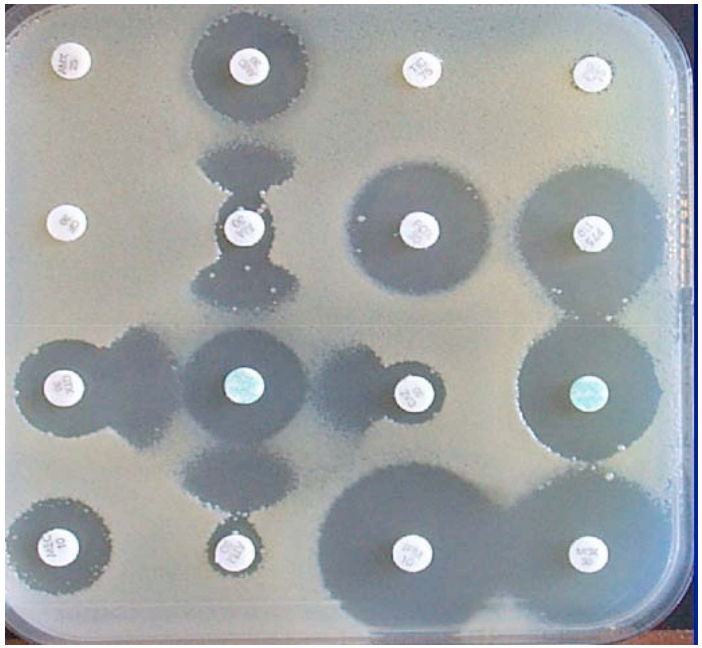
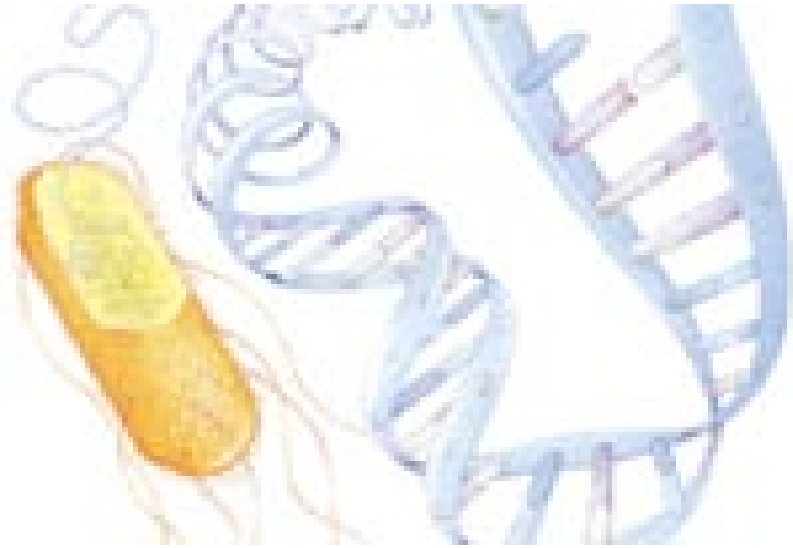
CENTRE HOSPITALIER UNIVERSITAIR E BICÊTRE

« Minor » ESBLs

ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS





T. NAAS,

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France

ß-lactamases

Serine b-lactamases

Metallo enzymes

Group 1	Group 2	Group 2d	Group 3
AmpC	TEM/SHV CTX-M Others	OXA	IMP,VIM, GIM, SPM, SIM
Cephs Inhib-R	Pens Cephs Inhib-S	Pens (Oxa++) Inhib-R/S	Carbapenems Inhib-R

ESBLs

K Bush, curr. Opin. Invest. Drugs 3-1284 (2002)

ß-lactamases (500)
> 50% ESBLs

Plasmid encoded

- 1983 SHV-type (> 88) } "classical ESBLs"
- 1985 TEM-type (> 150) } ESBLs of growing importance
- 1989 CTX-M-type (> 55)
- 1988 SFO-1 Serratia FOnticola
- 1991 TLA-1 TLAhuicas (indian tribe)
- 1991 PER (3) Pseudomonas Extended Resistance
- 1996 VEB (3) Vietnam Extended-spectrum ß-lactamase
- 1996 BES-1 Brazilian Extended-Spectrum ß-lactamase
- 1998 GES (9) Guyana Extended-Spectrum ß-lactamases
- 2005 BEL-1 Belgium Extended-spectrum ß-Lactamase
- 2005 TLA-2 ???? (Plasmid, waste water)

"infrequent ESBLs"

"peculiar ESBLs"

- 1998 KPC (4) Klebsiella pneumoniae Carbapenemase
- 1991 OXA-ESBL (OXA-1, OXA-2 and OXA-10-types)

http://www.lahey.org/studies/webt.asp

Chromosomally-encoded ESBLs

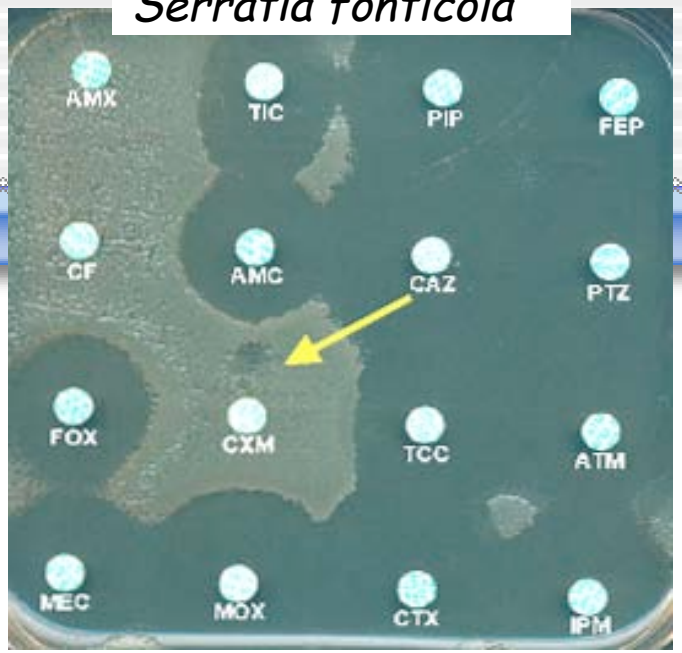
Bacteria	ß-lactamase	induction
Serratia fonticola	FONA	++
Kluyvera ascorbata	KLUA	
Kluyvera cryocrescens	KLUC-1	
Kluyvera georgiana	KLUG-1	
Citrobacter sedlakii	SED-1	++
Rhanella aquatilis	RAHN-1	
Erwinia percinina	ERP-1	
Chryseobacterium meningosepticum	CME-1/2	
Chryseobacterium gleum	CGA-1	
Stenotrophomonas maltophilia	L-2	
Desulfovibrio desulfuricans	DES-1	

Bacteroides uniformis, fragilis, vulgatus CBLA, CEPA,CFXA

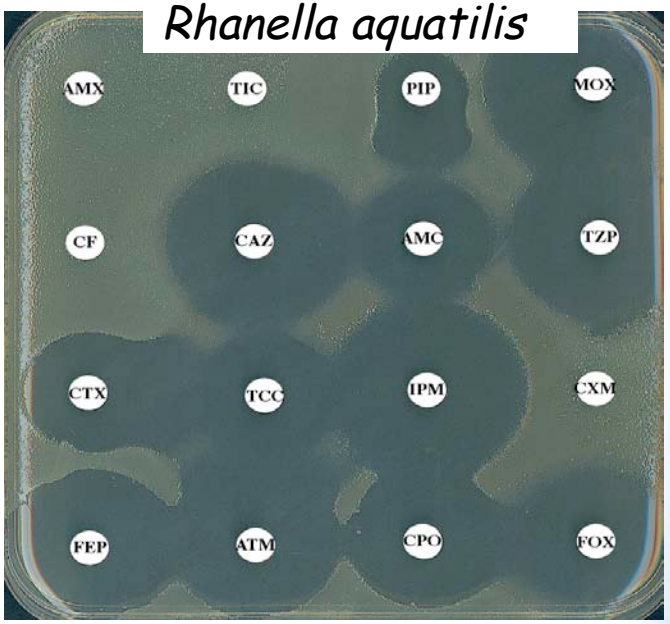
…

And many more……

Serratia fonticola

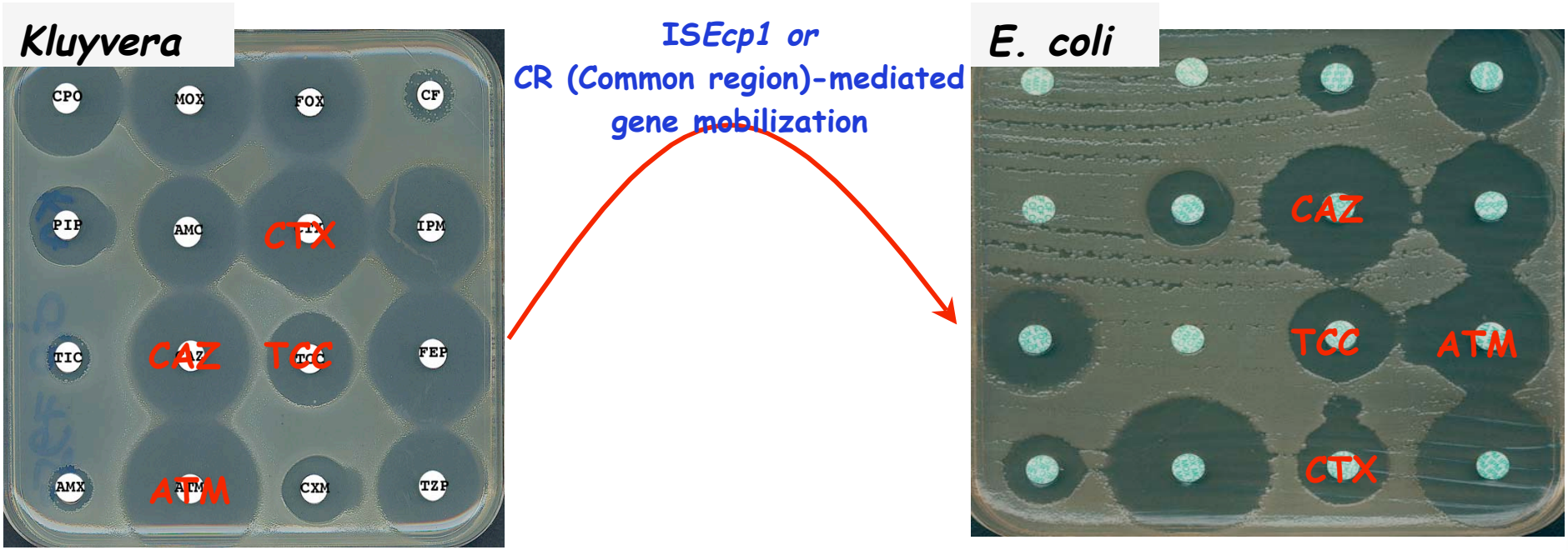


Rhanella aquatilis



Chromosomally-encoded ESBLs: progenitor of plasmid-encoded ESBLs

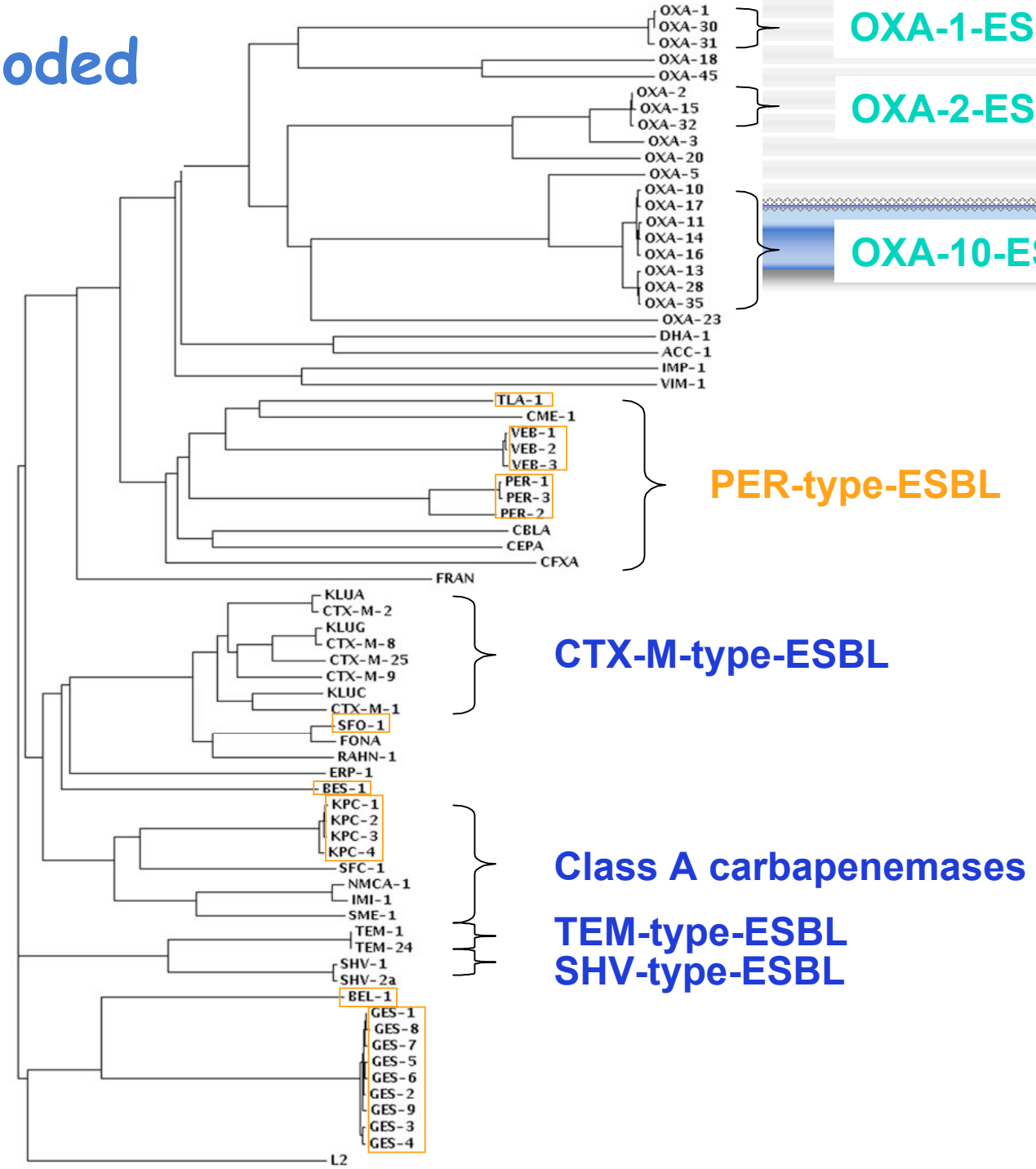
Bacteria	Chromosome	plasmid ß-lactamase
Serratia fonticola	FONA	SFO-1
Kluyvera ascorbata	KLUA	CTX-M
Kluyvera cryocrescens	KLUC-1	CTX-M
Kluyvera georgiana	KLUG-1	CTX-M



Plasmid-encoded ESBLs

Different ESBL families

Progenitors of these enzymes are unknown (except SFO-1)



OXA-1-ESBL

OXA-2-ESBL

OXA-10-ESBL

PER-type-ESBL

CTX-M-type-ESBL

Class A carbapenemases

TEM-type-ESBL

SHV-type-ESBL

MICs (µg/ml) for clinical strains and *E. coli* recombinant (rec) or transformant (Tc) strains

ß-Lactamase	*Species*	TIC	TCC	PIP	TAZ	CTX	CAZ	CAZ-Ac	FEP	ATM	IPM
SFO-1[a]	*E. cloacae*					128	4			16	1
	E. coli (Tc)					8	2			16	0,25
BES-1[b]	*S. marcescens*	>512	512	512	256	64	4	1	4	512	0,5
	E. coli (rec)	>512	32	512	256	64	16	0.25	8	512	0,25
TLA-1[d]	*E. coli*					>256	>256	4	64	>256	1
PER-1[e]	*P. aeruginosa*	>512	256	8	8	256	256		32	256	1
	S. typhimurium	>512	128	256	64	256	512		128	128	0.12
	E. coli (Tc)	>512	1	16	2	128	512		8	512	0,06
VEB-1[f]	*P. aeruginosa*	>512	8	256	8	>512	>512	16	256	512	0.5
	E. coli (Tc)	256	4	16	2	2	256	1	1	32	0.12
BEL-1[c]	*P. aeruginosa*	>512	128	16	8	32	32	4	4	32	1
	E. coli (rec)	>512	64	128	32	1	16	2	0.25	16	0.06
GES-1[g]	*K. pneumoniae*	>512	64	512	64	0.5	4	0.5	0.25	0.12	0.12
	E. coli (Tc)	256	8	16	2	0.5	8	1	0.25	0.25	0.06

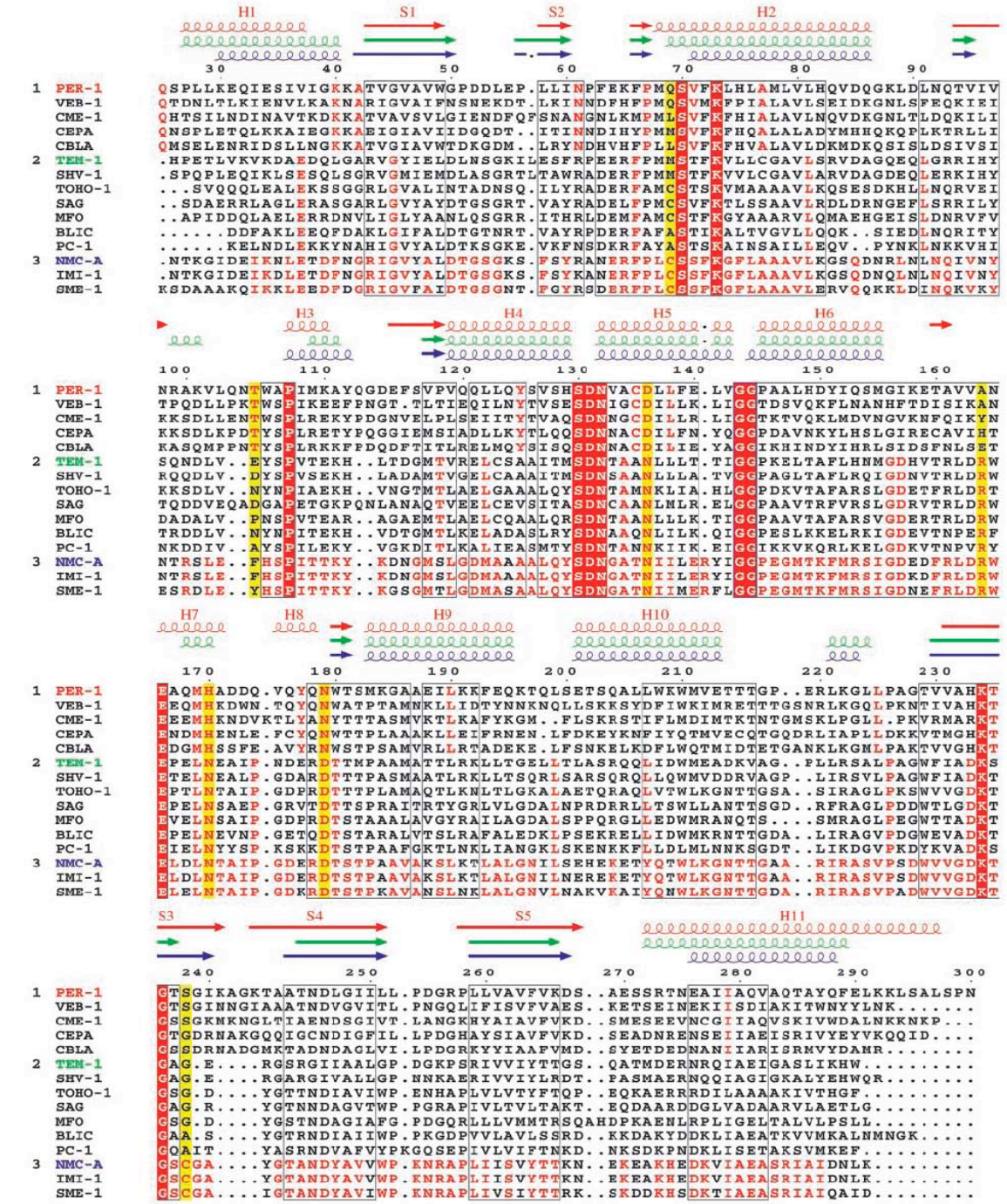
[a]Matsumoto & Inoue, AAC 1999, [b]Bonnet et al. AAC 2000; [c]Poirel et al. AAC 2005, [d] Silva et al, AAC, 2000, [e] Nordmann et al., AAC, 1993. [f] Naas et al. JAC 1999, [c]Poirel et al. AAC 2000.

Kinetic parameters

	*k*cat (S^{-1}) for : (Vmax rel)				*Km* (?M) for :				*k*cat/ Km_2 (mM-S^{-1}) for :			
	PER-1	VEB-1	GES-1	BEL-1	PER-1	VEB-1	GES-1	BEL-1	PER-1	VEB-1	GES-1	BEL-1
Benzylpenicillin	7	100	2.8	3	32	3	40	20	230	100	70	150
Ampicillin		110	13	10		6	200	30		50	65	330
Ticarcillin		8	0.3	6		1	400	8		22	0.7	750
Piperacillin			8	2			900	15			9	130
Cephalothin	12	700	179	150	46	6	3,400	280	269	325	52	540
Cefoxitin			0.9	<0.01			30	-			33	-
Cefotaxime	43	4,300	68	30	652	38	4,600	250	-	314	15	120
Ceftazidime	70	8,000	380	>1.5	3520	460	2000	>700	20	47	188	-
Cefepime			2.8	1			1,800	150			1.6	7
Imipenem			0.003	<0.01			45	-			0.007	-
Aztreonam	4	400	-	10	44	300	-	100	98	2	-	100

Kinetic parameters (IC50)

	IC50(?M) for :							
	PER -1	**VEB -1**	GES-1	BEL-1	TLA-1	TLA -2	BES-1	CME -2
Clavulanate	0.01	0.01	5	0.1	3.36	300	0.01	0.05
Tazobactam		0.02	2.1	2.2	5.37	100	0.1	1.5
Sulbactam	0.04	0.02		3	0.69	40	0.8	0.3
Cefoxitin	0.04	0.02		9		1.7		0.004
Imipenem	0.07	0.03	0.1	2		3.5		0.015
Moxalactam	0.03	0.02		7				0.02



Sequence alignment

Tranier et al., JBC, 2000, 275:29078

SFO-1, BES-1, and TLA-1

- Groupe 2be, Class A

- SFO-1 — *E. cloacae*, plasmid, inducible, Japan 1988
  Matsumoto & Inoue, AAC 1999

- BES-1 — *S. marcescens*, chromosome
  Hydrolyze ATM >> CTX >> CAZ
  Inhibition clavulanate > tazobactam
  Bonnet et al. 2000, AAC 44 3061-68

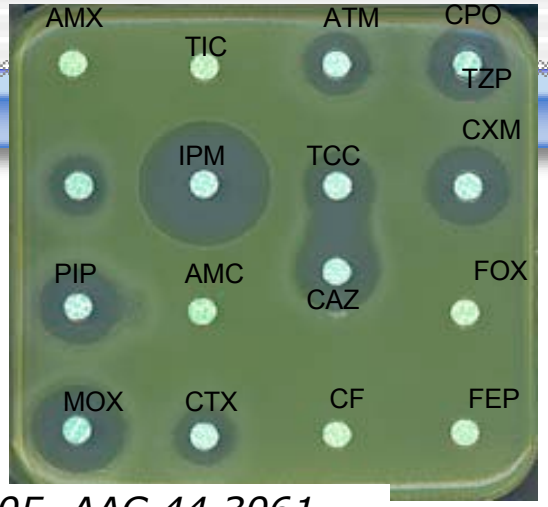
- TLA-1 — *E.coli*, plasmid
  Hydrolyze CTX <=> CAZ
  Inhibition tazobactam>clavulanate
  Outbreak of *K. pneumoniae* SHV-5 and TLA-1
  Silva et al. 2000, AAC,44 997-1003
  Alcantar-Curiel et al. 2004, CID,38:1067-74

BEL-1 and TLA-2

Groupe 2be, Class A

- BEL-1 *P. aeruginosa*, chromosome, integron
  Hydrolysis ATM >> CTX >> CAZ
  Inhibition clavulanate,tazobactam
  FOX, MOX, IMP

P. aeruginosa (BEL-1)



Poirel et al. 2005, AAC,44 3061

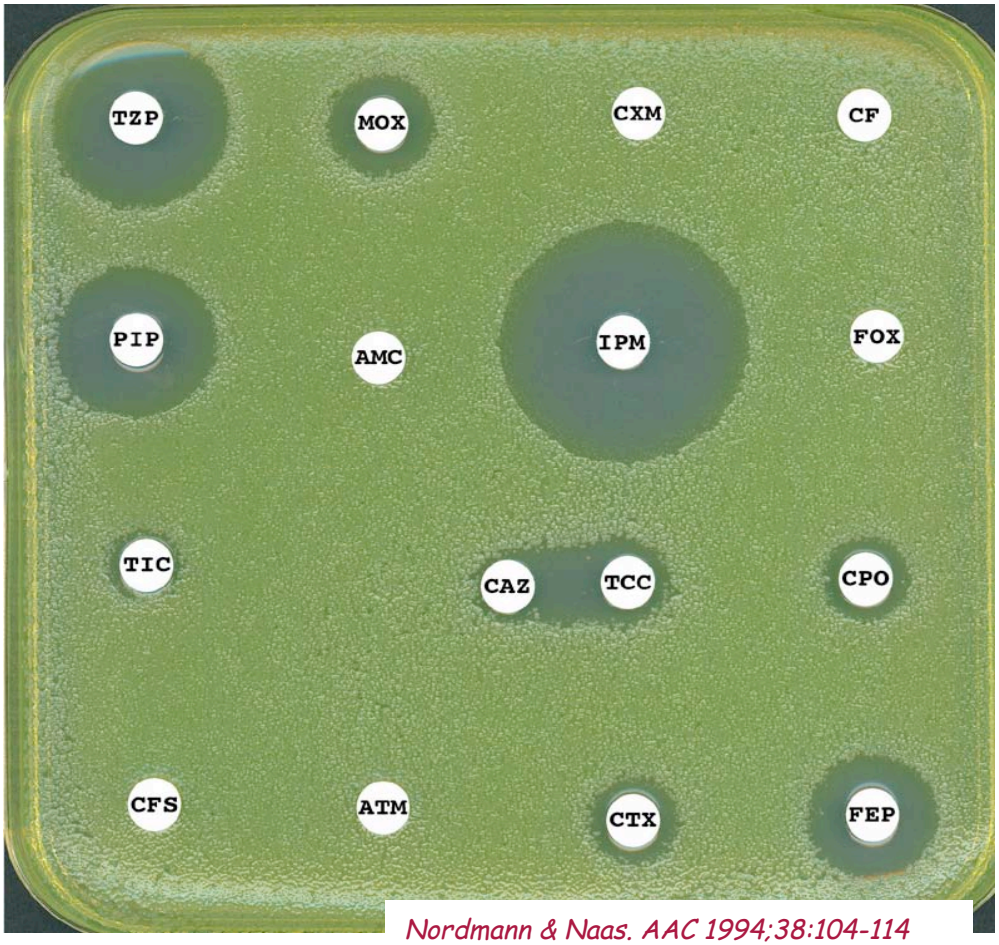
- TLA-2 ???, plasmid (only 51 % AA TLA-1)
  Waste water treatment
  No detectable hydrolysis AMX, TIC
  Strongly inhibited AMX, TIC, IMP, FOX
  Weakly inhibited clavulanate,tazobactam

E. coli (Topo-TLA-2)

Girlich et al. 2005, AAC,49 4767

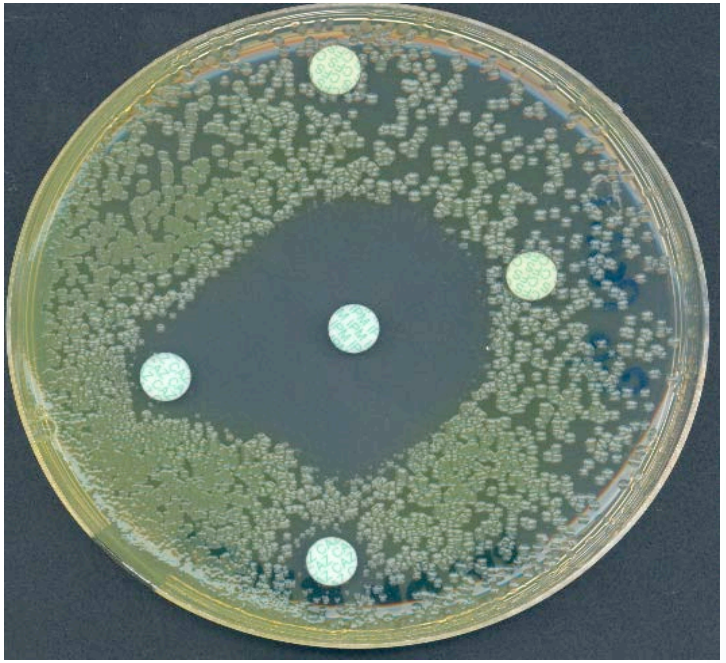
PER-1: Pseudomonas Extended Resistance

PER-1 *P.aeruginosa* in France (turkish patient) en 1991



Nordmann & Naas. AAC 1994;38:104-114

MH + cloxacilline



PER-1/3 and PER-2

- Endemic PER-1 strains in Turkey[1,2,3,4]

In ICUs	1997	1998	2000	2005
- *P. aeruginosa*	11%	24% (10%)	86% (41%)	55%
- *A. baumannii*	46%	62%		32%

() co-presence of OXA-10 like enzymes

- Enterobacteria: *E. coli, S. typhimurium*[3]

- PER-1 strains in the rest of the world

  A. baumannii in France, and Belgium, outbreaks in Korea

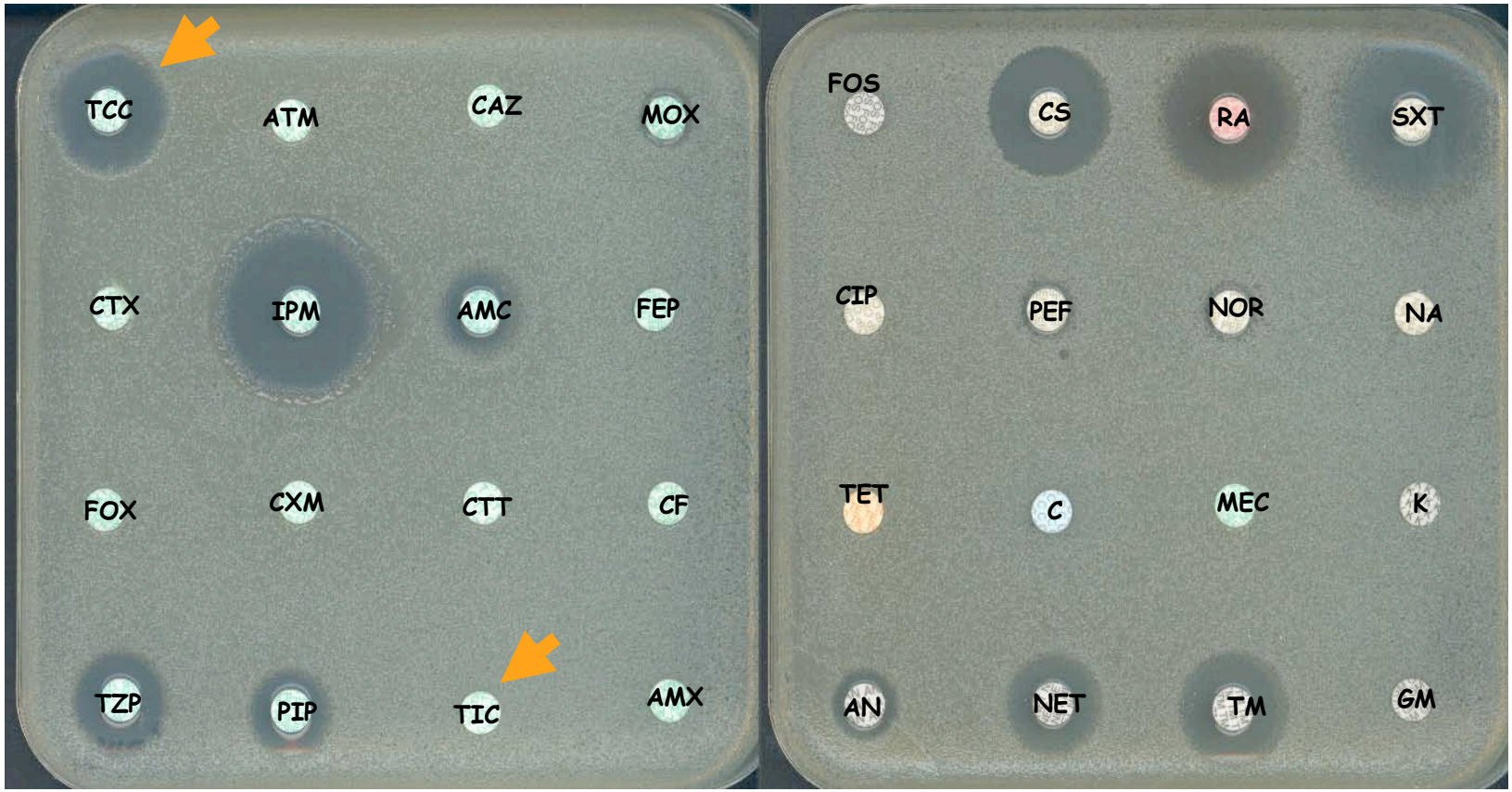
  Several enterobacterial species in Italy and Spain,

  P. aeruginosa outbreaks in France, Belgium and Italy

- PER-2 in *S. typhimurium, V. cholerae, K. pneumoniae, E. coli, Enterobacter, …* in Argentina, Uruguay, Bolivia (86% AA PER-1)

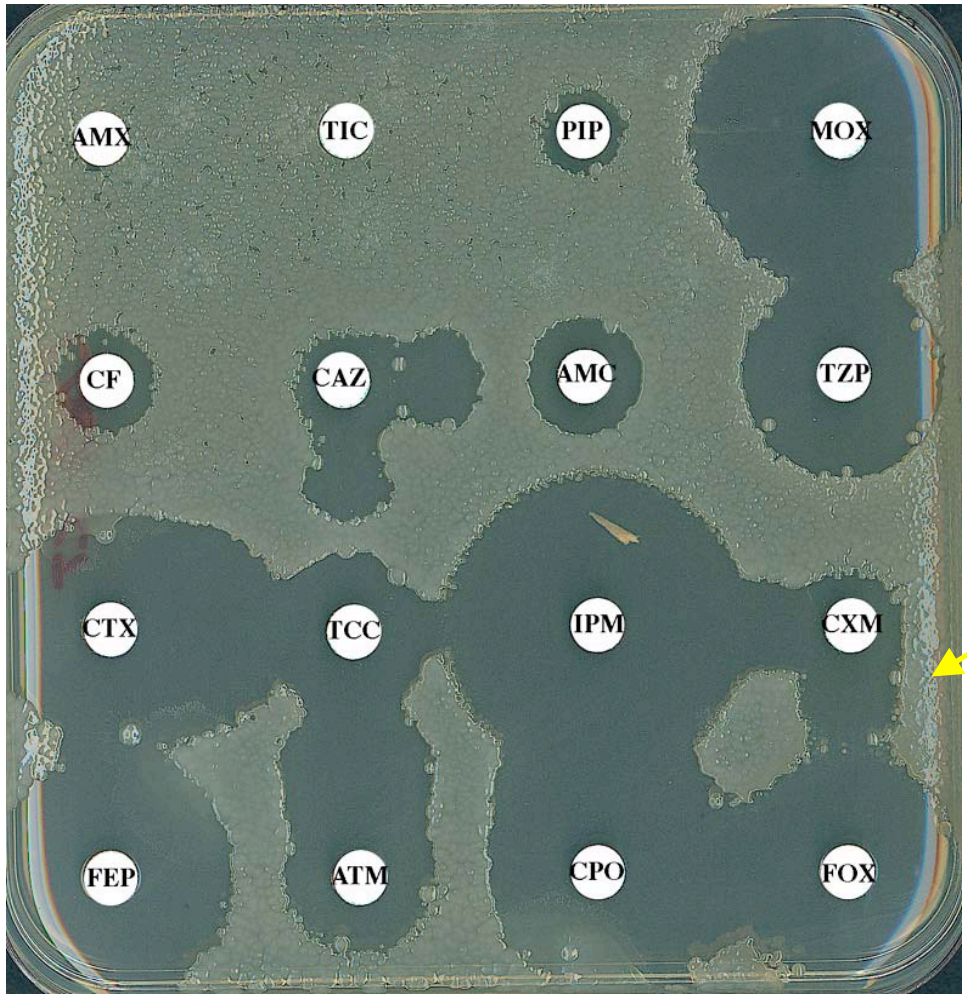
- PER-3 in *A. caviae* in France (point mutant of PER-1)

PER-1 in *A. baumannii*

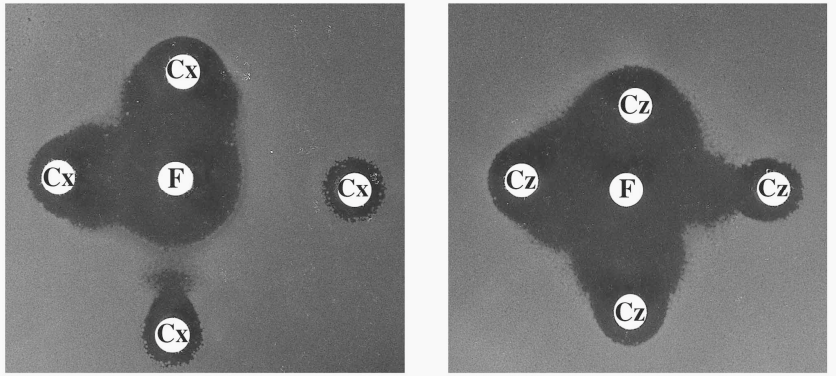


VEB-1 : Vietnamiese Extended β-lactamase

E. coli MG-1 (Vietnam) in 1996



F: cefoxitine
Cx: Cefuroxime
Cz: Ceftazidime



(Poirel et al. AAC, 1999, 43: 573)

Epidemiology of bla_{VEB-1}

1998: *P. mirabilis* (Vietnam), *P. aeruginosa* (Thailande), *P. aeruginosa* (Thailande); **2000:** *P. aeruginosa* (Kuwait), *P. aeruginosa* (India); **2002:** *E. cloacae (*France); **2003**: *E. coli* (France); *P. aeruginosa* (Bangladesh); **2004**: *P. stuartii* (Algeria) ; **2006:** *E. coli* (Canada)

Siriraj hospital , Bangkok, Thailande (1999)

- ➔ 40 % of enterobacteria CAZ^R are bla_{VEB-1} + *(Girlich et al. JCM, 2001, 39: 175)*
- ➔ 80 % of *P. aeruginosa* CAZ^R are bla_{VEB-1} + *(Girlich et al. CID, 2002, 34: 603)*

Srinagarind hospital , Khon Kaen, Thailande (1999)

- ➔3 % of enterobacteria CAZ^R are bla_{VEB-1} +
- ➔60 % of *P. aeruginosa* CAZ^R are bla_{VEB-1} + *(Chanawong et al., JAC, 2001, 48: 839)*

Seven hospital, Ho Chi Minh City, Vietnam (2000-2001)

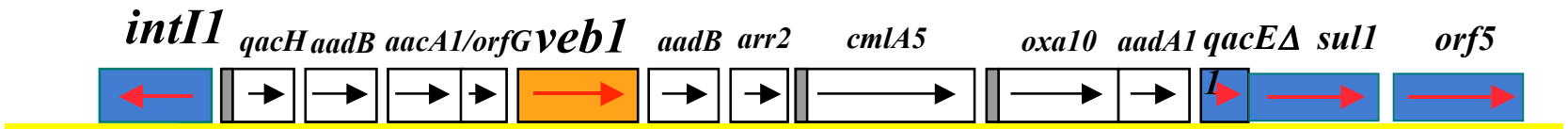
- ➔25 % of ESBL enterobacteria are bla_{VEB-1} + *(Cao et al., AAC, 2002, 46: 3739)*

Outbreaks

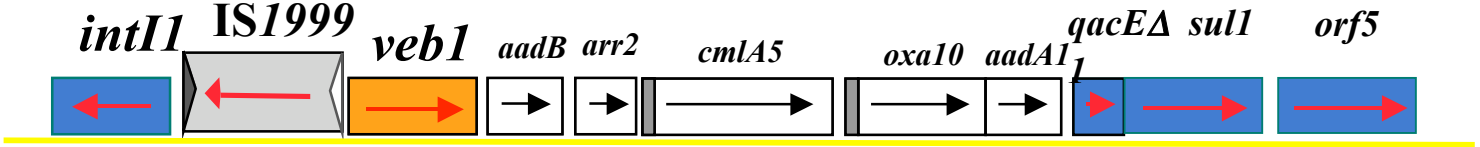
2001 VEB-1 *A. baumannii* in France (Poirel et al. JCM, 2003,41:3542)
2002 VEB-1 *P. mirabilis* in Korea (Kim et al. JAC, 2004,54:1144)
2003 VEB-3 *E. cloacae* in China (Jiang et al. JCM, 2005,43:826)
2003 VEB-1 *A. baumannii* in France (Naas et al. EID, 2006, in press)

Structure of *veb1* containing integrons

Enterobacteria



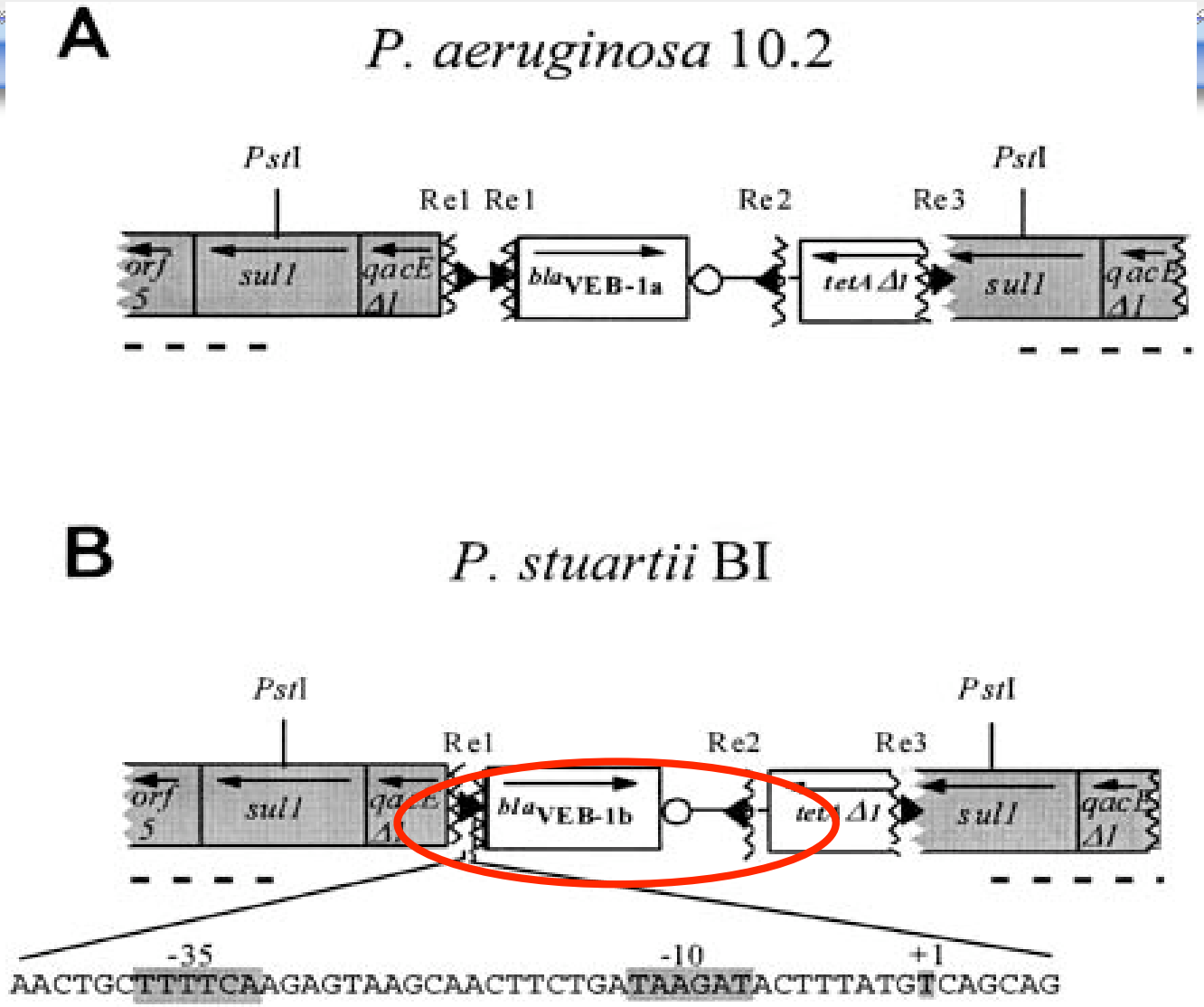
P. aeruginosa



=> bla_{VEB-1} is integron-located

- Consequences : co-resistance ; co-expression ; co-selection

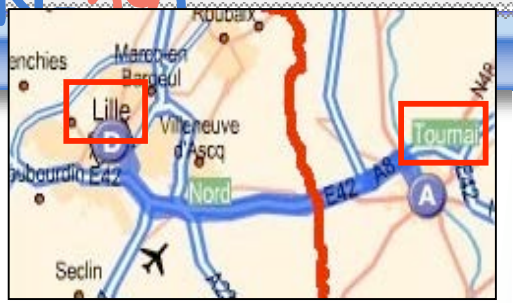
Novel genetic background with *bla*VEB gene



Acinetobacter baumannii (VEB-1)

- **2001** : First detection in *A. baumannii*
  - *A. baumannii* outbreak (36 cases in 1 hospital) in Northern France
  - one patient transferred to an hospital near Paris => ESBL: VEB-1
- **September 2003**
  - 4 hospitals from the same district (Nord) reporting 5 *A. baumannii* infection clusters (23 cases)
  - similar susceptibility profile (ESBL)
  - strains identical by PFGE to 2001 strain
- **November 2003** ===> National alert

Number of hospitals reporting VEB-1 ESBL producing *A. baumannii*, by district, France April 2003 à Septembre 2005 (N=59)



(Naas et al. JAC, 2006)

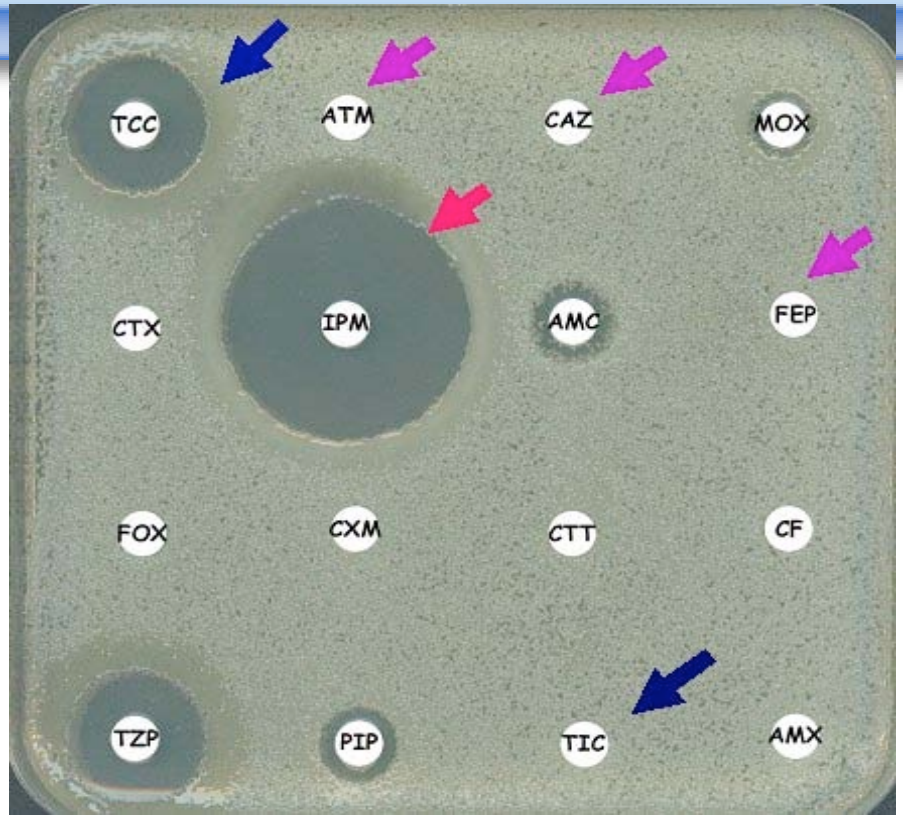
UK
Belgium
Lille
Reims
Paris
Switzerland
Lyon
Italy
Nice
Spain
Toulouse
Montpellier

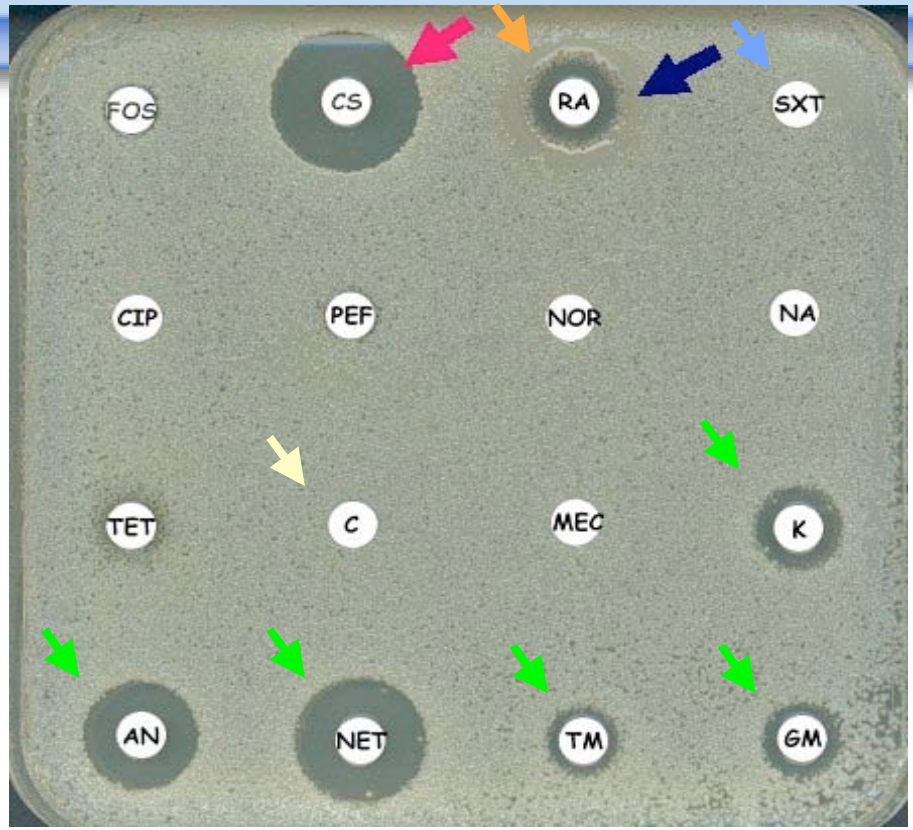
75 94 62 59 80 76 02 08 51 78 69 38 12 30 06 81 34 31

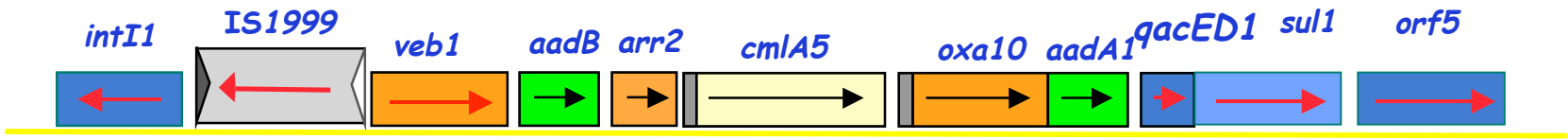
Number of hospitals
- 0
- 1
- 2-10
- 11-20

(Naas et al. EID, in press)

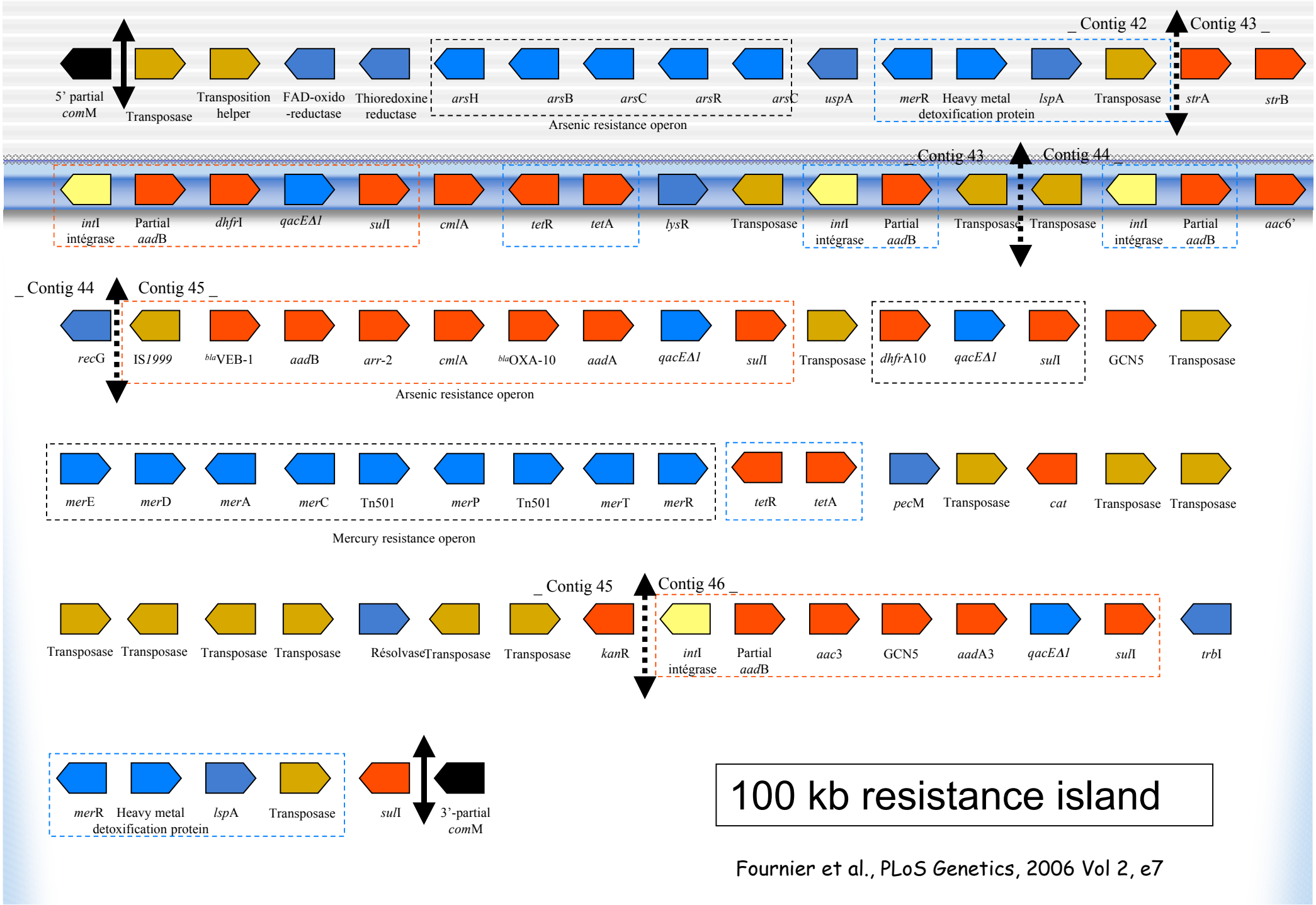
A. baumannii VEB-1







(Poirel et al., 2003, JCM, 41:3542)

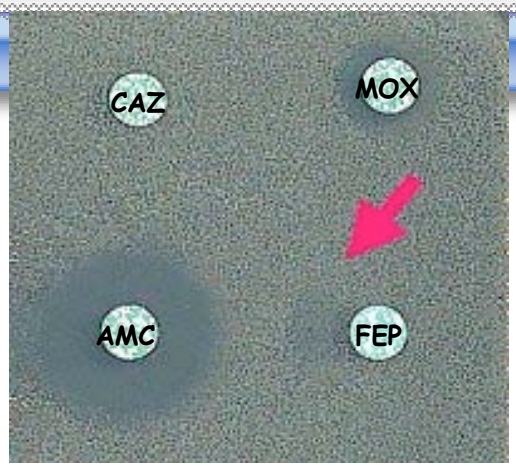


100 kb resistance island

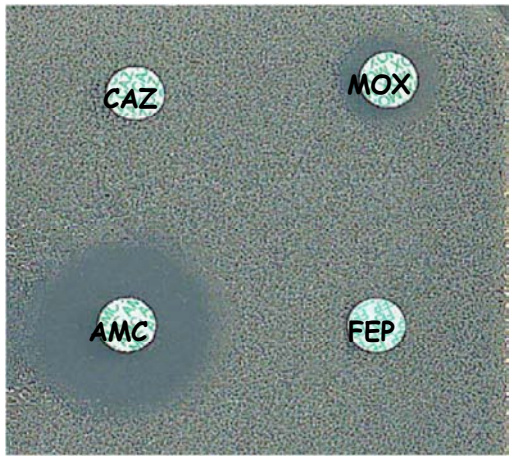
Fournier et al., PLoS Genetics, 2006 Vol 2, e7

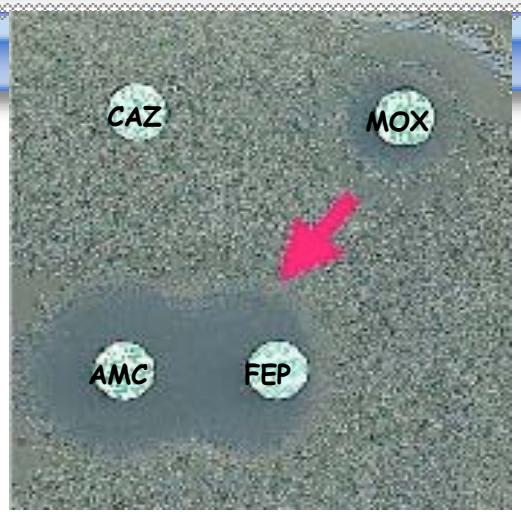
ESBL Laboratory Identification

Usefulness of disk diffusion methods

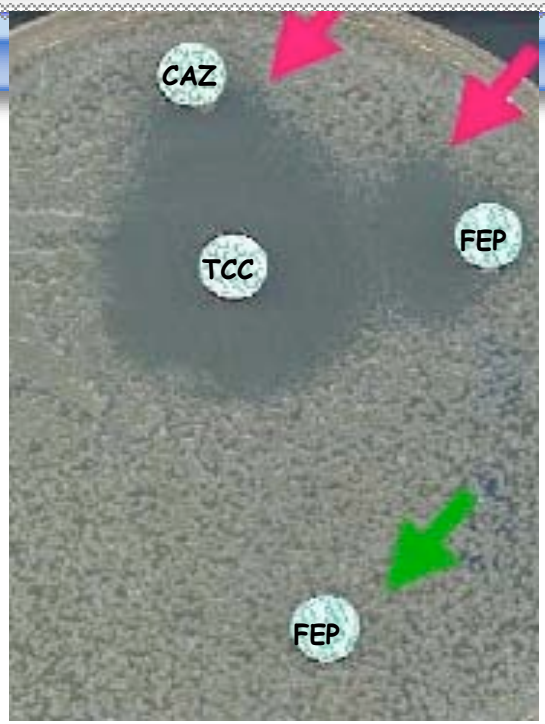


1. 25°C
standard disc diffusion
1. 37°C



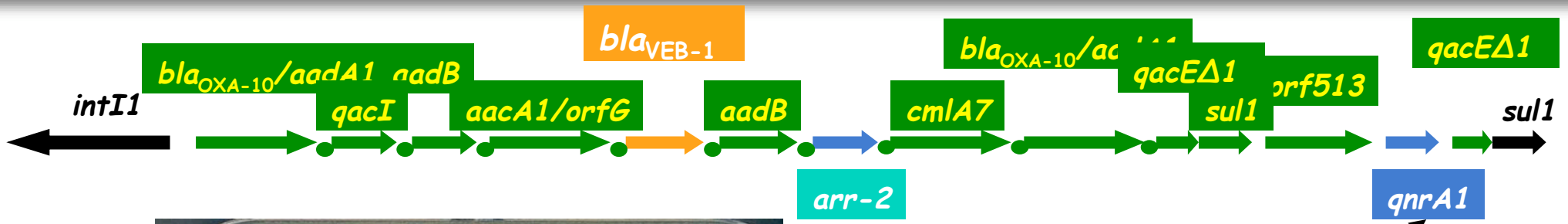


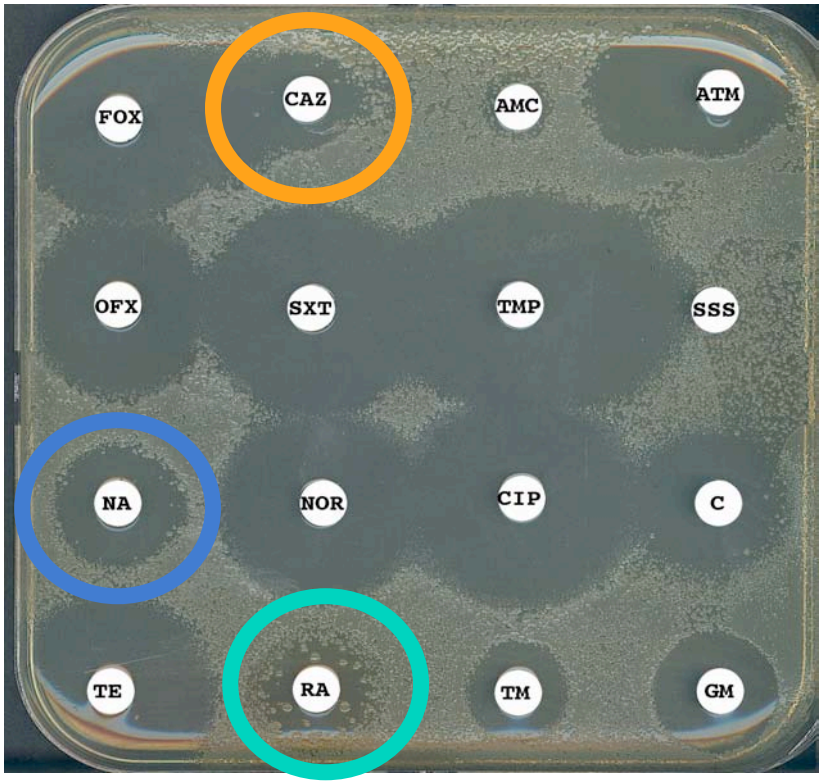
2. synergy when AMC & FEP disks brought closer



3. synergy between TCC & CAZ/FEP (cloxacillin-containing MH plates)

Association of *bla*VEB and *qnrA* in a *sul1*-type integron in *E. coli* from Canada



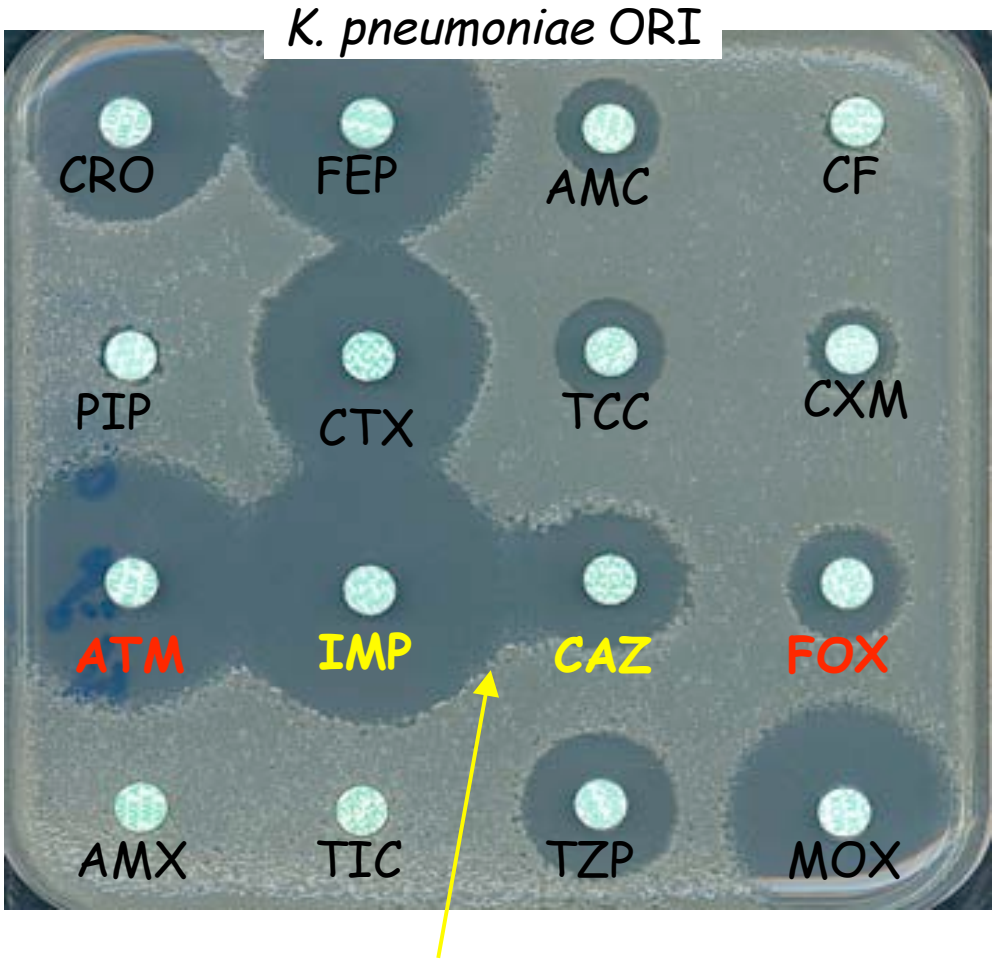


Plasmid mediated
Quinolone resistance gene

Plasmid mediated
Rifampin resistance gene

11 out of 23 VEB+ Enterobacteria from Thailand are qnr+
(Poirel et al. 2005, *AAC*, 49:3091)

GES: Guyana Extended-Spectrum ß-lactamases
IBC: Integron-Borne Cephalosporinase



GES-1 *K. pneumoniae* ORI, in Paris patient from French Guyana, 1998

IBC-1 *E. cloacae*, in Thessalonki From a Greek patient 1999

Strongly inhibited by imipenem
Plasmid encoded
Integron-borne

GES: Guyana Extended-Spectrum ß-lactamases

GES-1	France, Argentina Brazil, Portugal	*K. pneumoniae, P. aeruginosa*
GES-2	South Africa	*P. aeruginosa*
GES-3	Greece, Korea	*E. coli, K. pneumoniae*
GES-4	Greece	*K. pneumoniae*
GES-5	Japan, China	*K. pneumoniae*
GES-6	Japan	*K. pneumoniae*
GES-7 (IBC-1)	Greece	*E. cloacae*
GES-8 (IBC-2)	Greece	*P. aeruginosa*
GES-9	France	*P. ae*

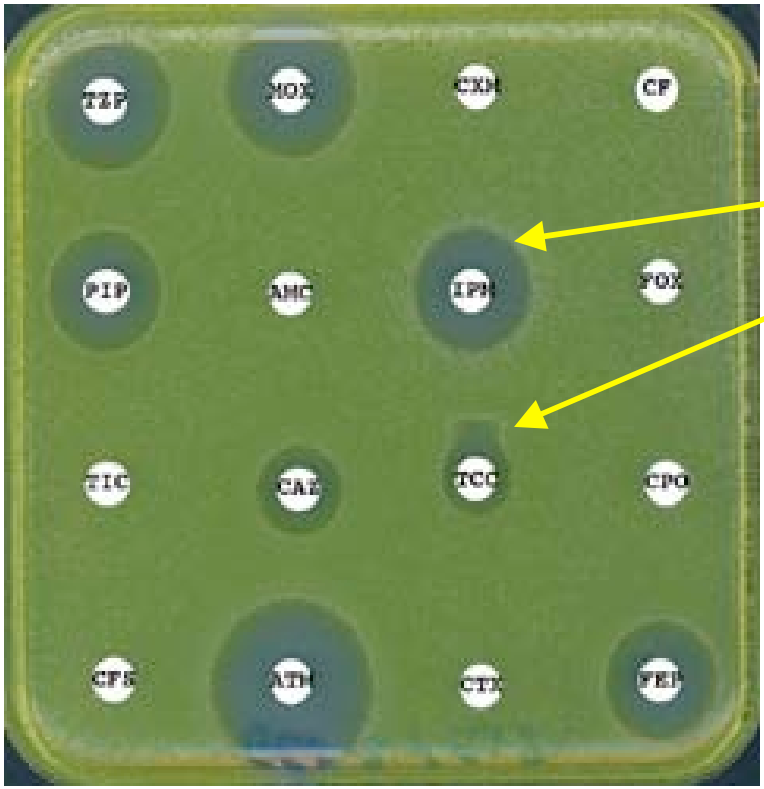
(Revised numbering per *AAC*, 2005, 49:2148)

Table 1. Key amino acid substitutions of GES variants in relation with their hydrolysis spectra

GES variant	Ambler position 170	Ambler position 243	Hydrolysis profile CAZ	FOX	ATM	IPM
GES-1	G	G	+[a]	-	-	-
GES-2	N	G	+	-	-	+
GES-3	S	G	+	+	-	+
GES-9	G	S	+	-	+	-

[a] + and – mean hydrolysis and no hydrolysis, respectively.

GES-2: Point mutant derivatives with reduced imipenem susceptibility



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2001, p. 2598–2603 Vol. 45, No. 9
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.9.2598–2603.2001

GES-2, a Class A β-Lactamase from *Pseudomonas aeruginosa* with Increased Hydrolysis of Imipenem

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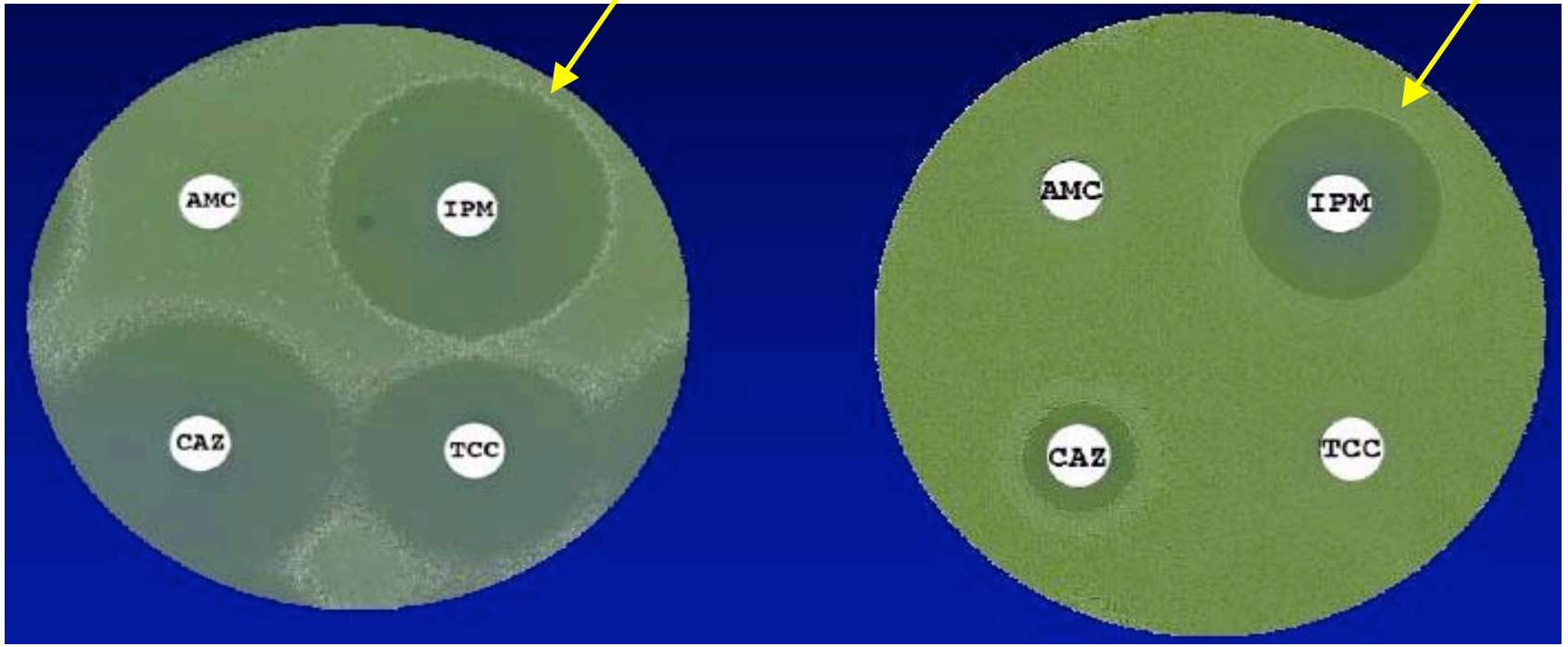
- May 2000 , *P. aeruginosa* GW-1 from blood culture of nosocomial pneumonia (Pretoria Academic hospital, South Afric)
- Outbreak of eight identical *P. aeruginosa* strains from hospitalized patients with a similar antibiotic resistance phenotype, CAZ_R, IMP_I
- 100-kb plasmid
- Integron located
- Point mutation in Omega loop (G170N) as compared to GES-1

Conjugation in *P. aeruginosa* PU21



P. aeruginosa PU21

P. aeruginosa PU21 (GES-2)

GES-2 versus GES-1

β-lactam(s)	*P. aeruginosa* isolate (GES-2)	*E.coli* DH10B (GES-2)	*E. coli* DH10B (GES-1)
	MICs (μg/ml)		
Piperacillin	64	8	64
Cephalothin	>512	32	256
Ceftazidime	16	8	128
Ceftazidime+Cla	16	0.5	8
Cefotaxime	128	1	4
Imipenem	**16**	**0.25**	**0.06**
Meropenem	2	0.06	0.06

Kinetics parameters GES-2/GES-1

Substrate	*K*cat(S^{-1})	*K*(μM)	*K*cat/*Km*/μM-S^{-1}	
Benzylpenicillin	0.4/2.8	4/40	96/70	1.4x
Ticarcillin	0.06/0.3	13/400	4/0.7	5.8x
Cefotaxime	2.2/68	890/4.600	2.5/15	0.15x
Imipenem	0.004/0.003	0.4/40	9/0.07	100x

Class A carbapenemases

- ✓ **Chromosome-encoded**
  - .NMCA → *Enterobacter cloacae*
  - . IMI-1, → *Enterobacter cloacae*
  - . Sme-1, -2 → *Serratia marcescens*
  - . SFC-1 → *Serratia fonticola*

  } Since 1982, 35 isolates

- ✓ **Plasmid-encoded** →
  - . IMI-2,-3 → *E. asburiae, E. cloacae*
  - . KPC-1-4 → *K. pneumoniae, Enterobacteriacae*

  } US Rivers, 1 in China

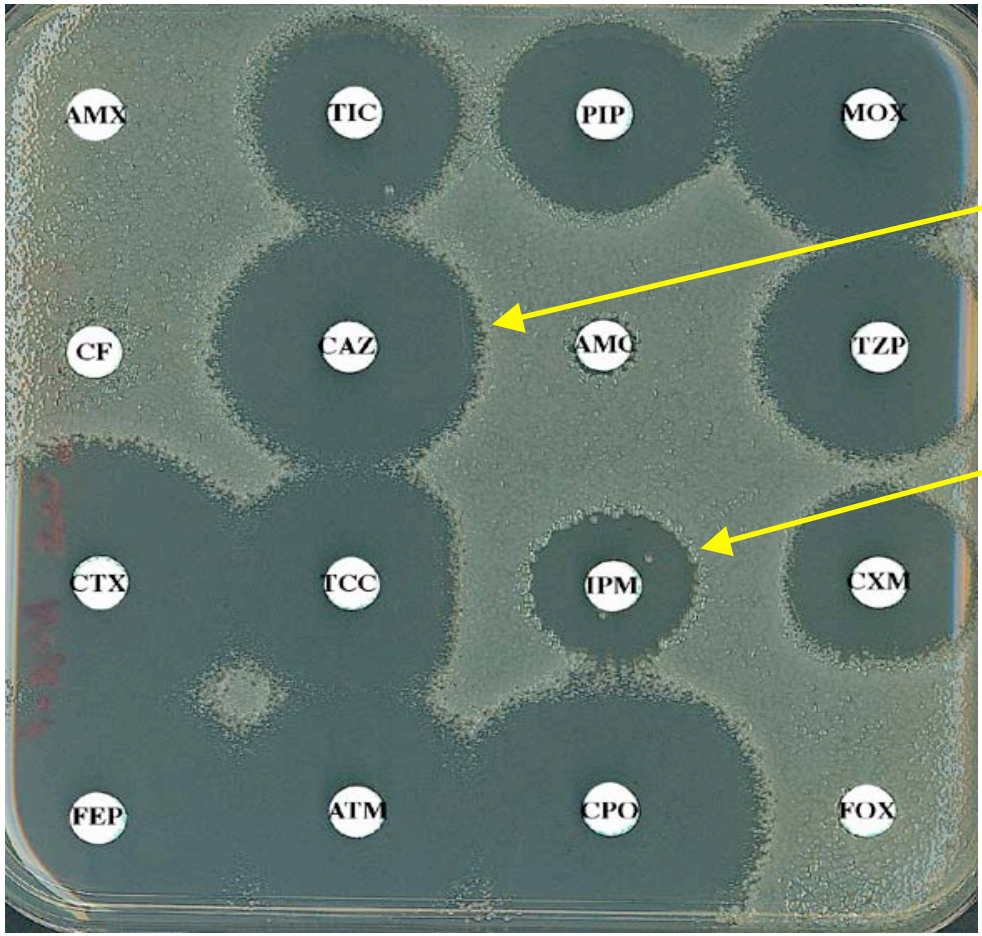
  } Since 1998, 260 isolates

  } ESBL

- ✓ **Point mutant with reduced imipenem susceptibility** →
  - . GES-2 → plasmid of *P. aeruginosa*

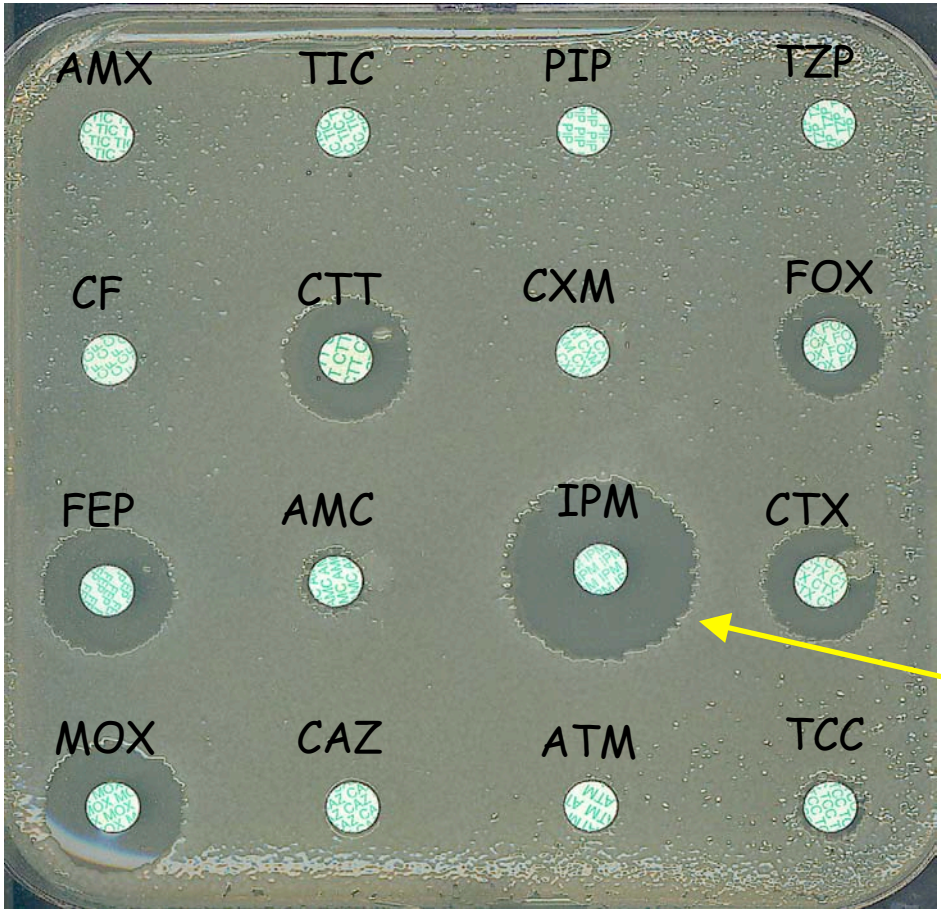
Chromosome-encoded class A carbapenemases

E. cloacae NOR-1 (NMC-A)



ESBLs of a novel type; the KPC enzymes

KPC in *K. pneumoniae* and *E. coli*; North East USA and Europe



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2001, p. 1151–1161 Vol. 45, No. 4
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.4.1151–1161.2001

Novel Carbapenem-Hydrolyzing β-Lactamase, KPC-1, from a Carbapenem-Resistant Strain of *Klebsiella pneumoniae*

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Received 19 September 2000/Returned for modification 21 November 2000/Accepted 23 January 2001

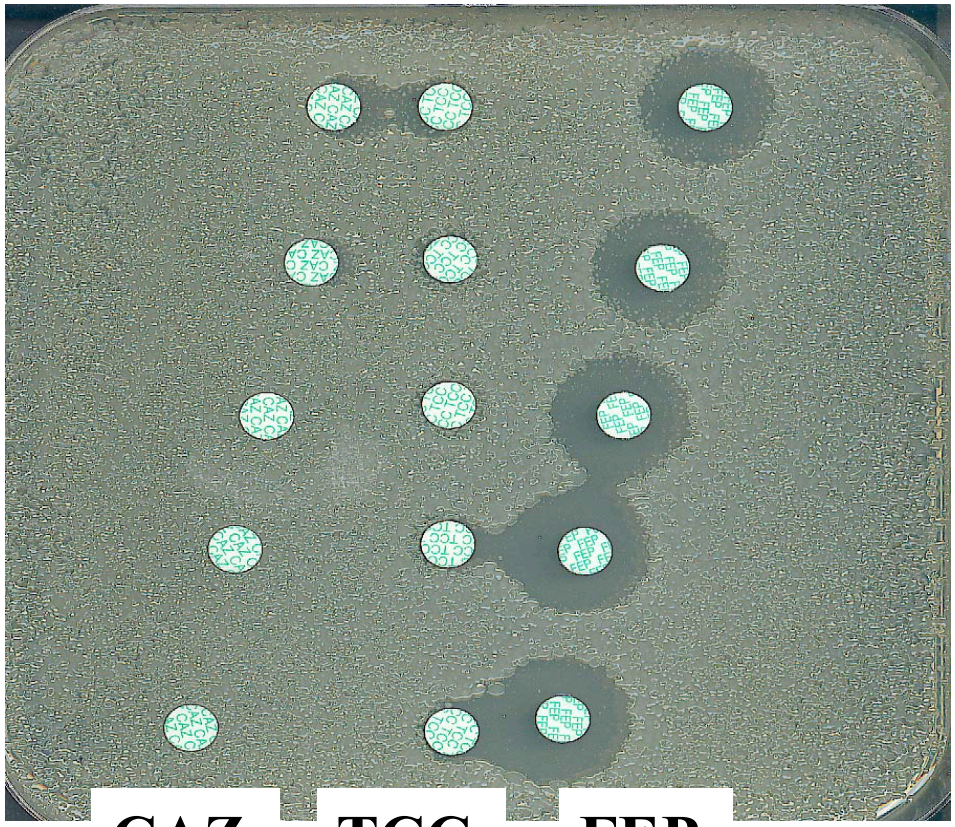
- ✓KPC-1:
- 45% Sme-1, 44% NMCA and IMI-1
- plasmid encoded (50-kb)

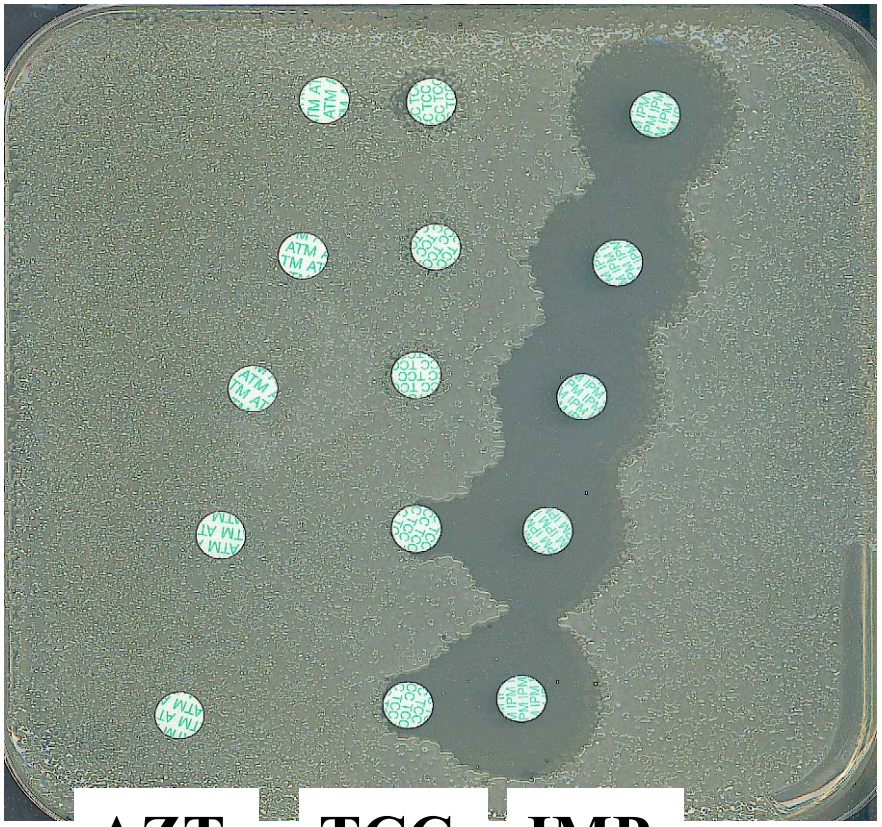
- ✓Imipenem resistance inhibited by clavulanic acid

MICs of β-lactams for KPC-1 producers

	MICs (μg/ml)		
	K. pneumoniae 1534	*E. coli* HB101 (KPC-1)	*E.coli* HB101 (KPC-1)
Ampicillin	>64	>64	4
Cefotaxime	64	8	≤ 1
Ceftazidime	32	8	≤ 2
Imipenem	16	8	≤ 0.25
Imipenem+cla	2	0.5	≤ 0.25
Meropenem	16	4	≤ 0.25
Aztreonam	>64	32	≤ 1

KPC detection





Extended Spectrum activity conferred by OXA (Group 2d, Class D) ß-lactamases

OXA

Resistance : ampicilline, cefalotine, cloxacilline, piperacilline

P. aeruginosa +++, Enterobacteria and *A. baumannii*

OXA-ESBL

- Resistance to ceftazidime and/or cefepime
- Usually not inhibited by clavulanate
  - Difficult to detect (except OXA-18, OXA-45)
- Most isolates are *P. aeruginosa*, (but also *E. coli*)
- Derive from OXA-1, OXA-2 or OXA-10 ==> integron located
- Occurrence ??: **Prévalence ++ in Turkey**
  - *OXA-11, -14, -15, -16, -17 first identification in Turkey (Gür, Livermore et al.)*
  - *OXA-10-like : 55% of P.aeruginosa CAZ-R ICU in Istambul, Turkey (Aktas et al. CMI, 2005, 11:193)*
  - *OXA-ESBLs : 2.9 % of P.aeruginosa CAZ-R in Tainan, Taiwan (Yan et al JMII, 2006, 39:130)*

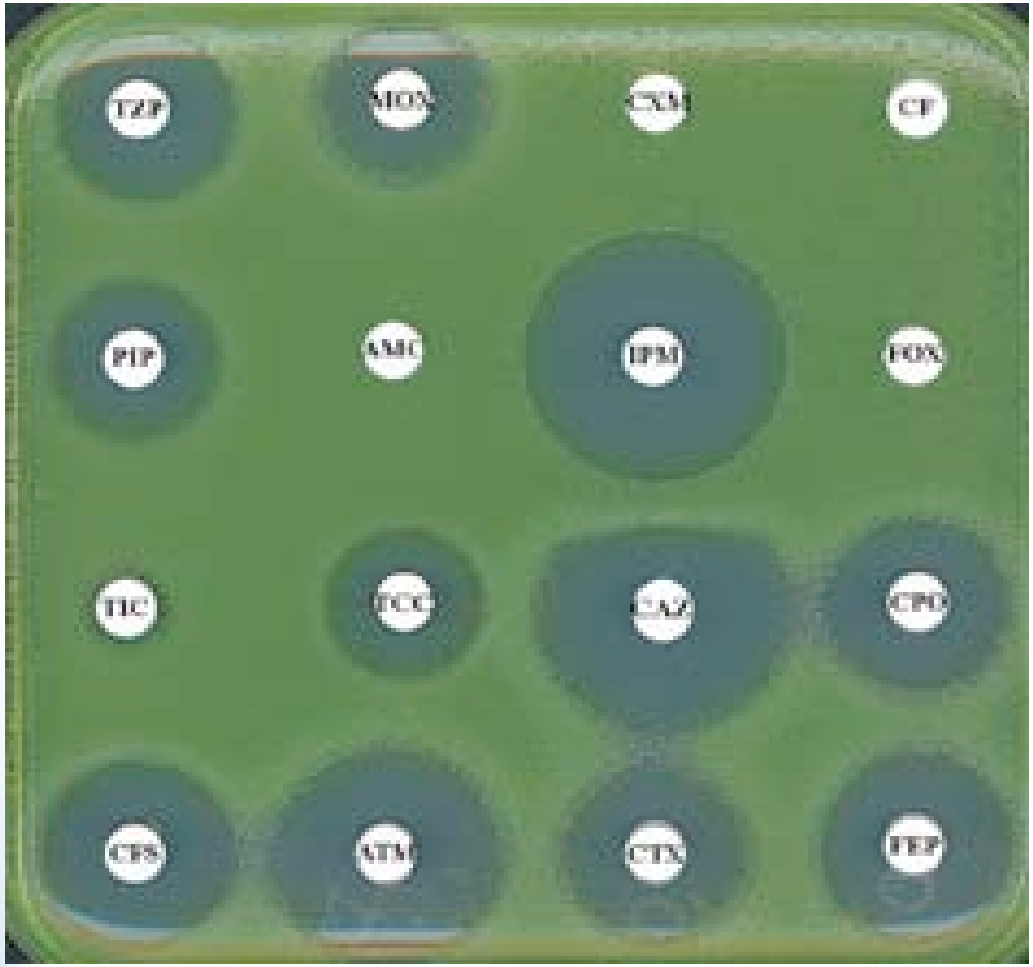
Extended Spectrum activity conferred by OXA (Group 2d, Class D) ß-lactamases

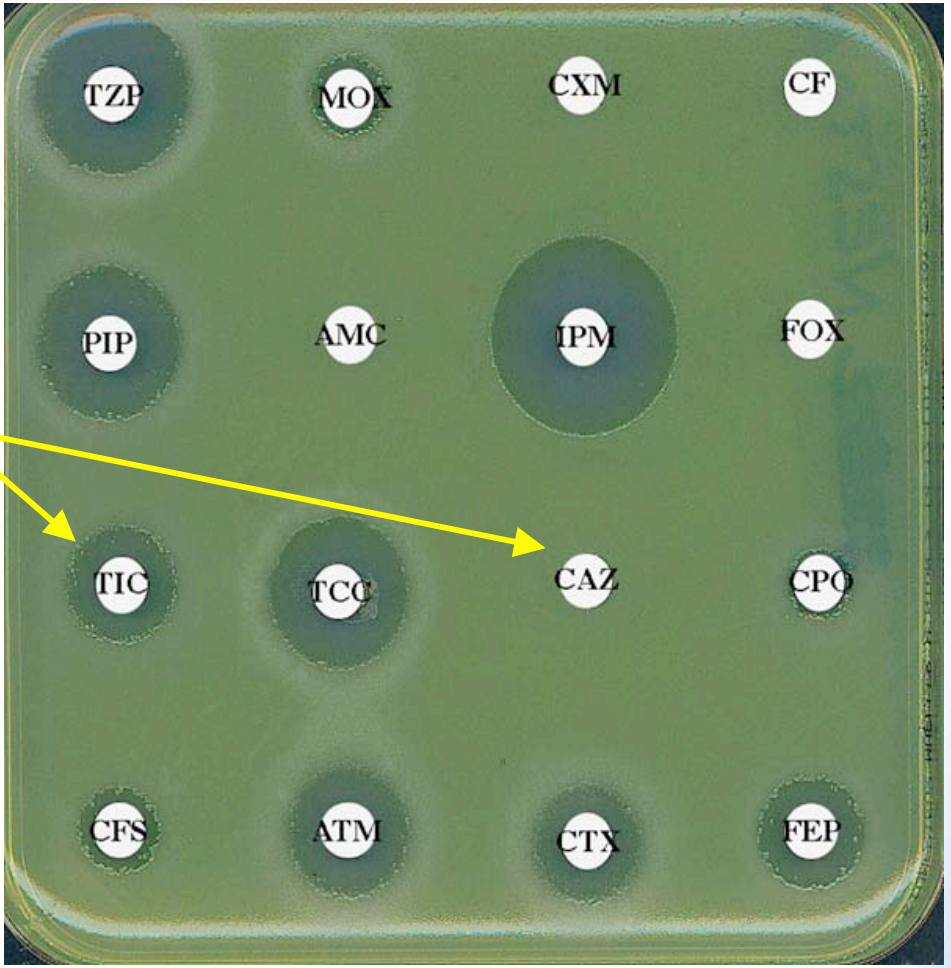
B-Lactamase	TIC	TCC	PIP	TAZ	CAZ	FEP	ATM	IMP
OXA-2	128	64	32	4	16	2	2	4
OXA-15	128	64	32	8	128	4	8	2
OXA-32	256	64	32	16	128	8	32	4
OXA-10	512		64		2	4	16	2
OXA-11	512		64		512	4	128	2
OXA-14	512	256	64	32	512	64	16	2
OXA-16	128	64	32	16	128	32	4	1
OXA-13	256	256	32	32	2		8	1
OXA-19	256	256	64	64	256		16	1
OXA-28	128	128	16	16	256	16	32	0,25
OXA-18	256	64	64	32	128	16	256	2
OXA-1	128	64	32	4	16	2	2	4
OXA-31	512	512	256	256	2	256	32	

TIC, ticarcilline, TCC, ticarcilline + clav.; PIP, piperacilline; TAZ, piperacilline + tazobactam; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam, IMP, imipénème

P. aeruginosa (OXA-10)

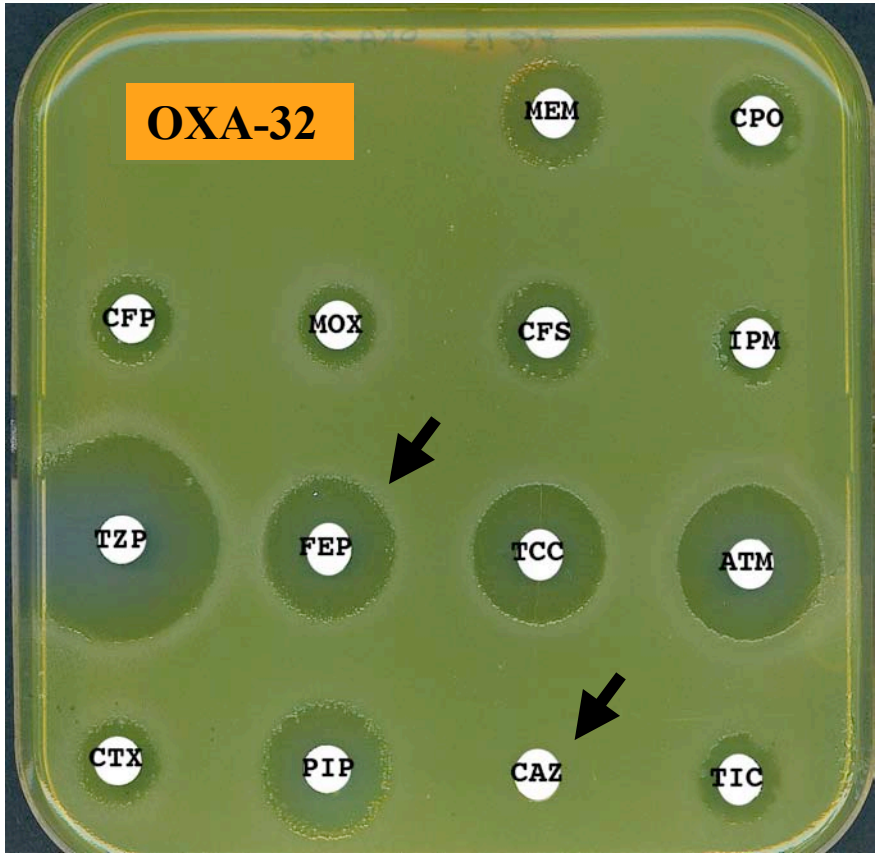
P. aeruginosa (OXA-28)

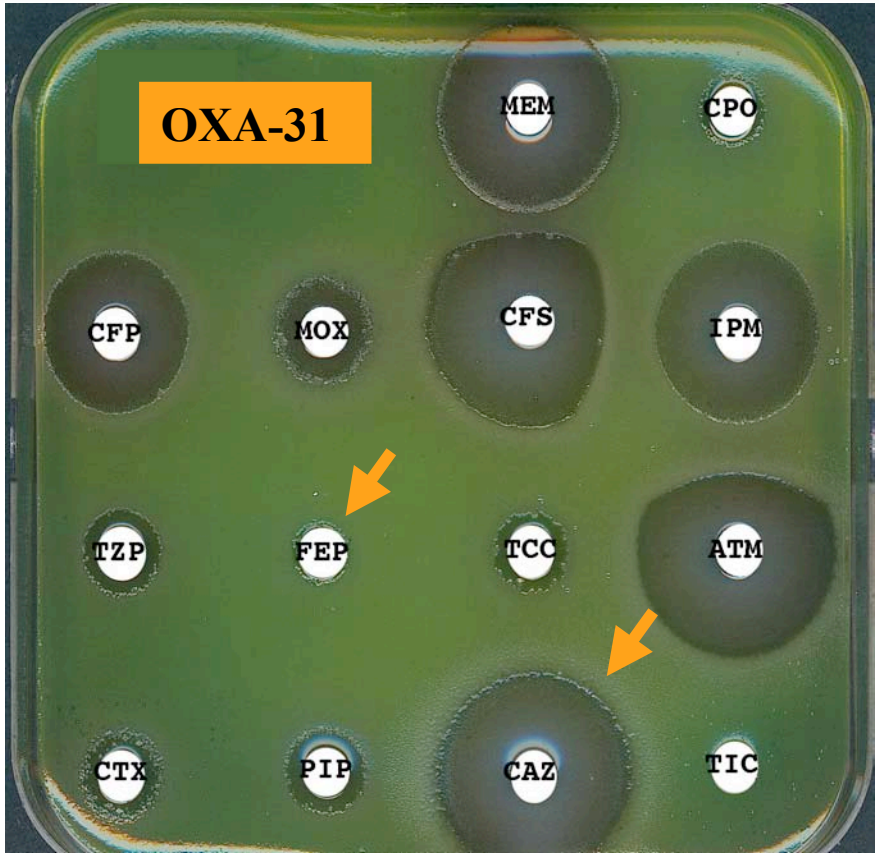




P. aeruginosa (OXA-32)

P. aeruginosa (OXA-31)





Conclusions

- Numerous chromosomally encoded ESBLs, waiting for a pick-up: Gene capture units
- Some of these "minor ESBLs": globalisation or regionalisation
- Point mutant derivatives of known enzymes: extension of spectrum toward carbapenems
- True carbapenem-hydrolyzing ESBLs
- Integron-located genes: co-selection, co-expression
  - ==> co-resistance
- Plasmid-encoded rifampin and quinolone resistance genes associated with bla VEB-1

Conclusions:
Minor problems may become rapidely major concerns

ORIGINAL INVESTIGATION

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

A New Threat to Our Antibiotic Armamentarium

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

Arch Intern Med. 2005;165:1430-1435

=> Transcontinental transfer of antibiotic resistance genes

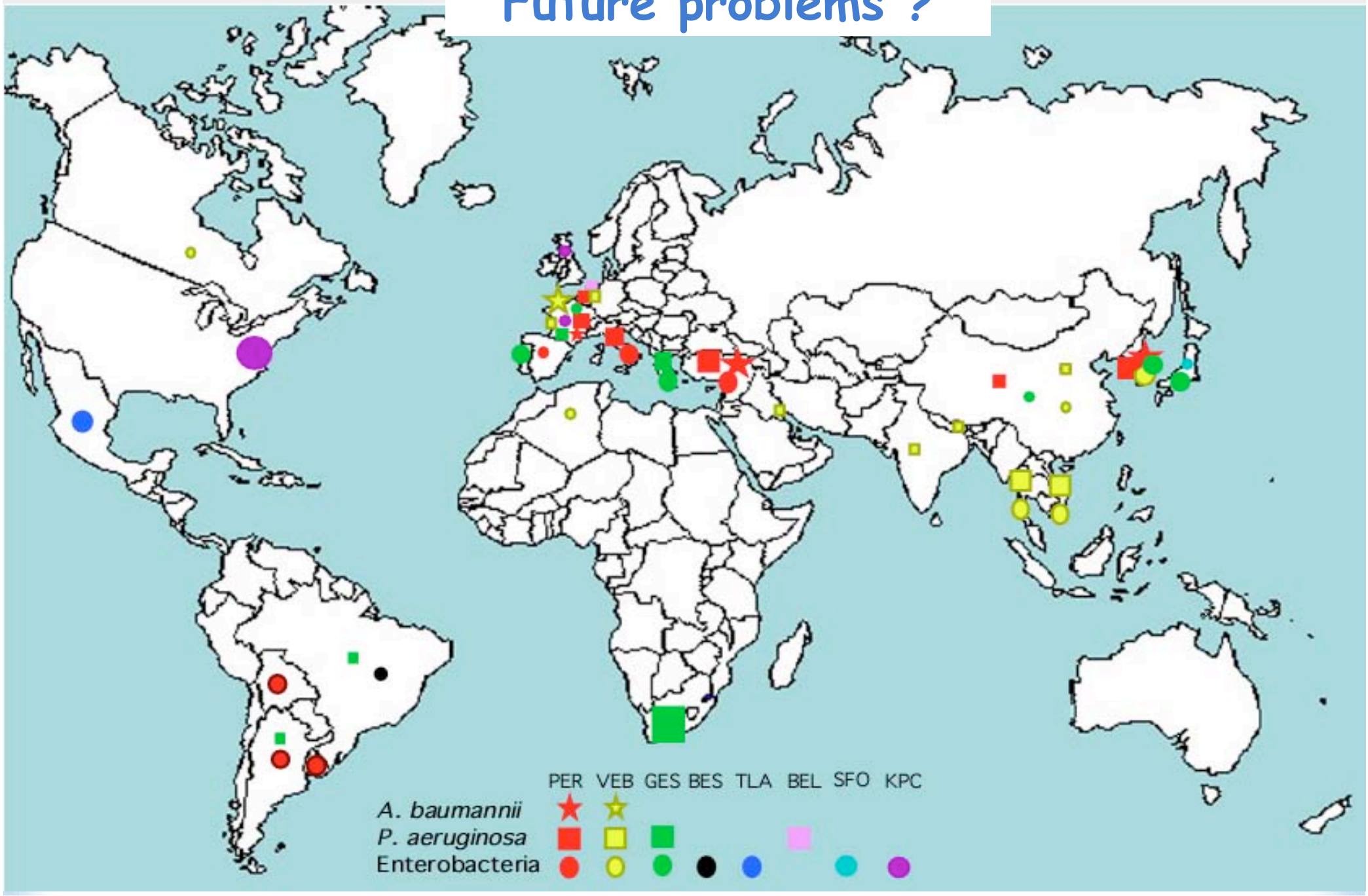
Plasmid-Mediated Carbapenem-Hydrolyzing β-Lactamase KPC in a *Klebsiella pneumoniae* Isolate from France

(Naas et al. *AAC* 2005, 49 ; 4423)

=> The *A. baumannii* VEB story

=> We find only what we are looking for …

Future problems ?



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