Méthodes de détection des entérobactéries productrices de carbapénèmases

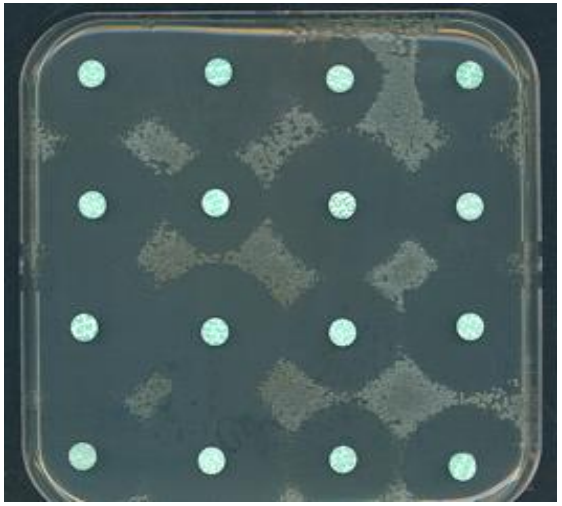
Dr Laurent DORTET

Laboratoire de Bactériologie-Hygiène, Hôpital de Bicêtre

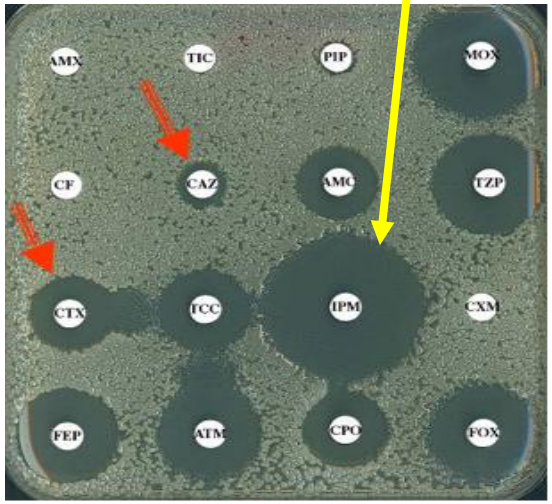
CNR associé Résistance aux Antibiotiques

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

E. coli
Of our youth

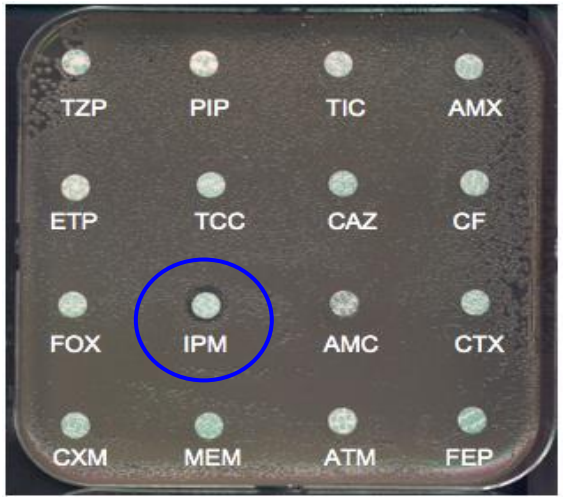


E. coli
of modern times



ESBLs

E. coli
of tomorrow



CRE (carbapenemase +++)

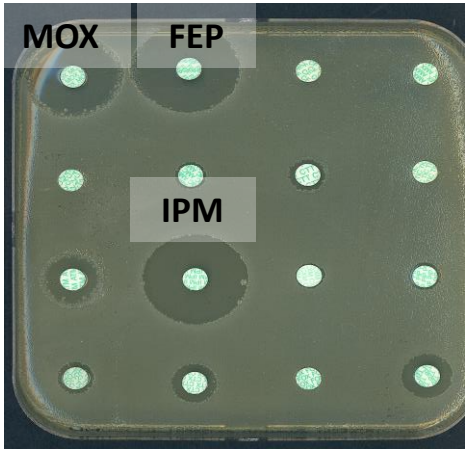
CRE : Carbapenem resistance in Enterobacteriaceae

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

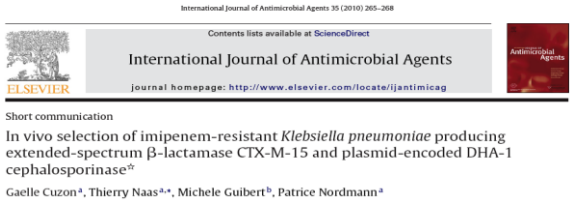
Resistance to Expanded spectrum cephalosporins BUT Carbapenem susceptible,

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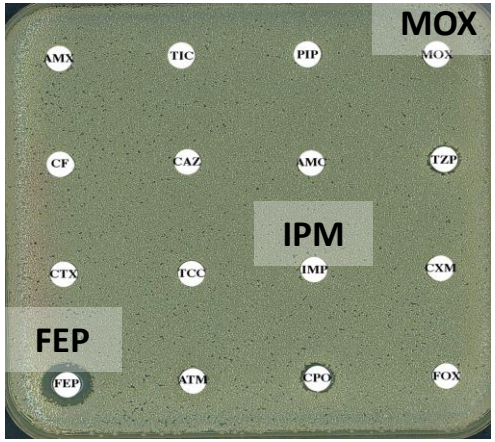
journal homepage: http://www.elsevier.com/locate/ijantimicag

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)

SUMMARY

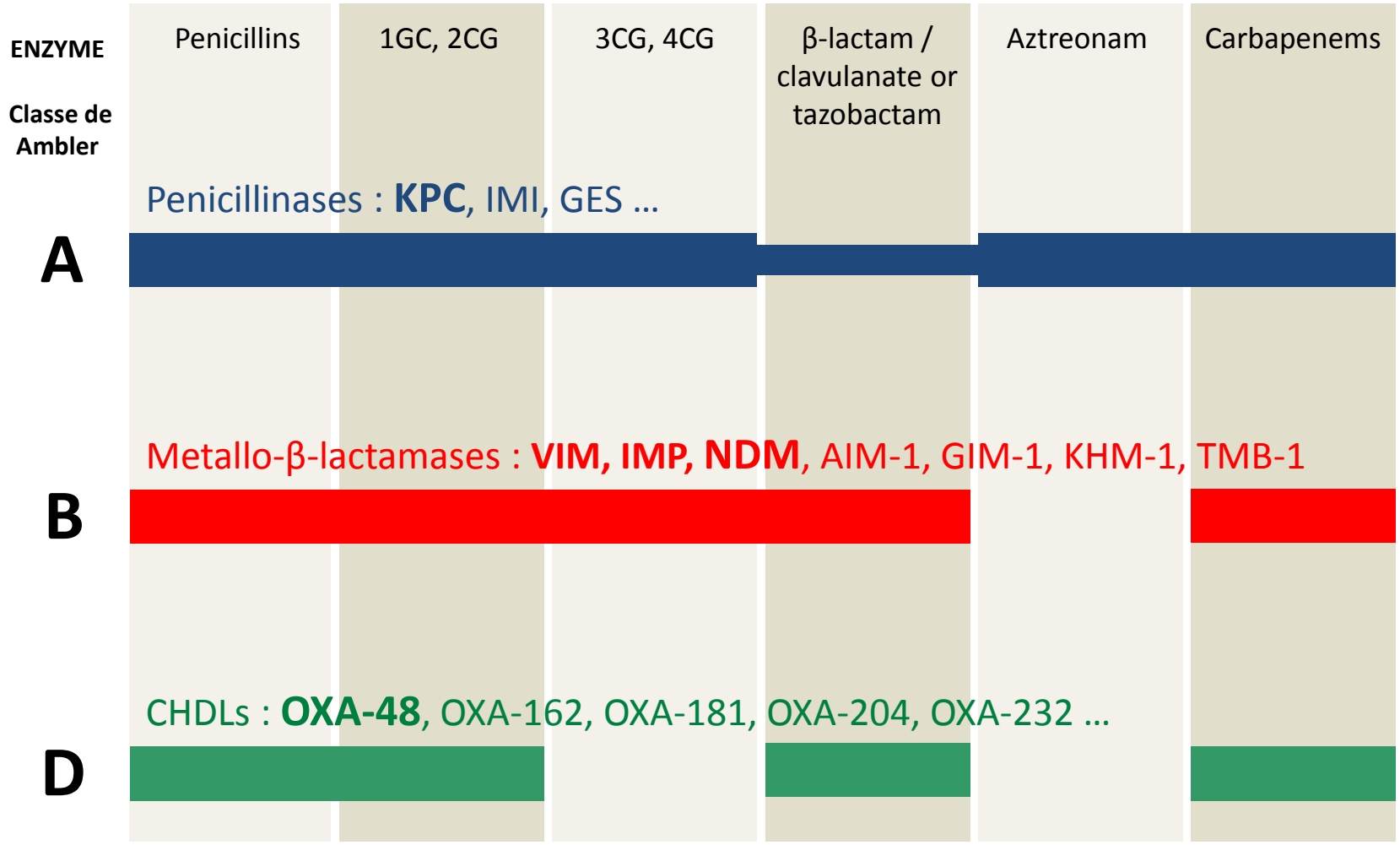
1) **Epidemiology and dissemination of carbapenemase producing Enterobacteriaceae (CPE)**

1) CPE Detection methods

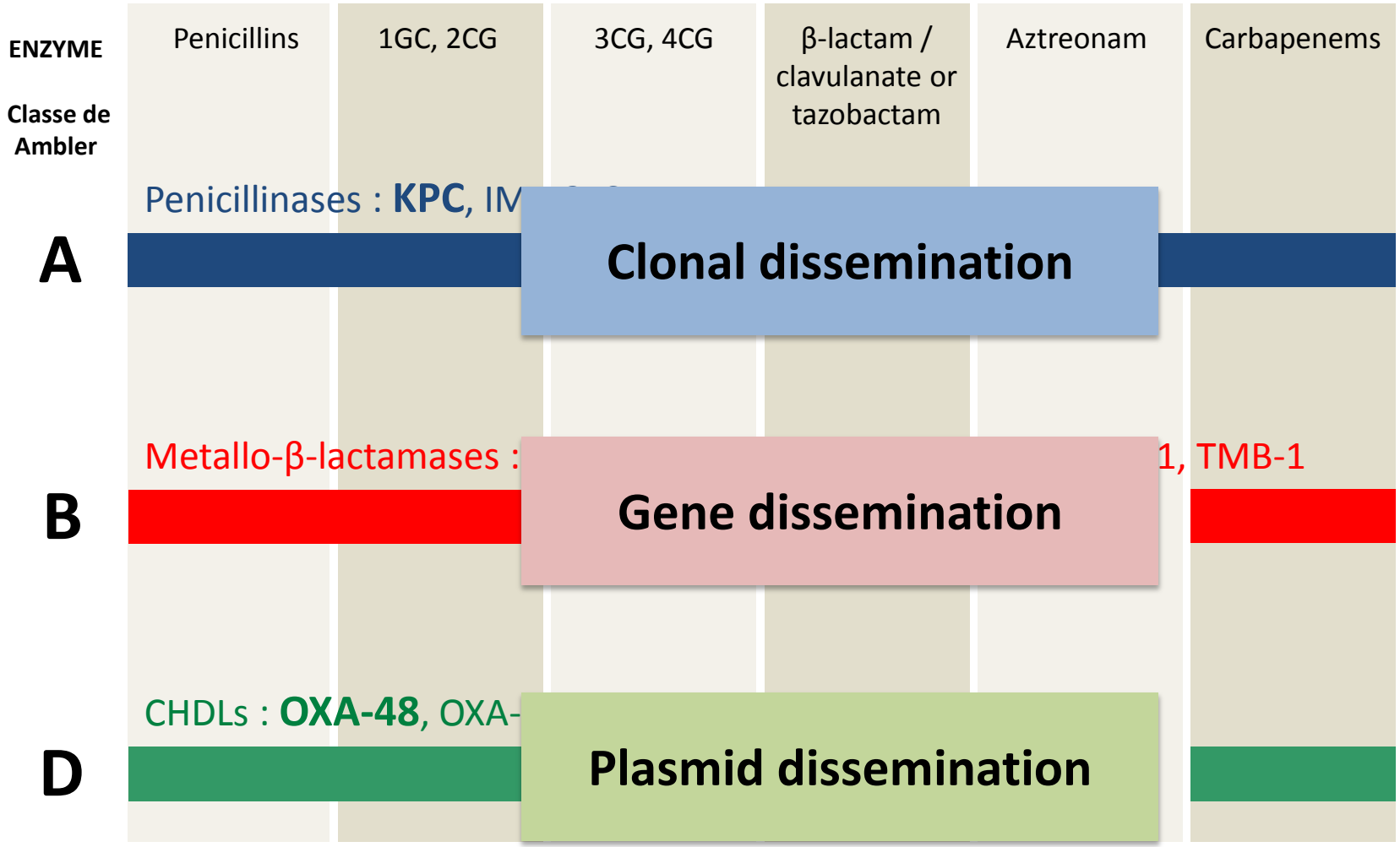
   a) From a clinical sample (infection)

   b) Screening of colonized patients

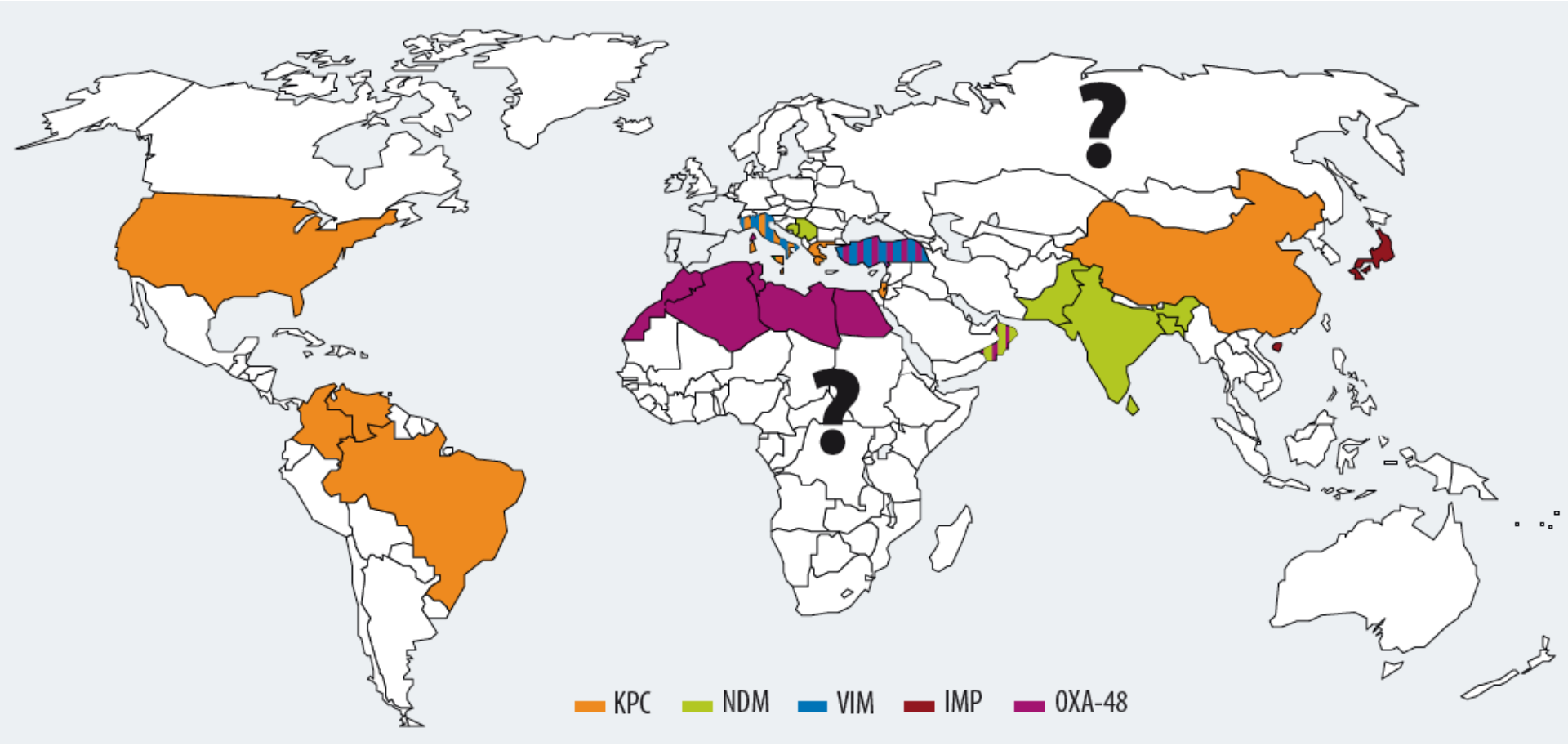
Carbapenemases in Enterobacteriaceae



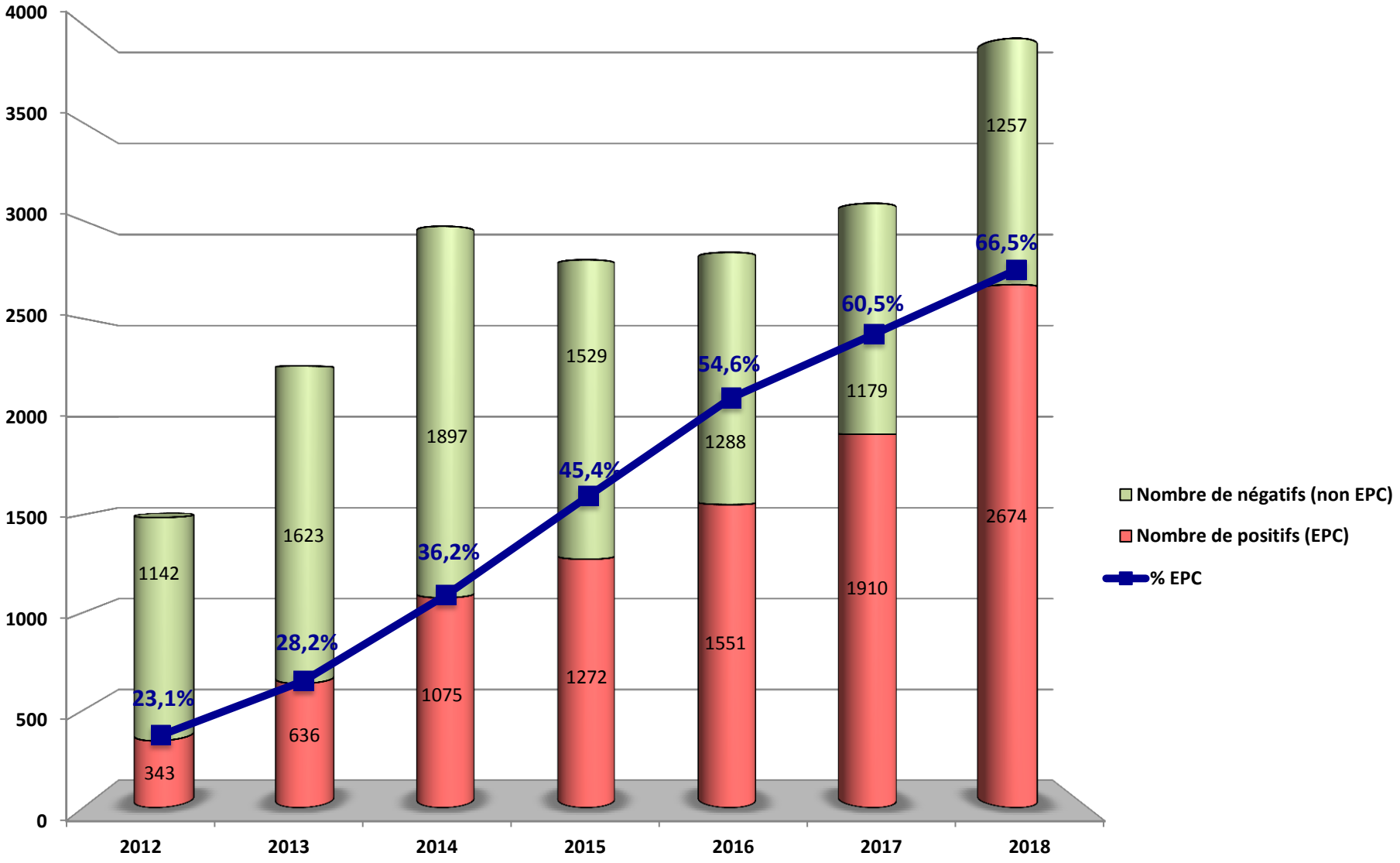
Carbapenemases in Enterobacteriaceae



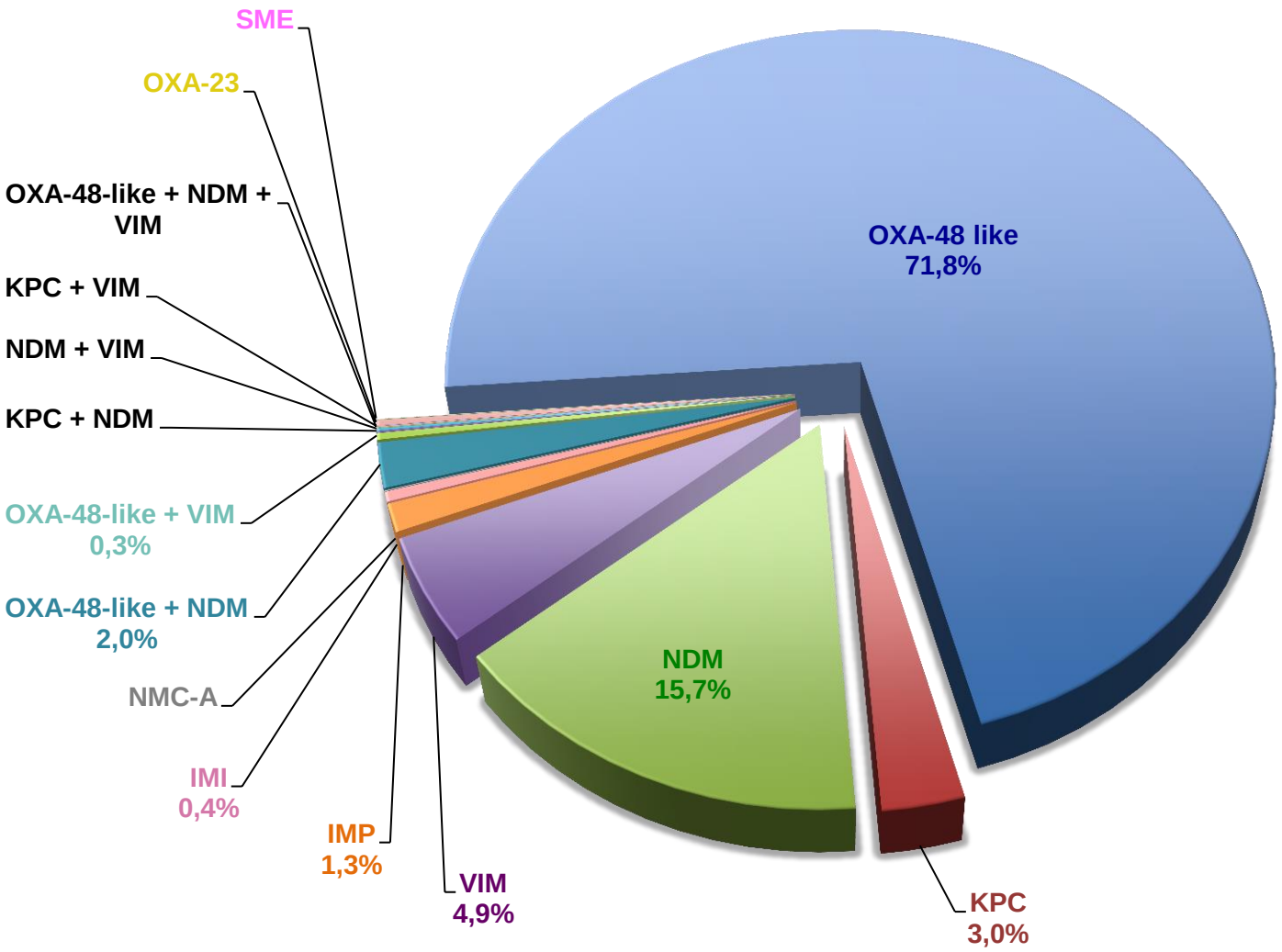
Global epidemiology of carbapenemase-producing *Enterobacteriaceae*



French epidemiology of carbapenemase-producing *Enterobacteriaceae*

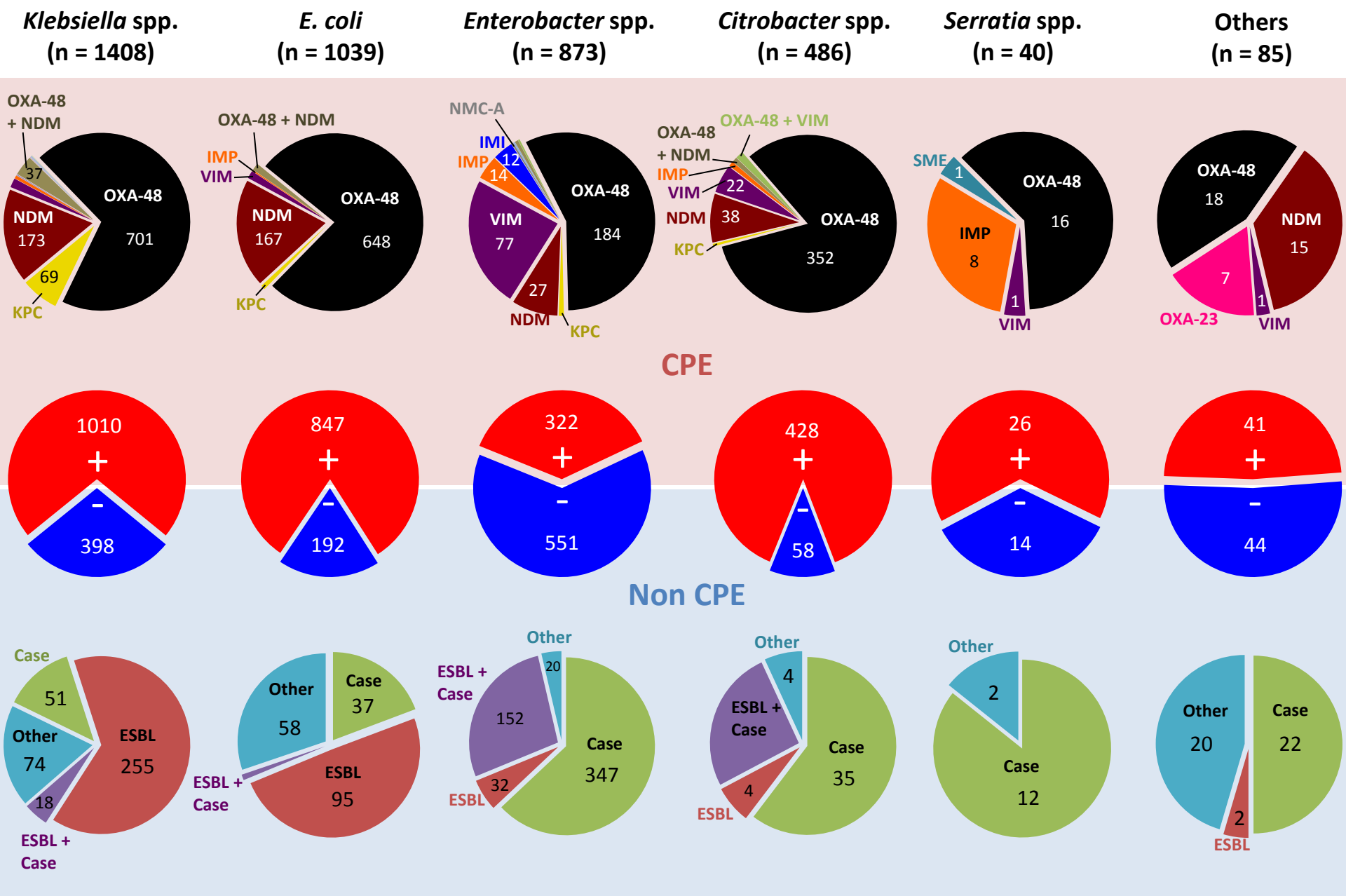


Distribution per carbapenemases type in 2018



Carbapenemase type	n
OXA-48-like	1919
KPC	81
NDM	420
VIM	130
IMP	34
IMI	12
NMC-A	1
OXA-48-like + NDM	54
OXA-48-like + VIM	8
KPC + NDM	2
NDM + VIM	3
OXA-48-like + NDM + VIM	1
KPC + VIM	1
OXA-23	7
SME	1
	2674

Carbapenem resistance mechanisms per bacterial species



SUMMARY

1) Epidemiology and dissemination of carbapenemase producing Enterobacteriaceae (CPE)

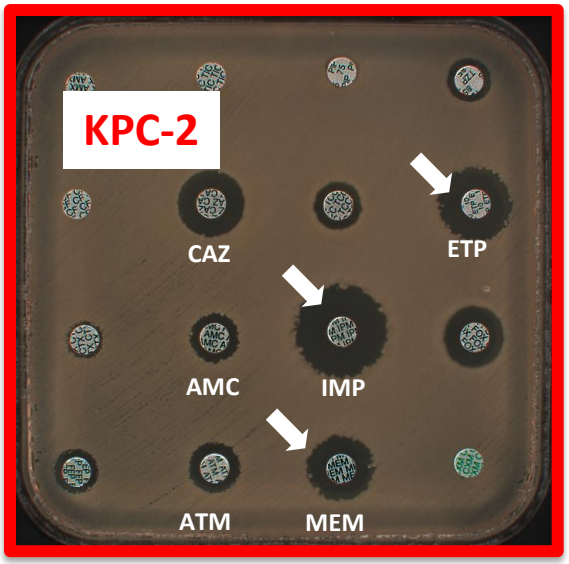
1) **CPE Detection methods**

   a) From a clinical sample (infection)

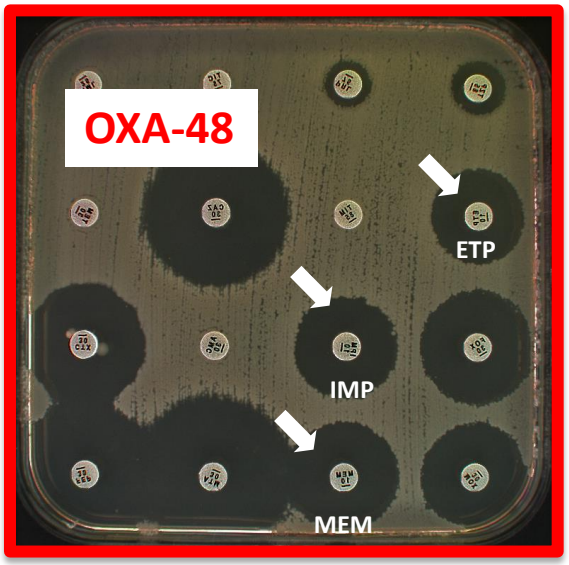
   b) Screening of colonized patients

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

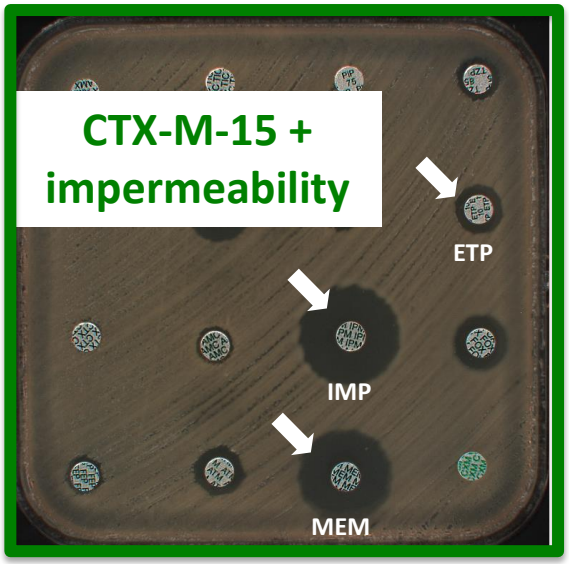
K. pneumoniae 1



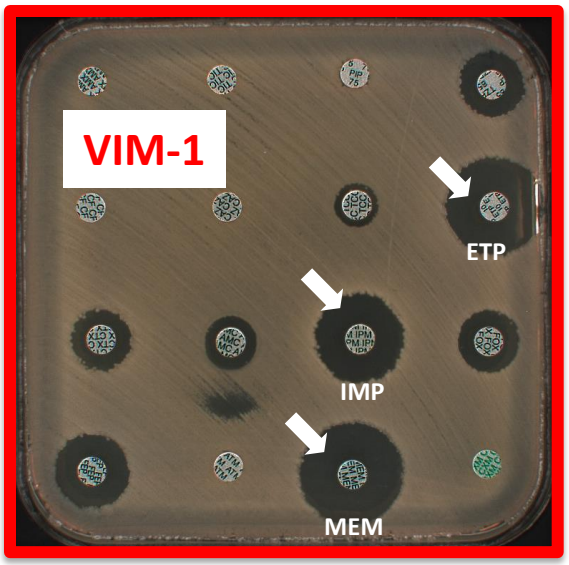
K. pneumoniae 2



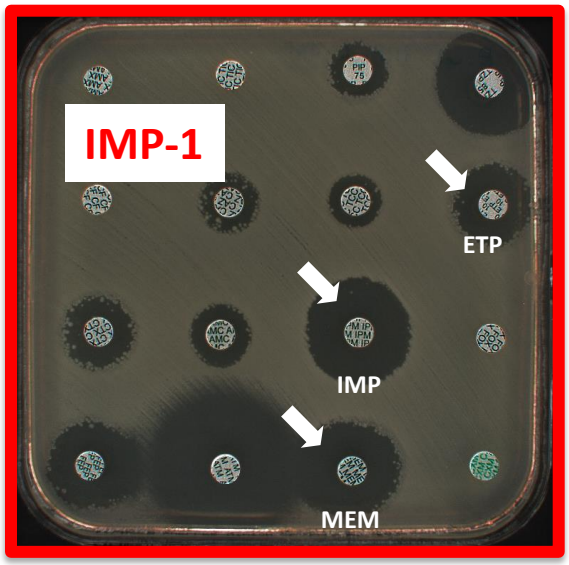
K. pneumoniae 3



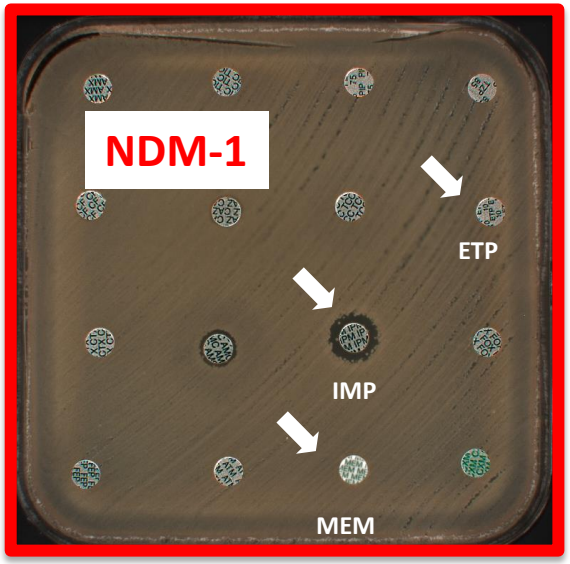
E. coli A



E. coli B



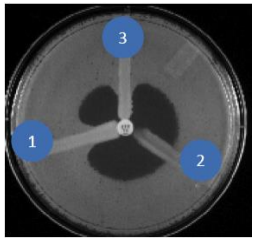
E. coli C



Methods of CPE detection : Phenotypic test

Phenotypic

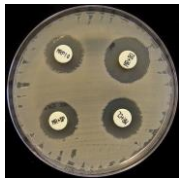
Hodge test



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

Inhibition tests

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



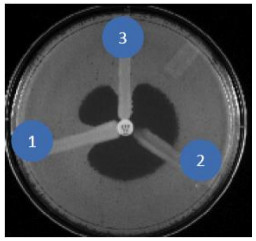
- ☑ Easy to perform
- ☑ Cheap
- ☒ Lack of sensitivity and specificity
- ☒ Not « rapid » (24h delay)

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Phenotypic

Hodge test



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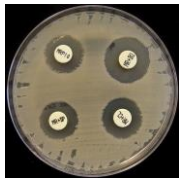
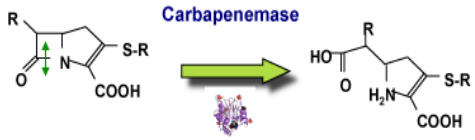


Carbapenem -hydrolysis

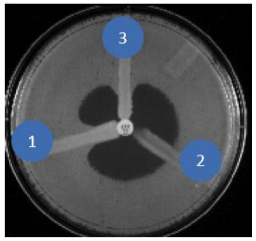


Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Phenotypic

Hodge test



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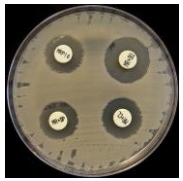
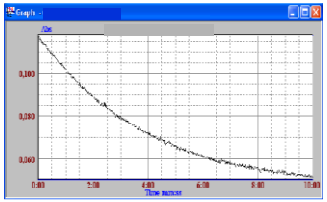


- ☑ Detect all carbapenemases
- ☒ Required experienced staff
- ☒ Specific material
- ☒ Not « rapid » (24h delay)

Carbapenem -hydrolysis

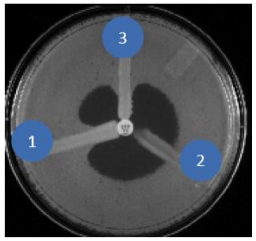
UV spectrophotometry



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

Methods of CPE detection : confirmation tests

Hodge test

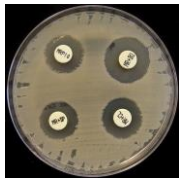


Girlich D. JCM 2012, **24h**

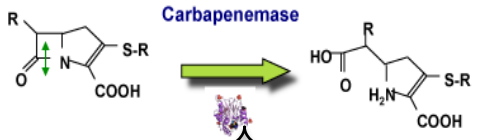
Phenotypic

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

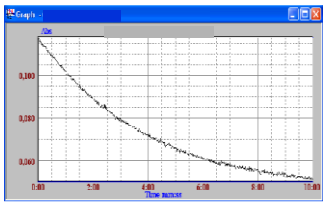
Inhibition tests



Carbapenem -hydrolysis

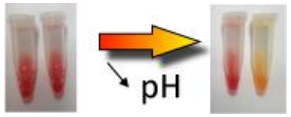


UV spectrophotometry



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

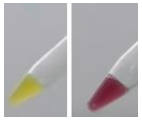
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

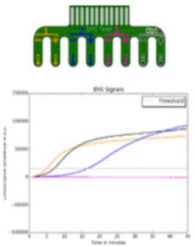
Dortet. JAC 2015, **2h**

β-Carba



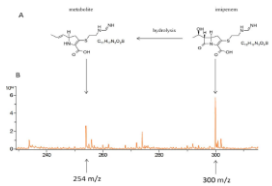
Bernabeu. JAC 2017 **<30'**

PH-metry



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

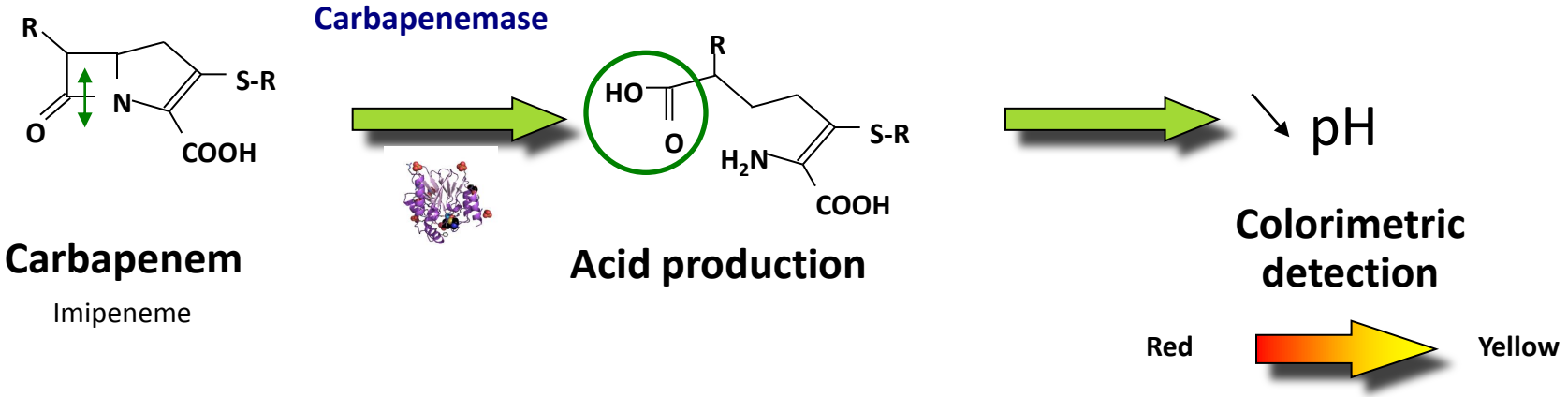
MALDI-TOF



Tandé D. JCM 2015, **30'**

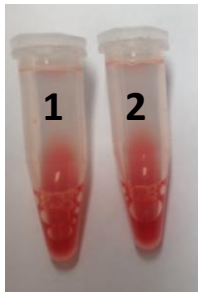
Carba NP test

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

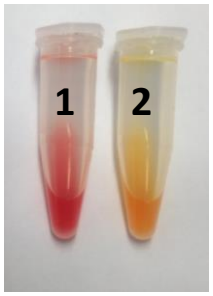


Interpretation:

No carbapenemase



Carbapenemase production

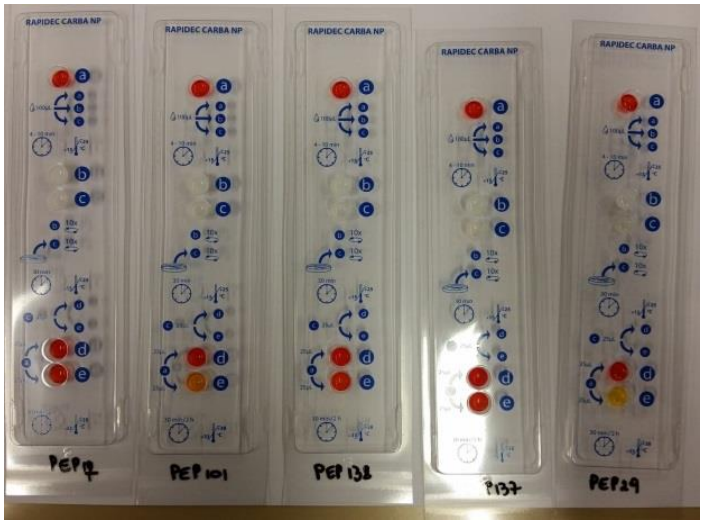


1 : Reveal solution
(internal negative control)

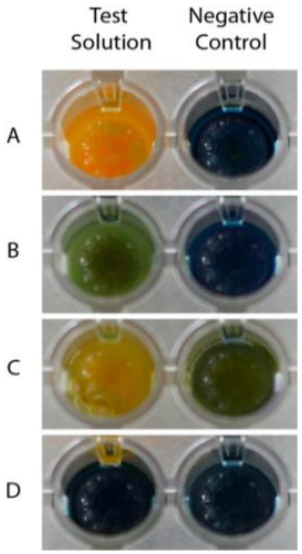
2 : Reveal solution + imipenem

Versions dérivées et versions commerciales du Carba NP test

RAPIDEC® CARBA NP (bioMérieux)

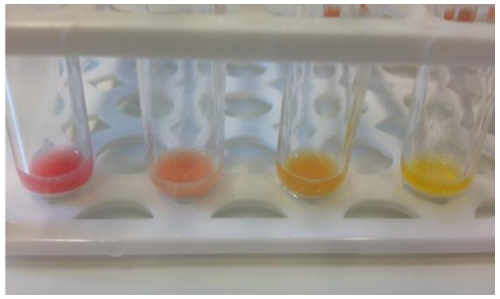


Blue Carba



Pires et al., JCM 2013
Pasteran, JCM 2015

RAPID CARB Screen® (ROSCO)



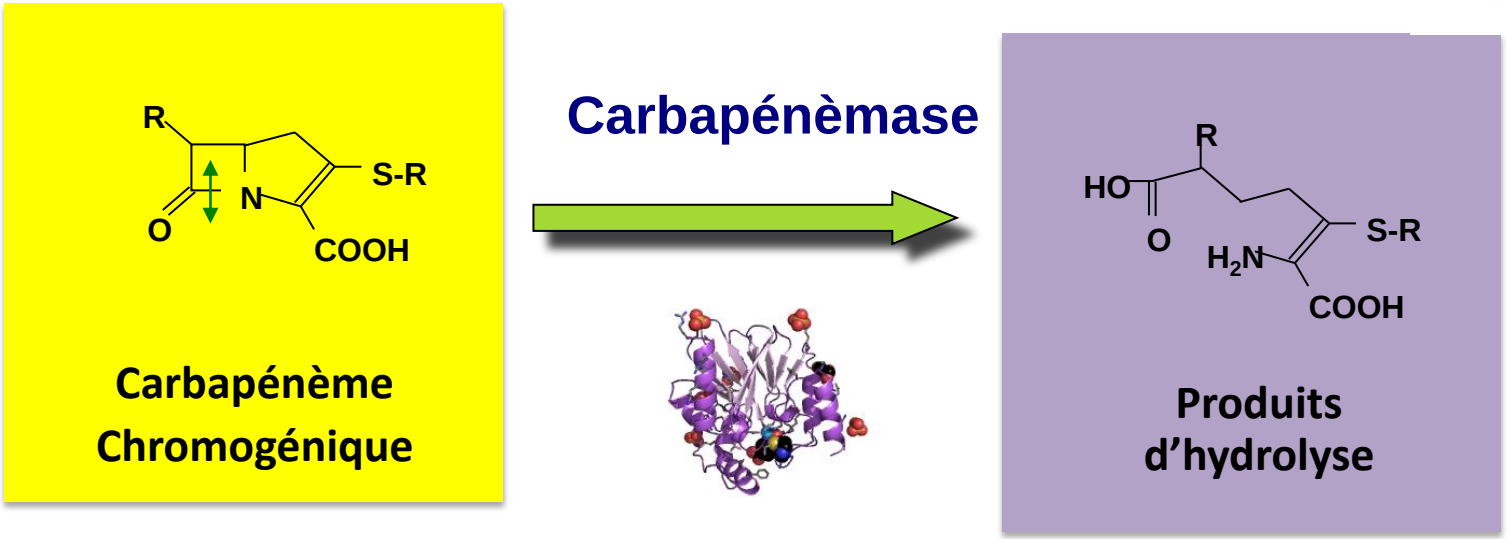
Neo RAPID CARB Screen® (ROSCO)

Evaluations Carba NP test et dérivés

Test	Company	n	Sen %	Spe %	PPV %	NPV %	NI %	reference
Carba NP test	-	235	97	100	100	95	0	Huang, JCM 2014
	-	150	96.8	100	-	-	0	Dortet, JAC 2015
	-	31	100	100	-	-	0	Denis, ECCMID 2015
RAPIDEC CARBA NP	bioMérieux	110	99	97	-	-	0	Poirel, ECCMID 2015
		150	99	100	-	-	0	Dortet, JAC 2015 submitted
Rapid CARB screen	ROSCO	235	98	83	81	95	11.8	Huang, JCM 2014
		150	89.5	70.9	-	-	16.7	Dortet, JAC 2015
Neo Rapid CARB screen	ROSCO	31	66.7	50	-	-		Denis, ECCMID 2015

β-CARBA test

Principe: Hydrolyse in vitro d'un carbapénème chromogénique



Avantages:

- Simple
- Rapide (30 min)

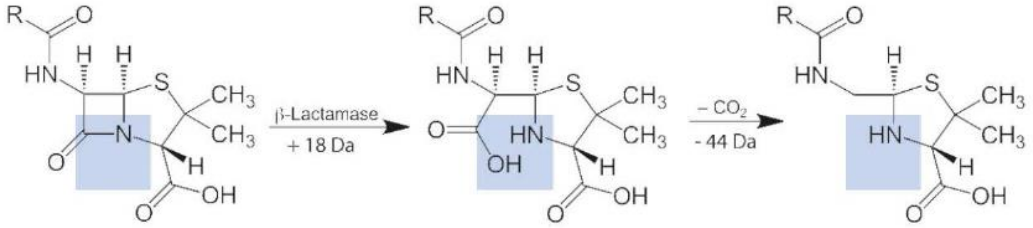
Inconvénients:

- **Absence de détection** des enzymes de type **IMI**, NmcA, SME
- Non utilisable directement à partir du prélèvement rectal
- Difficulté de lecture pour quelques souches OXA-48

Spectrométrie de masse : MALDI-TOF

Hydrolysis of β-lactam's ring leads to **mass shift** that can detected easily by MALDI-TOF MS

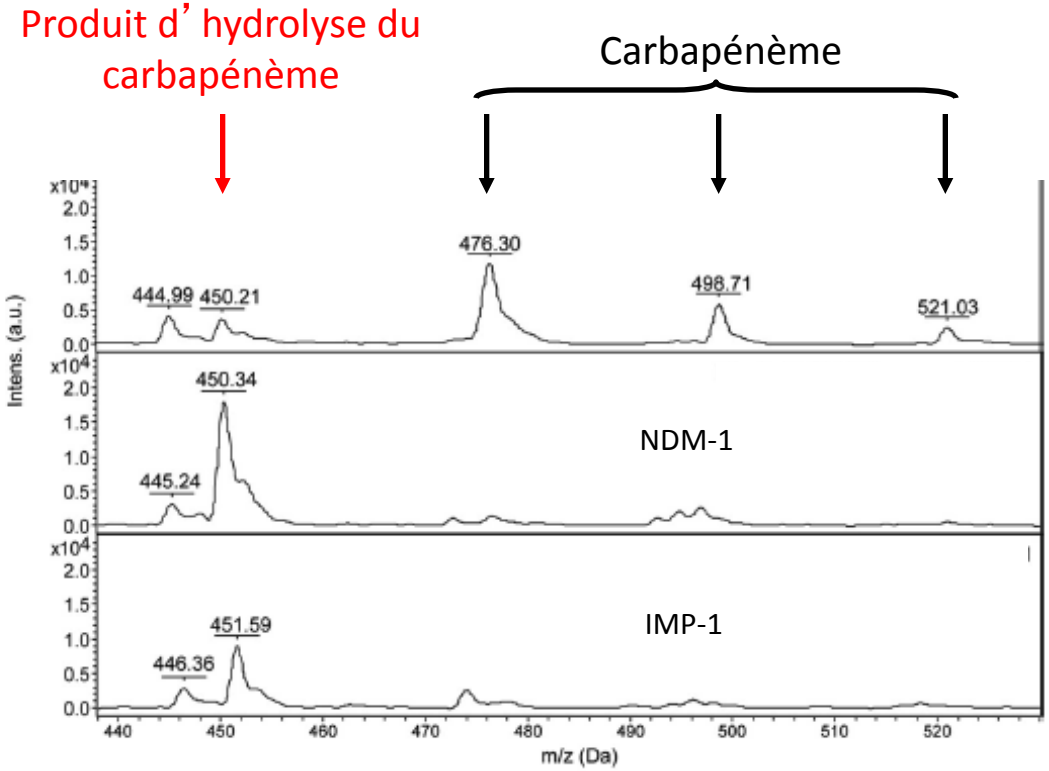
Hrabák et al. JCM. 2011
Burckhardt et al. JCM. 2011
Hrabák et al. JCM. 2012
Lasserre et al. JCM 2015



En pratique :

1) Bouillon contenant la bactérie à étudier + carbapénème : 20 min – 4h incubation
2) Spectromètre de masse

3) Si carbapénémase + : hydrolyse du carbapénème et apparition d'un produit de dégradation

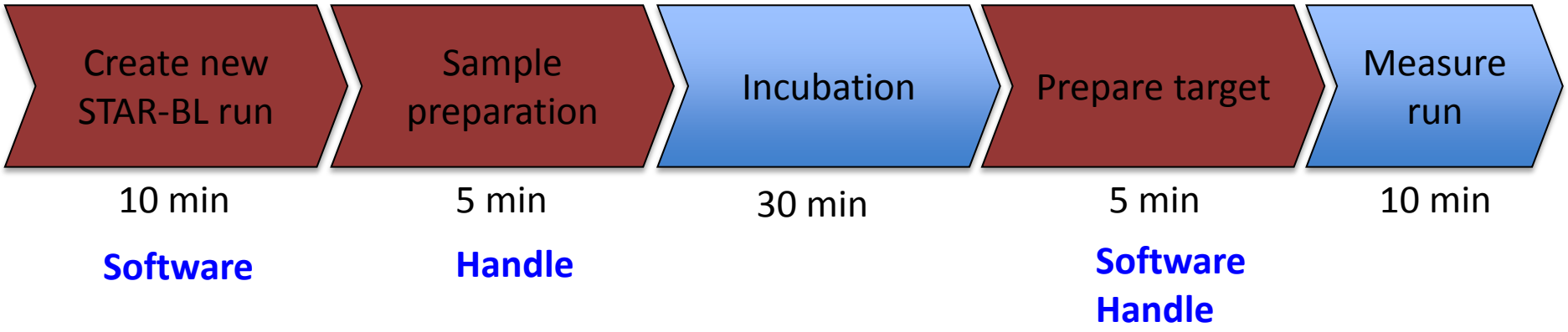


Spectrométrie de masse : MALDI-TOF

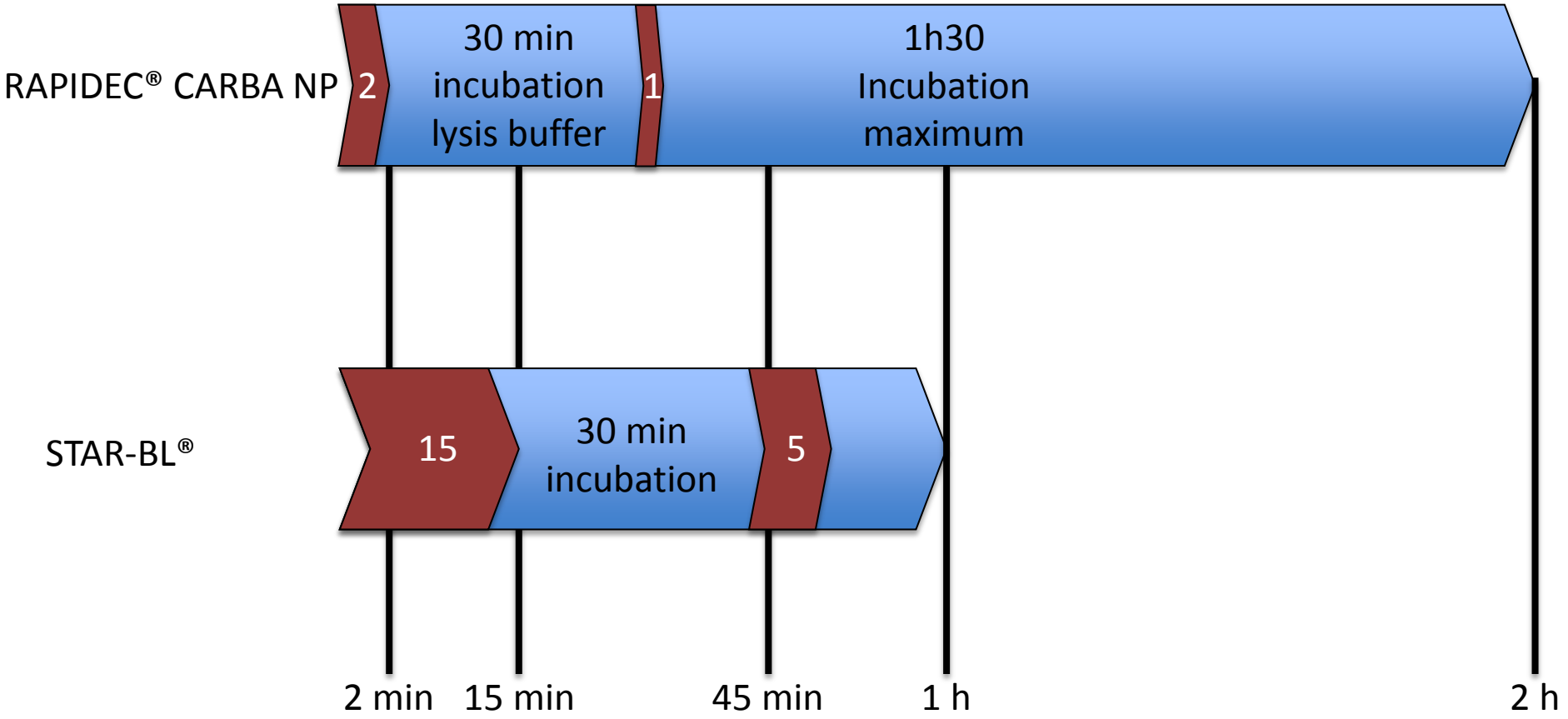
- **MALDI-TOF techniques** are reliable tools for the **rapid detection of CPE**

- Global performances :
  - **Sensitivity 96.5% to 100 %**
  - **Specificity 100 %**

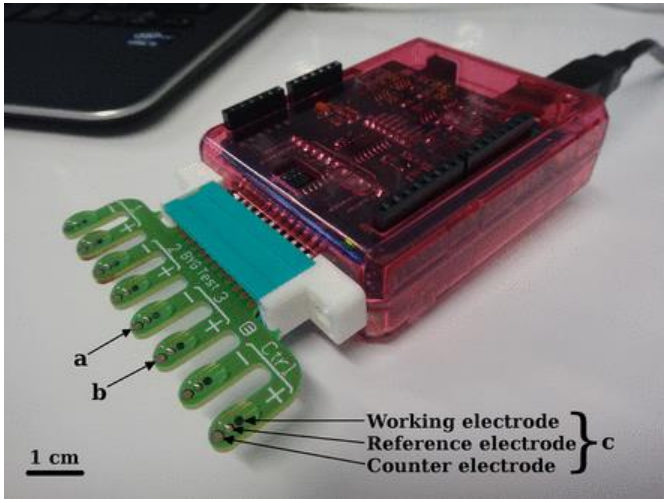
- Advantages of the **MALDI Biotyper STAR-BL®** are :
  - **Concomitant identification** of the bacterial species
  - **The unique CE IVD** assay for MALDI-TOF detection of CPE
  - **No subjective interpretation**
  - **Cost for already MALDI-TOF users ???**



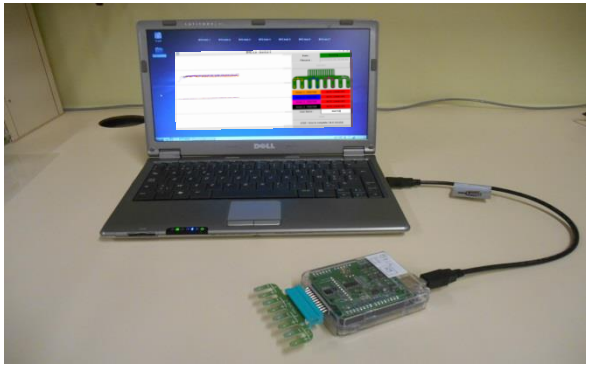
RESULTS : Workflow vs RAPIDEC® CARBA NP



BYG Carba test for CPE detection

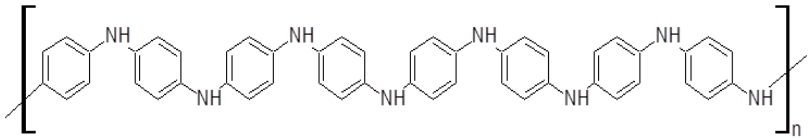


Sytème global incluant le software

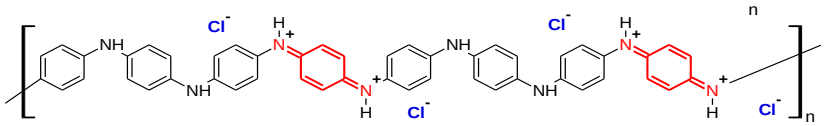


Détection Electrochimique de l'hydrolyse de l'imipénème

Senseur Polyaniline (PA)



Senseur Polyaniline



Mesure en temps réel des modifications de la conductivité de la Polyaniline

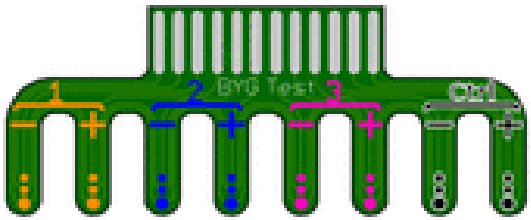
BYG Carba test for CPE detection

Mesure en temps réel des modifications de la conductivité de la polyaniline avec et sans imipénème

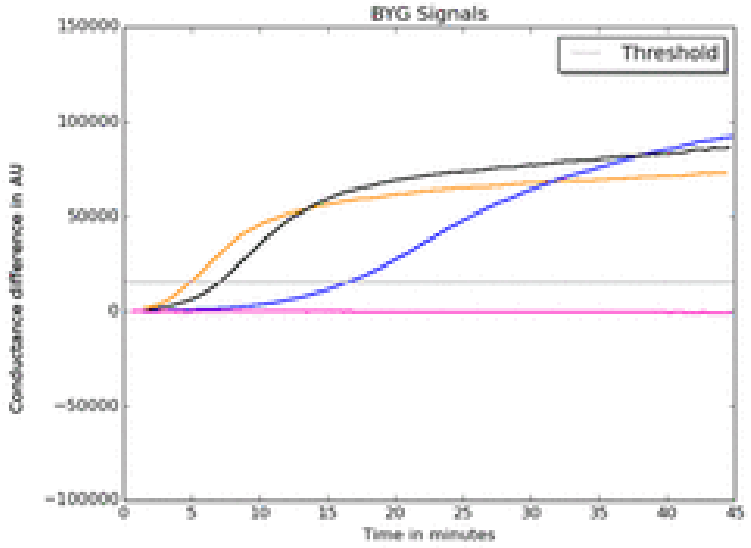
BYG Test Report

CHU Dinant Godinne UCL NAMUR

DATE : 18/03/2015
TIME : 08:46:02
User Name : MARTIN



Strain 1	:	NEQAS 1943	(POSITIVE)
Strain 2	:	NEQAS 1945	(POSITIVE)
Strain 3	:	CNR20150311	(NEGATIVE)
Strain 4 (Ctrl)	:	CNR20150325	(POSITIVE)



For any information concerning the BYG test please contact pierre.bogaert@uclouvain.be or wani.yunus@uclouvain.be

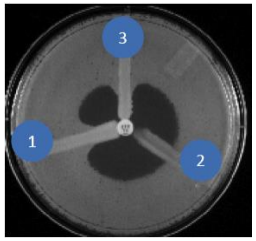
Bogaert *et al*. Evaluation of the BYG Carba Test, a New Electrochemical Assay for Rapid Laboratory Detection of Carbapenemase-Producing Enterobacteriaceae. J Clin Microbiol. 2016 Feb;54(2):349-58.

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

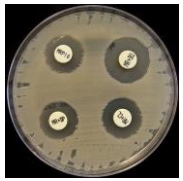
Phenotypic

Hodge test



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

Inhibition tests



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

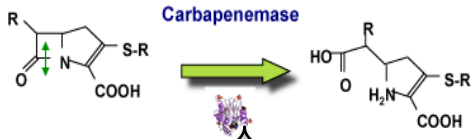
Immuno-chromatography

KPC
OXA-48,
NDM
(IMP
VIM)

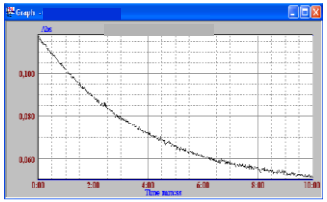
Bogaerts, JAC, 2016, in press 2017

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

Carbapenem -hydrolysis

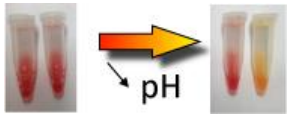


UV spectrophotometry



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

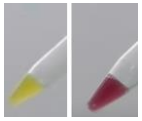
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

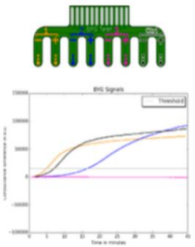
Dortet. JAC 2015, **2h**

β-Carba



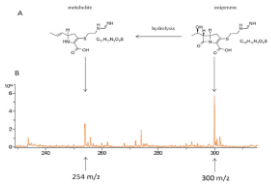
Bernabeu. JAC 2017 **<30'**

PH-metry



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

MALDI-TOF

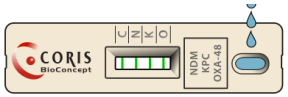


Tandé D. JCM 2015, **30'**

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

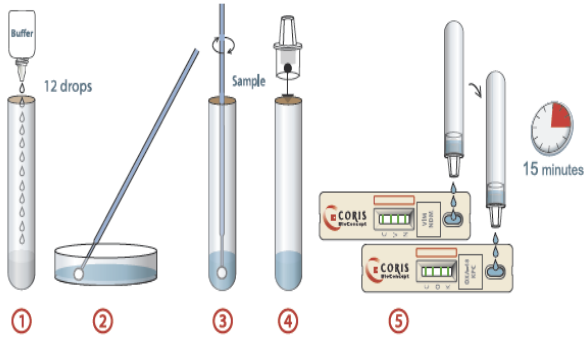
Coris Resist Oxa 48	OXA-48
Coris Resist KPC	KPC

Coris Resist-3 O.K.N.	OXA-48, KPC, NDM



Coris Resist-4 O.K.N.V.	OXA-48, KPC, NDM et VIM

Coris Resist-5 O.K.N.V.	OXA-48, OXA-163, KPC, NDM et VIM



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

Product	Specimen	Format	Packaging	Storage	Shelf-life	Cat. Ref.
NG-Test CARBA 5	Culture	Cassette	20 tests	4-30°C	24 months	NGB-CAR-S23-002

NG-Test CARBA 5

CE Q1 2018

Performance Characteristics

Detection limit

The detection limits were determined using purified recombinant enzymes:

NDM	150pg/mL
IMP	200pg/mL
VIM	300pg/mL
OXA	300pg/mL
KPC	600pg/mL

Validation on a reference strain bank

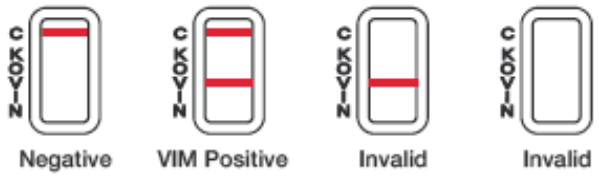
NG-Test CARBA 5 was evaluated on 167 clinical strains at the CNR of CHU Kremlin Bicêtre - Paris - France (AMR French Referent Center). Considering the carbapenemases targeted all the results were correlated with the genotype of the strains determined by PCR analysis.

Status	Positive	Negative	Total
Positive	116	0	116
Negative	0	51	51
Total	116	51	167

Sensitivity : 100% Confidence interval : 94,8% to 100%
Specificity : 100% Confidence interval : 96,5% to 100%

The NG test CARBA 5 detects at least the following variants:
NDM-1 / 4 / 5 / 6 / 7 and 9
KPC-2 and 3
IMP-1 / 8 and 11
VIM-1 / 2 / 4 and 19
OXA-48 / 181 / 204 / 232 /244 / 517 / 519 and 535
But also OXA-163 and OXA-405 (OXA-48-like extended spectrum oxacillinases)

Interpretation

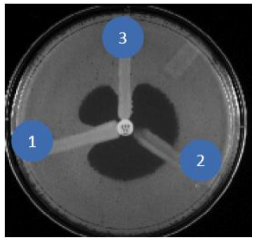


NOTE: Multiple lines or one line on K, O, V, I, N position must be considered as a positive result

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Hodge test

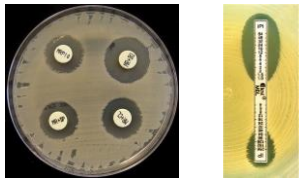


Girlich D. JCM 2012, **24h**

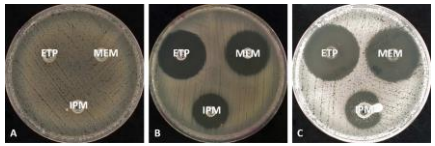
Phenotypic

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

Inhibition tests



CIM test



Gauthier, PlosOne, 2017
24h

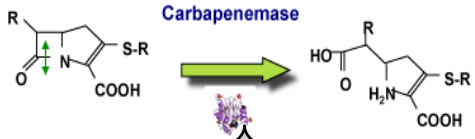
Immuno-chromatography

KPC
OXA-48,
NDM
(IMP
VIM)

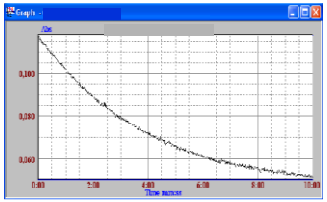
Bogaerts, JAC, 2016, in press 2017

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

Carbapenem -hydrolysis

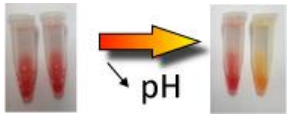


UV spectrophotometry



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

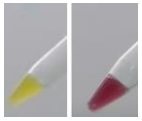
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

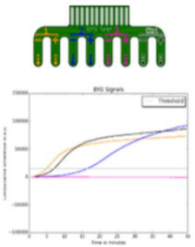
Dortet. JAC 2015, **2h**

β-Carba



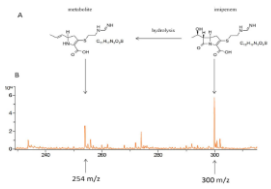
Bernabeu. JAC 2017 **<30'**

PH-metry



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

MALDI-TOF



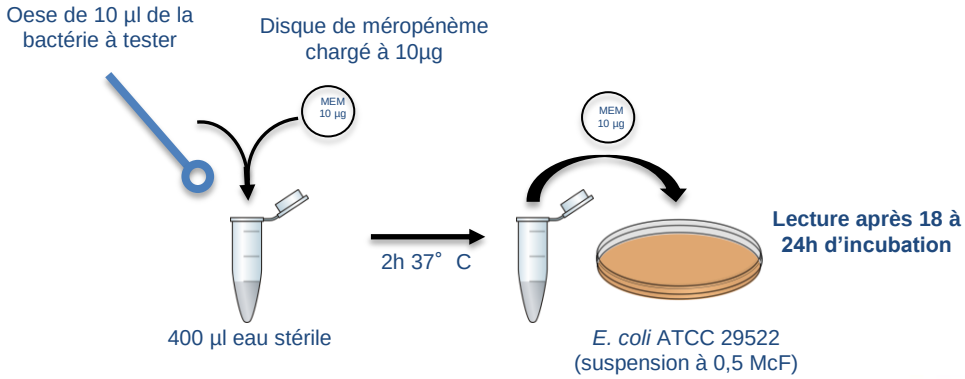
Tandé D. JCM 2015, **30'**

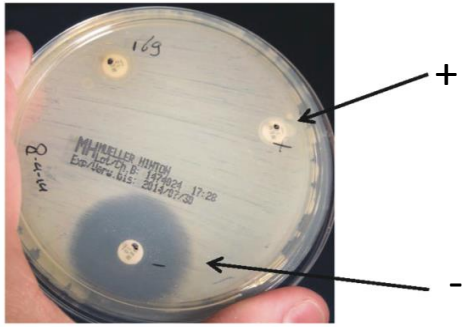
rCIM

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

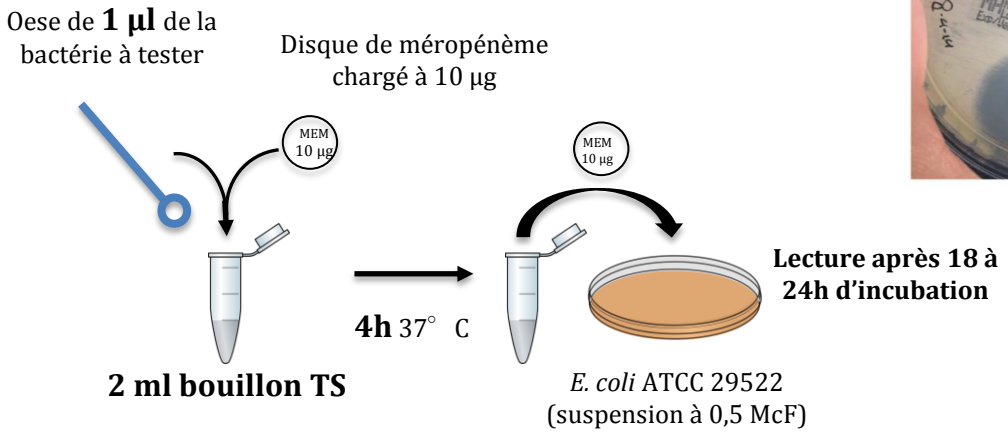
CIM tests et dérivés

CIM test





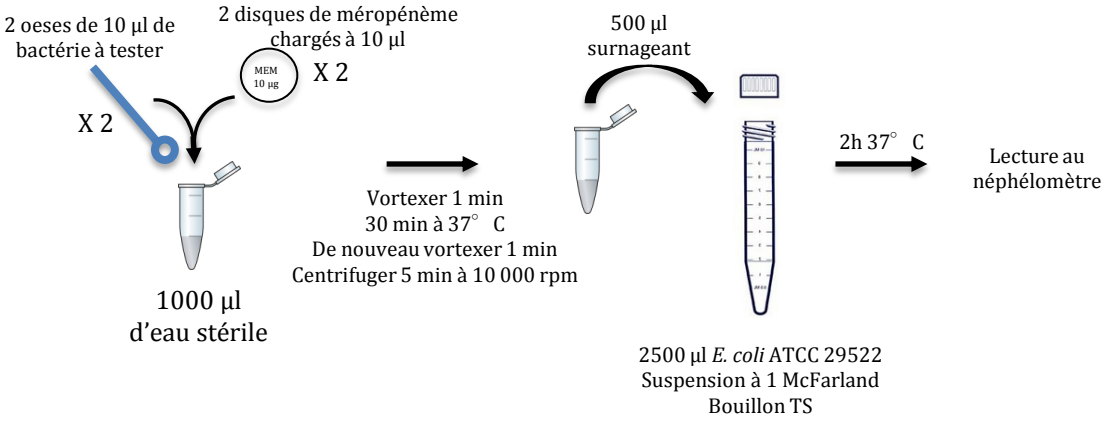
mCIM test



Meilleure sensibilité sur OXA-48-like

CIM tests et dérivés

rCIM (rapid CIM)



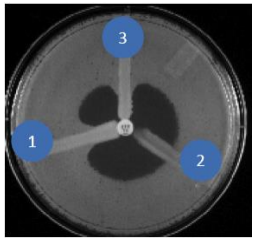
Croissance souche ATCC < 0,5 McF : absence de carbapénèmase
Croissance souche ATCC > 1 McF : présence d'une carbapénèmase
Croissance souche ATCC entre 0,5 et 1 McF : tests complémentaires

Methods of CPE detection : confirmation tests

CNR RÉSISTANCE AUX ANTIBIOTIQUES

Phenotypic

Hodge test



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

Girlich D. DMID 2013
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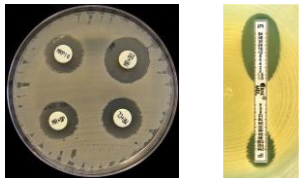
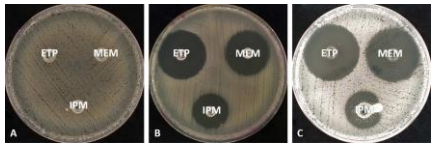
24h

Inhibition tests



CIM test



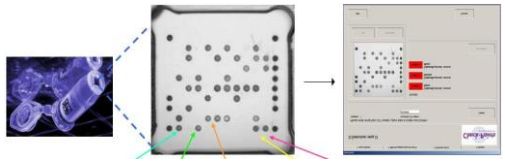
Gauthier, PlosOne, 2017
24h

Molecular

PCR et RT-PCR

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

DNA micro-array



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

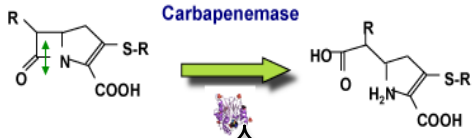
Immuno-chromatography

KPC
OXA-48,
NDM
(IMP
VIM)

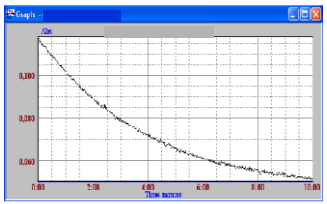
Bogaerts, JAC, 2016, in press 2017

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

Carbapenem -hydrolysis

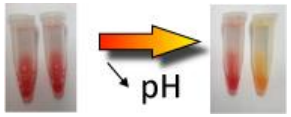


UV spectrophotometry



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

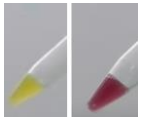
Colorimetry



Dortet, AAC, 2012
Pires J, JCM, 2013

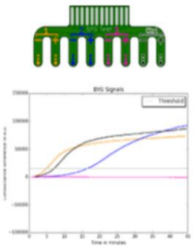
Dortet. JAC 2015, **2h**

β-Carba



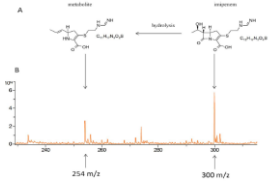
Bernabeu. JAC 2017 **<30'**

PH-metry



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

MALDI-TOF



Tandé D. JCM 2015, **30'**

rCIM

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

SUMMARY

1) Epidemiology and dissemination of carbapenemase producing Enterobacteriaceae (CPE)

1) **CPE Detection methods**

   a) From a clinical sample (infection)

   b) Screening of colonized patients

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]

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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.

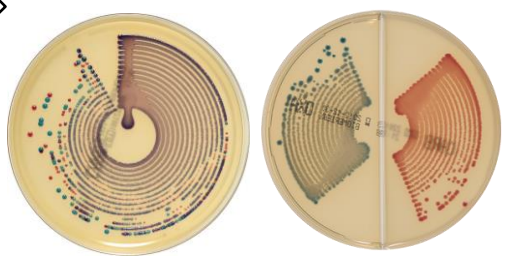
CPE colonisation detection

Stool, Rectal swabs

24-48h

Enrichment culture

Selective Chromogenic media

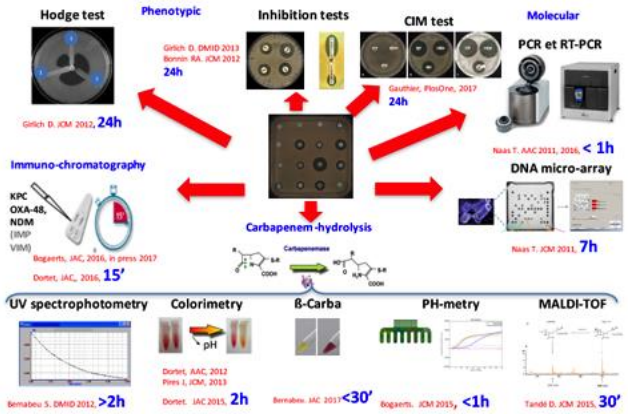


Girlich D. DMID. 2013
Réglier-Poupet H. JMM. 2008
Cuzon G. JCM. 2008

1-2h

Single- or Multi-plex Real time PCR

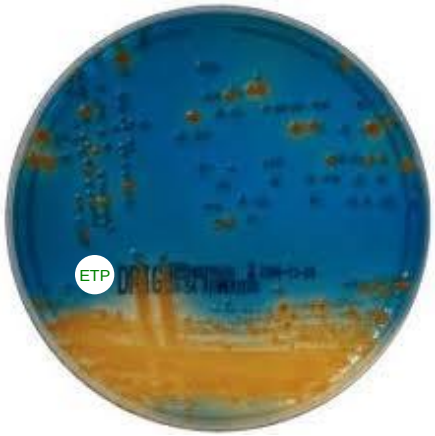
Naas T. AAC. 2013
Naas T. AAC. 2011
Oxacelay C. JAC. 2009
Hoyos, IJAA, 2017



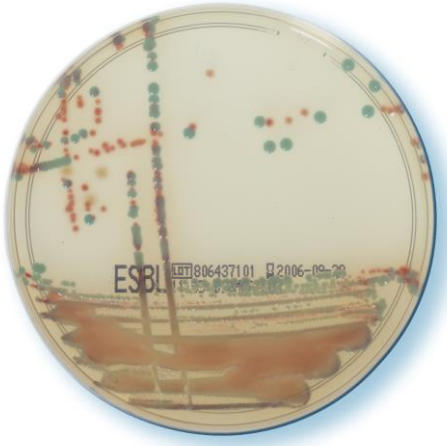
« Old » screening media

Before 2013

Drigalski + ertapenem disc



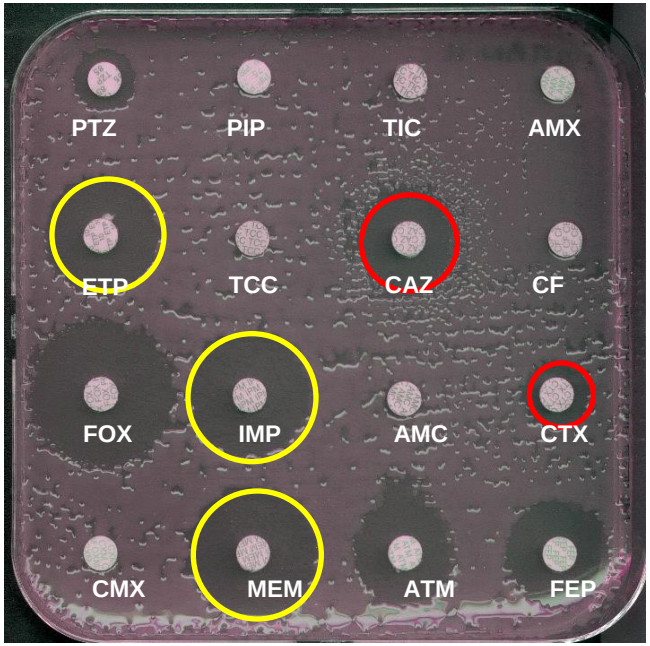
3CG supplemented media



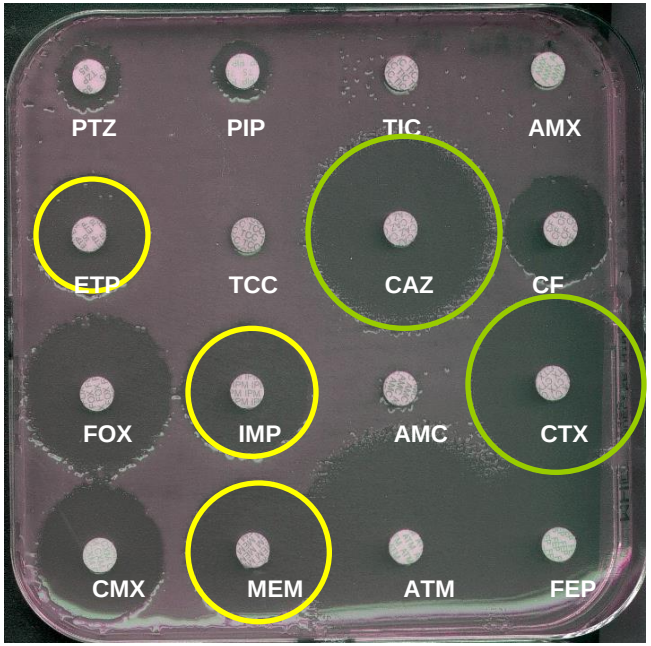
Ex : ChomID ESBL

3CG supplemented media

No detection of OXA-48 producer if no ESBL



K. pneumoniae OXA-48
BLSE +



K. pneumoniae OXA-48
BLSE -

New generation screening media

Carbapenem supplemented media

ChromID® CARBA

Brilliance CRE

- ☑ KPC
- ☑ NDM
- ☑ VIM
- ☑ IMP
- ☒ OXA-48

New generation screening media

Carbapenem supplemented media

ChromID® CARBA

- ☑ KPC
- ☑ NDM
- ☑ VIM
- ☑ IMP
- ☒ OXA-48

Brilliance CRE

Temocillin supplemented medium

ChromID® OXA

- ☒ KPC
- ☒ NDM
- ☒ VIM
- ☒ IMP
- ☑ OXA-48

New generation screening media

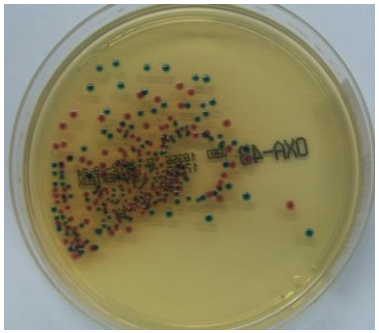
Carbapenem supplemented media

ChromID® CARBA

- ☑ KPC
- ☑ NDM
- ☑ VIM
- ☑ IMP
- ☒ OXA-48

Brilliance CRE

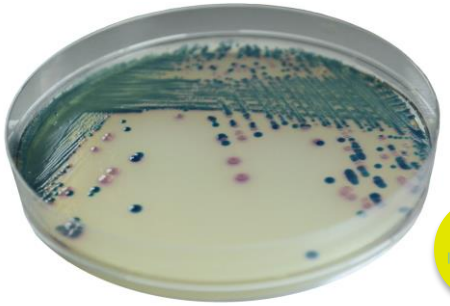
Temocillin supplemented medium



ChromID® OXA

- ☒ KPC
- ☒ NDM
- ☒ VIM
- ☒ IMP
- ☑ OXA-48

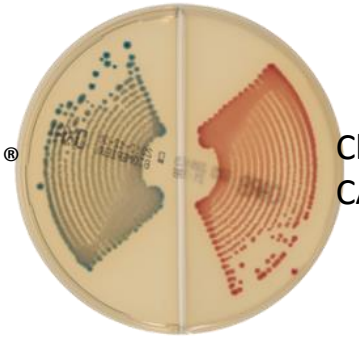
Optimal performance developed media



CHROMagar™ mSuperCARBA™

- ☑ KPC
- ☑ NDM
- ☑ VIM
- ☑ IMP
- ☑ OXA-48

ChromID® OXA-48

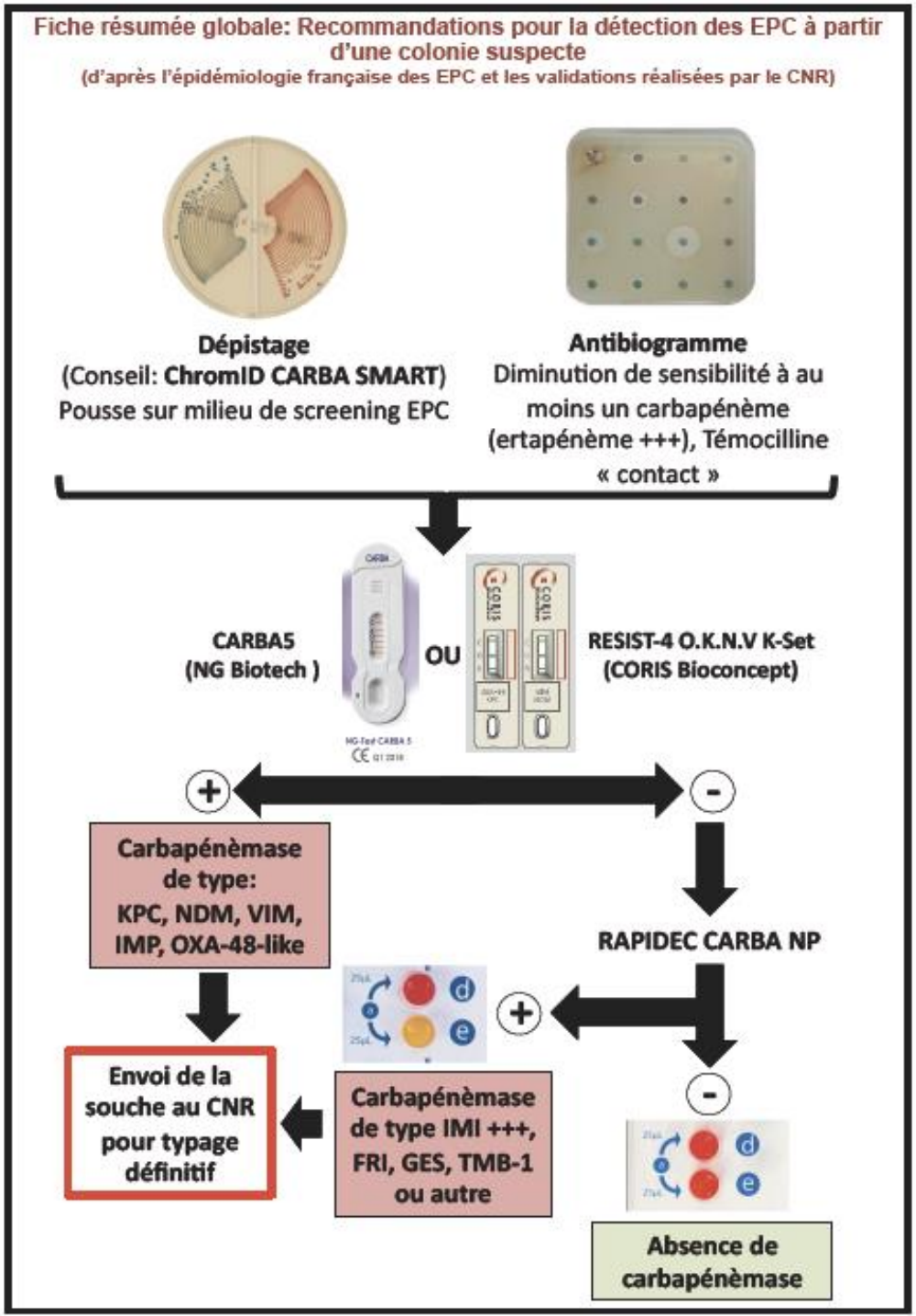


ChromID® CARBA

ChromID® CARBA SMART

CNR
RÉSISTANCE AUX ANTIBIOTIQUES

Version 2018



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

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 and the risk assessment

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

CNR RÉSISTANCE AUX ANTIBIOTIQUES

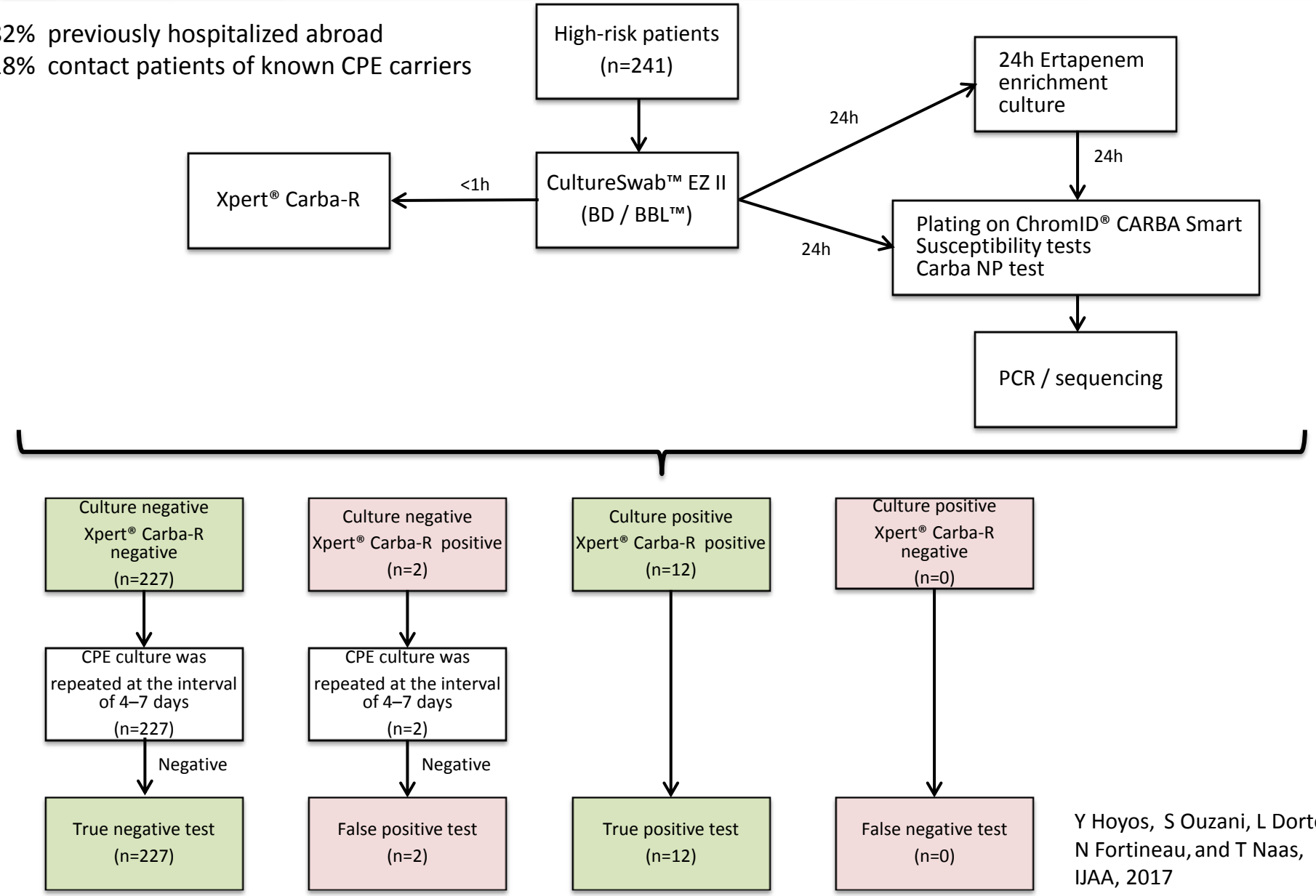
- **150 enterobacterial isolates** including 130 isolates with decreased susceptibility to at least one carbapenem)
- **61 non-carbapenemase** producers
- **89 carbapenemases** producers :

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

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

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	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)
=> 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

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			3 carrier)
9			
10		1	
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?

⇒ nothing? No diffusion to other 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 +++**

Acknowledgements

- Dr Agnès Jousset
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