51e JBP Journées de Biologie Praticienne

51ème Journées de Biologie Praticiennes

LA RÉSISTANCE BACTÉRIENNE AUX CARBAPÉNÈMES, UN PROBLÈME DE SANTÉ PUBLIQUE MONDIAL

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CNR RÉSISTANCE AUX ANTIBIOTIQUES

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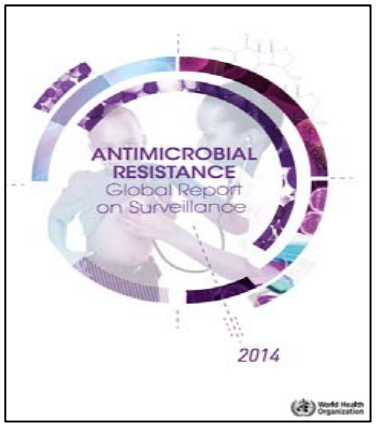
Le fardeau des bactéries multi-résistantes (BMR)

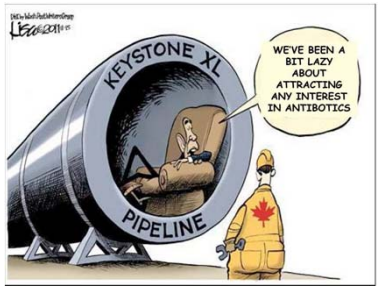
MDR
- 23,000 morts
- 2 million infections par an (USA).
=> 1,2 milliard $

- « Antibiotic Resistance Just Became Public Enemy Number One, Will Likely Kill More People Than Cancer By 2050 »
- MDR: 700,000 mort/ an globalement.
- En 2050 - 10 millions mort/ mort- cout de 100 000 billions $

En 2012
- 158 000 MDR infections
- 12 500 morts associées à ces infections. (étude Burden, InVS)



"Antimicrobial resistance: The problem is so serious that it threatens the achievements of modern medicine (WHO global report, 2014). « post-antibiotic era »



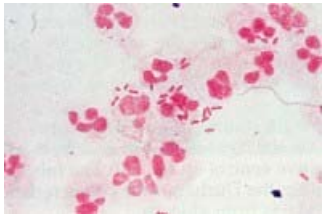
***Impact on PIB :* decrease of 1,1 % in an «optimistic» scenario (low impact on antimicrobial resistance) and 3,8 % in the worst scenario ; (even 5 % in low income countries)**

THE CLINICALLY-SIGNIFICANT BACTERIA (WHO)

- Echecs thérapeutiques et taux de mortalité élevés
- Maladies infectieuses restent la 2ème cause de mortalité dans le monde.

Enterobacteriaceae

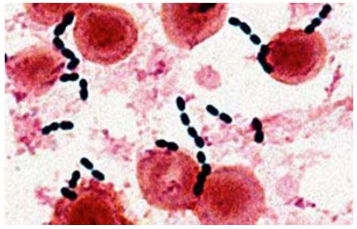
1) KPC, OXA-48, NDM
2) ESBL/AMPC + impermeability
3) VIM, IMP



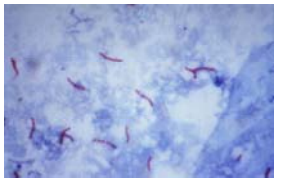
E. faecium

BHRe

1) VanA et VanB

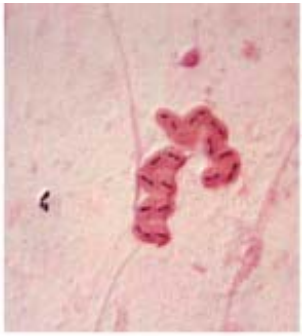


M. tuberculosis
MDR and XDR



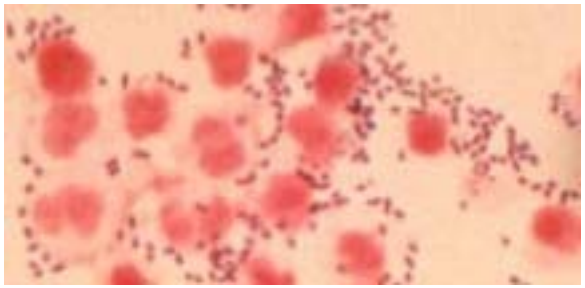
P. aeruginosa

1) VIM, IMP
2) KPC, NDM
3) ESBL,



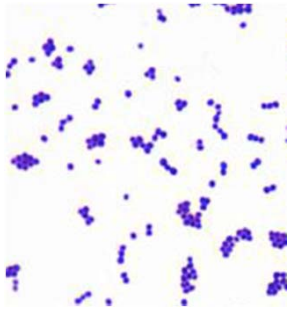
A. baumannii

1) Oxacillinases (OXA-23)
2) NDM,
3) GES, KPC

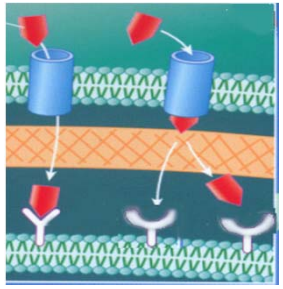


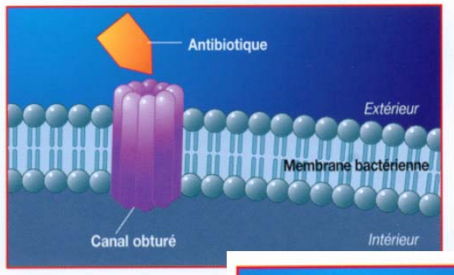
S. aureus

1) Methicilin R



Resistance to β-lactams in Gram negatives



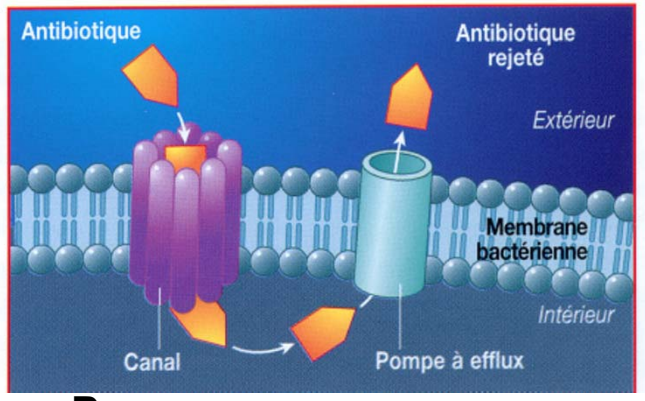


Decrease in permeability

Modification of PBPs

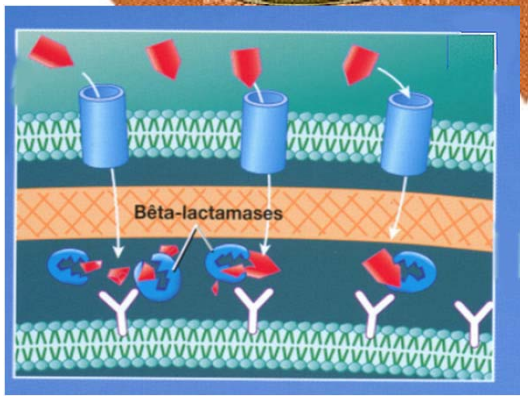
Overexpression Efflux pumps

Antibiotic

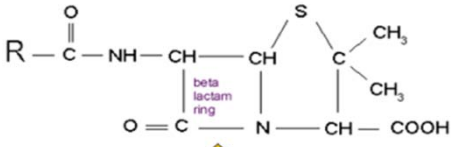


Pumps

Antibiotic



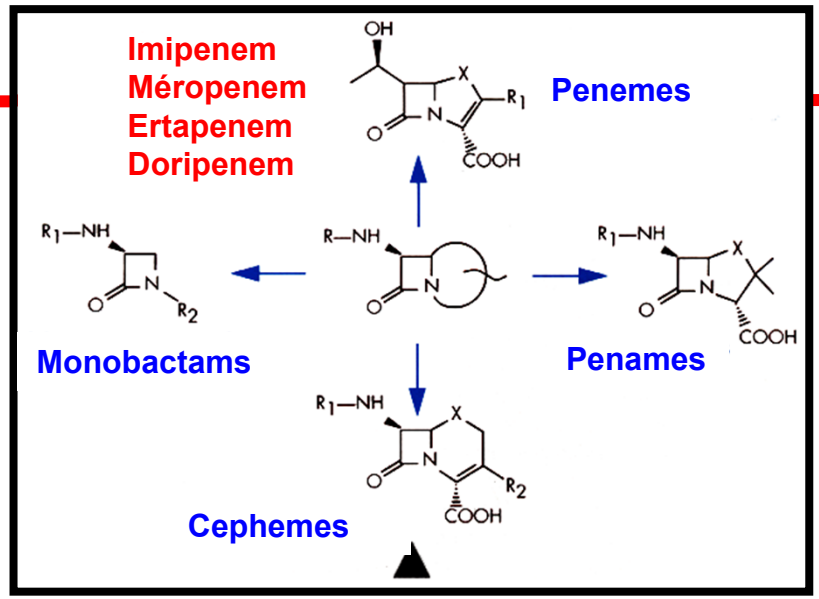
β-Lactamases



Site of penicillinase action (break in β lactam ring)

Resistance to β-lactams: β-lactamases

β-lactams

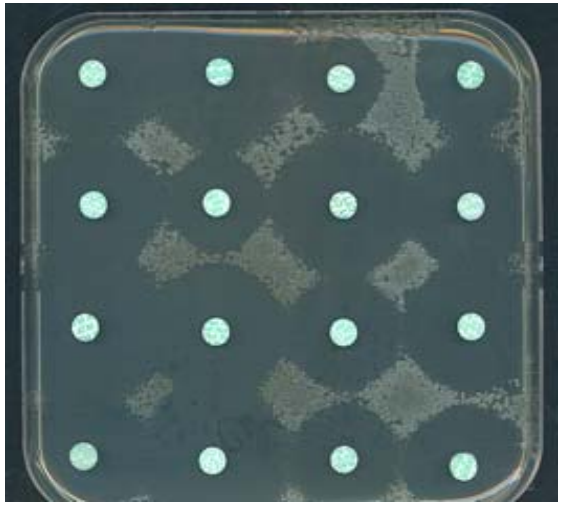


β-lactamases

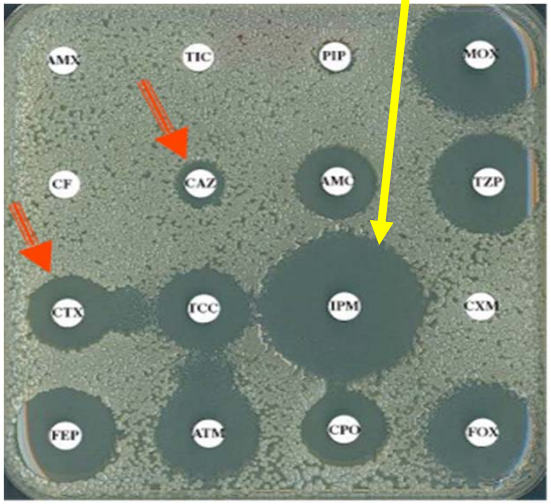
	Active site		G		KTG	Groupe	Inhibitors	
A	SXXK 70-73	SXN 130-132	156	Ωloop 164-179	234-236	Penicillinase	clavulanic acid	KPC
C	SXXK 64-67	YXN		Ωloop 208-213	315-317	Cephalosporinase	Cloxacillin	
D	SXXK 70-73	YGN 144-146		WxExxL 164-169	216-218	Oxacillinase	no inhibitor	OXA-48
B Zn++	61-65	Zn1 ligand His116, 118,196		Zn2 ligand Asp120, Cys221,His263		Metallo-enzyme	EDTA	NDM/VIM/IMP

Multi-resistance and therapeutic dead-ends
E. coli our best friend, and our worst ennemi

E. coli
Of our youth

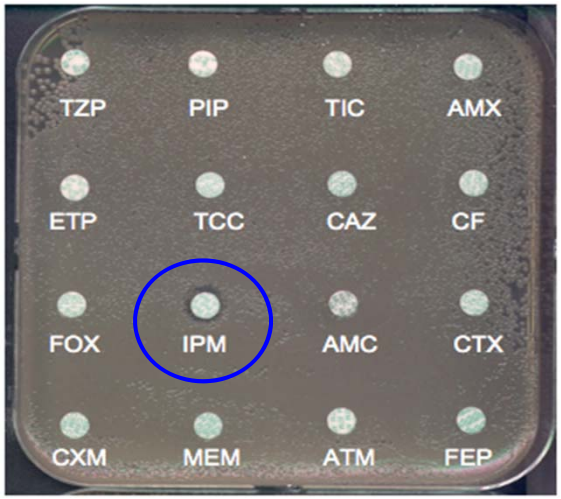


E. coli
of modern times



ESBLs

E. coli
of tomorrow

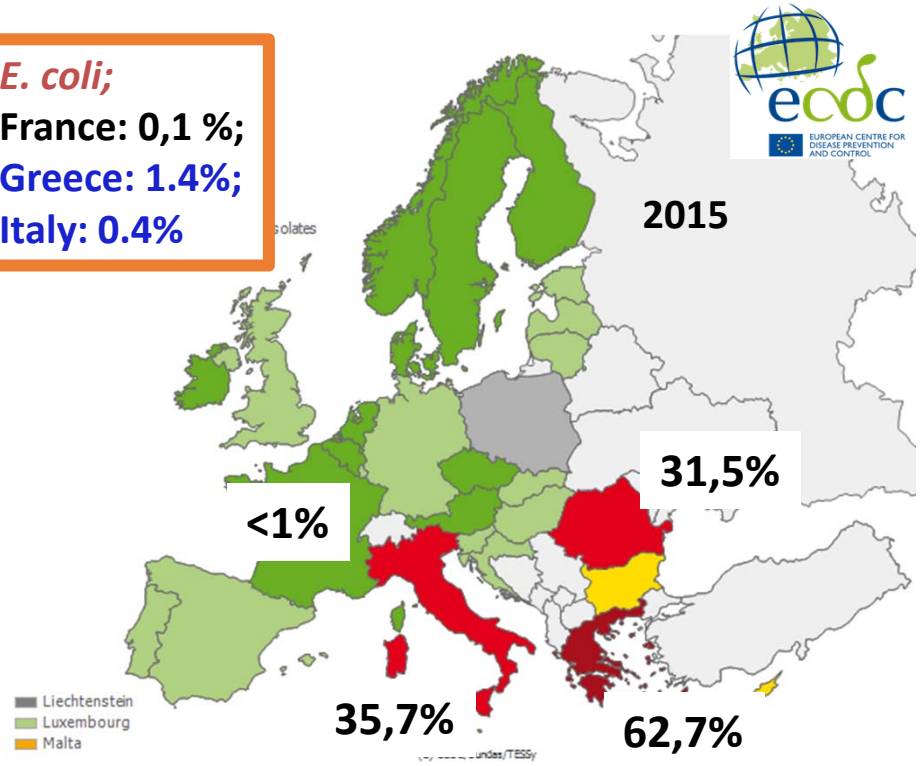


Carbapenemases

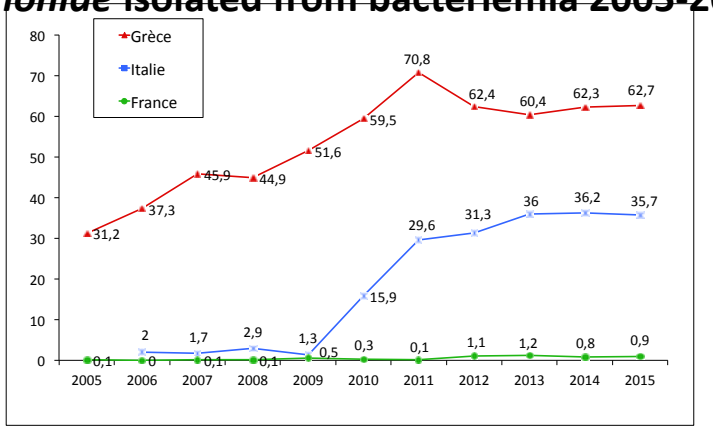
Carbapenem resistant Enterobacteriaceae (CRE)
Here we are !!!

Bacterimia with Enterobacteria resistant to carbapenems (CRE) in Europe 2015 (ECDC)

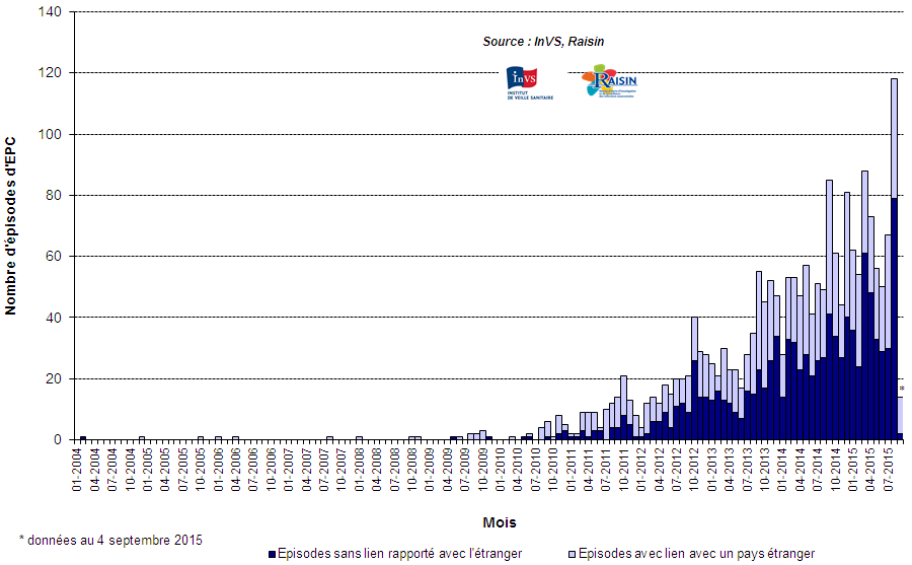
E. coli;
France: 0,1 %;
Greece: 1.4%;
Italy: 0.4%



Evolution of carbapenem-resistance in *K. pneumoniae* isolated from bacteriemia 2005-2015



Number of CRE Episodes, France, 2004 – 2015, per month of notification, 4 septembre 2015 (SPF; N= 2026 épisodes)



CRE remain susceptible to colistin, but frequent resistances described in Italy and Greece.

⇒ pan-resistance, therapeutic dead-end

⇒ High mortality rates (50-70%)

CRE : Carbapenem resistance in enterobacteriaceae

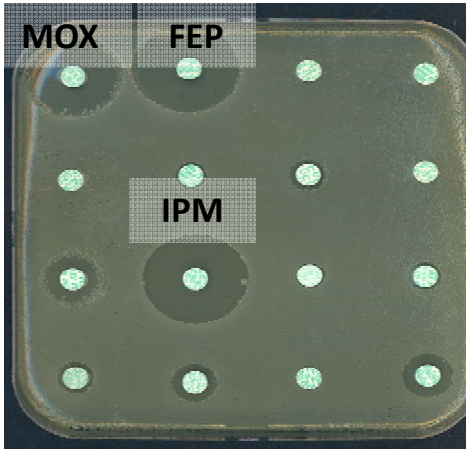
1) Decreased outer membrane permeability + β-lactamase with no (or very poor) hydrolytic activity against carbapenems

Resistance to Expanded spectrum cephalosporins BUT Carbapenem susceptible,

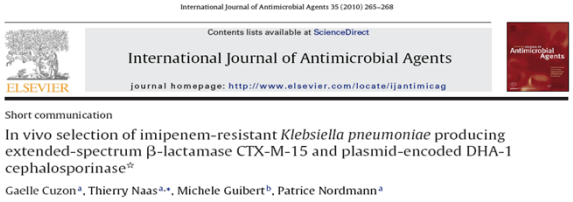
→

Lee EH, Nicolas MH, Kitzis MD, Pialoux G, Collatz E, Gutmann L. AAC 1991, 35:1093-8

Resistance to carbapenems by decreased permeability



after 21 days of imipenem mono therapy


International Journal of Antimicrobial Agents 35 (2010) 265–268

Contents lists available at ScienceDirect

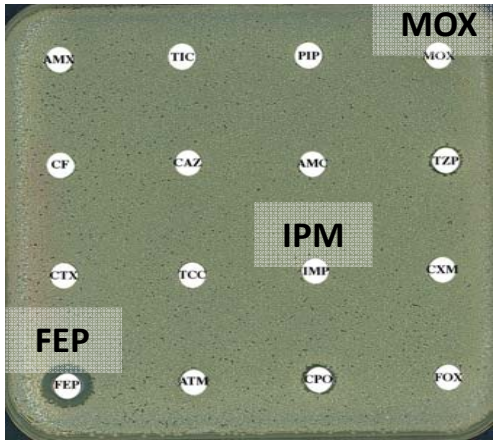
International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Short communication

In vivo selection of imipenem-resistant *Klebsiella pneumoniae* producing extended-spectrum β-lactamase CTX-M-15 and plasmid-encoded DHA-1 cephalosporinase☆

Gaelle Cuzon[a], Thierry Naas[a,*], Michele Guibert[b], Patrice Nordmann[a]

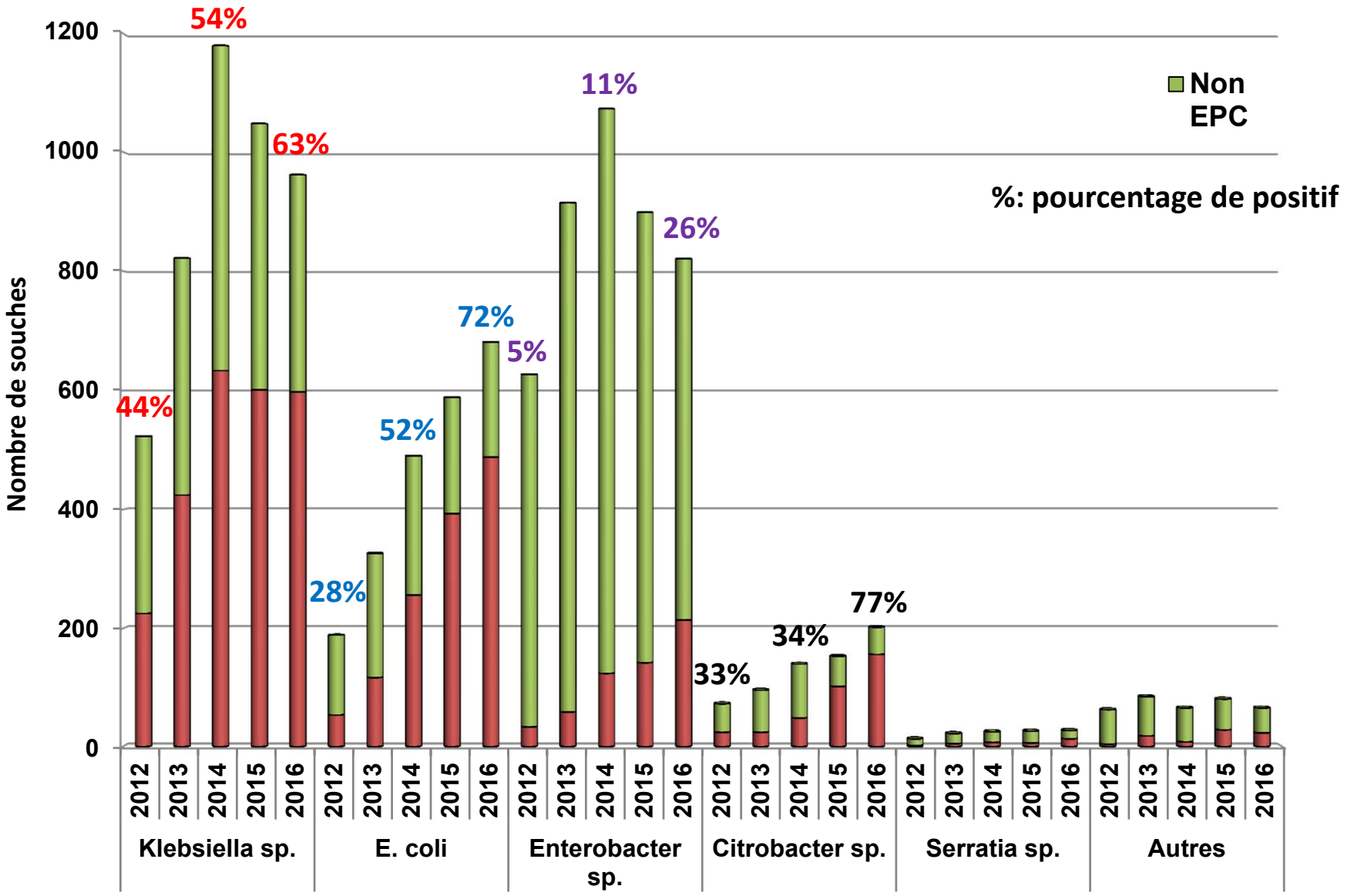


Important in terms of treatment issues, but no epidemic dissemination,

=> chromosomal mutations with important fitness cost

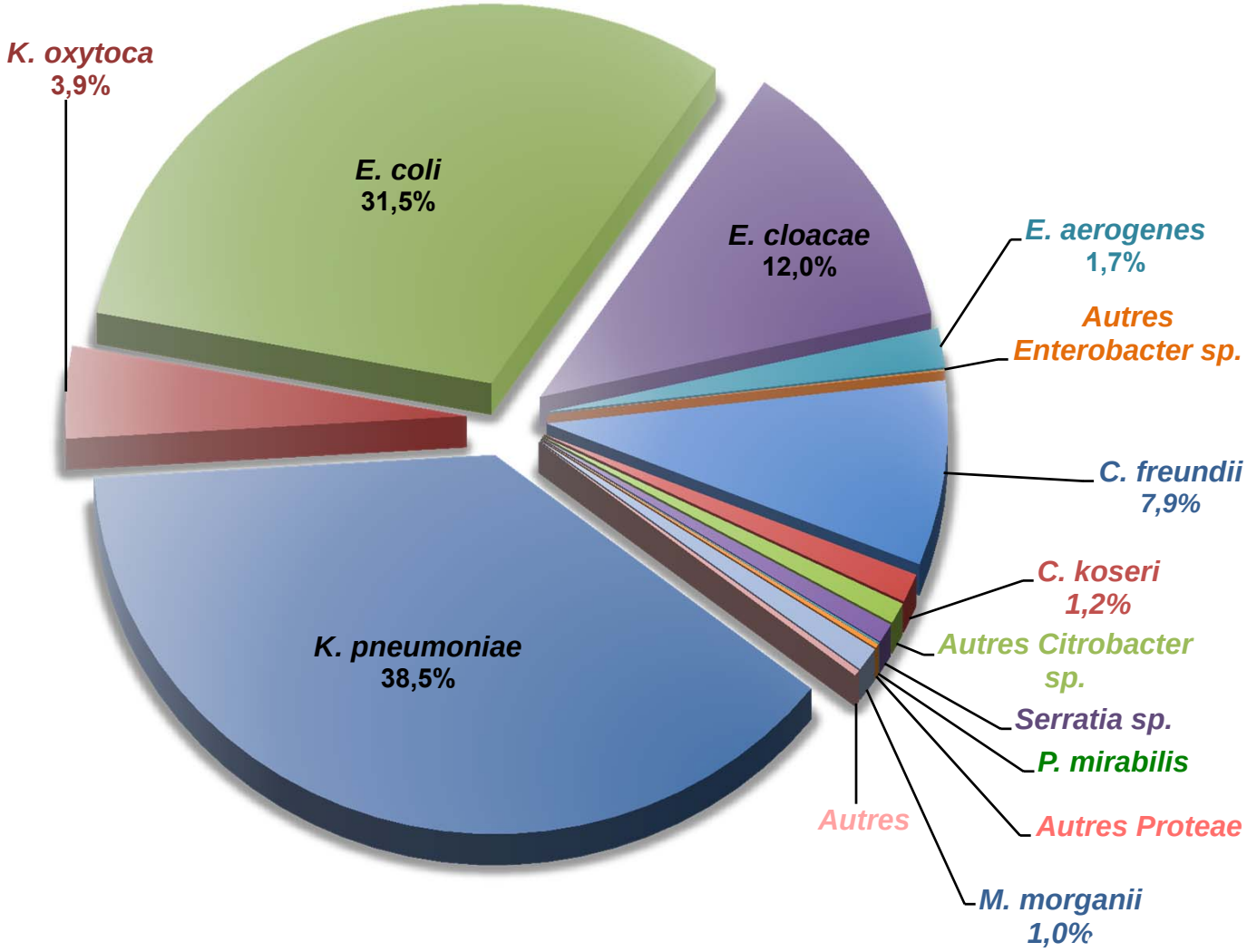
2) Carbapenemases (CPE)

Evolution of the number of CPEs received at the NRC between 2012 -2016 according to species



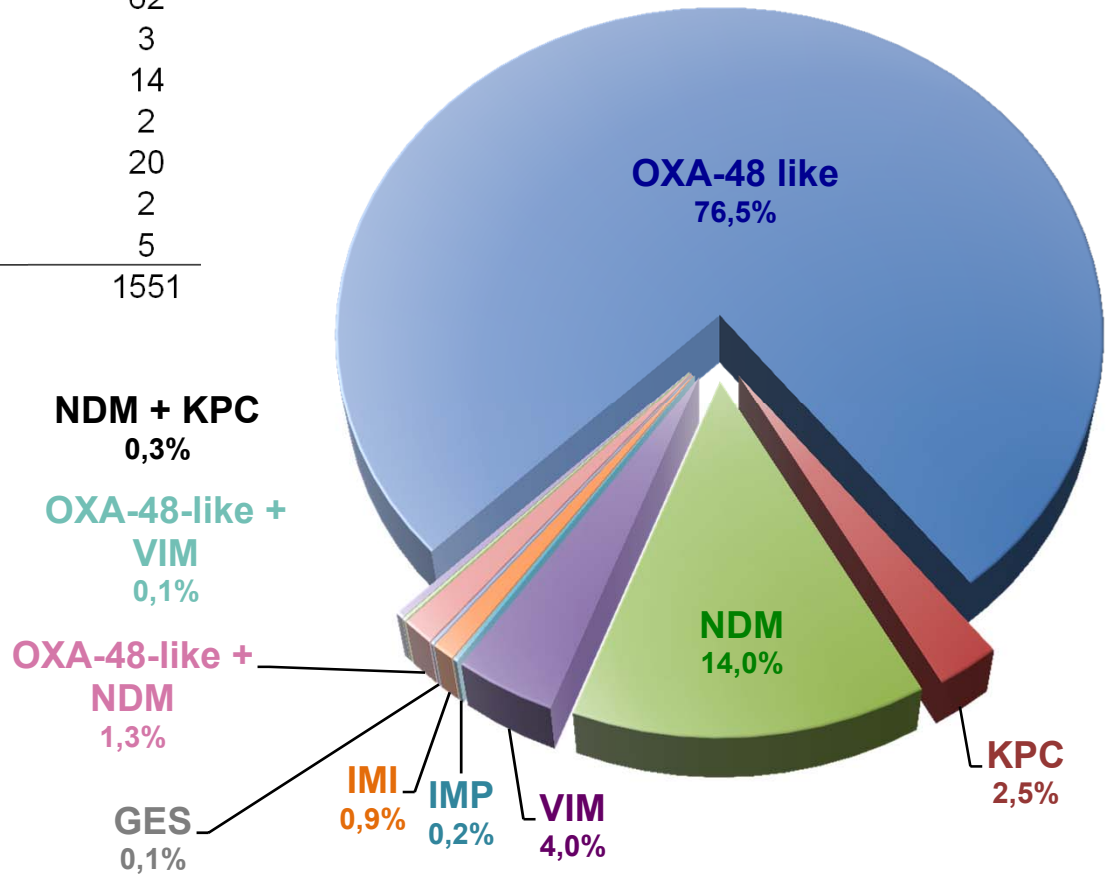
Distribution of CPEs per species in France (2016)

Espèce	n
K. pneumoniae	597
K. oxytoca	61
E. coli	488
E. cloacae	186
E. aerogenes	26
Autres *Enterobacter sp.*	1
C. freundii	122
C. koseri	19
Autres *Citrobacter sp.*	14
Serratia sp.	13
P. mirabilis	1
Autres *Proteae*	3
M. morganii	16
Autres	4
Total	1551



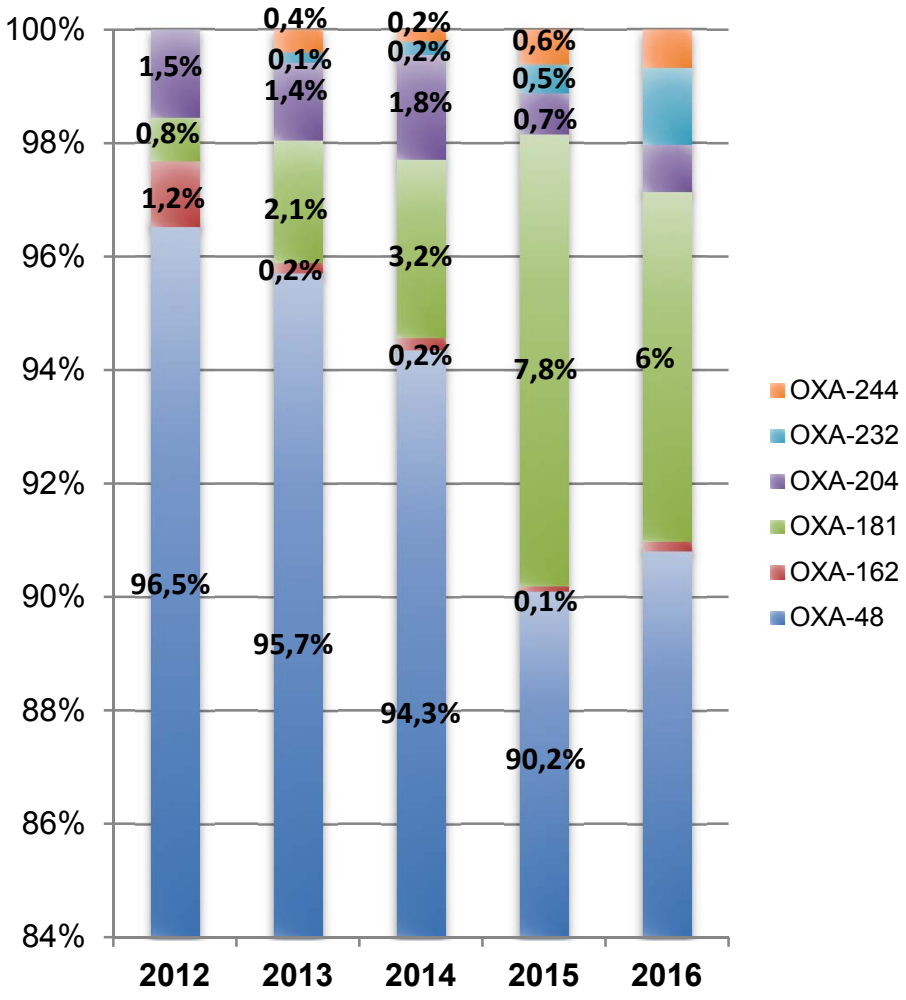
Distribution des CPEs per carbapenemase in France (2016)

Type of carbapenemase	n
OXA-48 like	1187
KPC	39
NDM	217
VIM	62
IMP	3
IMI	14
GES	2
OXA-48-like + NDM	20
OXA-48-like + VIM	2
NDM + KPC	5
Total	1551

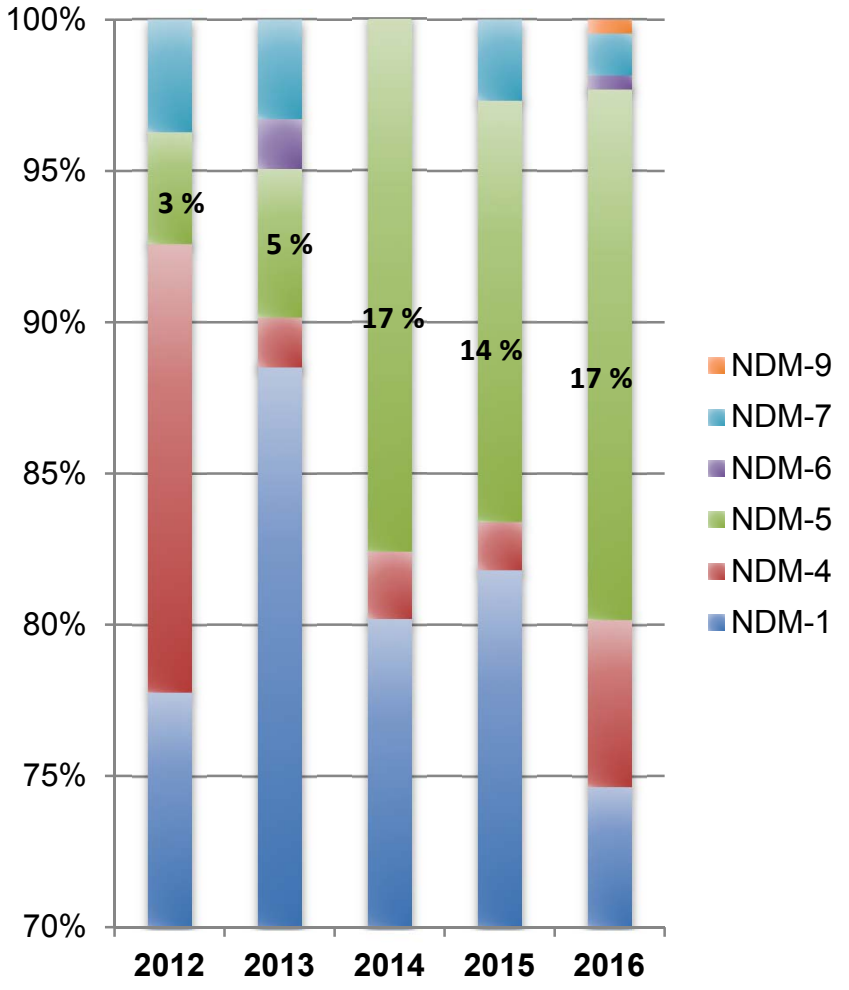


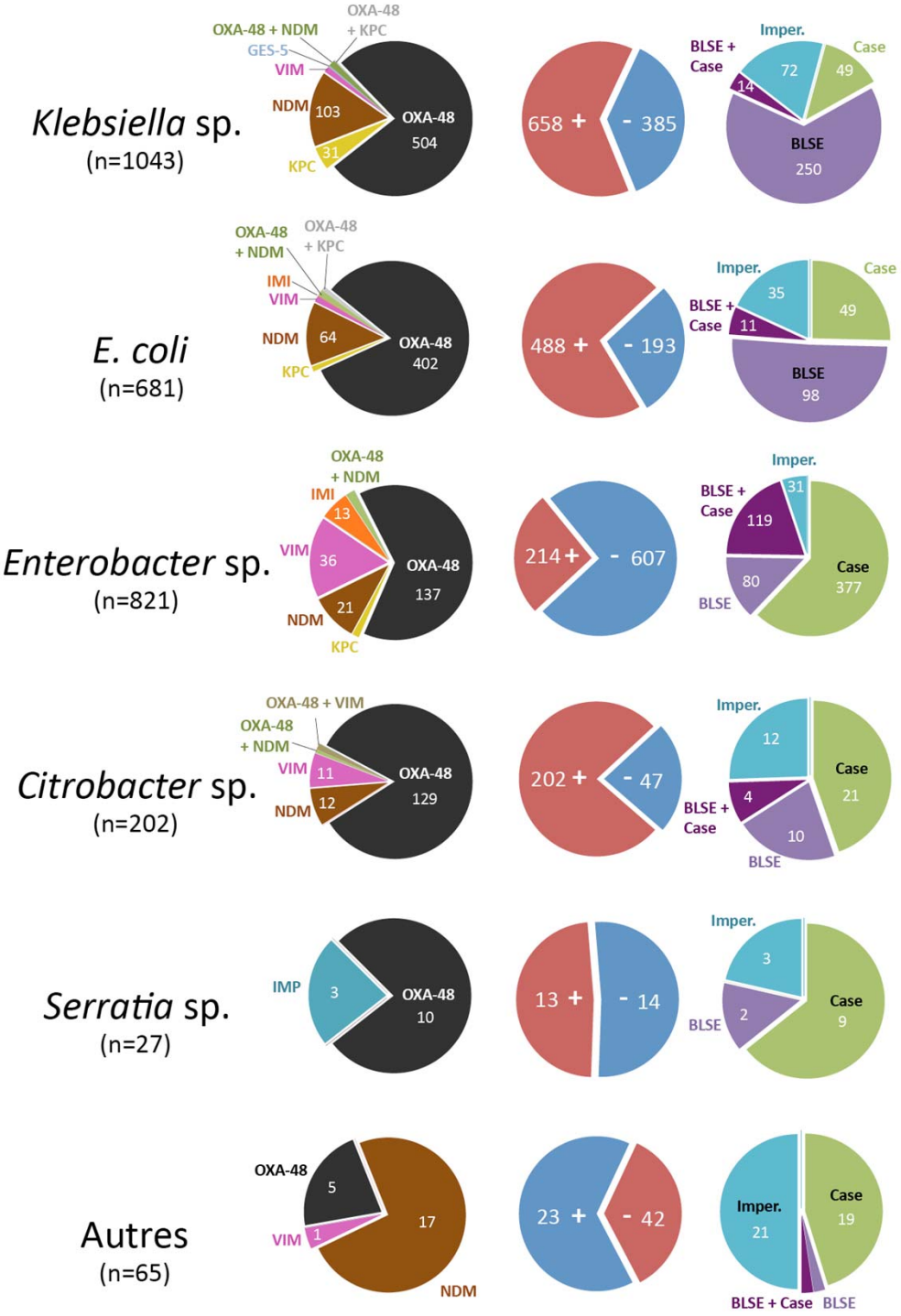
MBLs= 19,9 %

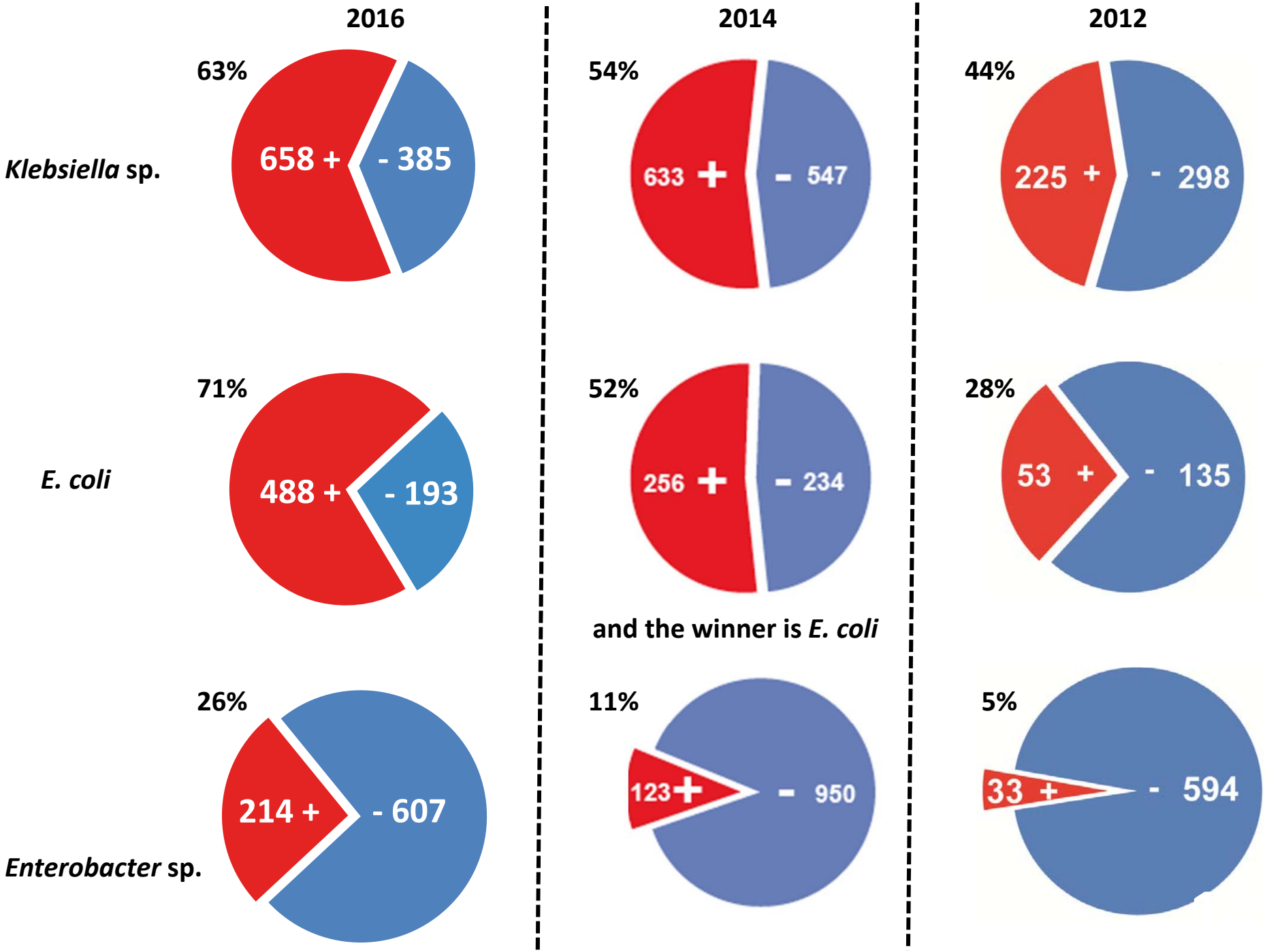
Variants OXA-48 : progression of OXA-181

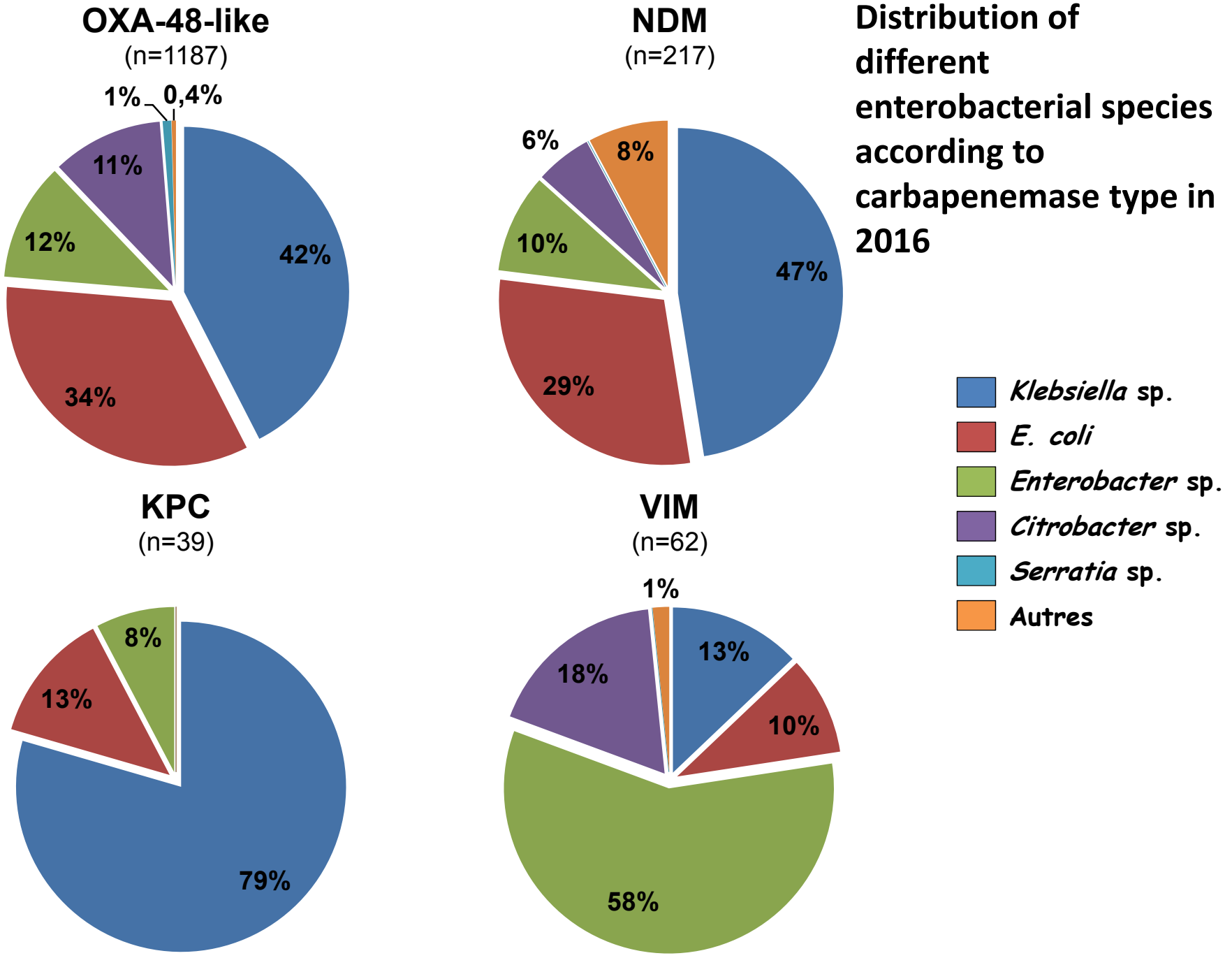


Variants NDM : progression of NDM-5

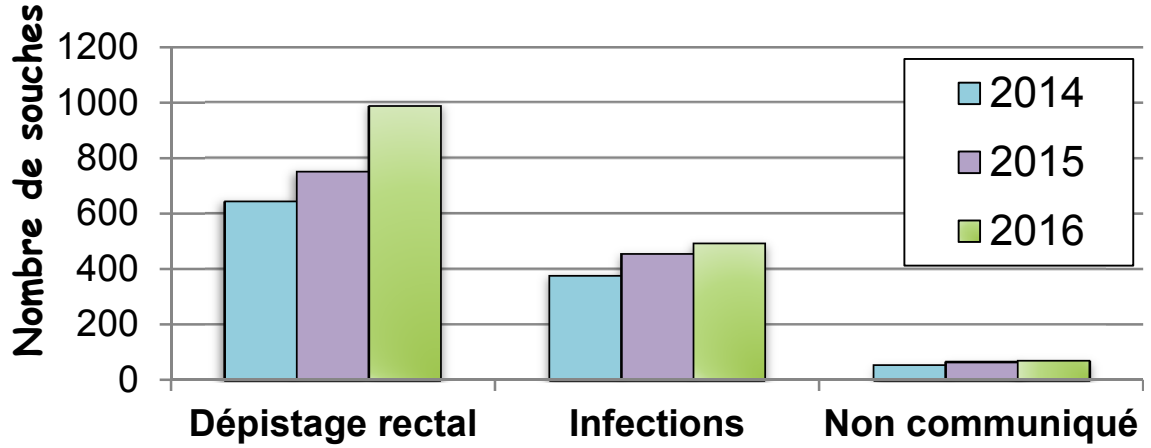


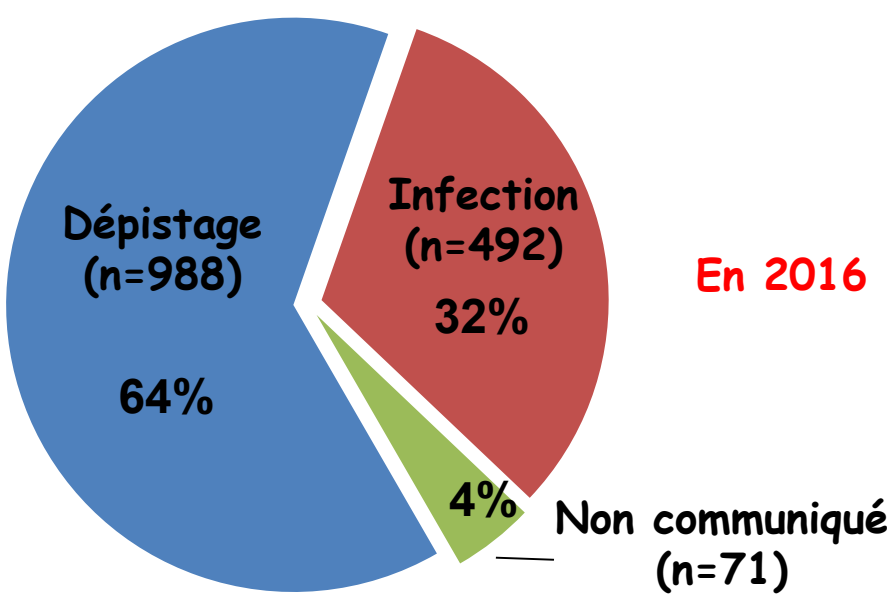

Klebsiella sp.
(n=1043)
OXA-48 + NDM
OXA-48 + KPC
GES-5
VIM
NDM
103
OXA-48
504
31
KPC
658 +
- 385
Imper.
BLSE + Case
72
49
Case
14
BLSE
250
E. coli
(n=681)
OXA-48 + NDM
OXA-48 + KPC
IMI
VIM
NDM
64
OXA-48
402
KPC
488 +
- 193
Imper.
35
Case
49
BLSE + Case
11
BLSE
98
Enterobacter sp.
(n=821)
OXA-48 + NDM
IMI
13
VIM
36
OXA-48
137
NDM
21
KPC
214 +
- 607
Imper.
31
BLSE + Case
119
Case
377
80
BLSE
Citrobacter sp.
(n=202)
OXA-48 + VIM
OXA-48 + NDM
VIM
11
OXA-48
129
NDM
12
202 +
- 47
Imper.
12
Case
21
4
BLSE + Case
10
BLSE
Serratia sp.
(n=27)
IMP
3
OXA-48
10
13 +
- 14
Imper.
3
Case
9
BLSE
2
Autres
(n=65)
OXA-48
5
17
VIM
1
NDM
23 +
- 42
Imper.
21
Case
19
BLSE + Case
BLSE


2016
2014
2012
63%
54%
44%
Klebsiella sp.
658 +
- 385
633 +
- 547
225 +
- 298
71%
52%
28%
E. coli
488 +
- 193
256 +
- 234
53 +
- 135
and the winner is E. coli
26%
11%
5%
Enterobacter sp.
214 +
- 607
123 +
- 950
33 +
- 594

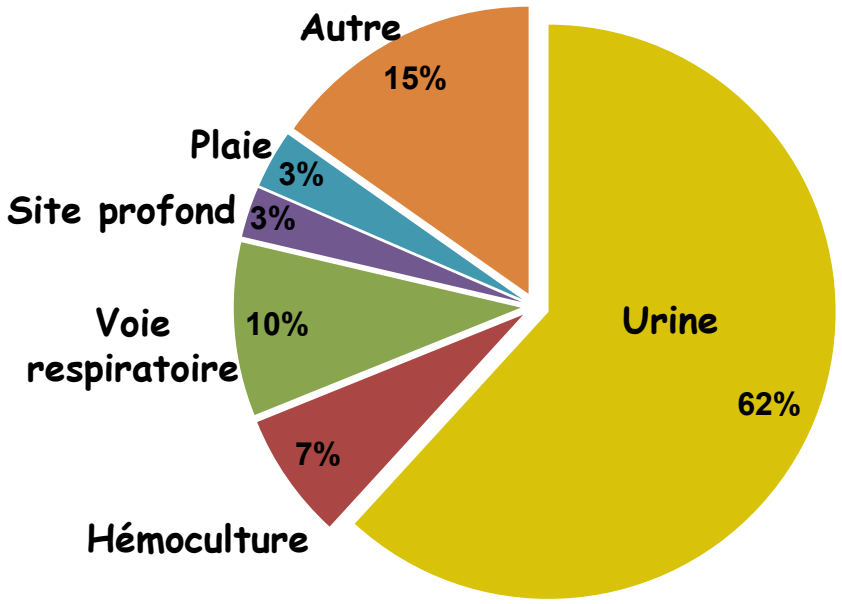

OXA-48-like
(n=1187)
1%
0,4%
11%
12%
42%
34%
NDM
(n=217)
6%
8%
10%
47%
29%
KPC
(n=39)
8%
13%
79%
VIM
(n=62)
1%
13%
18%
10%
58%
Distribution of different enterobacterial species according to carbapenemase type in 2016
Klebsiella sp.
E. coli
Enterobacter sp.
Citrobacter sp.
Serratia sp.
Autres

volution of the CPE per sample type: 2014-2016

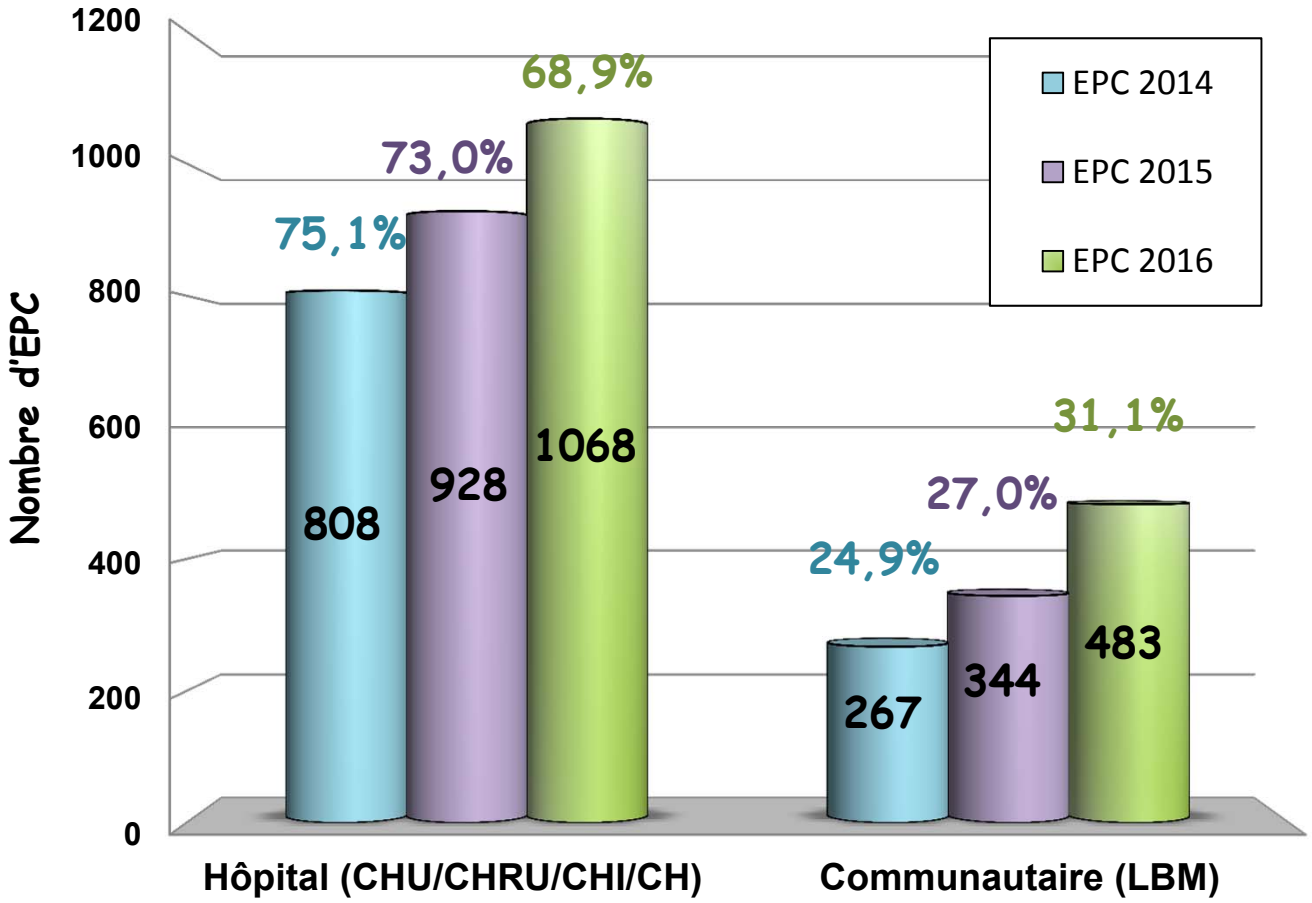




En 2016

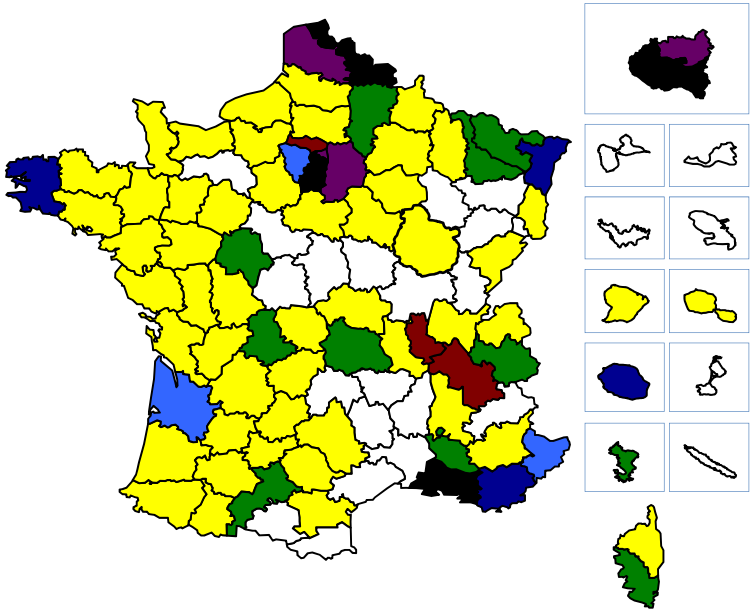


Hospitals / private labs 2014-2015



Répartition des EPC reçues au CNR Bicêtre en 2014 (n=1075), 2015 (n=1272) et 2016 (n=1551) en fonction du laboratoire expéditeur.

Number of CPEs according to departements in 2016



Number of isolates :

0	20 - 29	50 - 59
1 - 9	30 - 39	60 - 69
10 - 19	40 - 49	≥ 70

Epidodes of CPEs, France, 2004 – 2015, Per country of origin and type of carbapenemase, Dec 2015 (InVS; N= 971 episodes)

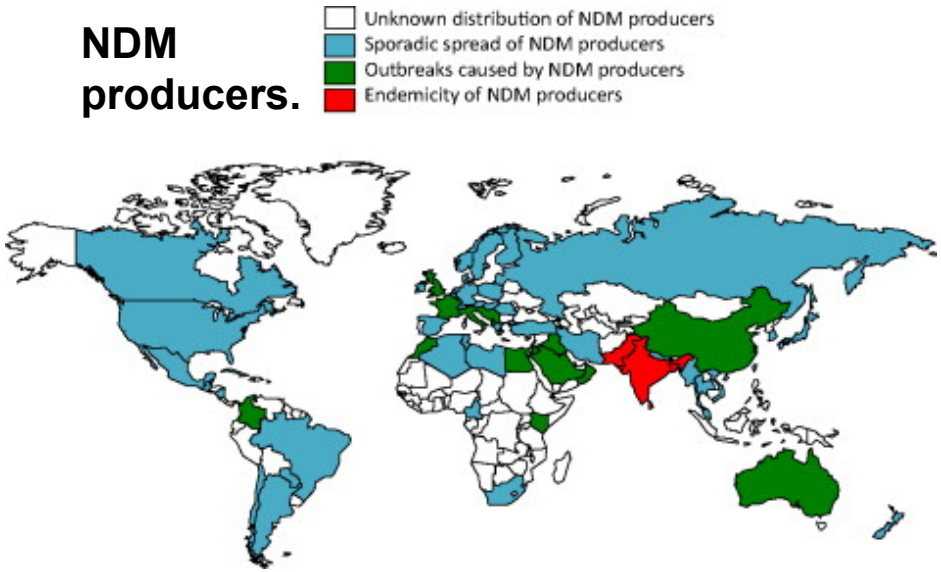
Pays	Mécanismes de résistance (carbapénèmases)					Nombre total d'épisodes*
	OXA-48 et OXA-48-like	NDM	KPC	VIM	IMI	
Maroc	237	19	2			246
Algérie	159	4	2	1		166
Tunisie	87	21	1	1		105
Inde	19	63	2			73
Égypte	44	16	1	3		59
Turquie	44	2	1	2		47
Sénégal	39	5				43
Grèce	1	1	28	10		39
Italie	5	1	27	6		39
Libye	22	1				22
Roumanie	14	2	1	1		18
Koweït	11	5	2	2		17
Cambodge	12	4				14
Congo	9	5				13
Ile Maurice	3	10				12
Vietnam	3	8	2		1	12
Espagne	7	1		2		10
Cameroun	8	1				9
Côte d'Ivoire	8				1	9
Israël	2	1	6			9
Liban	9					9

* *Nombre total d'épisodes pour lesquels le pays a été cité.*

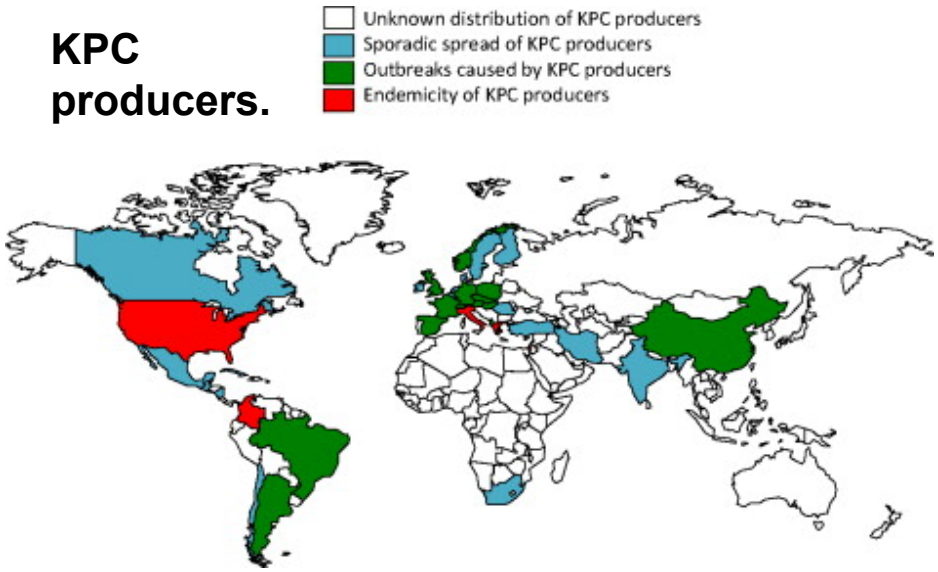
NB : pour un même épisode, plusieurs mécanismes de résistance différents peuvent être impliqués.

The tourist guide of carbapenemase-producing *Enterobacteriaceae*

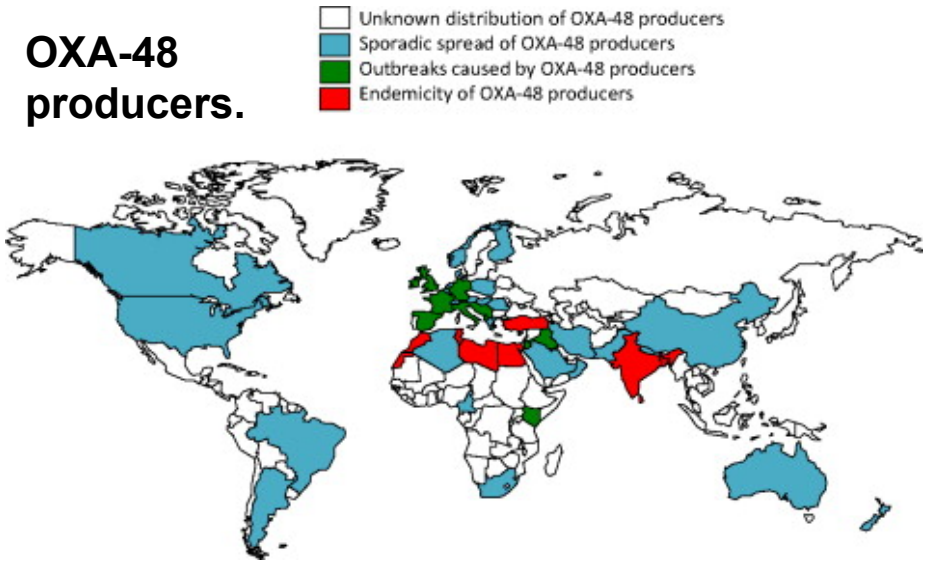
NDM producers.



KPC producers.

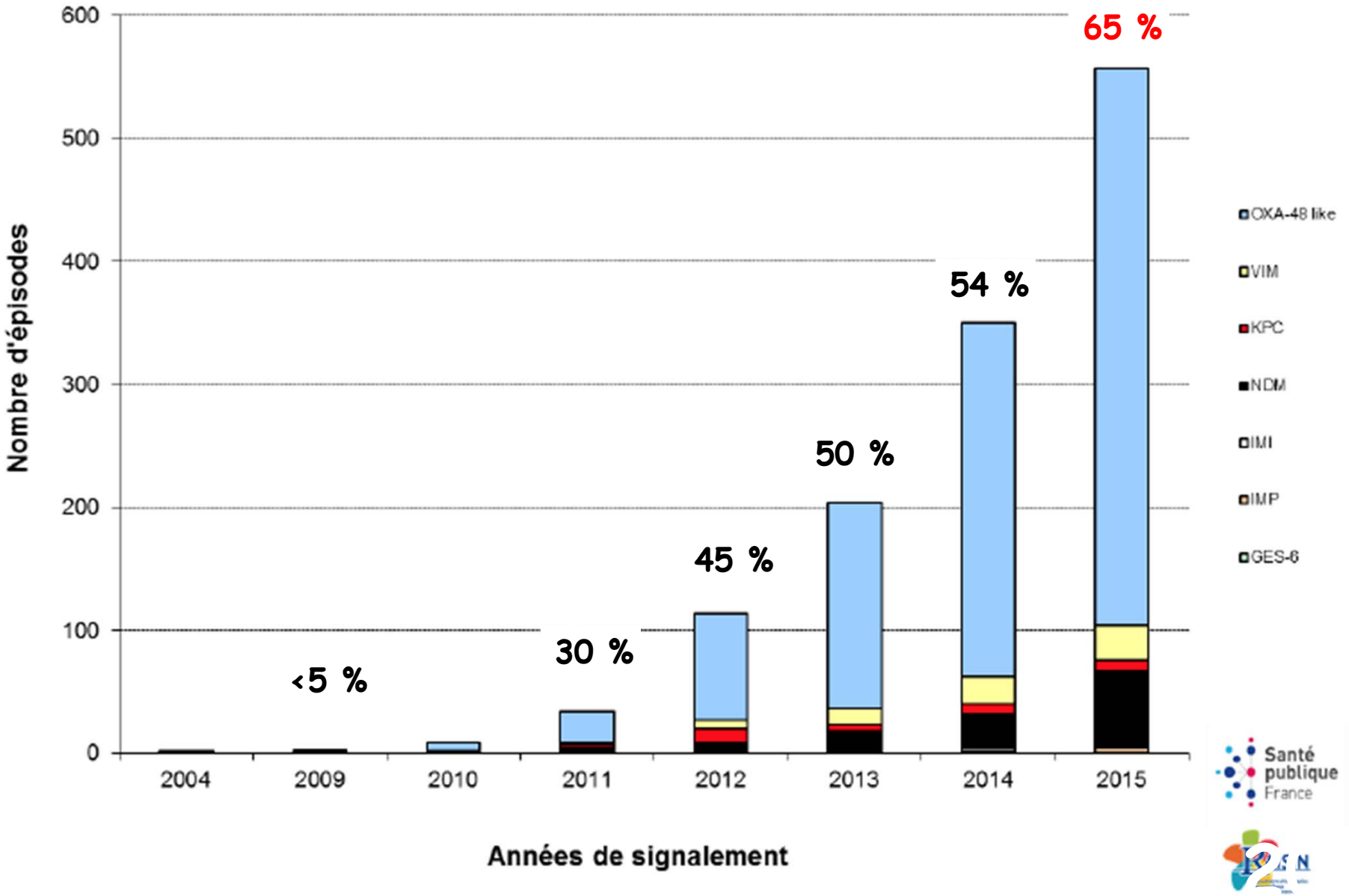


OXA-48 producers.



P. Nordmann, L. Poirel
CMI, 2014, 20, 821–830

Figure 10. Mécanismes de résistance impliqués dans les épisodes sans lien rapporté avec l'étranger, entre 2004 et 2015, par année de signalement (N=1 254 épisodes).



Colistin resistance ? Is the very last defence line about to fall?

- SENTRY study of the years **2014-2015**, have shown a prevalence of **0,4% among *E. coli*** isolates (n=13526) and **4,4% among *K. pneumoniae*** (n=7480) collected in 183 hospitals worldwide (Castaheira et al. AAC, 2016)

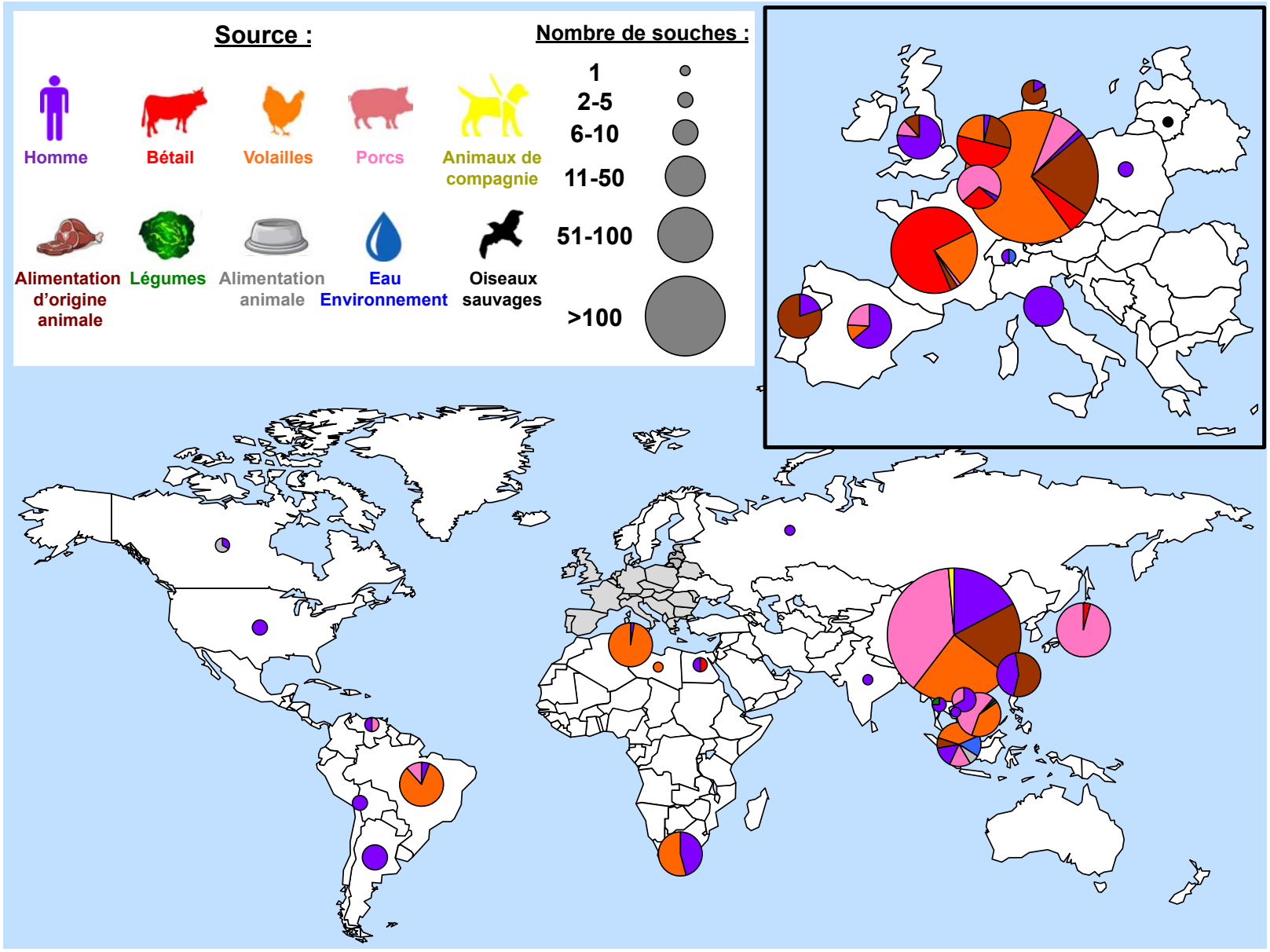
- This prevalence is however increasing as compared to the previous study performed in **Latin America between 2008 and 2010 (0,2% for *E. coli* and 3,0% for *K. pneumoniae)*** (Gales et al. DMID 2012)

- **In Europe 15% to 25% colistine resistance are reported in countries were carbapenem-resistance is already very high (Greece –Italy)**

- **In Greece colistin consumption has increased 6 times between 2009 -2013, while colistin resistant *K. pneumoniae* isolates from ICU patients rose from 0% over 2007-2010 to 21,8% over 2010-2013 period (Meletis et al, New Microbiol, 2015)**

- **Emergence of plasmid encoded colistine resistance mcr-1, mcr-1.2, mcr-2, mcr-5 ….. (Nov 2015)**

Geographic distribution of MCR-1-producing *Enterobacteriaceae* as of the 1st August 2016



What can be done?

IDENTIFICATION of CPEs

- As a source of infections

- As a source of gastro-intetinal colonisation
  How?, Who?, When?, Why?

Treatment of infections

Novel alternatives to antibiotics?

Numerous approaches (antimicrobial peptides, phagotherapy, immunotherapy, vaccination, bacterial cannibalism, phototherapy, essentila oils)

Almost no clinical phase studies, or non validated by authorities (EMA, FDA, etc..)

Many in early stage of develpment développement (proof of concept, need for clinical studies)

=> NOVEL ANTIBIOTICS

Méthodes de détection des entérobactéries productrices de carbapénèmases

1) A partir d'un prélèvement clinique (infection)

2) Dépistage des patients porteurs

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

WHEN TO SCREEN FOR A CRE

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

	MIC (mg/L)		Disk diffusion zone diameter (mm)	
Carbapenem	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.**

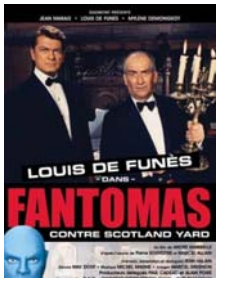
[3] **With imipenem the separation between the wild-type and carbapenemase-producers is relatively poor. Imipenem is therefore not recommended to use as a stand-alone screening test compound.**

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

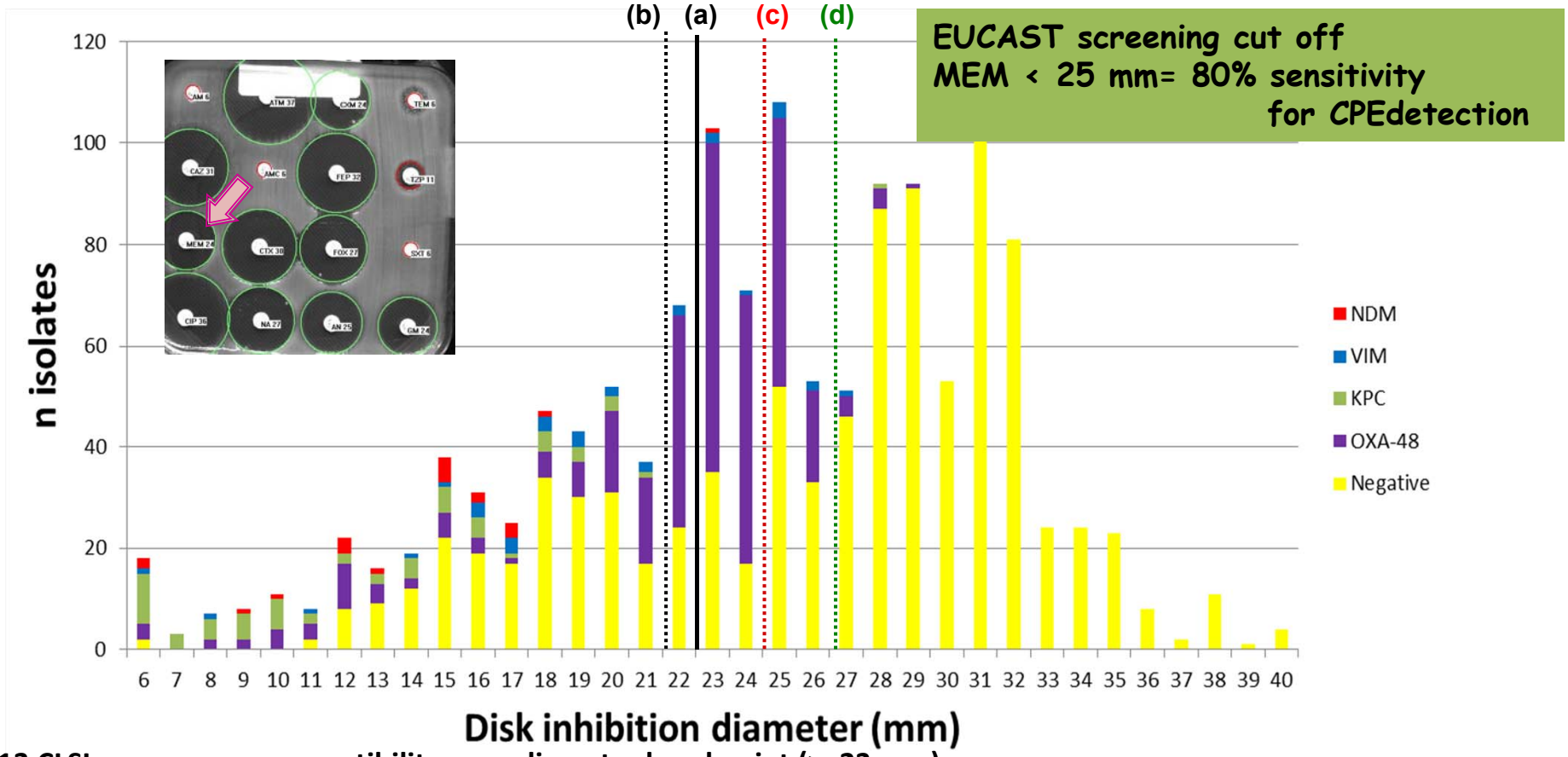
EUCAST Clinical breakpoints (v 6.0) (2016-01-01)

ARE EUCAST GUIDELINES SUFFICIENT?

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



« OXA-48 »



(a) 2013 CLSI meropenem susceptibility zone diameter breakpoint (>=23 mm)

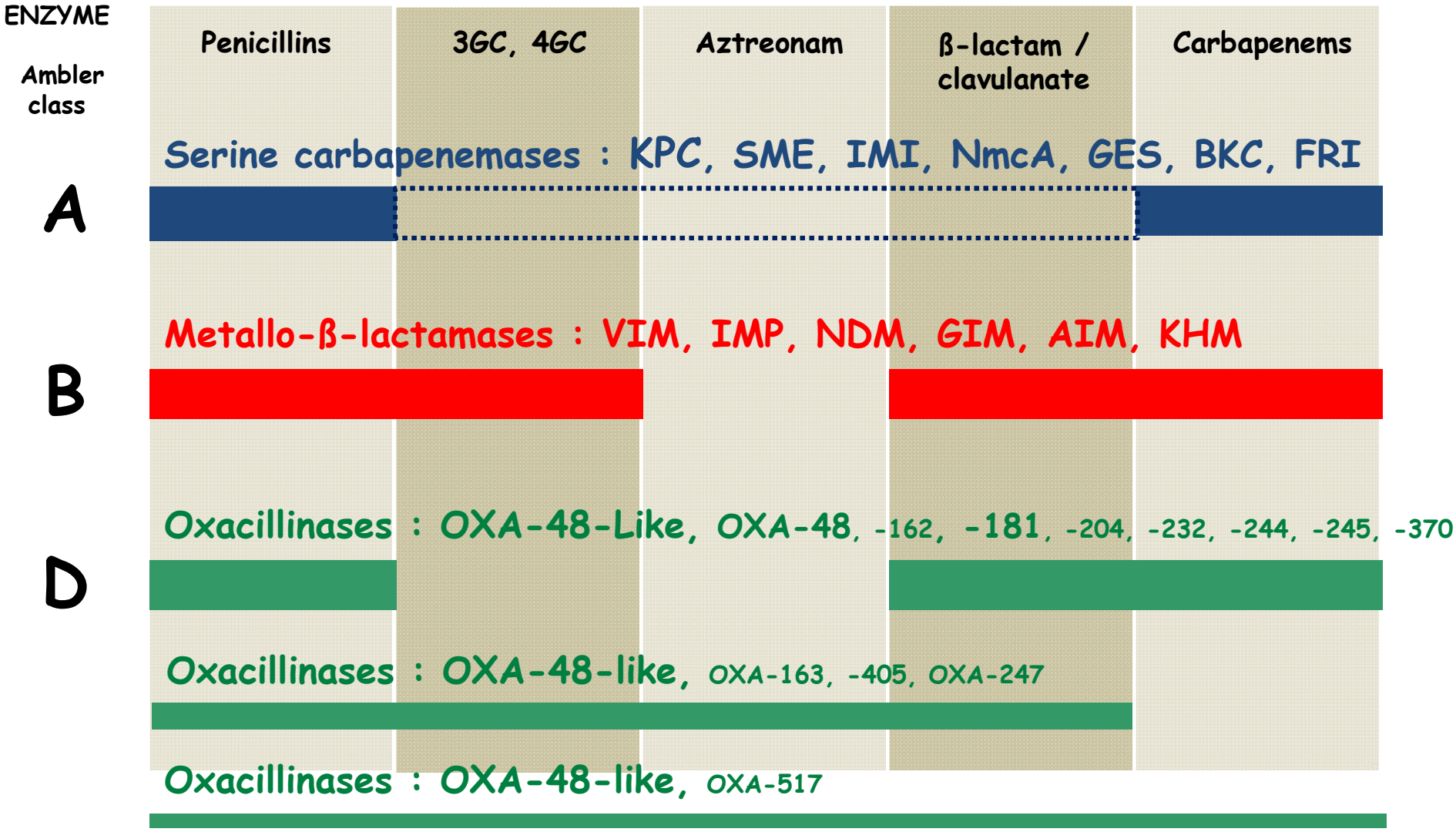
(b) 2013 EUCAST meropenem susceptibility zone diameter breakpoint (>=22 mm)

(c) 2013 EUCAST meropenem screening cut-off for the detection of CPE (<25 mm)

(d) 2013 EUCAST meropenem screening cut-off for the detection of CPE – epidemics (<27 mm)

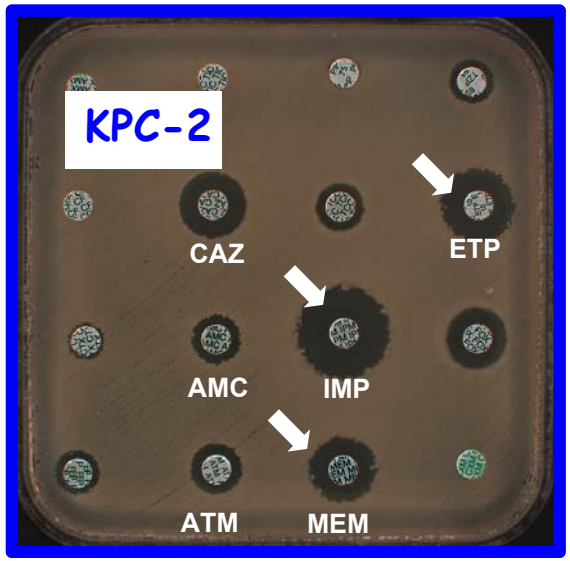
Huang TD et al., JAC 2013
(Courtesy Y Glupczynski)

Carbapenemases and *Enterobacteriaceae*
Hydrolysis profile

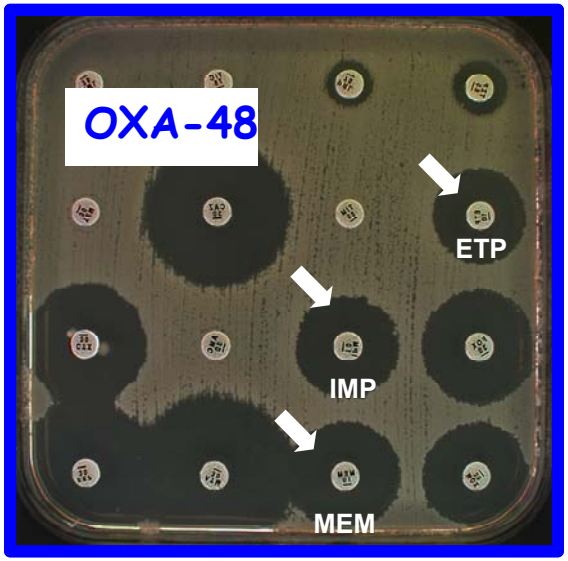


TO BE OR NOT TO BE A CARBAPENEMASE PRODUCER, THAT IS THE QUESTION IN CLINICAL MICROBIOLOGY !!!

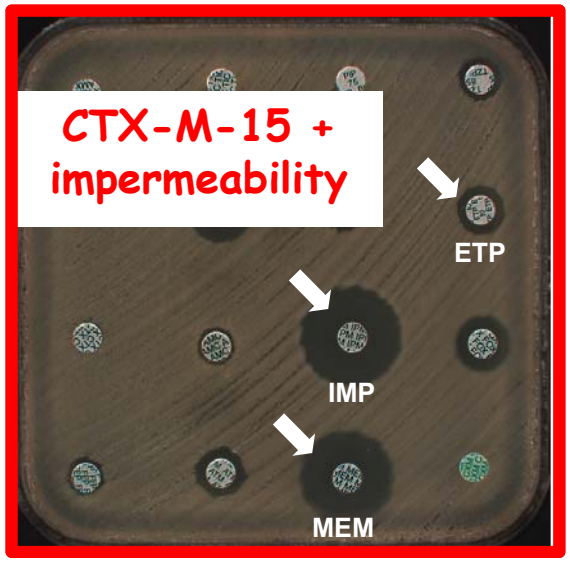
K. pneumoniae 1



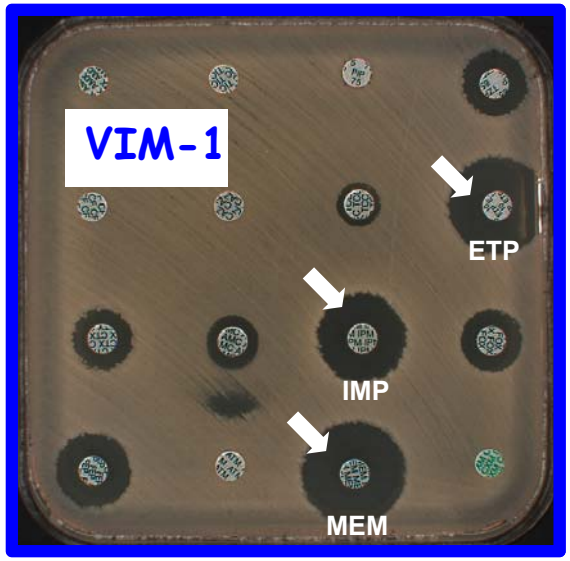
K. pneumoniae 2



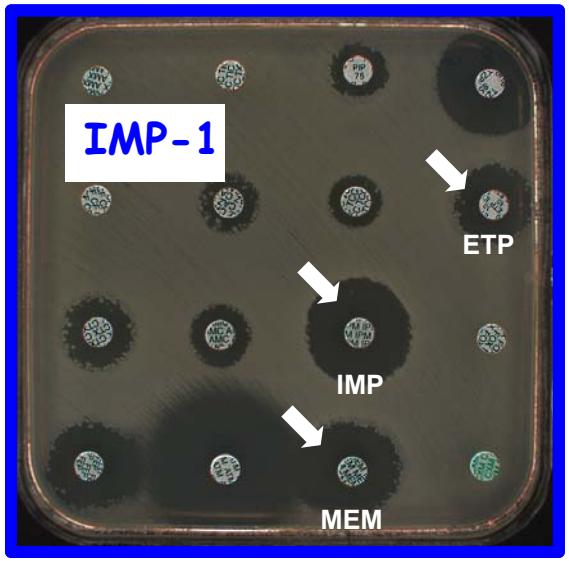
K. pneumoniae 3



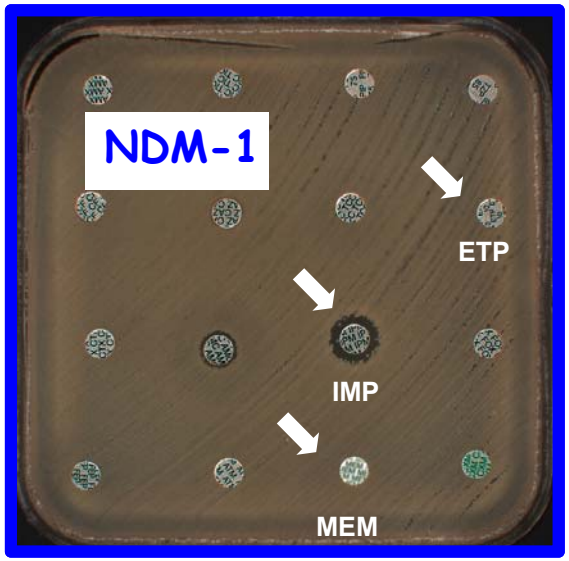
E. coli A



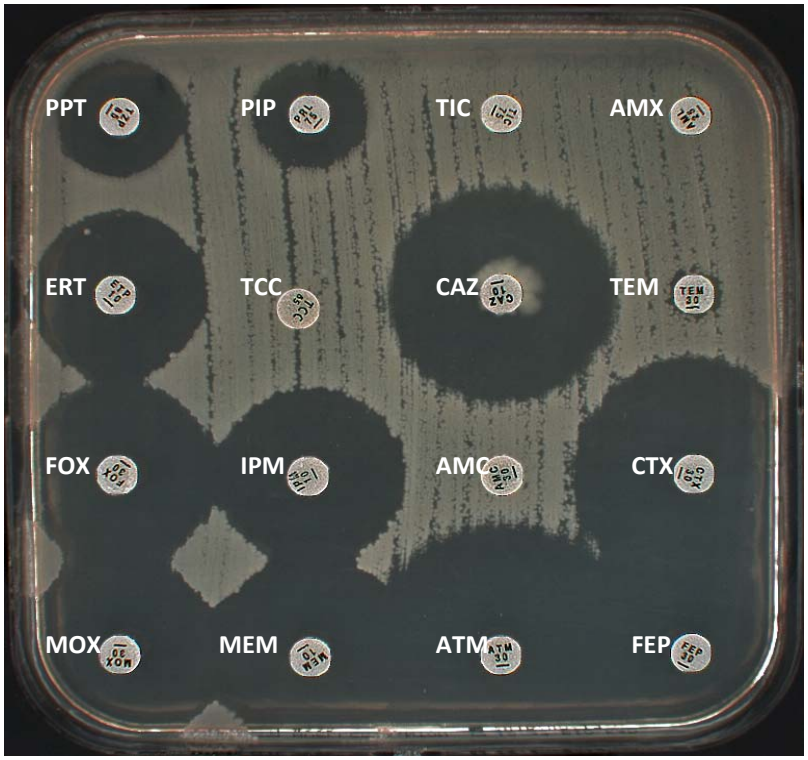
E. coli B

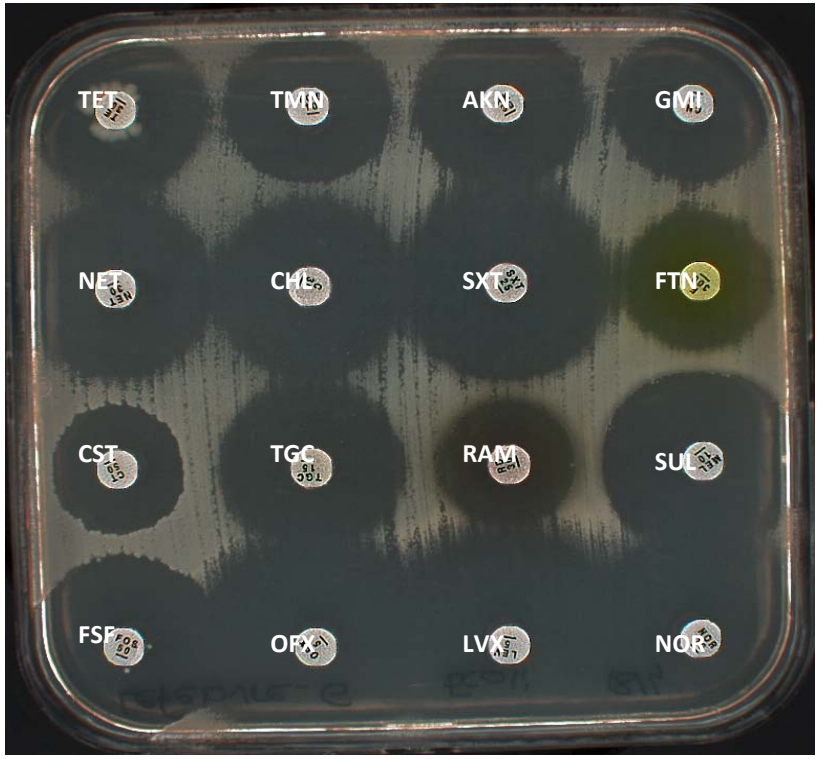


E. coli C



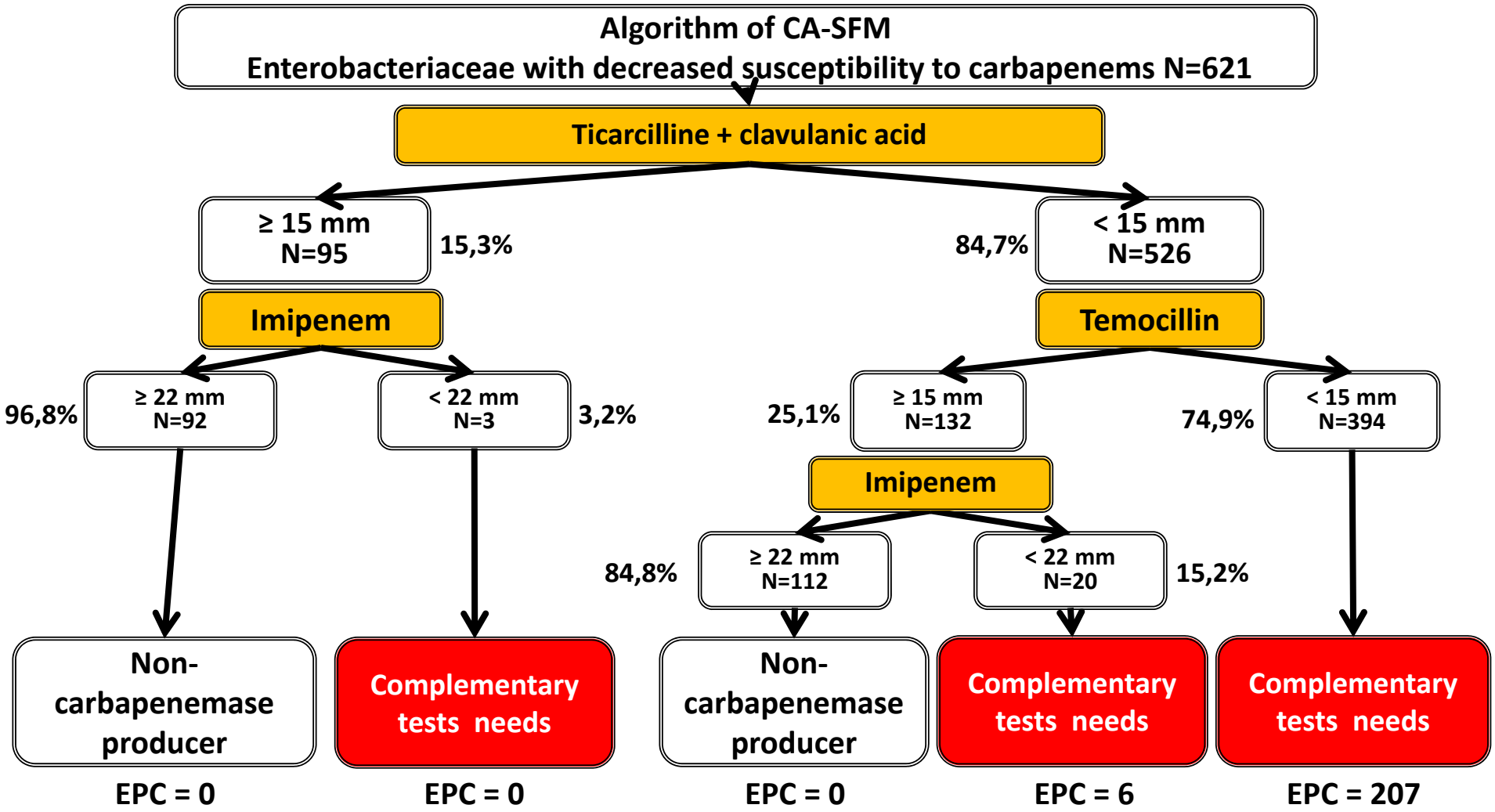
Urine with 10^7 *Escherichia coli*/ml.
Multi-susceptible





PPT : Pipéracilline-Tazobactam ; PIP : Pipéracilline ; TIC : Ticarcilline ; AMX : Amoxicilline ; ERT : Ertapénème; TCC : Ticarcilline-Acide clavulanique ; CAZ : Ceftazidime ; TEM : Temocilline ; FOX : Céfoxitine ; IPM : Imipénème; AMC : Amoxicilline-Acide clavulanique ; CTX : Céfotaxime ; MOX : Moxalactam ; ATM : Aztréonam; FEP : Céfépime ; TET : Tétracycline ; TMN : Tobramycine ; AKN : Amikacine ; GMI : Gentamycine ; NET : Netilmycine ; CHL : Chloramphénicol ; SXT : Bactrim ; FTN : Furanes ; CST : Colistine ; TGC : Tigécycline ; RAM : Rifampicine ; SUL : Sulfamide ; FSF : Fosfomycine ; OFX : Ofloxacine ; LVX : Lévofloxacine ; NOR : Norfloxacine

CMI ertapénème à 0,38 mg/l et imipénème 0,25mg/l)



5 NDM-1
1 NDM-5

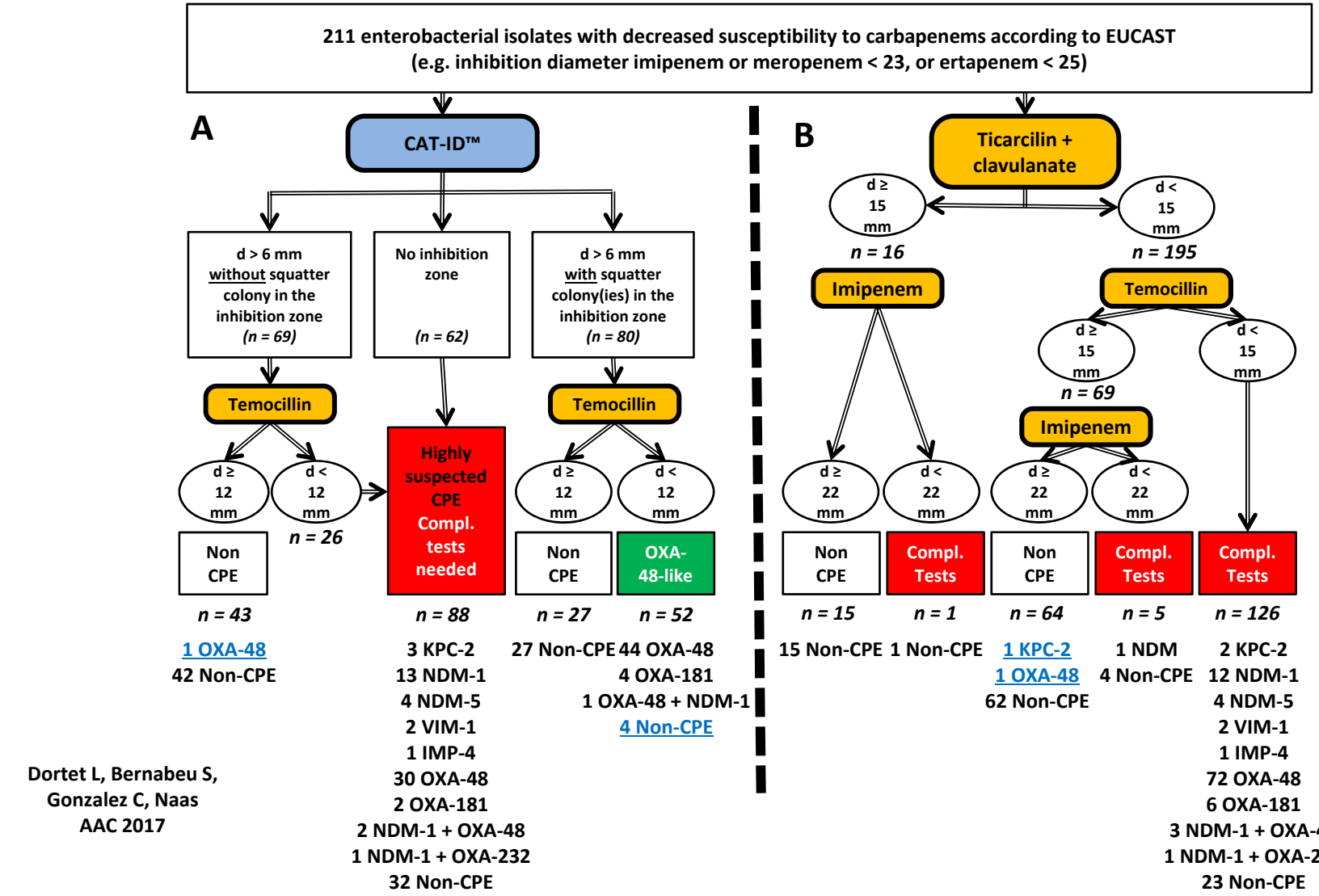
2 KPC-2, 1 KPC-3
10 NDM-1, 5 NDM-5
3 VIM-1, 1 VIM-4
166 OXA-48, 1 OXA-162
8 OXA-181, 9 OXA-204
1 OXA-232
1 NDM-1 + VIM-2
1 NDM-1 + OXA-48
1 NDM-1 + OXA-232

=> 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

Evaluation of an algorithm based on faropenem, and temocillin for the phenotypic detection and characterization of carbapenemase-producing *Enterobacteriaceae*



Dortet L, Bernabeu S, Gonzalez C, Naas
AAC 2017

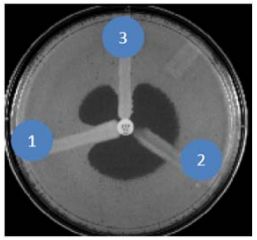
- Similar Performances (98.8% (Faro) *vs* 97.5% (CA-SFM) negative predictive value).
- Algorithm faropenem/témocillin detects well OXA-48-like producers => significant decrease of complementary tests (42.2% of isolates *vs* 62.6% with algorithme of CA-SFM).

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

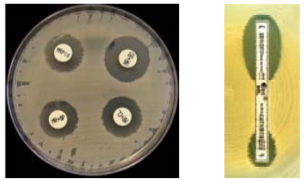
Phenotypic

Hodge test



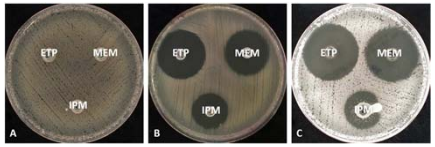
Girlich D. JCM 2012, **24h**

Inhibition tests



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

CIM test



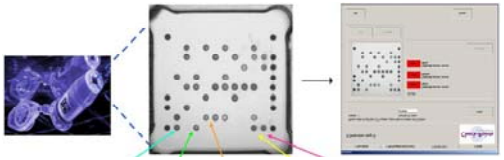
Gauthier, PlosOne, 2017
24h

Molecular

PCR et RT-PCR

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

DNA micro-array



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

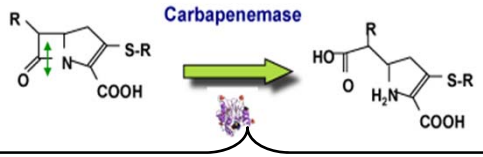
Immuno-chromatography

KPC
OXA-48,
NDM
(IMP
VIM)

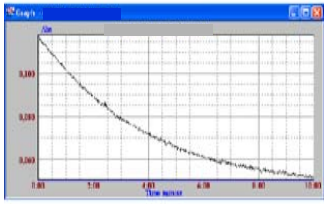
Bogaerts, JAC, 2016, in press 2017

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

Carbapenem -hydrolysis

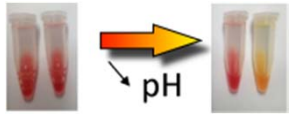


UV spectrophotometry



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

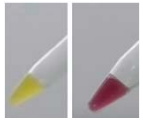
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

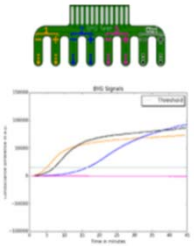
Dortet. JAC 2015, **2h**

β-Carba



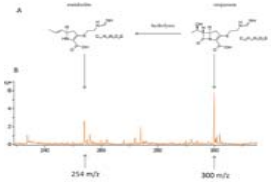
Bernabeu. JAC 2017 **<30'**

PH-metry



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

MALDI-TOF



Tandé D. JCM 2015, **30'**

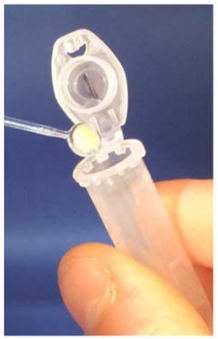
rCIM

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

rCIM
(rapid Carbapenemase Inactivation Method)

Protocol

Bacteria of interest



2 loopfulls

2 Meropenem discs

Incubate 30 minutes

Centrifuge 5 minutes

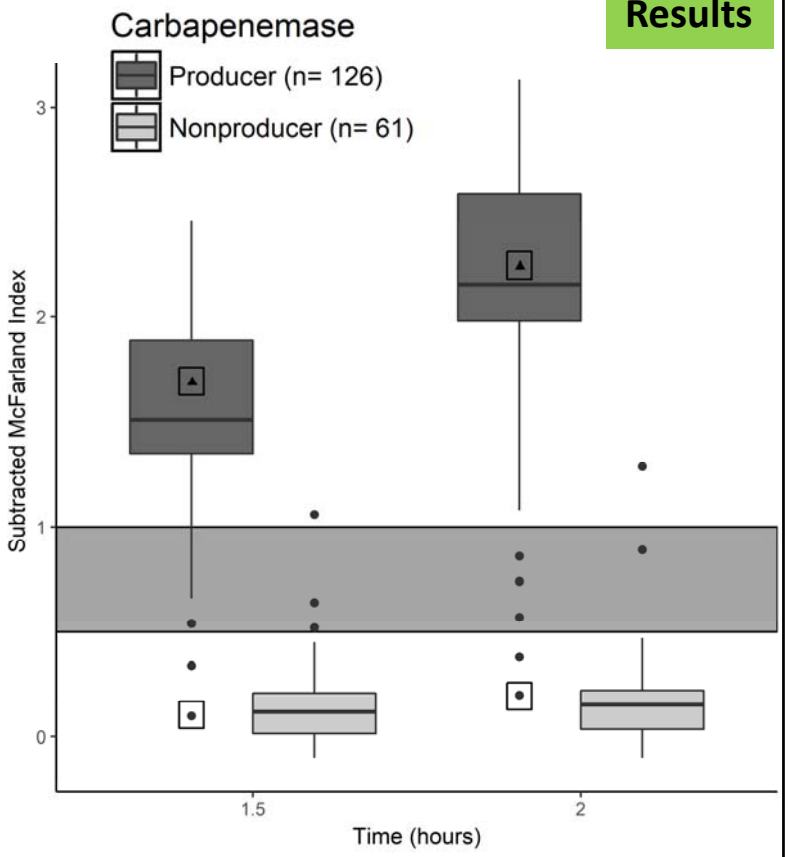
Supernatant w/ AB

IMF=1

E. coli ATCC 25922

Incubate 1.5 - 2h

Results



The rCIM correctly identified 62/63 of CPEs and 23/23 non-CPEs

⇒ rCIM: sensitivity of 98%, and specificity of 100%,

⇒ CIM and Carba NP test: 94% sensitivity and 100% specificity.

Muntean M. JAC, in press

Méthodes de détection des entérobactéries productrices de carbapénèmases

1) A partir d'un prélèvement clinique (infection)

2) Dépistage des patients porteurs

HOW? STOOLS / SWABS

AAC
Journals.ASM.org

Rectal Swabs Are Suitable for Quantifying the Carriage Load of KPC-Producing Carbapenem-Resistant *Enterobacteriaceae*

A. Lerner,[a,b] J. Romano,[a*] I. Chmelnitsky,[a] S. Navon-Venezia,[a] R. Edgar,[a] Y. Carmeli[a]

Molecular Epidemiology and Antimicrobial Resistance Laboratory, Division of Epidemiology, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel[a]; Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel[b]

It is more convenient and practical to collect rectal swabs than stool specimens to study carriage of colon pathogens. In this study, we examined the ability to use rectal swabs rather than stool specimens to quantify *Klebsiella pneumoniae* carbapenemase (KPC)-producing carbapenem-resistant *Enterobacteriaceae* (CRE). We used a quantitative real-time PCR (qPCR) assay to determine the concentration of the bla_{KPC} gene relative to the concentration of 16S rRNA genes and a quantitative culture-based method to quantify CRE relative to total aerobic bacteria. Our results demonstrated that rectal swabs are suitable for quantifying the concentration of KPC-producing CRE and that qPCR showed higher correlation between rectal swabs and stool specimens than the culture-based method.

***AAC*, 2013, 57: 1474–1479.**

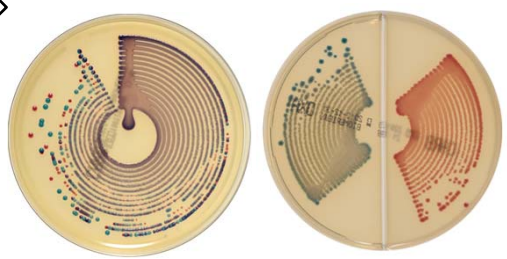
CPE colonisation detection

Stool, Rectal swabs

24-48h

Enrichment culture

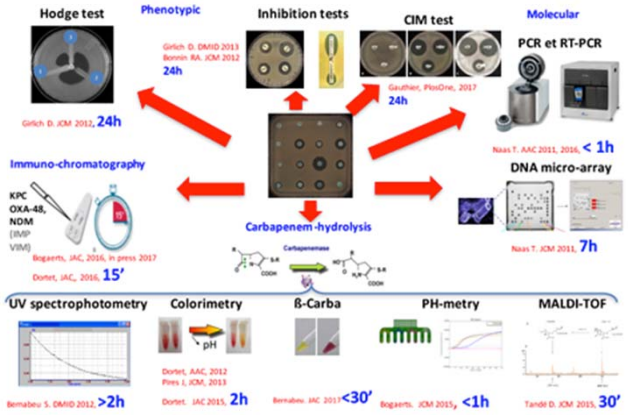
Selective Chromogenic media



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

HOW TO SCREEN? : CULTURE / MOLECULAR

- Culture: cheap, but lack of specificity and sensitivity, and LONG

- Molecular tools are often perceived as:

FASTER
Less then one hour

MORE ACCURATE:
Highly Sensitive and Specific

EASIER
Reduced hands-on-time, user friendly

MORE EXPENSIVE,
BUT WITH A MEDICAL ADDED VALUE

The answer is in the CPE prevalence

MOLECULAR METHODS FOR RAPID SCREENING OF CRE FROM RECTAL SWAB/STOOLS

Most studies in endemic areas with high prevalence, outbreak setting (infection control purposes)

Author (yr)	N° of specimens (pts)	Targeted bla genes	Method	Sens. %	Spec. %	Detection limit (CFU/PCR)	Process Time
Hindiyeh (2008)	189 (127)	KPC	RT PCR (TaqMan)	100	95	1	4h
Schechner (2009)	755 (650)	KPC	In house end -point PCR	92.6	99.6	-	30h* (culture: 144-192 h)
Giani (2012)	101 (65)	KPC	In house end-point PCR	100	86	1	3-4h
Pournaras (2012)	189 (NR)	KPC, VIM,	In house end-point PCR	94.4	86	NR	4h
Singh (2012)	95 (95)	KPC	RT PCR (TaqMan) FAM labeled reporter probes	97	96.6	1-10	24 h* (culture: 64-72h)
Richter (2012)	216 (125)	KPC-2/-12	Fast RT PCR (TaqMan) MGB probe	100	98	1	≤ 2h
Vasoo (2013)	126 (126)	KPC, NDM	RT PCR (Light Cycler) Simple lysis extraction (soiled spec.)	89.1 100	-	2-10	1.5-4 h

*** PCR performed from overnight enrichment broth culture**

CARBAPENEMASE GENE ASSAYS

	Check-Direct CPE on ABI 7500	Check-Direct CPE on BD MAX™	eazyplex SuperBug Complete	Xpert® Carba-R
Assay coverage	KPC, OXA-48-like, NDM/VIM	KPC, OXA-48-like, NDM, VIM	KPC, OXA-48, NDM, VIM	KPC, OXA-48, NDM, VIM, IMP-1-like
'Big 5' carbapenemases NOT detected	IMP family	IMP family	IMP family	some IMP subgroups
Hands on time per sample	<5 min	<5 min	<5 min	<5 min
Assay run time	~1.75 h	~2.5 h	20 min	~50 min
Sample throughput	Up to 94 tests in a batch	Up to 22 tests in a batch	1 or 2 independent tests	1 to 80 independent tests

Findlay J, et al. J Antimicrob Chemother. 2015 May;70(5):1338-42

Commercially available tests: the bloming of novel tests

- **Carbaplex (Brucker)**
  - IMP, KPC, NDM, OXA-48, VIM
  - (sens 96,3%, spec 99,5%) < 3h (de l'ADN extrait)
  - Ecouvillon
- **Carba Assay (Elitech)**
- **LightMix® modular carbapenemase kits (TIB Molbiol, Berlin, Germany)**
- **Amplidiag CarbaR-VRE (Mobidiag, Finlande)**
  - Détecte tous les variants IMP
  - Détecte les principales carbapénèmases des EPC, *P. aeruginosa* et *A. baumannii* (KPC, VIM, OXA-48/-181, IMP, NDM, ISAbal-OXA-51, OXA-23, OXA-40, OXA58, VanA, VanB)
  - Détecte toutes les BHREs en trois PCRs.
  - Sur colonies uniquement (nouvelle version incluant *mcr* et sur ecouvillon)

Extraction and independant PCR

⇒ **Avantages**

- **Open Systemes**
- **Good performances**

⇒ **But**

- **Long (4h),**
- **Trained personel**
- **Risk of contamination ++**

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

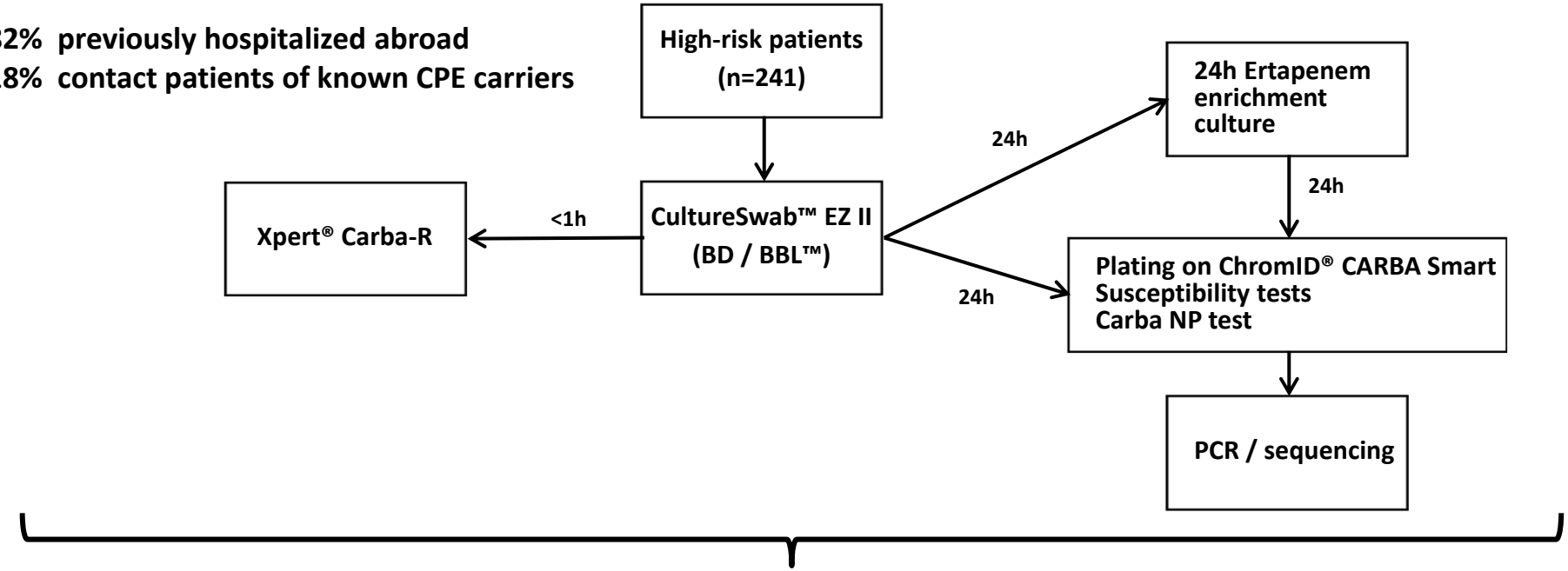
- **150 enterobacterial isolates including 130 isolates with decreased susceptibility to at least one carbapenem)**
- **61 non-carbapenemase producers**
- **89 carbapenemases producers :**

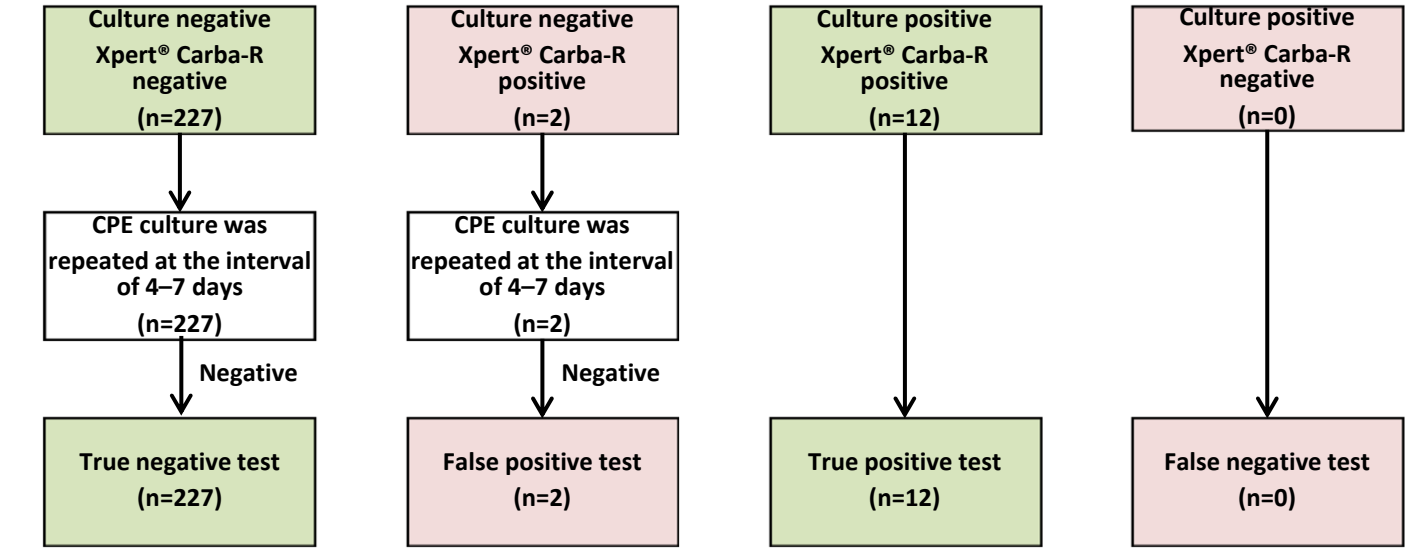
Performances	Xpert® Carba-R v2	
	This study	Global French CPE epidemiology (2012-2014)*
Sensitivity	97.8 %	99.61 %
Specificity	94.1 %	99.98 %
False positive	1 OXA-405 2 OXA-163	1 OXA-405
False negative	2 IMP-8	7 IMI 1 FRI-1

*** 2026 isolates**

Performances of the Xpert® Carba-R v2, in the daily workflow of a hygiene unit in a country with low prevalence of carbapenemase-producing *Enterobacteriaceae* (Sept 2015 - March 2016)

82% previously hospitalized abroad
18% contact patients of known CPE carriers





Y Hoyos, S Ouzani, L Dortet, N Fortineau, and T Naas, IJAA, 2017

Performances of the Xpert® Carba-R v2, in the daily workflow of a hygiene unit in a country with low prevalence of carbapenemase-producing *Enterobacteriaceae* (Sept 2015 - March 2016) (suite)

Patient	Xpert® Carba-R v2	Cultured CPE	Origin of patients
1	OXA-48 + VIM	*K. pneumoniae* OXA-48 and *E. cloacae* OXA-48 + NDM-1	Serbia
2	OXA-48	*K. pneumoniae* OXA-181	Algeria
3			France (contact patient of OXA-48 carrier)
4			
5			
6		OX	
7			
8			XA-48 carrier)
9			
10	OXA-48	*E. coli* OXA-181	India
11	OXA-48	*E. coli* OXA-204	France
12	OXA-48	*E. coli* OXA-48	Morocco
13	OXA-48	None	France (contact patient of OXA-48 carrier)
14	OXA-48	None	Cambodia
15	OXA-48	*E. aerogenes* OXA-48	France (patient contact)
16	OXA-48	*K. pneumoniae* OXA-48	Tunisie
17	OXA-48	*E. coli* OXA-48	Liban
18	NDM	*E. coli* NDM-5	Inde
19	OXA-48	*E. coli* OXA-48 *E. aerogenes* OXA-48	France (patient contact)
20	**NEG**	***C. freundii* OXA-48**	**France**

Performances biologiques
100% sensitivity,
99.13% specificity
85.71% positive predictive value
100% negative predictive value

2 false positives: What to do with these results?

⇒ **nothin? No diffusion to toher patients**
⇒ **Increased awareness (if antibiotherapy?)**

⇒ **Sept 2015 et nov 2016: 449 patients considered as high risk for CPE**
⇒ **Xpert® Carba-R v2 presents a sensibility of 94,44%, a specificity of 99.53%, a positive predictive value of 89.47% and a negative prédictive value of 99,76%.**
⇒ **CARE with false negatives: culture remains useful +++**

Explanations of PCR positive et culture negative

CNR RÉSISTANCE AUX ANTIBIOTIQUES

1. Antibiotic susceptibilities and phenotypic characteristics of the OXA-244-producing *E.coli* isolates.

Isolates	MLST[a]	Clones[b]	Plasmids size (c.a)	Date of isolation	Source of isolation	Origin	Susceptibility[c]					OXA-48 K-SeT®	Carba NP test[d]	RAPIDEC®Carba NP[d]	MALDI-TOF MS hydrolysis assay[d]	ChromID® ESBL[e]	Chrom ID® CARBA SMART[f]
							IMP (mg/L)	MEM (mg/L)	ETP (mg/L)	TEM (mg/L)	MOX (mm)						
86J1	ST-361	1	160 110 70	28/05/15	rectal	Egypt	0.5	0.5	2	>1024	7	+	+	+	+	+	+ | -
62D3	ST-1722	2	Abs	08/10/14	urine	unknown	0.38	0.38	1	128	21	+	+	+	+	+	- | -
69E6	ST-38	3	Abs	23/12/14	rectal	unknown	0.25	0.38	3	128	20	+	+/-	+	+	+	- | -
78B5	ST-38	3	Abs	15/04/15	rectal	unknown	0.38	0.5	3	256	21	+	+	+	+	+	- | -
35J9	ST-38	3	120 60 10	13/11/13	urine	France	0.5	0.75	2	96	21	+	-	+/-	-	-	- | -
73 G4	ST-3541	4	115	16/02/15	unknown	Egypt	0.25	0.19	0.75	128	20	+	+	+	+	+	- | -
85 H4	ST-3541	4	115	11/08/15	rectal	Egypt	0.38	0.25	2	384	20	+	+/-	+/-	-	+	- | -

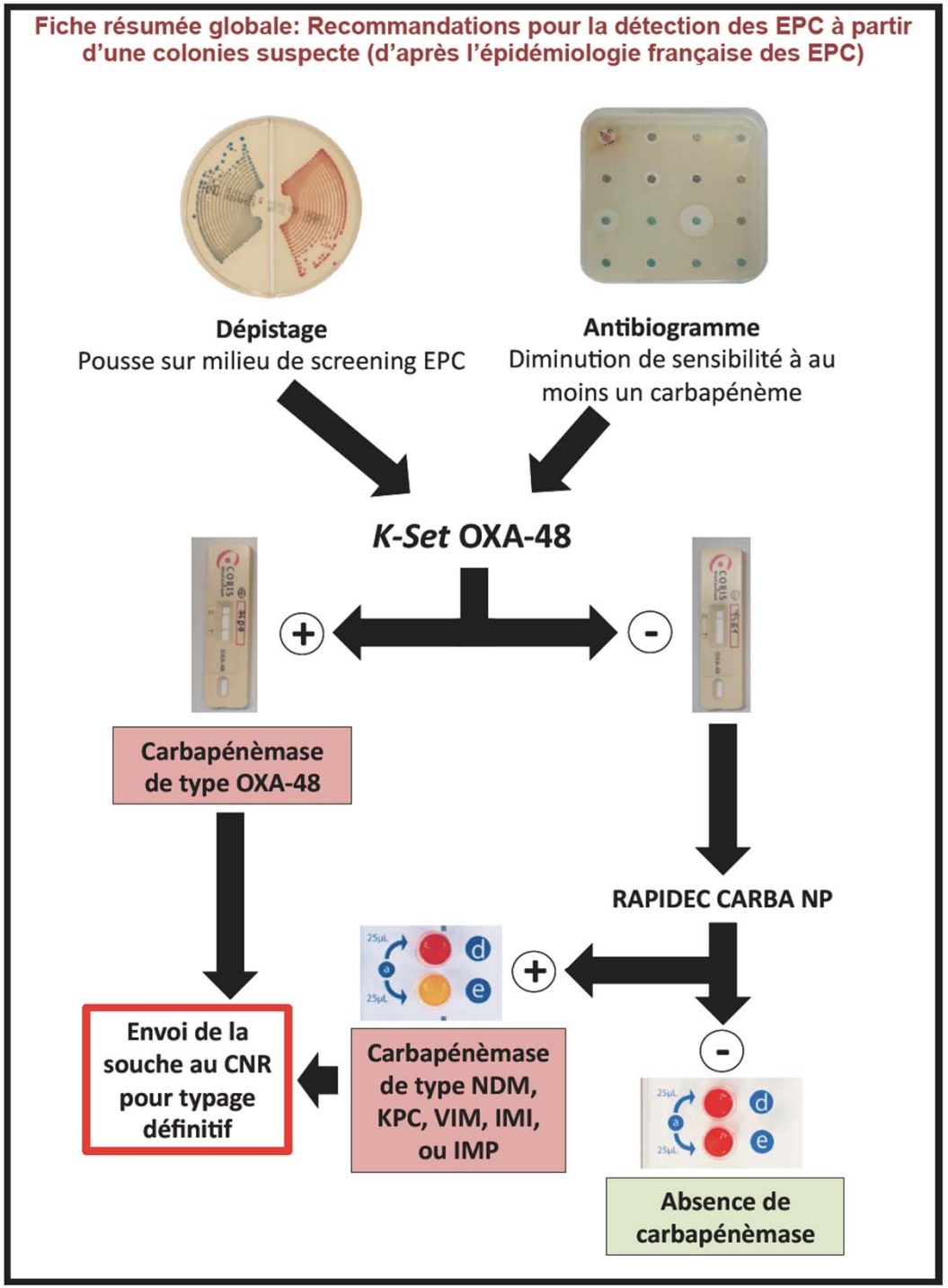
2. CTX-M-15-producing *Shewanella bicestrii* sp. nov. clinical isolate harboring a chromosome encoded OXA-48 variant, progenitor of plasmid encoded OXA-436. (Jousset et al. AAC submitted)

=> isolated from a 7-year-old immune-compromised child suffering from cholangitis.

3. *A. hermannii* VIM-1, no expression of the carbapenemase in that background, but PCR + , plasmid transferable

CNR
RÉSISTANCE AUX ANTIBIOTIQUES

Version mai 2016

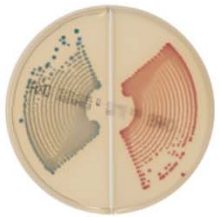


http://www.cnr-resistance-antibiotiques.fr/exp-des-souches-1.html

CNR
RÉSISTANCE AUX ANTIBIOTIQUES

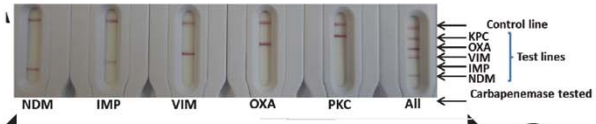
Version 2018

Fiche résumée globale: Recommandations pour la détection des EPC à partir d'une colonies suspecte (d'après l'épidémiologie française des EPC)



Dépistage
Pousse sur milieu de screening EPC

Antibiogramme
Diminution de sensibilité à au moins un carbapénème



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Carbapénèmase de type: KPC, NDM, VIM, IMP, OXA-48-like

\-

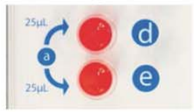
RAPIDEC CARBA NP

\+

Envoi de la souche au CNR pour typage définitif

Carbapénèmase de type IMI +++, FRI, GES ou autre

\-



Absence de carbapénèmase

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

CNR
RÉSISTANCE AUX ANTIBIOTIQUES

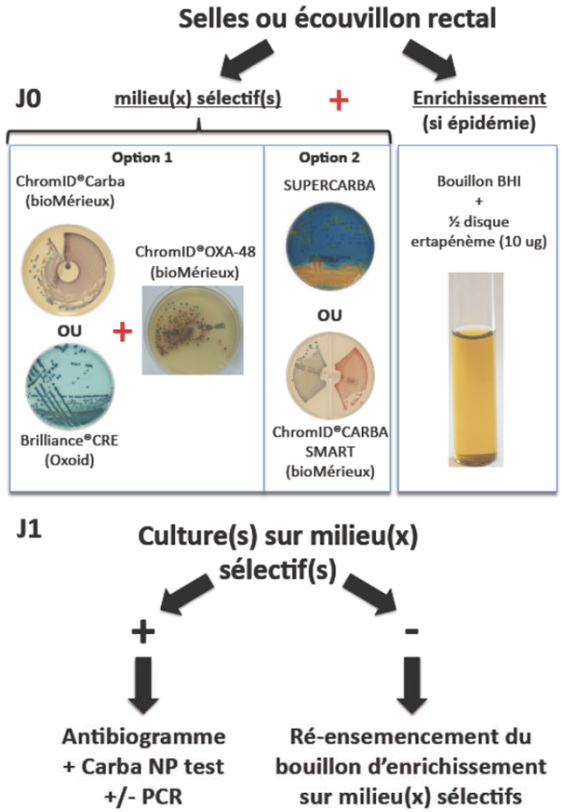
Fiche résumée : Recommandations pour le dépistage des patients porteurs d'une souche d'EPC (patients colonisés)

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.

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

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