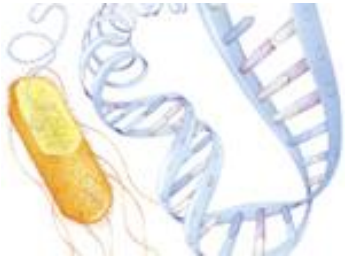


How to interprete the antibiogram ? What to do and not to do for Gram-negative organisms in 2017

Y. Glupczynski, MD, PhD

National Reference Centre for Antiotic Resistant Gram-Negative bacilli
Laboratory of Clinical Microbiology, CHU UCL Namur, Mont-Godinne

Microbiology Workshop, UZ Brussel 02/12/2016

Antimicrobial susceptibility testing methods

Diffusion

- Disc diffusion on agar (qualitative)
- Gradient strip diffusion on agar (quantitative variant of disc diffusion) (e.g.: Etest)

Dilution

- Agar dilution (reference)
- Broth dilution (reference)
  - macrodilution
  - microdilution

- Detection of inactivating enzymes

(e.g., beta-lactamases (ESBL, carbapenemases)

- Detection of resistance genes

(mecA gene↔ Methicillin/oxacillin-resistant Staphylococci; vanA/vanB gene ↔ Glycopeptide resistant Enterococci)

(several ESBL or carbapenemase coding genes,

Markers

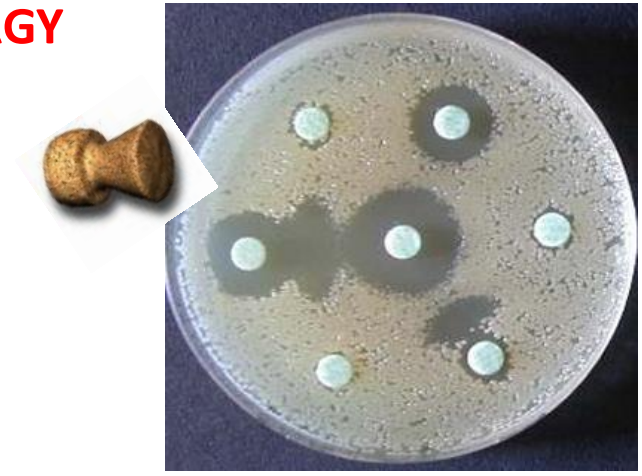
- Confirm bacterial identification/control purity of antibiogram
- Detect mechanisms of resistance
  - Cefoxitin:
    - GNB: AmpC
  - Ertapenem:
    - GNB: carbapEnemases

Interactions

« Champagne cork« image

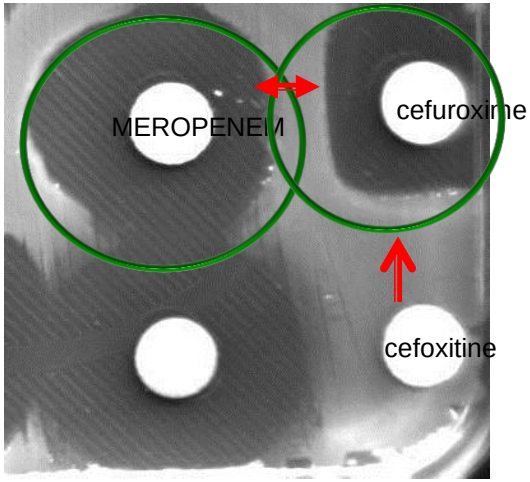
betweenamoxicillin/clavulate and C3G/C4G/Aztreonam = ESBL

SYNERGY



ANTAGONISM

AmpC



Different composition of culture media <-> microorganism type
Composition of AST panel: choice of drugs (for treatment and for detection of relevant resistance markers)
Positioning of discs may be important <-> drug interactions

Automated systems

Standardized
More rapid

Biomerieux Vitek 2/compact

BD Phoenix

MicroScan WalkAway

Enterobacteriaceae

LIST OF ANTIMICROBIAL AGENTS TO BE TESTED AGAINST *ENTEROBACTERIACEAE*

Liste standard	Liste complémentaire
Ampicilline ou Amoxicilline	Céfuroxime
Amoxicilline/acide clavulanique	Aztéonam
Ticarcilline	Netilmicine
Ticarcilline/acide clavulanique[1]	Tobramycine
Mécillinam	Lévofloxacine
Témocilline[1]	Chloramphénicol
Pipéracilline	Tigécycline
Pipéracilline/tazobactam	Triméthoprime
Cefadroxil ou céfalexine	Colistine
Céfoxitine	Azithromycine
Céfotaxime ou ceftriaxone	
Ceftazidime	
Céfépime	
Céfixime	
Imipénème ou méropénème ou doripénème	
Ertapénème	
Amikacine	
Gentamicine	
Acide nalidixique	
Ofloxacine ou norfloxacine	
Ciprofloxacine	
Cotrimoxazole	
Nitrofuranes	
Fosfomycine	

[1] Utile pour l'algorithme de détection des carbapénémases

Disc diffusion on solid medium
Medium: Mueller-Hinton
Inoculum : 0,5 McFarland
Incubation : Normal atmosph., 35±2ºC, 20±4H
Control strain: Escherichia coli ATCC 25922

LIST OF ANTIMICROBIAL AGENTS TESTED

Enterobacteriaceae

Second line molecules

-Colistin
-Tigecycline
-Fosfomycine

Méthodologie et interprétation: CLSI 2012

INTRINSIC RESISTANCE IN ENTEROBACTERIACEAE

Rule no.	Organisms	Ampicillin	Amoxicilin-Clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Cefazolin, Cefalotin Cefalexin, Cefadroxil	Cefoxitin[2]	Cefuroxime	Tetracyclines	Tigecycline	Polymyxin B, Colistin	Nitrofurantoin
1.1	*Citrobacter koseri, Citrobacter amalonaticus*[3]	R			R							
1.2	*Citrobacter freundii*[4]	R	R	R		R	R					
1.3	*Enterobacter cloacae* complex	R	R	R		R	R					
1.4	*Enterobacter aerogenes*	R	R	R		R	R					
1.5	*Escherichia hermannii*	R			R							
1.6	*Hafnia alvei*	R	R	R		R	R					
1.7	*Klebsiella pneumoniae*	R			R							
1.8	*Klebsiella oxytoca*	R			R							
1.9	*Morganella morganii*	R	R	R		R			R		R	R
1.10	*Proteus mirabilis*								R	R	R	R
1.11	*Proteus penneri*	R				R		R	R	R	R	R
1.12	*Proteus vulgaris*	R				R		R	R	R	R	R
1.13	*Providencia rettgeri*	R	R	R		R		R	R	R	R	R
1.14	*Providencia stuartii*	R	R	R		R		R	R	R	R	R
1.15	*Raoultella* spp.	R			R							
1.16	*Serratia marcescens*	R	R	R		R	R	R	R[5]		R	R
1.17	*Yersinia enterocolitica*	R	R	R	R	R	R					
1.18	*Yersinia pseudotuberculosis*										R	

Proteaceae

Enterobacteriaceae are also intrinsically resistant to benzylpenicillin, glycopeptides, fusidic acid, macrolides (some exceptions), lincosamides, streptogramins, rifampicin, daptomycin and linezolid.

EUCAST intrinsic resistance and exceptional phenotypes, Expert Rules version 3.1, September 2016

Ambler classification of β-lactamases

β-lactamases (>1000 variants)

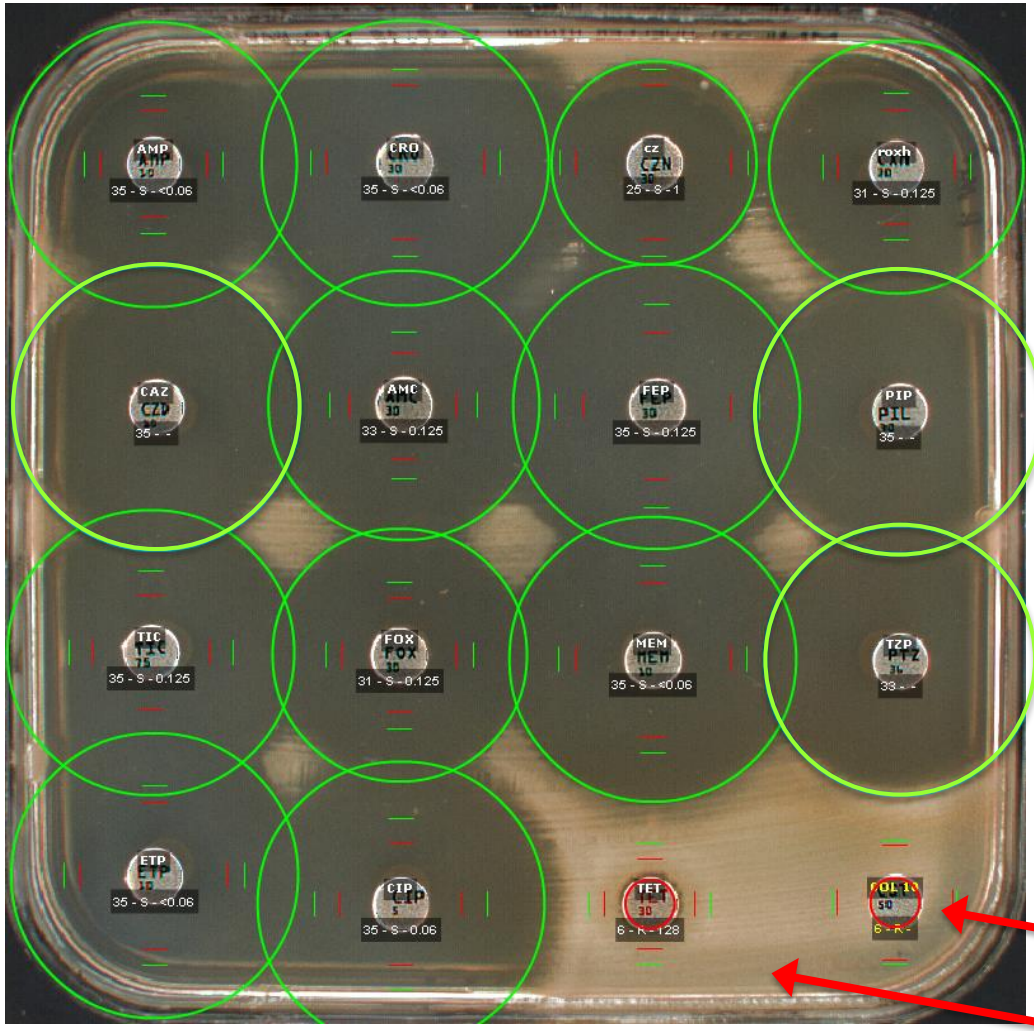
Serine			Zinc (metallo-β-lactamases)
Class A	**Class C**	**Class D**	**Class B**
TEM SHV CTX-M KPC GES VEB BEL …	cAmpC pAmpC - CMY - DHA - FOX - ACC - ACT …	OXA …	NDM VIM IMP GIM SIM SPM …
-Small spectrum penicillinases -Broad spectrum (ESBL) -Carbapenemases	-Cephalosporinases	-Small spectrum penicillinases -Broad spectrum (ESBL) -Carbapenemases (weak)	-Carbapenemases

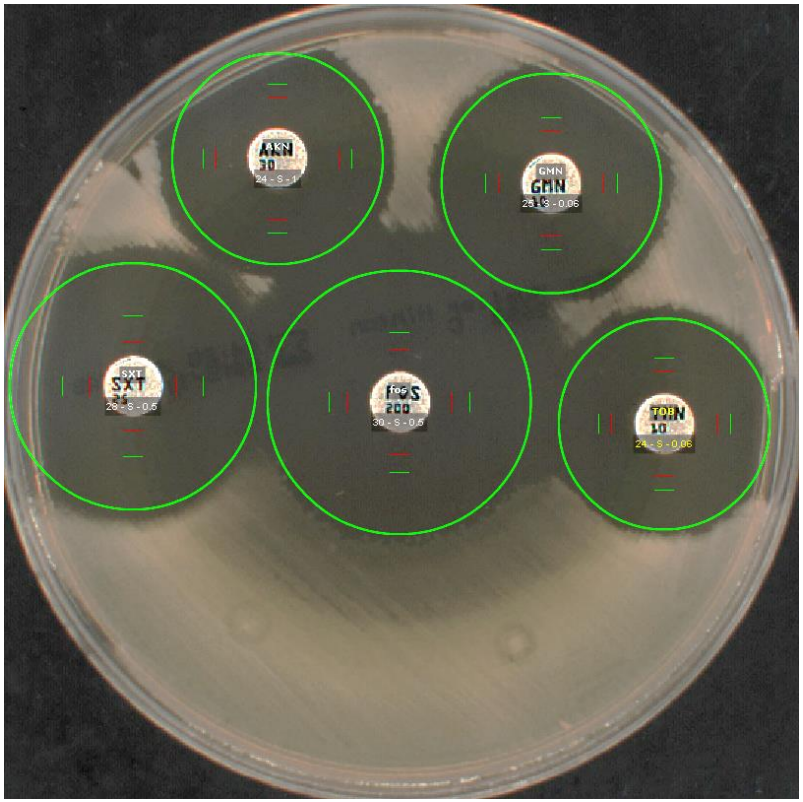
Enterobacteriaceae

- Natural/intrinsic susceptibility and resistance to β-lactam agents
  - Classification of Enterobacteriaceae

Groupe	Espèces	Enzyme naturelle
0	*P.mirabilis, Salmonella spp*	No enzyme
1	*E.coli, Shigella spp*	Low level Case (non inducible)
2	*K.pneumoniae, K.oxytoca, C.koseri*	Penicillinase
3	*E.aerogenes, E.cloacae, C.freundii, S.marcescens, Morganella morganii, Hafnia. alvei,*	Inducible Case R to beta-lactamase inhibitors (Clav./Taz.) (ampC)
4	*Yersinia enterocolitica*	Pase & Case (ampC)
5	*P. vulgaris, P.penneri*	Inducible Case (cefuroximase) S to inhibitors (Clav./Taz.)
6	*Kluyvera spp, Rhanella aquatilis, C.sedlakii, Erwinia persicina*	Chromosomal ESBL

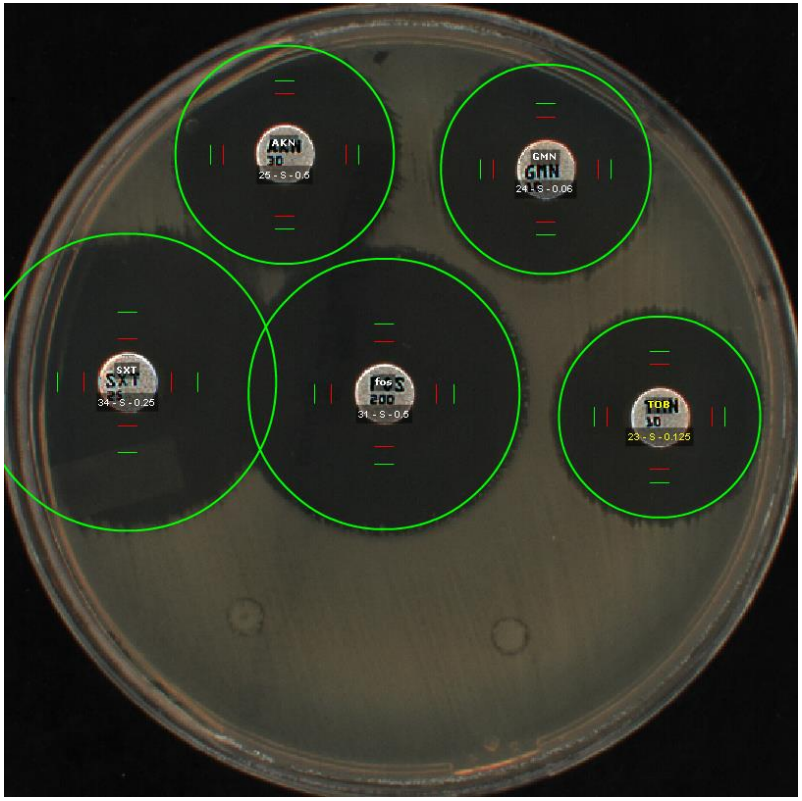
Proteus mirabilis (group 0)





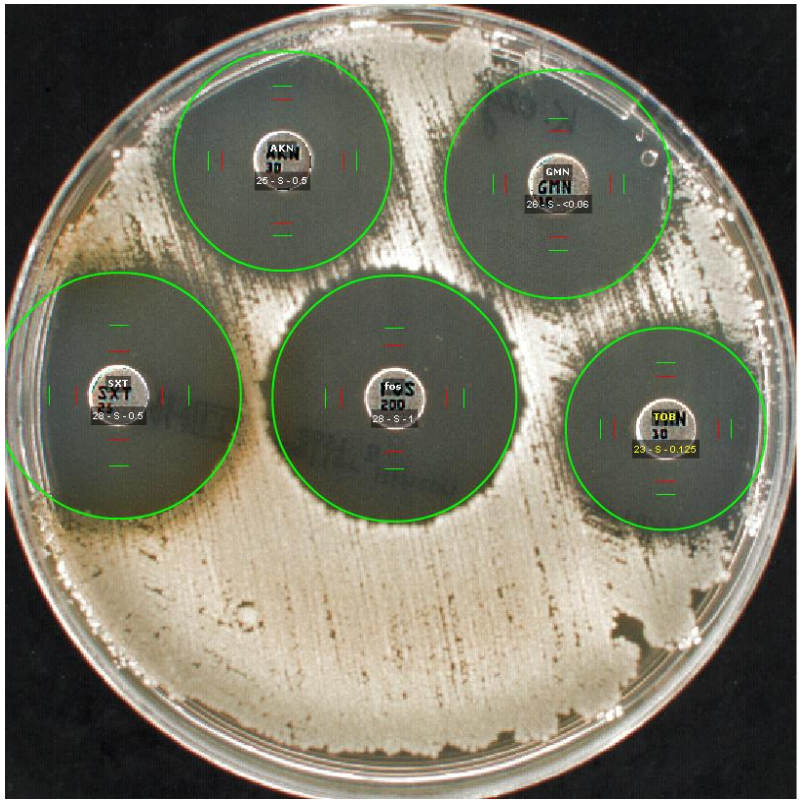
Intrinsic resistance

Escherichia coli (group 1)

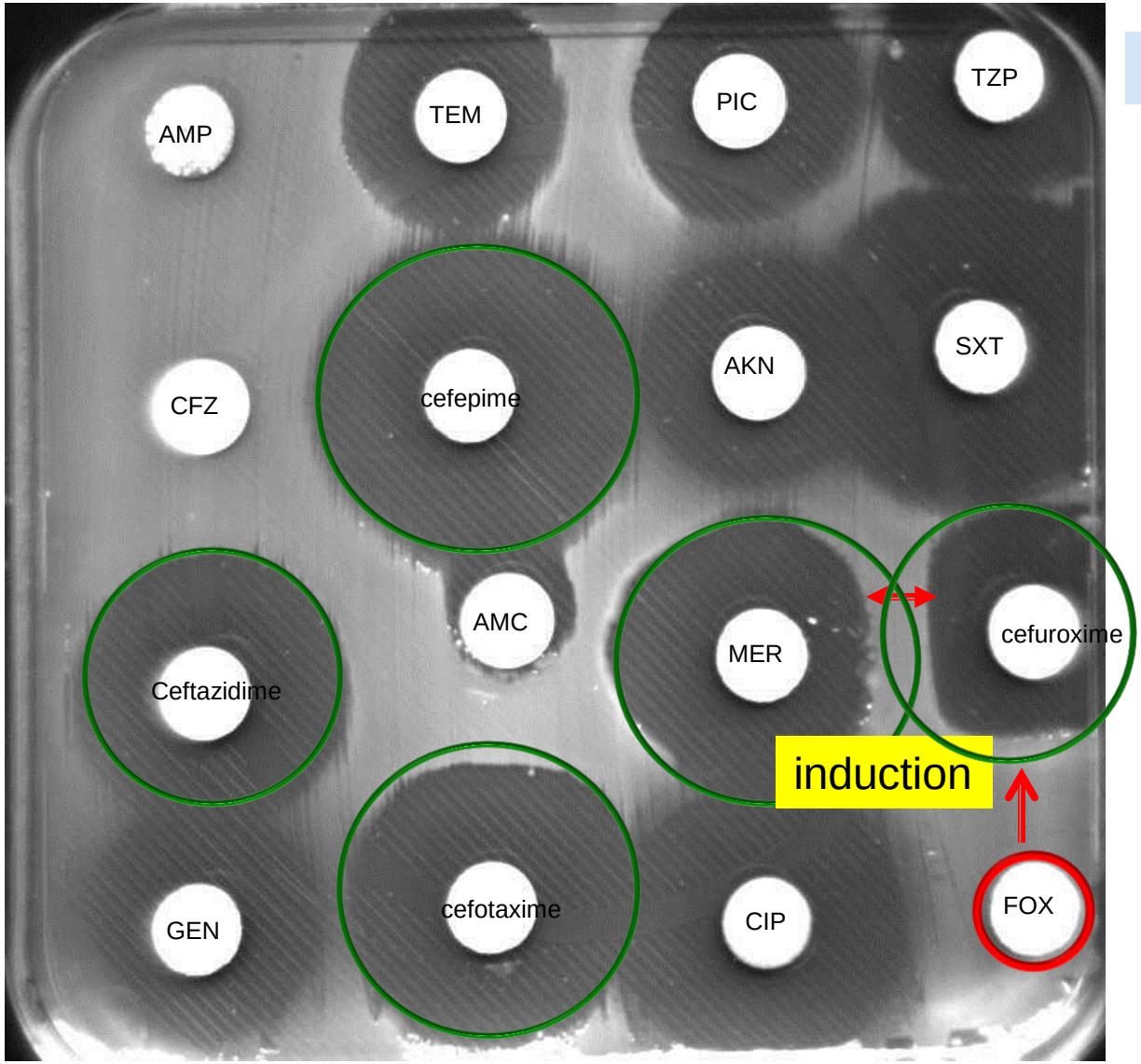


Klebsiella oxytoca et K. pneumoniae (groupe 2)

Small spectrum penicillinase = (R Ampi/Amox, Ticar/(Pip))



Group 3: *Enterobacter cloacae* wild type (Cephalosporinase low level)



Resistance CEFOX > CEFUR

Antibiotique	ENTBCLO CMI/*Conc*	SIR
Ampicilline	>8	R
Pipéracilline	<=4	S
Pipéracilline-Tazobactam	<=4/4	S
Céfazoline	>4	R
Céfuroxime	>8	R
Céfoxitine	>16	R
Ceftriaxone	<=1	S
Ceftazidime	<=1	S
Aztréonam	<=1	S
Céfépime	<=1	S
Méropénème	<=0.5	S
Témocilline	32	S
Lévofloxacine	<=0.5	S
Ciprofloxacine	<=0.125	S
Amikacine	<=4	S
Tobramycine	<=1	S
Gentamicine	<=1	S
Triméthoprime-Sulfaméthoxazole	<=1/19	S
Colistine	<=1	S
Amoxicilline-Clavulanate (f)	>8/2	R
Ertapenem	<=0.25	S

Resistance Cefoxitin (Amox/Clav.,(PTZ))

Induction of AmpC (Cefox, Carbapenems, BL inhibitors)

Morganella morganii (group 3)

Most potent β-lactam agents inducer of AmpC: carbapenems, cefoxitin, clavulanate

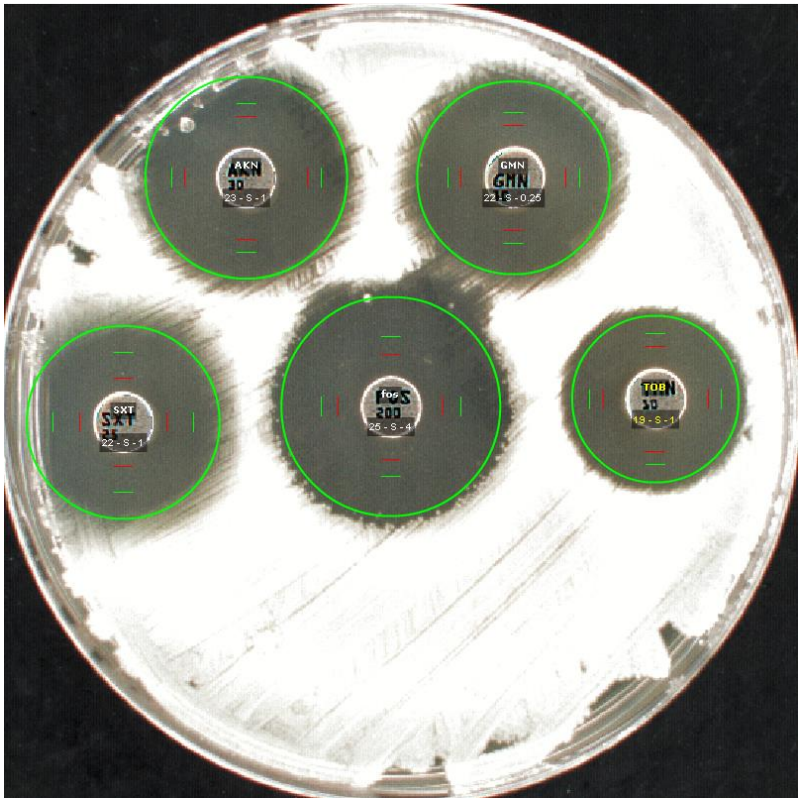
Cefur: R (R-CEFUR >CFOX)

Tetracycline R

Colistin R

Serratia spp. (group 3)

Cefur: R (CEFUR >CFOX)

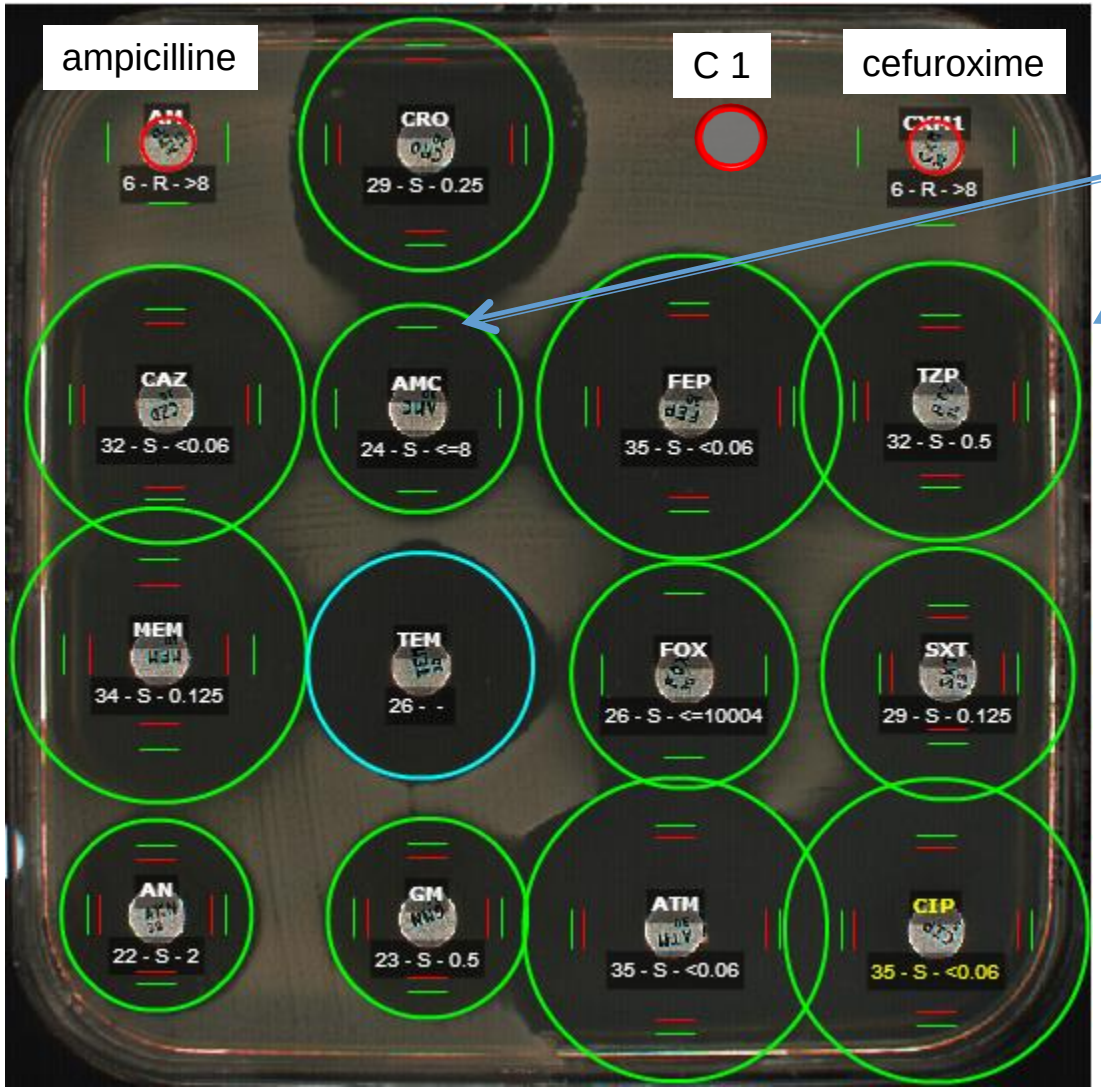


Tetracycline R

Colistin R (paradoxical resistance (double zone)

P. vulgaris (Groupe 5)

Wild type



Susceptible to inhibitors

Colistin R
Nitrofurantoine R
Tigecycline R

TIGE R: Proteus
Providencia

EXCEPTIONAL RESISTANCE PHENOTYPES OF GRAM-NEGATIVE BACTERIA

Rule no.	Organisms	Exceptional phenotypes
5.1	Any Enterobacteriaceae (except Proteeae and *Serratia marcescens*)	Resistant to colistin[1,2]
5.2	*Salmonella* Typhi	Resistant to fluoroquinolones and/or carbapenems
5.3	*Pseudomonas aeruginosa* and *Acinetobacter* spp.	Resistant to colistin[1]
5.4	*Haemophilus influenzae*	Resistant to any third-generation cephalosporin, carbapenems, fluoroquinolones
5.5	*Moraxella catarrhalis*	Resistant to any third-generation cephalosporin and/or fluoroquinolones
5.6	*Neisseria meningitidis*	Resistant to any third generation cephalosporins and/or fluoroquinolones
5.7	*Neisseria gonorrhoeae*	Resistant to spectinomycin and/or azithromycin

[1] Except in countries where colistin resistance is not rare. [2] Colistin MICs for some *Salmonella* serotypes are slightly above the breakpoint (S ≤2; R >2 mg/L).

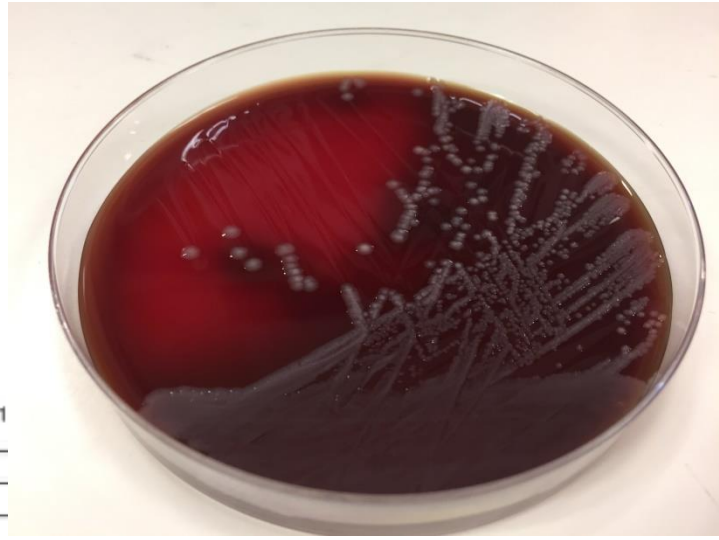
Exceptional resistance phenotypes may be exceptional at some places but not in others (e.g. colistin-resistance in Klebsiella in Greece/Italy (->20%) and may change over time (e.g: resistant to carbapenem in Enterobacteriaceae)
Also possible occurrence of wide local, regional or national differences

EUCAST intrinsic resistance and exceptional phenotypes, Expert Rules version 3.1, September 2016

The occurrence of « exceptional phenotypes » should always raise the possibility of technical problem in AST, check for bacterial ID an/or presence of antibiogram contaminations (purity check !)

My own recommendation ! (unpublished)

Escherichia coli



Germe sélectionné : Escherichia coli

Source : U

Prélevé : 21 nov. 2016 1

Commentaires :	

Résultats Antibiogramme	Heure de l'analyse :	9,25 heures		État : Final	
Antibiotique	**CMI**	**Interprétation**	**Antibiotique**	**CMI**	**Interprétation**
Témocilline	<= 4	S	Méropénème	<= 0,25	S
Ampicilline	>= 32	R	Amikacine	<= 2	S
Amoxicilline/acide clavulanique	16	*R	Gentamicine	<= 1	S
Pipéracilline/tazobactam	64	I	Ciprofloxacine	<= 0,25	S
Céfuroxime	>= 64	R	Tigecycline	<= 0,5	S
Céfuroxime axétil	>= 64	R	Fosfomycine	32	S
Céfotaxime	<= 1	S	Nitrofurantoïne	<= 16	S
Ceftazidime	<= 1	S	Colistine	>= 16	R
Céfépime	<= 1	S	Triméthoprime/sulfaméthoxazole	>= 320	R
Ertapénème	<= 0,5	S			

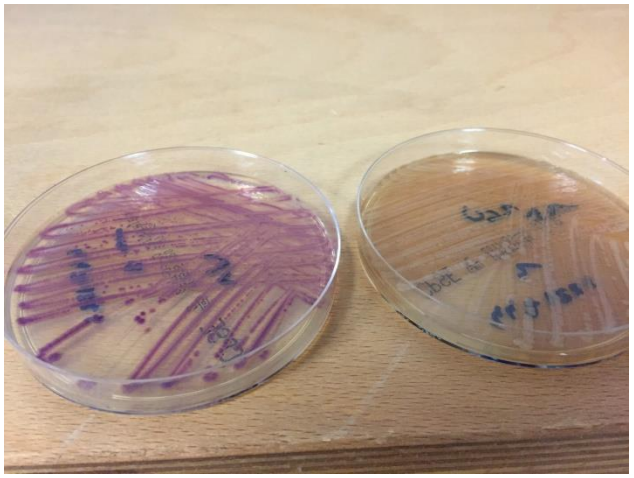
+= Médicament déduit *= Modification AES **= Modification Utilisateur

Résultats AES		
Fiabilité :	Concordant	
Phénotype :	BÊTA-LACTAMINES	CÉPHALOSPORISNASE (AmpC),PENICILLINASE ACQUISE + CÉPHALOSPORINASE (AmpC)

Urine from outpatient

- $>10^5$ CFU/ml *E. coli*
- Two types of colonies on subculture

Mixed culture



Résultats Antibiogramme	Heure de l'analyse :	8,50 heures		État :	Final
Antibiotique	**CMI**	**Interprétation**	**Antibiotique**	**CMI**	**Interprétation**
Témocilline	<= 4	S	Méropénème	<= 0,25	S
Ampicilline	>= 32	R	Amikacine	<= 2	S
Amoxicilline/acide clavulanique	>= 32	R	Gentamicine	<= 1	S
Pipéracilline/tazobactam	<= 4	**S	Ciprofloxacine	<= 0,25	S
Céfuroxime	>= 64	R	Tigecycline	2	*R
Céfuroxime axétil	>= 64	R	Fosfomycine	>= 256	R
Céfotaxime	4	R	Nitrofurantoïne	64	*R
Ceftazidime	4	*R	Colistine	>= 16	R
Céfépime	<= 1	**S	Triméthoprime/sulfaméthoxazole	<= 20	S
Ertapénème	<= 0,5	S			

+= Médicament déduit *= Modification AES **= Modification Utilisateur

Résultats AES		
Fiabilité :	Concordant	
Phénotype :	BÊTA-LACTAMINES	BÊTA-LACTAMASE À SPECTRE ÉTENDU,CÉPHALOSPORINASE À HAUT NIVEAU (AmpC)

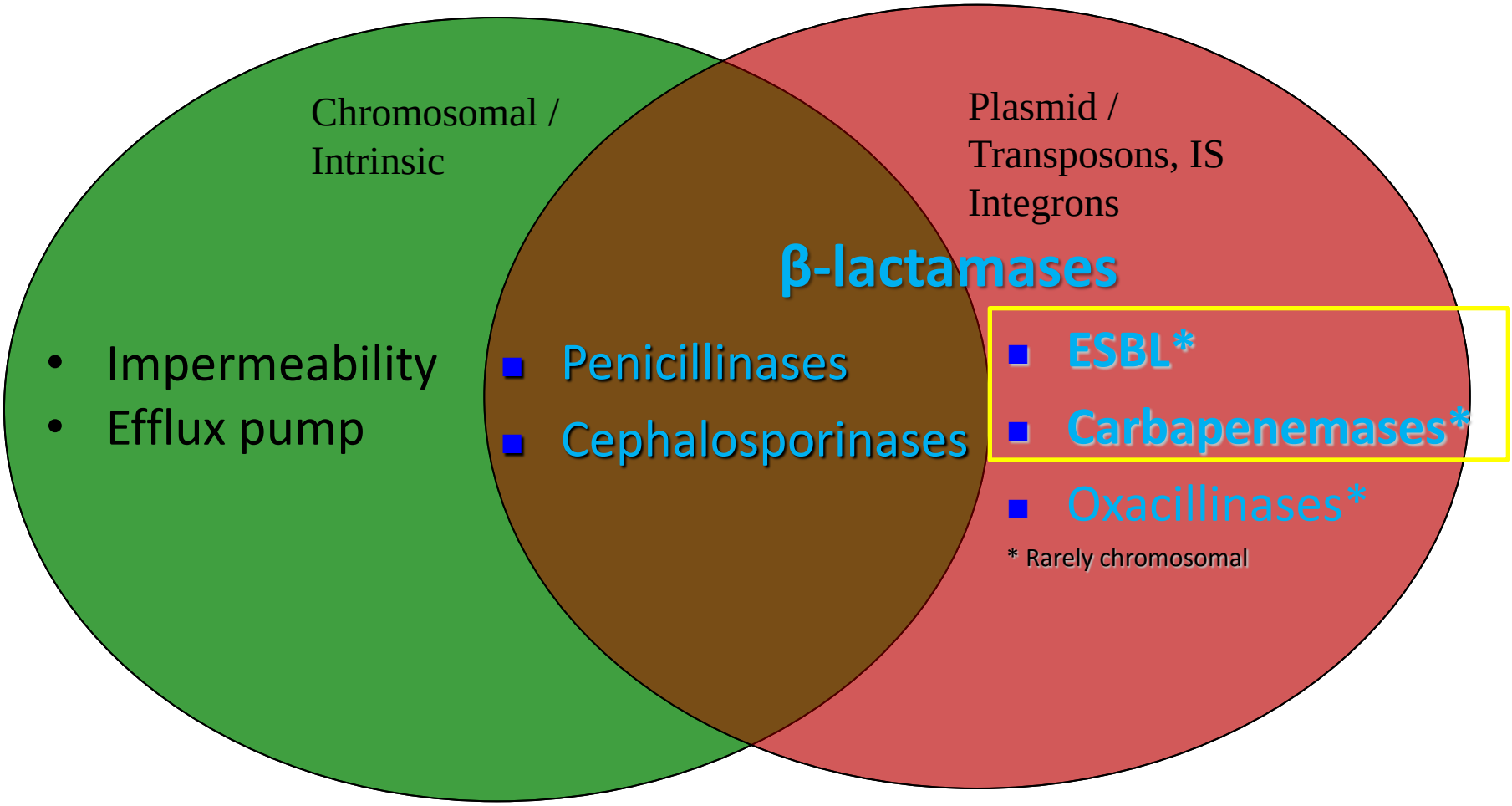
Résultats Antibiogramme	Heure de l'analyse :	8,25 heures		État :	Final
Antibiotique	**CMI**	**Interprétation**	**Antibiotique**	**CMI**	**Interprétation**
Témocilline	<= 4	S	Méropénème	<= 0,25	S
Ampicilline	>= 32	R	Amikacine	<= 2	S
Amoxicilline/acide clavulanique	16	**S	Gentamicine	<= 1	S
Pipéracilline/tazobactam	<= 4	S	Ciprofloxacine	<= 0,25	S
Céfuroxime	2	S	Tigecycline	<= 0,5	S
Céfuroxime axétil	2	S	Fosfomycine	<= 16	S
Céfotaxime	<= 1	S	Nitrofurantoïne	<= 16	S
Ceftazidime	<= 1	S	Colistine	<= 0,5	S
Céfépime	<= 1	S	Triméthoprime/sulfaméthoxazole	>= 320	R
Ertapénème	<= 0,5	S			

+= Médicament déduit *= Modification AES **= Modification Utilisateur

Résultats AES	
Fiabilité :	Concordant

E. coli and *M. morgannii*

Acquired resistance mechanisms to β-lactams



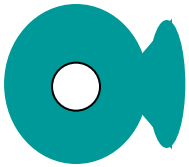
Laboratory detection of β-lactamases

Different substrates profiles and different susceptibility to inhibitor compounds

Serine			Zinc (metallo-β-lactamases)
Class A	**Class C**	**Class D**	**Class B**
TEM **SHV** **CTX-M** KPC GES VEB BEL …	**cAmpC** **pAmpC** - CMY - DHA - FOX - ACC - ACT …	**OXA** OXA-penicillinases (1, -2, -10,….) OXA-ESBL (-15, -18,-32,….) OXA-carba (-48, -162, -181, -204, 244,…) (-23,-24,-58,…)	**VIM** **NDM** IMP GIM SIM SPM …
Clavulanate Tazobactam Boronic acid (KPC)	Cloxacillin Boronic acid	No specific inhibitors !	EDTA, DPA

Enterobacteriacae : Detection of ESBLs

- DOUBLE DISC METHOD:
  - synergy between clavulanate - C3/C4/aztreonam
  - Interpretation (champagne cork image)

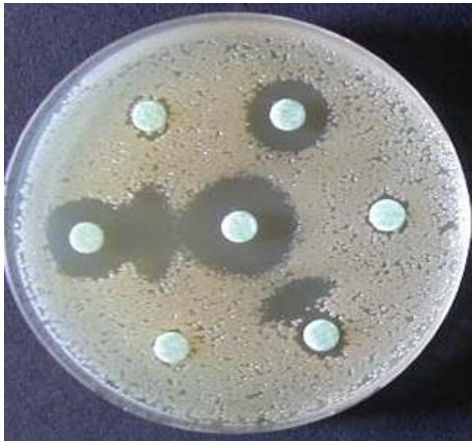


C3 (ceftazidime, cefotaxime)
C4 (cefepime..)
Aztreonam

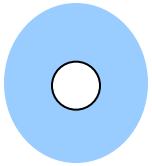
Acide clavulanique

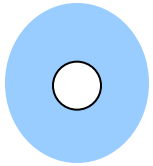
Champagne cork image=
partial/complete restoration of activity
lthrough beta-lactamase inhibitor

ESBL



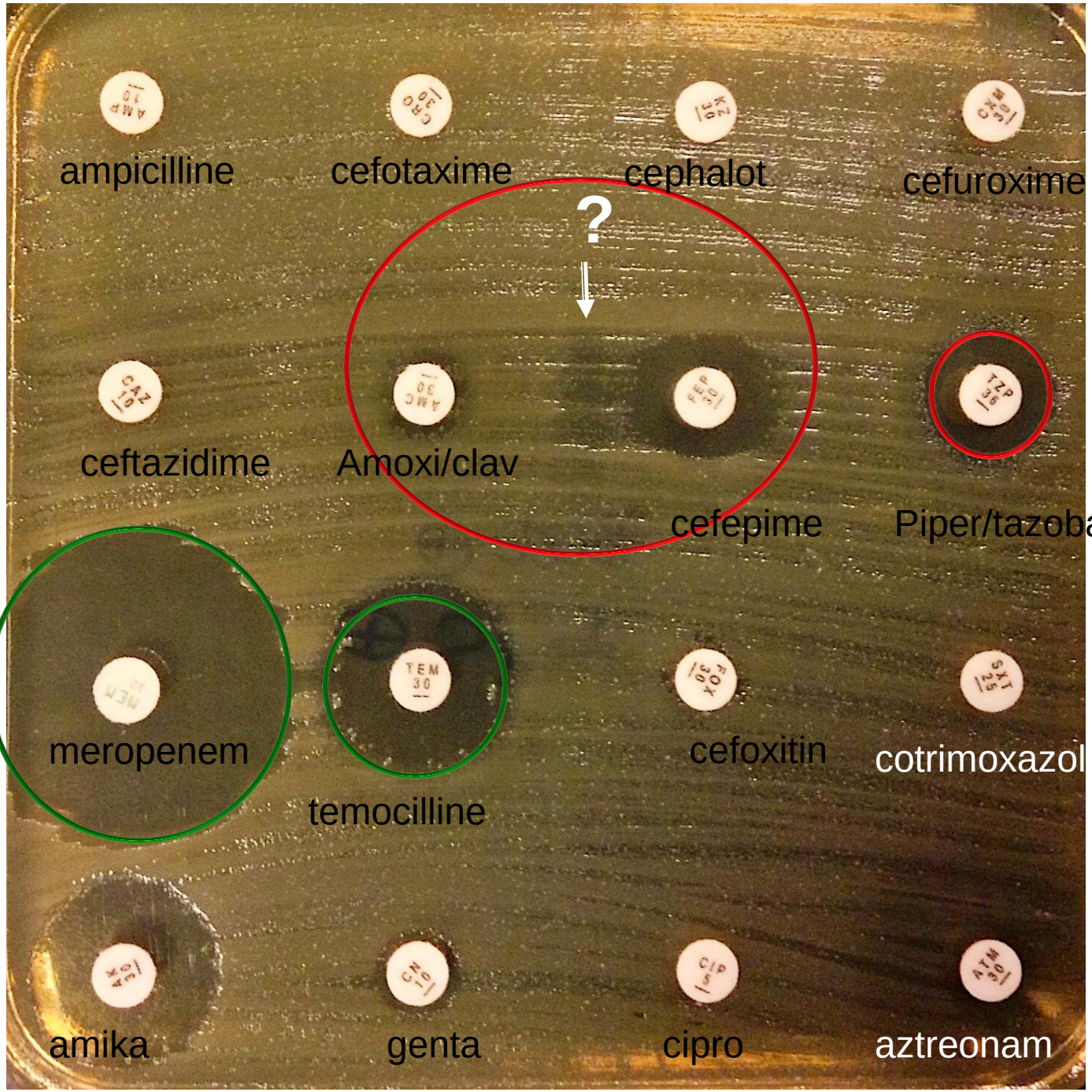
Test necessitate >1 substrate
(at least 2; CAZ/CTAX)





No « champagne cork » image = No
effect of inhibitory compound

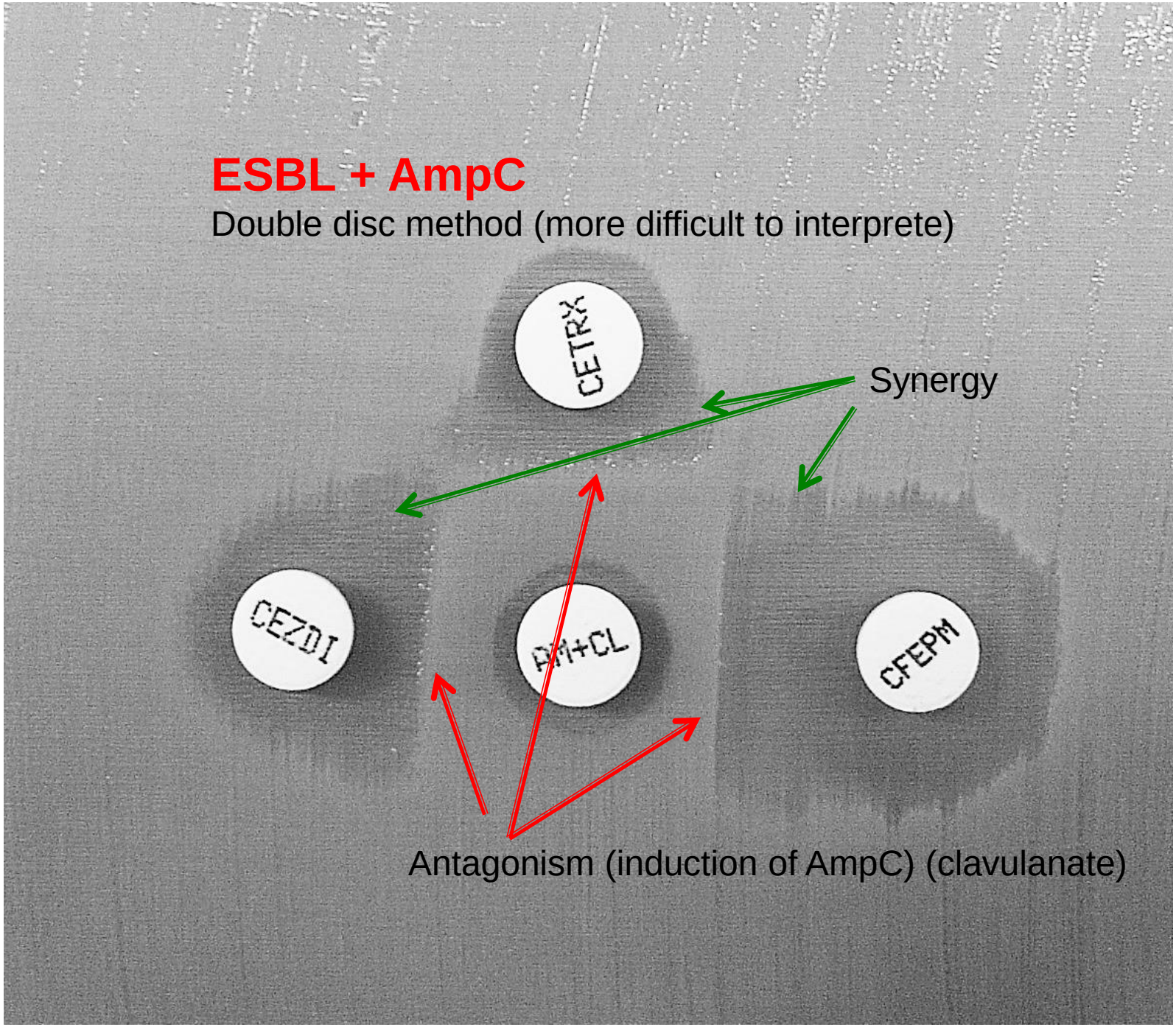
Cephalosporinase or Pénicillinase



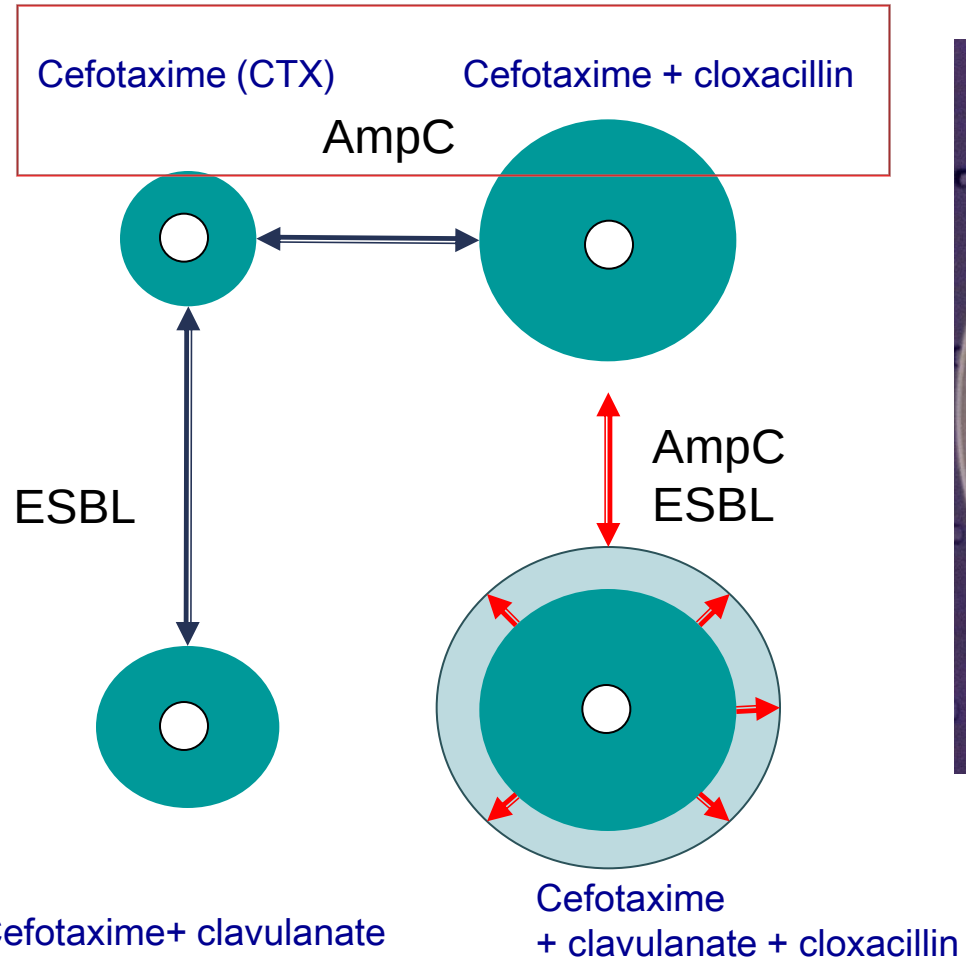
E. cloacae
AmpC HN
+ ESBL ??

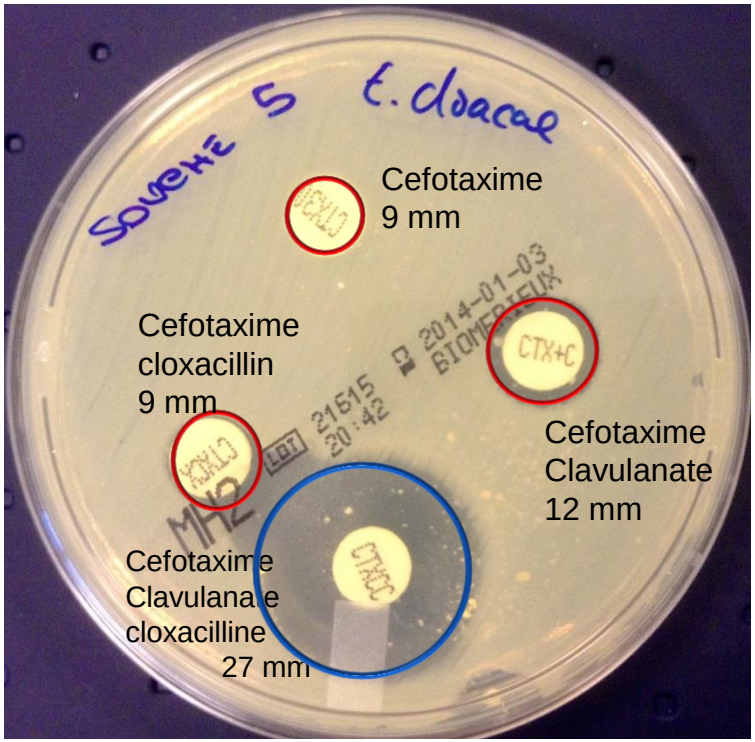
ESBL + AmpC

Double disc method (more difficult to interprete)



Detection of ESBL in AmpC producing organisms



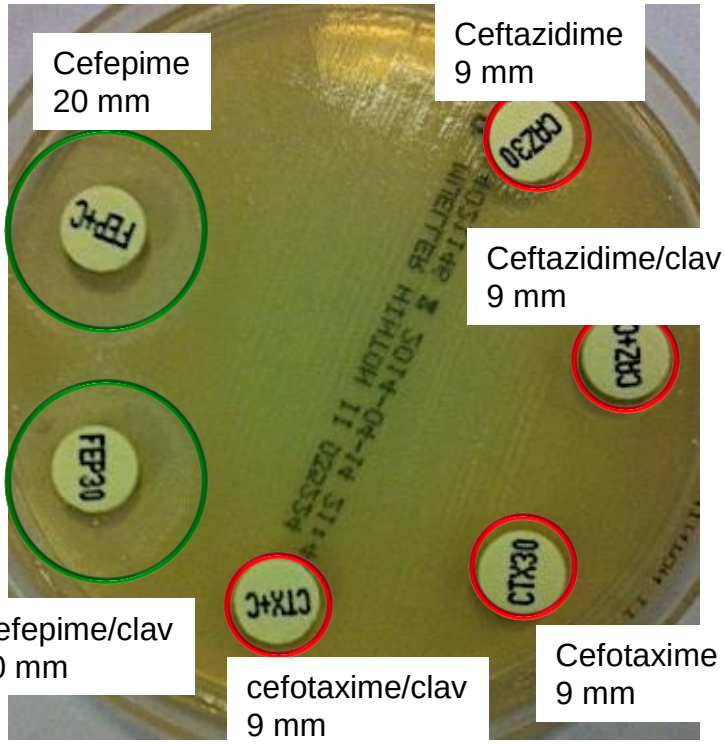


Cloxacillin inhibits AmpC and allows the detection of synergy with clavulanate

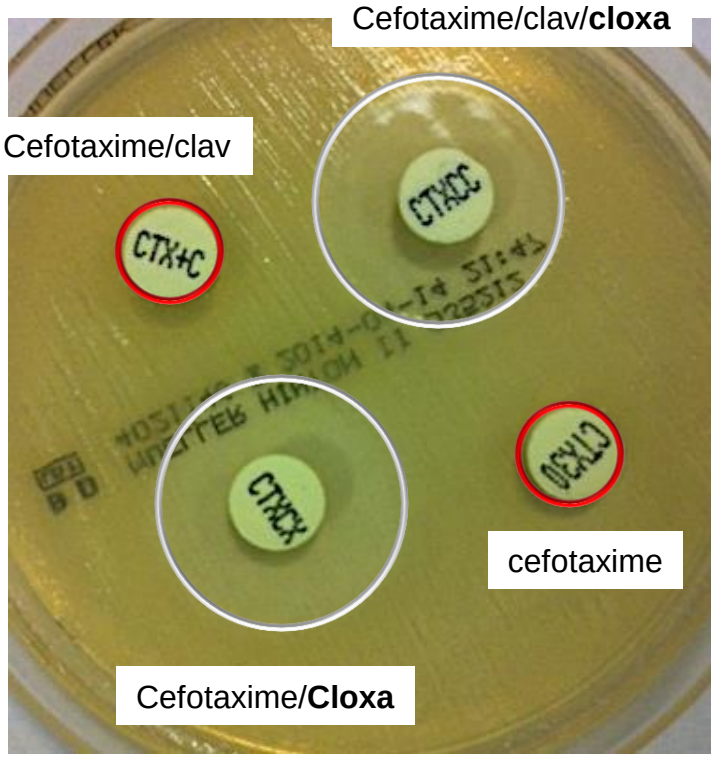
Exemple : *Citrobacter freundii* AmpC +, ESBL -

Use of combined disks with Cefepime/Clav in organisms of group 3

Combined disc tests
ESBL: NEG



Test AmpC positive but ESBL negative



INTERPRETATIVE GUIDELINES FOR ESBLS

- **Perform laboratory tests for detection of extended spectrum β-lactamase** (ESBLs) (epidemiology/Infection control)

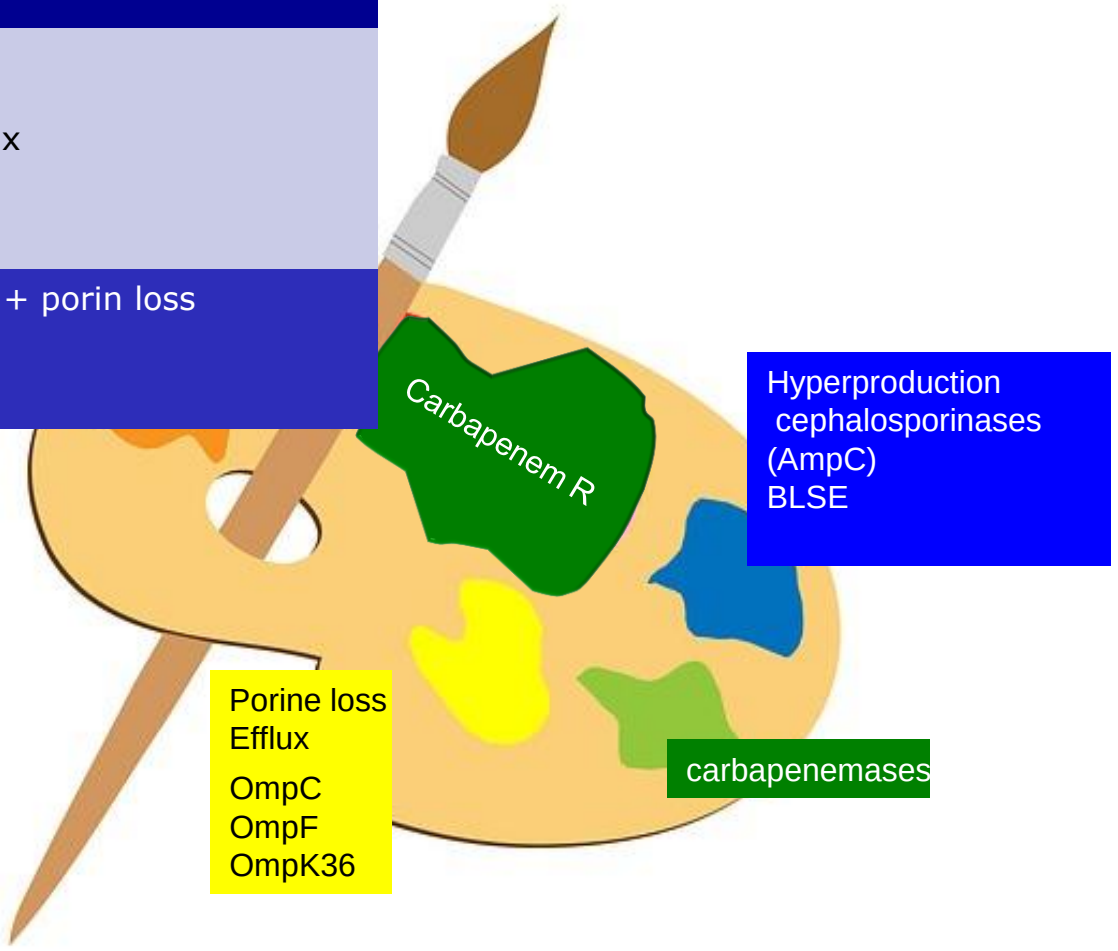
- **Do not edit results for C3G or C4G and for aztreonam** (AZT) for ESBL-producing Enterobacteriaceae isolates (categorize as S, I, R on the basis of antibiogram result)

- **When an ESBL-producing isolate is categorized as « S » to C3G/C4G or AZT**, an MIC should be performed against these agents when used for treatment of infection.

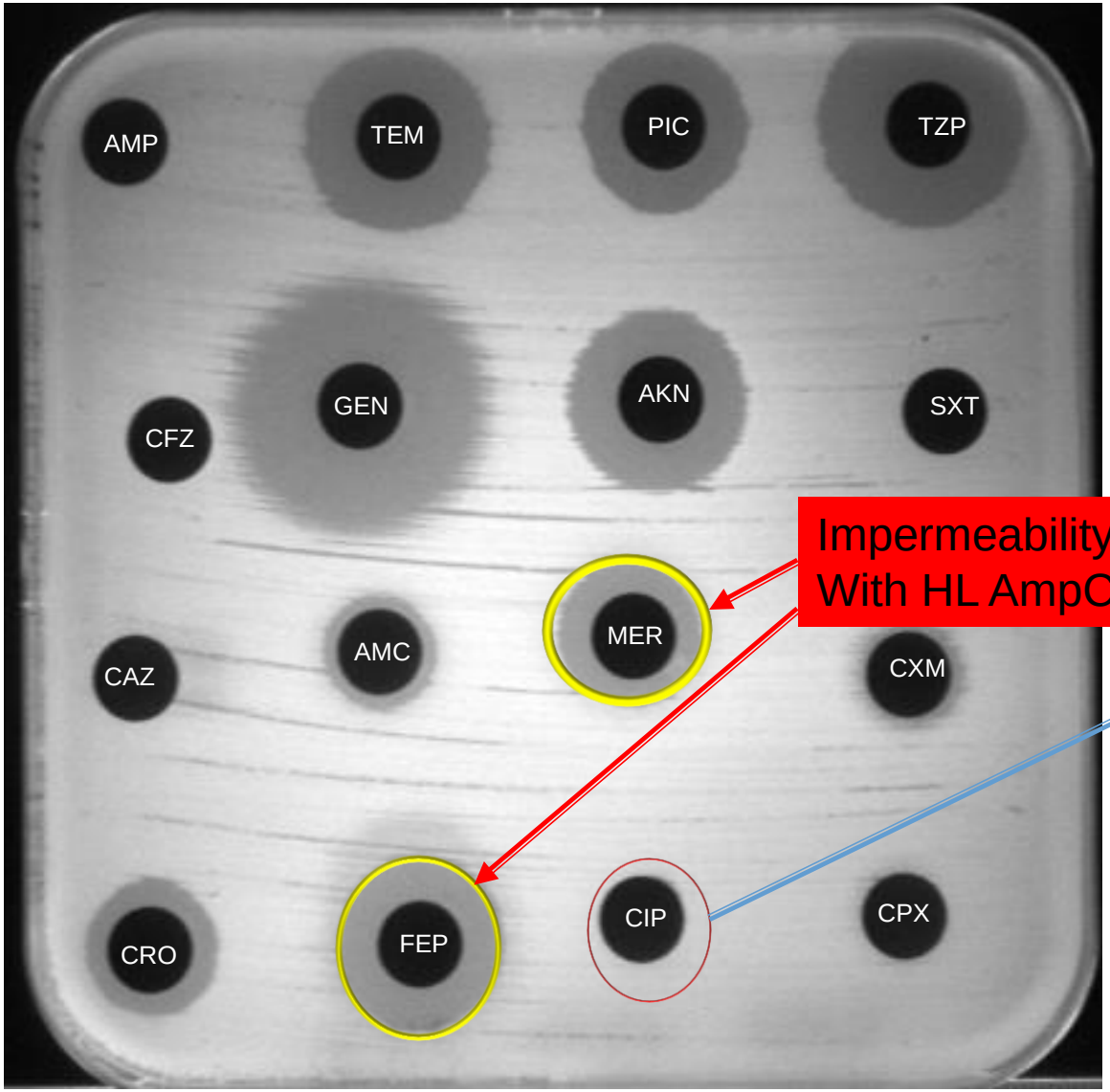
- **When an ESBL-producing isolate is categorized as « S » to a penicillin/beta-lactamase inhibitor association** (clavulanate, tazobactam), an MIC should be performed before using this agents for treatment of infection other than UTI or urosepsis.

Resistance to carbapenems

Enterobacteriaceae	Cephalosporinase + porin loss Carbapenemase
P. aeruginosa	Porin loss Up-regulated efflux Carbapenemase
Acinetobacter spp.	Cephalosporinase + porin loss Carbapenemase



Lee *et al.* J Appl Microbiol, 2003
Kim *et al.* Mol Microbiol, 2006
Nordman trends Mol Med 2012



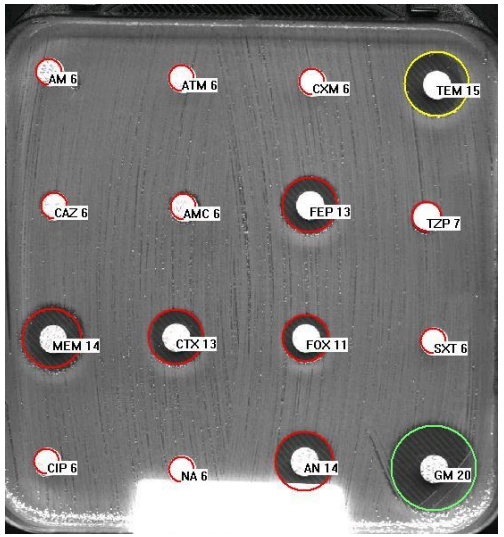
***Enterobacter aerogenes* (Case HN + ↓ permeability)**

Perform ESBL test
Perform MIC to meropenem

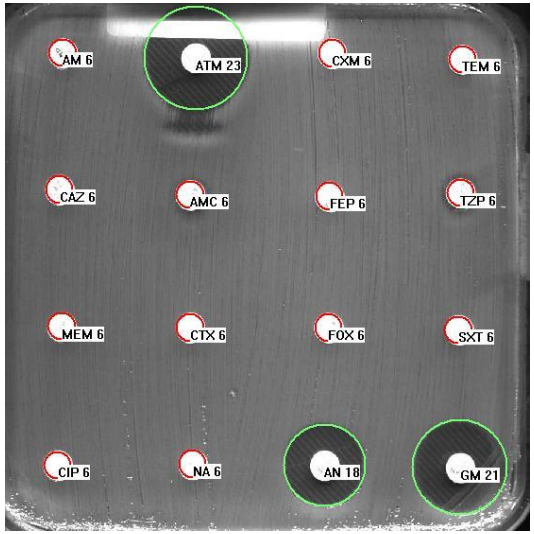
Antimicrobial resistance patterns of CPE

MDR
XDR

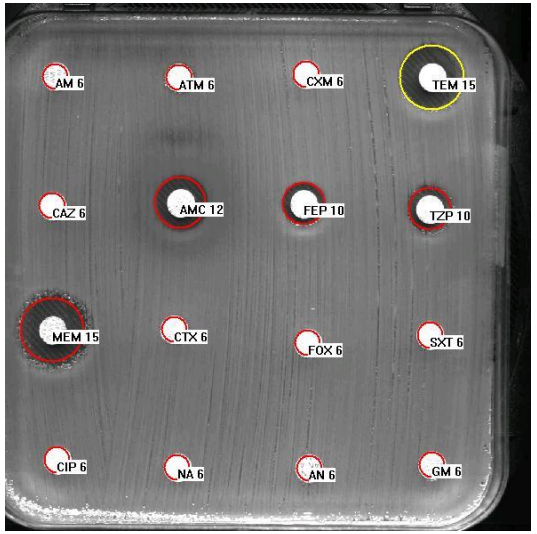
K. pneumoniae KPC-2

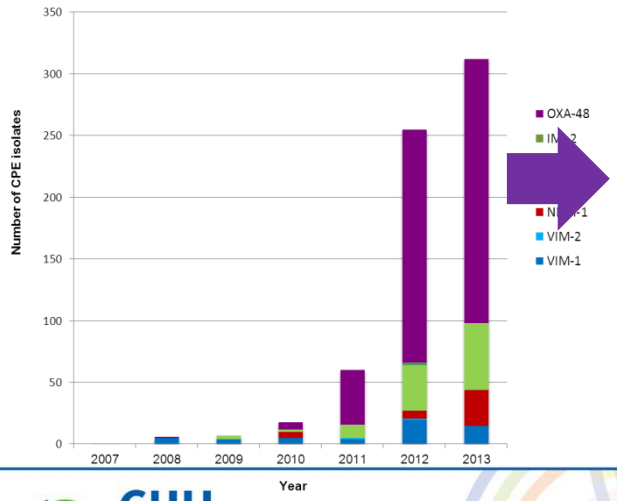


K. pneumoniae VIM-1



E. coli NDM-1





E. coli OXA-48

MDR ?
Carba R?

→ Difficult to detect!

→ Unnoticed spread!

OXA-48 carbapenemase (Class D)

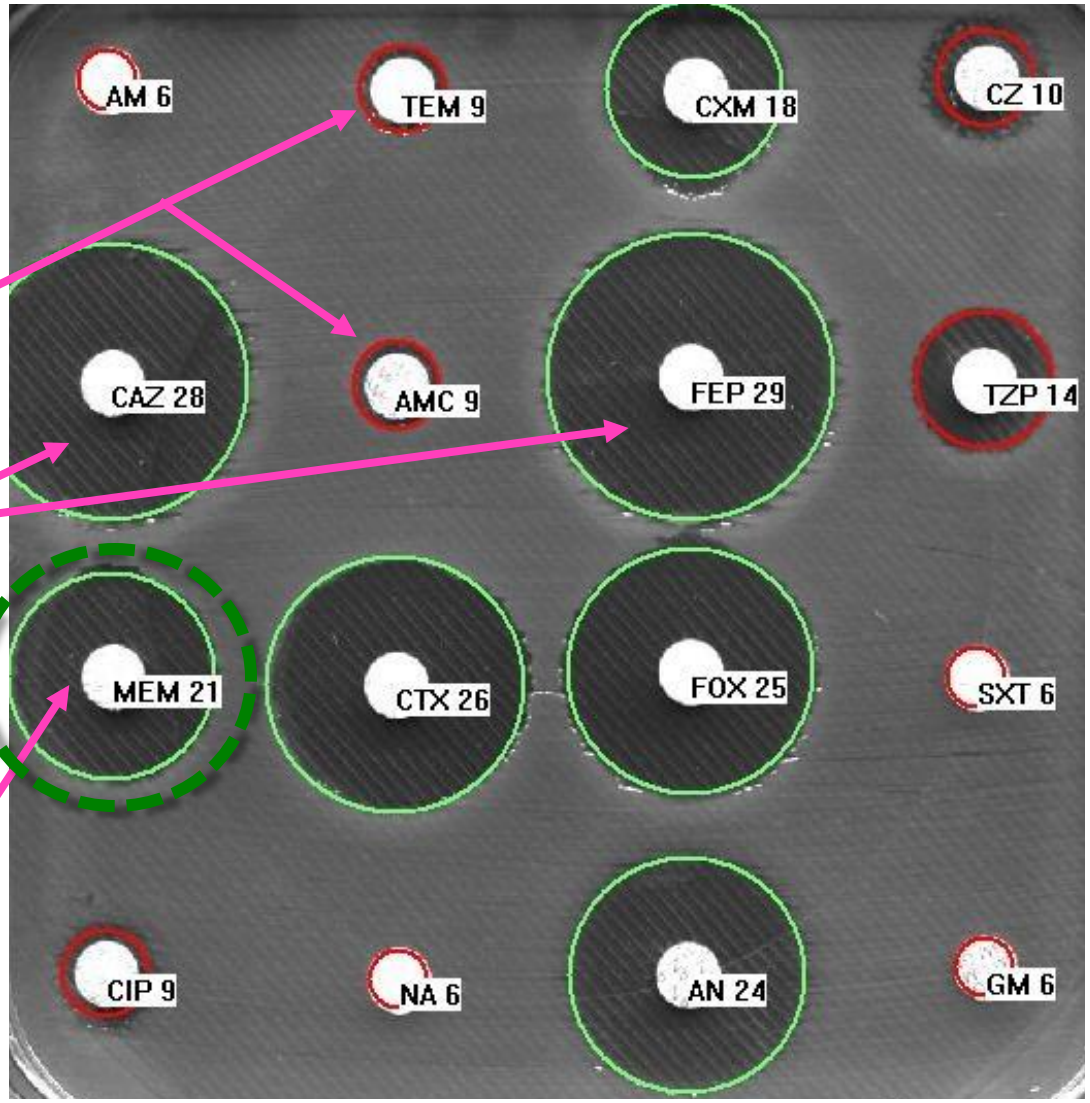
R penicillins (TEMO) and R AMC/PTZ (no activity of BL inhibitors)

C3G-C4G S if no associated ressitance mechanisms

variable level of R to carbapenems (CMI MEM 0.25->128) but most often low level R

Zone <25mm EUCAST (screening)

<28 mm in Belgium



Clinical breakpoints of selected carbapenems and EUCAST proposals for screening cut-off of carbapenemases

	Clinical breakpoints (susceptible category)				Screening cut-off	
	MIC (mg/L)		Disk diffusion inhibition zone diameter (mm)			
	EUCAST	CLSI	EUCAST	CLSI	MIC (mg/L)	Disk diffusion inhibition zone diameter (mm)
Ertapenem	≤0.5	≤0.25	≥25	≥23	>0.125	<25
Imipenem	≤2	≤1	≥22	≥23	>1	<23
Meropenem	≤2	≤1	≥22	≥23	>0.125	<25

[1] **Meropenem**: best balance of sensitivity and specificity.

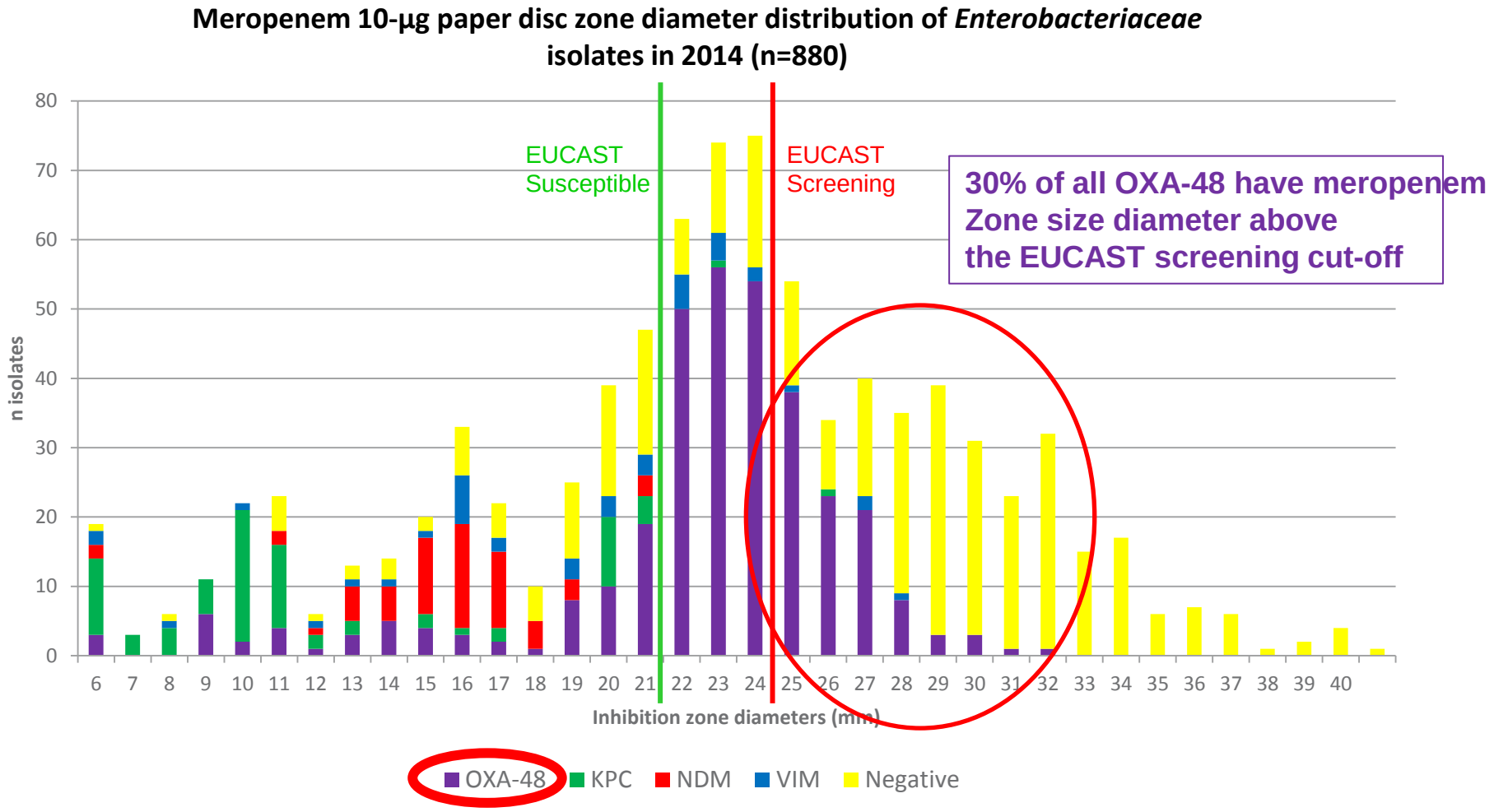
[2] In some cases **OXA-48** producers have zone diameters up to 26 mm, **so <27 mm** may be used as a screening cut-off during outbreaks caused by OXA-48-producing Enterobacteriaceae, but with reduction in specificity.

[3] 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] **Ertapenem**: high sensitivity, but low specificity, and therefore not routinely recommended. **!?**

EUCAST clinical breakpoints (v4.0; 2014-01-01)
EUCAST guidelines for detection of resistance mechanisms (v 1.0; 2013-12-11)

EUCAST meropenem disk (MEM10) screening breakpoint

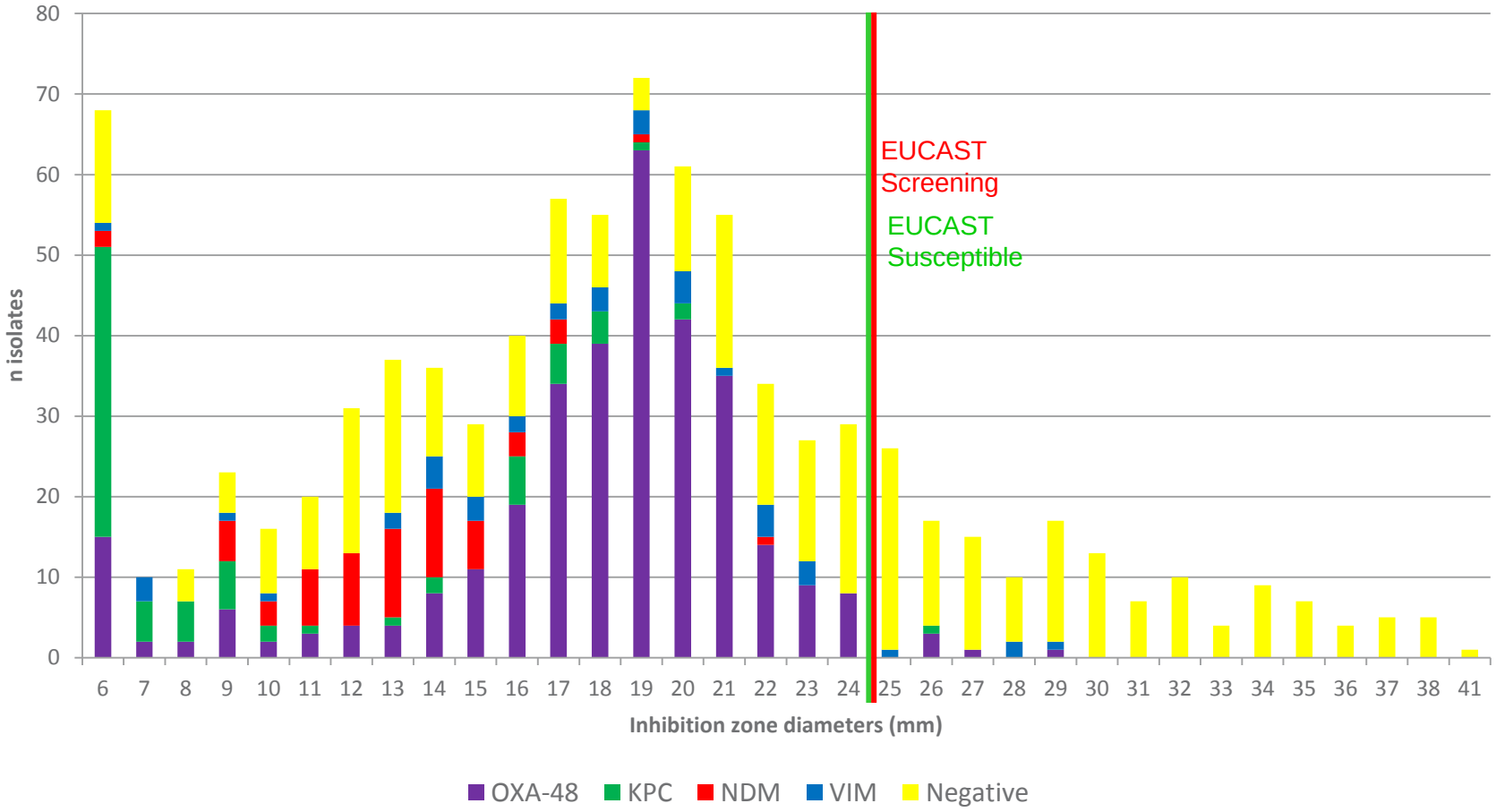


- **EUCAST recommended screening breakpoint (MEM10 <25 mm): Sensitivity =80% for CPE overall**

Huang et al. ECCMID 2015. Oral session O096

EUCAST ertapenem disk (ETP10) screening breakpoint

Ertapenem 10-µg paper disc zone diameter distribution of *Enterobacteriaceae* isolates in 2014 (n=880)

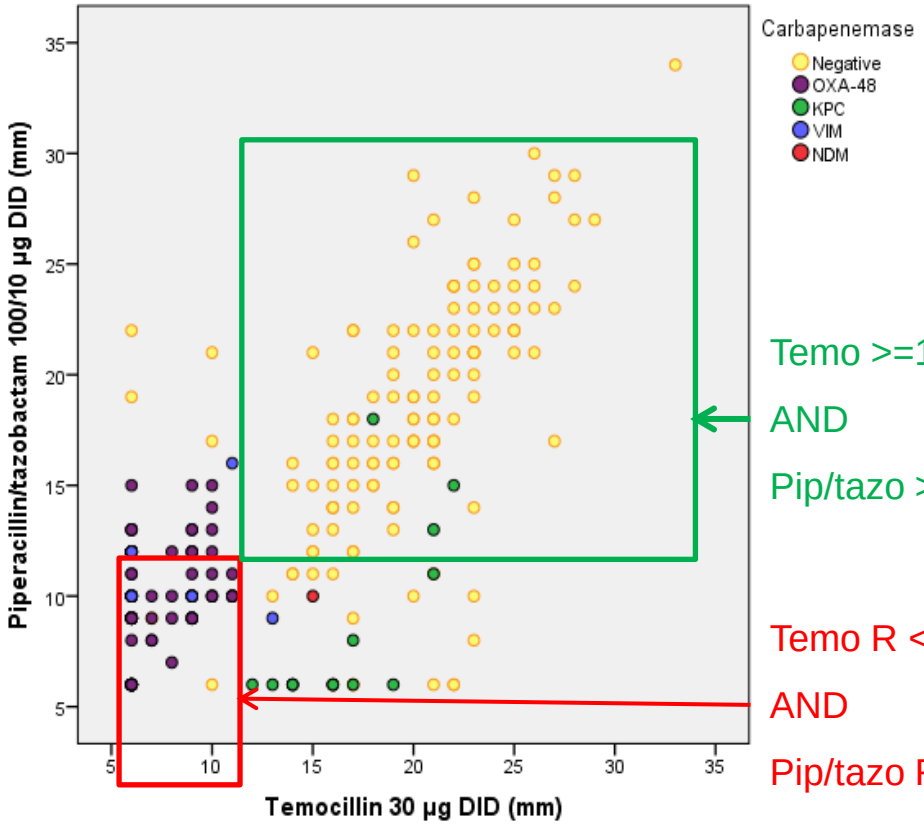


- **EUCAST screening breakpoint (ETP10 <25 mm): Sensitivity =97% for CPE overall**

Huang et al. ECCMID 2015. Oral session O096

Need for surrogate markers for the detection of CPE: temocillin and piperacillin/tazobactam resistance

Scattergram of pip/tazo and of temocillin disks inhibition zones (DID) for Enterobacteriaceae isolates referred to the **Belgian and French** national reference laboratories in 2012 (**n=1354**)



Temo >=12 mm

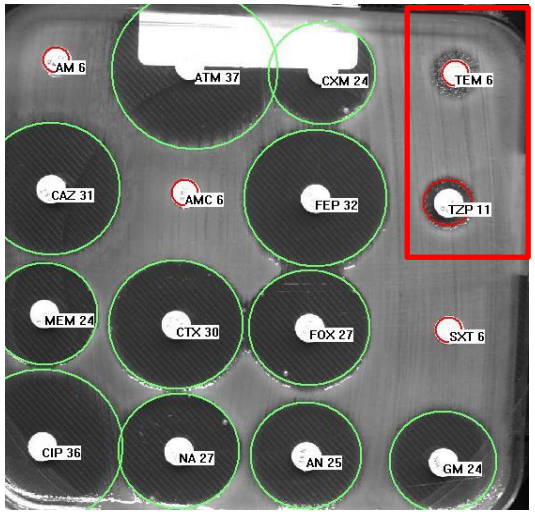
AND

Pip/tazo >=16 mm

Temo R <12 mm

AND

Pip/tazo R <16 mm



NPV =99% exclude a CPE preventing unnecessary additional testing (40% of referred isolates in 2012)

PPV =95% in *K. pneumoniae* in areas where OXA-48 producers are predominant

Selection criteria for suspicion of CPE

- **Decreased susceptibility to at least one carbapenem using screening breakpoint:**
  - Automated systems*:
    - Ertapenem (Vitek2 > 0.5 /Phoenix > 0.25) OR
    - Meropenem (Vitek2 > 0.25 /Phoenix > 0.125)

  → **should be verified by one diffusion method (one of the following)**

  - Diffusion methods:
    - Disk diffusion:
      - Ertapenem (10 µg) < 25 mm (Sensitivity of 97%) OR
      - Meropenem (10 µg) **<29 mm** (Sensitivity of 98%)

    OR

    - MIC gradient diffusion:
      - Ertapenem MIC > 0.12 mg/L

Most recent automates AST galleries will have lower range limits
-> increase sensitivity of detection

- **In area with high prevalence of OXA-48-like CPE → test temocillin:**
  - Disk diffusion: Temocillin (30 µg) <12 mm

  OR

  - MIC methods*: Temocillin > 128 mg/L

*Current **automated systems use concentration ranges not adapted** for screening breakpoints for:

- Carbapenem [(ERTA, MERO)] not low enough to detect CPE isolates with very low resistance levels (MIC of 0.25-0.5 mg/l) →suboptimal sensitivity for detection of some CPE producers (**OXA-48 !**).
- [Temocillin] not high enough (max >32 mg/l) to be used as indicator of **OXA-48** CPE → suboptimal specificity

→ algorithm proposed for disk diffusion may constitute a suitable alternative

INTERPRETATIVE GUIDELINES FOR CARBAPENEMASES

- **Perform laboratory tests for detection of carbapenemases** (CPE) (epidemiology/Infection control)

- **Do not edit results for carbapenems** (MERO, IMI, ERTA) for CPE (categorize as S, I, R on the basis of antibiogram result)

- **When an CPE isolate is categorized as « S » to MERO, (IMI, ERTA)**, an MIC should be performed against these agents when used for treatment of infection.
- (association Rx of 2-3 active drugs including a carbapenem if MIC ≤8 µg/ml)

EUCAST guidelines
CA-SFM 2015, version 2.0

Phenotypic confirmation of carbapenemases

- **Modified Hodge Test (MHT):**
  - **CLSI** 2010; M100-S20: Table 2A-S2
  - Sensitivity: designed for **KPC** (90%), lower for other CPE (MBLs such as NDM)
  - Specificity low
  - Difficulties: QC+/- strains, standardization, reproducibility of interpretation, long time to result (24h)…

Seah et al. JCM 2011
Doyle et al., JCM 2012

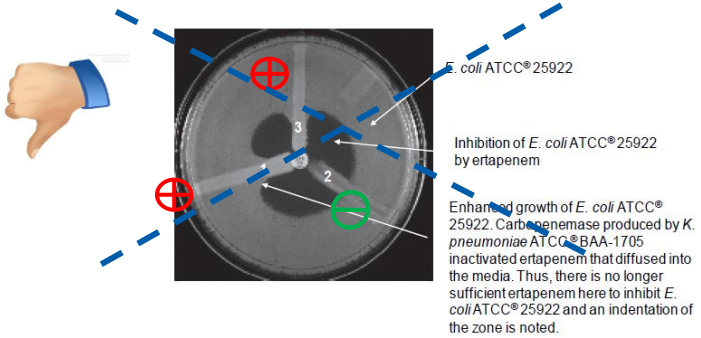
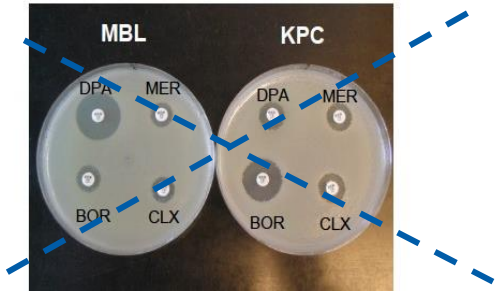


Figure 1. The MHT Performed on a Small MHA Plate.
(1) *K. pneumoniae* ATCC®BAA-1705, positive result;
(2) *K. pneumoniae* ATCC®BAA-1706, negative result;
and (3) a clinical isolate, positive result.

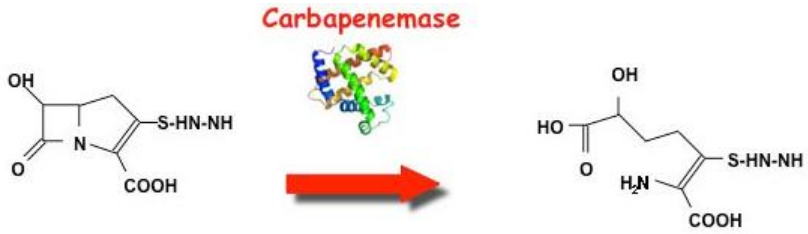


- **Inhibitor-based combination disk tests (IBT):**
  - **EUCAST**; *Giske et al. CMI 2011; 17: 552–556*
  - Good sensitivity, specificity for MBL/KPC
  - Lengthy time to result (24 h, same as MHT)
  - No inhibitor-based positive confirmation test for OXA-48 CPE

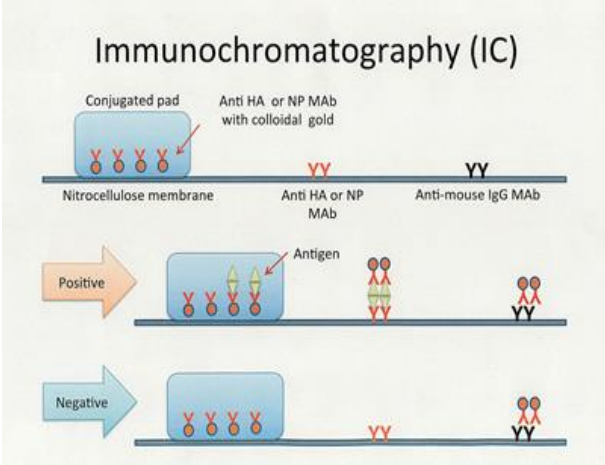
Carbapenemase (β-lactamase)	Inhibited by		
	EDTA/DPA	Boronic acid	Cloxacillin
MBL (class B)	Y	N	N
KPC (class A)	N	Y	N
OXA-48 (class D)	N	N	N
Negative (ESBL)	N	N	N
Negative (AmpC)	N	Y	Y

Novel rapid phenotypic tests for detection of CPE

- **Carbapenem hydrolysis-based tests (CHBT):**
  - **Colorimetric assays:**
    - Home-made:
      - Carba NP test, (Blue Carba test)
    - Commercial kits:
      - Rapid CARB Screen (ROSCO)
      - Rapid CARB Blue (ROSCO)
      - RAPIDEC (BioMérieux)
      - Beta-CARBA test (BioRad)
  - **Device-assisted tests:**
    - MALDITOF-MS: MBT Star BL (Bruker)



- **Immunochromatographic tests (ICT):**
  - OXA-48, KPC K-SeT (ICTO48; Coris BioConcept)
  - Upcoming other carbapenemases (NDM, VIM…)
  - …and ESBL
  - Multiplex tests (OXA-48/KPC/NDM + CTX-M15)



Comparative performances of commercial chromogenic screening tests for detection of carbapenem hydrolysis

		BYG	BCT	RCNP	NRCK
***Enterobacteriaceae* (n=198)**	Sensitivity (%)	100	97.3	91.9	89.2
	[CI 95%]	[100-100]	[93.4-100]	[85.4-98.4]	[81.8-96.6]
111 CPE + (69 OXA-48)	Specificity (%)	98.9	97.7	83.9	89.7
	[CI 95%]	[96-100]	[93.7-100]	[74.1-93.8]	[81.5-97.8]
	PPV (%)	99.1	98.2	87.9	91.7
	[CI 95%]	[96.9-100]	[95-100]	[80.4-95.5]	[85-98.3]
	NPV (%)	100	96.6	89	86.7
	[CI 95%]	[100-100]	[91.8-100]	[80.4-97.7]	[77.7-95.6]
***Pseudomonas* spp. (n=89)**	Sensitivity (%)	87.3	90.9	90.9	92.7
	[CI 95%]	[76-98.5]	[81.2-100]	[81.2-100]	[84-100]
55 CP + (45 VIM/IMP)	Specificity (%)	94.1	94.1	88.2	88.2
	[CI 95%]	[84-100]	[84-100]	[74.4-100]	[74.4-100]
***Acinetobacter* spp. (n=41)**	Sensitivity (%)	72.7	75.8	36.4	27.3
	[CI 95%]	[53.3-92.1]	[57.1-94.4]	[15.4-57.3]	[7.9-46.7]
21 CP + (OXA-23/-24/-58)	Specificity (%)	100	87.5	75	100
	[CI 95%]	[100-100]	[58.3-100]	[36.7-100]	[100-100]

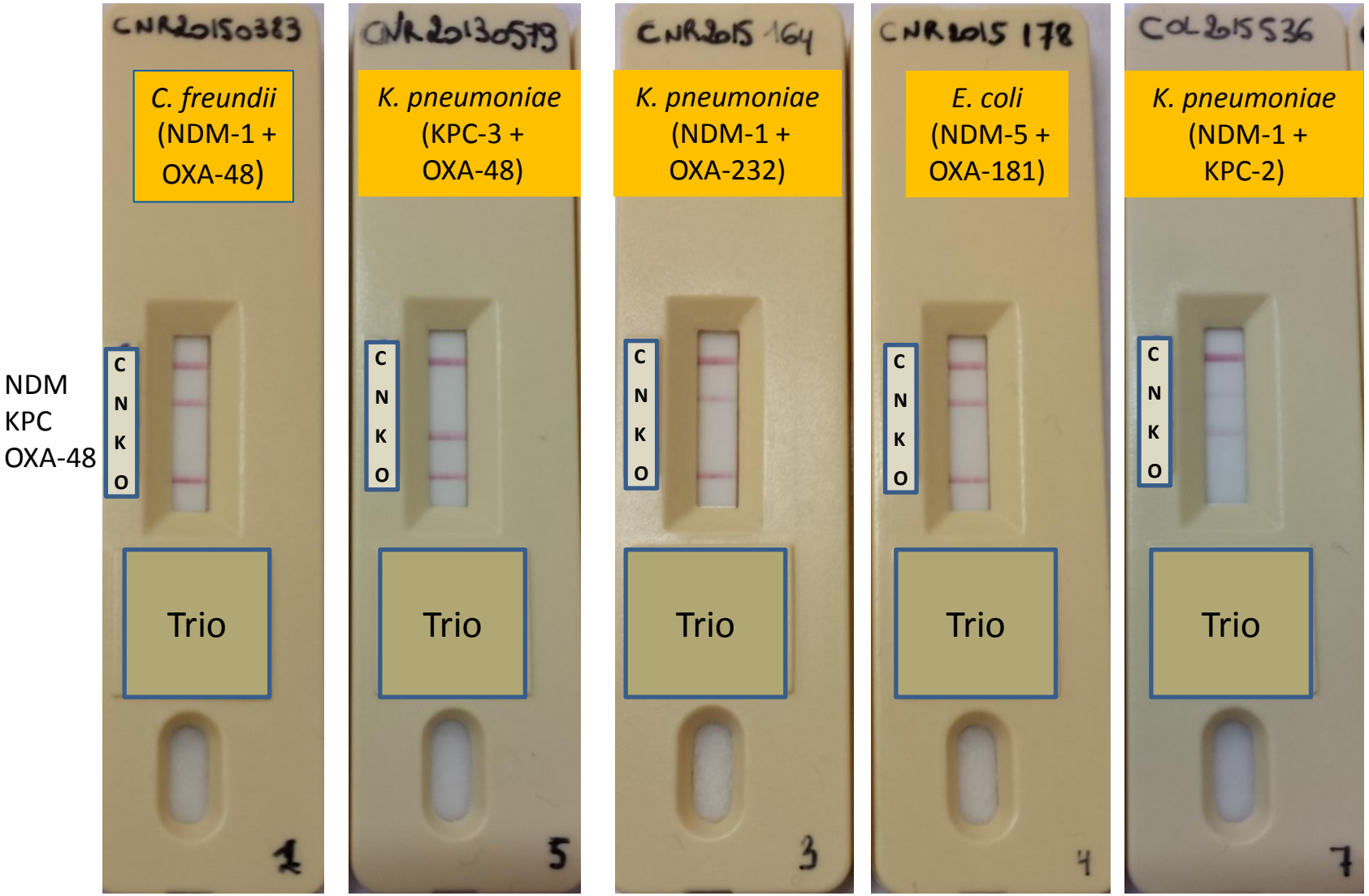
CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value

Non interpretable results: (3-9%)

Beta-CARBA: excellent performances for screening CPE (OXA-48 !) and CP-*Pseudomonas* spp.

A. Noel et al., JCM 2016 accepted

New triplex immunochromatographic assay (RESIST 3 O.K.N *K*-SeT) for the rapid detection of OXA-48 like, NDM and KPC carbapenemases from cultured bacteria



Evaluation of RESIST 3 O.K.N *K*-SeT assay for detection of OXA-48, NDM and KPC carbapenemases from culture colonies

Retrospective

200 collection strains	27 strains tested positive for KPC with the RESIST 3 K-SeT	27 strains carrying a KPC carbapenemase	Escherichia coli, Klebsiella ozaenae, Enterobacter cloacae, Citrobacter freundii, Serratia marcescens (positive for KPC-2 or KPC-3).
	30 strains tested positive for NDM with the RESIST 3 K-SeT	30 strains carrying a NDM carbapenemase	*E. coli*, *K. pneumoniae*, *E. cloacae*, *Morganella morganii*, *Providencia* spp., *Salmonella* spp. (positive for NDM-1, NDM-4,-5,-6,-7 or -9)
	43 strains tested positive for OXA-48 with the Trio K-SeT	43 strains carrying a OXA-48carbapenemase	*E. coli*, *E. cloacae*, *Enterobacter aerogenes*, *C. freundii*, *C. koseri*, *K. pneumoniae* (positive for OXA-48, -162, -181, -204, 232, -244)
	100 strains tested negative with theTrio K-SeT	40 strains carrying non-KPC, non NDM, non OXA-48 like carbapenemases	IMI-1/-2, Sme-1/-2, NmcA, GES-5, FRI-1, GIM-1, IMP-1,IMP-8, IMP-10, IMP-11 , VIM-1, VIM-2,-VIM-4, VIM-19
		60 strains not carrying a carbapenemase	

Evaluation on 200 collection isolates including 140 CPE and 60 non CPE strains with variable resistance mechanisms to carbapenems:

- **100% sensitivity** (OXA-48, NDM, KPC)
- **100% specificity** (non CPE)

Prospective

175 non-duplicated, consecutive suspected CPE referred to the Belgian NRC (July-Sept. 2016)
Gold-standard reference PCR/seq.

CPE isolates (n=107) (61.8%)
OXA-48 like: [n=68]; NDM: [n=18]; KPC: [n=8];
VIM: [n=8]; OXA-181 + NDM [n=1]; KPC+ VIM [n=1]
others [n=3]

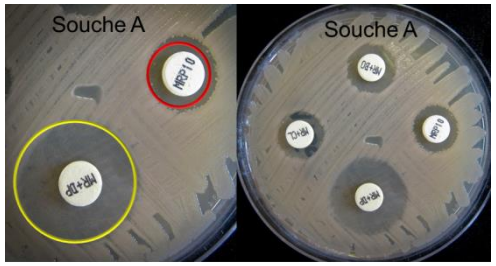
100% Sensitivity (OXA-48, NDM, KPC)
100% specificity (other CPE; non-CPE)

Confirmation of carbapenemases from culture colonies in 15 min
OXA-48/NDM/KPC account for 85-90% of all carbapenemases in countries like Belgium and France

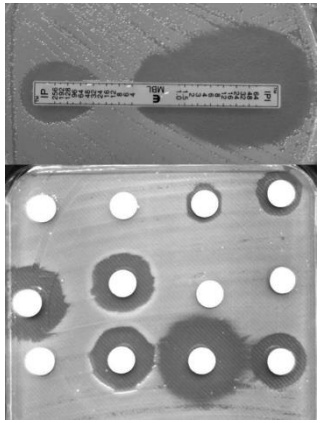
Y. Glupczynski et al;
abstract submitted to ECCMID 2017

Second-line confirmatory tests (optional)

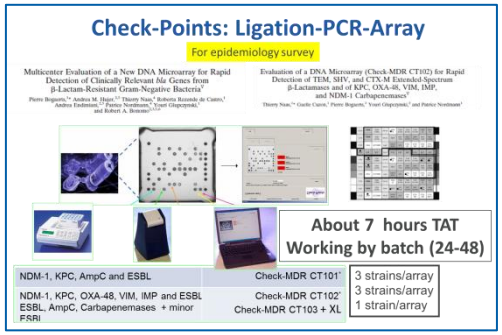
- **Inhibitor-based combination disk tests (IBT) tests for MBL** (mainly VIM/NDM):
  - Paper disks: IMIP +/- EDTA
  - Rosco tablets: MEM +/- DPA
  - Etest (gradient MIC): IMIP +/- EDTA…

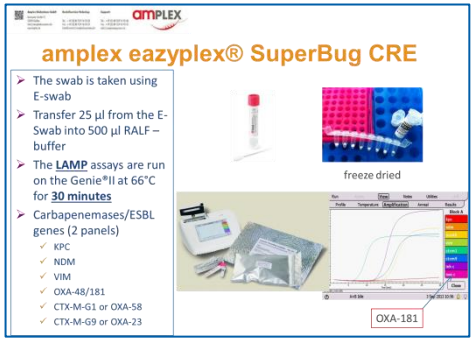


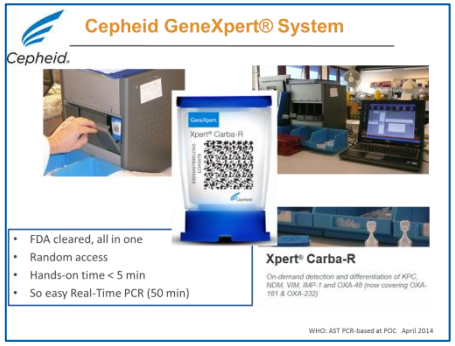
K. Pneumoniae NDM-1



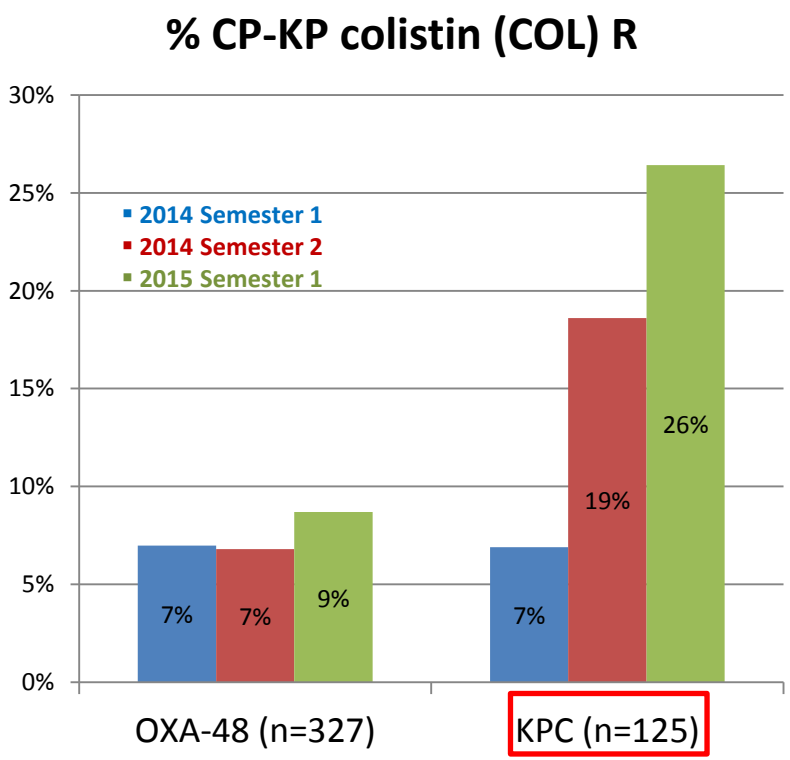
- **Molecular tests** (commercial PCR multiplex assays, DNA microarray, LAMP,…)

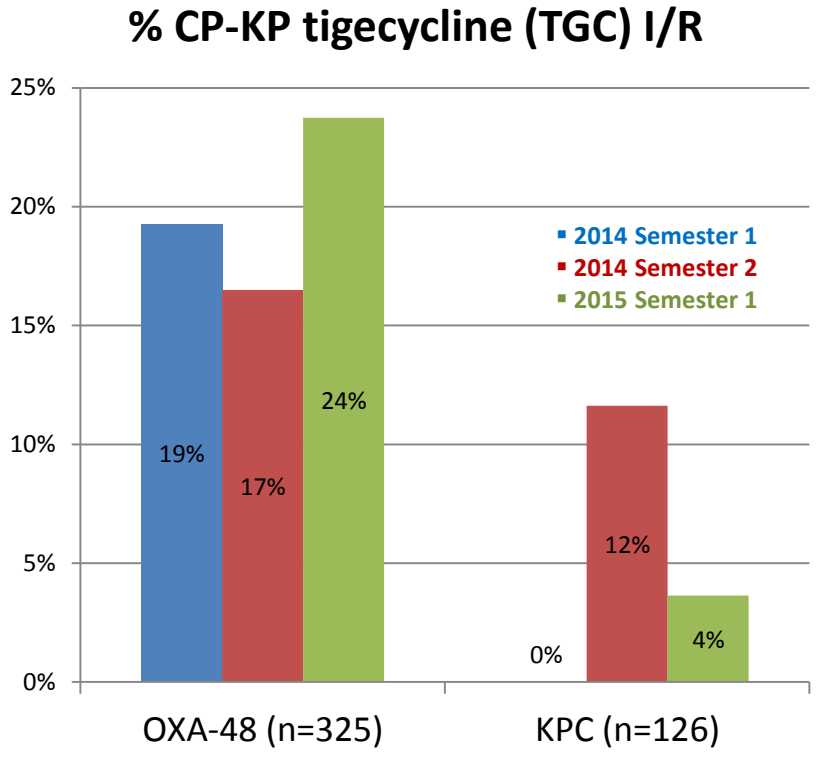






Colistin and tigecycline R in CP-*K. pneumoniae* (CP-KP)





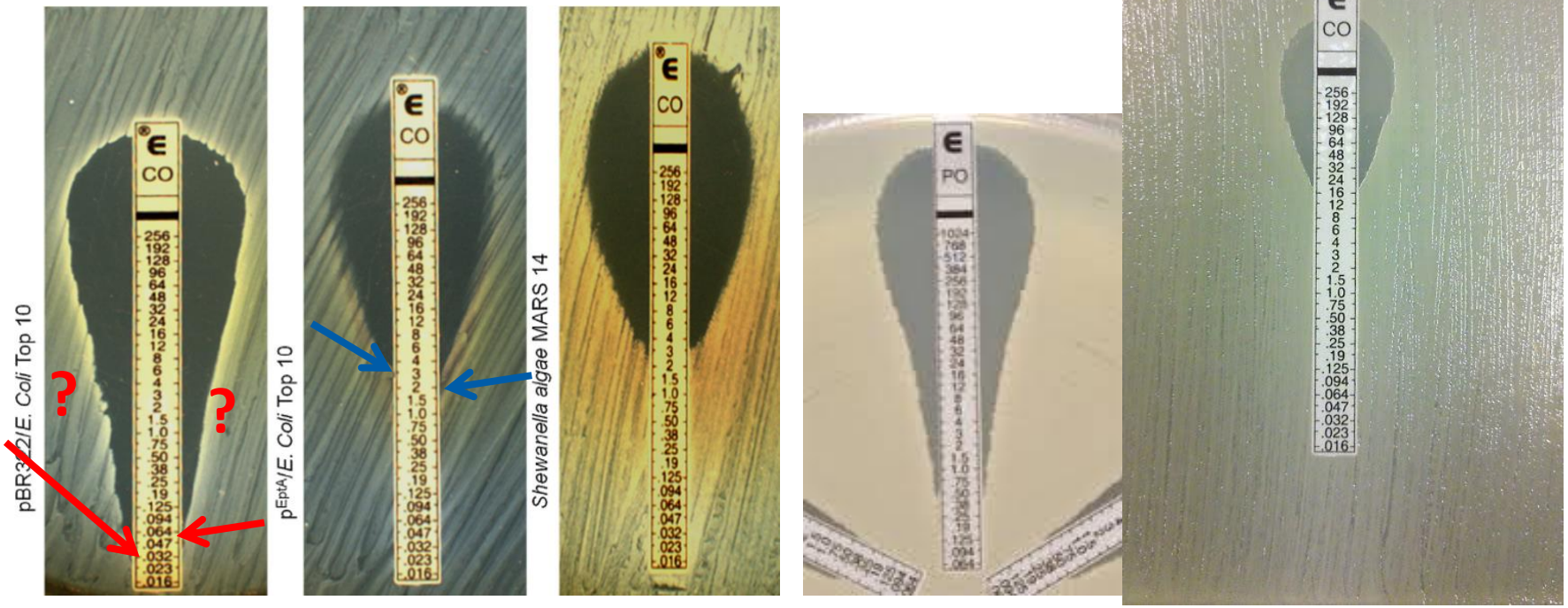
- **COL-R rate higher in KPC KP** (19%) vs OXA-48 KP (8%); p<0.001
- **TGC-I/R rate higher in OXA-48 KP** (20%) vs KPC KP (6%); p<0.001
- **Global increase of I/R rates in KP** from 2014 to 2015 for:
  - TGC-I/R (17% to 21%; p NS)
  - **COL-R** (6% to 14%; p=0.007) especially in **KPC KP** (p<0.001)

- 2 *E. coli* OXA-48 isolates also COL-R and mcr-1 positive referred at NRC (1 in 2014, 1 in 2015)

AST methods for colistin

Several methodological issues (EUCAST Working group)
No diffusion methods (discs, gradient strip diffusion) but only microdilution MIC

Bad/irregular diffusion in agar
Lectures difficult, test poorly reproducible



News from EUCAST

Breakpoints for Ceftazidime-Avibactam

Ceftazidime-avibactam is a combination of ceftazidime and a novel β-lactamase inhibitor, avibactam, which inhibits Ambler class A, class C and some class D enzymes but not metallo-β-lactamases (class B). The indications are for treatment of the following infections in adults:

- complicated intra-abdominal infections
- complicated urinary tract infections including pyelonephritis
- nosocomial pneumonia, including ventilator associated pneumonia
- infections caused by aerobic Gram-negative organisms in patients with limited treatment options

Dosage of ceftazidime-avibactam is 2 g-500 mg three times daily by intravenous infusion over 2 h.

OK for OXA-48 (class D)
OK for KPC (class A)
Not for MBL (NDM, VIM)

Clinical breakpoints for ceftazidime-avibactam[1]

Organism group	MIC breakpoints (mg/L)		Disk content (µg)	Zone diameter breakpoints (mm)	
	S ≤	R >		S ≥	R <
Enterobacteriaceae	8	8	10-4	13	13
Pseudomonas aeruginosa	8	8	10-4	17	17
PK-PD breakpoints	8	8	-	-	-

[1] For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L.

Quality control
Quality control of ceftazidime-avibactam must be performed with a susceptible control strain (*Escherichia coli* ATCC 25922 or *Pseudomonas aeruginosa* ATCC 27853) to control the ceftazidime component and with *Klebsiella pneumoniae* ATCC 700603 (ESBL-producing strain) to control the avibactam component.

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

News from EUCAST

Methods recommandation for fosfomycin

Methodological testing problems for fosfomycin both with MIC (microdilution !) and disc diffusion (Growth of colonies within the inhibition zone).

For antimicrobial susceptibility testing of fosfomycin, EUCAST recommends:

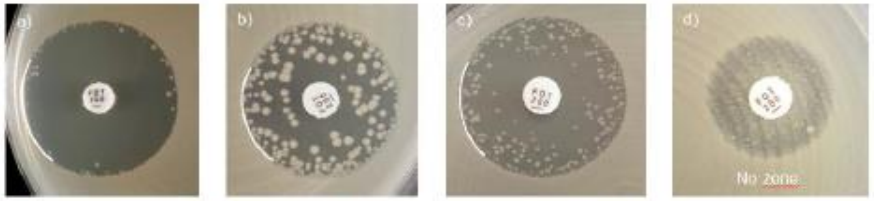
- The reference method for MIC determination is agar dilution with 25 mg/L glucose-6-phosphate in the medium.
- Follow the manufacturers' instructions for commercial MIC systems.
- Disk diffusion shall be performed according to EUCAST methodology using fosfomycin 200 µg disks with 50 µg glucose-6-phosphate.
  - Disk diffusion for fosfomycin is only validated for *Escherichia coli*.
  - When reading fosfomycin 200 µg inhibition zones for *E. coli*, ignore isolated colonies within the inhibition zone and read the outer zone edge (see pictures below).

Disc diffusion only validated for *E. coli*

EUCAST breakpoints for fosfomycin and Enterobacteriaceae
(to be included in EUCAST Breakpoint Table v. 7.0, January 2017)

Indication	MIC breakpoints (mg/L)		Disk content (µg)	Zone diameter breakpoints (mm)	
	S ≤	R >		S ≥	R <
Fosfomycin iv	32	32	200[1]	24[2,3]	24[2,3]
Fosfomycin oral (uncomplicated UTI only)	32	32	200[1]	24[2,3]	24[2,3]

[1] Fosfomycin 200 µg disks with 50 µg glucose-6-phosphate.
[2] Fosfomycin zone diameter breakpoints apply to *E. coli* only.
[3] Ignore isolated colonies within the inhibition zone and read the outer zone edge (as illustrated in pictures a-c below).



Examples of inhibition zones for Escherichia coli with fosfomycin.
a-c) Ignore all colonies and read the outer zone edge.

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

EUCAST breakpoint table v. 7.0 (January 2017)

Pseudomonas aeruginosa
Acinetobacter spp.

INTRINSIC RESISTANCE IN GRAM-NEGATIVE NON FERMENTERS

Organisms	Ampicillin	Amoxicillin-Clavulanic acid	Ampicillin-sulbactam	Ticarcillin	Ticarcillin-clavulanic acid	Piperacillin	Piperacillin-tazobactam	Cefazolin, Cefalothin Cefalexin, Cefadroxil	Cefotaxime	Ceftriaxone	Ceftazidime	Cefepime	Aztreonam	Ertapenem	Imipenem	Meropenem	Ciprofloxacin	Chloramphenicol	Aminoglycosides	Trimethoprim	Fosfomycin	Tetracyclines	Tigecycline	Polymyxin B/Colistin
Acinetobacter baumannii, *Acinetobacter pittii*, *Acinetobacter nosocomialis* and *Acinetobacter calcoaceticus* complex	R	R	Note[1]					R	R	R			R	R						R	R	R[2]	Note[2]	
Achromobacter xylosoxydans	R							R	R	R				R										
Burkholderia cepacia complex[3]	R	R	R	R	R	R	R	R	R	R			R	R			R	R	R[4]	R	R			R
Elizabethkingia meningoseptica	R	R	R	R	R	R		R	R	R	R	R	R	R	R	R								R
Ochrobactrum anthropi	R	R	R	R	R	R	R	R	R	R	R	R	R	R										
Pseudomonas aeruginosa	R	R	R					R	R	R				R				R	Note[5]	R		R	R	
Stenotrophomonas maltophilia	R	R	R	R		R	R	R	R	R			R	R	R	R			R[4]	R[6]	R	R[7]		

istant

NB: Pseudomonas aeruginosa normally resistant to Ertapenem and Temocillin
Acinetobacter spp. normally resistant to Ertapenem, Aztreonam and Temocillin

EUCAST intrinsic resistance and exceptional phenotypes, Expert Rules version 3.1, September 2016

LIST OF ANTIMICROBIAL AGENTS TO BE TESTED AGAINST *PSEUDOMONAS AERUGINOSA*

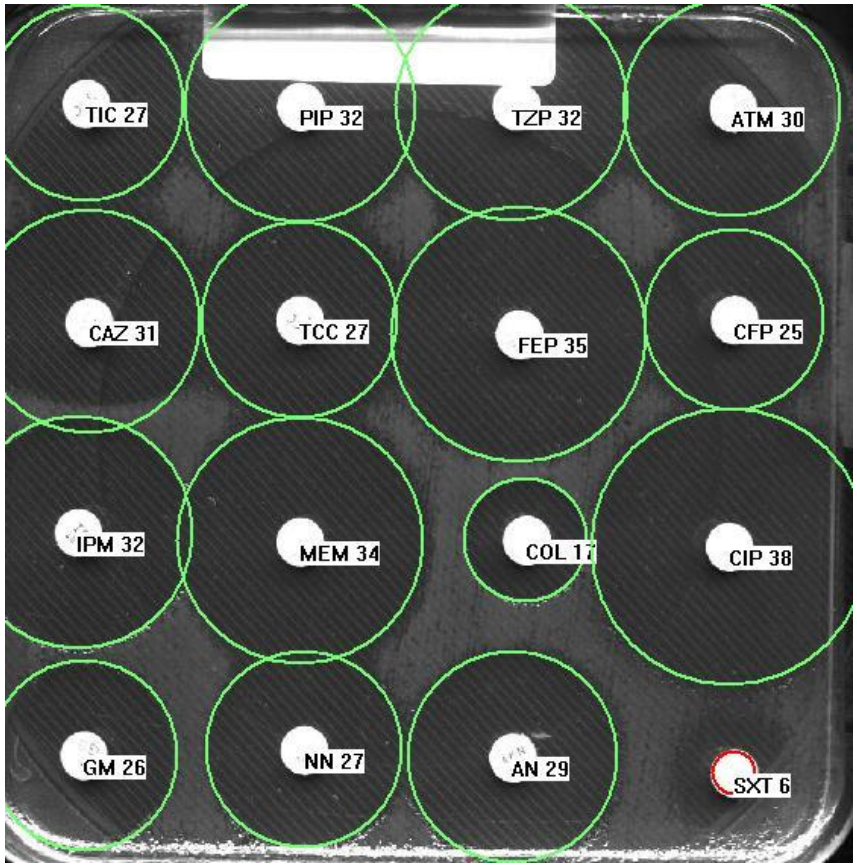
Liste standard	Liste complémentaire
Ticarcilline	Doripénème
Ticarcilline - acide clavulanique	Nétilmicine
Pipéracilline	Lévofloxacine
Pipéracilline - tazobactam	Colistine
Ceftazidime	Fosfomycine
Céfépime	
Imipénème	
Méropénème	
Tobramycine	
Amikacine	
Ciprofloxacine	
Aztréonam	
Gentamicine	

Disc diffusion on solid medium
Medium: Mueller-Hinton
Inoculum : 0,5 McFarland
Incubation : Normal atmosph., 35±2ºC, 20±4H
Control strain: Pseudomonas aeruginosa ATCC 27853

Guidelines 2015; v 2.0

Wild-type profile of *P. aeruginosa*

- **Intrinsic resistance**:
  - Minocycline
  - Cotrimoxazole
- **Susceptibility:**
  - Ceftaz, Cefep, Azt, Cefoperazone
  - Ticar±Clav, Pipera±Tazo
  - Imip, Merop
  - Cipro
  - Amika, Tobra, Genta
  - Colimycine
  - Fosfomycine



Mechanisms of acquired resistance to ß-lactam agents in *P. aeruginosa*

- **Enzymatic Inactivation :** different β-lactamases
  - **Overproduced AmpC (chromosomal)**
  - Penicillinases: small spectrum (OXA/PSE),
  - ESBL (OXA, other uncommon types (GES, PER, VEB, BEL)
  - Carbapenemases (mostly MBLs (VIM>>> all others)
- **Cell wall impermeability :** Mutations/deficiencies in porins
  - **↓ OprD2 → R to carbapenems only (IMI > MERO)**
- **Over expression of active efflux pump systems**
  - Active efflux pump systems (MexAB, MexXY, MexCD, MexEF) involved in resistance (constitutive presence/expression)
  - Cross-resistance to several class of antimicrobials (quinolones +++); low level of resistance

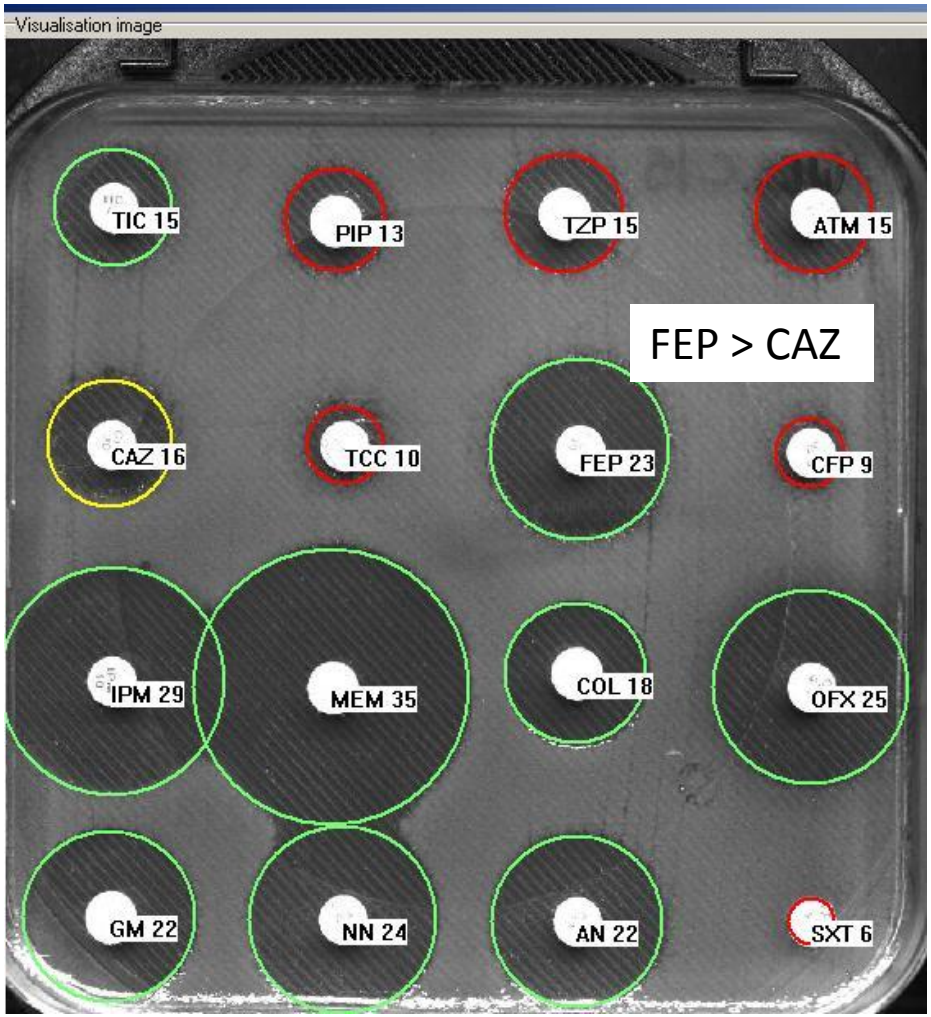
Distribution of acquired resistance mechanisms to ß-lactam agents

Resistance to Ceph3/Ceph4/Pip-Tazo	%
No mechanism	62
Acquired Pen-ase (PSE type, TEM2, OXA type)	3
BLSE (SHV, OXA type)	1
Acquired Pen-ase + Ceph-ase	2
Ceph-ase	9
Ceph-ase + non enzymatic resistance	6
Non enzymatic resistance (efflux)	16
Other mechanisms	1
Resistance to IPM (essentially porine D2)	
porine D2 modification	16,6
Carbapenemase (VIM-type)	0,4

GERPA; RICAI 2004 400/81P

Overproduced cephalosporinase

AmpC



Resistance tous all β-lactam agents included association with BL inhibitors (clav./tazol)

Remains susceptible to Carbapenems

Mutation in regulator/repressor genes (AmpD/AmpR)

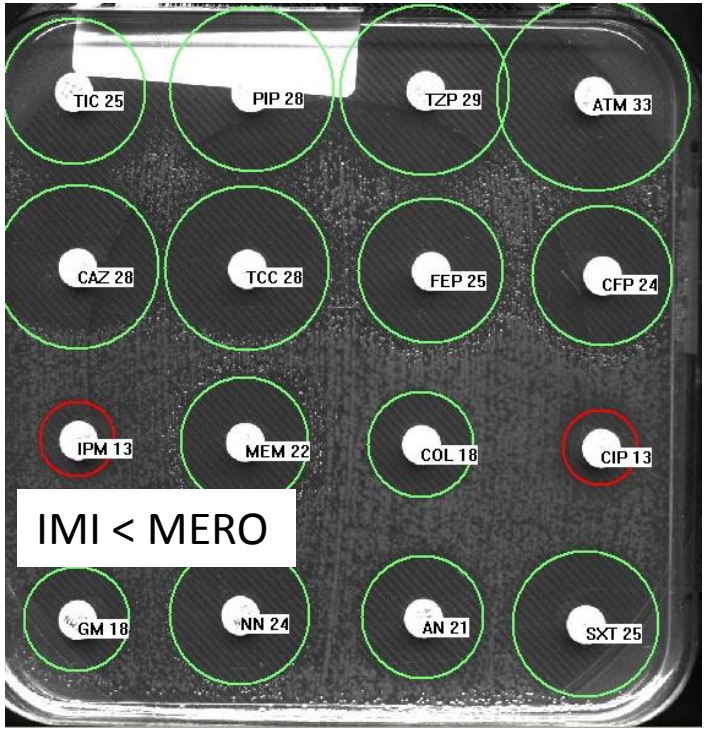
Stable derepression/ overproduction of AmpC

+/- 10 % P. aeruginosa isolates (most frequent resistance mechanisms in treated patients)

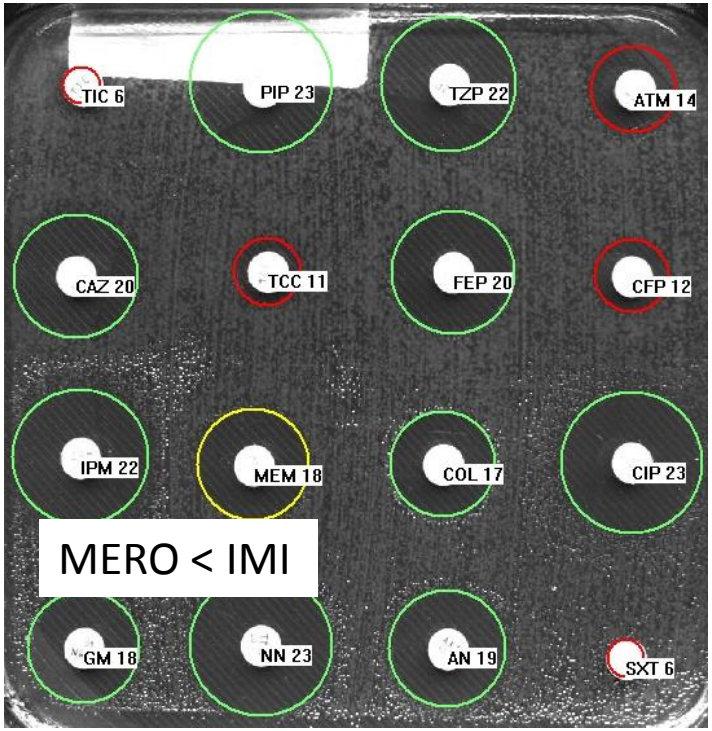
Non enzymatic intrinsic resistances in *P.aeruginosa*

Efflux/AB	Tic	Azt	Cefep	Imip	Merop	FQ	AG
MexAB-OprM (mexR) ↑	+	+			+	+	
MexCD-OprJ (nfxB) ↑			+			+	
MexXY-OprM ↑			+			+	+
MexEF-OprN (nfxC) ↑				++	+	+	
Porine OprD2 ↓				++	+		

OprD (porin)



MexAB-OprM (efflux)



Carbapenemases in *Pseudomonas aeruginosa*

R: most B-lactams (inoculum dependent)

Carbapenems (IMI +++ and MERO ++++)

S: Aztreonam

I/S: Pip + tazo

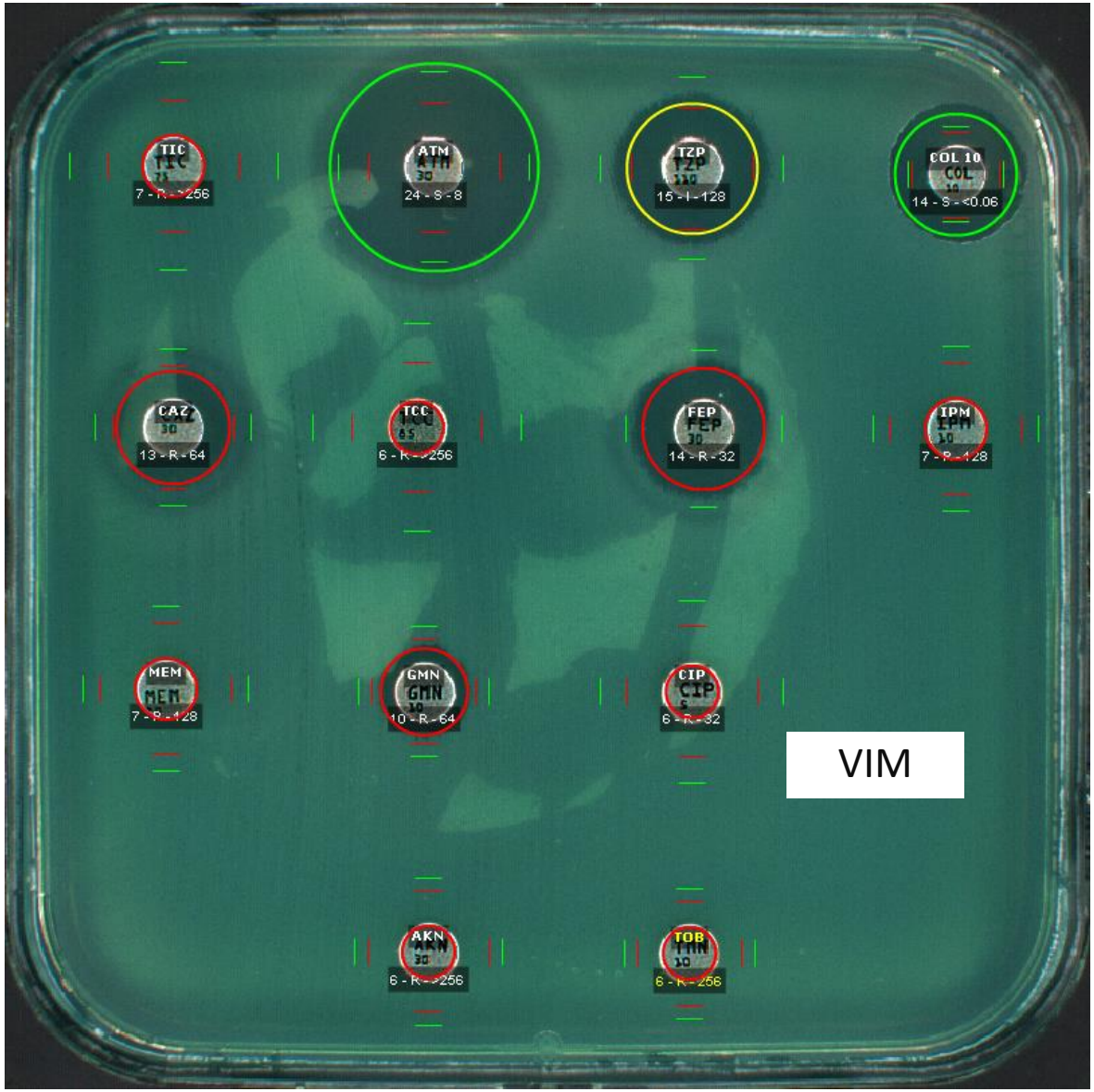
Genes located on transferable elements (Integrons/transposons/plasmids)

Frequent multiple associated resistance

- aminosides (+++)
- quinolones

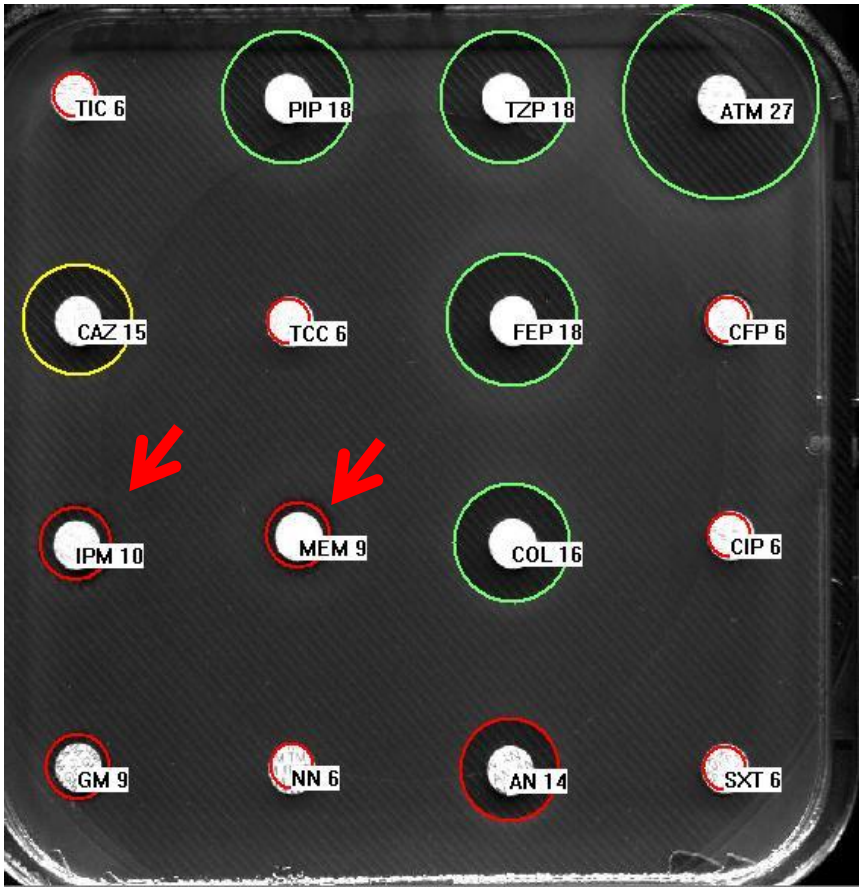
Beta-lactamases of MBL type (classe B)

- VIM >>> IMP, GIM, SPM, NDM…)

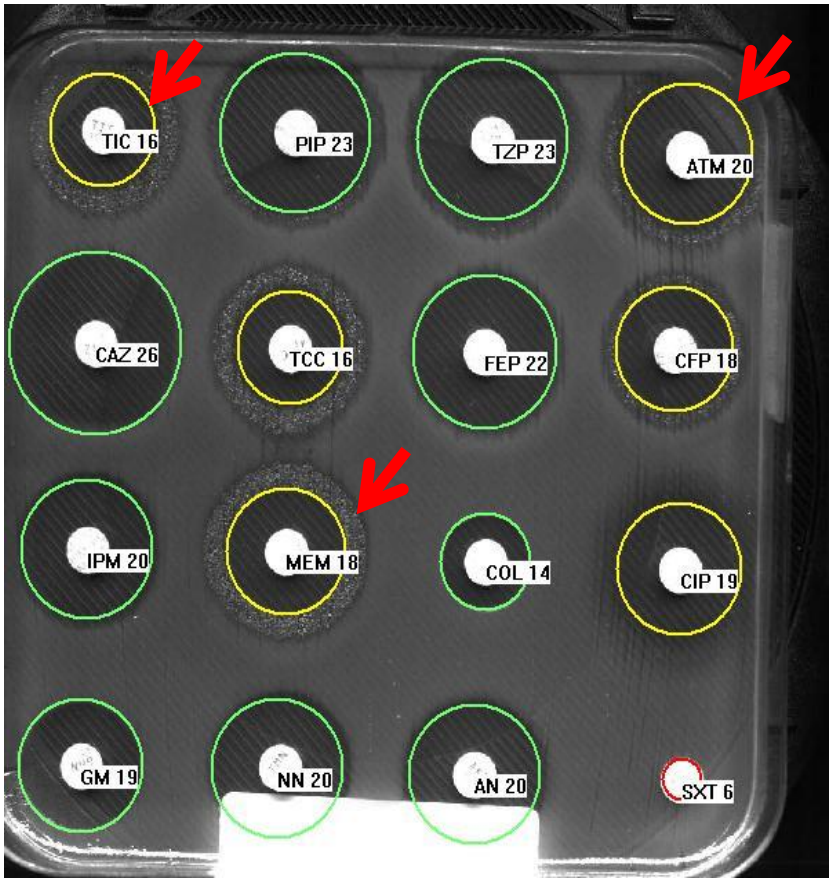


Difference in resistance profiles of *P. aeruginosa* isolates wit intrinsic and acquired resistance

MBL of VIM type



MexAB OprM (efflux)

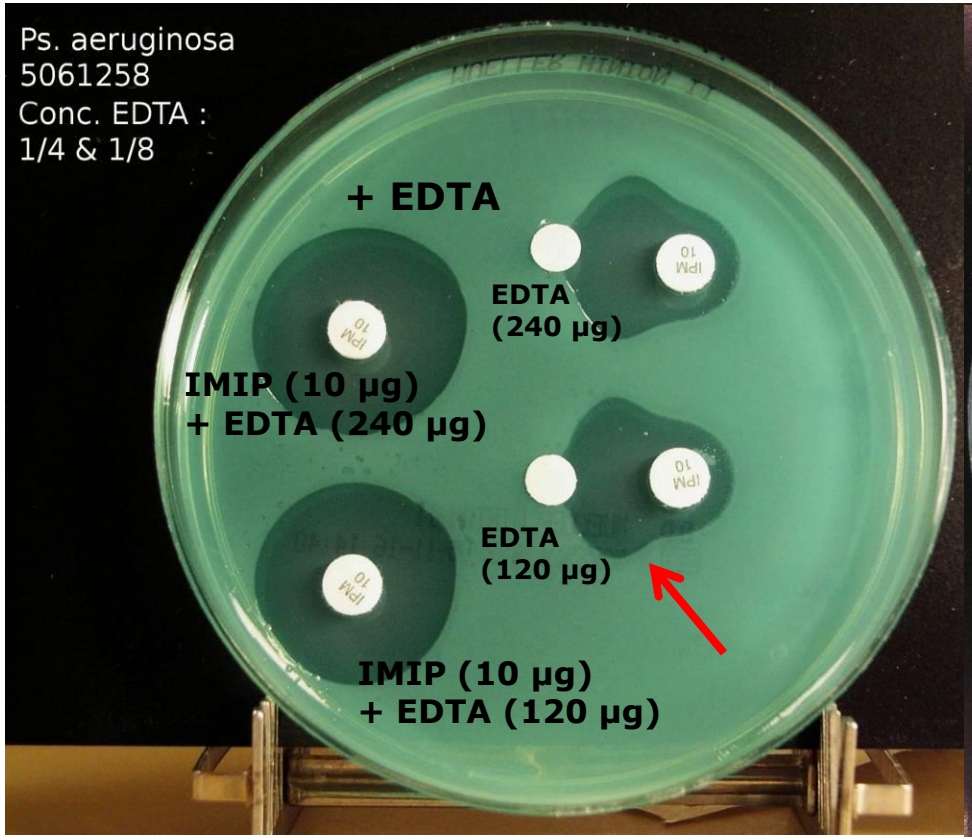


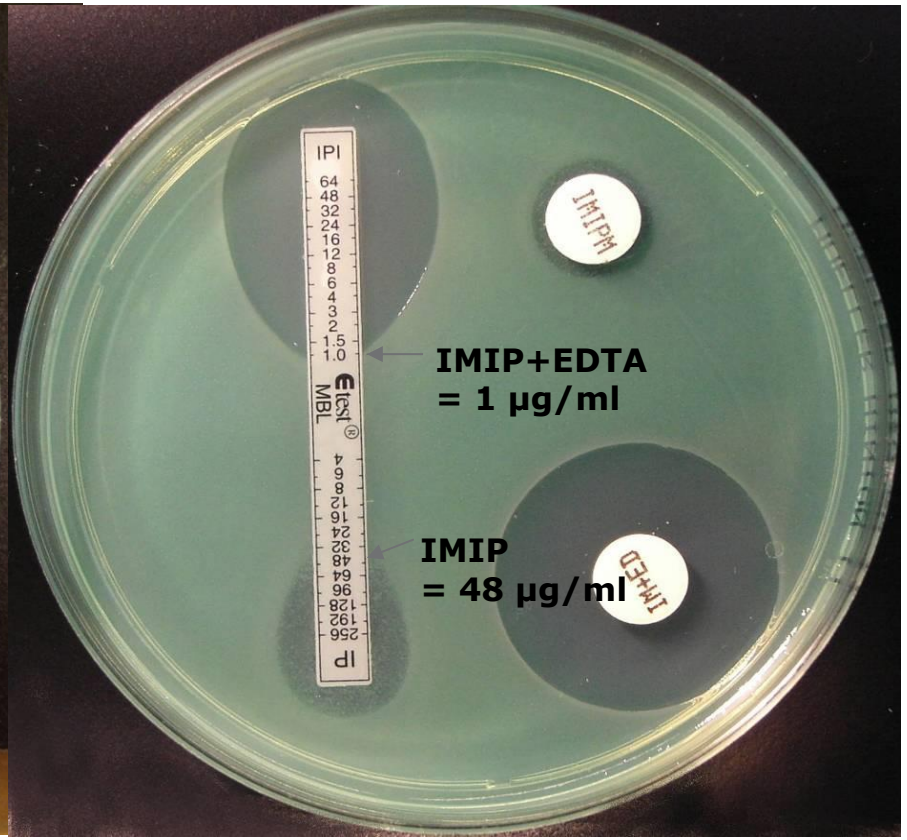
High level Resistance to both MERO & IMI
Resistance to > 3 classes (Peni, ceph3, Ag, FQ)

Low level resistance to peni, aztreonam, FQ
Low level Mero-R (IMI-S)

Detection of MBL (class B) in *P. aeruginosa*

P. aeruginosa VIM-2





∅Zone IMIP+EDTA vs. IMIP ≥ 6 mm

and

Synergy between discs

Ratio MIC IMIP+EDTA/IMIP >8

Confirmatory tests for carbapenemase detection in *P. aeruginosa*

Phenotypical tests

- DO NOT USE Commercial tests for with inihibitors (phenyl boronic acid) of class A carbapenemases (KPC) not validated in Non-fermenters (KPC extremely rare in P. aeruginosa !!)

  (frequent false-positive results in *P. aeruginosa* (AmpC overproduction)

- **Synergy test with inhibitors of class B MBL (EDTA/DPA)**:
  - Paper disks : IMI +/- EDTA
  - Tablets (ROSCO): IMI +/- EDTA ou IMI +/- DPA ou MERO +/- DPA
  - MIC Gradient diffusion strips: IMI +/- EDTA ou MERO +/- DPA…

NB: these tests do not allow precise characterization of MBL type (VIM, IMP, NDM,…)

Molecular tests

- PCR monoplex, PCR multiplex (in house, commercial)
- DNA microarray
- Isothermal amplification assays [LAMP],

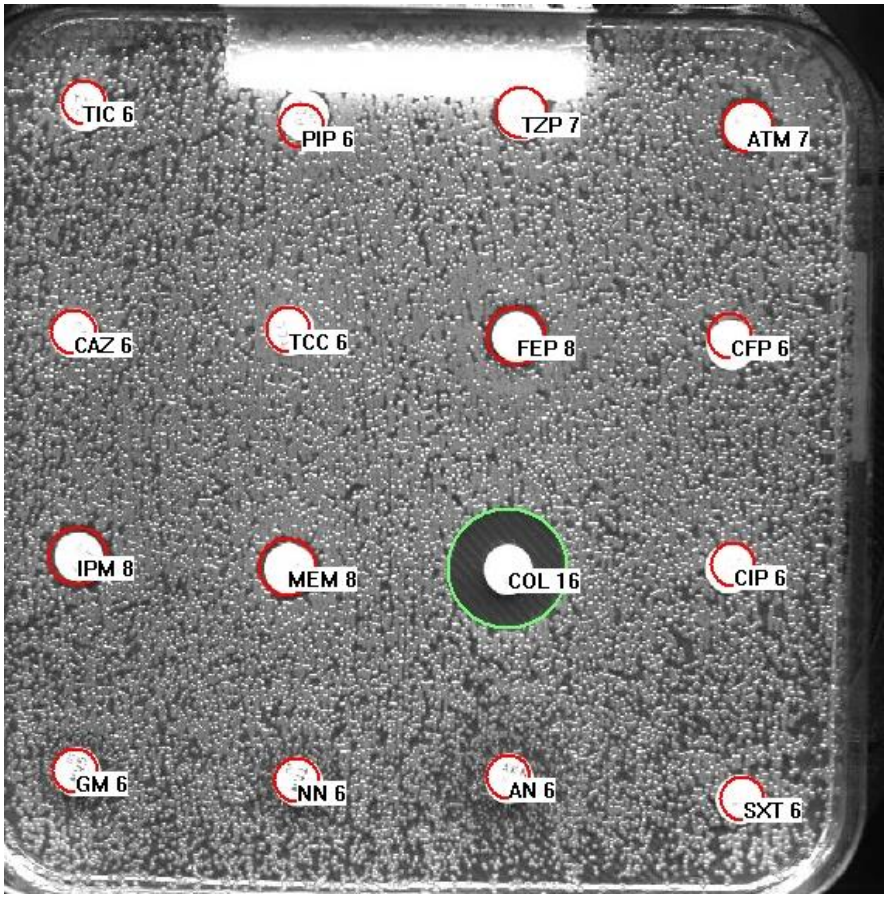
Phenotypes of acquired resistance to ß-lactam drugs in *P. aeruginosa*

Mécan R	Porine	Efflux		Pen-ase	Ceph-ase	ESBL	MBL
	OprD-	MexXY	MexAB				
TIC	S	S	**IR**	**R**	**R**	**R**	**R**
TIC/CLAV	S	S	**IR**	**IR**	**R**	SI	**R**
PIP	S	S	S	**R**	**R**	**R**	SI
PIP/TAZO	S	S	S	**IR**	**R**	SI	SI
AZTR	S	S	**IR**	S	**IR**	**R**	S
CEFTAZ	S	S	S	S	**R**	**IR**	**IR**
CEFEP	S	**IR**	S	V	**SI**	**IR**	**IR**
IMIP	**IR**	S	S	S	S	S	**R**
MEROP	SI	S	**IR**	S	S	S	**R**

Carbapenemases in *Acinetobacter* spp.

- **R: Ticar±Clav, Pipera±Tazo, Cefta, Cefep (High level), carbapenems (variable level)**
- **Frequent multiple associated résistance** (almost pan-resistant or extremely-resistant)
- Carbapenemases
  - **OXA (Class D): OXA-23, -40, -58…**
    - Outbreaks +++
  - Class B: NDM (rare) VIM, IMP (exceptional)
  - Class A: GES-14 (very rare)

OXA-23

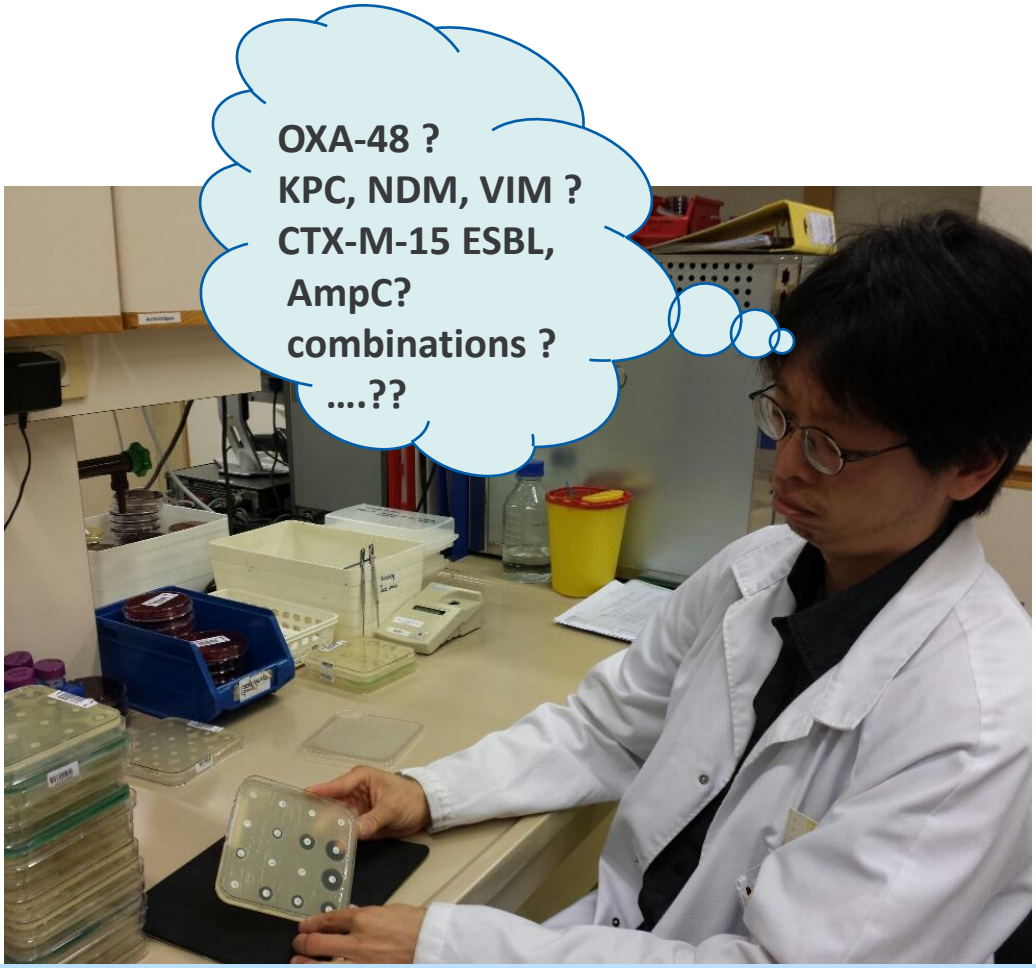


OXA-23 + ArmA (aminoglycoside R)

No existing phenotypical test for confirming Class D carbapenemase in Acinetobacter spp
Do not use tests with class B inhibitors (EDTA/DPA) -> false positives in OXA-23, OXA-58

Need to adapt and to optimize the strategies and laboratory detection tools for AB resistance

Microbiologist of the 20th century



Microbiologist of the 21th century