Epidémiologie et détection des entérobactéries productrices de carbapénèmases

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Laboratoire de Bactériologie-Hygiène, Hôpital de Bicêtre
CNR associé Résistance aux Antibiotiques

PLAN

1. Epidémiologie et dissémination des carbapénèmases

1.1. Les carbapénèmases de la classe A de Ambler

1.2. Les métallo-β-lactamases

1.3. les carbapénèmases de la classe D de Ambler

2. Méthodes de détection des entérobactéries productrices de carbapénèmases

2.1. A partir d'un prélèvement clinique (infection)

2.2. Dépistage des patients porteurs

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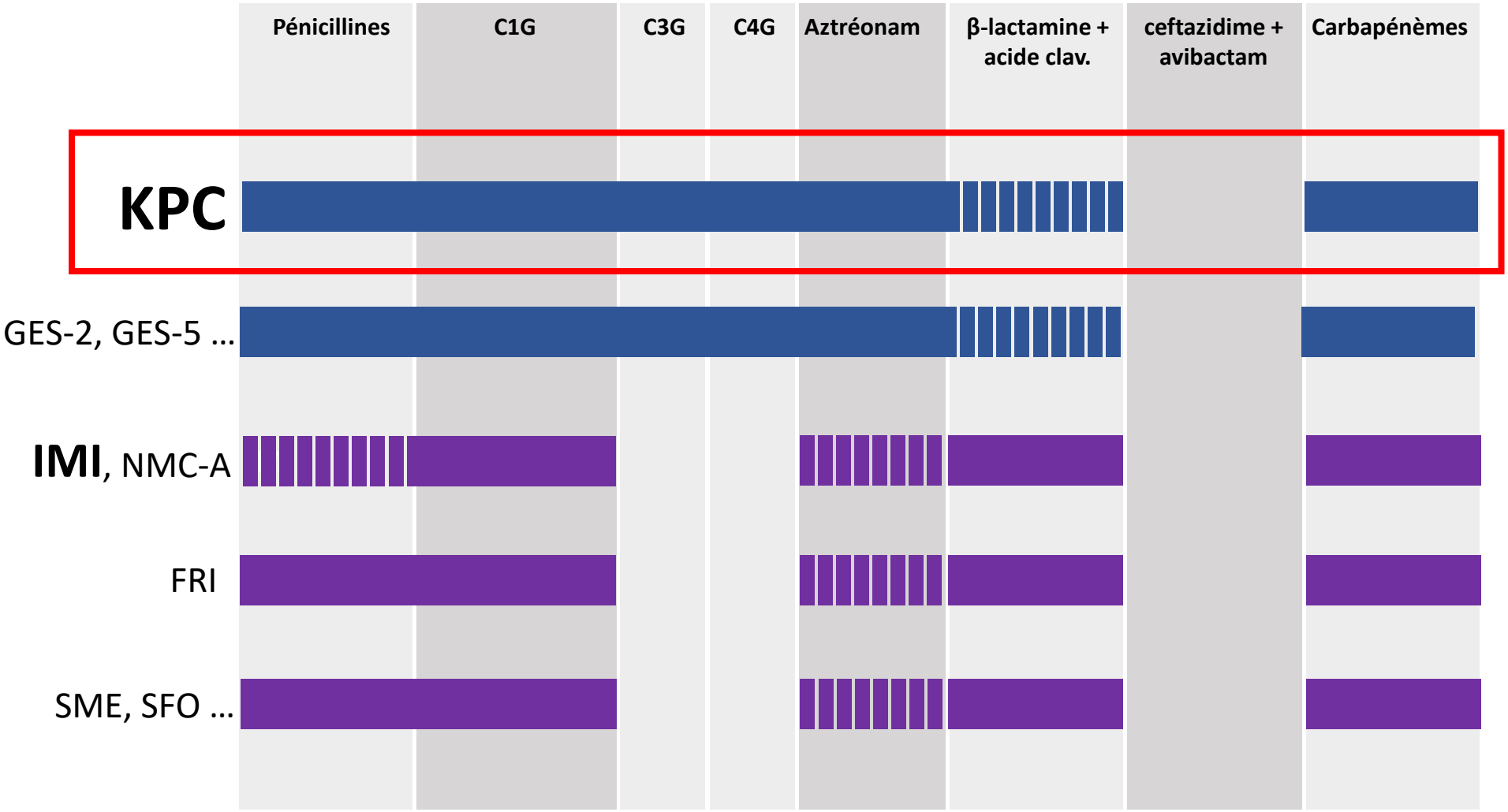
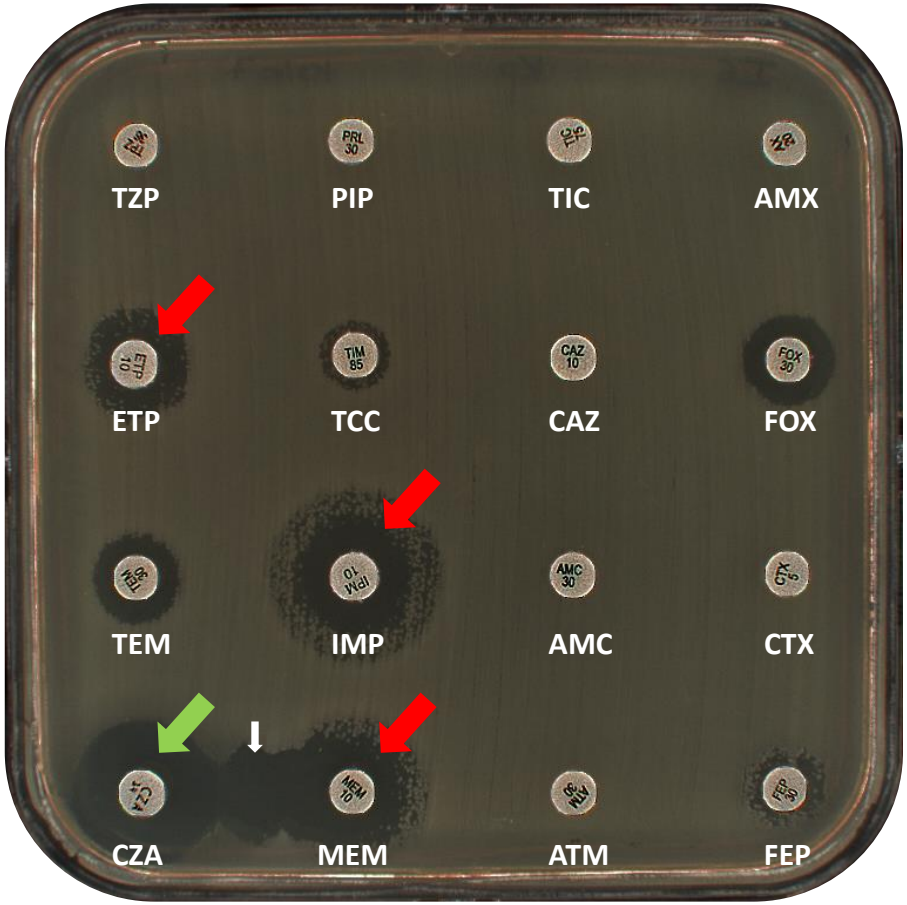
Class A carbapenemases in Enterobacterales



KPC-3 producing *K. pneumoniae*



KPC : ***K****lebsiella* ***P****neumoniae* **C**arbapenemase

2001

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2001, p. 1151–1161 Vol. 45, No. 4
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.4.1151–1161.2001
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Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of *Klebsiella pneumoniae*

HESNA YIGIT,[1] ANNE MARIE QUEENAN,[2] GREGORY J. ANDERSON,[1]
ANTONIO DOMENECH-SANCHEZ,[3] JAMES W. BIDDLE,[1] CHRISTINE D. STEWARD,[1]
SEBASTIAN ALBERTI,[4] KAREN BUSH,[2] AND FRED C. TENOVER[1]*

Hospital Infections Program, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333[1]; The R. W. Johnson Pharmaceutical Research Institute, Raritan, New Jersey 08869[2]; and Unidad de Investigacion, Hospital Son Dureta, Andrea Doria, Palma de Mallorca, 07014,[4] and Área de Microbiologia, Universidad de las Islas Baleares, Crtra. Valldemosa, Palma de Mallorca, 07071,[3] Spain

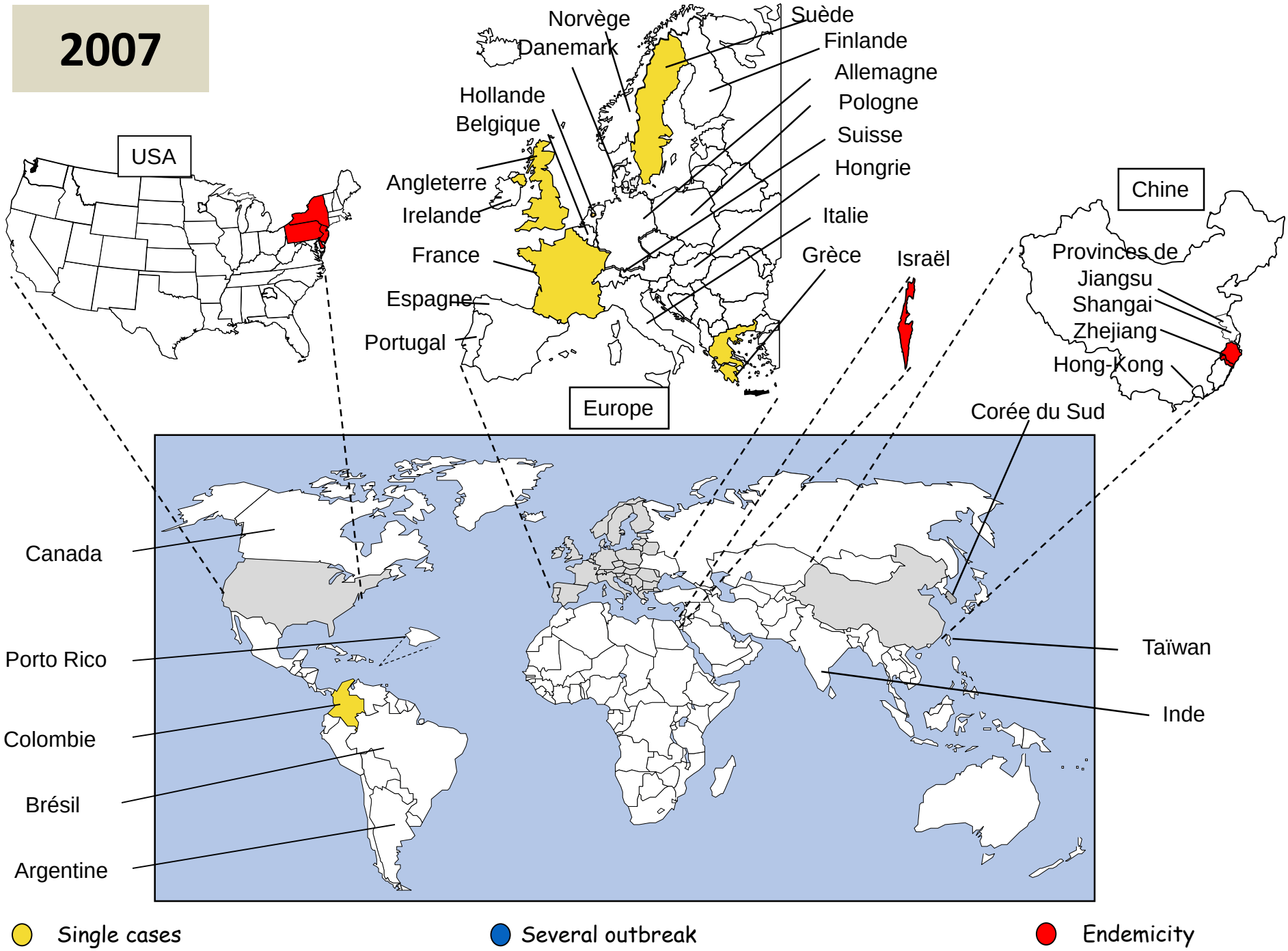
ORIGINAL INVESTIGATION

Rapid Spread of Carbapenem-Resistant *Klebsiella pneumoniae* in New York City

A New Threat to Our Antibiotic Armamentarium

Simona Bratu, MD; David Landman, MD; Robin Haag, RN; Rose Recco, MD; Antonella Eramo, RN; Maqsood Alam, MD; John Quale, MD

2007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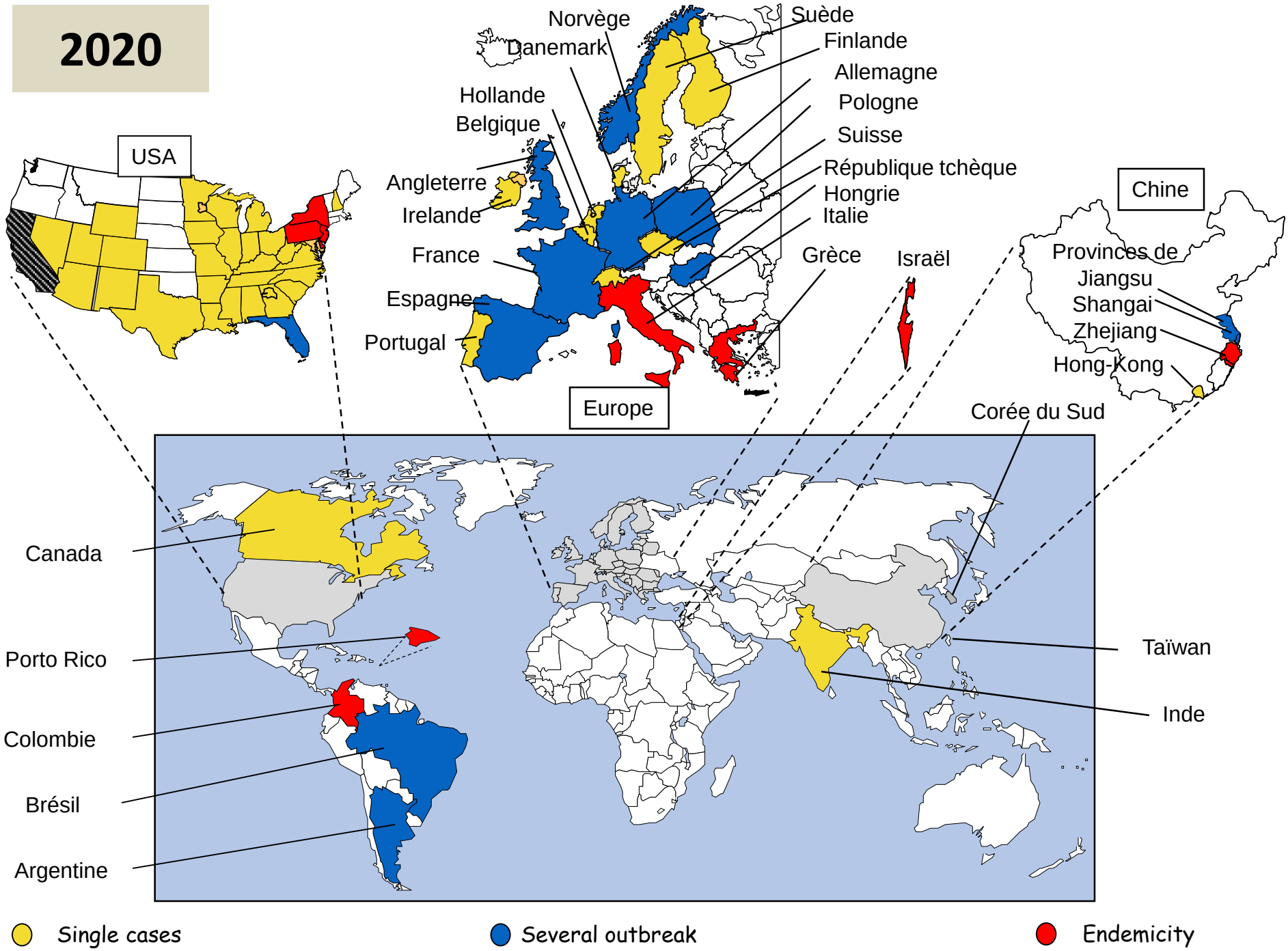
Angleterre
Irelande
France
Espagne
Portugal
Europe
Chine
Provinces de
Jiangsu
Shangai
Zhejiang
Hong-Kong
Corée du Sud
Canada
Porto Rico
Colombie
Brésil
Argentine
Taïwan
Inde
Single cases
Several outbreak
Endemicity

2020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Grèce
Israël
Hollande
Belgique
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Irelande
France
Espagne
Portugal
Europe
Chine
Provinces de
Jiangsu
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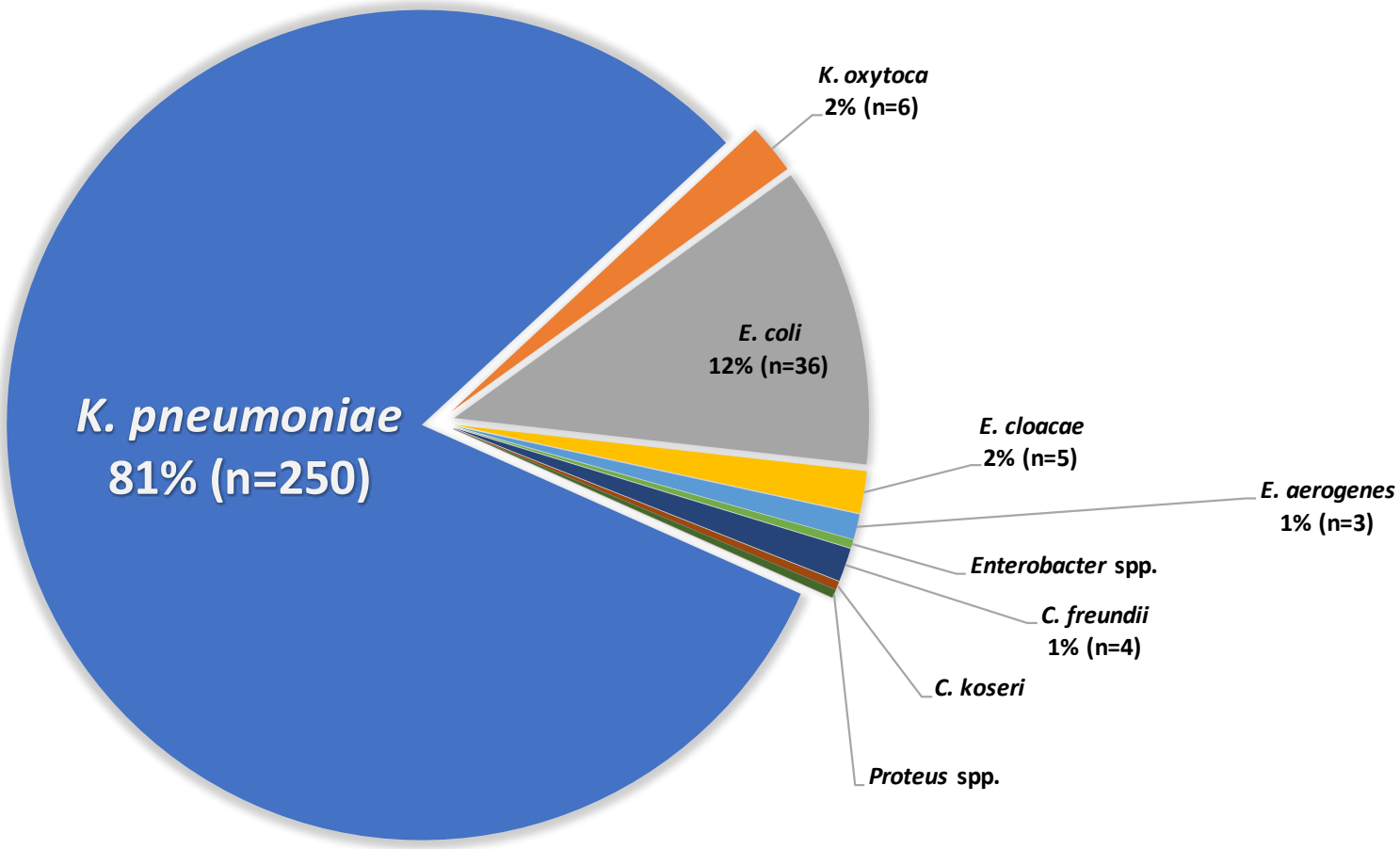
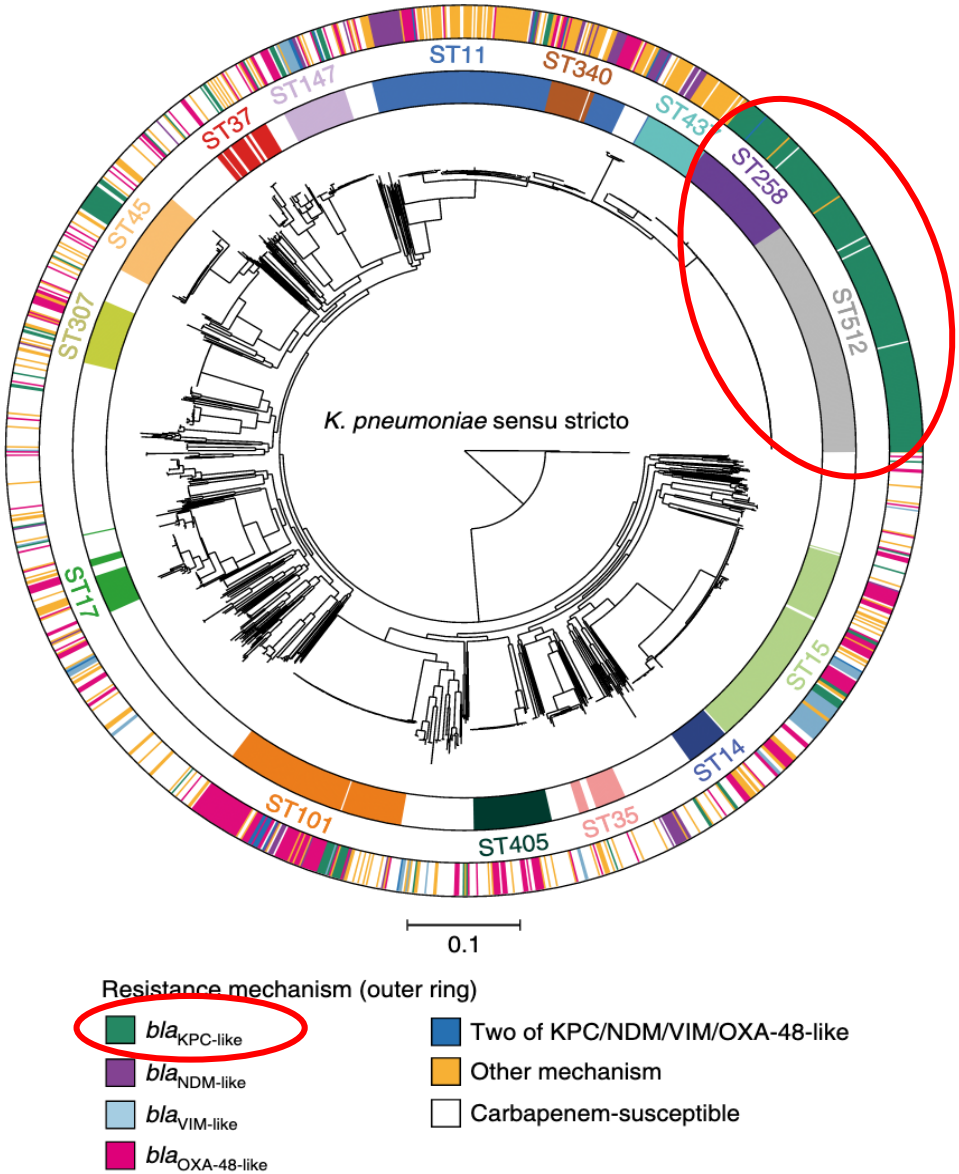
Argentine
Taïwan
Inde
Single cases
Several outbreak
Endemicity

KPC carbapenemases are strongly associated with *K. pneumoniae*



KPC-type carbapenemase producing Enterobacterales from France 2014-2019

Dissemination of ST258/512 *K. pneumoniae* in Europe

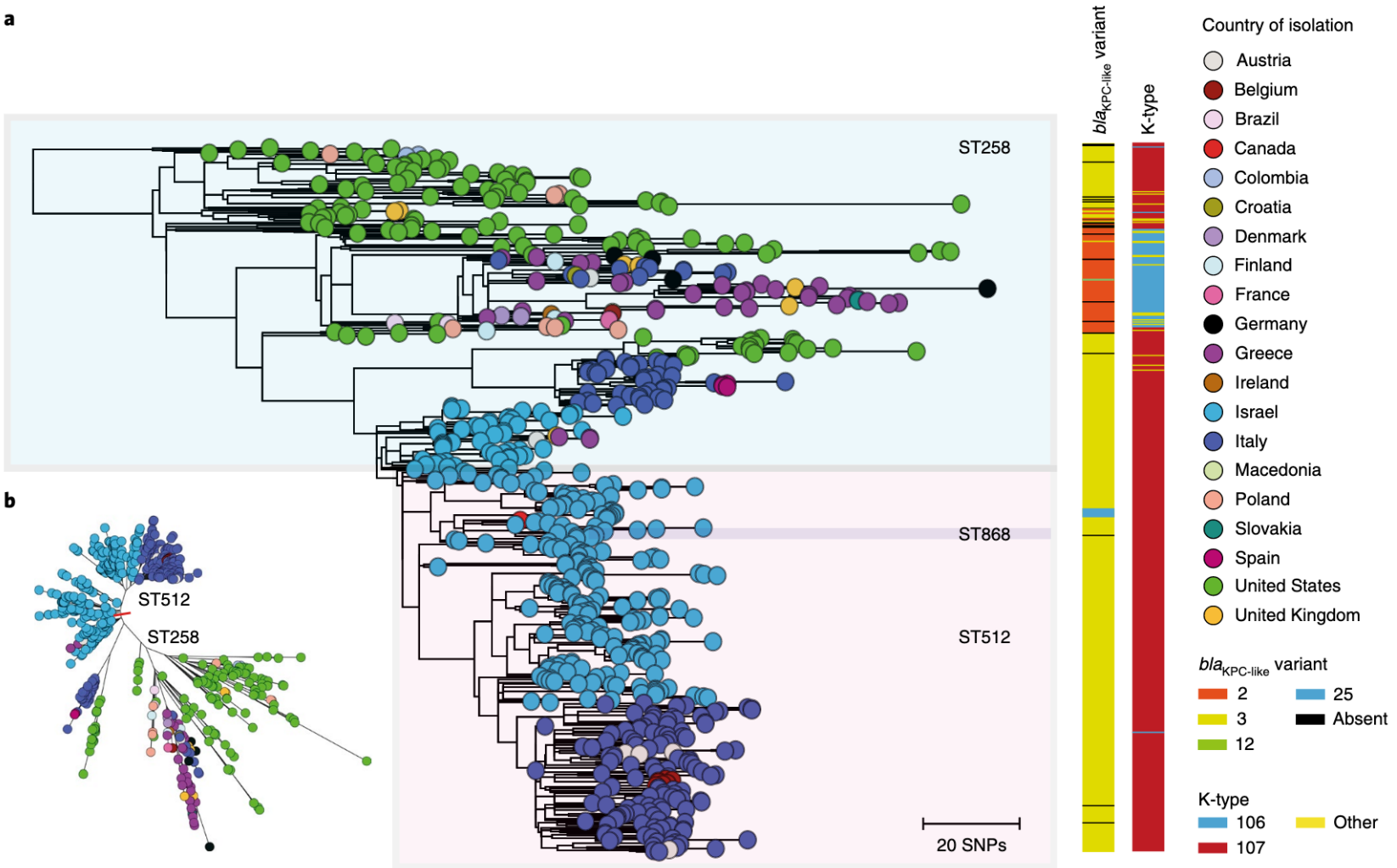


In 2013, in Europe most of KPC-producing *K. pneumoniae* were of ST258/512

Phylogenetic tree of 1,649 isolates belonging to *K. pneumoniae* of the EuSCAPE study (2013)

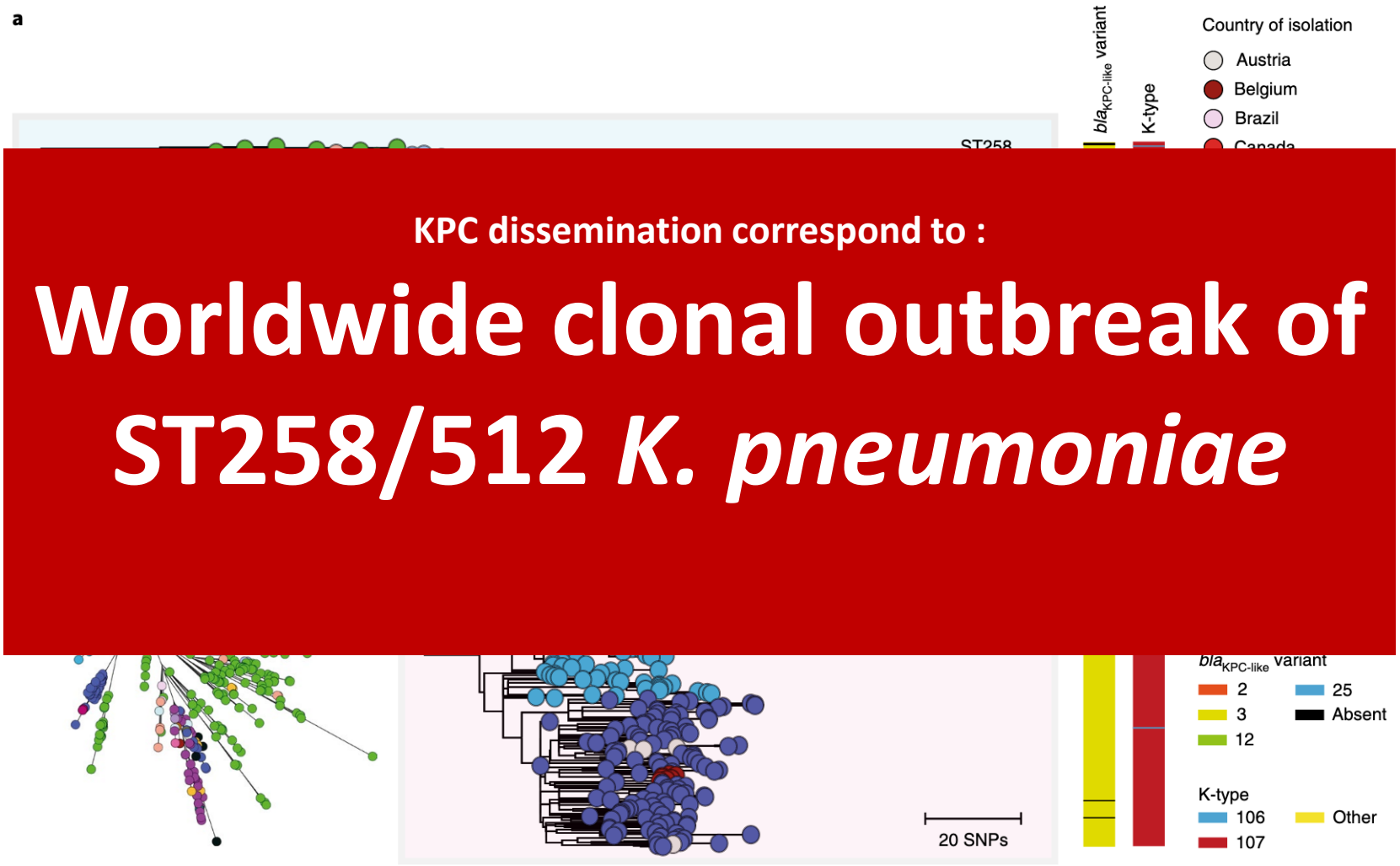
Davis S et al. Nat Microbiol 2019

International spread of the epidemic ST258/512 clone of *K. pneumoniae*



Davis S et al. Nat Microbiol 2019

International spread of the epidemic ST258/512 clone of *K. pneumoniae*

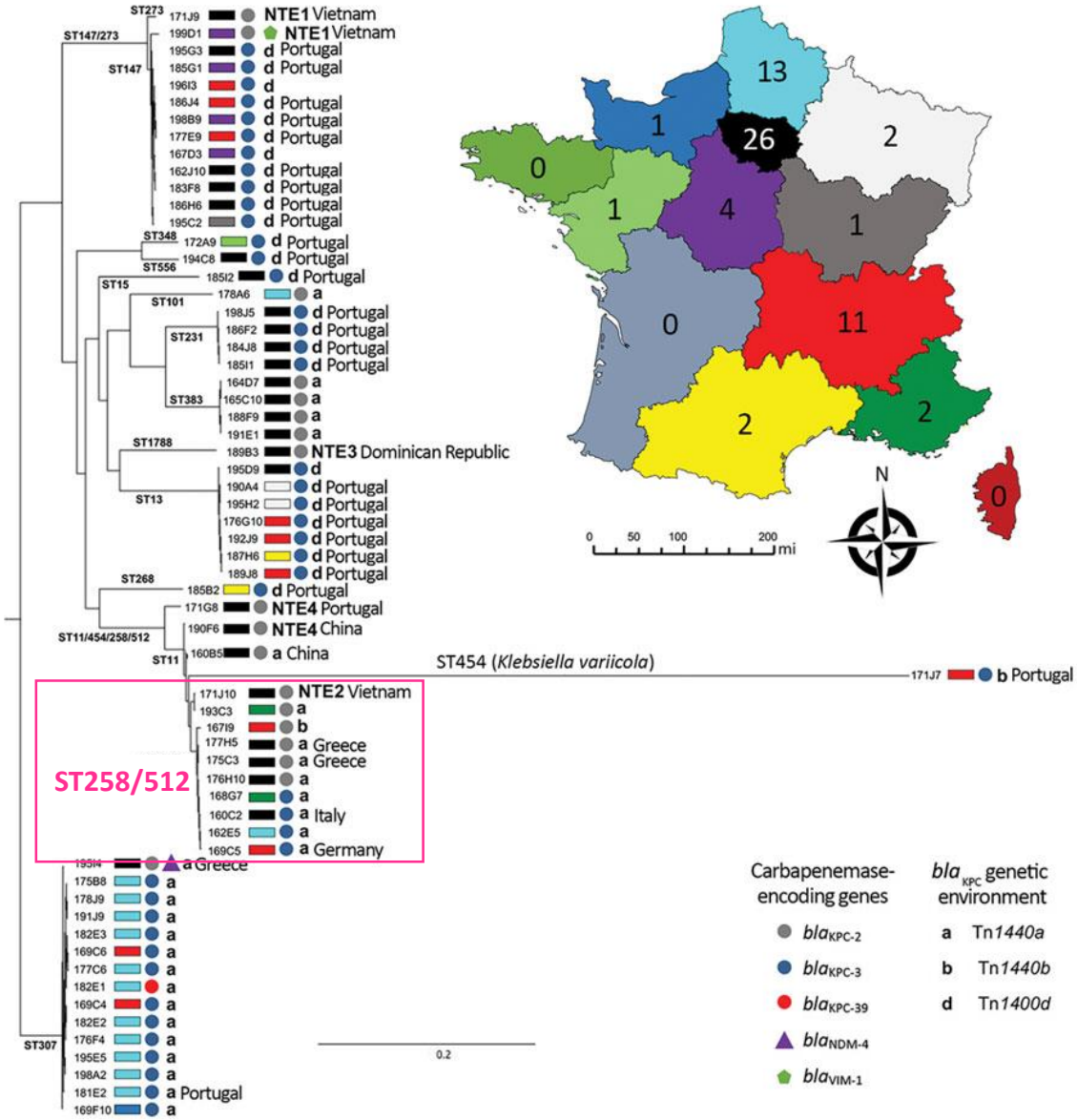


KPC dissemination correspond to :

Worldwide clonal outbreak of ST258/512 *K. pneumoniae*

Davis S et al. Nat Microbiol 2019

KPC-producing *K. pneumoniae* : the French and « Portuguese » paradox



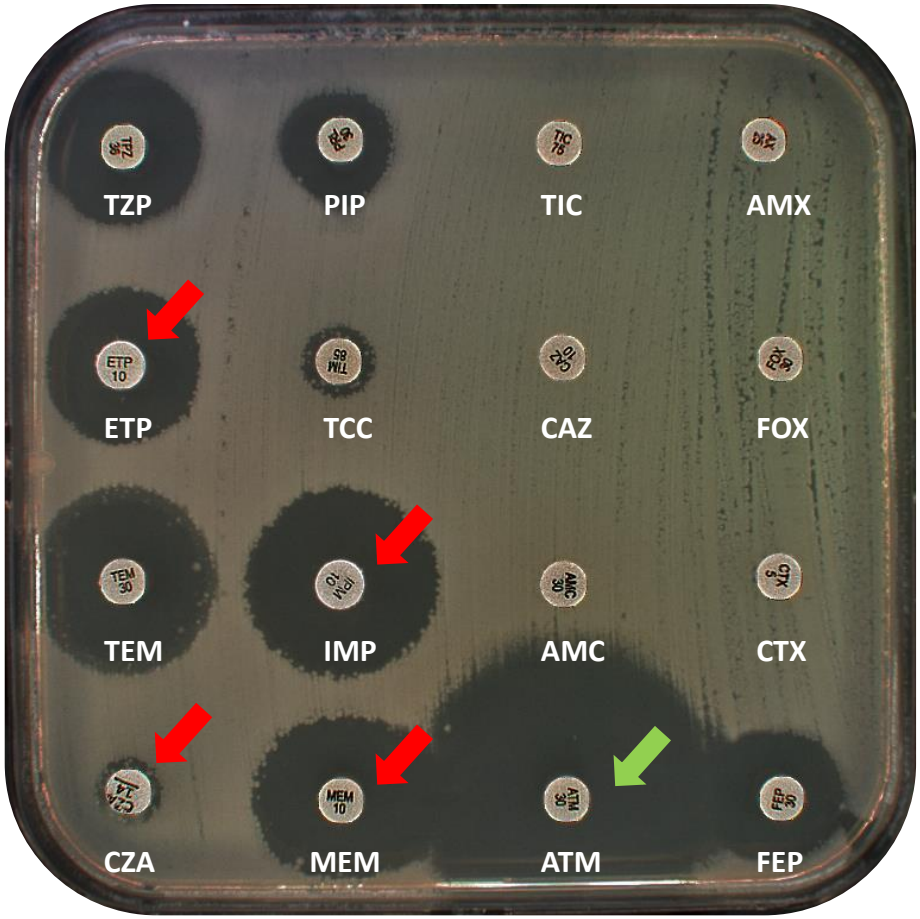
In 2018, ST258/512 KPC-producing *K. pneumoniae* are not the most prevalent STs in France and Portugal

Bonnin RA et al. Emerg Infect Dis 2020

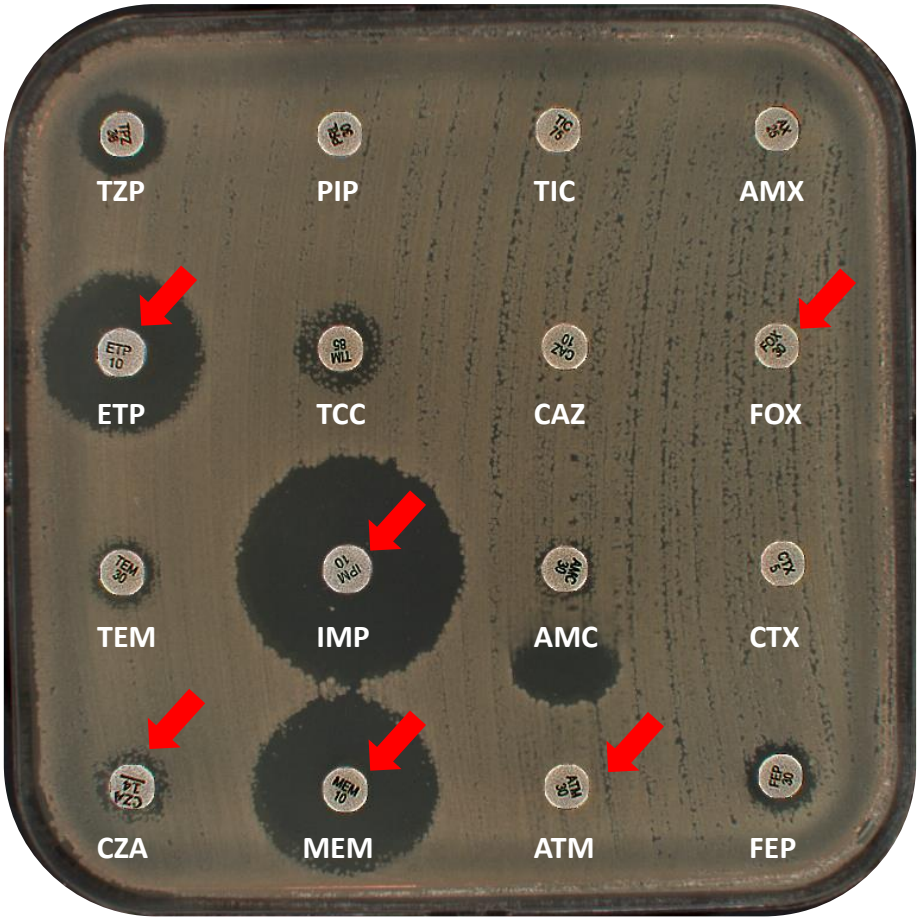
Metallo-β-lactamases in Enterobacterales

Pénicillines	C1G	C3G	C4G	Aztréonam	β-lactamine + acide clav.	ceftazidime + avibactam	Carbapénèmes
Métallo-β-lactamases : **NDM, VIM,** IMP							

IMP-4 producing *E. cloacae*



IMP-4 + CTX-M-15 producing *K. pneumoniae*



VIM, IMP and GIM-type metallo-β-lactamases

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1999, p. 1584–1590 Vol. 43, No. 7
0066-4804/99/$04.00+0
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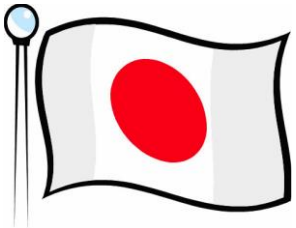
Cloning and Characterization of *bla*$_{VIM}$, a New Integron-Borne Metallo-β-Lactamase Gene from a *Pseudomonas aeruginosa* Clinical Isolate

LAURA LAURETTI,[1] MARIA LETIZIA RICCIO,[1] ANNARITA MAZZARIOL,[2] GIUSEPPE CORNAGLIA,[2] GIANFRANCO AMICOSANTE,[3] ROBERTA FONTANA,[2] AND GIAN MARIA ROSSOLINI[1]*

Dipartimento di Biologia Molecolare, Sezione di Microbiologia, Università di Siena, 53100-Siena,[1] Istituto di Microbiologia, Università di Verona, 37134-Verona,[2] and Dipartimento di Scienze e Tecnologie Biomediche e Biometria, Università dell'Aquila, 67100-L'Aquila,[3] Italy

VIM 1999



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1995, p. 824–829 Vol. 39, No. 4
0066-4804/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Plasmid-Mediated Dissemination of the Metallo-β-Lactamase Gene *bla*$_{IMP}$ among Clinically Isolated Strains of *Serratia marcescens*

HIDEO ITO,[1,2] YOSHICHIKA ARAKAWA,[1]* SHINJI OHSUKA,[1] ROCHAPORN WACHAROTAYANKUN,† NOBUO KATO,[1] AND MICHIO OHTA[1]

Department of Bacteriology, Nagoya University School of Medicine, Nagoya 466,[1] and College of Medical Technology, Nagoya University, Nagoya 461,[2] Japan

IMP 1995

AAC
Journals.ASM.org

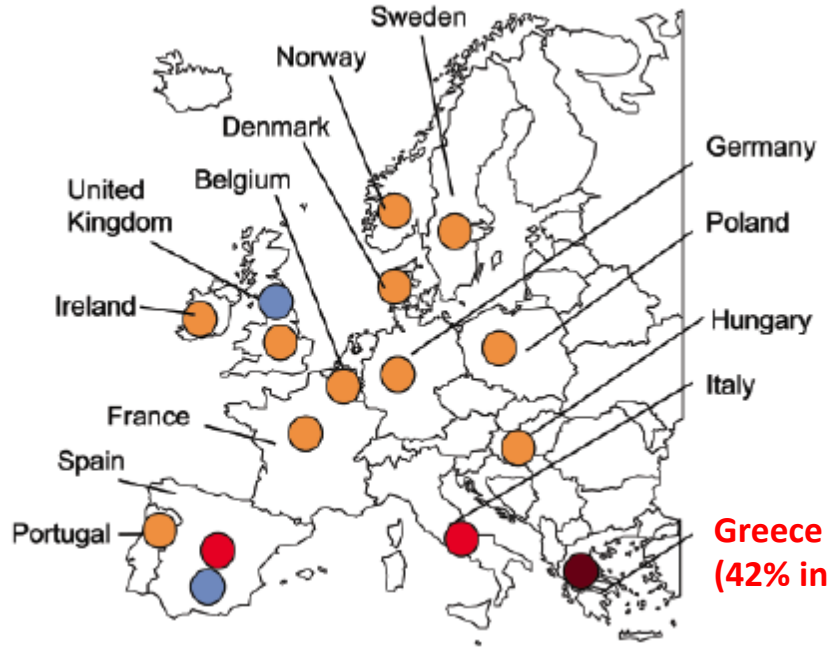
Emergence of Metallo-β-Lactamase GIM-1 in a Clinical Isolate of *Serratia marcescens*

Heike Rieber,* Andre Frontzek,* and Yvonne Pfeifer[b]

GIM 2012

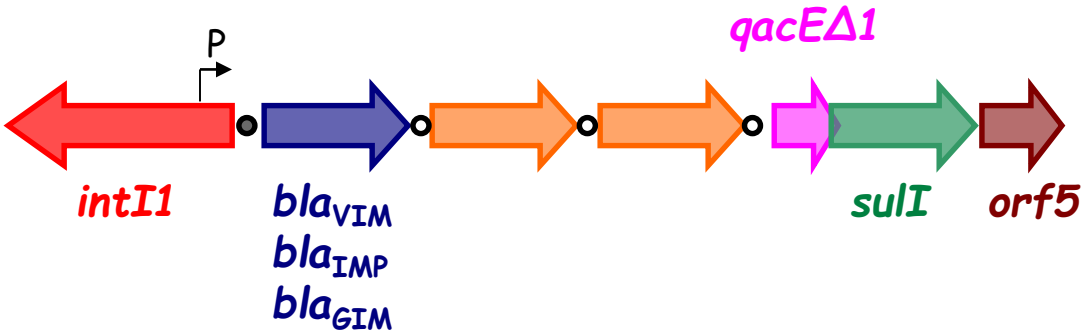
Dissemination of IMP and VIM carbapenemases

- VIM-producing isolates/outbreaks
- VIM interhospital spread
- VIM high prevalence
- IMP-producing isolates/outbreaks
- IMP high prevalence



Genetic environment of *bla*$_{VIM}$, *bla*$_{IMP}$ et *bla*$_{GIM}$

- Class 1 integron



- Diversity of plasmids

- ***Pseudomonas aeruginosa* and** Enterobacterales

NDM : **N**ew **D**ehli **M**etallo-β-lactamase

2009

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2009, p. 5046–5054 Vol. 53, No. 12
0066-4804/09/$12.00 doi:10.1128/AAC.00774-09
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Characterization of a New Metallo-β-Lactamase Gene, *bla*$_{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]*

Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study

Timothy R Walsh, Janis Weeks, David M Livermore, Mark A Toleman

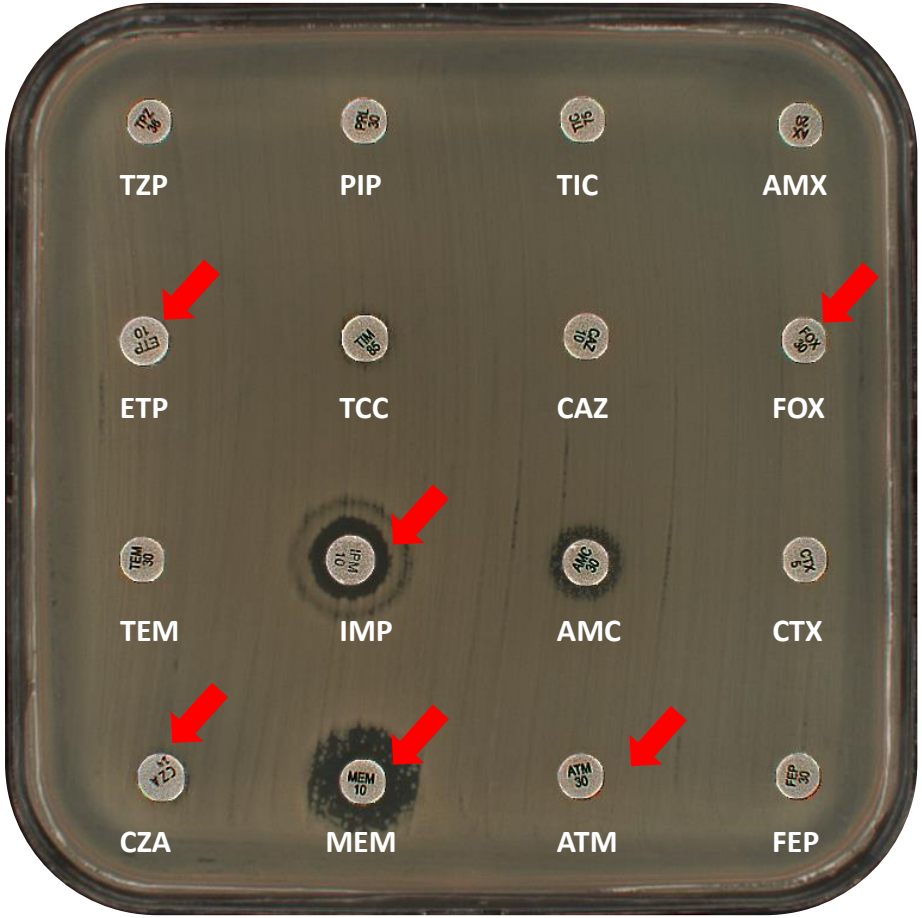
Lancet Infectious Diseases 2011

Carriage in population prevalence = 18,5%

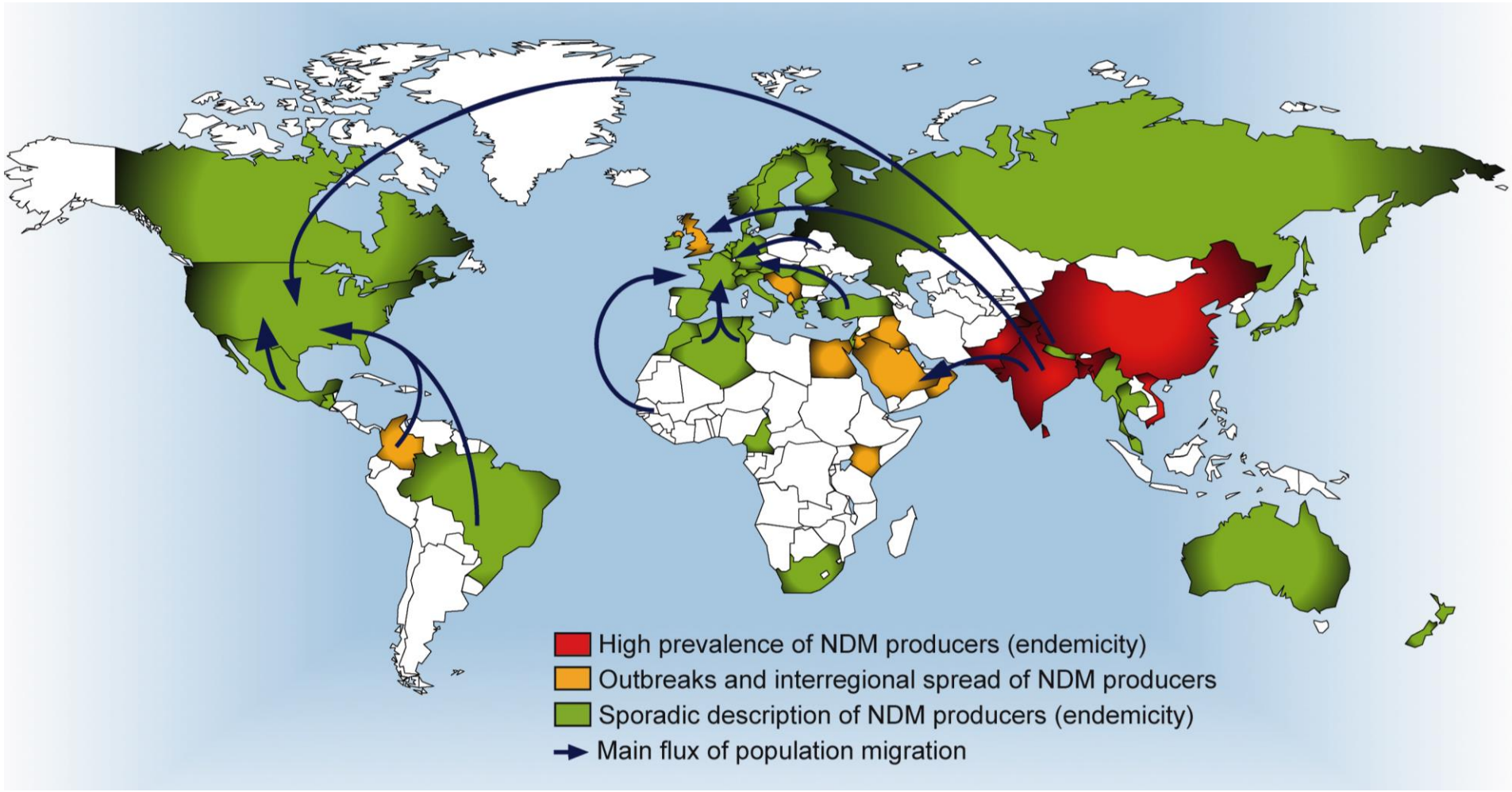
Prevalence of faecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media

John D. Perry[1]*, Sakeenah Hussain Naqvi[2], Irfan Ali Mirza[2], Shehla Ambreen Alizai[2], Aamir Hussain[2], Sandrine Ghirardi[3], Sylvain Orenga[3], Kathryn Wilkinson[1], Neil Woodford[4], Jiancheng Zhang[4], David M. Livermore[4], Shahid Ahmad Abbasi[2] and Muhammad W. Raza[1]

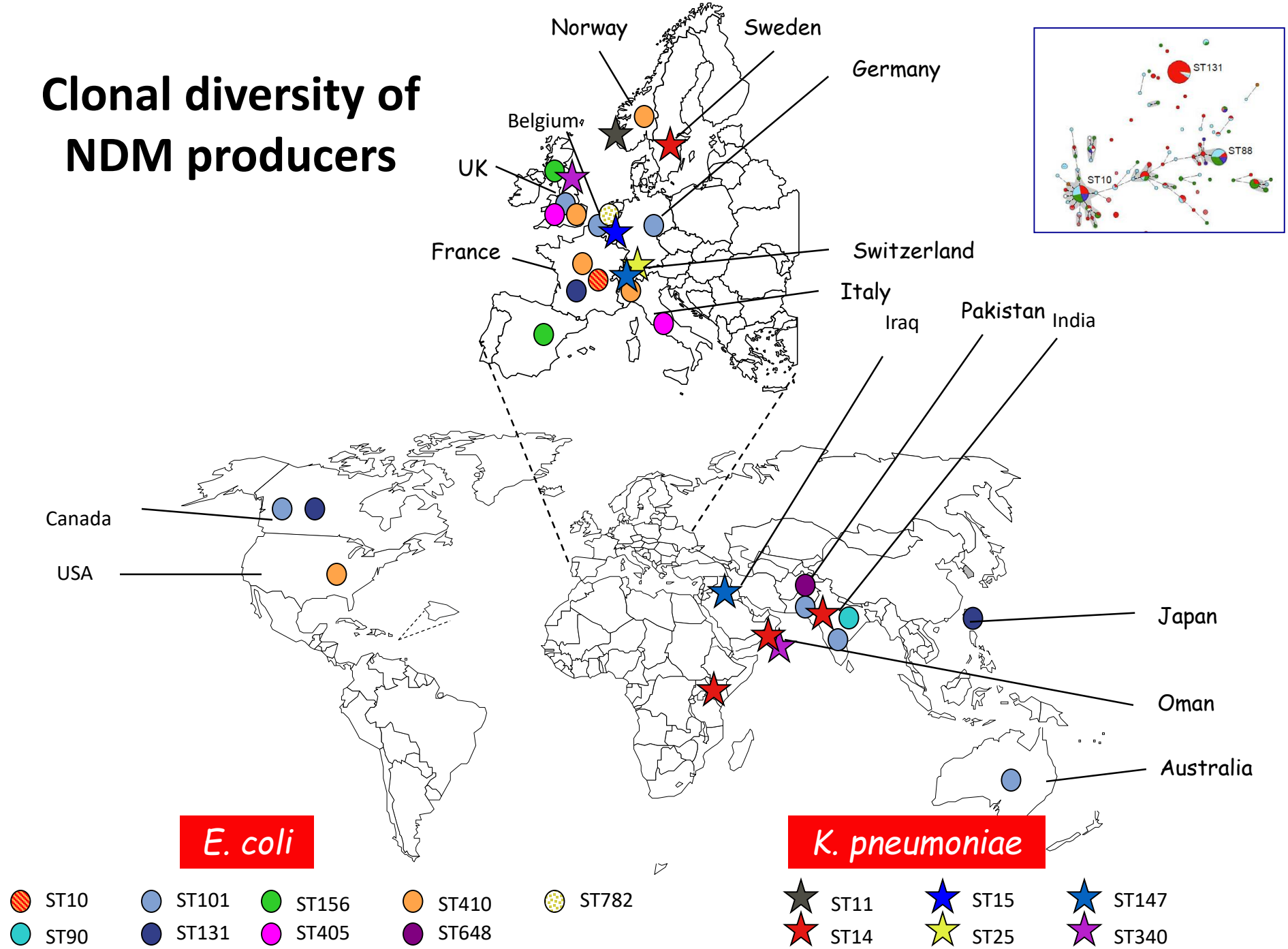
NDM-1 + CTX-M-15 producing *E. coli*



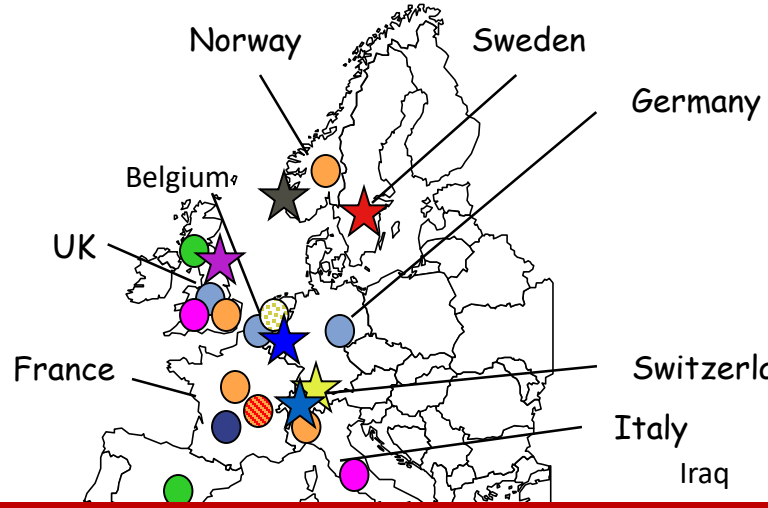
Dissemination of NDM producers from the Indian subcontinent

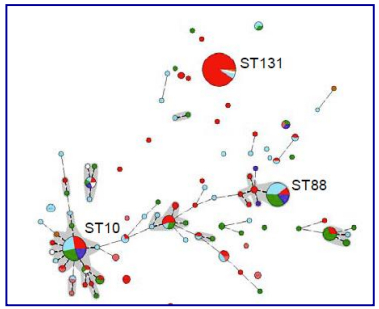


Clonal diversity of NDM producers



Clonal diversity of NDM producers





Canad

USA

n

n

Australia

Gene outbreak

Worldwide dissemination of diverses strains with diversity of plasmids

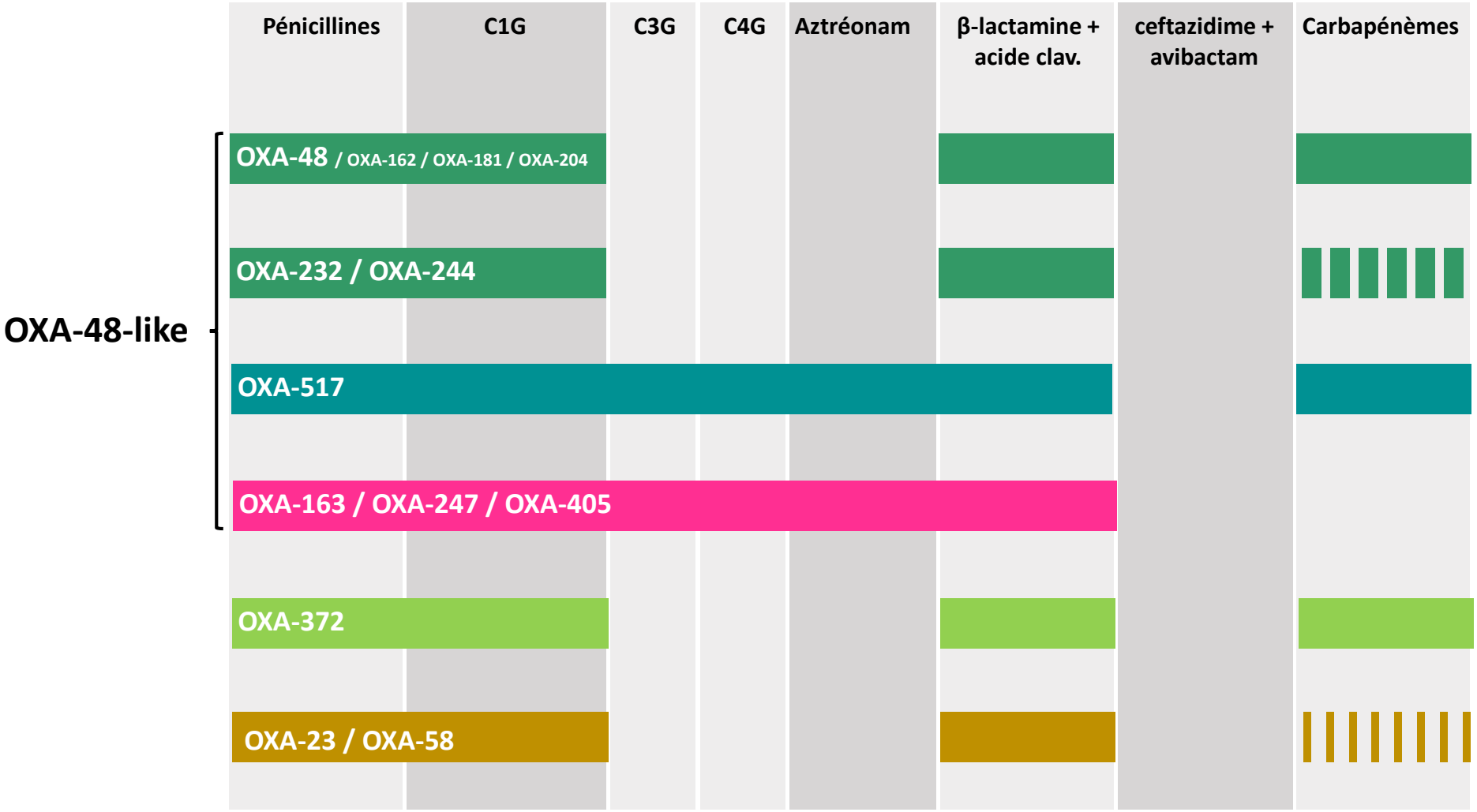
E. coli

ST10 ST101 ST156 ST410 ST782
ST90 ST131 ST405 ST648

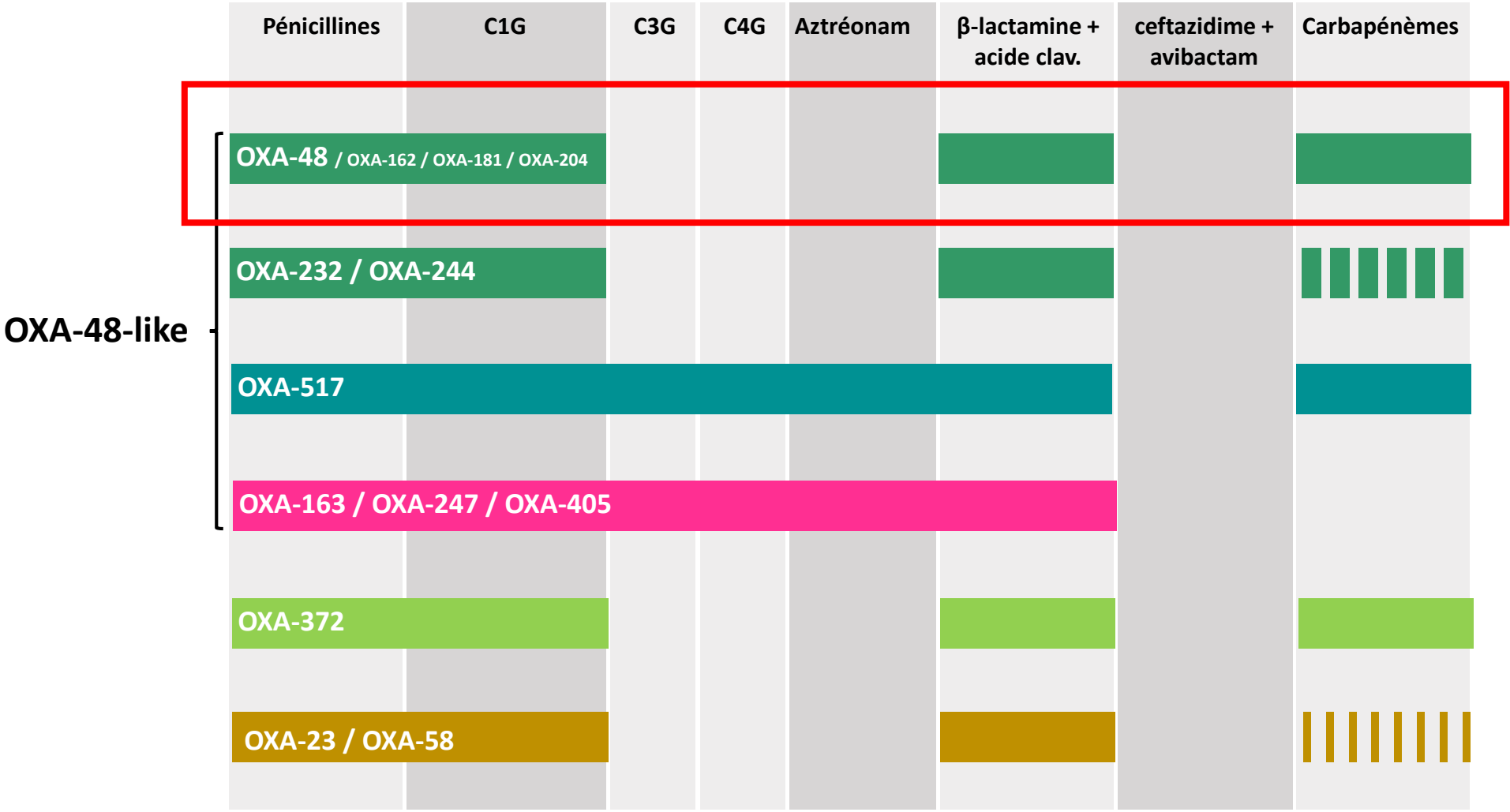
K. pneumoniae

ST11 ST15 ST147
ST14 ST25 ST340

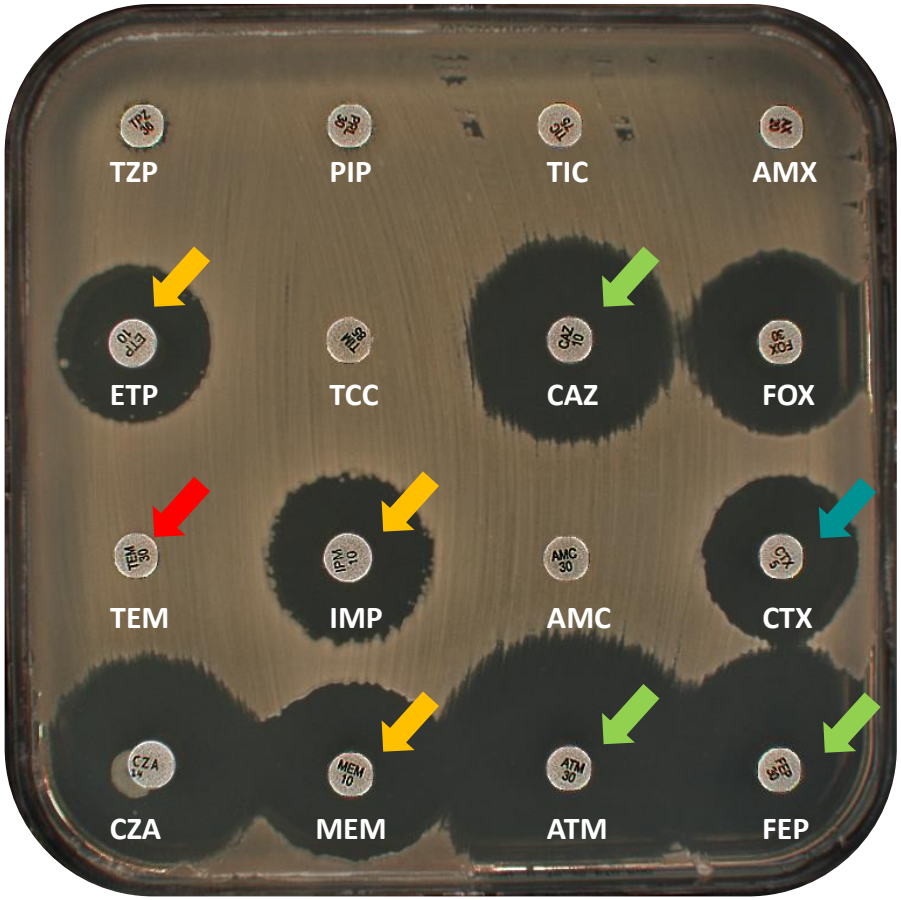
Class D carbapenemases in Enterobacterales



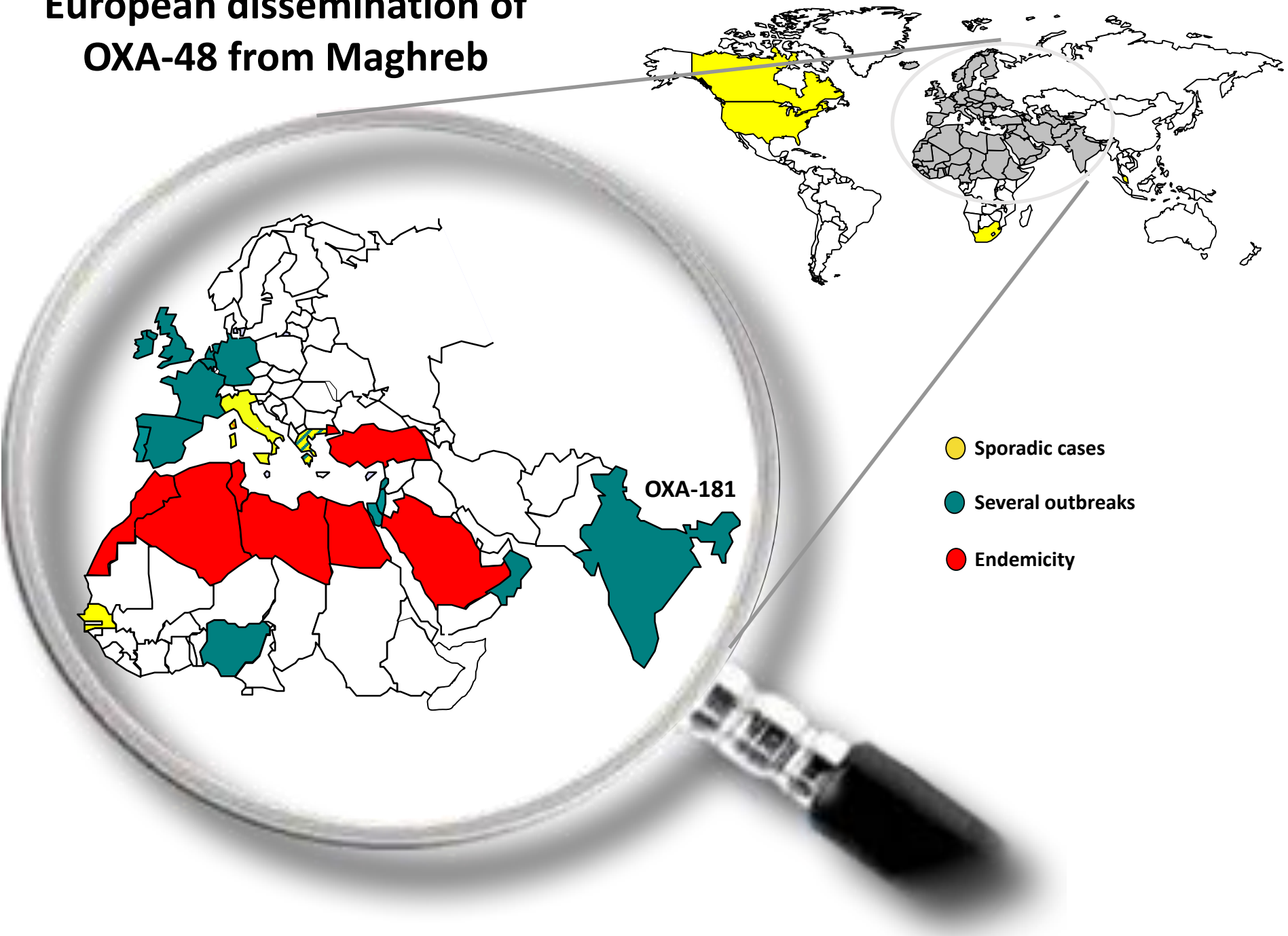
Class D carbapenemases in Enterobacterales



OXA-48 producing *E. coli*

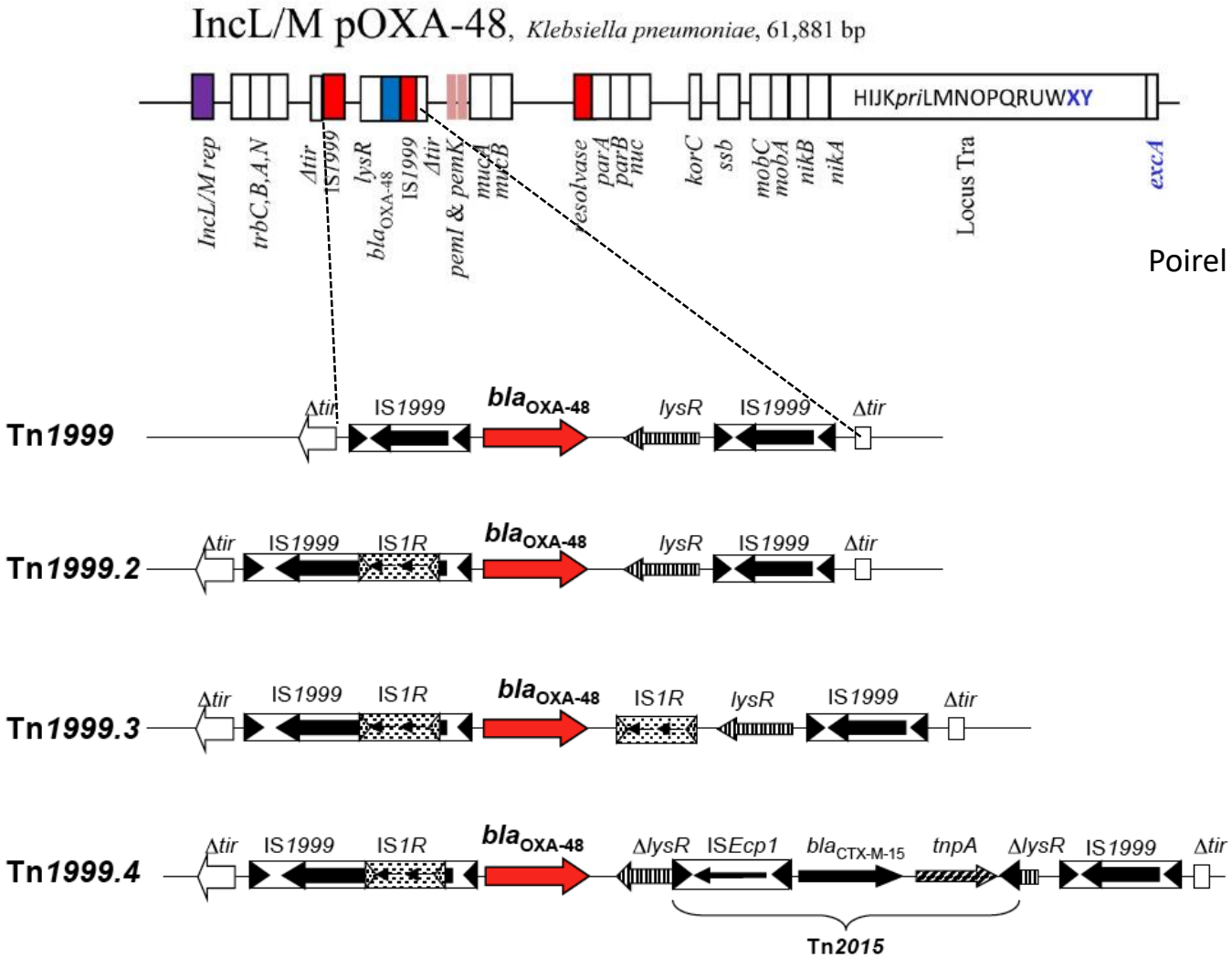


European dissemination of OXA-48 from Maghreb



Genetic environment of *bla*$_{OXA-48}$

same 62,5 kb plasmid of Inc L-type



Poirel et al. AAC 2012

AAC Journals.ASM.org

Derepressed Transfer Properties Leading to the Efficient Spread of the Plasmid Encoding Carbapenemase OXA-48

January 2014 Volume 58 Number 1

Potron A. et al.

Tn*1999*

TABLE 2 Transfer frequencies in *E. coli* JM109 and *E. cloacae* SB[a]

Donor strain	Recipient strain	Mean transfer frequency ± SD	
E. coli TOP10(pOXA-48a)	*E. coli* JM109	$1.1 \times 10^{-1} \pm 0.02$	**Δ*tir* (Tn*1999*)**
E. coli TOP10(pNDM-OM)	*E. coli* JM109	$2.6 \times 10^{-3} \pm 0.016$	
E. coli TOP10(pOXA-48a, pTOPO-Nc)	*E. coli* JM109	$1.7 \times 10^{-1} \pm 0.03$	**Δ *tir* + control DNA**
E. coli TOP10(pOXA-48a, pTOPO-TIR)	*E. coli* JM109	$1.6 \times 10^{-3} \pm 0.0005$	**Δ *tir* + *tir***
E. coli TOP10(pOXA-48a, pTOPO-Nc)	*E. cloacae* SB	$4.9 \times 10^{-2} \pm 0.018$	
E. coli TOP10(pOXA-48a, pTOPO-TIR)	*E. cloacae* SB	$1.2 \times 10^{-3} \pm 0.00004$	

% 100

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Derepressed Transfer Properties Leading to the Efficient Spread of the Plasmid Encoding Carbapenemase OXA-48

January 2014 Volume 58 Number 1

Potron A. et al.

Plasmid outbreak

Dissemination of the same hyper conjugative plasmid among diverse species of Enterobacterales

% 100

TAB

Don

E. coli		
E. coli		
E. coli pT		
E. coli pT		
E. coli pTOPO-Nc)		
E. coli TOP10(pOXA-48a, pTOPO-TIR)	*E. cloacae* SB	$1.2 \times 10^{-3} \pm 0.00004$

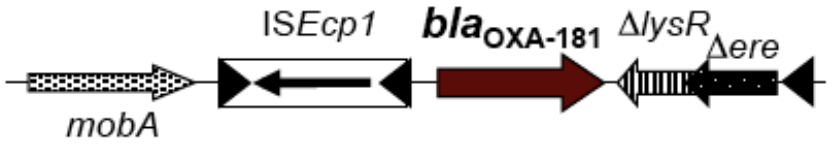
OXA-48 variants with carbapenemase activity similar to OXA-48: OXA-162, OXA-181, OXA-204, OXA-245

Plasmides différents de celui qui porte *bla*$_{OXA-48}$

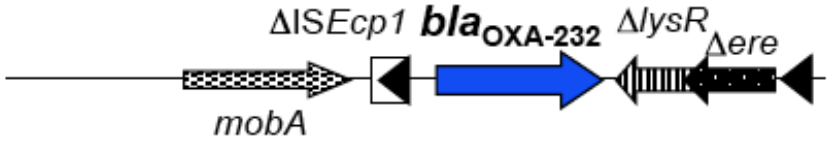
Environnement génétique différent de celui de *bla*$_{OXA-48}$

Mobilisation par ***ISEcp1*** probable comme décrit pour *bla*$_{CTX-M}$

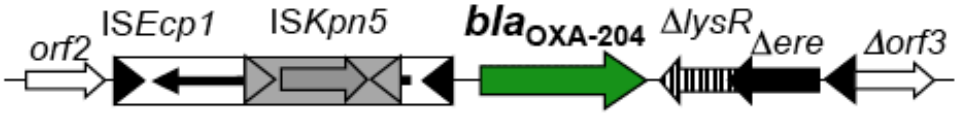
OXA-181 **Tn*2013***



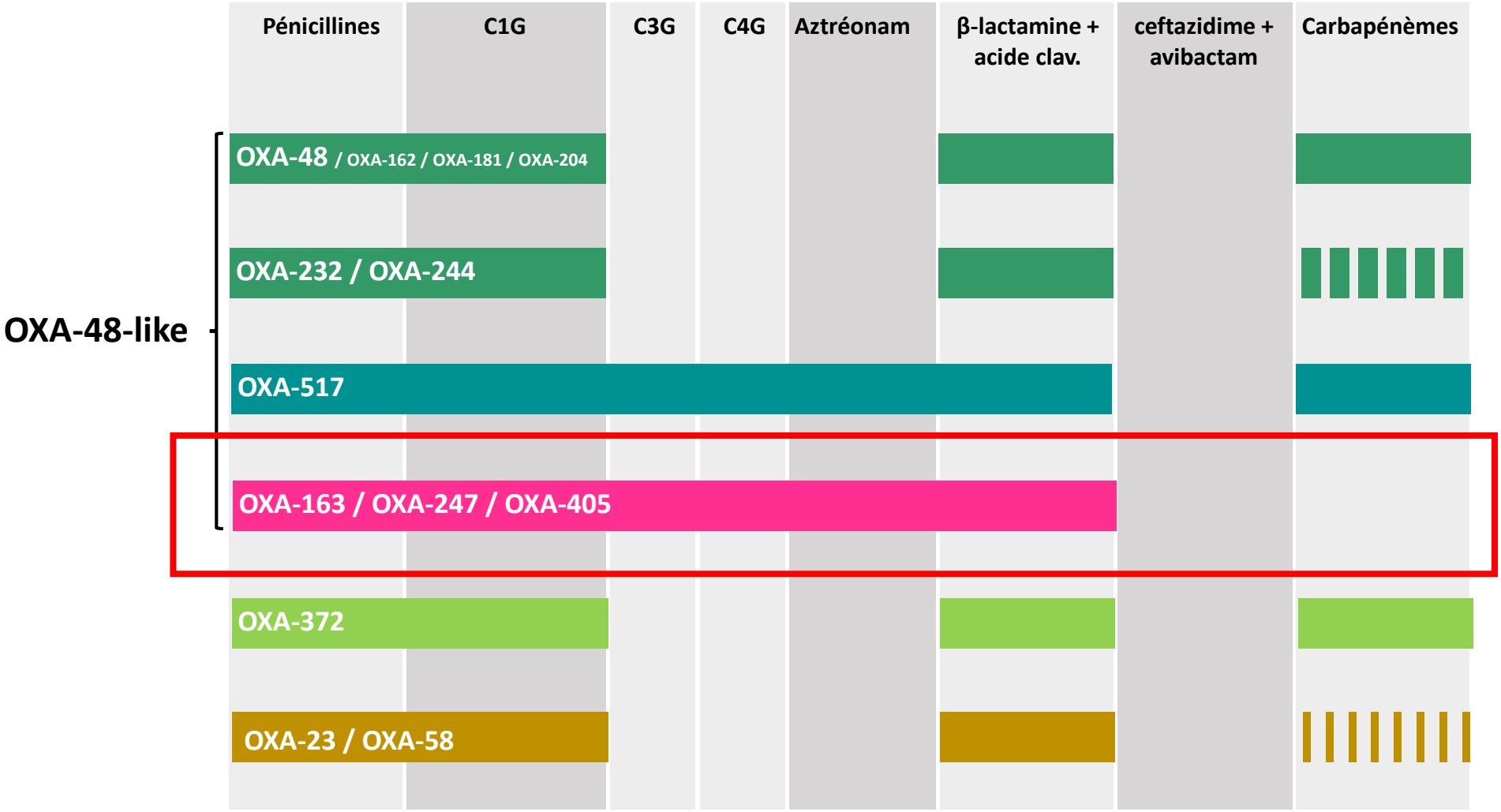
OXA-232



OXA-204 **Tn*2016***



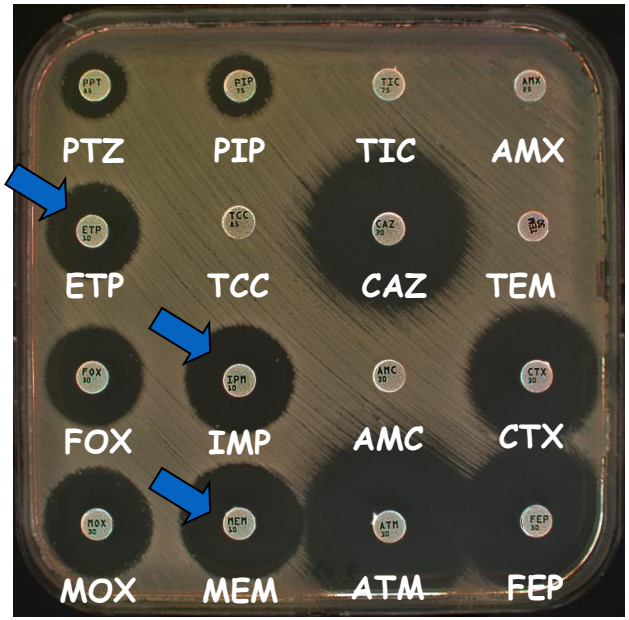
Class D carbapenemases in Enterobacterales



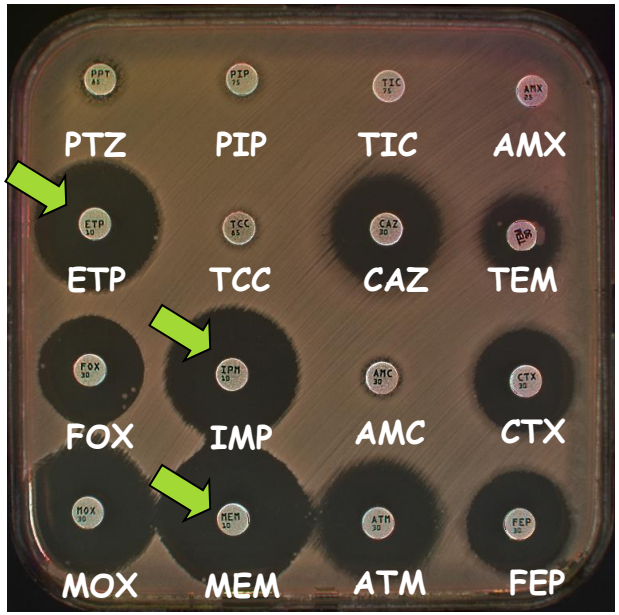
OXA-48 variants without carbapénèmase activity : OXA-163, OXA-247, OXA-405

- **No carbapenemase activity** : deletion in the active site

OXA-48

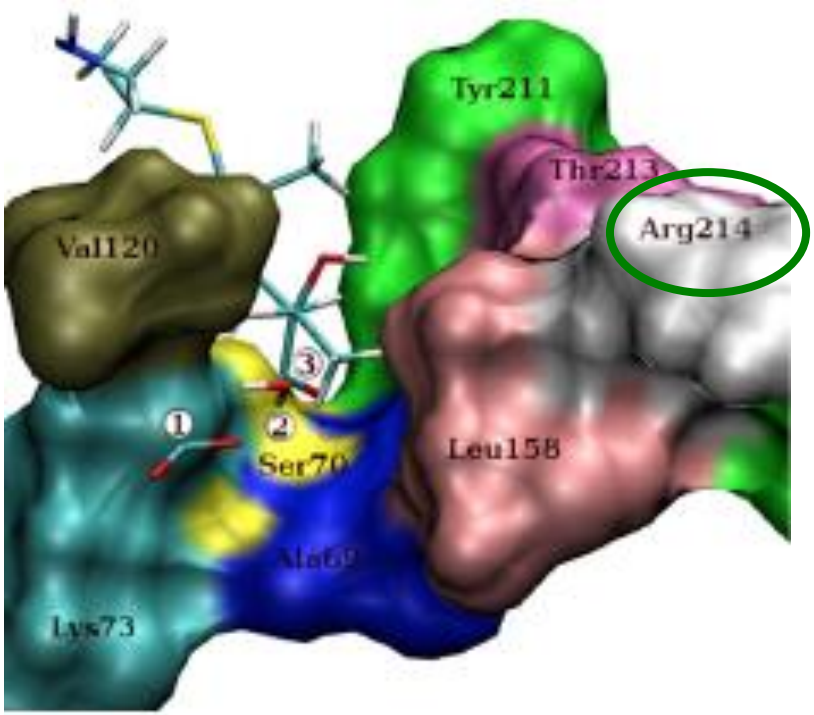


OXA-163



```
                                  210       220       230       240       250       260
                                   |         |         |         |         |         |
Carbapenemase   OXA-204   GDYIIRAKTGYSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-245   GDYIIRAKTGYSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-48    GDYIIRAKTGYSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-370   GDYIIRAKTGYETRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-162   GDYIIRAKTGYSARIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-181   GDYIIRAKTGYSTRIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-232   GDYIIRAKTGYSTSIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-244   GDYIIRAKTGYSTGIEPKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
Non             OXA-163   GDYIIRAKTGYDT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
Carbapenemase   OXA-247   GDYIIRAKTGSNT----KIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                OXA-405   GDYIIRAKTGYS-----PKIGWWVGWVELDDNVWFFAMNMDMPTSDGLGLRQAITKEVLKQEKIIP
                          **********..      ********************************************
```

OXA-48 active site

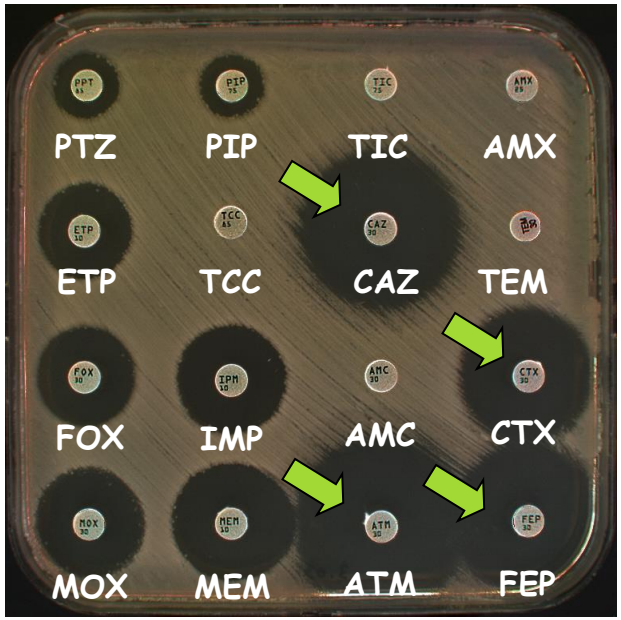


Deletion in OXA-163, OXA-247 and OXA-405

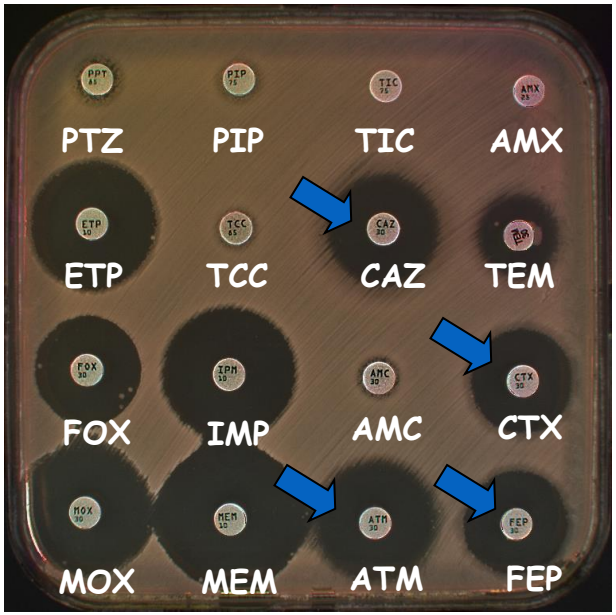
OXA-48 variants without carbapénèmase activity : OXA-163, OXA-247, OXA-405

- **No carbapenemase activity** : deletion in the active site
- **3rd generation cephalosporins hydrolysis** (ESBL-like)

OXA-48



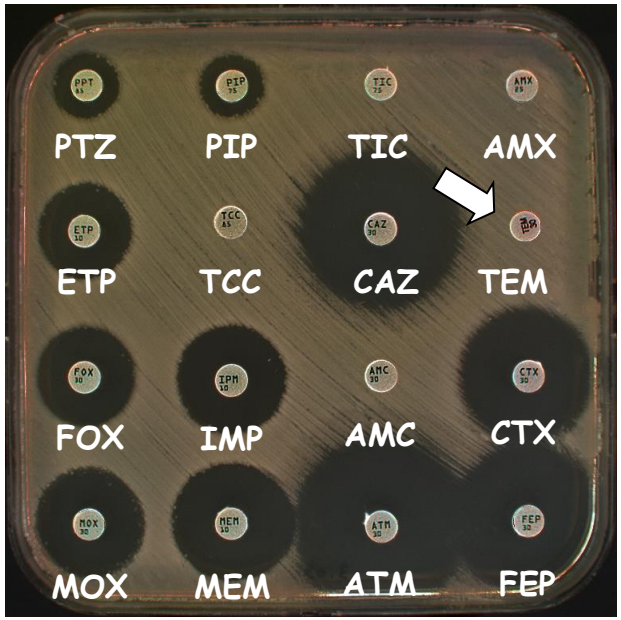
OXA-163



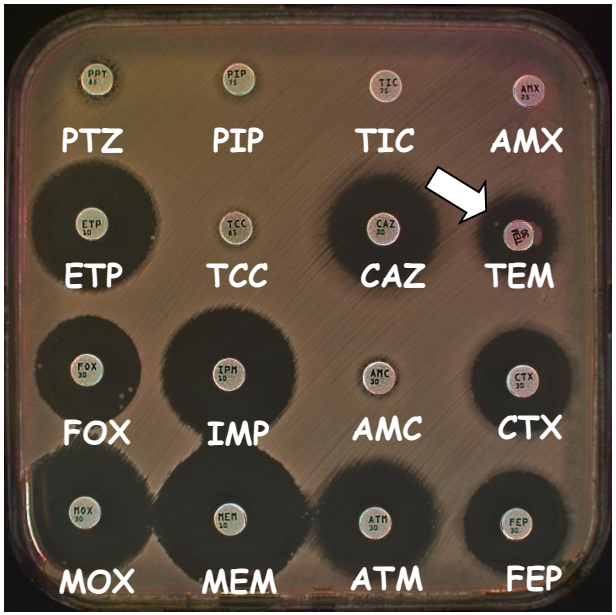
OXA-48 variants without carbapénèmase activity : OXA-163, OXA-247, OXA-405

- **No carbapenemase activity** : deletion in the active site
- **3rd generation cephalosporins hydrolysis** (ESBL-like)
- **Temocillin increased susceptibility**

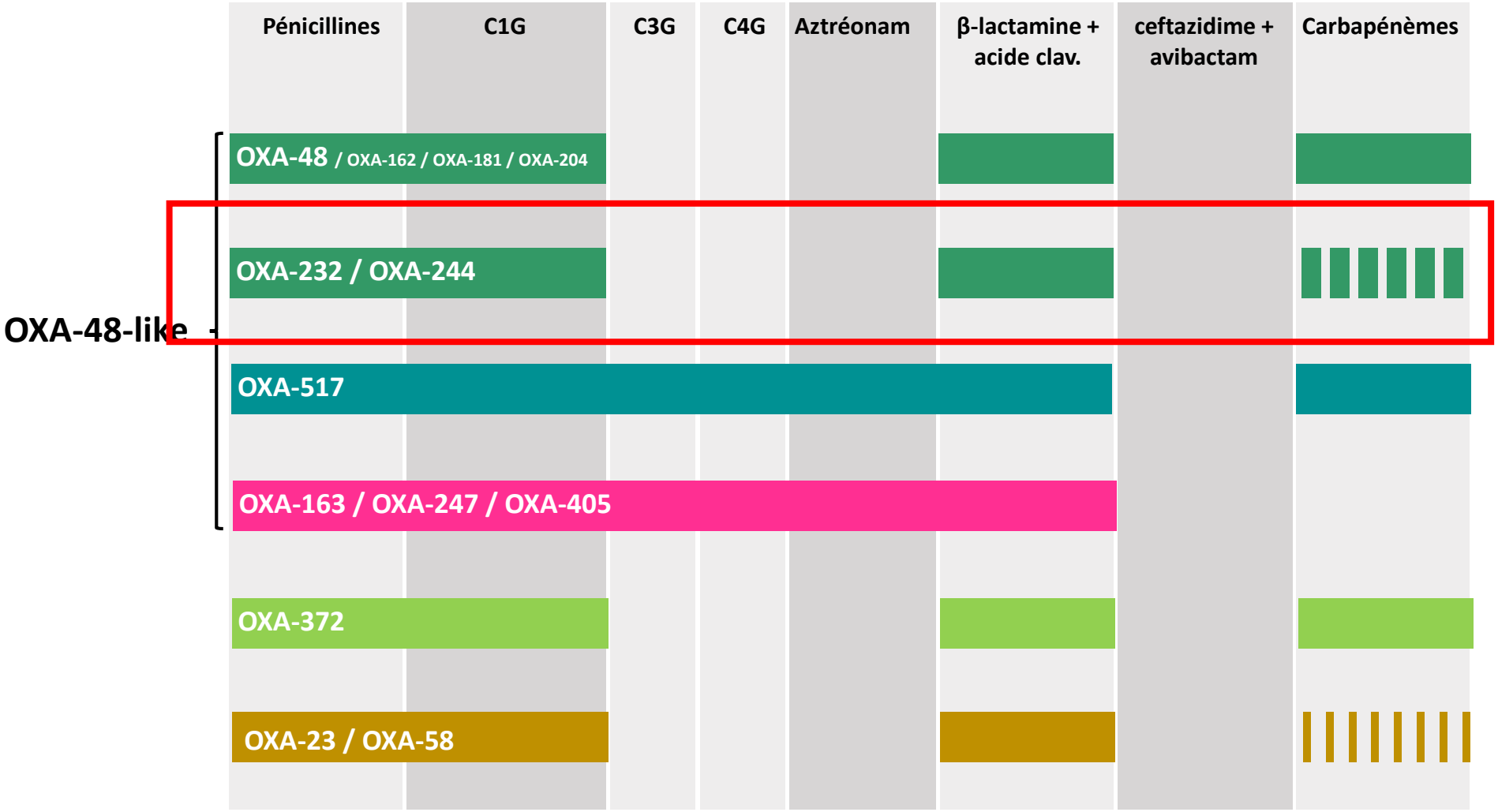
OXA-48



OXA-163



Class D carbapenemases in Enterobacterales



OXA-244

OXA-48

Low hydrolytic activity compare to OXA-48

Variant	Amino acid at position (DBL numbering system)															Hydrolysis	
	98	99	104	110	120	125	175	179	219	220	221	222	223	224	225	IMI	CAZ
OXA-48	Q	T	T	N	V	E	E	S	Y	S	T	R	I	E	P	+	-
OXA-162											A					++	-
OXA-244												G				+/-	-
OXA-245						T										+	-
OXA-204	H	R														+	-
OXA-181			A	D			Q	A								+	-
OXA-232			A	D			Q	A				S				+/-	-
OXA-163										D		*	*	*	*	-	+
OXA-247									S	D		*	*	*	*	-	+
OXA-405											*	*	*	*		-	+

Loop β5- β6

Shewanella spp. are progenitors of OXA-48-like enzymes

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2004, p. 348–351 Vol. 48, No. 1
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.1.348–351.2004
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Chromosome-Encoded Ambler Class D β-Lactamase of *Shewanella oneidensis* as a Progenitor of Carbapenem-Hydrolyzing Oxacillinase

Laurent Poirel, Claire Héritier, and Patrice Nordmann*

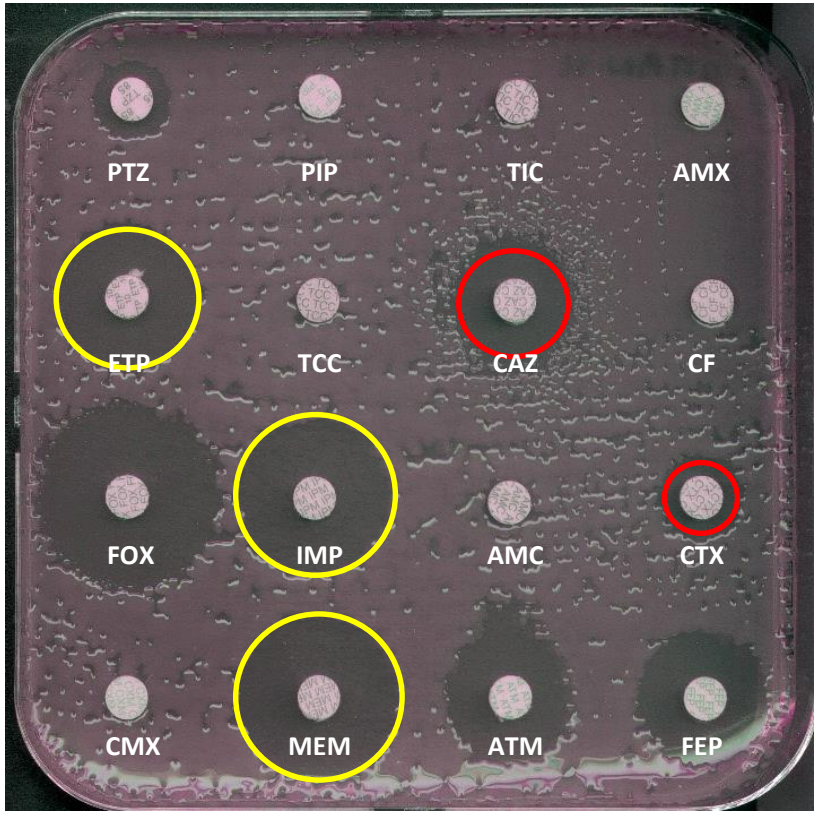
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4405–4407 Vol. 55, No. 9
0066-4804/11/$12.00 doi:10.1128/AAC.00681-11
Copyright © 2011, American Society for Microbiology. All Rights Reserved.

Origin of OXA-181, an Emerging Carbapenem-Hydrolyzing Oxacillinase, as a Chromosomal Gene in *Shewanella xiamenensis*▽

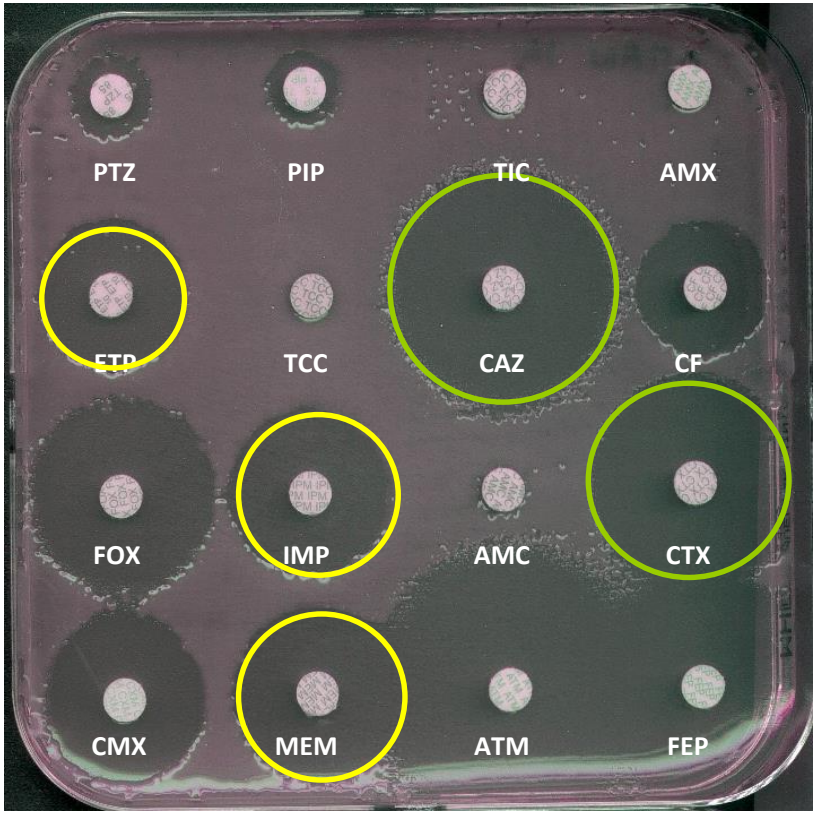
Anaïs Potron, Laurent Poirel, and Patrice Nordmann*

OXA-48 success story

Low hydolysis of carbapenems + no hydolysis of 3GC ⇒ Detection difficulties



***K. pneumoniae* OXA-48**
BLSE +

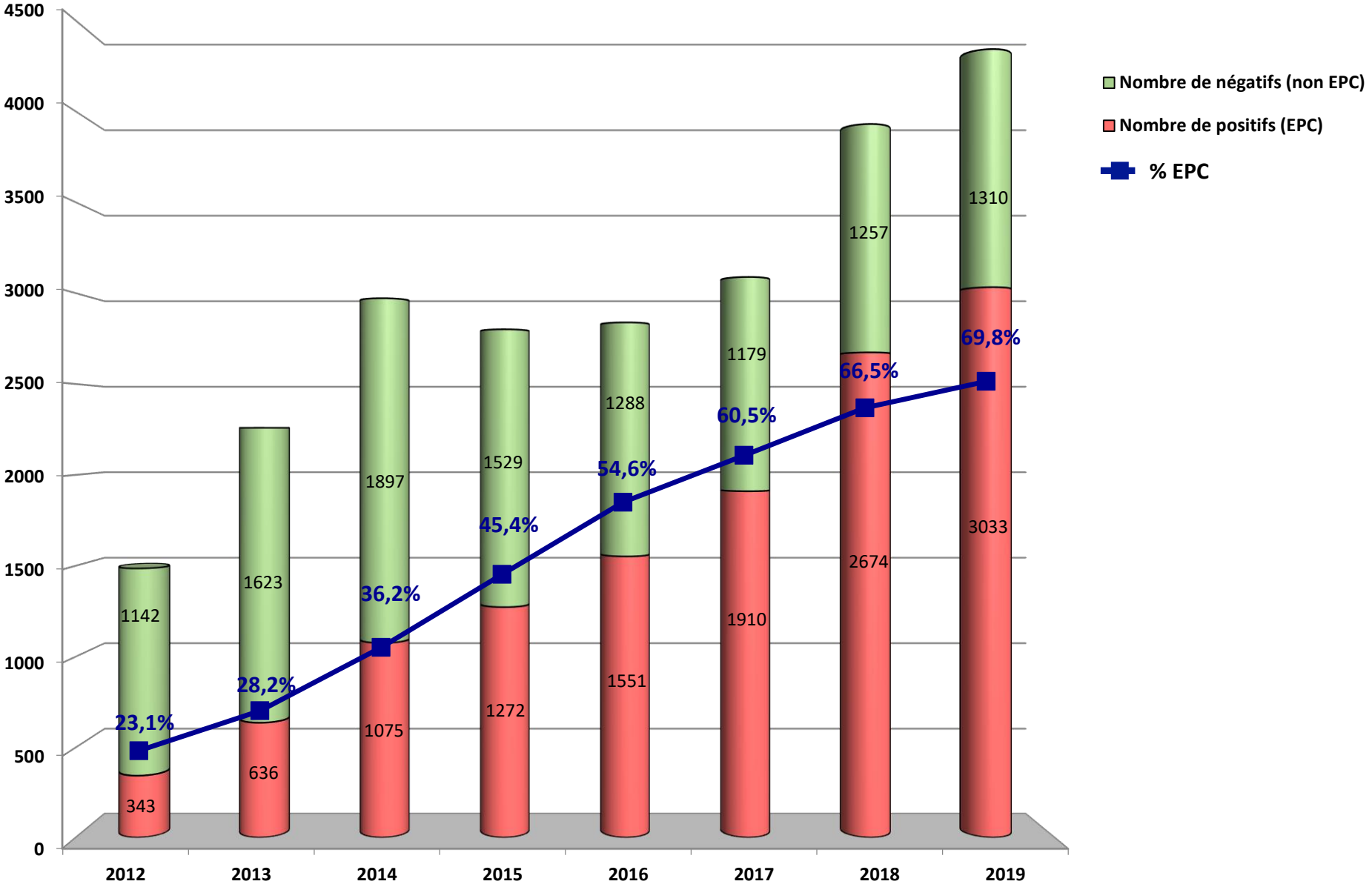


***K. pneumoniae* OXA-48**
BLSE -

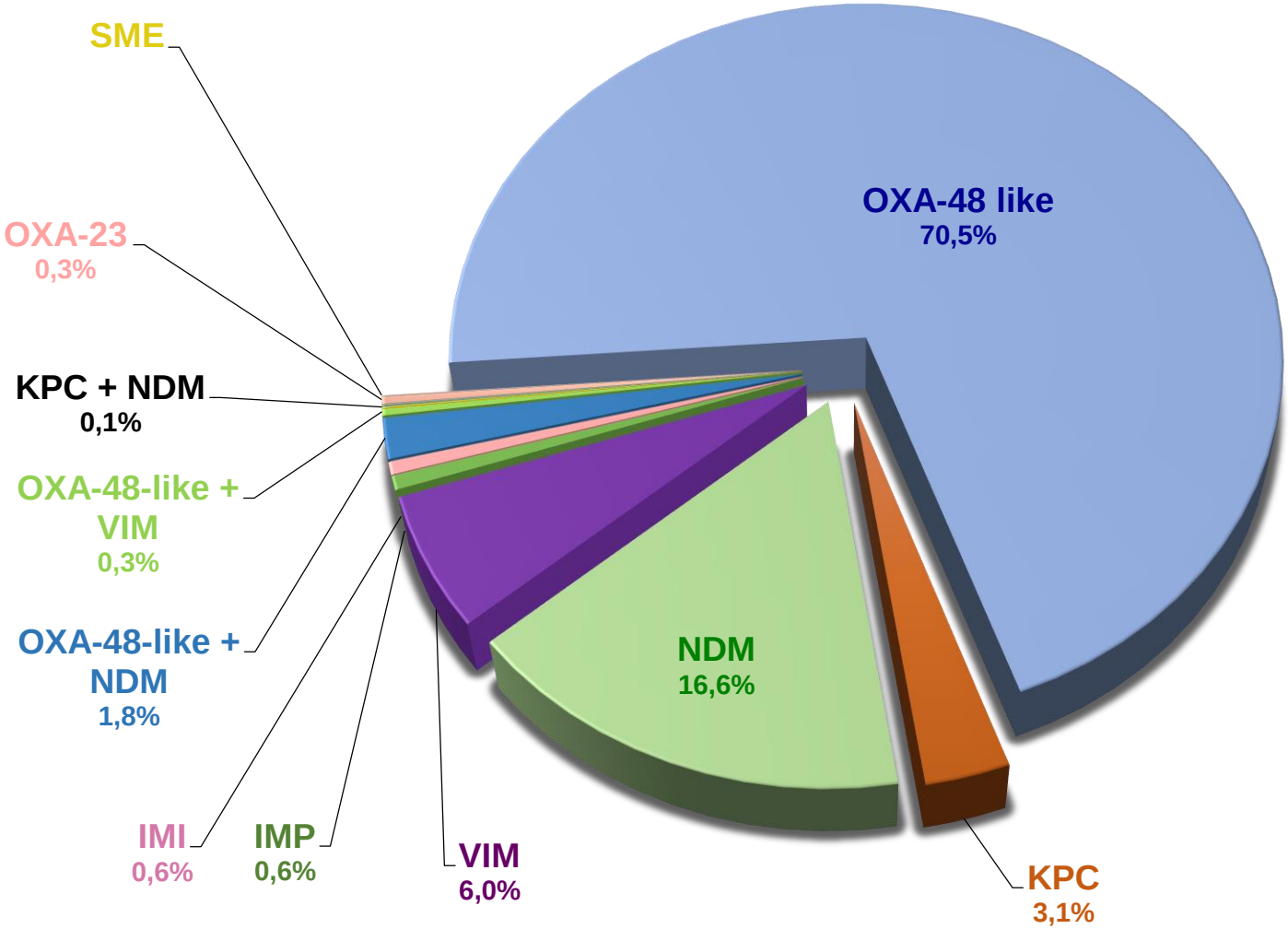
Epidemiology of CPE in France

2019

Distribution of carbapenem non-susceptible Enterobacteral isolates expertised by the NRC from 2012 to 2019

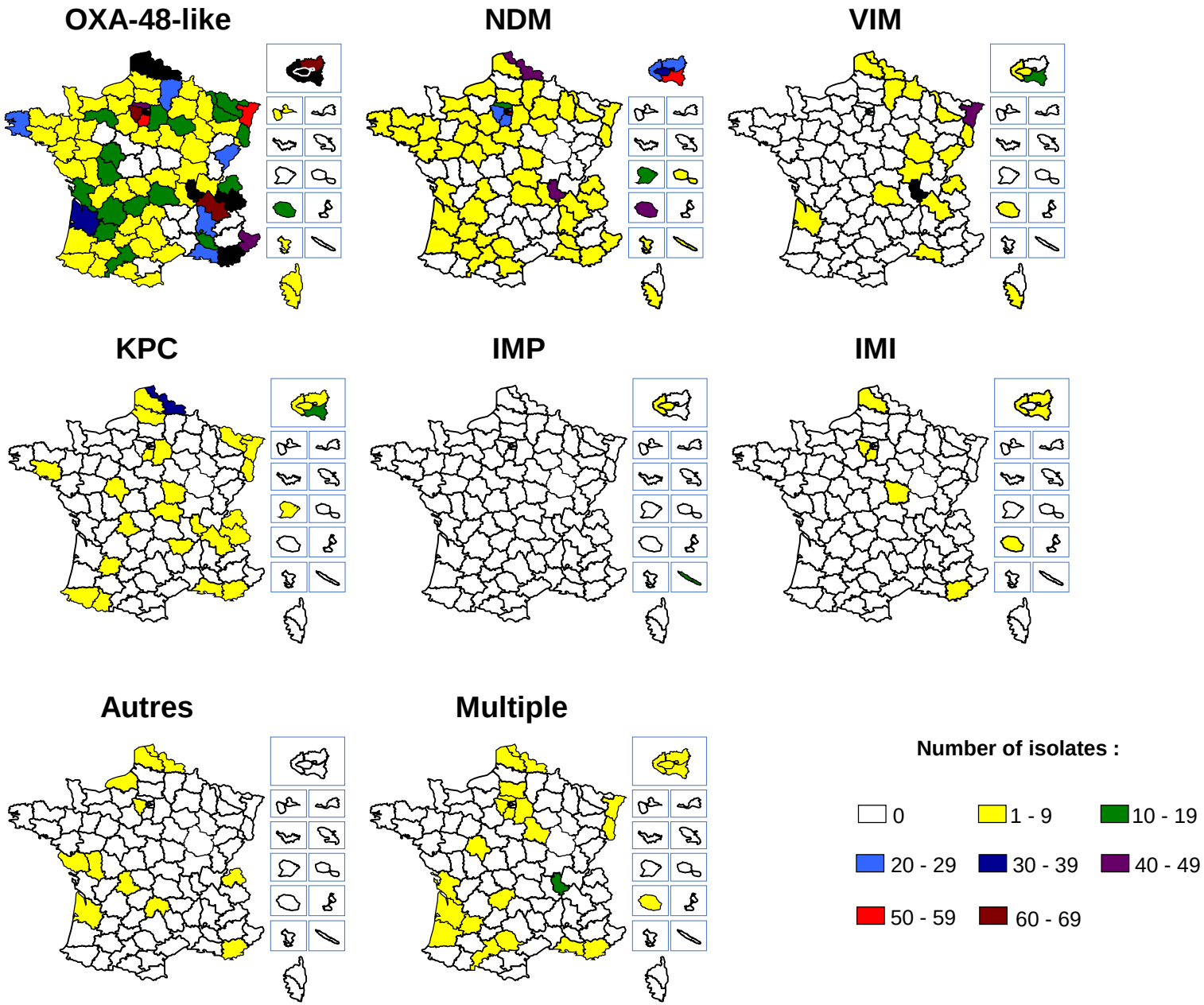


Distribution of CPE per type of carbapenemase

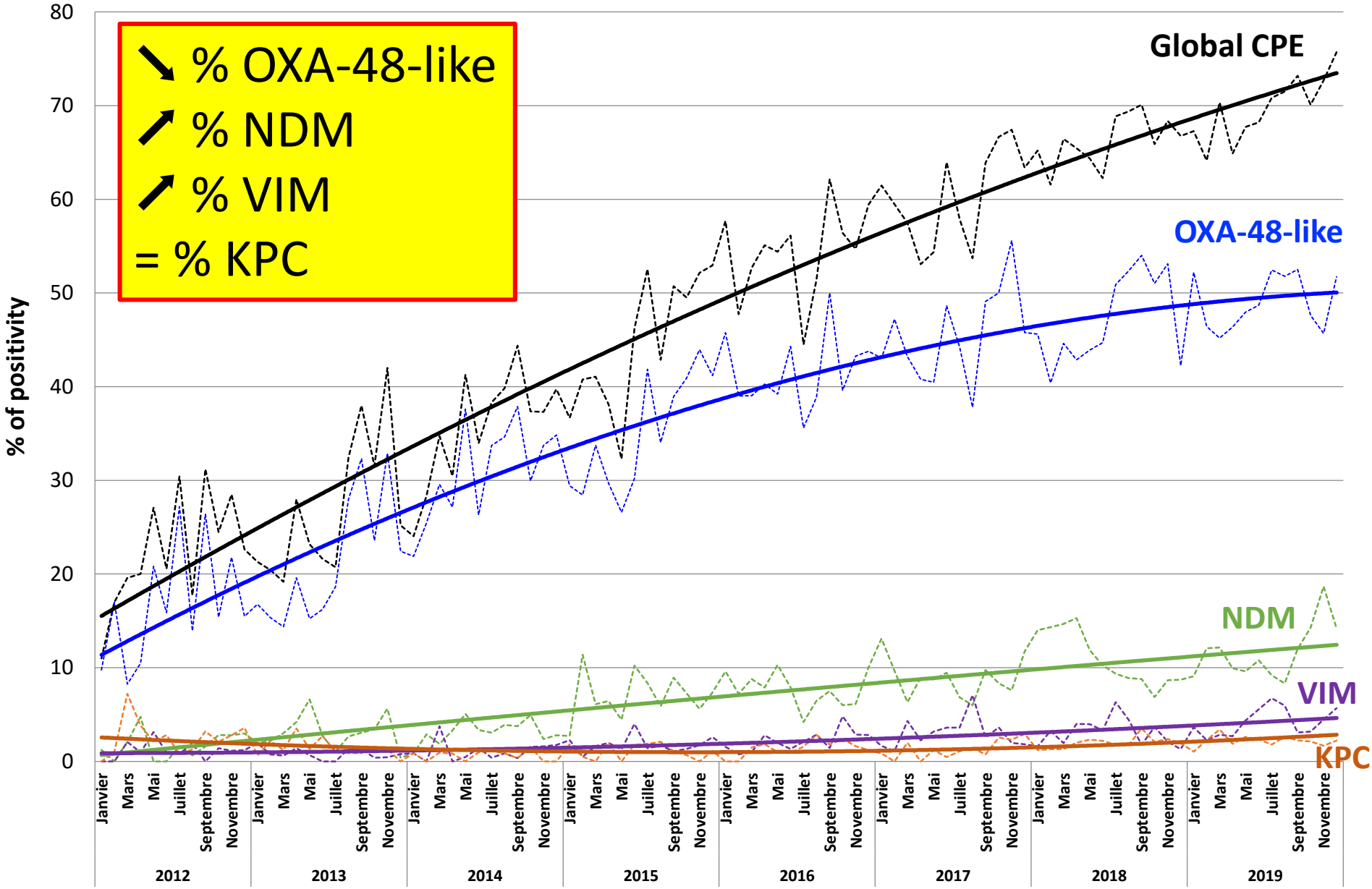


Type de carbapenemase	n	%
OXA-48-like	2138	70,5
KPC	94	3,1
NDM	502	16,6
VIM	183	6,0
IMP	19	0,6
IMI	17	0,6
NMC-A	0	0,0
OXA-48-like + NDM	55	1,8
OXA-48-like + VIM	9	0,3
KPC + NDM	2	0,1
KPC + OXA-48-like	1	0,0
NDM + IMP	1	0,0
SFH	1	0,0
OXA-23	10	0,3
SME	1	0,0
	3033	100

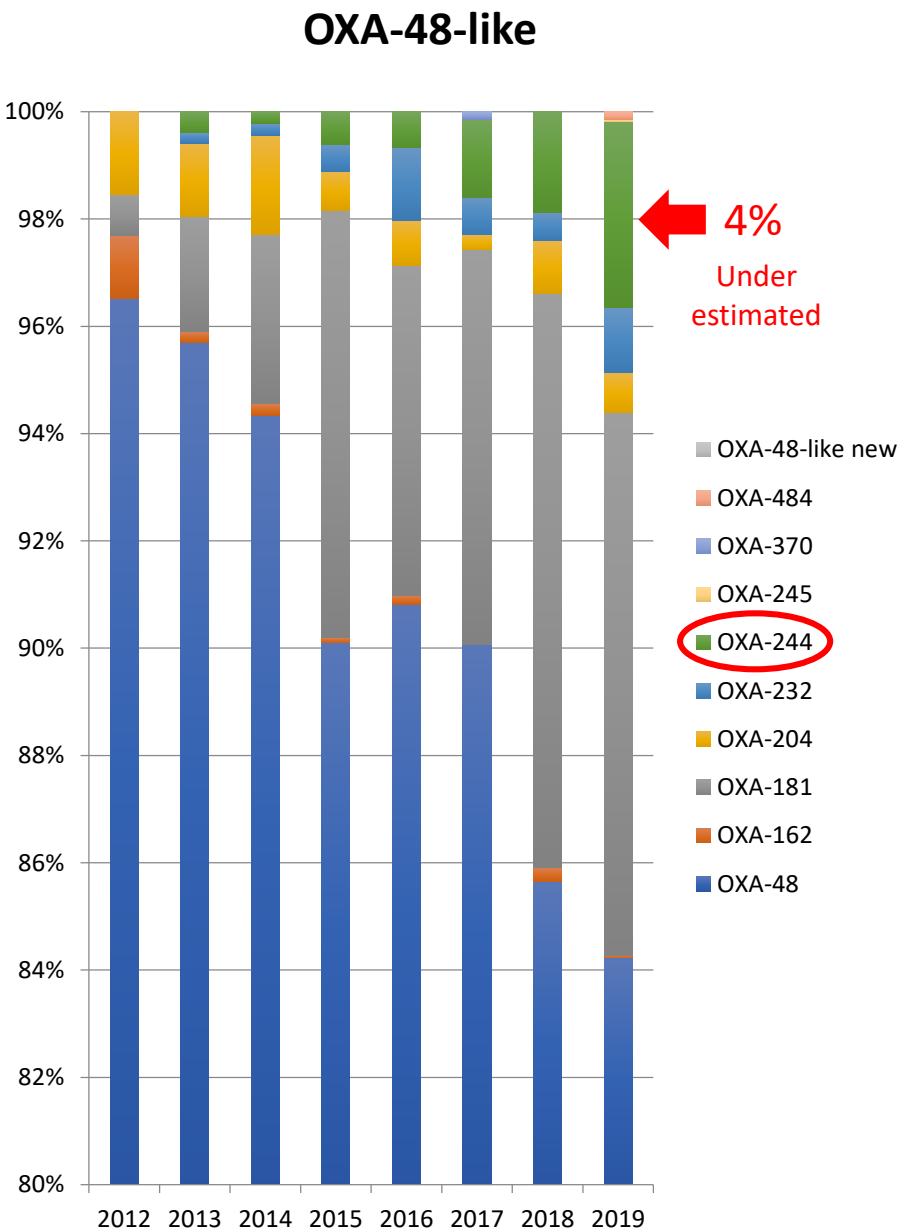
Geographic distribution of CPE per type of carbapenemase

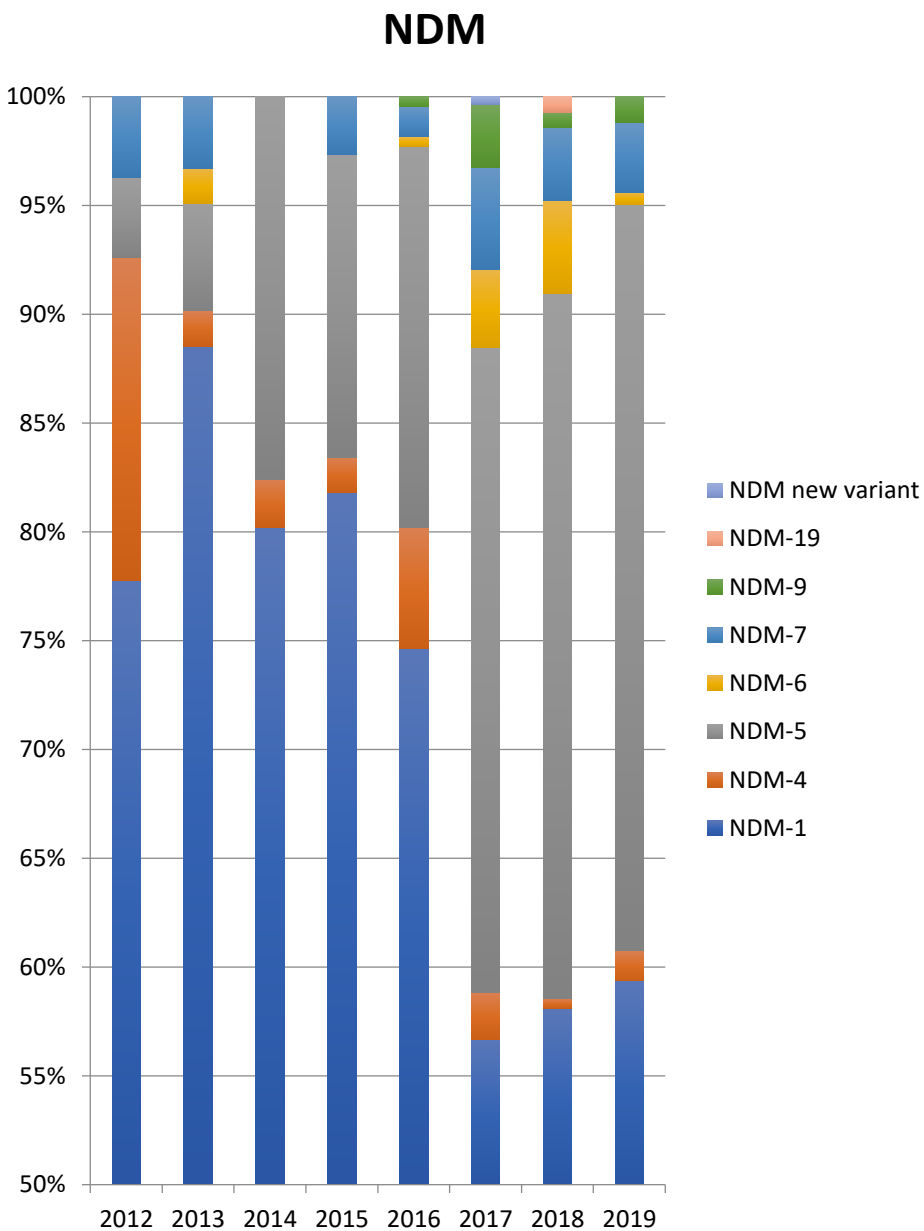


% of CPE and main carbapenemases among strains expertised by the NRC from 2012 to 2019

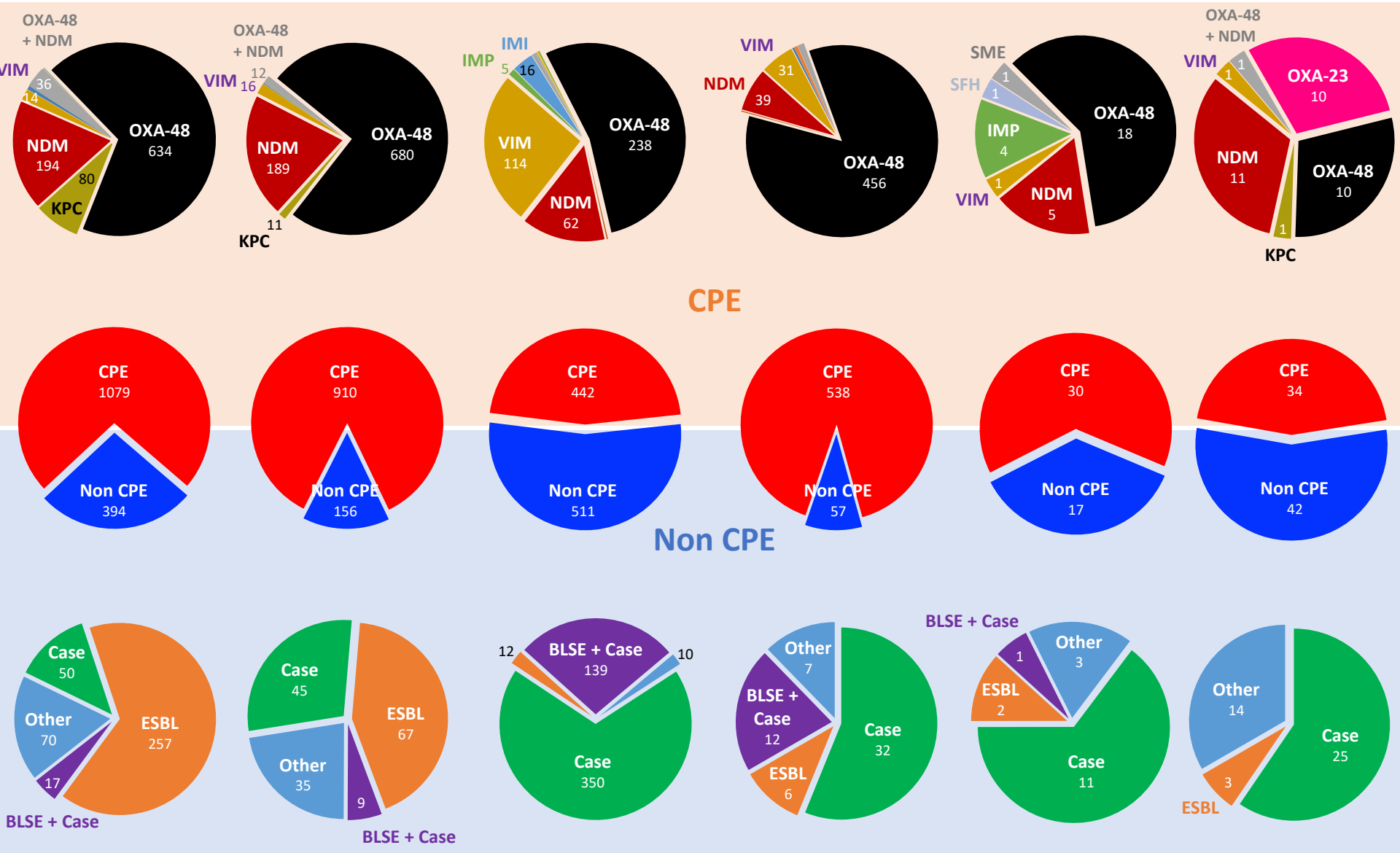


Diversification of OXA-48-like and NDM

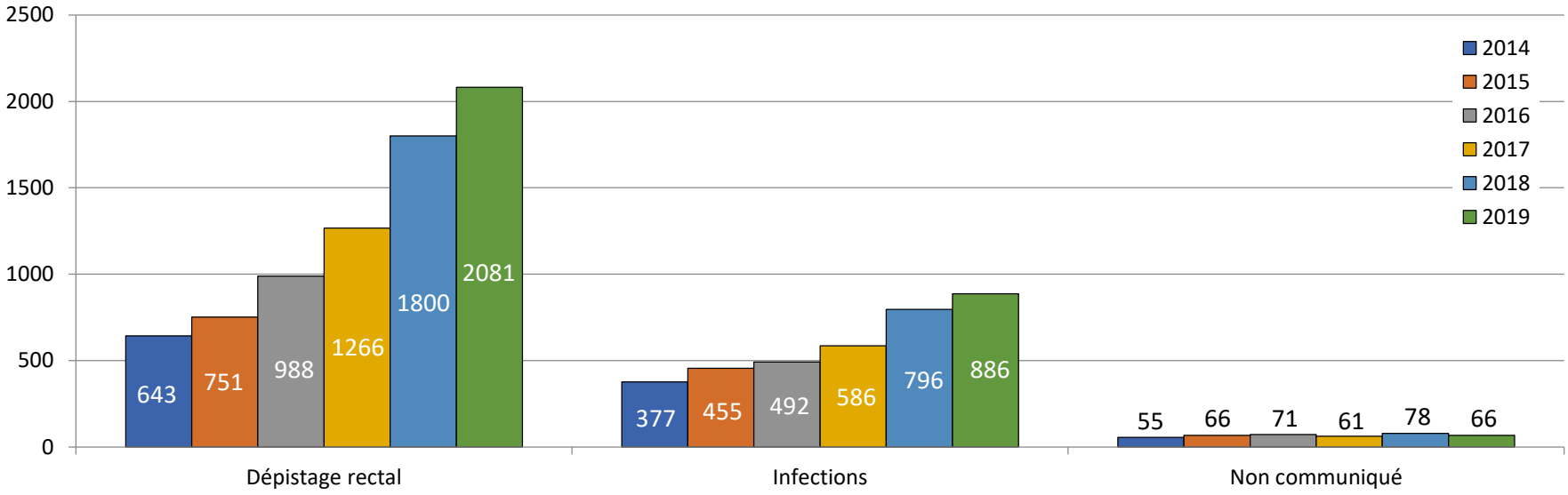




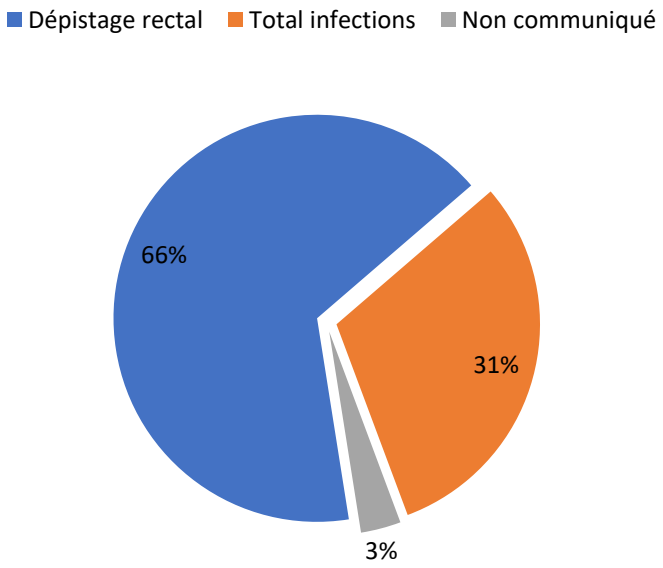
Carbapenem resistance mechanisms per species



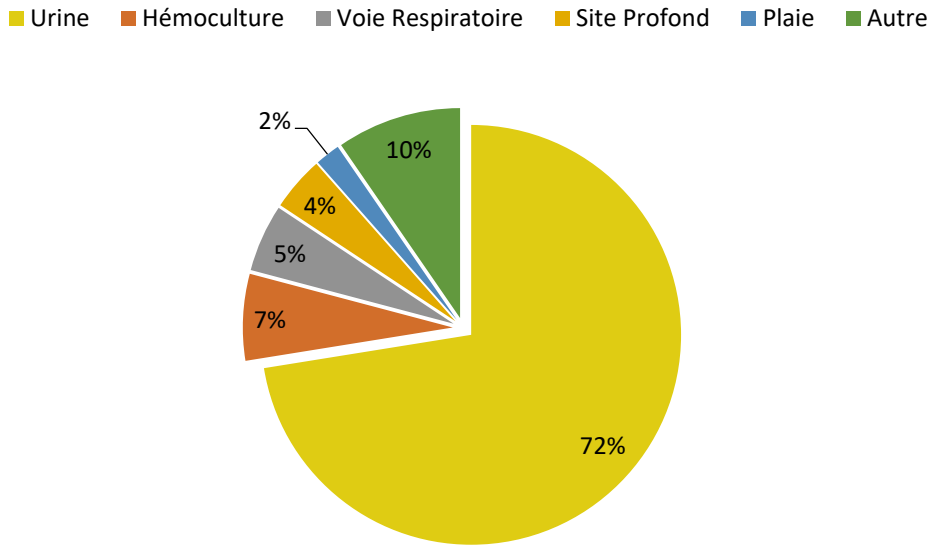
Répartition des EPC par type de prélèvement et par année

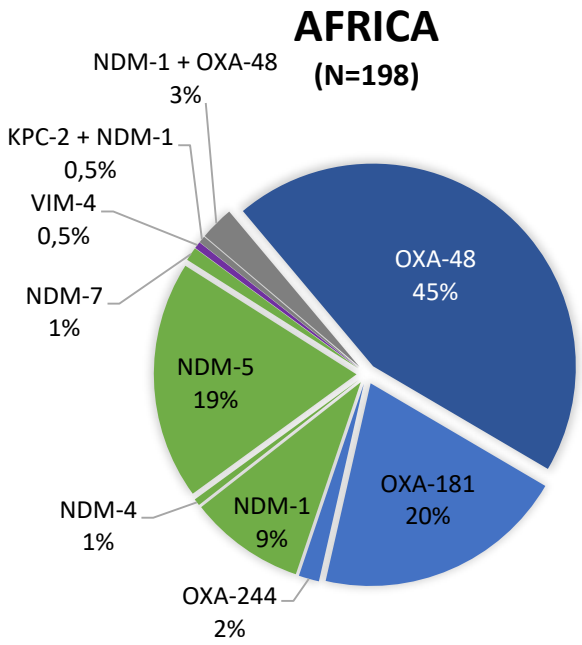


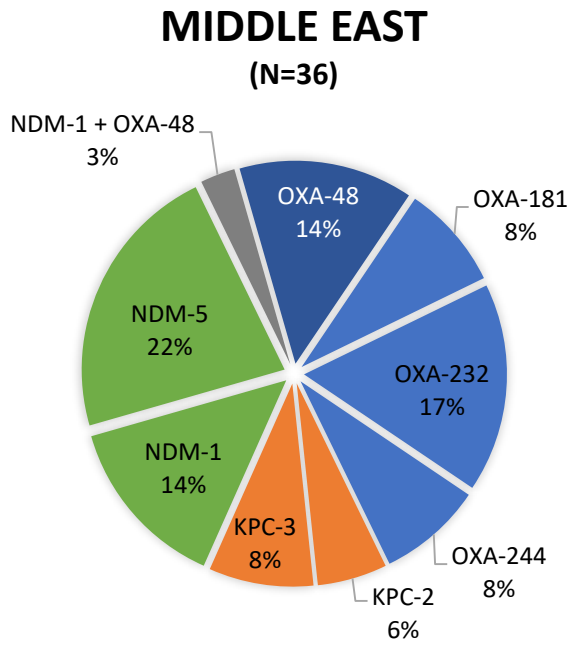
Répartition des EPC par type de prélèvement en 2019

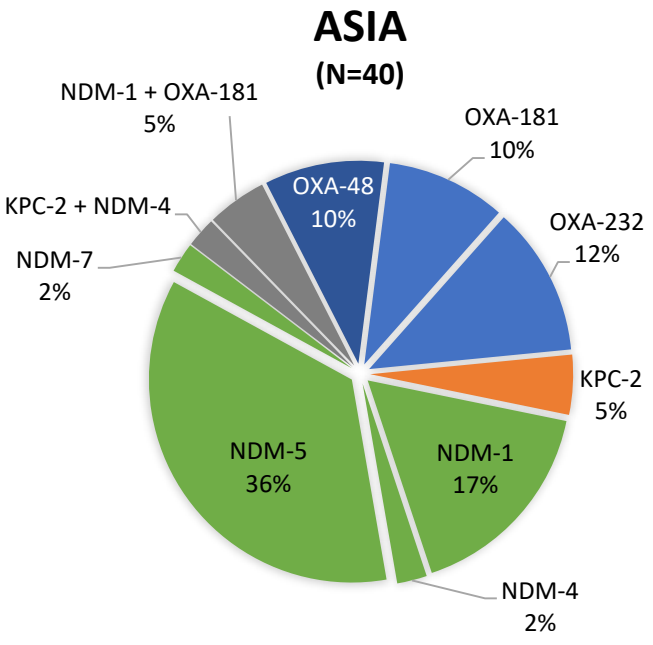


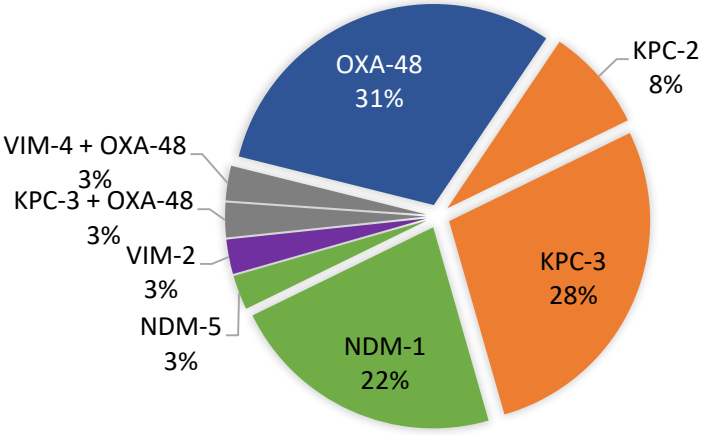
Répartition des EPC responsables d'infections en 2019

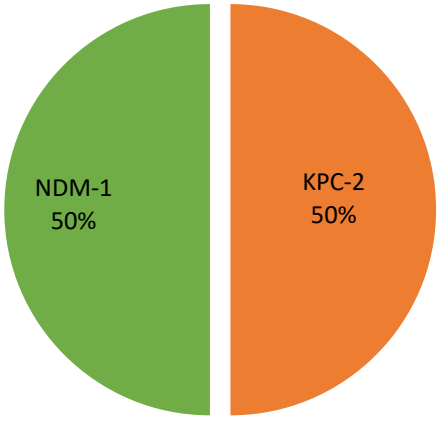



AFRICA
(N=198)
NDM-1 + OXA-48
3%
KPC-2 + NDM-1
0,5%
VIM-4
0,5%
NDM-7
1%
OXA-48
45%
NDM-5
19%
NDM-4
1%
NDM-1
9%
OXA-181
20%
OXA-244
2%


MIDDLE EAST
(N=36)
NDM-1 + OXA-48
3%
OXA-48
14%
OXA-181
8%
NDM-5
22%
OXA-232
17%
NDM-1
14%
KPC-3
8%
OXA-244
8%
KPC-2
6%


ASIA
(N=40)
NDM-1 + OXA-181
5%
OXA-181
10%
KPC-2 + NDM-4
OXA-48
10%
OXA-232
12%
NDM-7
2%
KPC-2
5%
NDM-5
36%
NDM-1
17%
NDM-4
2%


EUROPE
(N=28)
OXA-48
31%
KPC-2
8%
VIM-4 + OXA-48
3%
KPC-3 + OXA-48
3%
VIM-2
3%
NDM-5
3%
KPC-3
28%
NDM-1
22%


AMERICA
(n=2)
NDM-1
50%
KPC-2
50%

PLAN

1. Epidémiologie et dissémination des carbapénèmases

1.1. Les carbapénèmases de la classe A de Ambler

1.2. Les métallo-β-lactamases

1.3. les carbapénèmases de la classe D de Ambler

2. Méthodes de détection des entérobactéries productrices de carbapénèmases

2.1. A partir d'un prélèvement clinique (infection)

2.2. Dépistage des patients porteurs

When to screen for a CPE?

Clinical breakpoints and screening cut-off values for CPE (according to EUCAST methodology)

Carbapenem	MIC (mg/L)		Disk diffusion zone diameter (mm)	
	S/I breakpoint	Screening cut-off	S/I breakpoint	Screening cut-off
Meropenem (10 µg)[1]	**≤2**	**>0.125**	**≥22**	**<25[2]**
Imipenem (10 µg)[3]	≤2	>1	≥22	<23
Ertapenem (10 µg)[4]	≤0.5	>0.125	≥25	<25

[1] Best balance of sensitivity and specificity.

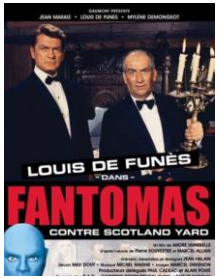
[2] In some cases OXA-48 producers have zone diameters up to 26 mm, so <27 mm may be used as a screening cut-off during outbreaks caused by OXA-48-producing Enterobacteriaceae, but with reduction in specificity.

[3] With imipenem the separation between the wild-type and carbapenemase-producers is relatively poor. Imipenem is therefore not recommended to use as a stand-alone screening test compound.

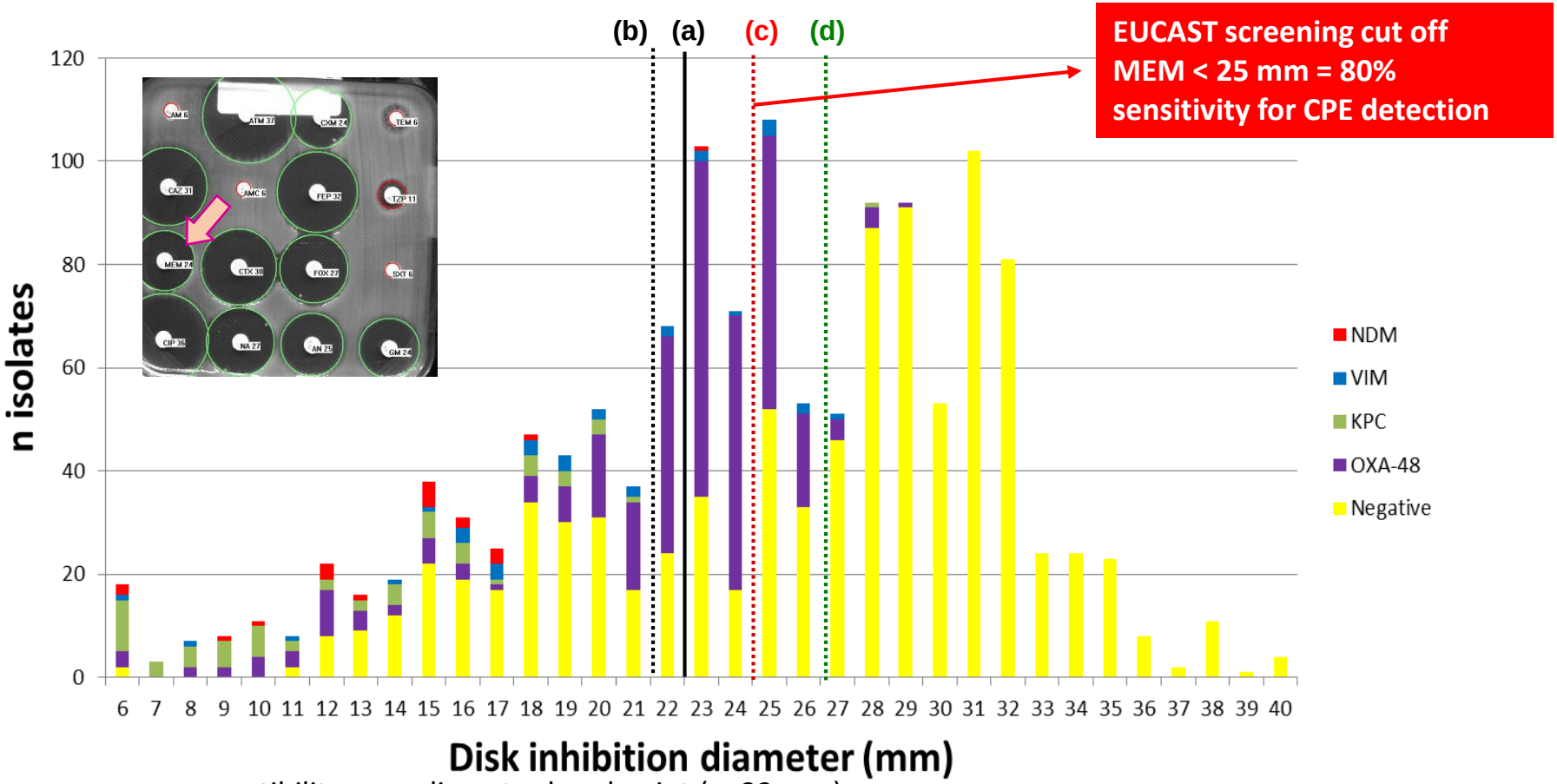
[4] High sensitivity, but low specificity, and therefore not routinely recommended.

ARE EUCAST GUIDELINES SUFFICIENT?

Meropenem zone size distribution for suspected CPE isolates referred at two NRLs in Belgium and in France



« OXA-48 »



(a) CLSI meropenem susceptibility zone diameter breakpoint (>=23 mm)

(b) EUCAST meropenem susceptibility zone diameter breakpoint (>=22 mm)

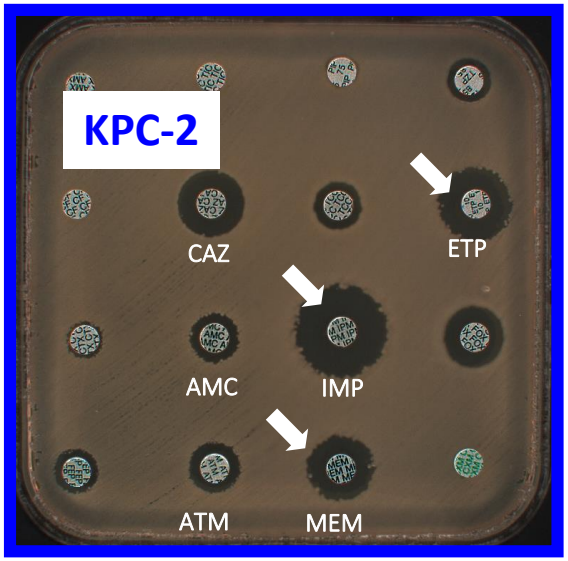
(c) EUCAST meropenem screening cut-off for the detection of CPE (<25 mm)

(d) 2EUCAST meropenem screening cut-off for the detection of CPE – epidemics (<27 mm)

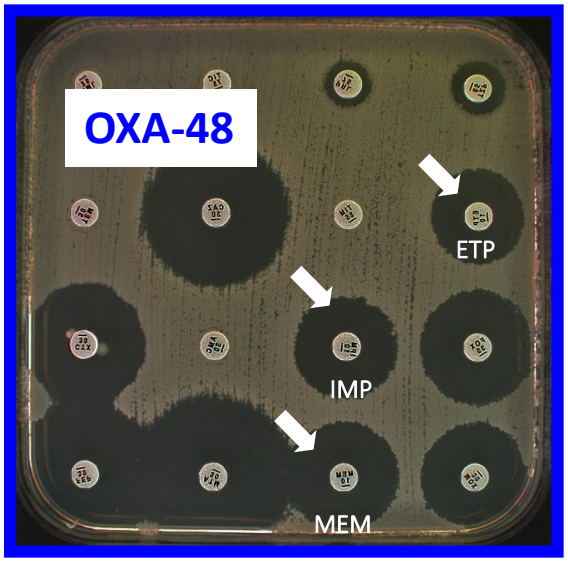
Huang TD et al., JAC 2013
(Courtesy Y Glupczynski)

TO BE OR NOT TO BE A CARBAPENEMASE PRODUCER, THAT IS THE QUESTION IN CLINICAL MICROBIOLOGY !!!

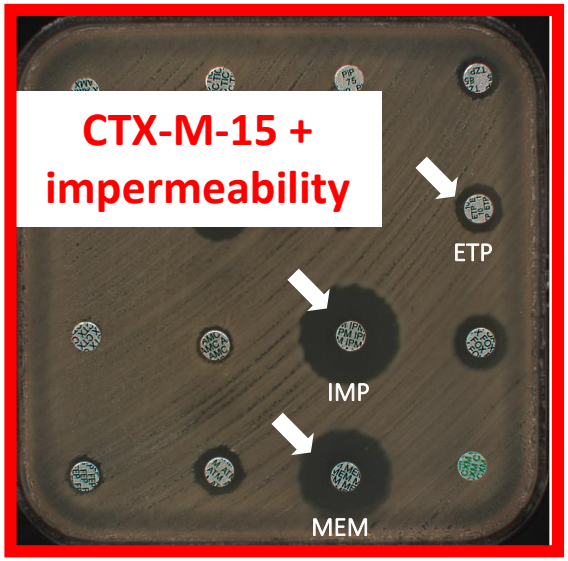
K. pneumoniae 1



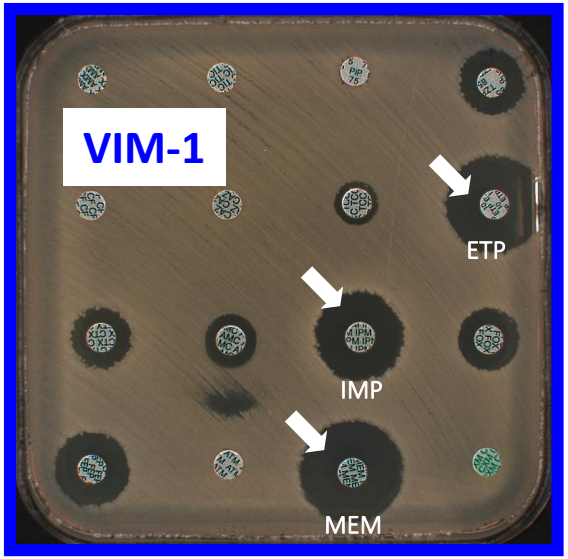
K. pneumoniae 2



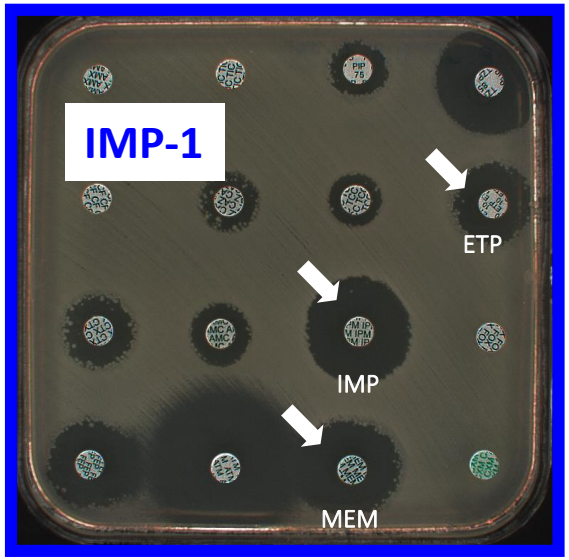
K. pneumoniae 3



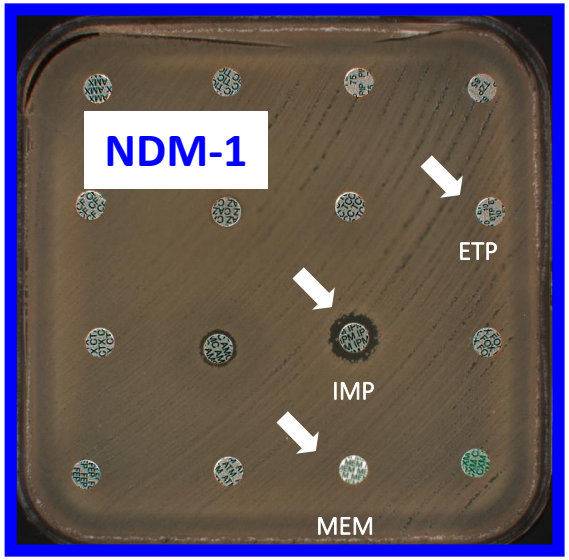
E. coli A

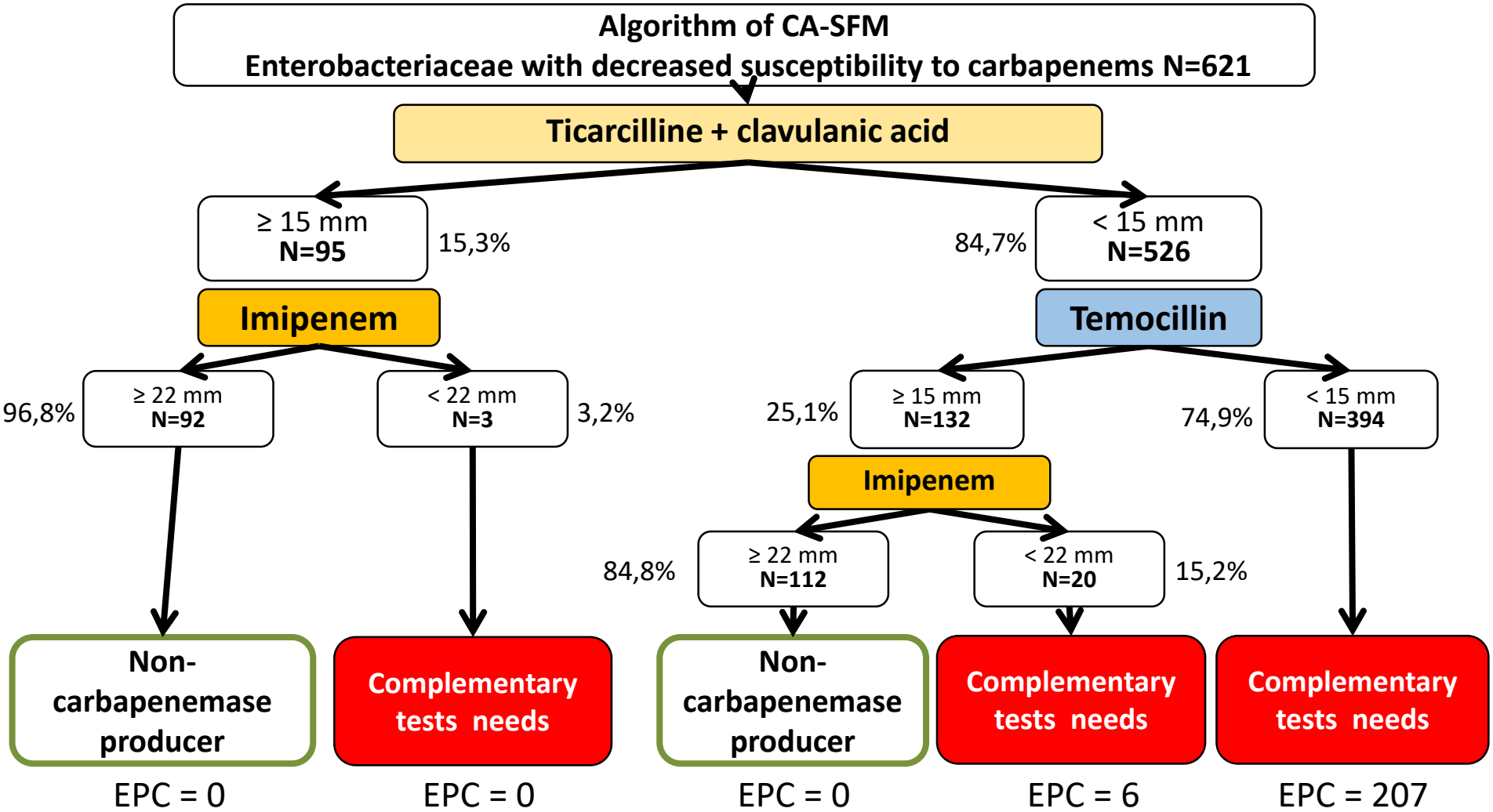


E. coli B



E. coli C





5 NDM-1
1 NDM-5

2 KPC-2, 1 KPC-3
10 NDM-1, 5 NDM-5
3 VIM-1, 1 VIM-4
166 OXA-48, 1 OXA-162
8 OXA-181, 9 OXA-204
1 OXA-232
1 NDM-1 + VIM-2
1 NDM-1 + OXA-48
1 NDM-1 + OXA-232

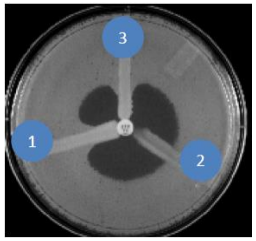
=> Eliminates 1/3 of putative CPEs (watch out for SME; IMI)

Prospective evaluation of an algorithm for the phenotypic screening of carbapenemase-producing Enterobacteriaceae

Dortet L, Cuzon G, Plésiat P, Naas T, JAC, 2016

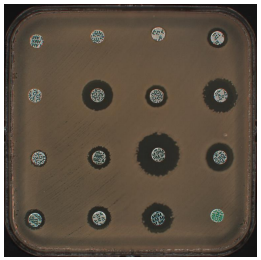
Methods of CPE detection : Phenotypic test

Hodge test



Girlich D. JCM 2012, **24h**

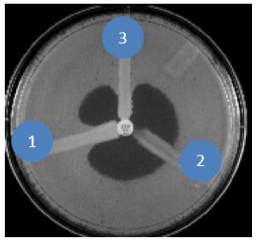
Phenotypic



Methods of CPE detection : Phenotypic test

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Hodge test

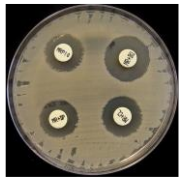


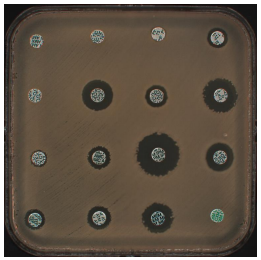
Girlich D. JCM 2012, **24h**

Phenotypic

Girlich D. DMID 2013
Bonnin RA. JCM 2012
24h

Inhibition tests



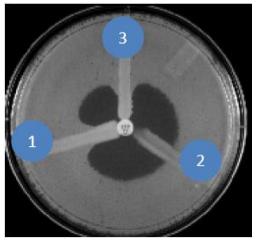


Methods of CPE detection : Phenotypic test

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Phenotypic

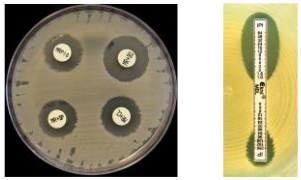
Hodge test



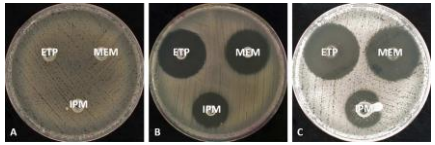
Girlich D. JCM 2012, **24h**

Inhibition tests

Girlich D. DMID 2013
Bonnin RA. JCM 2012
24h



CIM test



Gauthier, PlosOne, 2017
24h

Advantages

- Easy to perform
- Cheap

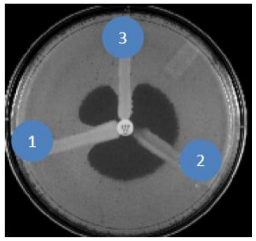
Limits

- Lack of sensitivity and specificity
- 24h delay after antimicrobial susceptibility testing

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Hodge test



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Phenotypic

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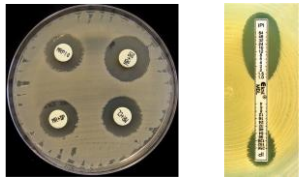
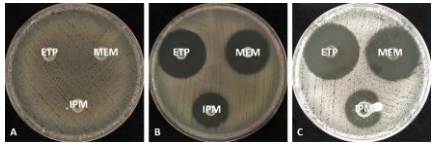
24h

Inhibition tests



CIM test



Gauthier, PlosOne, 2017
24h

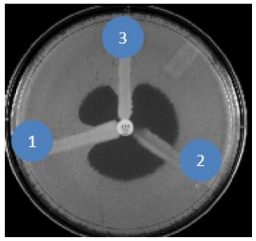
Carbapenem -hydrolysis

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Phenotypic

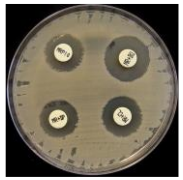
Hodge test



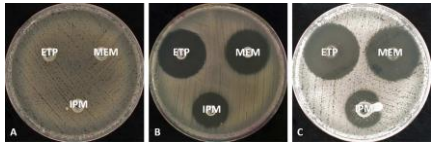
Girlich D. JCM 2012, **24h**

Inhibition tests

Girlich D. DMID 2013
Bonnin RA. JCM 2012
24h



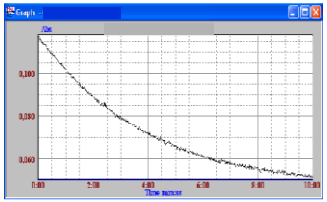
CIM test



Gauthier, PlosOne, 2017
24h

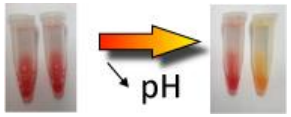
Carbapenem -hydrolysis

UV spectrophotometry



Bernabeu S. DMID 2012, **>2h**

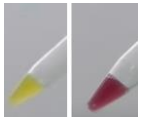
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

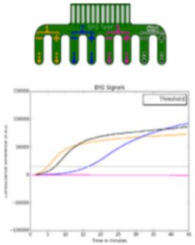
Dortet. JAC 2015, **2h**

β-Carba



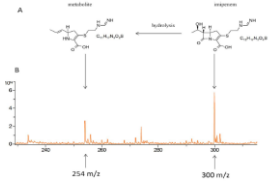
Bernabeu. JAC 2017 **<30'**

PH-metry



Bogaerts. JCM 2015, **<1h**

MALDI-TOF



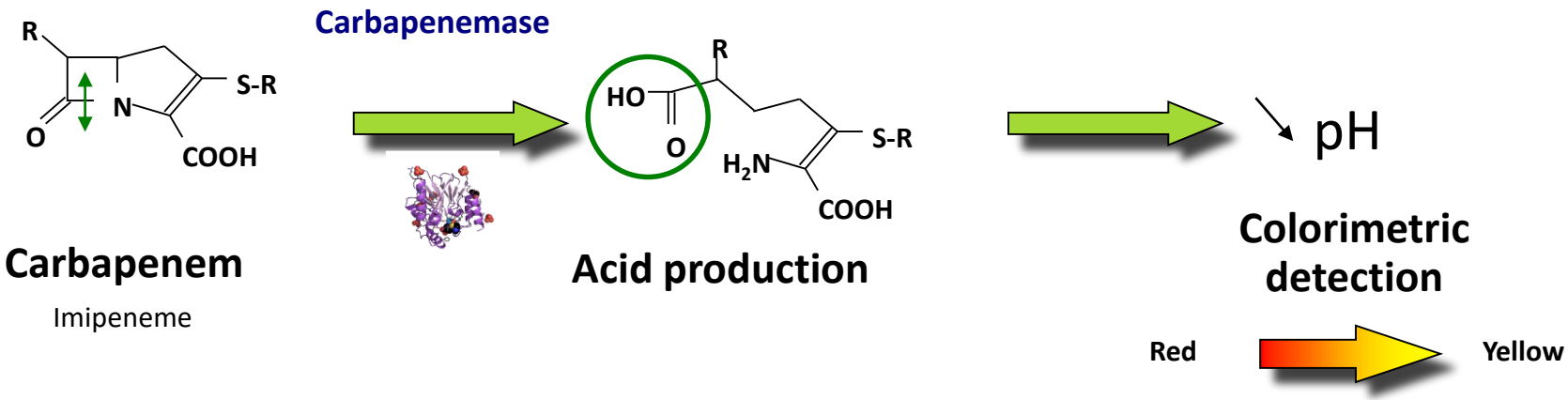
Tandé D. JCM 2015, **30'**

rCIM

Muntean M. JAC, in press, **3h**

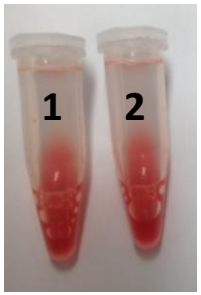
Carba NP test

Principle: *In vitro* hydrolysis of a carbapenem (imipenem)

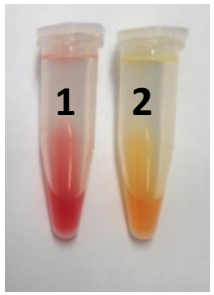


Interpretation:

No carbapenemase



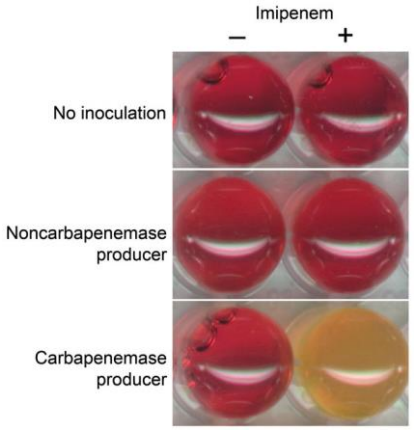
Carbapenemase production



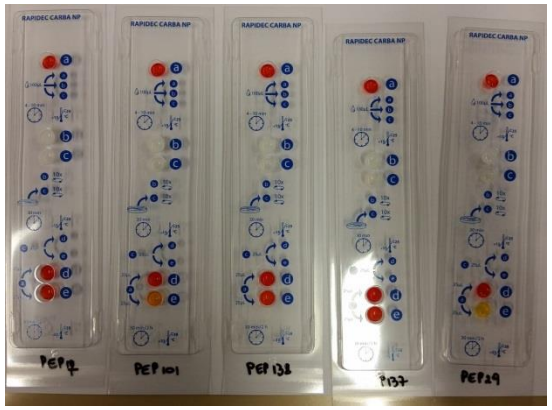
1 : Reveal solution
(internal negative control)

2 : Reveal solution + imipenem

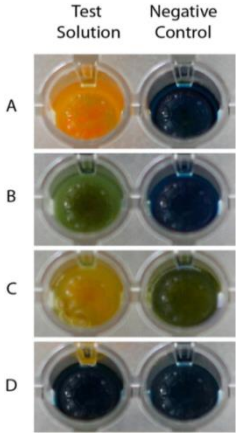
Carba NP test derivatives



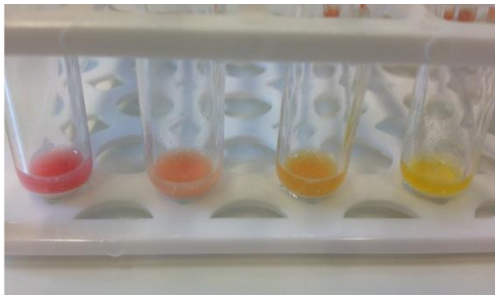
Carba NP
Nordmann et al., EID 2012



RAPIDEC® CARBA NP
bioMérieux



Blue Carba
Pires et al., JCM 2013
Pasteran, JCM 2015



Negative

Positive

Rapid CARB Screen, ROSCO Diagnostica

β-Carba test (Biorad)

Principle:

In vitro hydrolysis of a chromogenic **broad spectrum cephalosporin** which remain stable towards ESBLs and cephalosporinases

Interpretation:

No carbapenemase

Carbapenemase production

β-Carba test (Biorad)

- Detection problems with 10% of OXA-48 (low hydrolysis)
- No detection of Non KPC Ambler class A carbapenemase

- OXA-48 variant with no carbapenemase activity falsely detected as CPE

J Antimicrob Chemother
doi:10.1093/jac/dkx061

Journal of Antimicrobial Chemotherapy

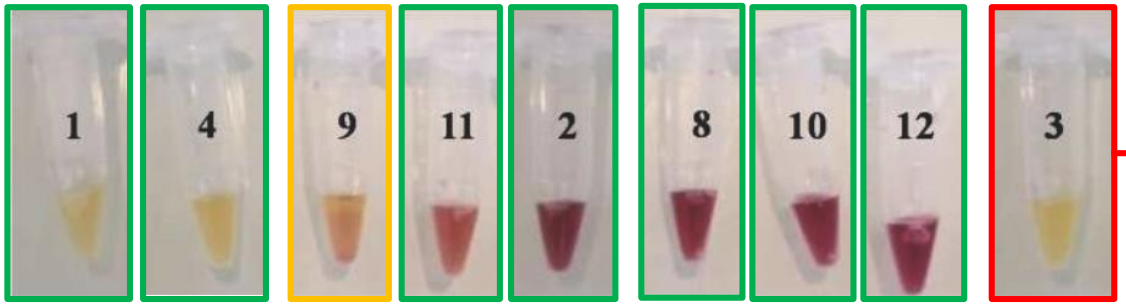
Evaluation of the β-CARBA™ test, a colorimetric test for the rapid detection of carbapenemase activity in Gram-negative bacilli

Sandrine Bernabeu[1–4], Laurent Dortet[1–4] and Thierry Naas[1–4]*

American Society for Microbiology | Journal of Clinical Microbiology®

Noncarbapenemase OXA-48 Variants (OXA-163 and OXA-405) Falsely Detected as Carbapenemases by the β Carba Test

Laurent Dortet, Thierry Naas



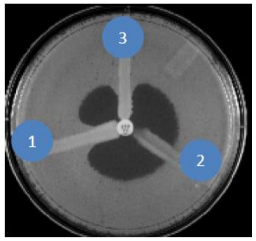
No hydrolysis of cephalosporins

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Phenotypic

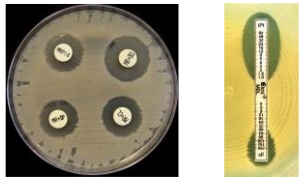
Hodge test



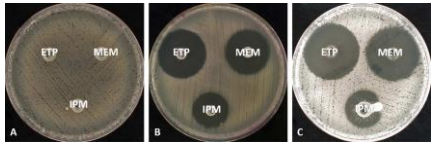
Girlich D. JCM 2012, **24h**

Inhibition tests

Girlich D. DMID 2013
Bonnin RA. JCM 2012
24h



CIM test



Gauthier, PlosOne, 2017
24h

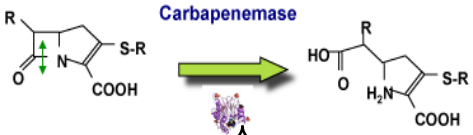
Immuno-chromatography

KPC
OXA-48,
NDM
(IMP
VIM)

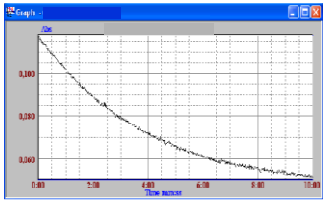
Bogaerts, JAC, 2016, in press 2017

Dortet, JAC,, 2016, **15'**

Carbapenem -hydrolysis

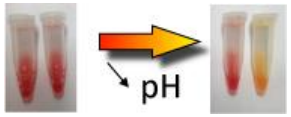


UV spectrophotometry



Bernabeu S. DMID 2012, **>2h**

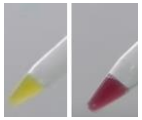
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

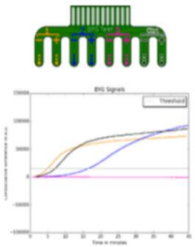
Dortet. JAC 2015, **2h**

β-Carba



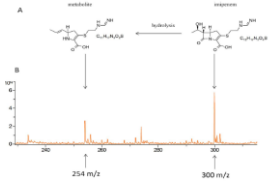
Bernabeu. JAC 2017 **<30'**

PH-metry



Bogaerts. JCM 2015, **<1h**

MALDI-TOF

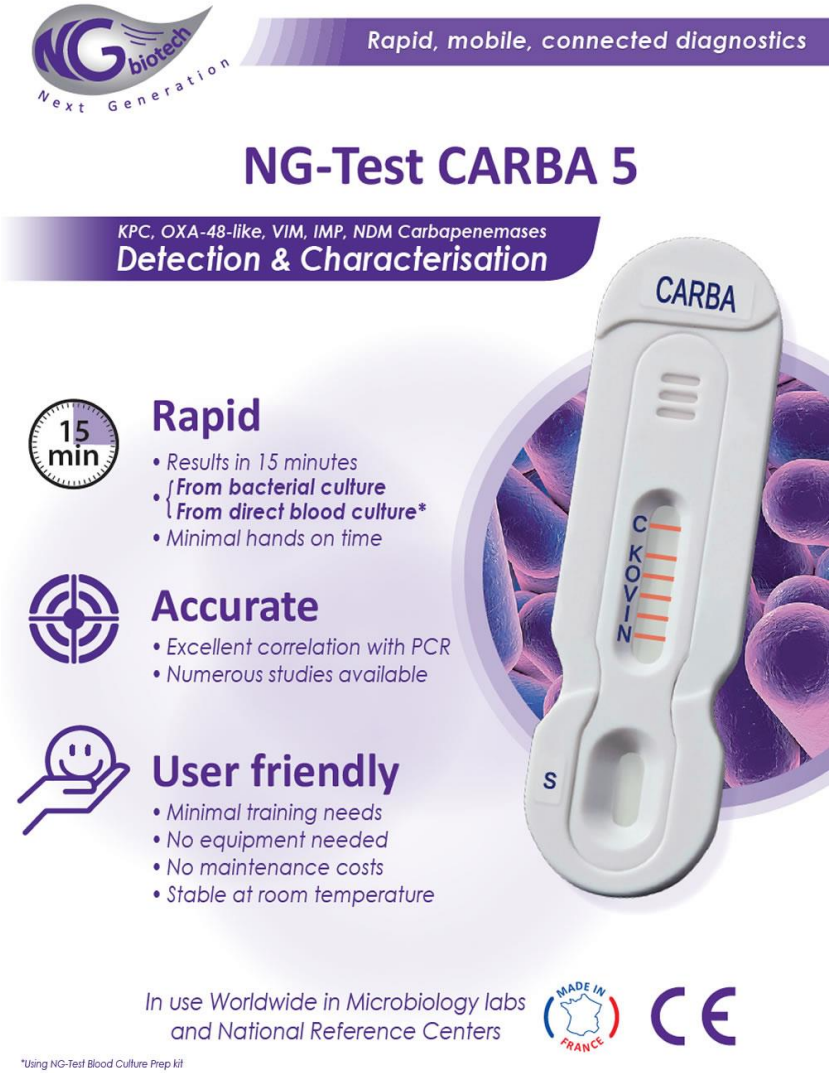


Tandé D. JCM 2015, **30'**

rCIM

Muntean M. JAC, in press, **3h**

Methods of CPE detection : Immunochromatography **confirmation tests**



Coris Resist-5 O.O.K.N.V.

OR **+**

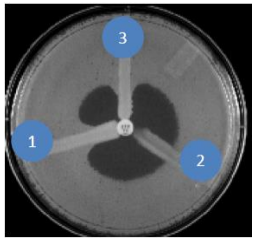
Coris IMP K-set

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

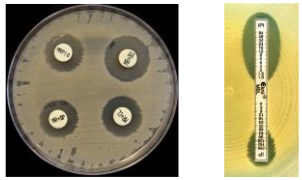
Phenotypic

Hodge test



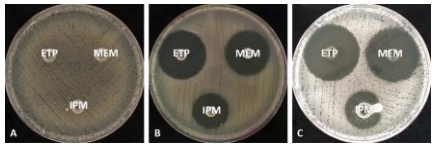
Girlich D. JCM 2012, **24h**

Inhibition tests



Girlich D. DMID 2013
Bonnin RA. JCM 2012
24h

CIM test



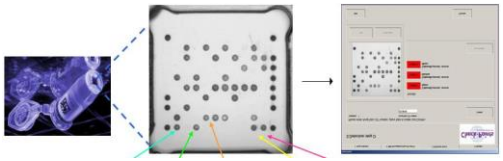
Gauthier, PlosOne, 2017
24h

Molecular

PCR et RT-PCR

Naas T. AAC 2011, 2016, **< 1h**

DNA micro-array



Naas T. JCM 2011, **7h**

Carbapenem -hydrolysis

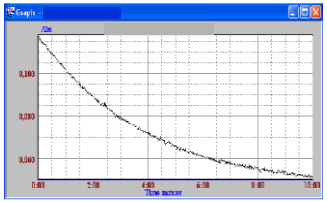
Immuno-chromatography

KPC
OXA-48,
NDM
(IMP
VIM)

Bogaerts, JAC, 2016, in press 2017

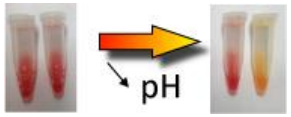
Dortet, JAC,, 2016, **15'**

UV spectrophotometry



Bernabeu S. DMID 2012, **>2h**

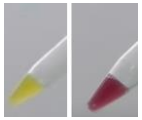
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

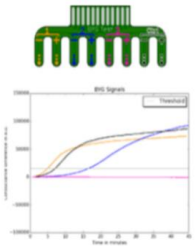
Dortet. JAC 2015, **2h**

β-Carba



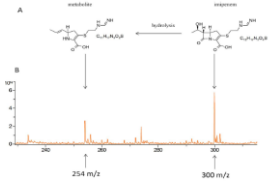
Bernabeu. JAC 2017 **<30'**

PH-metry



Bogaerts. JCM 2015, **<1h**

MALDI-TOF



Tandé D. JCM 2015, **30'**

rCIM

Muntean M. JAC, in press, **3h**

PLAN

1. Epidémiologie et dissémination des carbapénèmases

1.1. Les carbapénèmases de la classe A de Ambler

1.2. Les métallo-β-lactamases

1.3. les carbapénèmases de la classe D de Ambler

2. Méthodes de détection des entérobactéries productrices de carbapénèmases

2.1. A partir d'un prélèvement clinique (infection)

2.2. Dépistage des patients porteurs

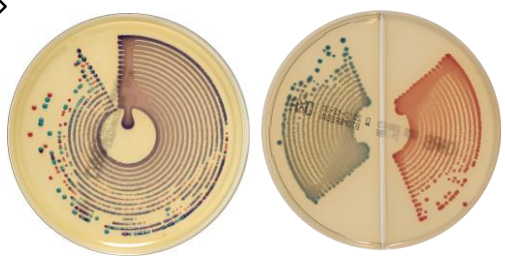
CPE colonisation detection

Stool, Rectal swabs

24-48h

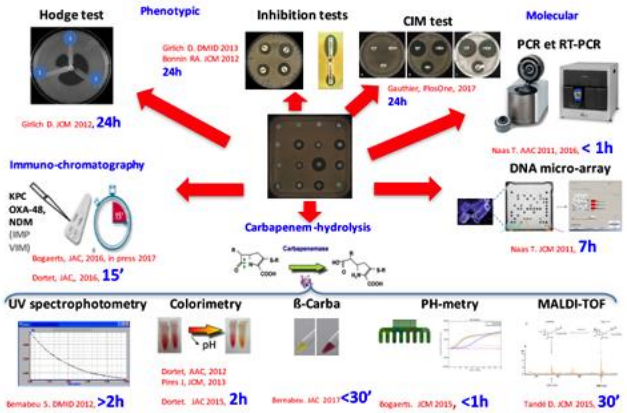
Enrichment culture

Selective Chromogenic media



1-2h

Single- or Multi-plex Real time PCR

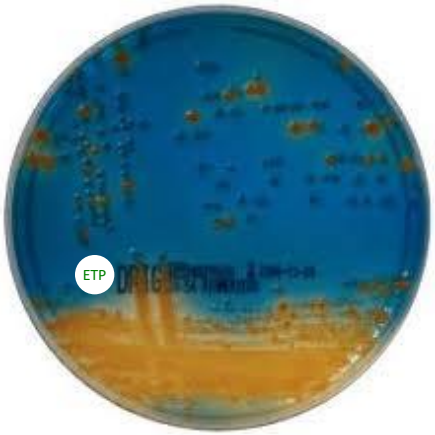


Girlich D. DMID. 2013
Réglier-Poupet H. JMM. 2008
Cuzon G. JCM. 2008

Naas T. AAC. 2013
Naas T. AAC. 2011
Oxacelay C. JAC. 2009
Hoyos, IJAA, 2017

Screening Media

Drigalski + ertapenem disc

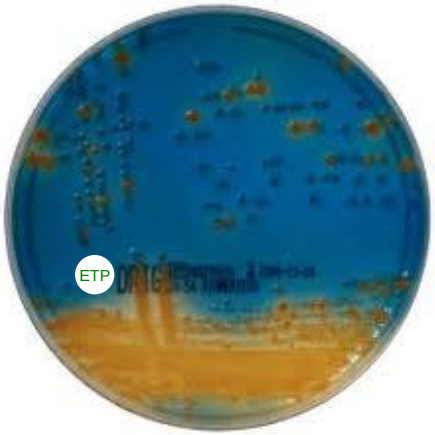


Avantages : Simple, Faible coût

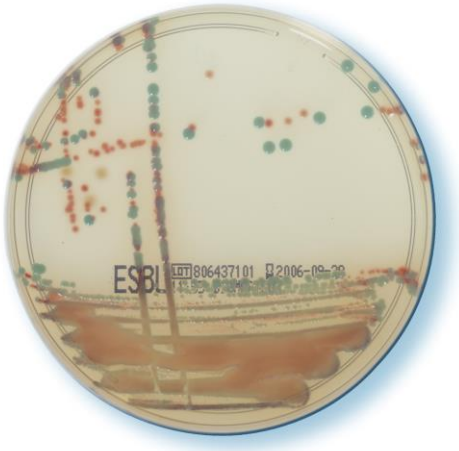
Inconvénients : Milieu non recommandé pour Carba NP test
Faible inoculum

Screening Media

Drigalski + ertapenem disc



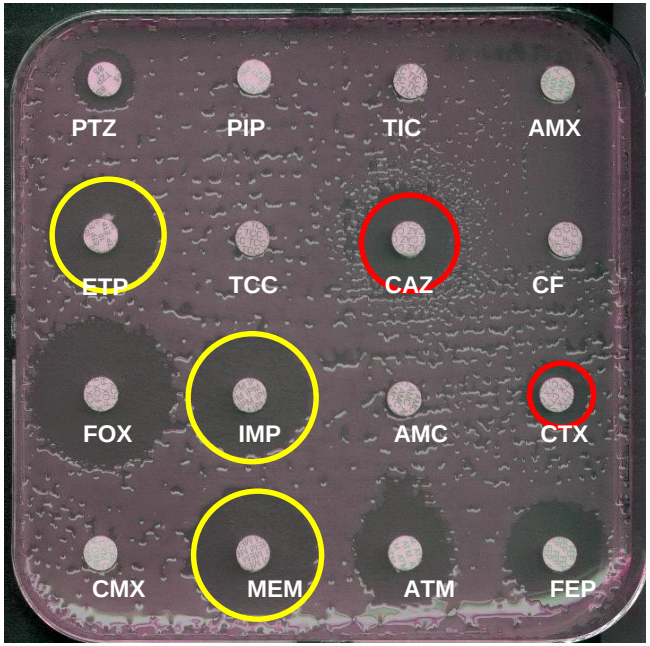
3CG supplemented media



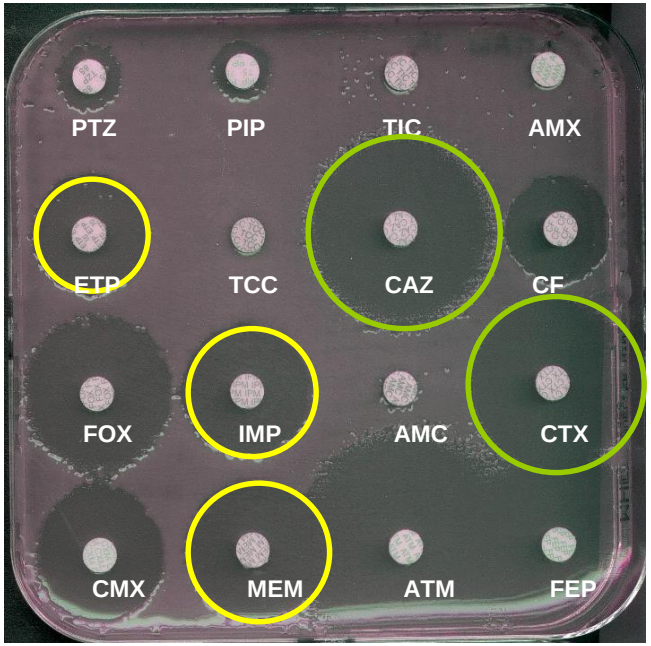
Ex : ChomID ESBL

3CG supplemented media

No detection of OXA-48 producer if no ESBL



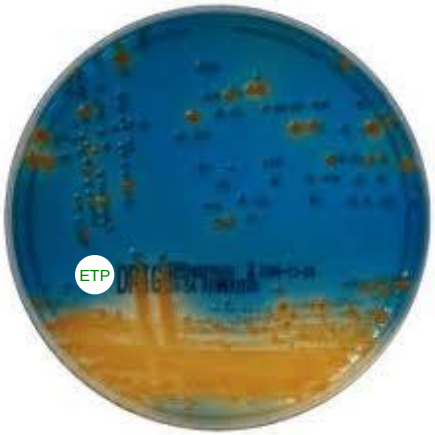
K. pneumoniae OXA-48
BLSE +



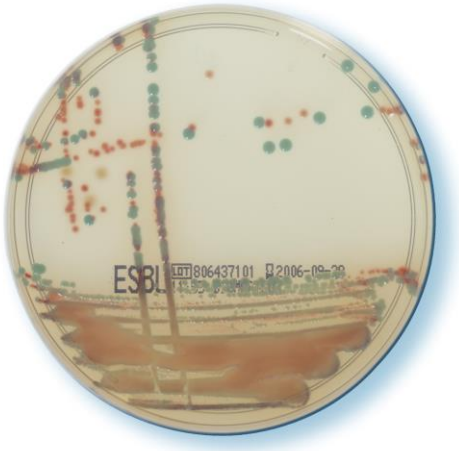
K. pneumoniae OXA-48
BLSE -

Screening Media

Drigalski + ertapenem disc



3CG supplemented media



Carbapenem supplemented media

CHROMAGAR KPC
ChromID Carba
Brillance CRE
SUPERCARBA

Carbapenem supplemented media

- CHROMAGAR KPC (Chromagar) : Méropénème + chromogenes NO
- ChromID Carba (Biomérieux) : Carbapenem (?) + chromogèenes OK except OXA-48
- Brillance CRE agar (Oxoid) : Carbapenem (?) + chromogènes OK except OXA-48
- SUPERCARBA medium (made in Bicêtre) : Etapenem + cloxacillne + Zinc OK for all

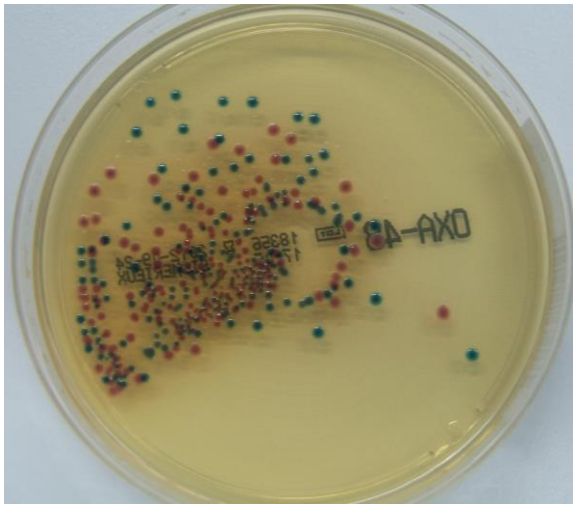
	SUPERCARBA	Brillance CRE	CHROMagar KPC
Sensibilité (%)	**96.5**	76.3	43
Spécificité (%)	60.7	57.1	67.8
Sensibilité classe A	**100**	85	70
Sensibilité classe B	**92**	78.4	58.8
Sensibilité classe D	**100**	69.8	11.6

Girlich, Poirel and Nordmann. Diag Microb Infect Dis. 2012

OXA-48 screening medium

Containing temocillin

+ chromogenes



chromID by bioMérieux OXA-48

Avantages :

Easy to use, commercialized standardized medium

Problems :

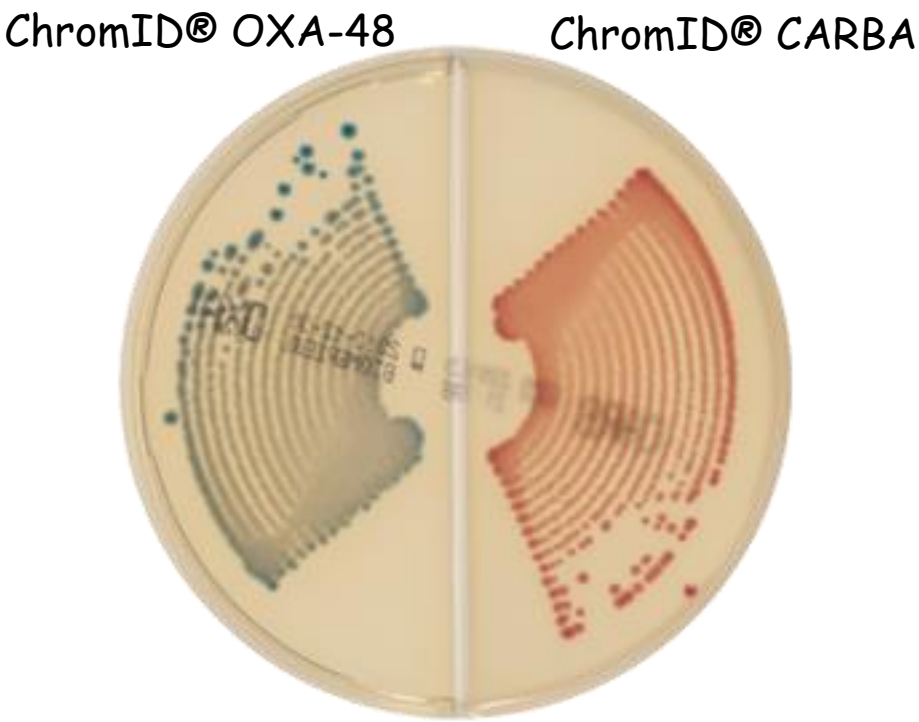
No detection of other cabapenemases

Global CRE screening medium

Avantages :

Easy , commercialized standardized medium

Detect all carbapenemases



ChromID® CARBA SMART

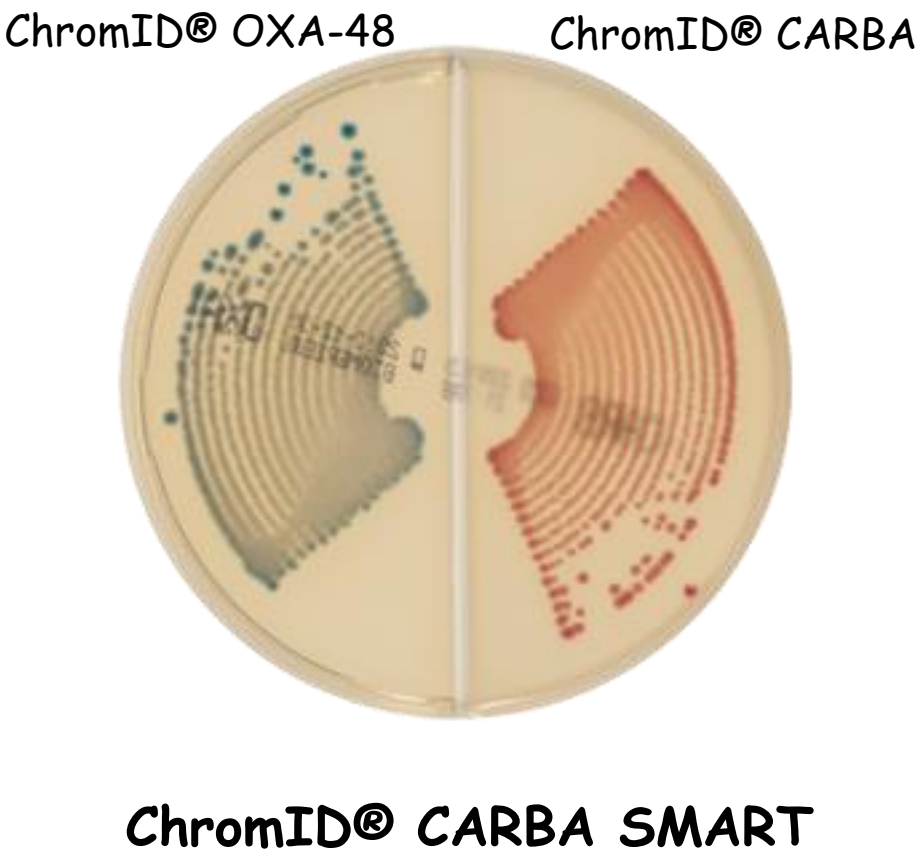
Global CRE screening medium

Avantages :

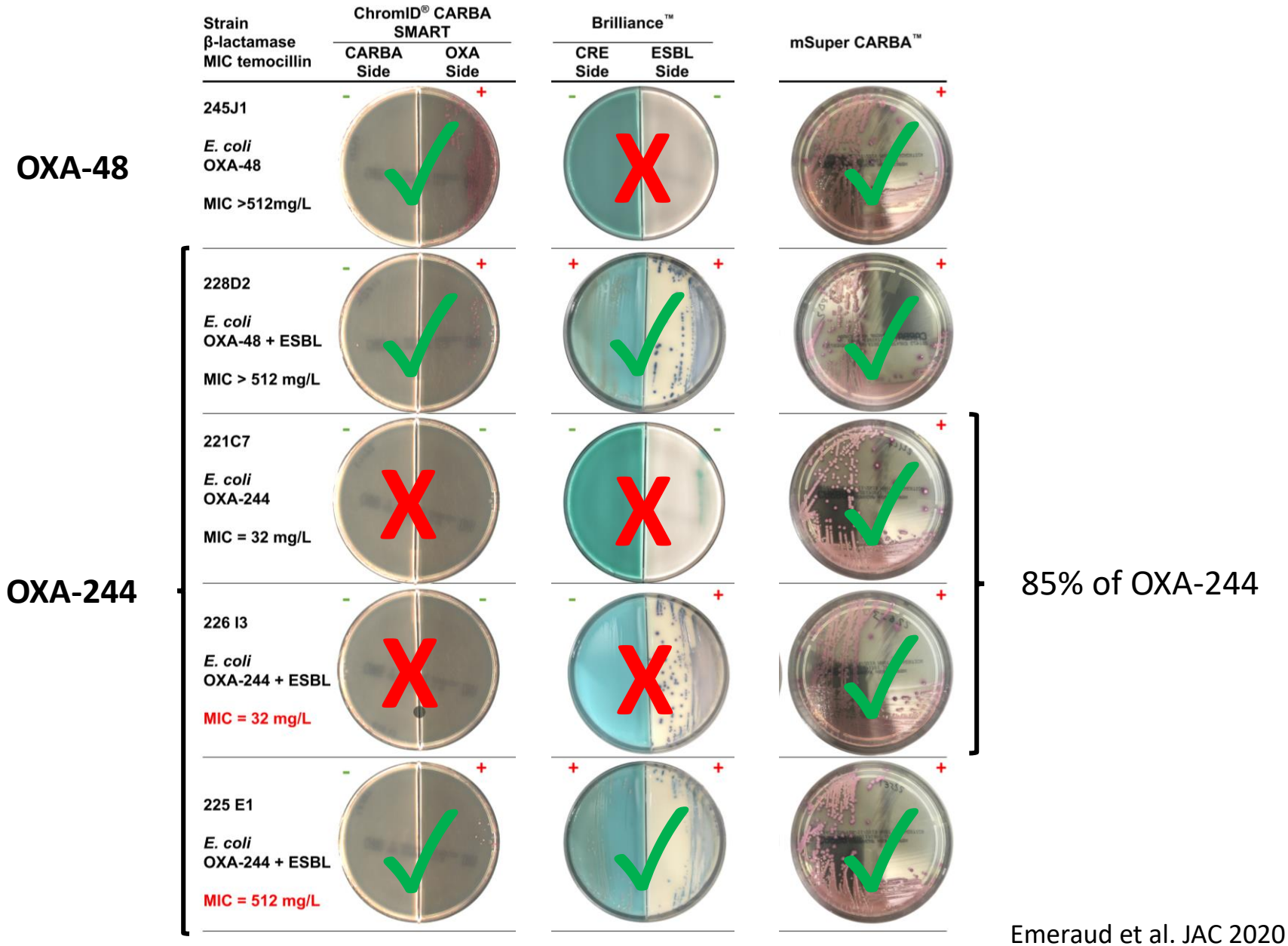
Easy , commercialized standardized medium

Detect all carbapenemases

Except …



Screening of OXA-244-producers, a difficult to detect and emerging OXA-48 variant?



Emeraud et al. JAC 2020

XPERT® CARBA-R

Détection simultanée de **bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP-1} and $bla_{OXA-48\ like}$ incluant les carbapénèmase OXA-181, OXA-232.**

RAPIDE

Résultats en: 48 min

PRÉCIS:

Sensibilité 96 %

Spécificité 98 %

FACILE

Temps de manip. < 1 minute

« PCR pour les nuls »

Xpert Carba-R package insert

XPERT® CARBA-R V2 KIT FOR THE DETECTION OF CARBAPENEMASE-PRODUCING ENTEROBACTERIACEAE

CNR RÉSISTANCE AUX ANTIBIOTIQUES

- **150 enterobacterial isolates** including 130 isolates with decreased susceptibility to at least one carbapenem)
- **61 non-carbapenemase** producers
- **89 carbapenemases** producers :

Performances	Xpert® Carba-R v2	
	This study	Global French CPE epidemiology (2012-2014)*
Sensitivity	97.8 %	99.61 %
Specificity	94.1 %	99.98 %
False positive	1 OXA-405 2 OXA-163	1 OXA-405
False negative	2 IMP-8	7 IMI 1 FRI-1

* 2026 isolates

Molecular detection of CPE :
False positive and False negative results

Reference = Culture after enrichment in ertapenem supplemented BHI

False positive results of the molecular biology (culture -)

- Transitory fecal carriage of ***Shewanella*** = OXA-48-like progenitor (no expression of $bla_{\text{OXA-48-like}}$ gene)

  Jousset AB et al. AAC 2018
  Antonelli A et al. DMID 2015

- **VIM-producing *P. aeruginosa*** and **NDM-producing *Acinetobacter*** ≠ CPE

  Diene SM et al. CMI 2014

False negative results of the molecular biology (culture +)

- Low level carriage of CPE

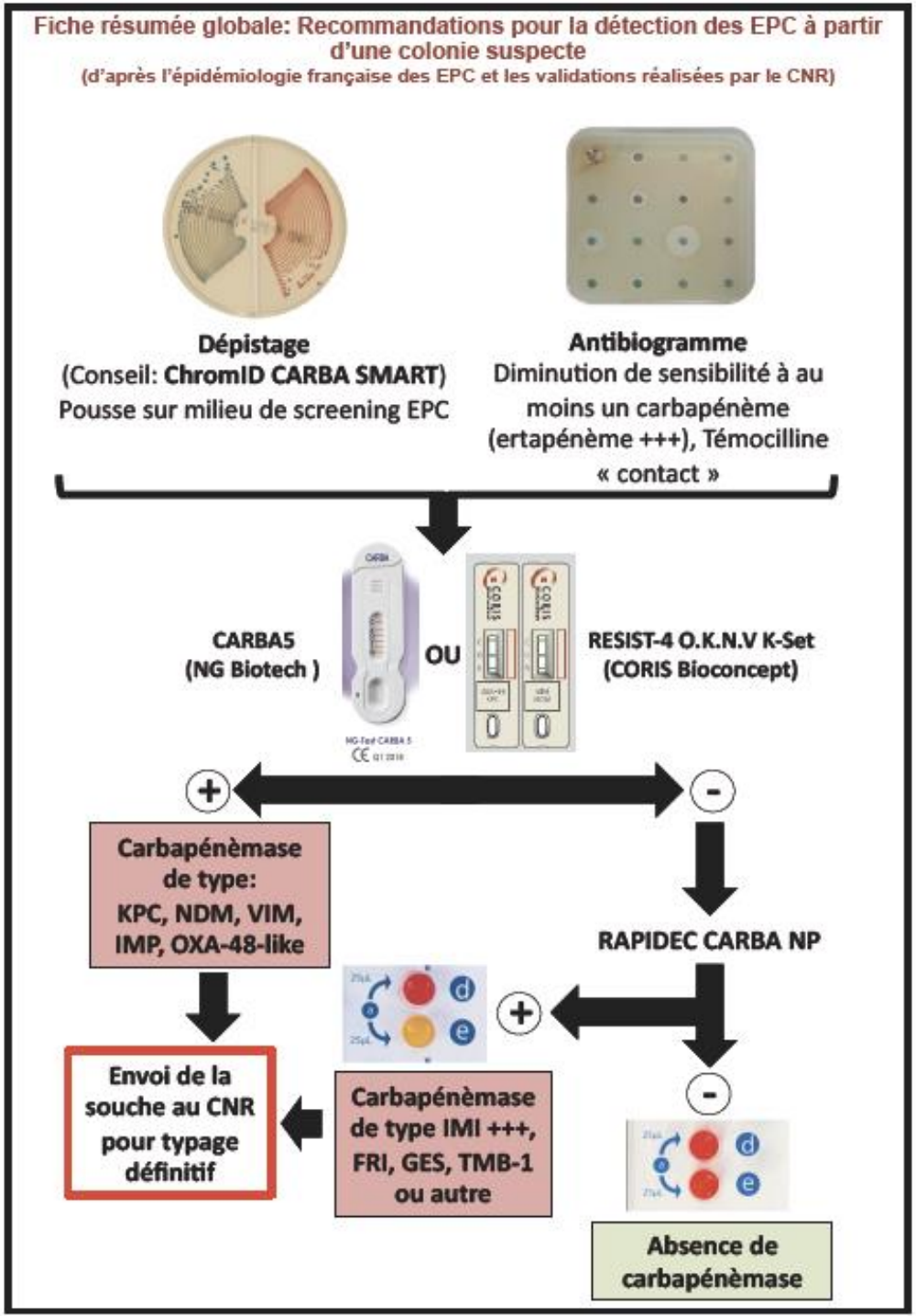
  Decousser JW et al. CMI 2015
  Girlich et al. DMID 2019

How to identify false positive and false negative results of molecular biology

- ❑ Perform **culture ± enrichment**
- ❑ Repeat molecular biology on another sample
- ❑ Think **OXA-244** which is not a false positive result of the molecular biology but a **false negative result of the culture** if using ChromID CARBA SMART

CNR
RÉSISTANCE AUX ANTIBIOTIQUES

Version 2018



http://www.cnr-resistance-antibiotiques.fr/expertise-des-souches-1.html

CNR RÉSISTANCE AUX ANTIBIOTIQUES

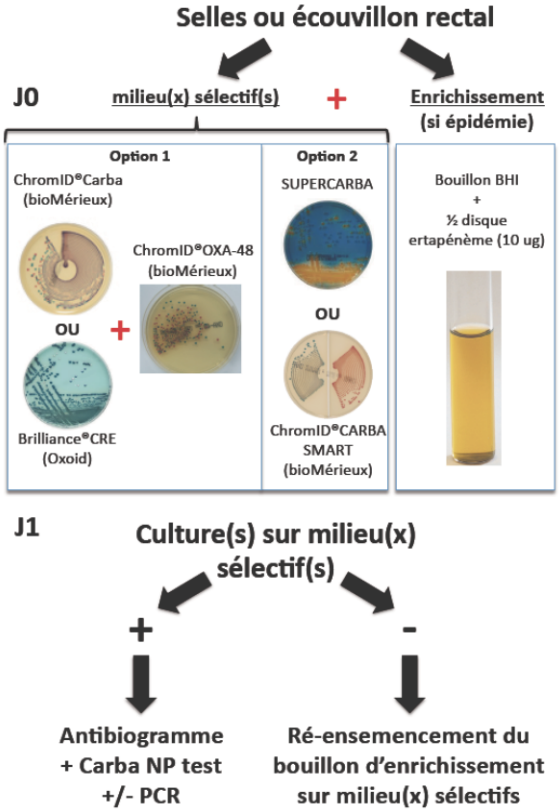
Fiche résumée : Recommandations pour le dépistage des patients porteurs d'une souche d'EPC (patients colonisés)

1) Patient ayant eu dans les 12 derniers mois une hospitalisation de plus de 24 h quel que soit le secteur ou de prise en charge dans une filière de soins spécifique (dialyse) à l'étranger.

2) Types de prélèvements : **selles** ou **écouvillonnages rectaux.** il est important de **vérifier visuellement la présence de matières fécales sur l'écouvillon.**

3) Il est conseillé de **répéter les prélèvements** en cas de forte suspicion de colonisation par une EPC (3 prélèvements à 3-4 jours d'intervalle). Ne pas hésiter à réaliser un nouveau dépistage après la mise sous antibiothérapie.

4) Méthodologie recommandée pour le dépistage des patients porteur d'une EPC :



5) **La détection moléculaire de EPC directement à partir du prélèvement** permet de gagner une journée sur la détection des EPC. Etant donné la non détection de certaines carbapénèmases par biologie moléculaire il est conseillé de **réserver ce type de technique au dépistage des patients contact lors d'épidémies**. Il conviendra alors de vérifier que le kit de biologie moléculaire est capable de détecter efficacement la souche épidémique avant utilisation directe sur les prélèvements cliniques.

En cas d'épidémie
Patients « contact »

http://www.cnr-resistance-antibiotiques.fr/expertise-des-souches-1.html

Acknowledgements

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- Garance Cotellon

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Inserm

Hôpital Bicêtre AP-HP