

# Evaluation du BD Phoenix CPO Detect pour la détection et la classification des carbapénémases chez les Gram négatifs

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**CORATA**  
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# WHO : Priority pathogens



## WHO PRIORITY PATHOGENS LIST FOR R&D OF NEW ANTIBIOTICS

### Priority 1: CRITICAL<sup>#</sup>

*Acinetobacter baumannii*, carbapenem-resistant  
*Pseudomonas aeruginosa*, carbapenem-resistant  
*Enterobacteriaceae*<sup>\*</sup>, carbapenem-resistant, 3<sup>rd</sup> generation cephalosporin-resistant

### Priority 2: HIGH

*Enterococcus faecium*, vancomycin-resistant  
*Staphylococcus aureus*, methicillin-resistant, vancomycin intermediate and resistant  
*Helicobacter pylori*, clarithromycin-resistant  
*Campylobacter*, fluoroquinolone-resistant  
*Salmonella spp.*, fluoroquinolone-resistant  
*Neisseria gonorrhoeae*, 3<sup>rd</sup> generation cephalosporin-resistant, fluoroquinolone-resistant

### Priority 3: MEDIUM

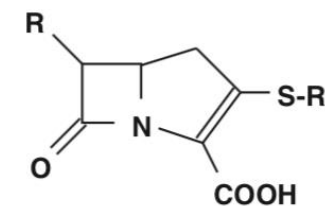
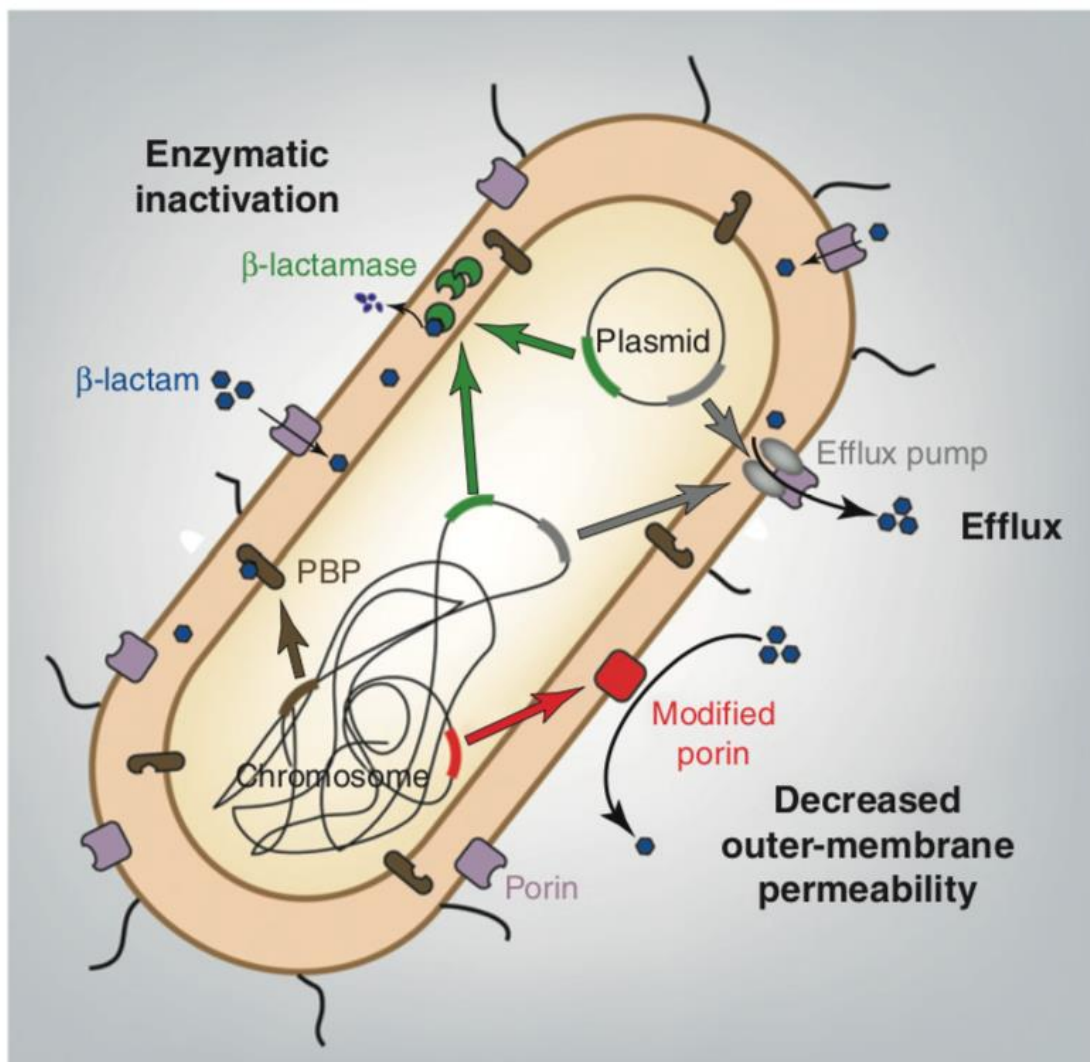
*Streptococcus pneumoniae*, penicillin-non-susceptible  
*Haemophilus influenzae*, ampicillin-resistant  
*Shigella spp.*, fluoroquinolone-resistant

<sup>#</sup> *Mycobacteria* (including *Mycobacterium tuberculosis*, the cause of human tuberculosis), was not subjected to review for inclusion in this prioritization exercise as it is already a globally established priority for which innovative new treatments are urgently needed.

### Priority 1: CRITICAL<sup>#</sup>

*Acinetobacter baumannii*, carbapenem-resistant  
*Pseudomonas aeruginosa*, carbapenem-resistant  
*Enterobacteriaceae*<sup>\*</sup>, carbapenem-resistant, 3<sup>rd</sup> generation cephalosporin-resistant

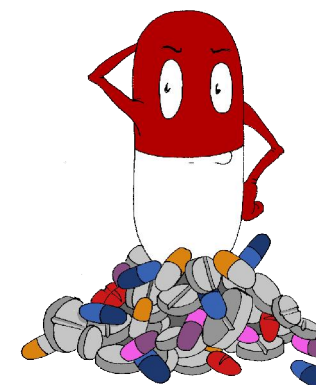
# Carbapenems Resistance



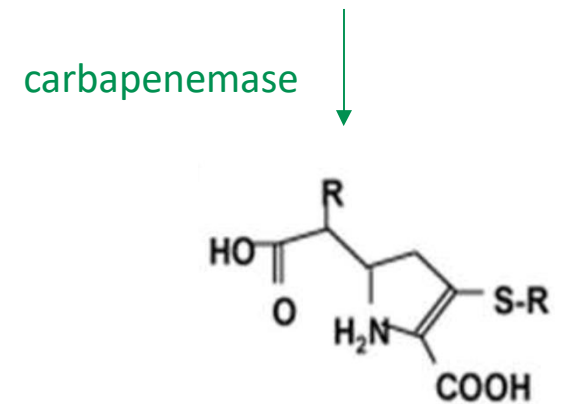
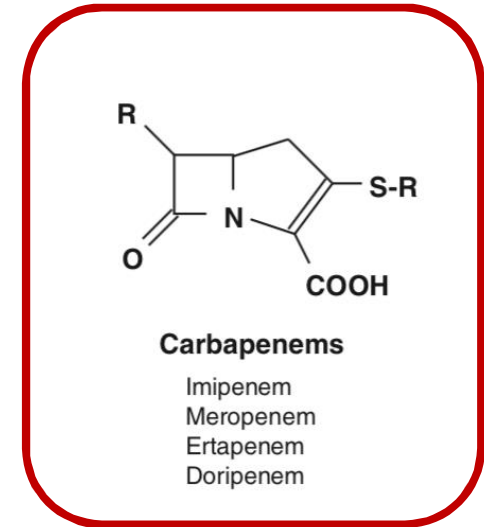
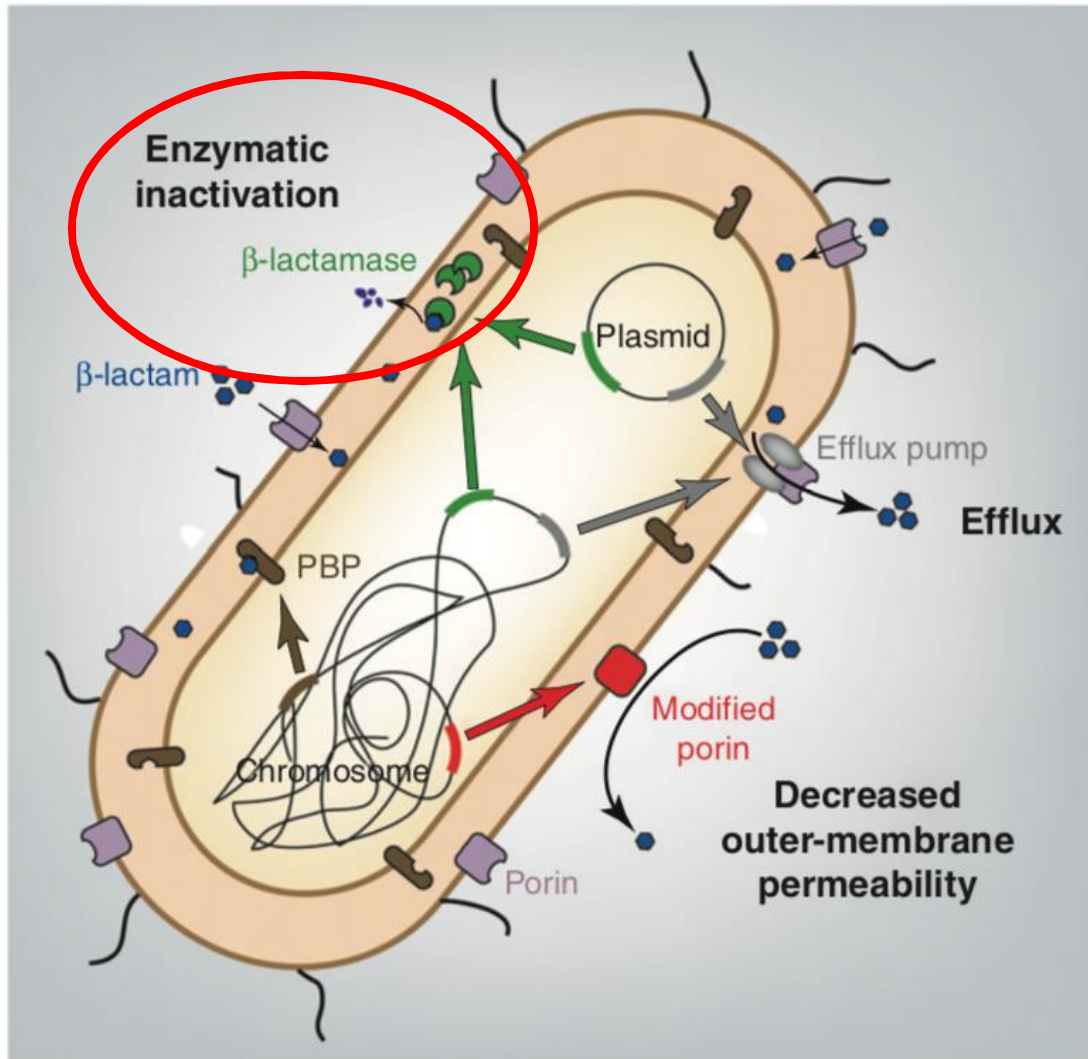
## Carbapenems

Imipenem  
Meropenem  
Ertapenem  
Doripenem

*TRENDS in Molecular Medicine*



# Carbapenemase Production



# Carbapenemase Production

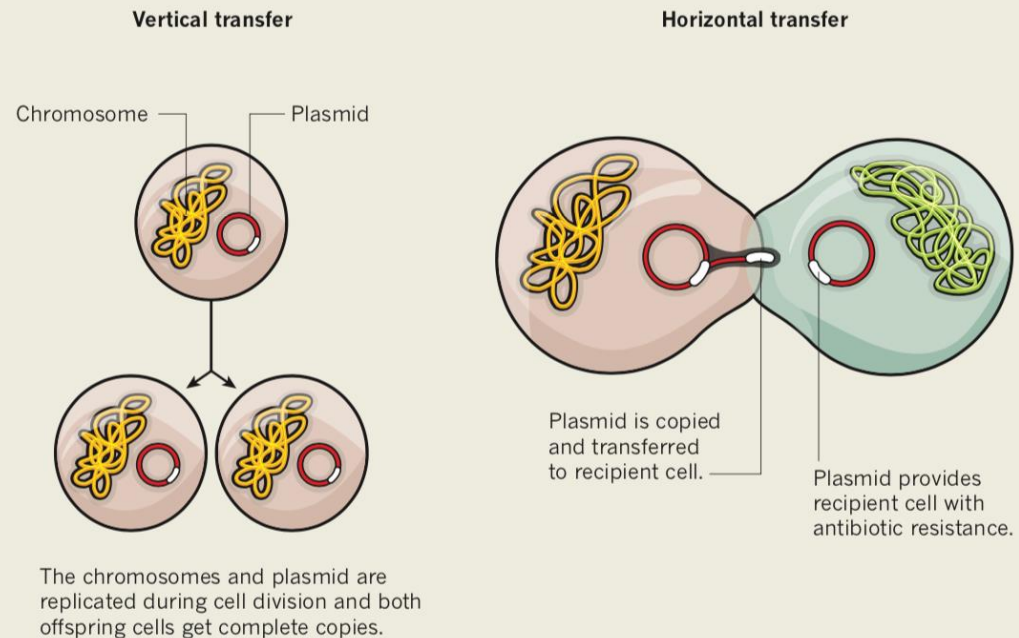


**Plasmid-mediated carbapenems resistance**  
↳ mobile genetic elements

- “ **Rapid dissemination of carbapenem resistance** among different bacterial strains and species.
- “ **Co-resistances** (aminoglycosides, fluoroquinolones..)

## TWO WAYS TO SPREAD

Vertical and horizontal transfer mechanisms can spread resistance to antibiotics in bacteria.



# Carbapenemase Production



**Plasmid-mediated carbapenems resistance**  
↳ mobile genetic elements

” **Rapid dissemination of carbapenem resistance** among different bacterial strains and species.

” **Co-resistances** (aminoglycosides, fluoroquinolones..)

- ⇨ Multi-resistance
- ⇨ Therapeutic alternatives limited
- ⇨ Poor pronostic and high morbidity (inappropriate treatment...)
- ⇨ Cross transmission to other patients

# Ambler classification of $\beta$ -lactamases

## Carbapenemases

Serine

Zinc  
(metallo- $\beta$ -lactamases)

Class A

Class C

Class D

Class B

**KPC**

GES  
SME  
IMI  
NMC-A  
...

**OXA-48**

OXA-23  
OXA-24/-40  
OXA-58  
OXA-198  
OXA-427  
...

**NDM**

**VIM**  
**IMP**  
GIM  
SIM  
SPM  
...

- Large number of carbapenemases of different classes
- Resistance to most  $\beta$ -lactams

# Ambler classification of $\beta$ -lactamases

## Carbapenemases

Serine

Zinc  
(metallo- $\beta$ -lactamases)

Class A

Class C

Class D

Class B

**KPC**

GES  
SME  
IMI  
NMC-A  
...

Ceftazidim-  
avibactam  
Meropenem-  
vaborbactam

**OXA-48**

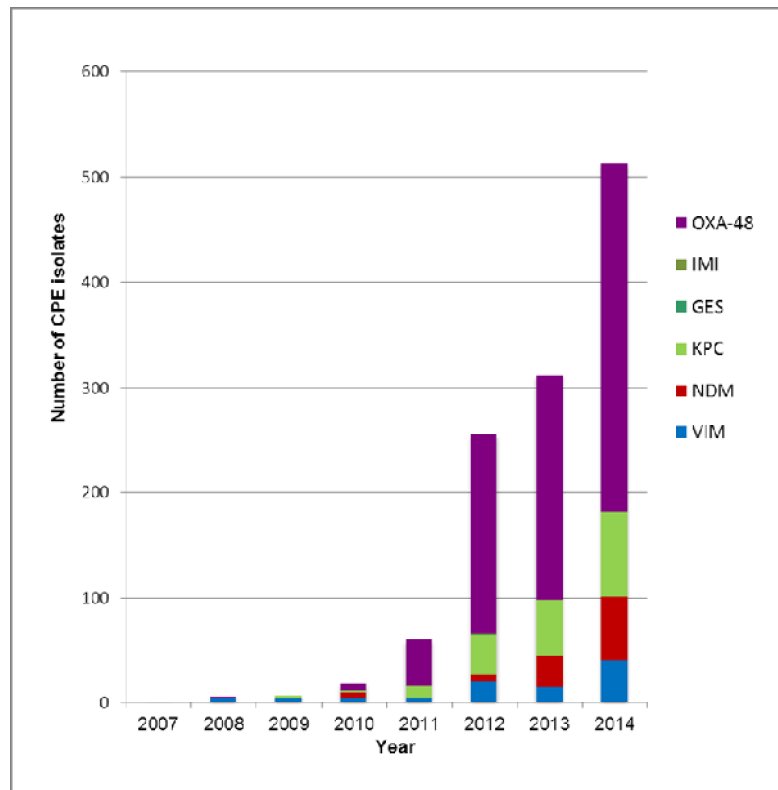
OXA-23  
OXA-24/-40  
OXA-58  
OXA-198  
OXA-427  
...

**NDM**

**VIM**  
**IMP**  
GIM  
SIM  
SPM  
...

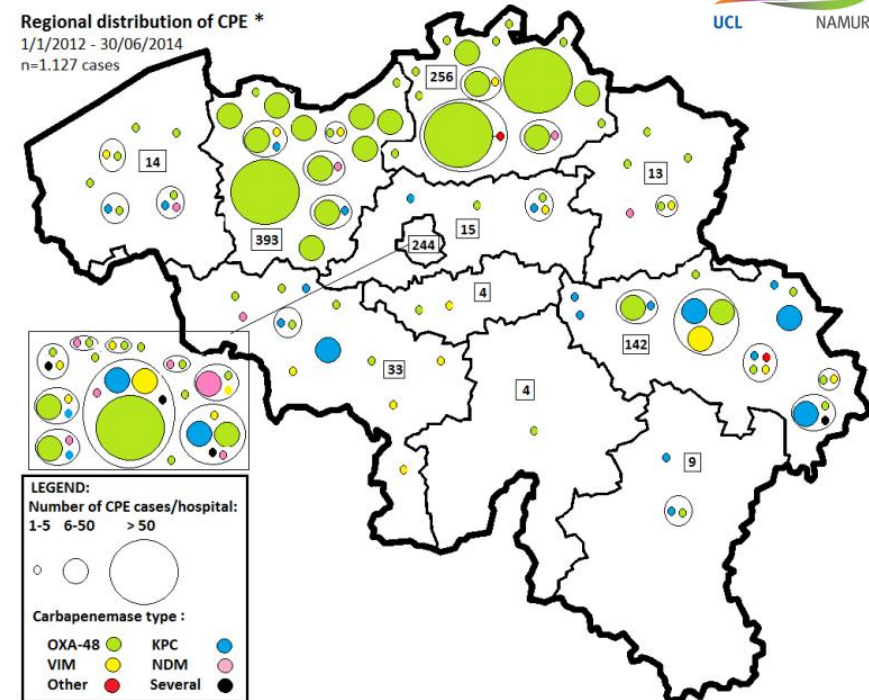
- Large number of carbapenemases of different classes
- Resistance to most  $\beta$ -lactams

# Belgium CPE epidemiology



Evolution of the distribution of resistance mechanisms of carbapenemase-producing Enterobacteriaceae isolates, National Reference Centre, Belgium, January 2007-December 2014 (n=1169)

Regional distribution of CPE \*  
1/1/2012 - 30/06/2014  
n=1.127 cases



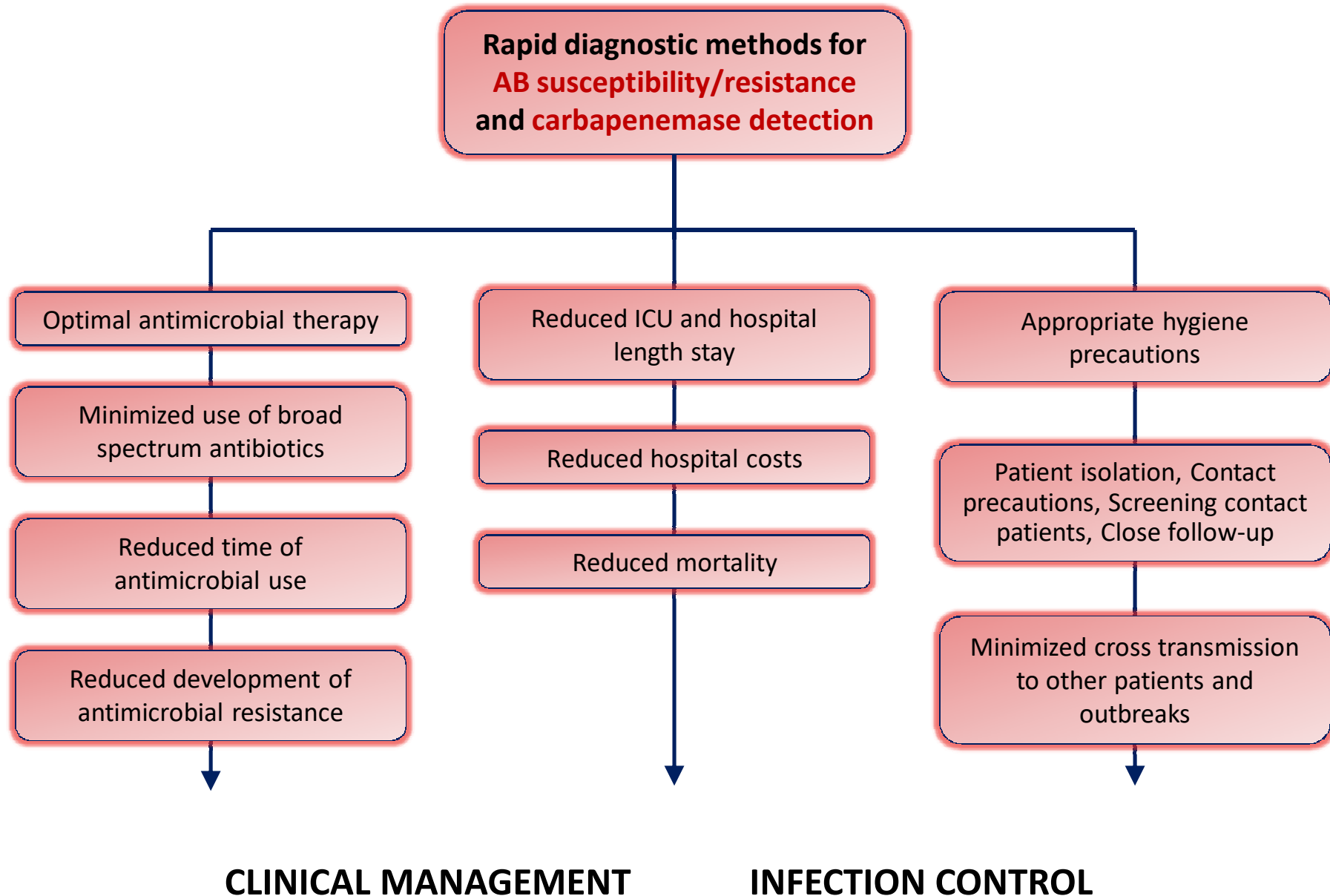
**Remark:**

For confidentiality reasons, the locations of the bullets representing individual hospitals do not correspond to the real location of the hospitals in the province.

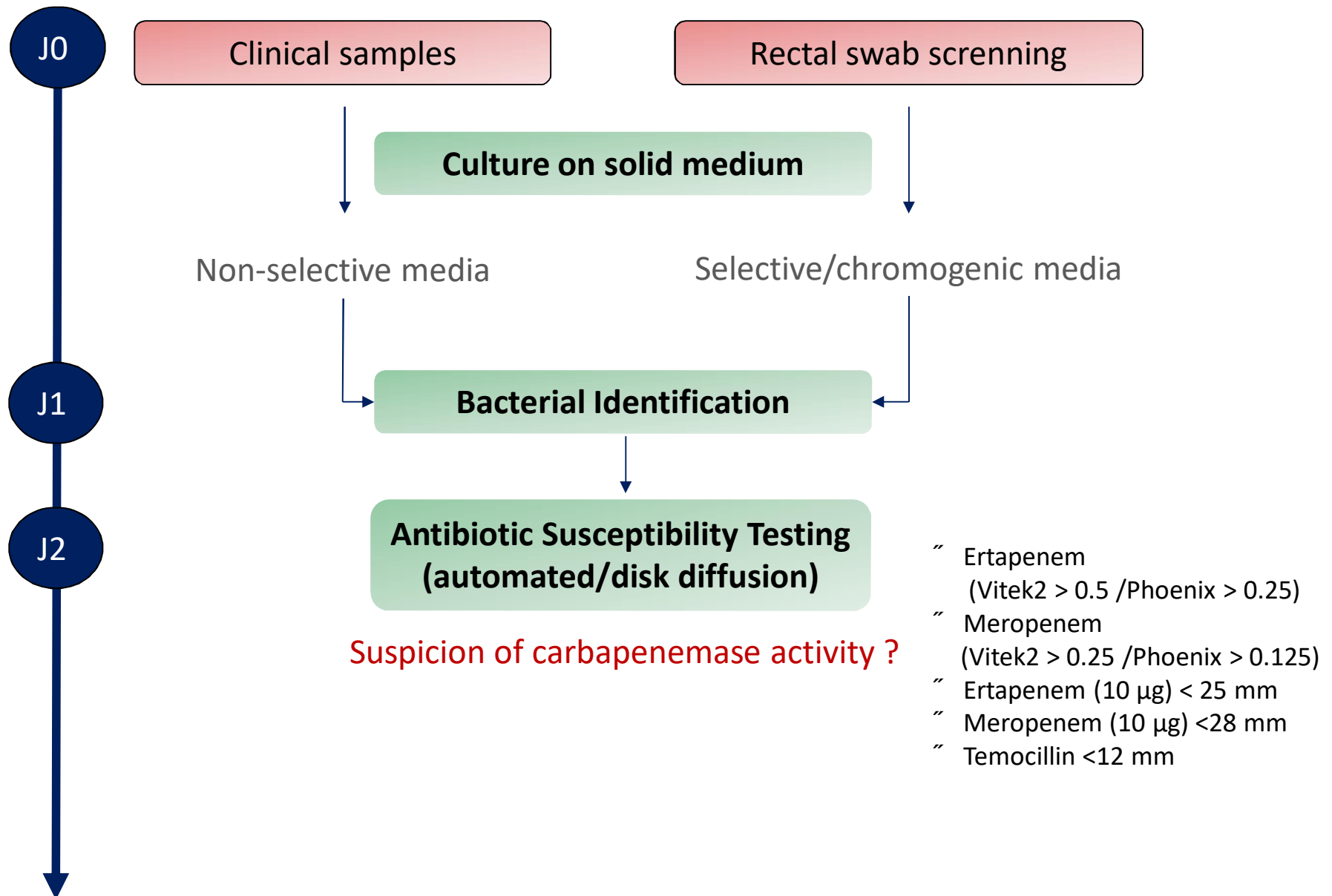
\*Données cumulées (30 mois de surveillance)

- ↗ CPO (Carbapenemases Producing Organisms) prevalence
- Major carbapenemases in *Enterobacterales* [OXA-48 > KPC > NDM > VIM]
- 30% of MDR *P.aeruginosa* are carbapenemase producers (mostly VIM-2)  
≠ most frequent mechanism of carbapenem resistance in *A. baumannii* : OXA-23 (and OXA-58)

# Clinical microbiology laboratory : Objectives



# Carbapenemase Detection

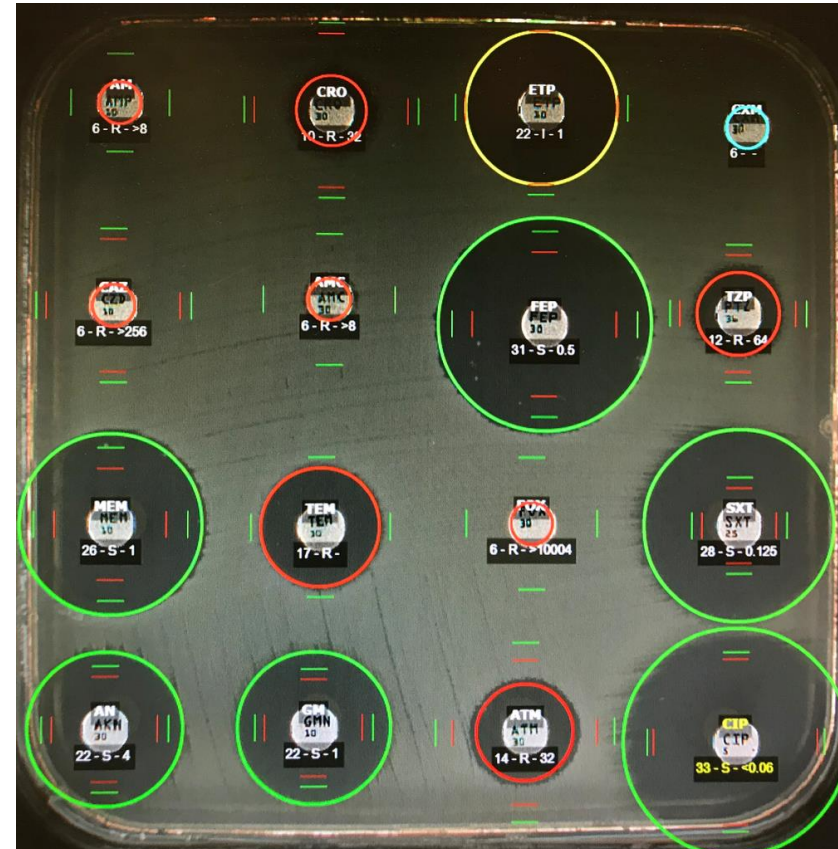
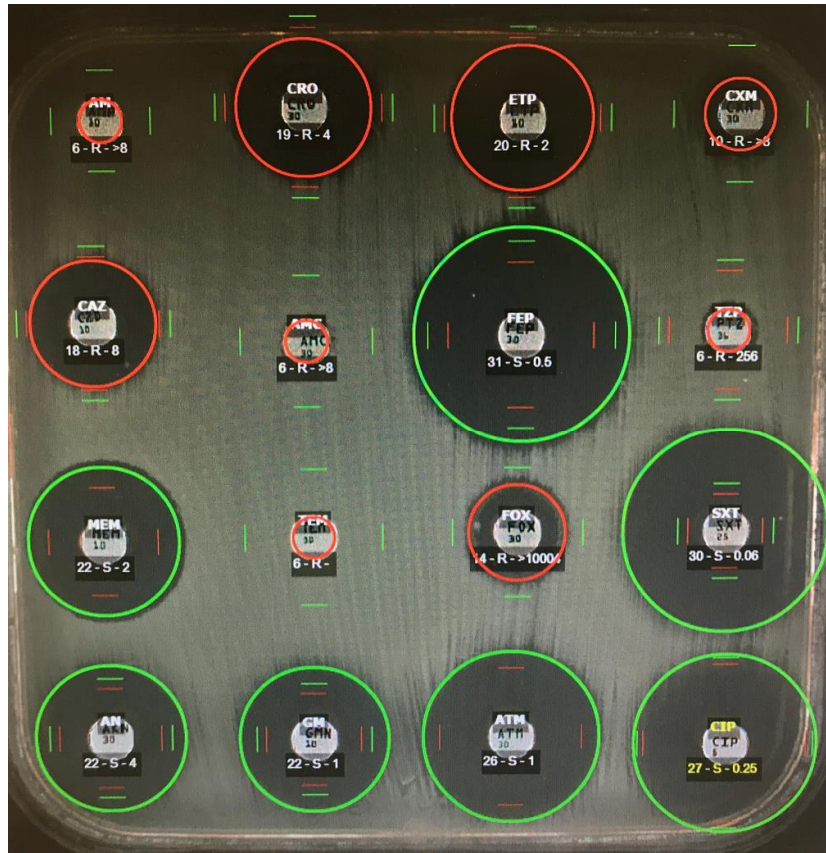






# Carbapenemase Detection

Enterobacteriaceae : Multi-resistant? Carbapenemase producer?

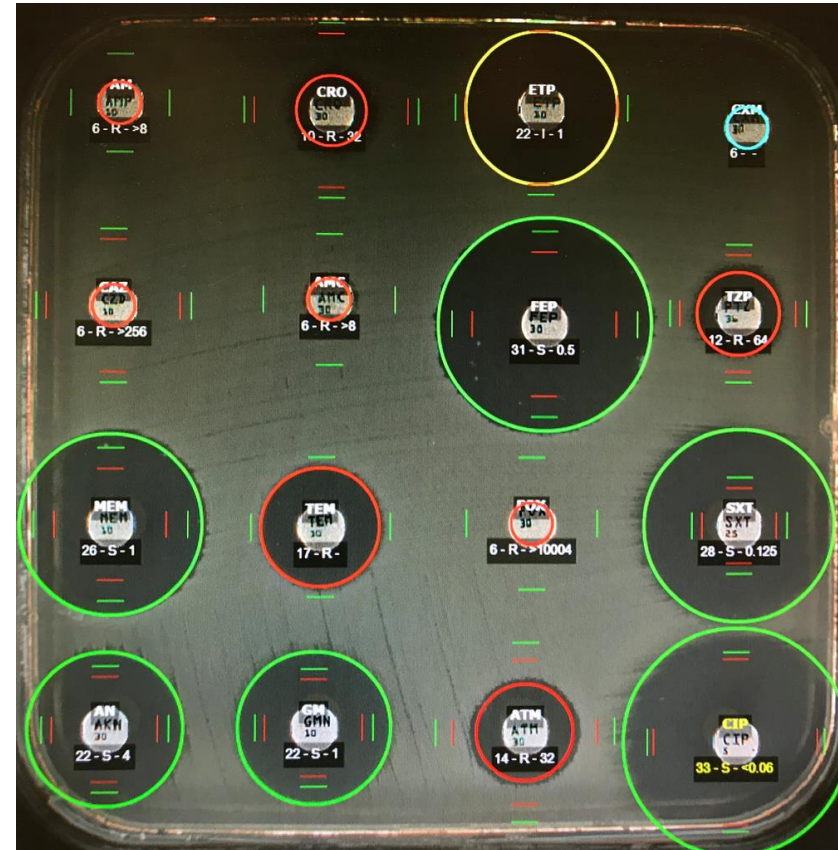


# Carbapenemase Detection

Enterobacteriaceae : Multi-resistant? Carbapenemase producer?



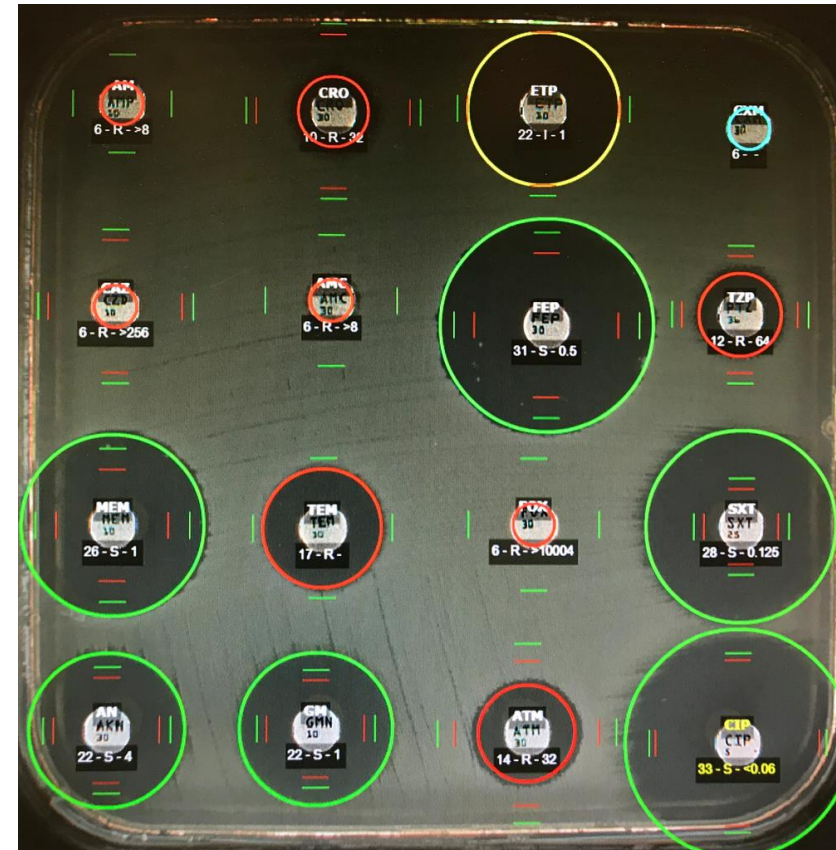
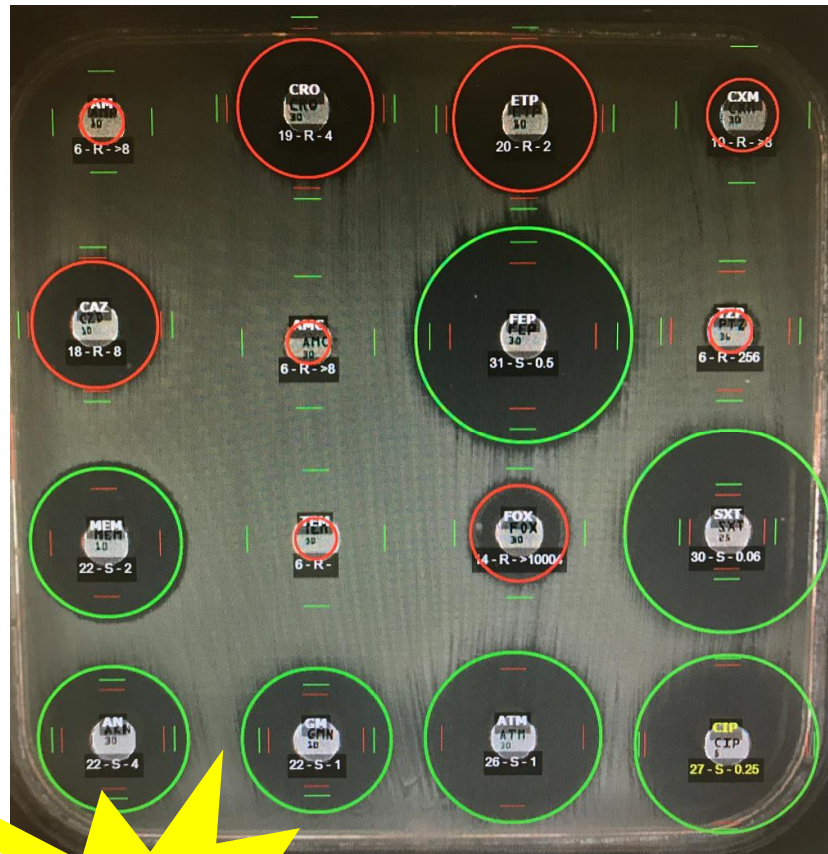
!! *K.pneumoniae*  
OXA-48



*E.cloacae*  
NO carbapenemases

# Carbapenemase Detection

Enterobacteriaceae : Multi-resistant? Carbapenemase producer?



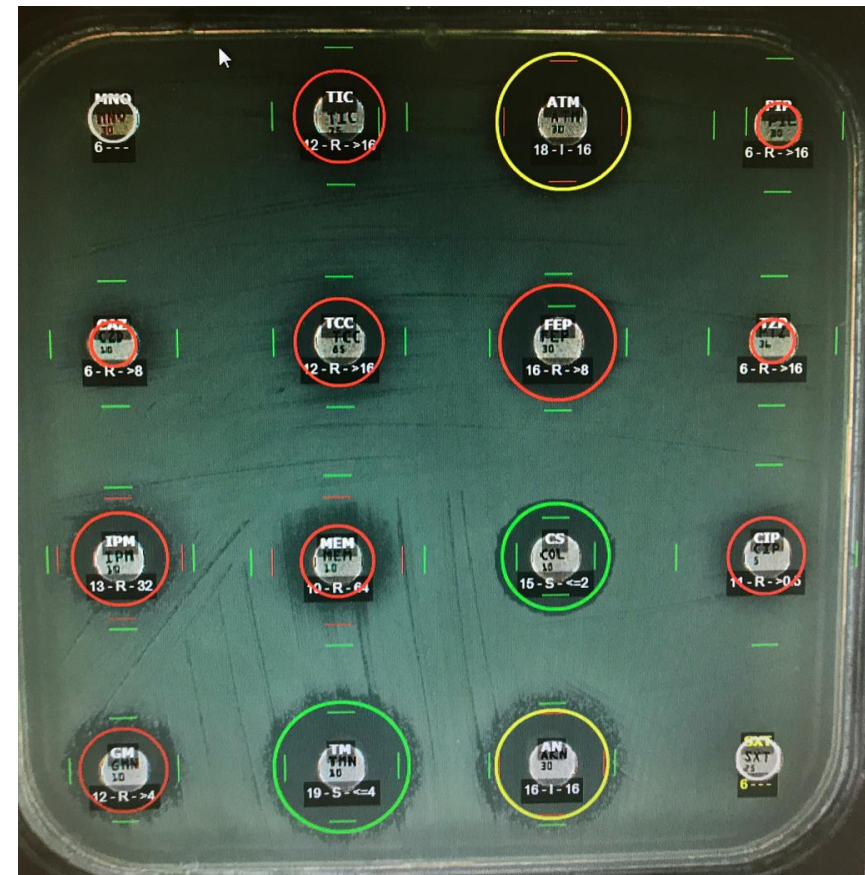
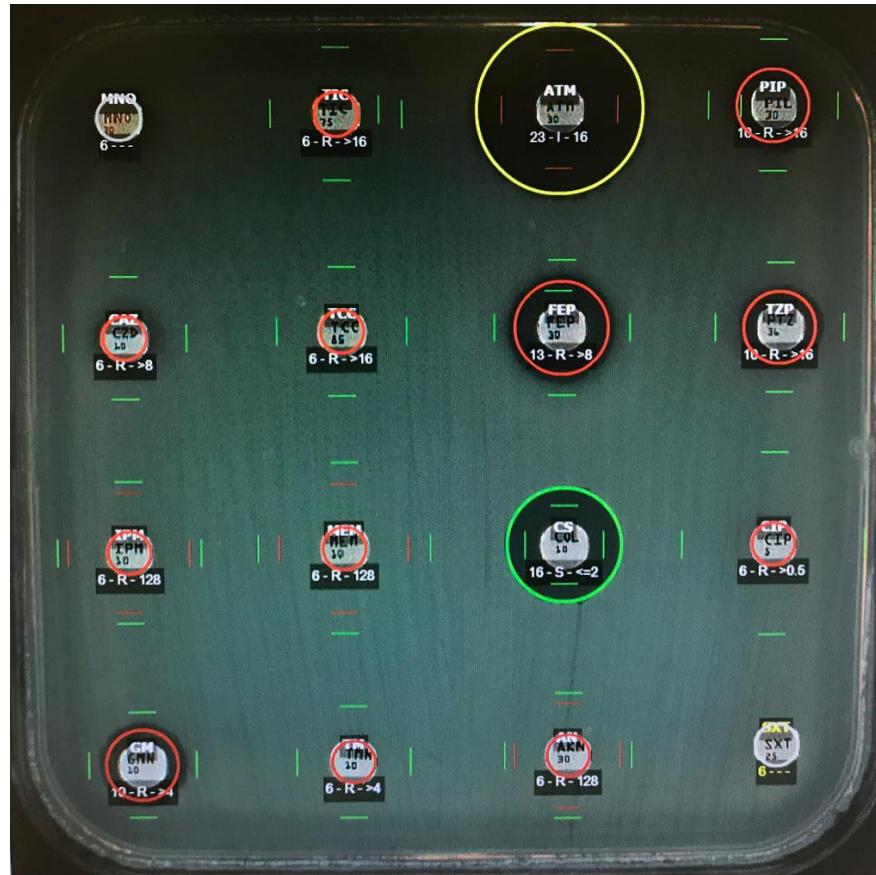
**HYGIENE  
ALERT**

*K. pneumoniae*  
OXA-48

*E. cloacae*  
NO carbapenemases

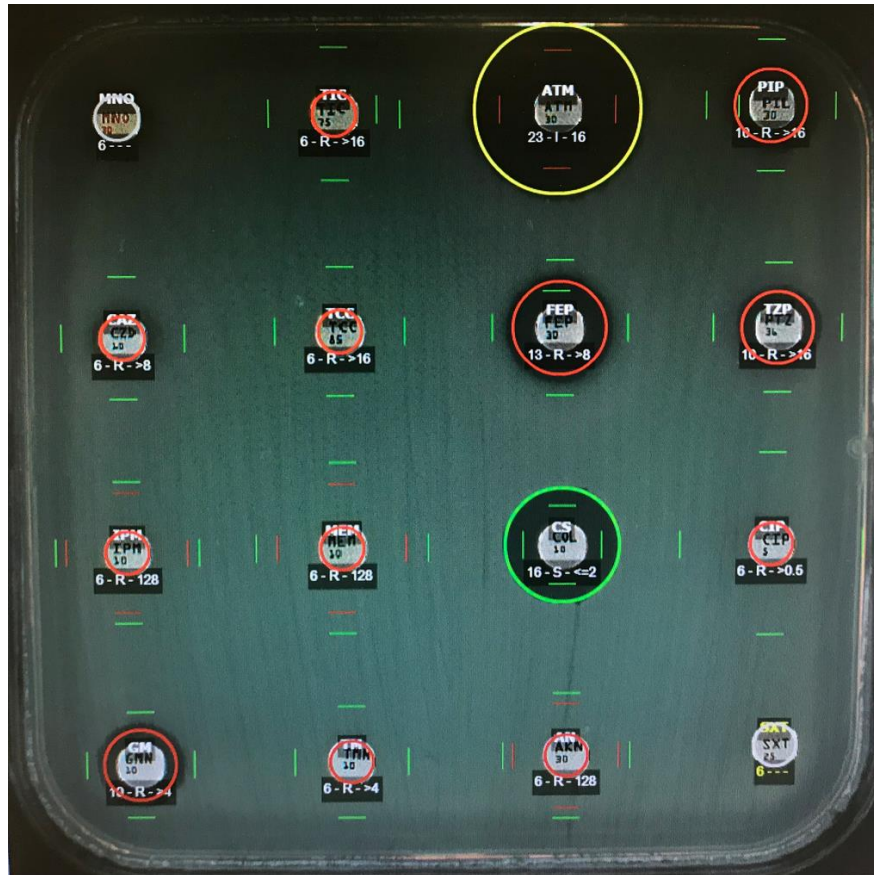
# Carbapenemase Detection

*P.aeruginosa* : Multi-resistant? Carbapenemase producer?

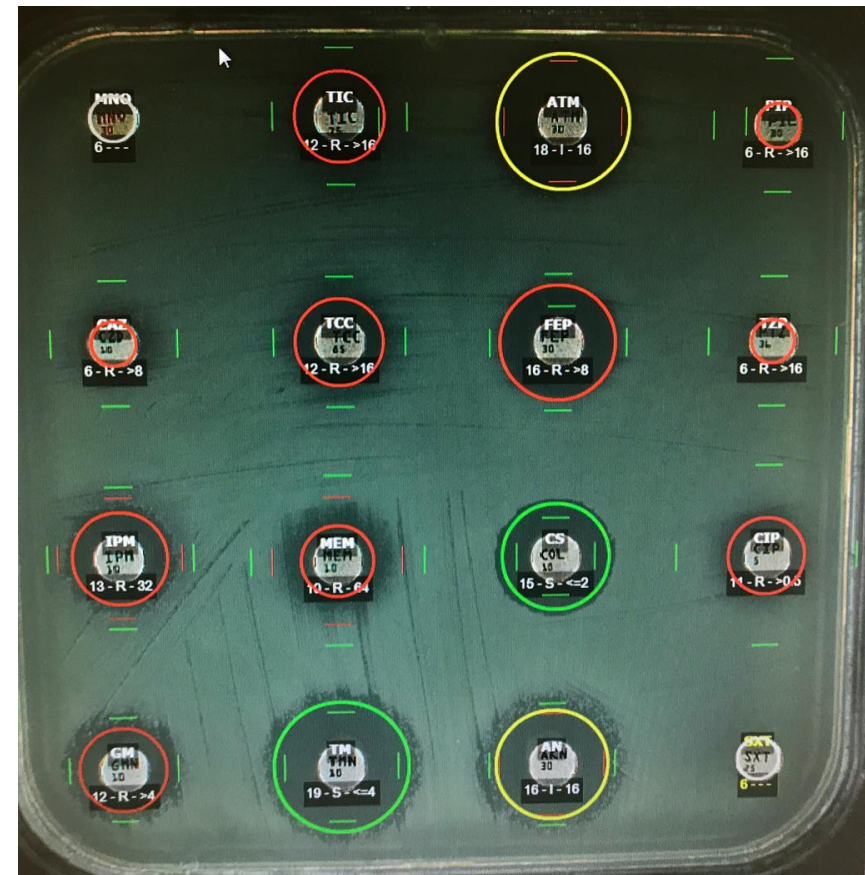


# Carbapenemase Detection

*P.aeruginosa* : Multi-resistant? Carbapenemase producer?



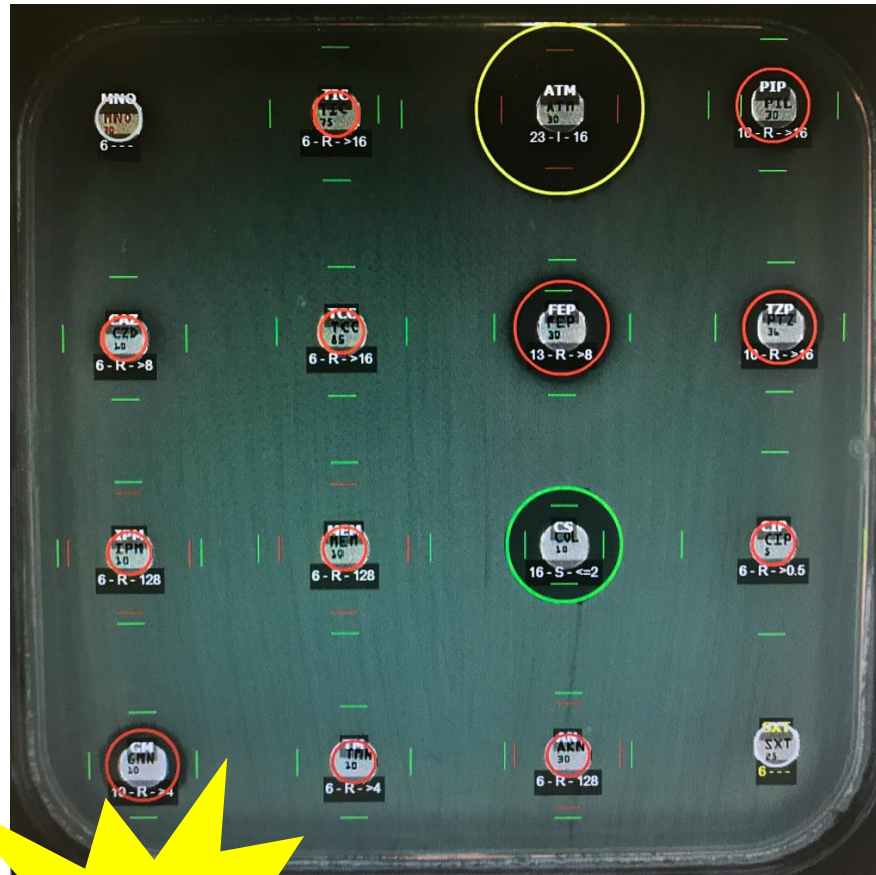
VIM



NO carbapenemases

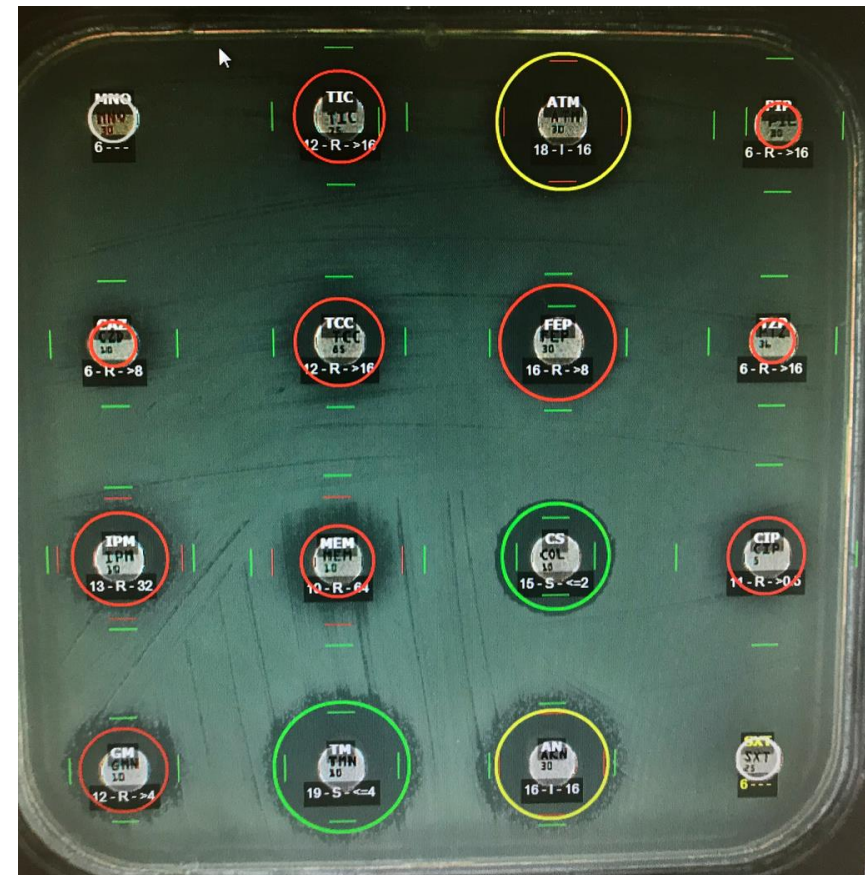
# Carbapenemase Detection

*P.aeruginosa* : Multi-resistant? Carbapenemase producer?



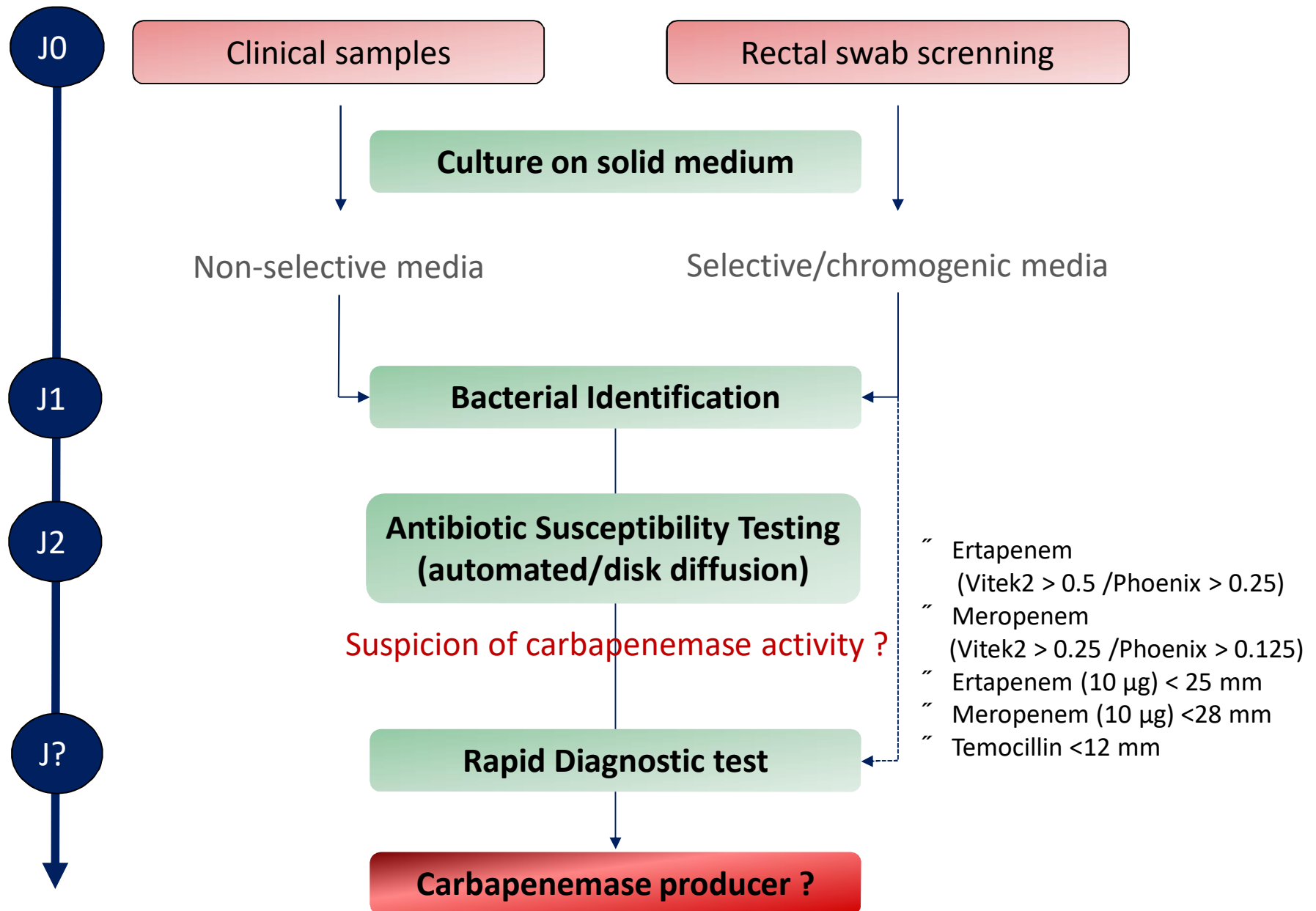
**HYGIENE  
ALERT**

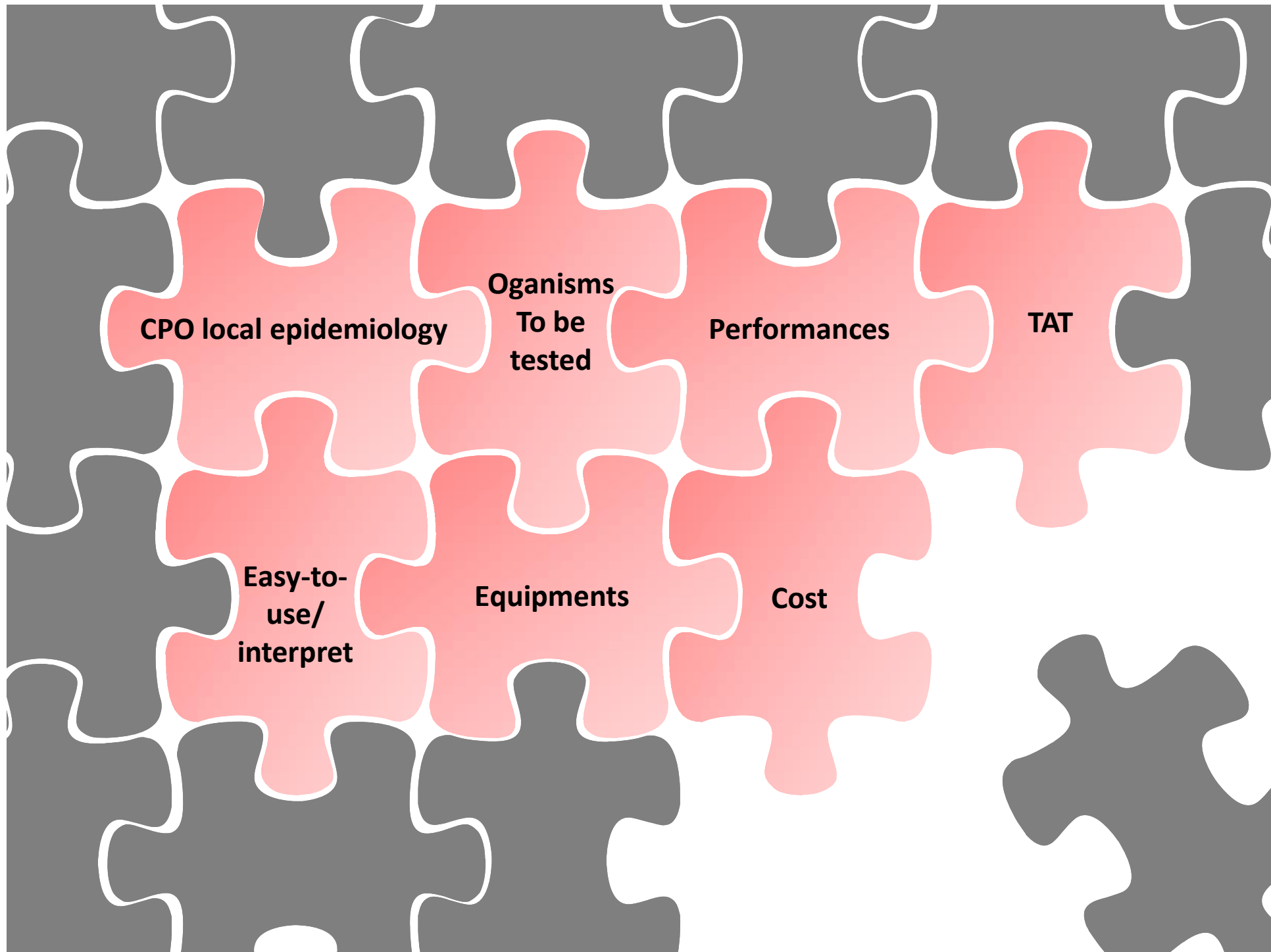
**VIM**



**NO carbapenemases**

# Carbapenemase Detection





**CPO local epidemiology**

**Oganisms  
To be  
tested**

**Performances**

**TAT**

**Easy-to-  
use/  
interpret**

**Equipments**

**Cost**



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ELSEVIER

Bacteriology

### Evaluation of the automated BD Phoenix CPO Detect test for detection and classification of carbapenemases in Gram negatives

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<sup>b</sup> Department of Microbiology, Laboratoire Hospitalier Universitaire de Bruxelles (LHUB-ULB), Hôpital Erasme-Cliniques universitaires de Bruxelles, Université Libre de Bruxelles, Brussels, Belgium

<sup>1</sup> Both authors contributed equally to this work.



# BD CPO Phoenix Detect



“ Large Antimicrobial Susceptibility Testing (24 antibiotics)

NMIC-408	NMIC-502
Amikacin	Amikacin
Amoxicillin/Clavulanate	Amoxicillin-Clavulanate (f)
Ampicillin	Ampicillin
	<b>Aztreonam</b>
Cefepime	Cefepime
<b>Cefoxitin</b>	
	<b>Cefixime</b>
Ceftazidime	Ceftazidime
	<b>Ceftazidime-Avibactam</b>
Ceftriaxone	Ceftriaxone
Cefuroxime	Cefuroxime
	<b>Cephalexin</b>
Ciprofloxacin	Ciprofloxacin- Other than Salmonella species
	<b>Ciprofloxacin- Salmonella species</b>
Colistin	Colistin
Ertapenem	Ertapenem
	<b>Fosfomycin</b>
Gentamicin	Gentamicin
Imipenem	Imipenem
Levofloxacin	Levofloxacin
	<b>Mecillinam</b>
Meropenem	Meropenem
	Nitrofurantoin
Piperacillin	Piperacillin
Piperacillin/Tazobactam	Piperacillin-Tazobactam
Temocillin	Temocillin
Tigecycline	Tigecycline
Tobramycin	Tobramycin
Trimethoprim/Sulfamethoxazole	Trimethoprim-Sulfamethoxazole
	<b>CPO detect</b>
ESBL	ESBL
Cefotaxime/Clavulanate (ESBL)	Cefotaxime/Clavulanate (ESBL)
Ceftazidime/Clavulanate (ESBL)	Ceftazidime/Clavulanate (ESBL)
Cefpodoxime-proxetil (ESBL)	Cefpodoxime-proxetil (ESBL)
Ceftazidime (ESBL)	Ceftazidime (ESBL)
Ceftriaxone/Clavulanate (ESBL)	Ceftriaxone/Clavulanate (ESBL)



# BD CPO Phoenix Detect



- “ Large Antimicrobial Susceptibility Testing (24 antibiotics)
- “ Carbapenemase detection and characterization according Ambler classification (A, B, D)
  - “ 9 wells containing a  $\beta$ -lactam antibiotic, alone and in combination with various chelators and  $\beta$ -lactam inhibitors
- “ 3 specific alarms by the expert system:
  - “ Resistant to one or more carbapenems
  - “ Carbapenemase producer
  - “ Class A, B or D carbapenemase producer



**Nom organisme**

- 1 ESCCOL Escherichia coli
- 2 ESCCOL Escherichia coli

**Marqueurs de résistance**

- 2 CARBD Producteur de carbapénémase de classe D
- 2 CBPEN Isolot testé résistant à un ou plus carbapénèmes

Antibiotique	ESCCOL	
	CMI/Conc	SIR
Ampicilline	>8	R
Pipéracilline	>64	R
Pipéracilline-Tazobactam	>64/4	R
Céfuroxime	>8	R
Ceftriaxone	>4	R
Ceftazidime	>8	R
Aztréonam	>16	R
Céfépime	<=1	S
Imipénème	2	S
Méropénème	1	S
Lévofoxacine	<=0.5	S
Ciprofloxacine	0.5	S
Amikacine	<=4	S
Tobramycine	<=1	S
Gentamicine	<=1	S
Triméthoprim-Sulfaméthoxazole	<=1/19	S
Colistine	<=0.5	S
Tigécycline	<=0.5	S
Amoxicilline-Clavulanate (f)	>32/2	R
Ceftazidime-Avibactam	<=0.25/4	S
Ertapenem	>1	R
Fosfomicine avec G6P	<=16	S

**Nom organisme**

- PSEAER Pseudomonas aeruginosa

**Marqueurs de résistance**

- 1 CARBB Producteur de carbapénémase de classe B
- 1 CBPEN Isolot testé résistant à un ou plus carbapénèmes

Antibiotique	PSEAER	
	CMI/Conc	SIR
Pipéracilline	32	R
Pipéracilline-Tazobactam	32/4	R
Ceftazidime	>8	R
Aztréonam	16	I
Céfépime	>16	R
Imipénème	>8	R
Méropénème	>8	R
Lévofoxacine	>2	R
Ciprofloxacine	>1	R
Amikacine	>16	R
Tobramycine	>4	R
Gentamicine	>4	R
Colistine	1	S
Ceftazidime-Avibactam	>8/4	R

**Marqueurs de résistance**

- 1 CARB Producteur de carbapénémase
- 1 CBPEN Isolot testé résistant à un ou plus carbapénèmes

Antibiotique	PSEAER	
	CMI/Conc	SIR
Pipéracilline	>64	R
Pipéracilline-Tazobactam	>64/4	R
Ceftazidime	>8	R
Aztréonam	>16	R
Céfépime	>16	R
Imipénème	>8	R
Méropénème	>8	R
Lévofoxacine	>2	R
Ciprofloxacine	>1	R
Amikacine	16	I
Tobramycine	>4	R
Gentamicine	2	S
Colistine	1	S
Ceftazidime-Avibactam	>8/4	R

**Nom organisme**

- ACINBAU Acinetobacter baumannii

**Marqueurs de résistance**

- 1 CARBD Producteur de carbapénémase de classe D

Antibiotique	ACINBAU	
	CMI/Conc	SIR
Imipénème	>8	R
Méropénème	>8	R
Lévofoxacine	>2	R
Ciprofloxacine	>1	R
Amikacine	>16	R
Tobramycine	>4	R
Gentamicine	>4	R
Triméthoprim-Sulfaméthoxazole	>4/76	R
Colistine	1	S

# Clinical isolates - Species

## Clinical Isolates (n=287)

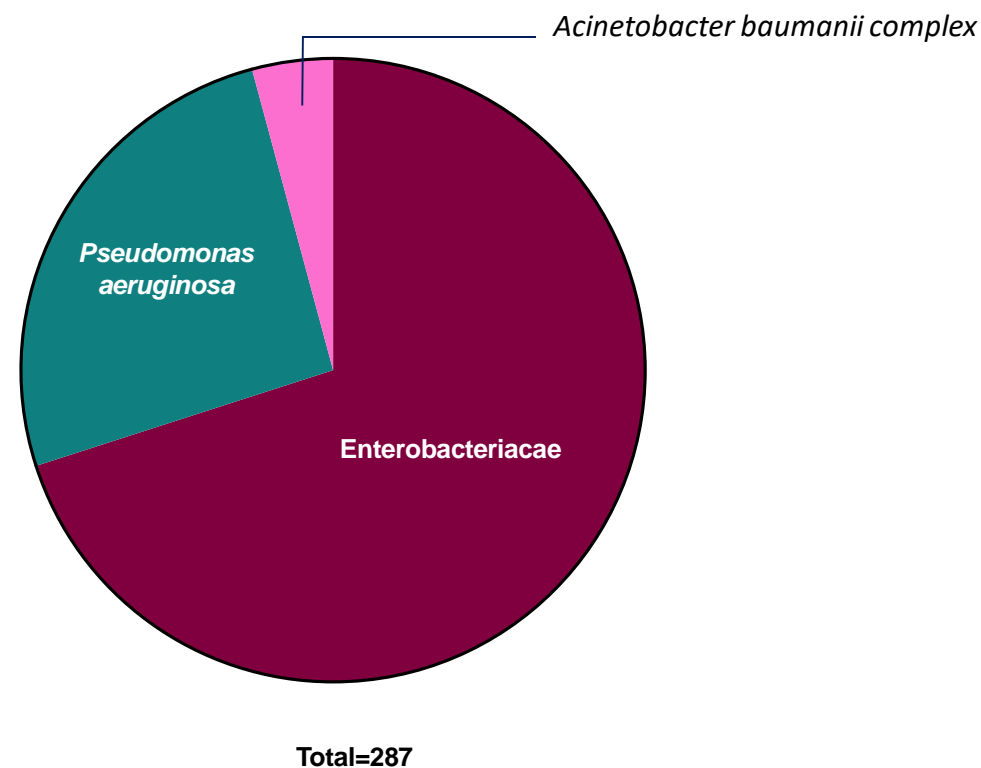
### Enterobacteriaceae (n=201)

- K.pneumoniae* (n=93)
- E.coli* (n=42)
- E.cloacae* complex (n=25)
- K.oxytoca* (n=10)
- C.freundii* (n=9)
- K.aerogenes* (n=9)
- S.marcescens* (n=4)
- C.amalonaticus* (n=3)
- M.morganii* (n=2)
- C.koseri* (n=1)
- K.variicola* (n=1)
- P.stuartii* (n=1)
- Shigella sp* (n=1)

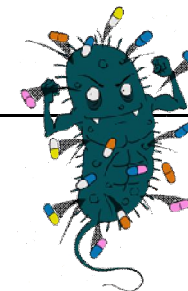
### *Pseudomonas aeruginosa* (n =74)

### *A. baumannii* complex (n=12)

- 70.03% (201) Enterobacteriaceae
- 25.78% (74) *Pseudomonas aeruginosa*
- 4.18% (12) *Acinetobacter baumannii* complex



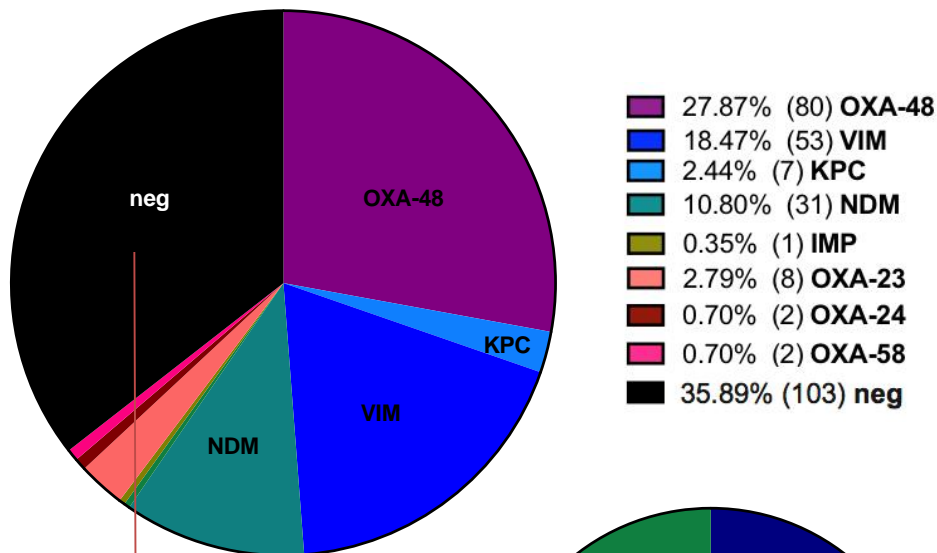
# Clinical isolates - carbapenemases



## Clinical Isolates (n=287)

### Enterobacteriaceae (n=201)

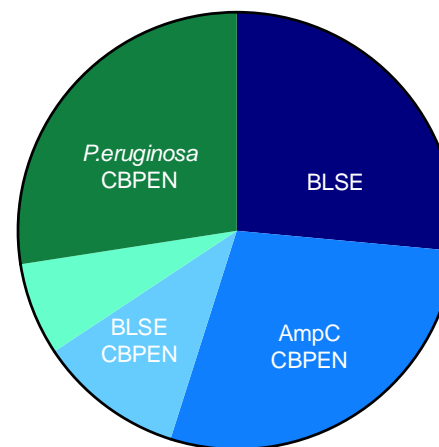
- K.pneumoniae* (n=93)
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  - C.amalonaticus* (n=3)
  - M.morganii* (n=2)
  - C.koseri* (n=1)
  - K.variicola* (n=1)
  - P.stuartii* (n=1)
  - Shigella sp* (n=1)



Total=287

### *Pseudomonas aeruginosa* (n =74)

#### *A. baumannii* complex (n=12)



Total=103

- 26.97 % (28) Carbapenems-resistant *P.aeruginosa*
- 10.78% (11) Carbapenems-resistant BLSE
- 26.47% (28) ESBL
- 28.43% (29) AmpC
- 6.86% (7) Others

Enterobacteriaceae

**Table 1**

Performances of the BD-CPO test for carbapenemase detection and Ambler classification.

CPO type	Species	No of isolates tested	Carbapenemase detection				Carbapenemase Ambler classification				
			No of positive	No of negative	Sensitivity% (95% CI*)	Specificity% (95% CI*)	A	B	D	Unclassified positive	Sensitivity% (95% CI*)
All CPO		184	165	19	89.7 (84.4–93.3)	-	1	58	79	27	83.6 (77.2–88.5)
Class A	<i>Enterobacteriaceae</i>	7	7	-	100.0 (64.6–100.0)	-	1	-	-	6	14.3 (2.6–51.3)
Class B	All Class B	85	70	15	82.4 (72.9–89.0)	-	-	58	-	12	82.9 (72.4–89.9)
	<i>Enterobacteriaceae</i>	40	39	1	97.5 (87.1–99.6)	-	-	32	-	7	82.1 (67.3–91.0)
	<i>P. aeruginosa</i>	45	31	14	68.9 (54.3–80.5)	-	-	26	-	5	83.9 (67.4–92.9)
Class D	All Class D	92	88	4	95.7 (89.3–98.3)	-	-	-	79	9	89.8 (81.7–94.5)
	<i>Enterobacteriaceae</i>	80	79	1	98.8 (93.2–99.8)	-	-	-	71	8	89.9 (81.3–94.8)
	<i>A. baumannii</i> complex	12	9	3	75.0 (46.8–91.1)	-	-	-	8	1	88.9 (56.5–98.0)
negative	All non-CPO <sup>‡</sup>	103	17	86	-	83.5 (75.2–89.4)	7	-	7	3	-
	<i>Enterobacteriaceae</i>	74	6	68	-	91.9 (83.4–96.2)	-	-	3	3	-
	<i>P. aeruginosa</i>	29	11	18	-	62.1 (42.4–78.7)	7	-	4	-	-

<sup>‡</sup> CPO = carbapenemase producing-organisms.

\* 95% CI = 95% confidence interval.

# Results – carbapenemase detection

Clinical Isolates (n=287)

## Detection of Carbapenemase Production

### CPO n=184

N° of true positive	165
N° of false negative	<b>19</b>

### NON CPO n=103

N° of true negative	86
N° of false positive	<b>17</b>

### Performance

Sensitivity %	89.7
Specificity %	83.5
PPV %	90.7
NPV %	81.9

# Results – carbapenemase Ambler classification

**Clinical Isolates (n=287)**  
**CPO (n=184)**  
**NON CPO (=103)**

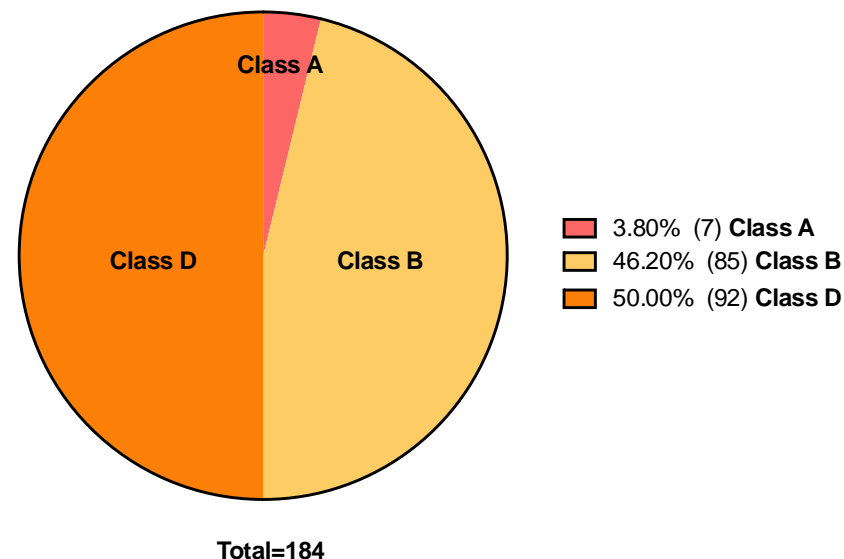
## Detected CPO n=165/184

Correctly classified	138
Unclassified	27
Misclassified	0
<b>Sensitivity %</b>	<b>83.6</b>

## NON CPO false positive n=17/103

Classified	<b>14</b>
Unclassified	3

## Detection of Carbapenemase Class



Amber Class	N° of isolates	Correctly detected	Correctly classified	Unclassified	<b>Sensitivity %</b>
<b>A</b>	7	7	1	6	<b>14.3</b>
<b>B</b>	85	70	58	12	<b>82.9</b>
<b>D</b>	92	88	79	9	<b>89.8</b>

# Results – *Enterobacteriaceae*

## Enterobacteriaceae (n=201)

CPO type	Amber Class	No of isolates	BD phoenix CPO Detect Carbapenemase Detection				BD phoenix CPO Detect Carbapenemase Classification					
			Positive	Negative	Sensitivity %	Specificity %	PPV %	NPV %	A	B	D	Unclassified positive
<b>all CPOs</b>		127	125	2	98.4	95.4	97.1	1	32	71	21	83.2
<b>OXA-48</b>	D	80	79	1	98.8			-	-	71	8	89.9
<b>KPC</b>	A	7	7	-	100.0			1	-	-	6	14.3
<b>VIM</b>	B	9	8	1	88.9			-	8	-	-	100.0
<b>NDM</b>	B	31	31	-	100.0			-	24	-	7	77.4
<b>Negative</b>		74	6	68	91.9			-	-	3	3	-

“ Detection of Carbapenemase production detection :

125/127 CPO (98.4%) correctly identified

**High sensitivity and specificity**

“ Detection of Carbapenemase class :

104/125 CPO (83.2%) correctly classified

KPC (Class A) detection (low sensitivity 1/7)

# Results – *Acinetobacter baumannii*

***A.baumannii* (n=12)**

CPO type	Amber Class	No of isolates	BD phoenix CPO Detect Carbapenemase Detection				BD phoenix CPO Detect Carbapenemase Classification				
			Positive	Negative	Sensitivity %	Specificity %	A	B	D	Unclassified positive	Sensitivity %
all CPOs		12	9	3	75.0	-	-	8	1	88.9	
OXA-23	D	8	7	1	87.5	-	-	6	1	85.7	
OXA-24	D	2	2	0	100.0	-	-	2	-	100.0	
OXA-58	D	2	0	2	0.0	-	-	-	-	-	

“ Detection of Carbapenemase production :

9/12 CPO (75.0%) correctly identified

Specificity?

OXA-58 detection?

“ Detection of Carbapenemase class :

8/9 CPO correctly classified

⇒ Promising results, to be confirmed with more strains

# Results – *Pseudomonas aeruginosa*

## *P.aeruginosa* (n=74)

CPO type	Amber Class	No of isolates	BD phoenix CPO Detect Carbapenemase Detection				BD phoenix CPO Detect Carbapenemase Classification						
			Positive	Negative	Sensitivity %	Specificity %	PPV %	NPV %	A	B	D	Unclassified positive	Sensitivity %
all CPOs		45	31	14	68.9			-	26	-	5	83.9	
VIM	B	44	30	14	68.2	73.8	56.2	-	25	-	5	83.3	
IMP	B	1	1	-	100.0			-	1	-	-	-	100.0
GES (ESBL)	A	1	-	1	100.0			-	-	-	-	-	-
Negative		28	11	17	60.7			7	-	4	-	-	-

“ Detection of Carbapenemase production detection :

31/45 CPO (68.9%) correctly identified

**LOW sensitivity and specificity**

“ Detection of Carbapenemase class :

26/31 CPO (83.9%) correctly classified

! Misclassification of non-CPO (*P.aeruginosa* multiR)

# Results – *Pseudomonas aeruginosa*

## *P.aeruginosa* (n=74)

CPO type	Amber Class	No of isolates	BD phoenix CPO Detect Carbapenemase Detection				BD phoenix CPO Detect Carbapenemase Classification						
			Positive	Negative	Sensitivity %	Specificity %	PPV %	NPV %	A	B	D	Unclassified positive	Sensitivity %
<b>all CPOs</b>		45	31	14	68.9			-	26	-	5	83.9	
VIM	B	44	30	14	68.2	73.8	56.2	-	25	-	5	83.3	
IMP	B	1	1	-	100.0			-	1	-	-	-	100.0
GES (ESBL)	A	1	-	1	100.0			-	-	-	-	-	-
<b>Negative</b>		28	11	17	60.7			7	-	4	-	-	-

### Resistance to Ceftazidim/avibactam

CPO n=45	
N° of CAZ/AVIR	45
N° of CAZ/AVIS	0

NON CPO n=28	
N° of CAZ/AVIR	5*
N° of CAZ/AVIS	23

Performance	
Sensitivity %	100,0
Specificity %	82.1
PPV %	90.1
NPV %	100.0

\* 3 Class A and 2 Class D

# Results – *Pseudomonas aeruginosa*

## *P.aeruginosa* (n=74)

CPO type	Amber Class	No of isolates	BD phoenix CPO Detect Carbapenemase Detection				BD phoenix CPO Detect Carbapenemase Classification					
			Positive	Negative	Sensitivity %	Specificity %	PPV %	NPV %	A	B	D	Unclassified positive
all CPOs		45	31	14	68.9			-	26	-	5	83.9
VIM	B	44	30	14	68.2			-	25	-	5	83.3
IMP	B	1	1	-	100.0	73.8	56.2	-	1	-	-	100.0
GES (ESBL)	A	1	-	1				-	-	-	-	-
Negative		28	11	17				7	-	4	-	

### Resistance to Ceftazidim/avibactam

CPO n=45	
N° of CAZ/AVIR	45
N° of CAZ/AVIS	0

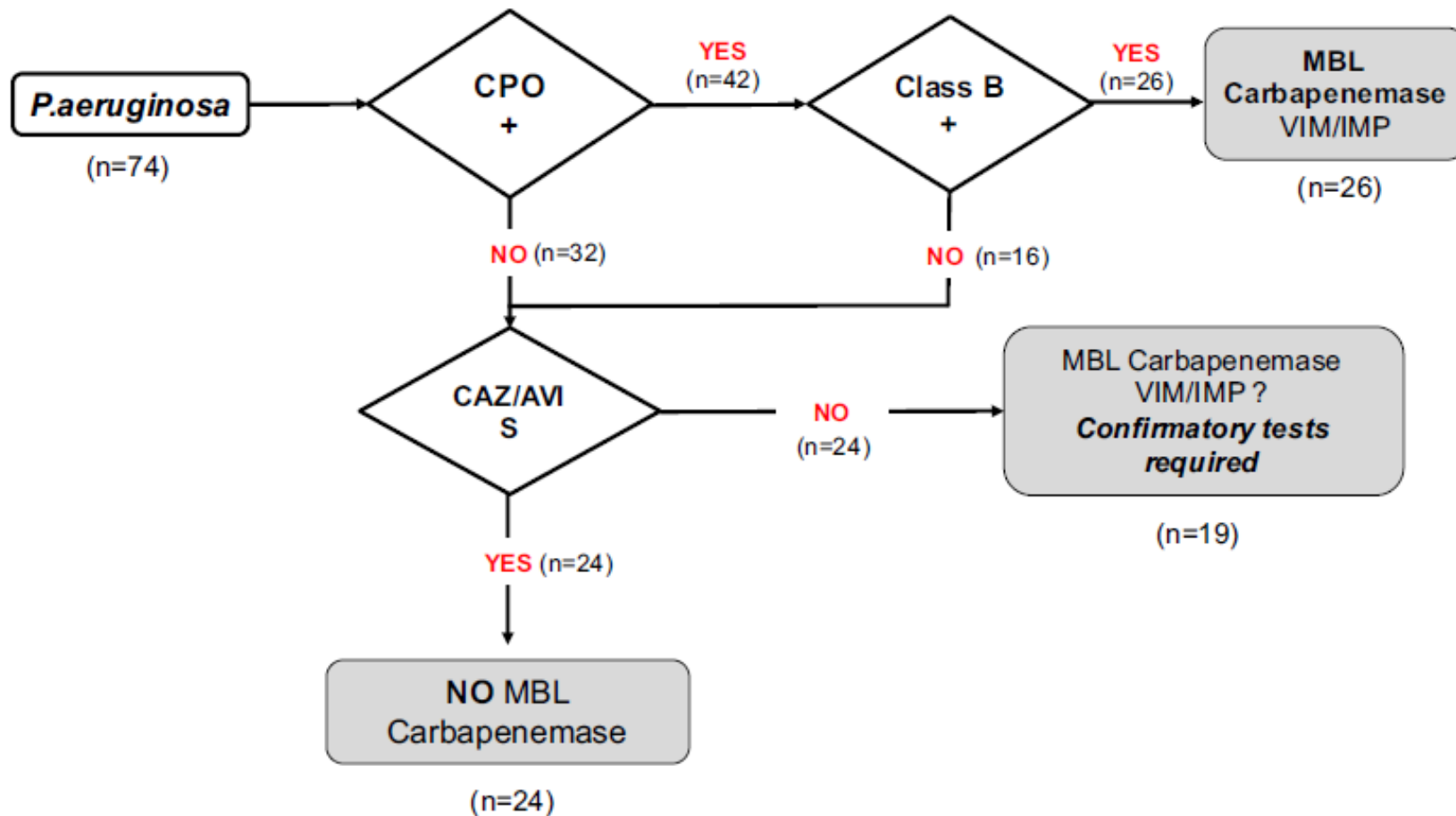
NON CPO n=28	
N° of CAZ/AVIR	5*
N° of CAZ/AVIS	23

Performance	
Sensitivity %	100,0
Specificity %	82.1
PPV %	90.1
NPV %	100.0

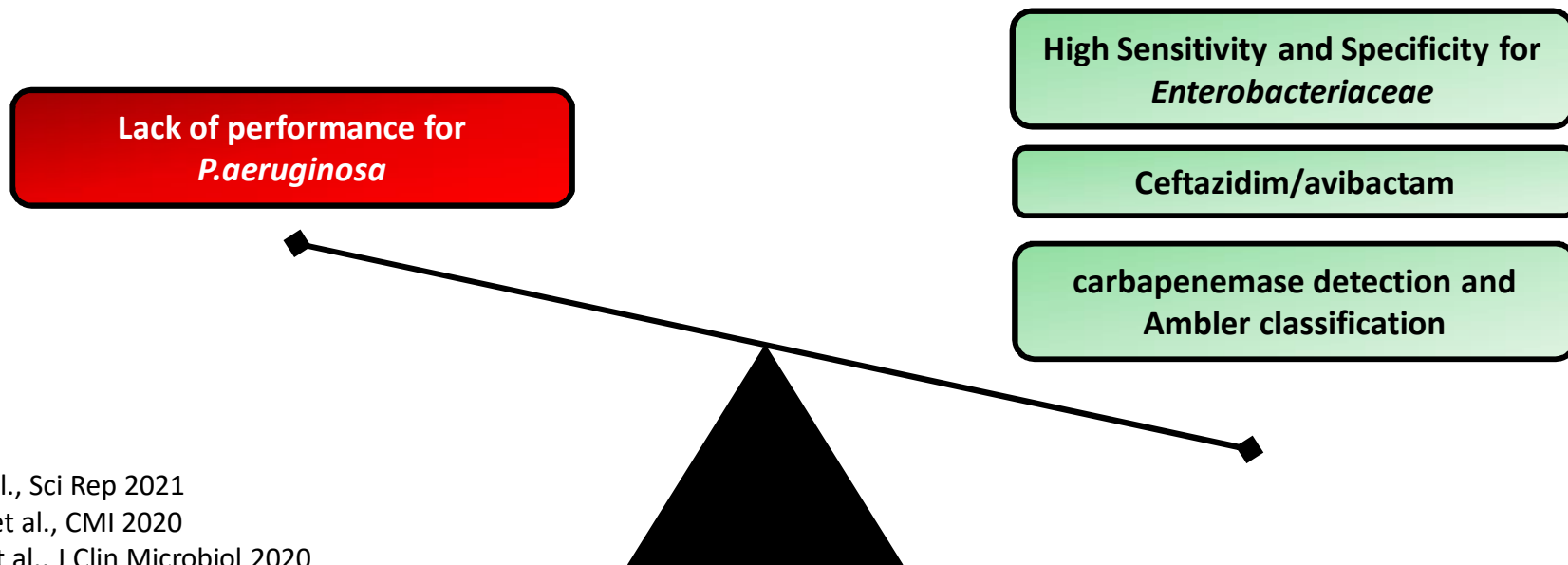
Including susceptibility to ceftazidim/avibactam increases sensitivity and specificity in carbapenemase production/class B detection

\* 3 Class A and 2 Class D

# Results – *Pseudomonas aeruginosa*

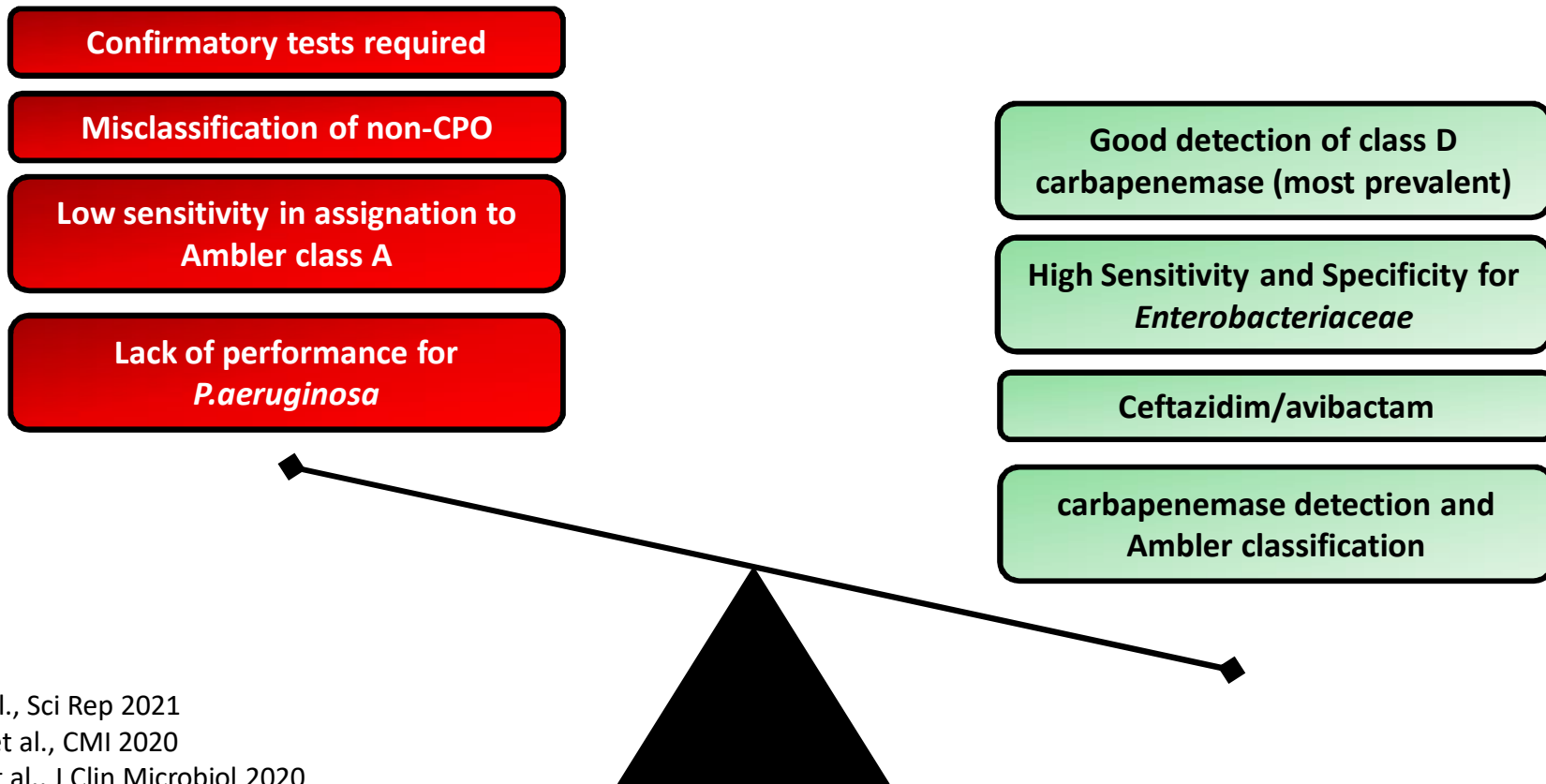


# Conclusion

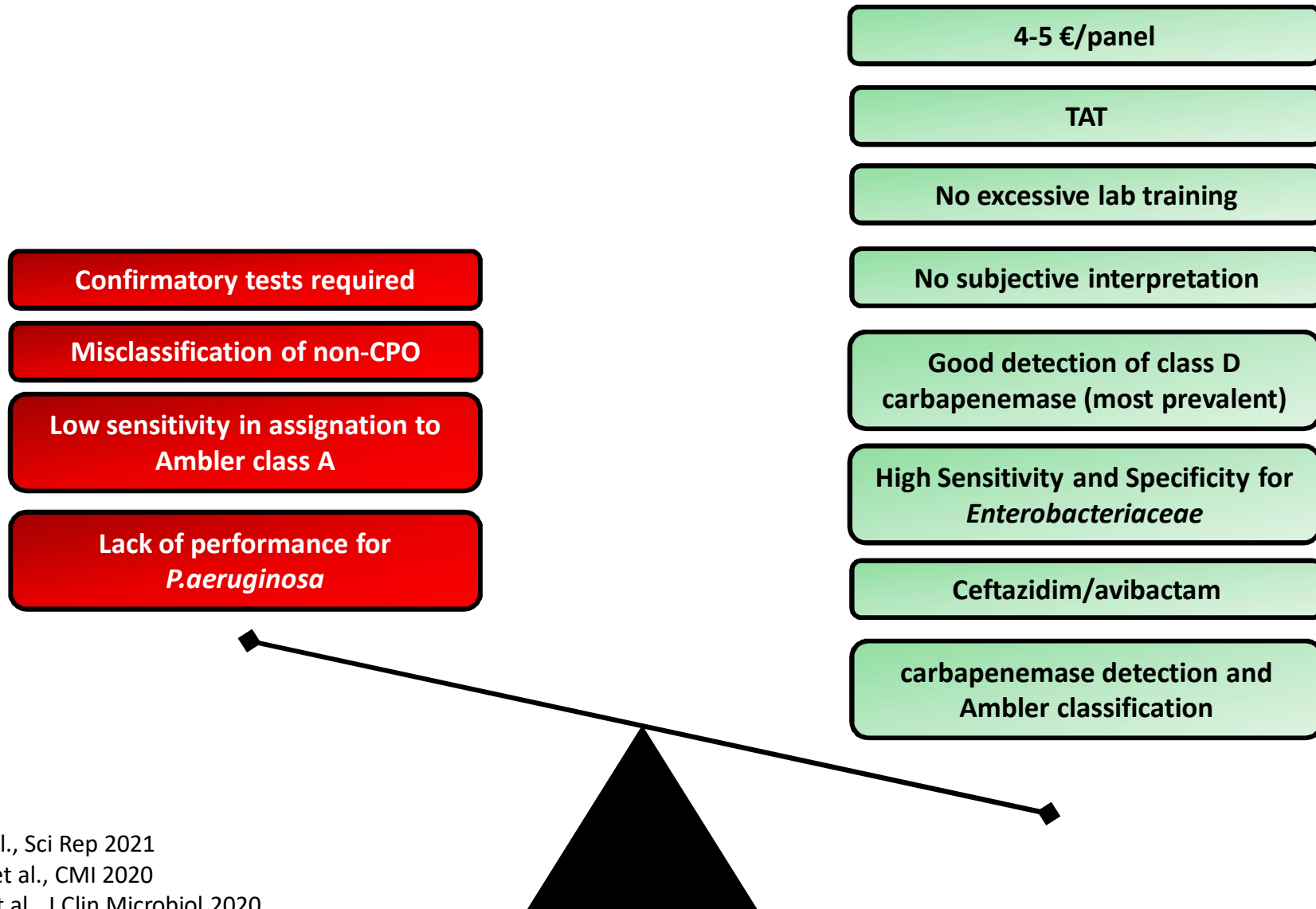


Jonas et al., Sci Rep 2021  
Croxatto et al., CMI 2020  
Whitley et al., J Clin Microbiol 2020

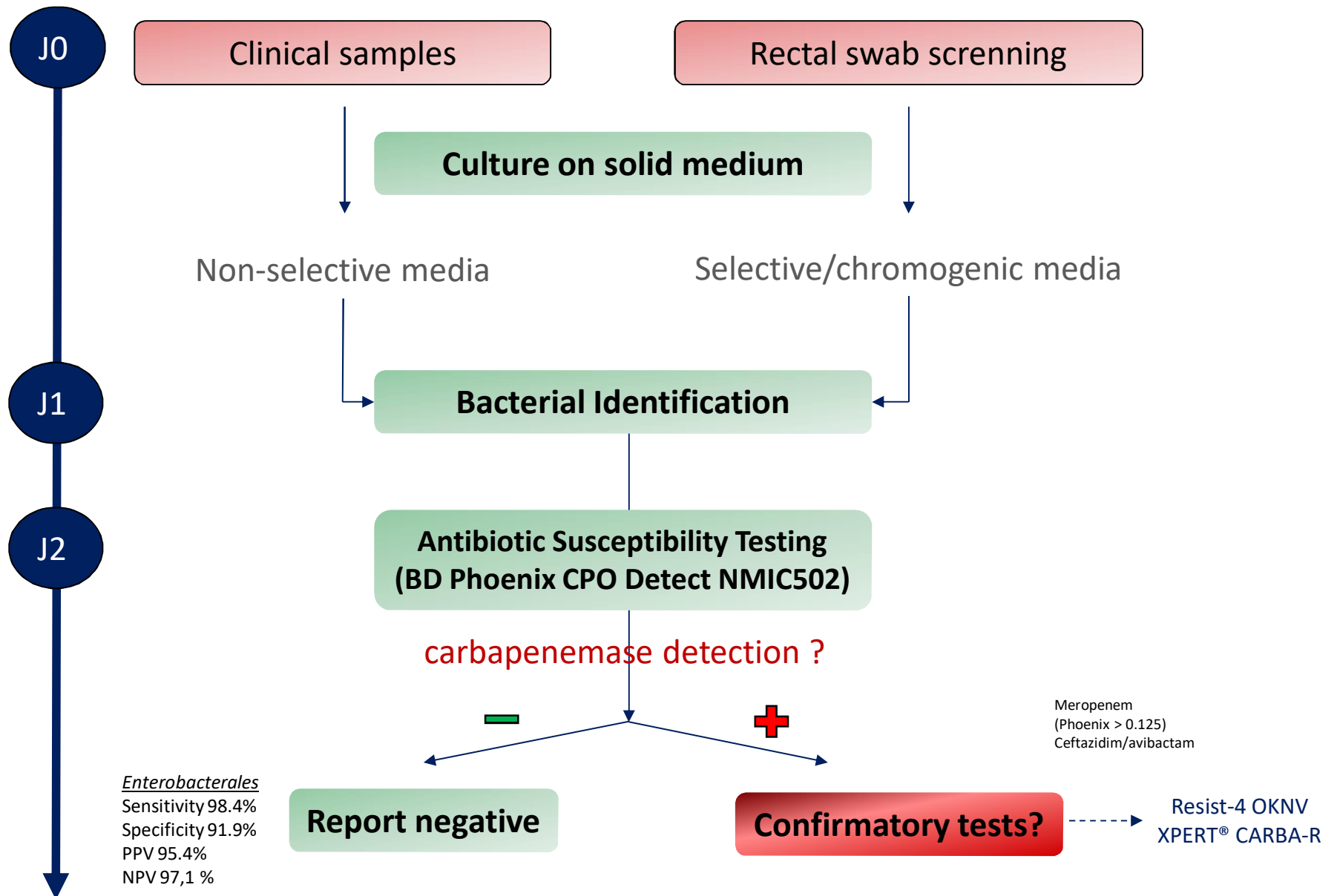
# Conclusion



# Conclusion



# Conclusion



*Merci de votre attention*



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# Conclusions

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## Good global sensitivity and specificity

- Very accurate for *Enterobacteriaceae*
- Promising results for *A.baumannii complex sp.* Need of further investigation with more positive and negative CPO
- Lack of performance for *P.aeruginosa* BUT increased by including the susceptibility to Ceftazidim/avibactam in the algorithm
- Good detection of class D carbapenemase (Detection of OXA-48 sensitivity of 89%, the most prevalent in Belgium)
- When CPO are detected : no misclassification. However misclassification among non-CPO
- Lack of performance for the classification of class A carbapenemase (1/7) in our setting (to be confirmed with more Belgian isolates)