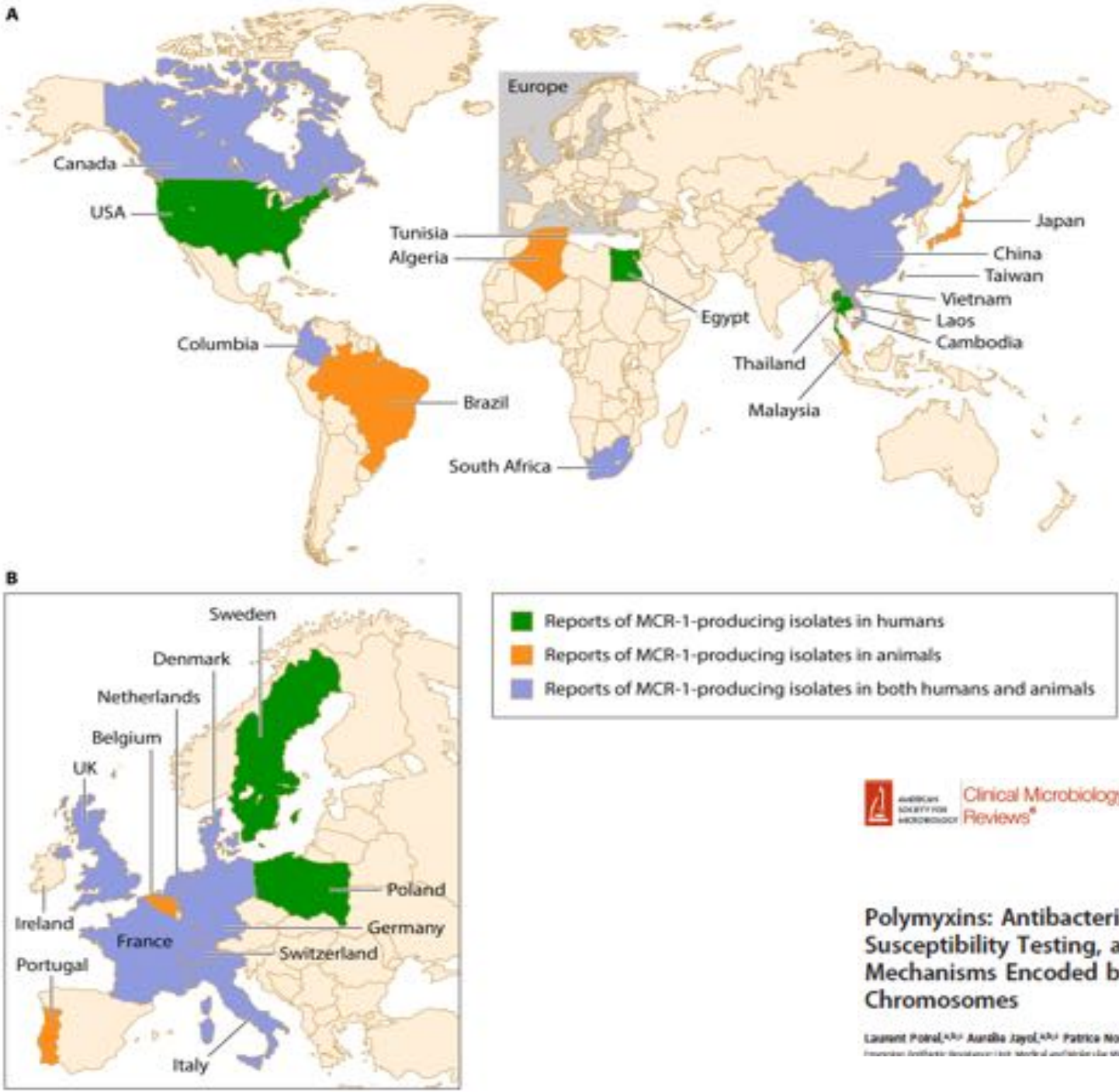
## Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

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




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]

LA LIBERTÉ
QUOTIDIEN ROMAND ÉDITÉ À FRIBOURG
MARDI 30 JANVIER 2024


**FRIBOURG** • C'est une découverte d'importance qui a été faite à Fribourg en décembre, mais ô combien inquiétante. «Nous allons tout droit vers une résistance à tous les antibiotiques», s'alarme le professeur Patrice Nordmann, chef de la chaire de microbiologie de l'Université de Fribourg. A la suite d'une infection urinaire contractée par un patient genevois, Patrice Nordmann et son collègue Laurent Poirel ont identifié la souche bactérienne la plus coriace au monde. Celle-ci résiste aux deux familles d'antibiotiques utilisés en dernier recours pour traiter certaines infections chez l'homme et l'animal. Patrice Nordmann appelle à restreindre rapidement l'usage de ces antibiotiques pour traiter le bétail. > 3

# Ces bactéries résistent à tout

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

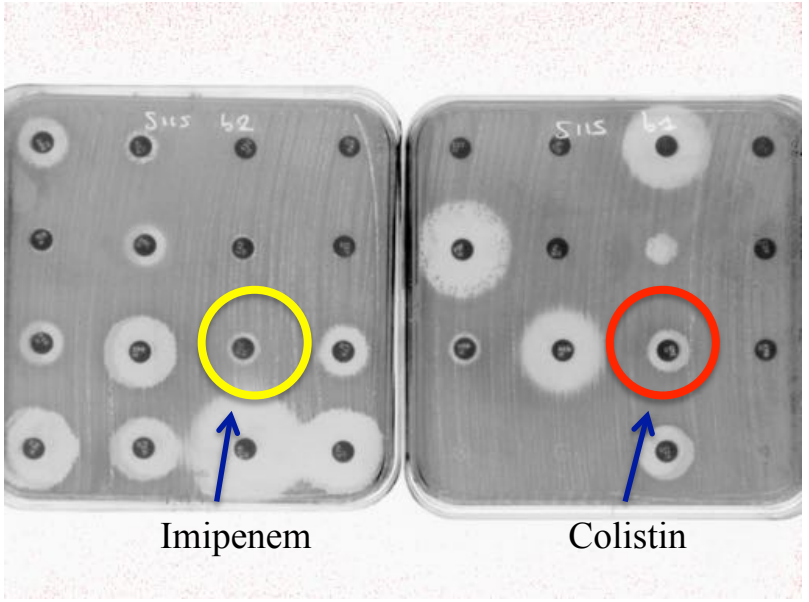
- Patient from Switzerland, December 2015

- No history of travel abroad

- No colistin-based treatment

- Urinary tract infection-Community-acquired

- *E. coli* isolate being 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, 2016

1 Antimicrobial Agents and Chemotherapy – New Data Letter

2

# 3 Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in ESBL-producing
# 4 Enterobacteriaceae in river water and imported vegetable samples in Switzerland

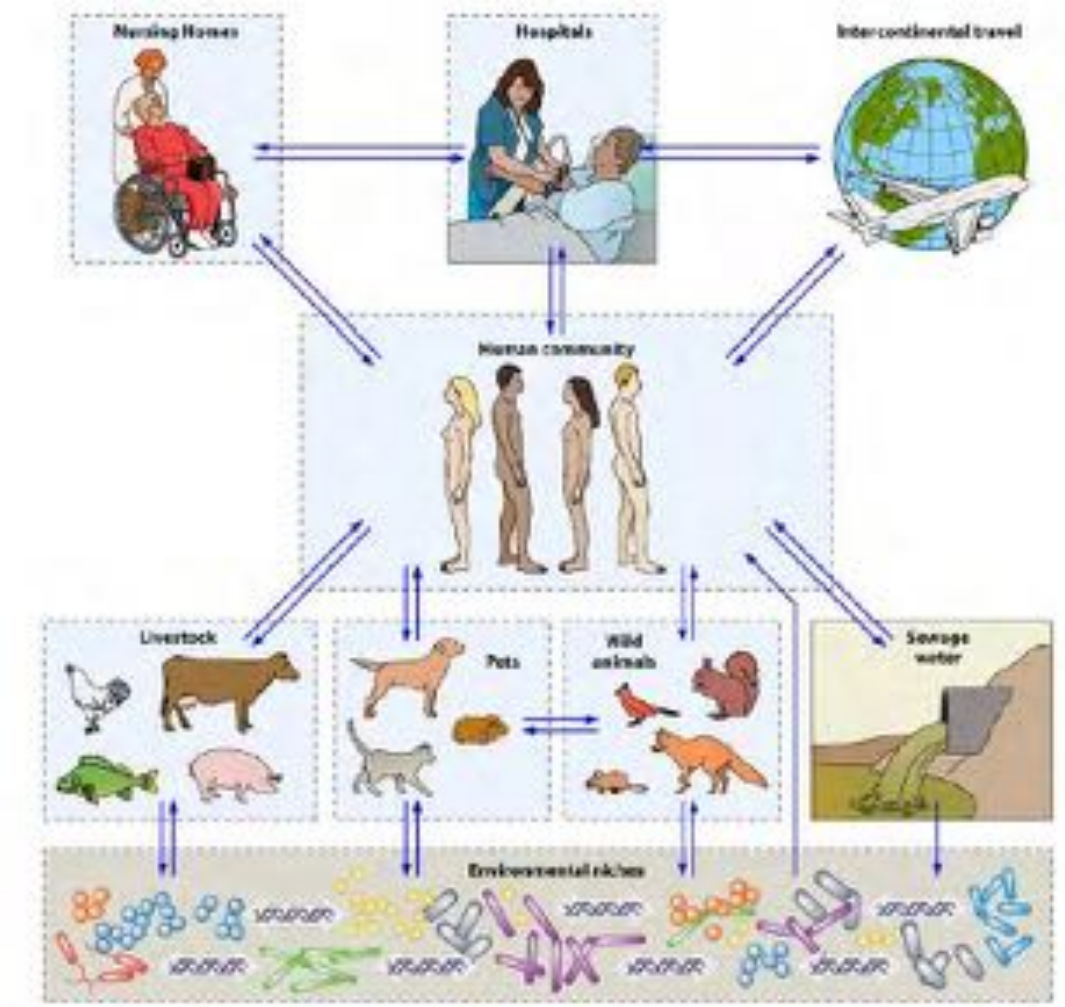
5

6 Katrin Zurfluh[1], Laurent Poirel[2], Patrice Nordmann[2,3], Magdalena Nüesch-Inderbinen[1],
7 Herbert Hächler[1], Roger Stephan[1]*

8

9 [1]*Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zurich*
10 *Winterthurerstrasse 272, 8057 Zurich, Switzerland*

# A One-health world; plasmid-mediated colistin resistance

nature microbiology

LETTERS

PUBLISHED: 6 FEBRUARY 2017 | VOLUME: 2 | ARTICLE NUMBER: 16260

# Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production

Yang Wang[†], Rongmin Zhang[†], Jiyun Li[1], Zuowei Wu[2], Wenjuan Yin[1], Stefan Schwarz[3,4], Jonathan M. Tyrrell[5], Yongjun Zheng[6], Shaolin Wang[1], Zhangqi Shen[1], Zhihai Liu[7], Jianye Liu[7], Lei Lei[7], Mei Li[5,7], Qidi Zhang[8], Congming Wu[1], Qijing Zhang[2], Yongning Wu[9], Timothy R. Walsh[5]* and Jianzhong Shen[1]*

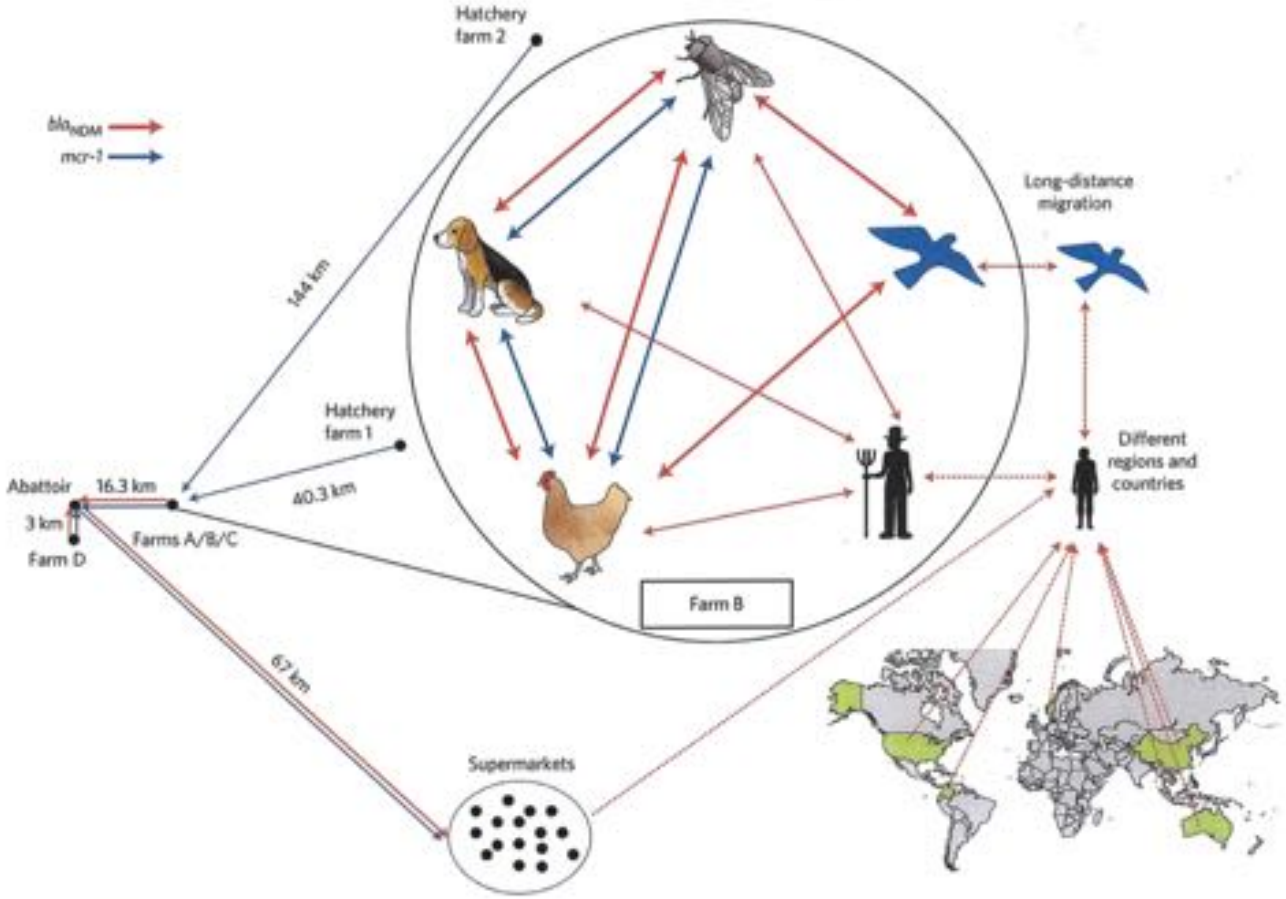
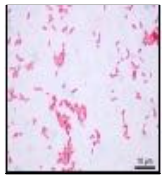


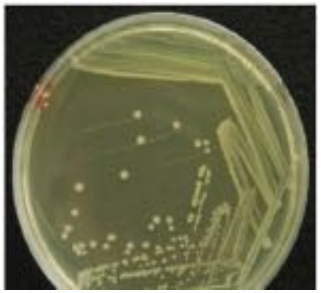

**Figure 1 | Sampling diagram and transmission routes of $bla_{NDM}$ and mcr-1.** Black circles represent different sections in the poultry-producing chain. The

# Classical scheme for diagnostic in microbiology

Direct examination
Gram staining

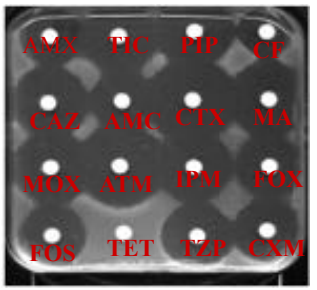


Culture
18h (*E. coli*)
to three weeks
(Mycobacteria)



Phenotypic identification

Antibiogram



# 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 135 isolates expressing various mechanisms of colistin resistance (intrinsic, chromosomally encoded, and plasmid-mediated MCR-1) and 65 colistin-susceptible isolates. Sensitivity and specificity were 99.3% and 95.4%, 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 32%) (*4*,*5*).

Acquired resistance to colistin in *Enterobacteriaceae* results mostly from modification of lipopolysaccharide (*6*). Addition of phosphoethanolamine, 4-amino-L-arabinose cationic groups, 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 200 isolates collected from clinical samples worldwide. This collection included 135 *Enterobacteriaceae* isolates resistant to polymyxin: 5 isolates of intrinsically polymyxin-resistant species (*Morganella morganii*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia stuartii*, and *Serratia marcescens*) and 130 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 65 enterobacterial isolates belonged to various species and were susceptible to polymyxins (online Technical Appendix).

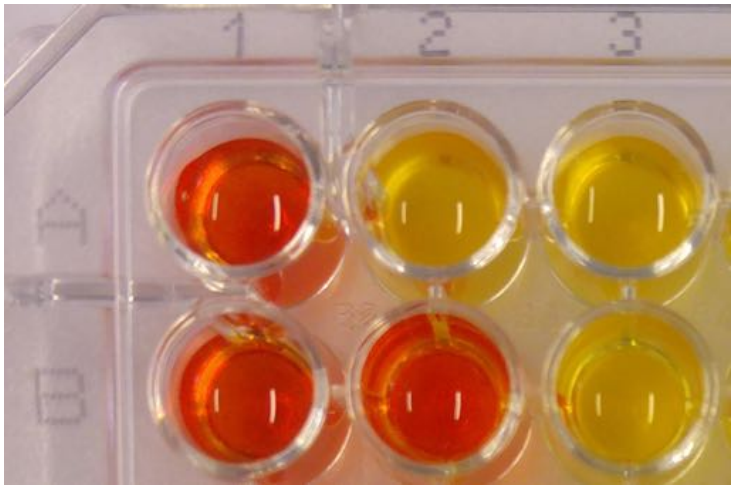
#### MIC Determination

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

Author affiliation: University of Fribourg, Fribourg, Switzerland

DOI: http://dx.doi.org/10.3201/eid2206.151840






**1.Results: < 2 h, currently 24 h à 48 h**

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

**3.Sensibility 99%, specificity 99%**

# Rapid Polymyxin NP test



Patented on behalf of the University of Fribourg,
Marketed in Europe
since Nov 15, 2016

# WHO's 'priority pathogens' list highlights urgent need for new drugs

*Feb27, 2017*

**Priority 1: Critical**

•*Acinetobacter baumannii*, carbapenem-resistant.

• *Pseudomonas aeruginosa*, carbapenem-resistant.

• Enterobacteriaceae, carbapenem-resistant, ESBL-producing.

**Priority 2: High**

• *Enterococcus faecium*, vancomycin-resistant.

• *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant.

• *Helicobacter pylori*, clarithromycin-resistant.

• Campylobacter spp., fluoroquinolone-resistant.

• Salmonella, fluoroquinolone-resistant.

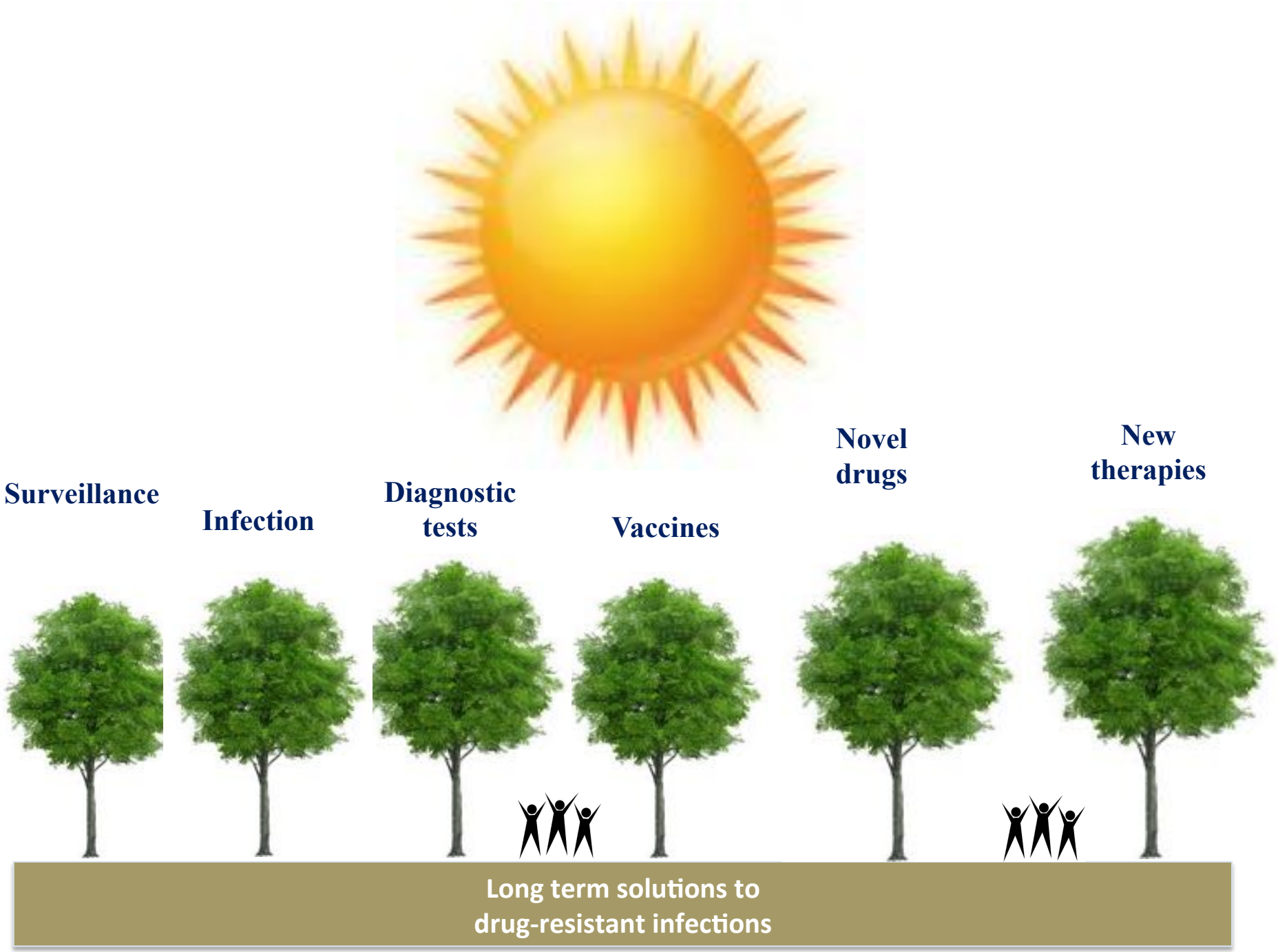
•*Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant.

**Priority 3: Medium**

•*Streptococcus pneumoniae*, penicillin-non-susceptible.

•*Haemophilus influenzae*, ampicillin-resistant.

•Shigella spp., fluoroquinolone-resistant.


Surveillance
Infection
Diagnostic tests
Vaccines
Novel drugs
New therapies
Long term solutions to drug-resistant infections