4 th Arab Symposium for Antimicrobials Agents
16 th National Congresss of Infectiology

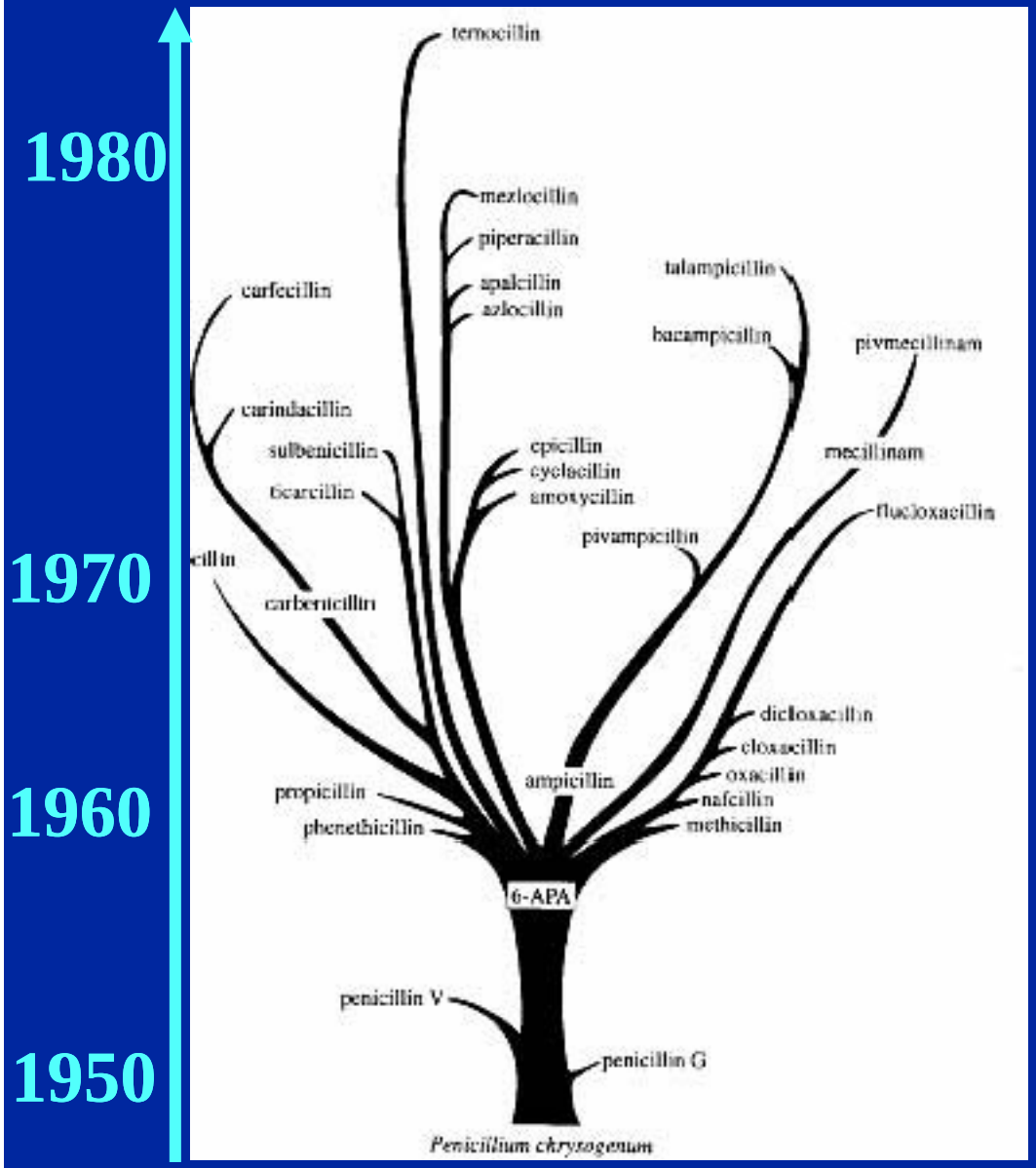
Multidrug resistance of Gram negative bacilli :

Evolution of ß-Lactamases

Pr. A. PHILIPPON
Faculté de Médecine Descartes
Paris V - France

????

ß-LACTAMS



1/ Major family

2/ High diversity

Penicillins

- Amoxicillin AMX
- Ticarcillin TIC
- Piperacillin PIP

Competitive inhibitors

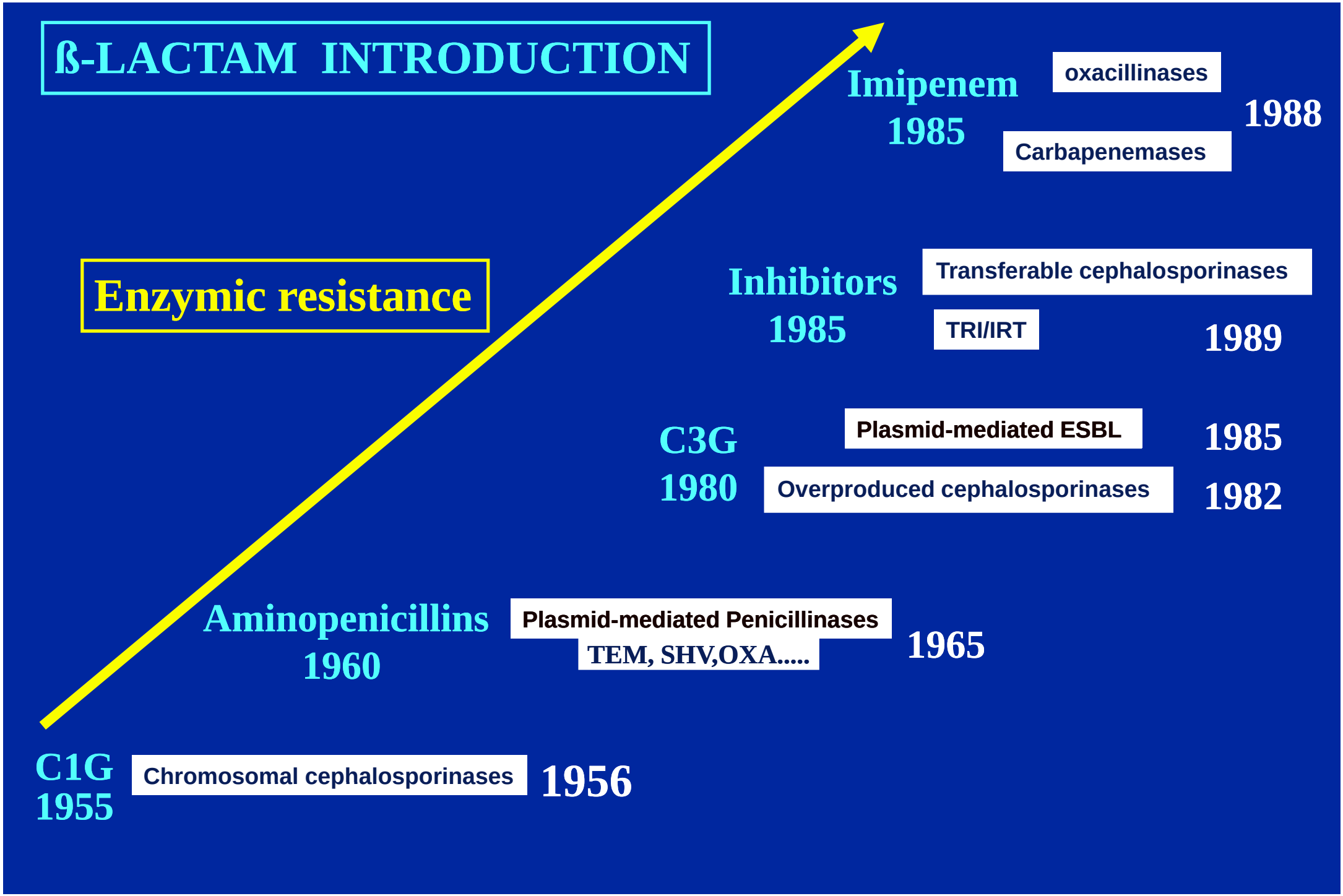
- clavulanic acid CA
- tazobactam PTZ

Cephalosporins

- C1G
- C2G
- C3G
- C4G

Carbapenems IMP

3/ But no new drugs since 1990


ß-LACTAM INTRODUCTION
Enzymic resistance
C1G
1955
Chromosomal cephalosporinases
1956
Aminopenicillins
1960
Plasmid-mediated Penicillinases
TEM, SHV,OXA.....
1965
C3G
1980
Plasmid-mediated ESBL
1985
Overproduced cephalosporinases
1982
Inhibitors
1985
Transferable cephalosporinases
TRI/IRT
1989
Imipenem
1985
oxacillinases
Carbapenemases
1988

Plasmid-mediated Penicillinases (1965)

TEM : wordlwide distributed,
> 40% (*E. coli, H. influenzae, N. gonorrhoeae...*)
SHV : low prevalence
OXA : low prevalence

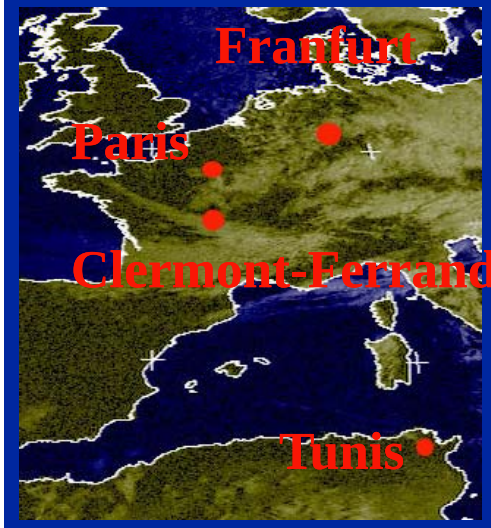
Unknown origin of gene ?

Broad-spectrum enzymes

	Penicillins	
High	AMX	+
High	TIC	+
High	PIP	+
	Cephalosporins	
Low	C1G	+
Low	C2G	±

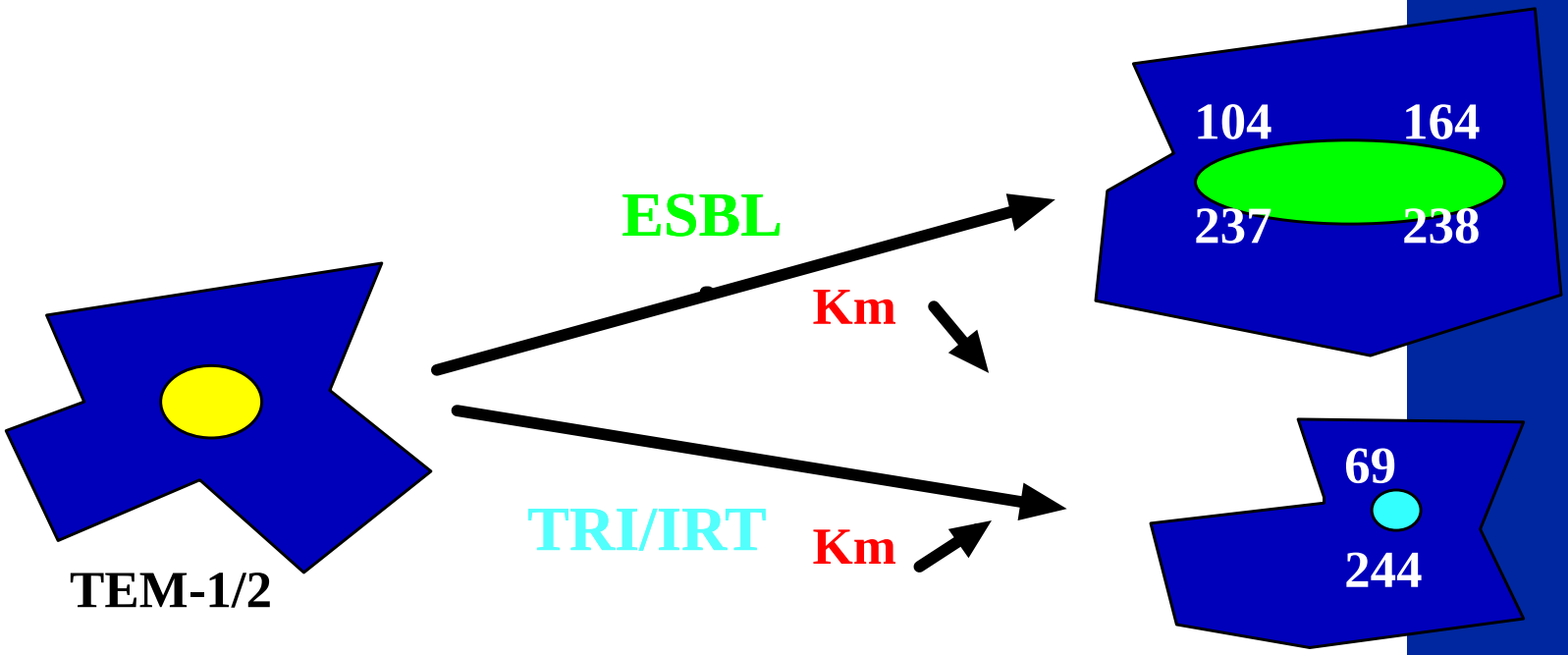
+ hydrolysis

NEW THERAPEUTIC ALTERNATIVES 1980-1985



1983-1985

1/ Introduction of C3G ⟶ ESBL



2/ Introduction of inhibitors ⟶ TRI/IRT

ESBL: wider spectrum of inactivation

E. coli

Before

TEM-2

After

TEM-3

Penicillins

AMX	+
TIC	+
PIP	+

Cephalosporins

C1G	+
C2G	+
FOX	-
C3G	+
C4G	+

Inhibitors	-
Carbapenems	-

+ hydrolysis

ESBL SINCE 1990

MANY OTHER BLSE

TEM -150
SHV-88
OXA-87

widely distributed

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

NOVEL TRANSFERABLE-ENCODED ESBL

SFO-1 **S**erratia **FO**nticola
1988
TLA-1 **TLA**huicas 1991
PER-1 **P**seudomonas **E**xtended-spectrum **R**esistance
1991 **VEB-1** **V**ietnam **E**xtended-spectrum **B**eta-
lactamases 1996
BES-1 **B**razilian **E**xtended-**S**pectrum ß-lactamases
1996
GES-1 **G**uyana **E**xtended-**S**pectrum ß-lactamases
1998
IBC-1 **I**ntegron-**B**orne **C**ephalosporinase 9

CTX-M-1 **C**ef
1989




CTX-M = Cefotaximase

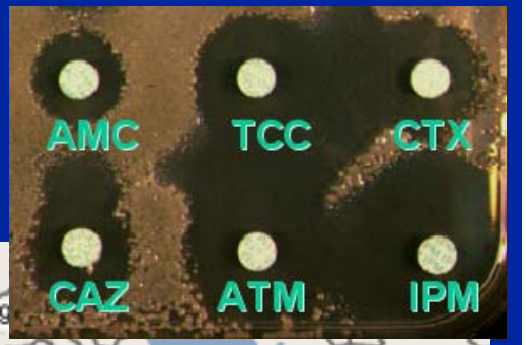
CTX-M-53

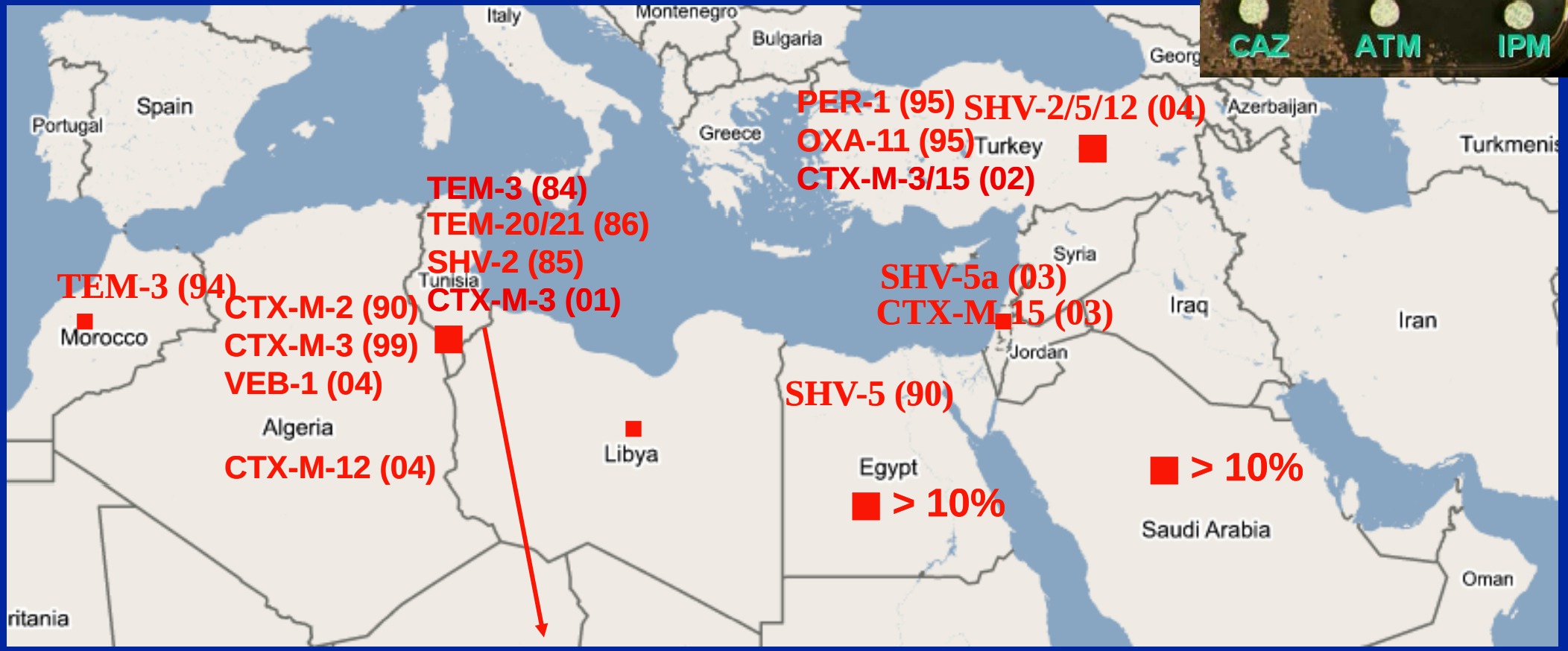
Bonnet R. AAC, 2004

Enzyme	pI	host	Country of isolation (origin of patient) (year)
FEC-1		E.coli	Japon (88)
MEN-1	8,4	E.coli	France (Italie) (89)
CTX-M-1	8,4	E.coli	Allemagne (89)
CTX-M-2	7,9	S.typhimurium V.cholerae	Argentina (92)
		Entérobactéries	Argentina (92) Israel (92), Brazil (97), France (97)
Toho-1	7,8	E.coli	Japan (93)
Toho-2		E.coli	Japan (1995)
CTX-M-3	8,4	E.coli C. freundii	Pologne (96)
		Entérobactéries	Taiwan (98) France (98) China (99)
CTX-M-4	8,4	S.typhimurium	Russia (96) Hungary (98) Greece (98)
CTX-M-5	8,8	S.typhimurium	Lituania (96)
		A.baumannii	China
CTX-M-6	8,4	S.typhimurium	Greece (97)
CTX-M-7	8,4	S.typhimurium	Greece (96)
CTX-M-8	7,6	C.amalonaticus E. cloacae	Brazil (98)
CTX-M-9	8,0	E.coli	Spain (96)
		Enterobactéries	France (94) Brazil (96) China (97)
CTX-M-10	8,1	E.coli	Spain (97)
CTX-M-11		K.pneumoniae	Japan (00)
CTX-M-12	9,0	K.pneumoniae	Kenya (00)
CTX-M-13	8,1	K.pneumoniae	China (97)
CTX-M-14	8,0	E.coli	China (97)
		Entérobactéries	Korea (96) Taiwan (98) France (00), Spain (00)
CTX-M-15	8,9	E.coli K.pneumoniae	
		E.cloacae	India (00) Pologne (98)
CTX-M-16		E.coli	Brazil (96)
CTX-M-17		K.pneumoniae	Vietnam (96)
CTX-M-18		E.coli K.pneumoniae	France (Vietnam) (99)
CTX-M-19		K.pneumoniae	France (Vietnam) (99)
CTX-M-20		P.mirabilis	France (98)
CTX-M-21		E.coli	France (00)
CTX-M-26		K.pneumoniae	UK (01)
CTX-M-27		E.coli	France (00)

TYPES OF BLSE

■ Synergy test +





PER-1 (95)
OXA-11 (95)
CTX-M-3/15 (02)
SHV-2/5/12 (04) ■

TEM-3 (84)
TEM-20/21 (86)
SHV-2 (85)
CTX-M-3 (01) ■

TEM-3 (94) ■

CTX-M-2 (90)
CTX-M-3 (99) ■
VEB-1 (04)

CTX-M-12 (04)

SHV-5a (03)
CTX-M-15 (03) ■

SHV-5 (90)

■ > 10%

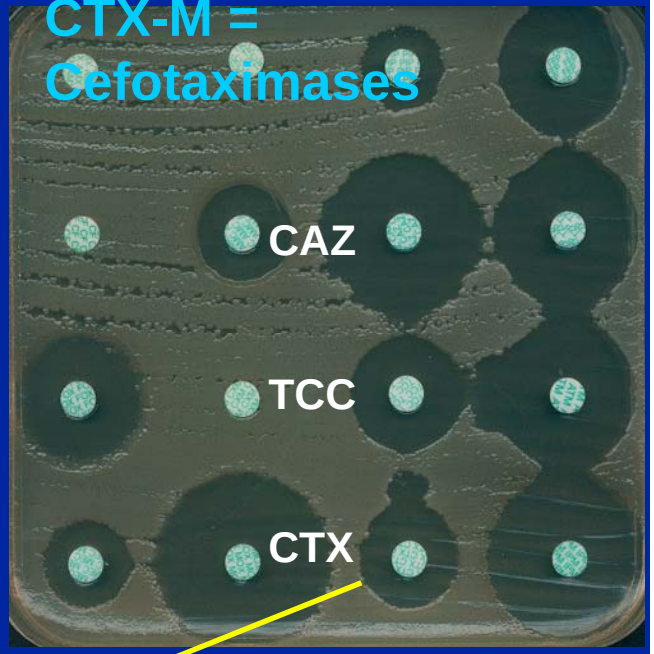
■ > 10%

CTX-M-15
CTX-M-16
CTX-M-27
TEM-4 (04)

Hammani A. personal communication

SUSCEPTIBILITY PATTERNS (MICs, mg/L)

ceftazidimase



Bla	Species	TIC	TCC	PIP	TAZ	CTX	CAZ	FEP	ATM	IMP
CTX-M-13	E. coli (Tc)	>512	32	128	2	64	0,5	16	16	0,25
CTX-M-15	E. coli	>512	32	128	8	>512	128	32	128	0,12
	K.pneumoniae		>512	256	>512	8	256	128	32	512
	0,12									
CTX-M-16	E. coli (Tc)	>512	16	256	2	16	8	2	8	0,12
CTX-M-19	E. coli (Tc)	>512	32	128	2	1	64	0,5	4	0,12

single mutation

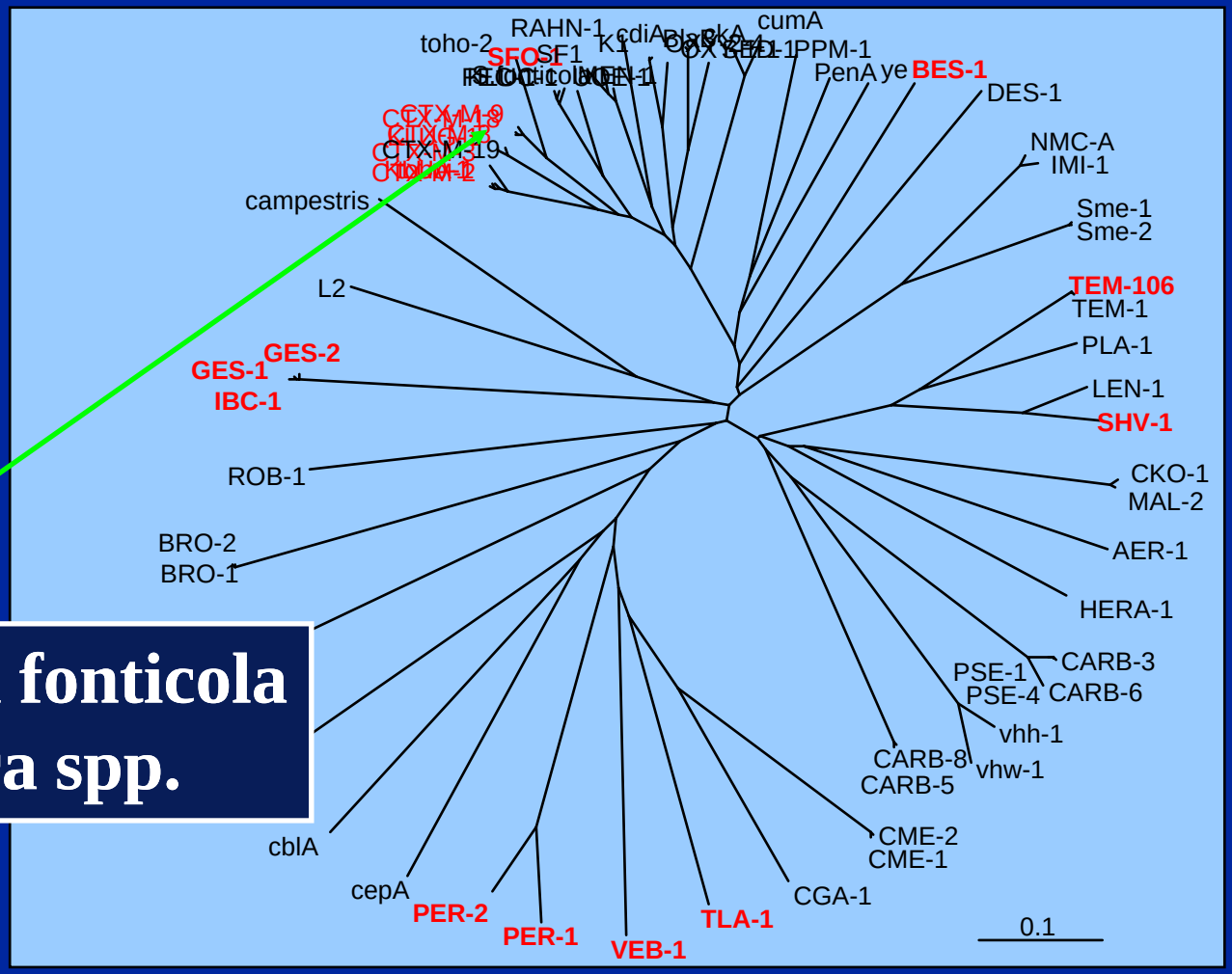
NEW ESBL : Progenitors ??????

Orphans

PER-1
VEB-1
BES-1
TLA-1
GES-1

Except
SFO-1
CTX-M

Serratia fonticola
Kluyvera spp.



Dendogramm (alignment of aminoacid sequences)

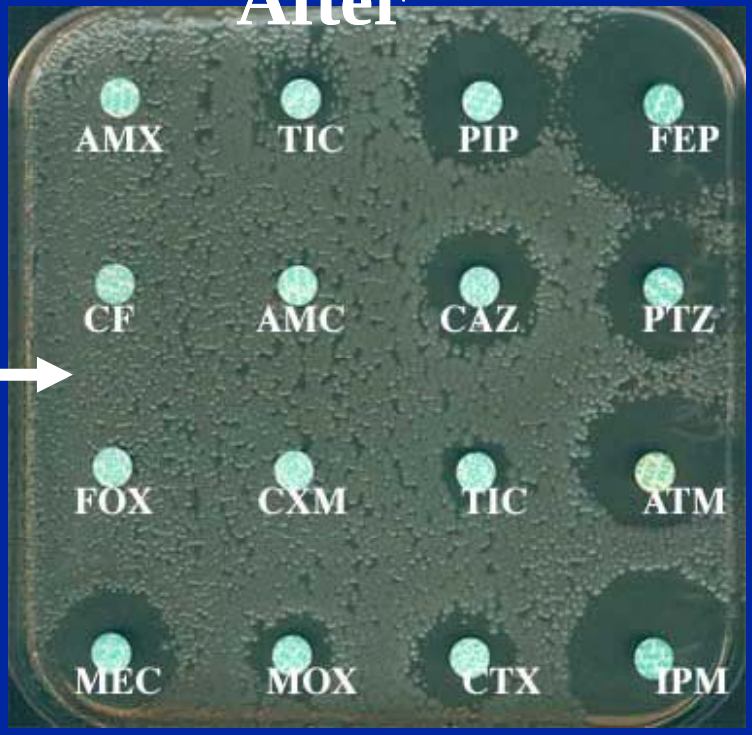
Matsumoto S. AAC 1999
Humeniuk C. AAC 2002

OTHER EVOLUTION: C3G + FOX + INHIBITORS

1/ Cephalosporinase overproduced

Before

After



Enterobacter, Serratia, P. aeruginosa……

Single mutation (AmpD gene)

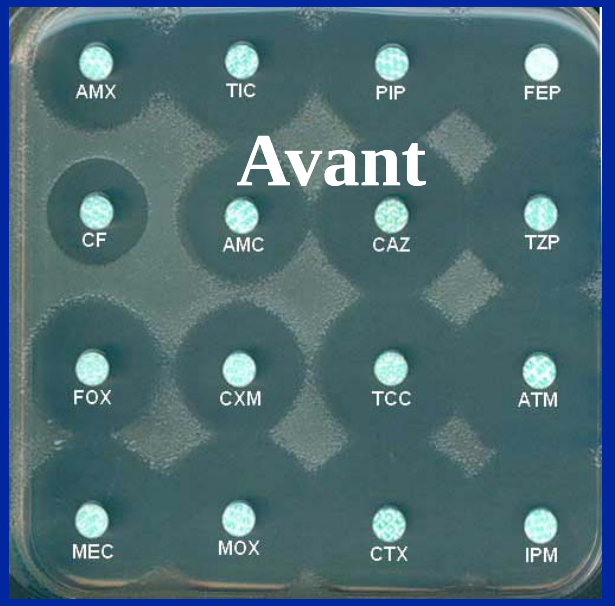
Penicillins	
AMX	+
TIC	+
PIP	+
Inhibitors	+
Cephalosporins	
C1G	+
C2G	+
FOX	+
C3G	+
C4G	-
Carbapenems	-

+ hydrolysis

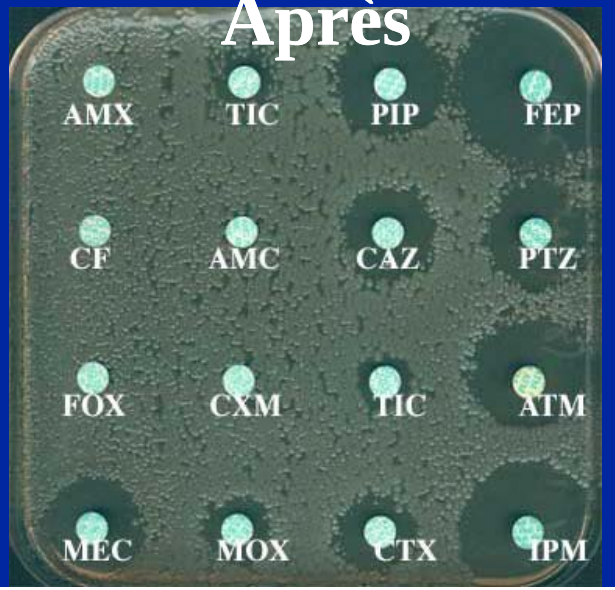
2/ Cephalosporinase mobilized = plasmid-encoded AmpC

Morganella morganii

Gene transfer



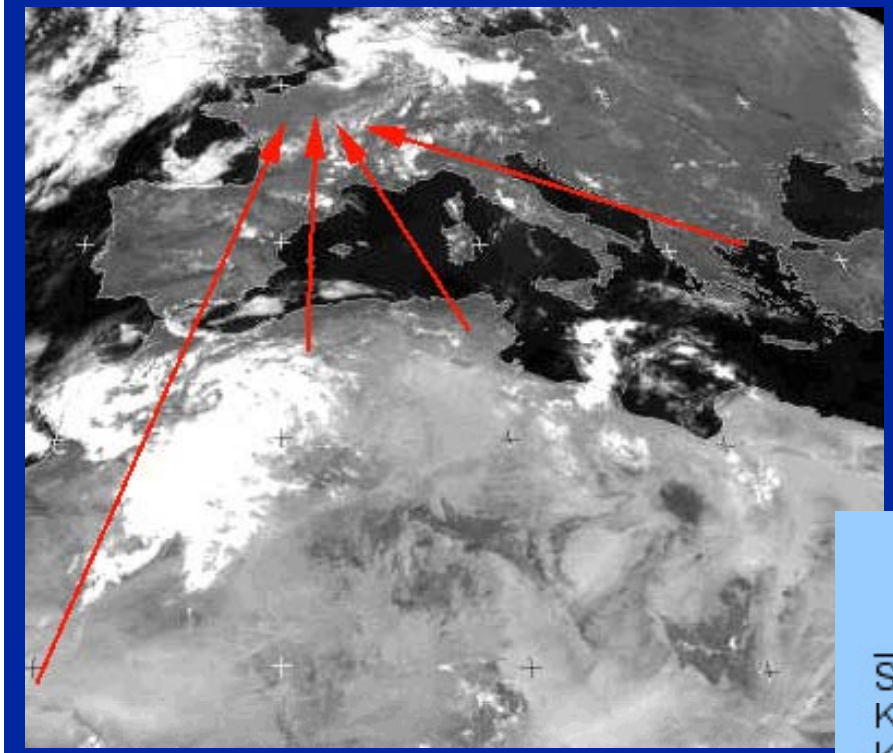
Salmonella enterica



PLASMID-ENCODED CEPHALOSPORINASES

Type (Year)	Denomination	Species	Country
MIR-1	Miriam hosp.	K. pneumoniae	USA (1988)
ACT-1	AmpC Type	K. pneumoniae, E. coli	USA (1994)
CMY-1 (1989)	Cephamycin	K. pneumoniae	Korea
LAT-1	Latamoxef	K. pneumoniae	Greece (1993)
DHA-1 (1992)	Dharan hosp.	S. enteritidis	Saudi Arabia
FOX-1	Cefoxitin	K. pneumoniae	[illegible]
MOX-1 (1991)	Moxalactam	K. pneumonia	
ACC-1 (1997)	Ambler Class C	K. pneumonia	

risk factors = ESBL



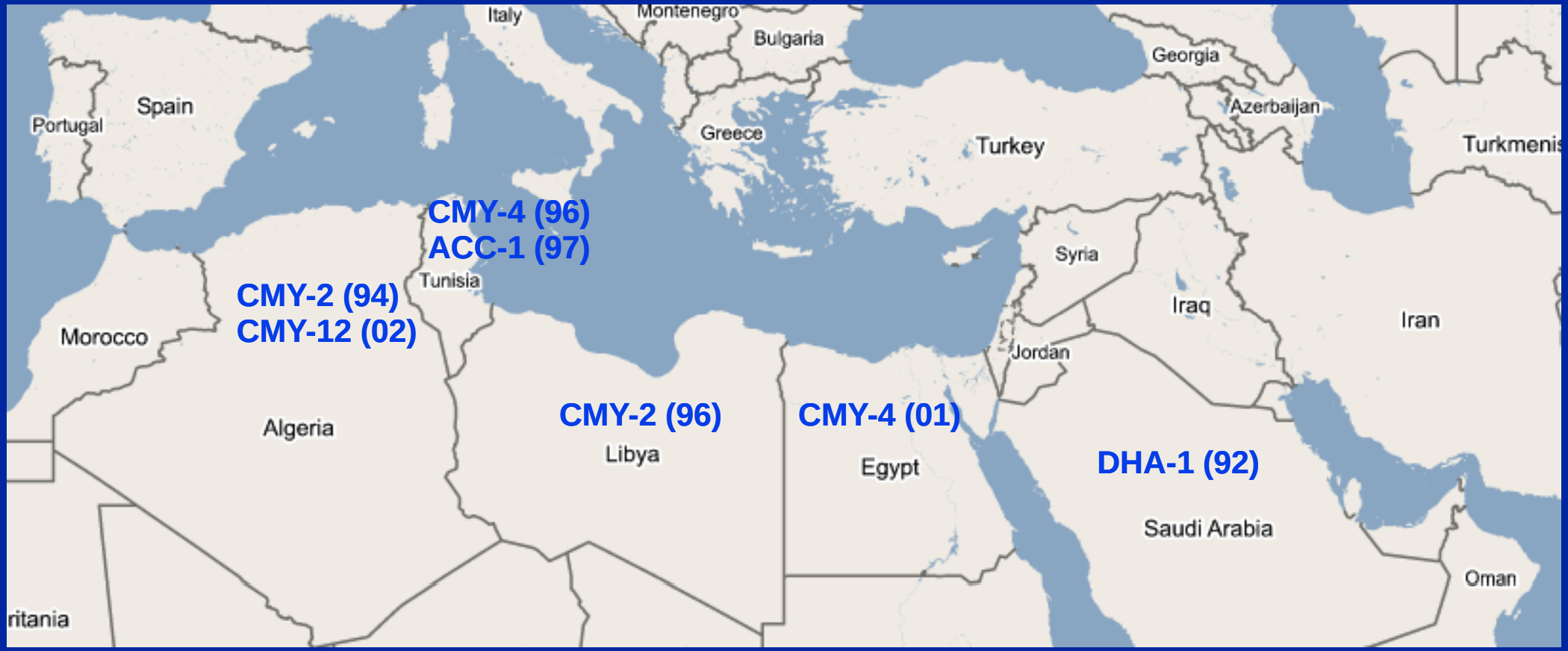
Imported Cases in Paris (199-2001)

Origine des AmpC plasmidiques isolées à Paris depuis 1990

S. senftenberg (selles)	CMY-2b	Algérie
K. pneumoniae (ECBU)	MOX-2	Grèce
K. pneumoniae, P. mirabilis, E. coli (divers)	ACC-1	Tunisie (2 hôpitaux)
K. pneumoniae (urines)	CMY-2	Paris, France
K. pneumoniae (urines)	CMY-4	Le Caire, Egypte
P. mirabilis (Bile)	CMY-4	Athènes, Grèce
P. mirabilis (selles)	CMY-4	Athènes, Grèce
P. mirabilis (urines, cathéter)	CMY-X	Constantine, Algérie
S.agona (selles)	CMY-2	Abidjan, Côte d'Ivoire

Decré D., Verdet C., Raskine L., Blanchard H., Burghoffer B., Philippon A., Sanson-Le-Pors M.J., Petit J.C., Arlet G. Characterization of CMY-type ß-lactamases in clinical strains of *Proteus mirabilis* and *Klebsiella pneumoniae* isolated in four hospitals in the Paris area, J. Antimicrob. Chemother. 50 (2002) 681-688.

Plasmid-encoded cephalosporinases



Detection ????????

ORIGIN OF GENES ?

group CMY-2, CFE-1 (*C.freundii*)

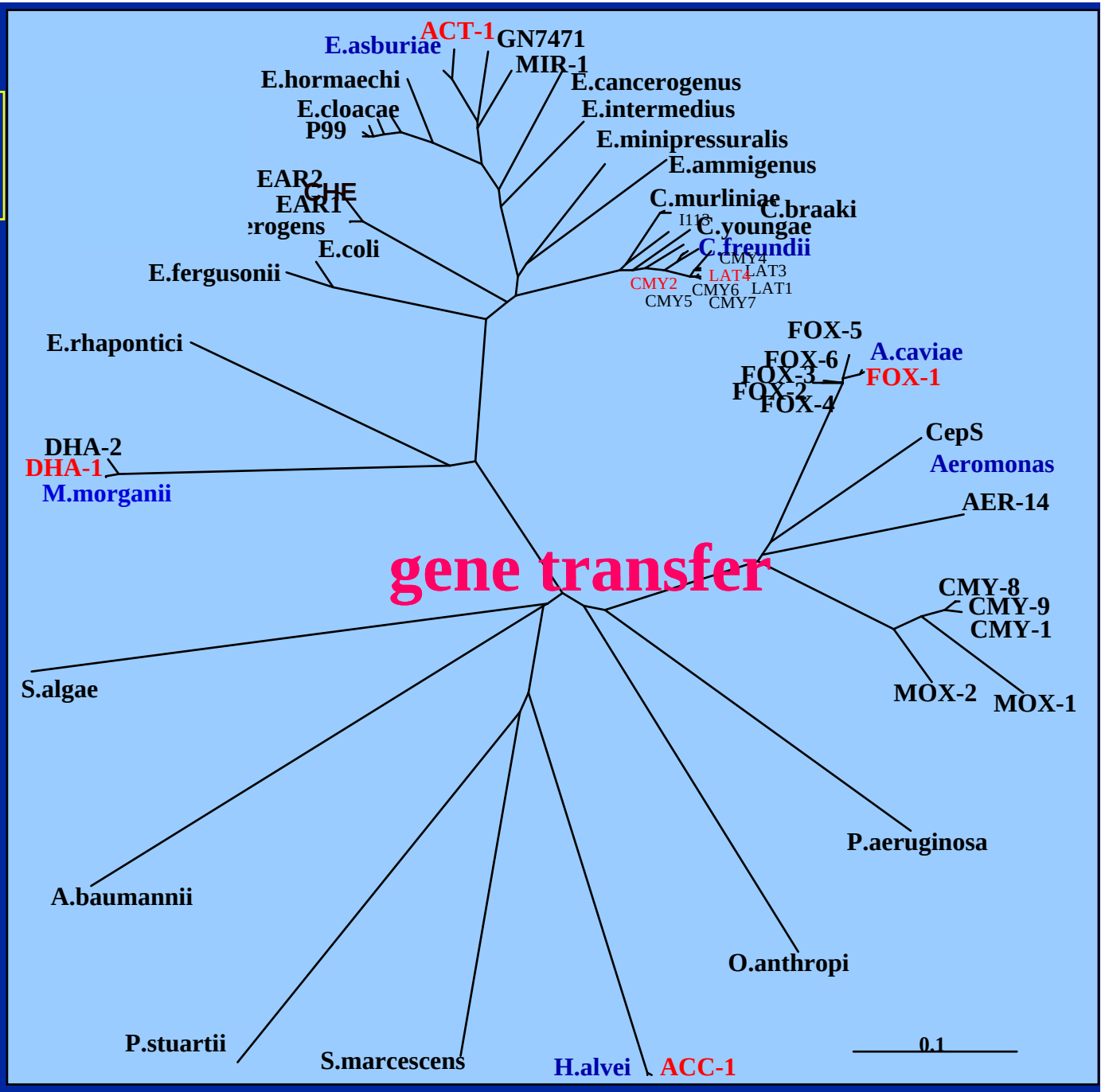
DHA-1 (*M. morganii*)

group FOX-1 (*A. caviae*)

ACT-1 (*E. asburiae*)

ACC-1 (*H. alvei*)

Environmental bacteria ?

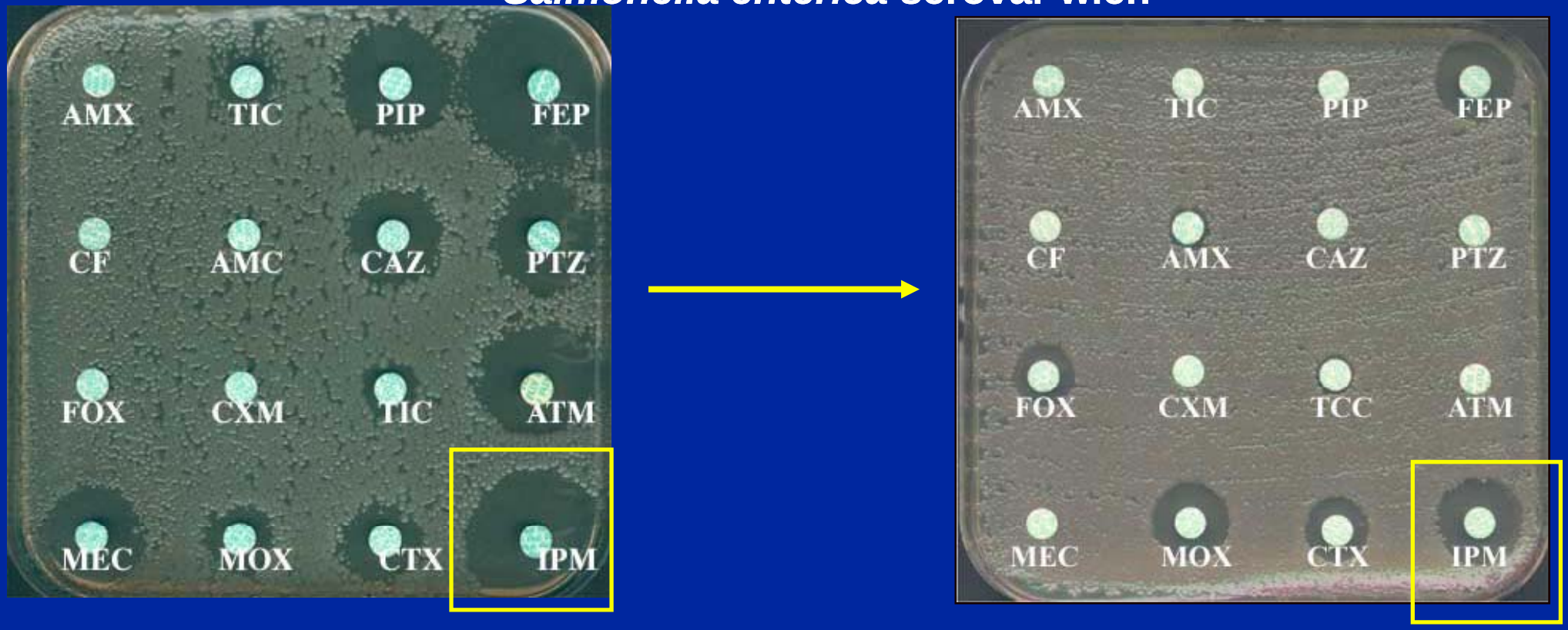


Comparison of amino acid sequences

The calm before the storm !

RESISTANCE TO CARBAPENEMS

***Salmonella enterica* serovar wien**



Armand-Lefèvre L., Leflon-Guibout V., Bredin J., Barguellil F., Amor A., Pagès J.M., Nicolas-Chanoine M.H. Imipenem resistance in *Salmonella enterica* serovar wien related to porin loss and CMY-4 ß-Lactamase production, Antimicrob. Agents Chemother. 47 (2003) 1165-1168.

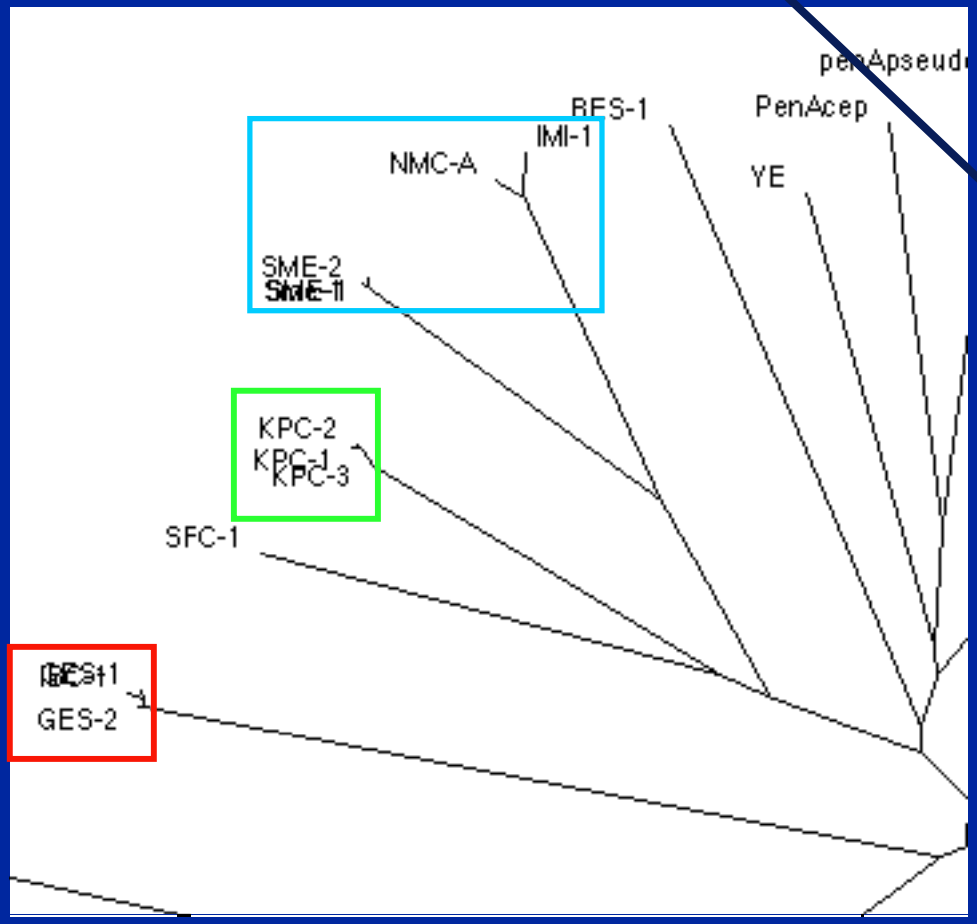
RESISTANCE TO CARBAPENEM

Class A Carbapenemases

NMC-A	E. cloacae	France (1990)
SME-1	S. marcescens	UK (1982)
SME-2	S. marcescens	USA (1994-1999)
IMI-1	E. cloacae	USA (1984)

R = IMP, ATM but C3G S

Some clinical isolates
but emergence .....



GES = Guyana Extended-Spectrum ß-lactamases

Poirel et al. AAC 2000

KPC = Klebsiella Pneumoniae Carbapenemase

Ygit et al. AAC 2001,2003; Miriagou et al 2003

Origin of genes ???

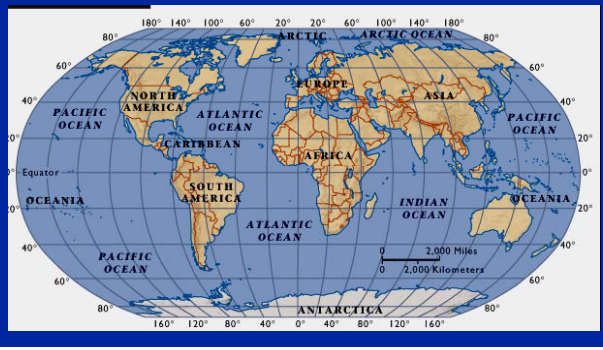
OTHER CARBAPENEMASES (class B)

Enzyme	Species	Country (isolation)
IMP-1	S. marcescens	Japan (>91)
	P. aeruginosa	Japan
	A. xylosoxydans	Japan
	P. putida	Japan
	C. freundii	Japan
	K. pneumoniae	Japon, Singapore(99)
	A. baumannii	Japan
	P. stutzeri, P. putida	Taiwan
	A. junii	UK (00)
IMP-2	A. baumannii	Italy (97)
IMP-3	S. flexneri	Japan (96)
IMP-4	Acinetobacter	Hong-Kong (>94)
	C. youngae	China (98)
IMP-5	A. baumannii	Portugal (98)
IMP-6	S. marcescens	Japan (96)
IMP-7	P. aeruginosa	Canada (95) Malaisia (99)
IMP-8	K. pneumoniae	Taiwan (98)
IMP-9	P. aeruginosa	China
IMP-10	A. xylosoxydans	Japan (00)
	P. aeruginosa	Japan (97)

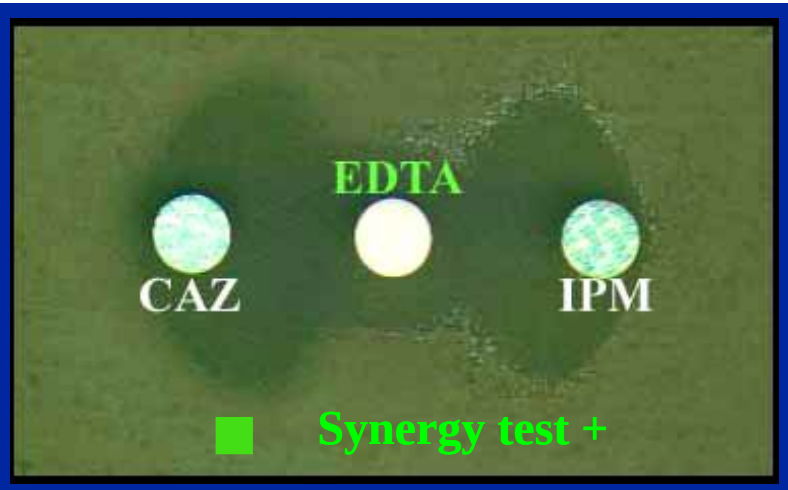
IMP-22 , VIM-12 April 2006

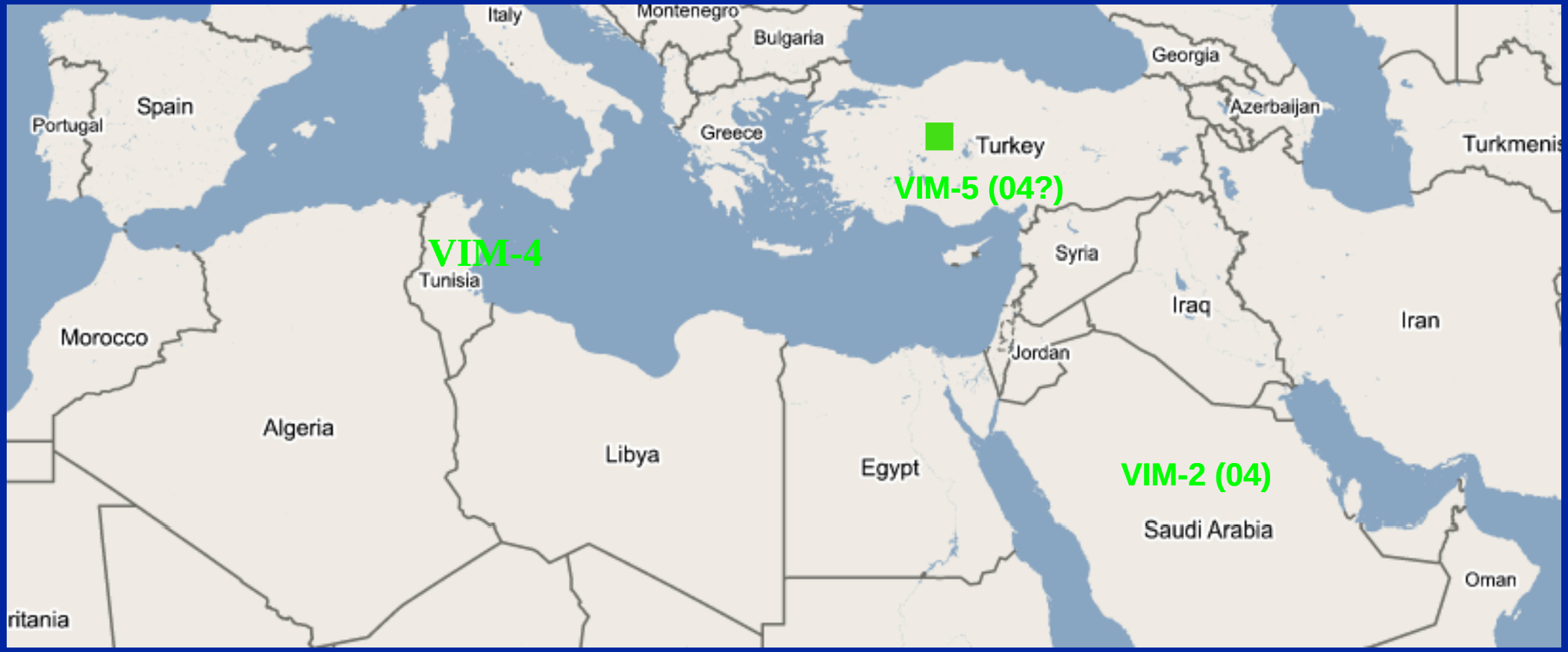
Enzyme	Species	Country (isolation)
VIM-1	P. aeruginosa	Italy (1997)
	A. baumannii	Italy (1997)
	P. aeruginosa	Greece (1996)
	E. coli	Greece (2001)
	A. xylosoxydans	Italy (1997)
VIM-2	P. aeruginosa	France (1996)
	P. aeruginosa	Greece (1996)
	P. aeruginosa	Italy (1998)
	S. marcescens	Korea (2000)
	A. baumannii	Korea (1998)
	P.putida stutzeri	Taiwan (>1997)
VIM-3	P. aeruginosa	Taiwan (>1997)
VIM-4	P. aeruginosa	Greece (2001)

SPM-1	P. aeruginosa	Brazil (1997)
GIM-1	P. aeruginosa	Germany (2003)

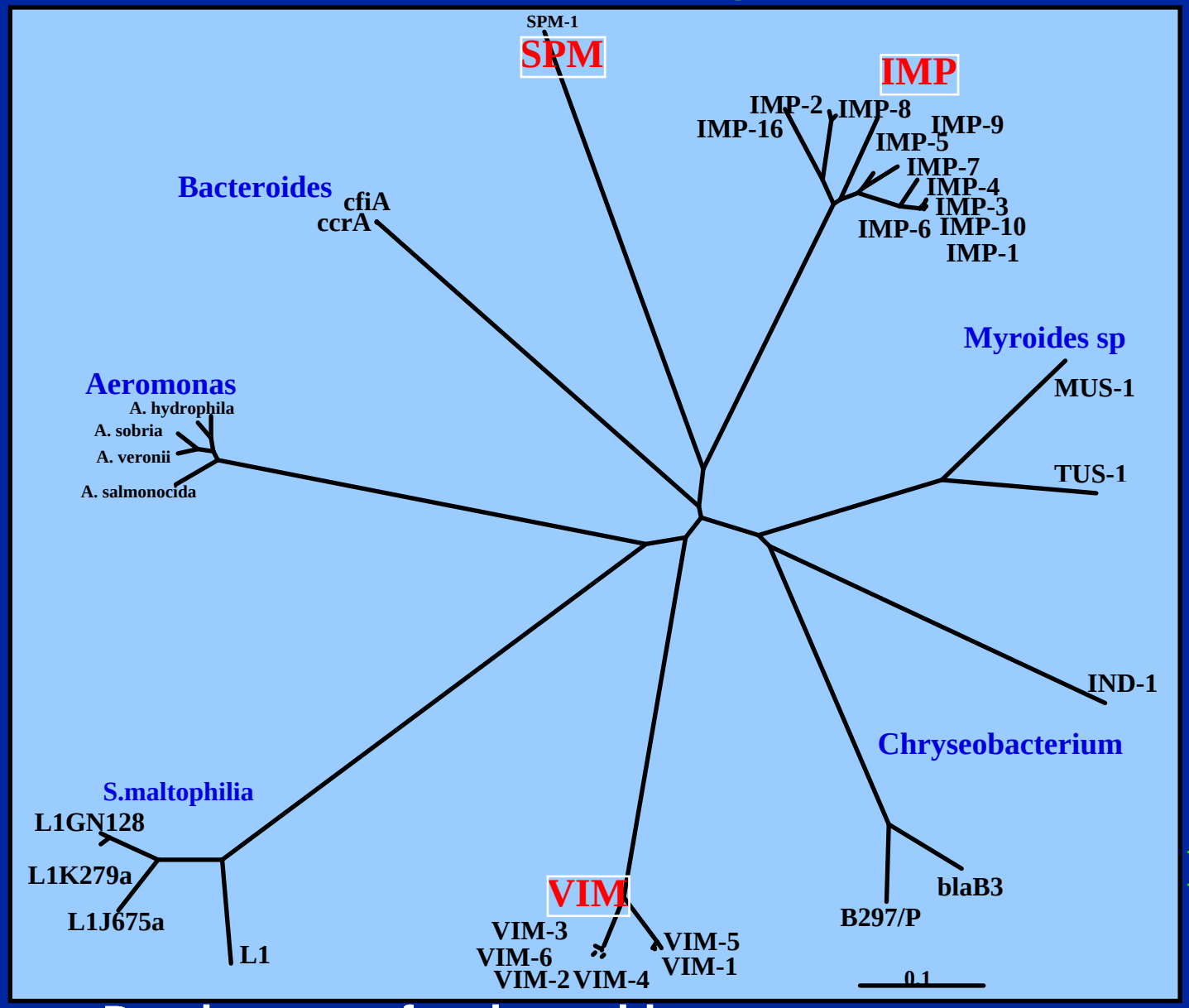


Carbapenemases (metalloenzymes, class B)





Progenitors ?



Dendogram of amino acid sequences

Plasmid-encoded Bla

Chromosomal

Environmental bacteria

> 350 ß-lactamases

OXACILLINASES class D

Enzyme (Year)	Species	Country
OXA-23 (ARI-1)	A. baumannii	United Kingdom (1985)
OXA-27	Acinetobacter spp.	Singapore (1995-1997)
OXA-24	A. baumannii	Spain (1997)
OXA-25	Acinetobacter spp.	Spain (1995-1997)
OXA-26	Acinetobacter spp.	Belgium (1995-1997)
OXA-40	A. baumannii	France (Portugal) (2001)
	A. baumannii	Spain (1998) .....

Expression (mg/l)

Bla	Host	TIC	TCC	PIP	TAZ	CAZ	FEP	ATM	IPM
OXA-27	A. baumannii			>128		>128	16	32	16
OXA-24	A. baumannii	>512				>256	256	>256	128
OXA-25	A. baumannii			>128		>128	>128	>128	64
OXA-26	A. baumannii			>128		>128	8	128	64
OXA-40	A. baumannii	512	512	512	512	512	64	128	256

TIC, ticarcillin, TCC, ticarcillin + ac. clavulanic acid; PIP, piperacillin; TAZ, piperacillin + tazobactam; CAZ, ceftazidime; FEP, cefepime; ATM, aztreonam, IMP, imipenem

ß-lactamases : model of integration

bla

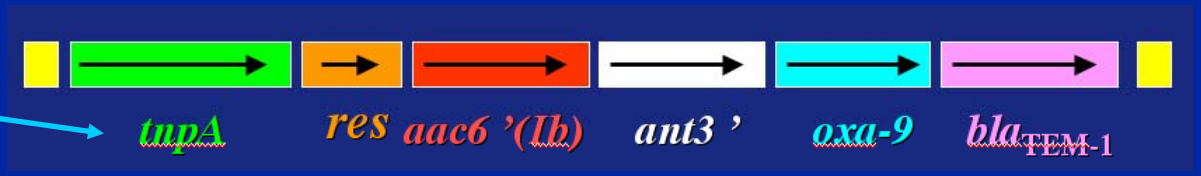
Plasmid (1965)

Transposon (1974)

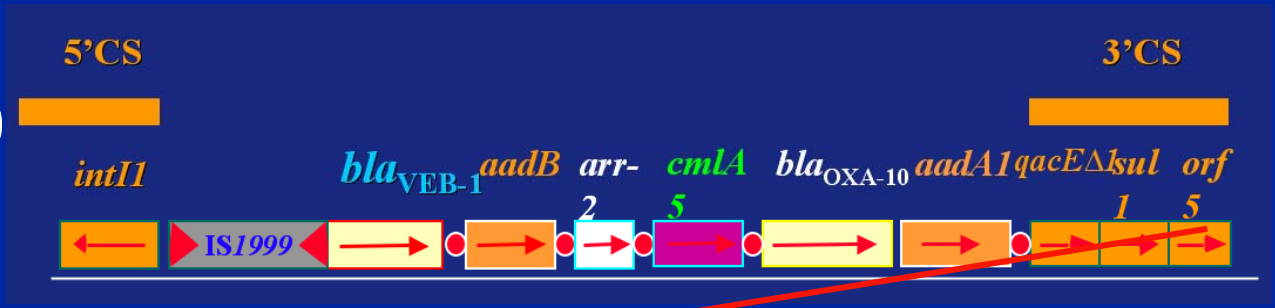
Cassette gene (1985)

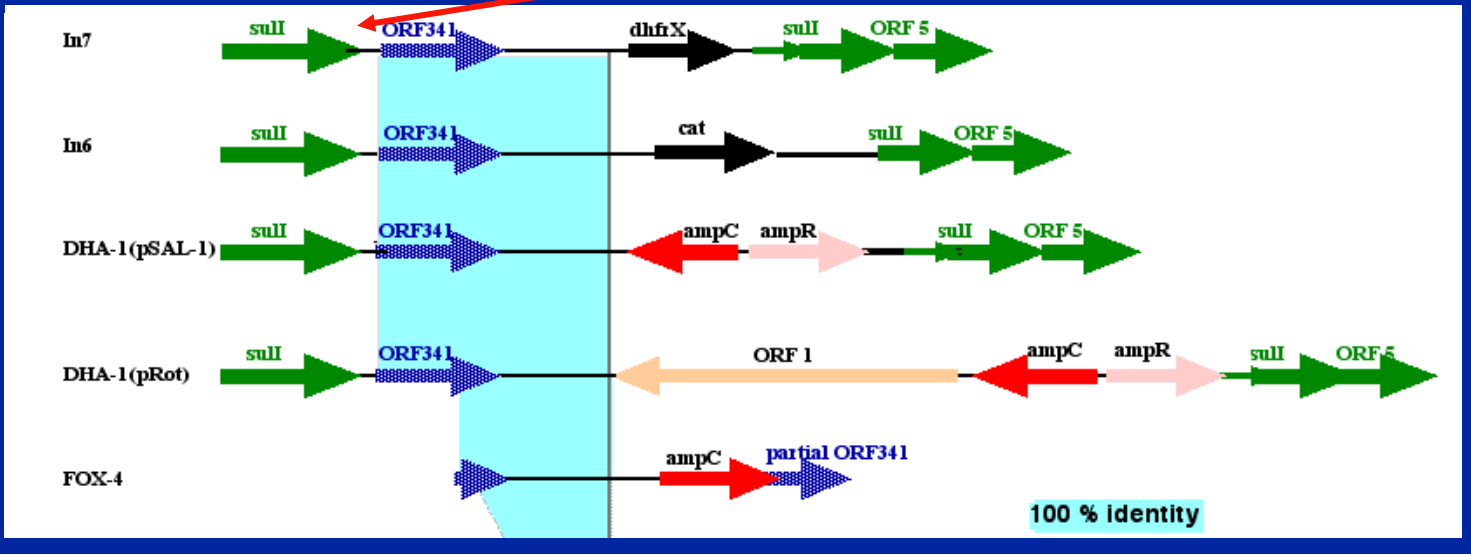
Integron (1992)

CR (recombinases) (2002)

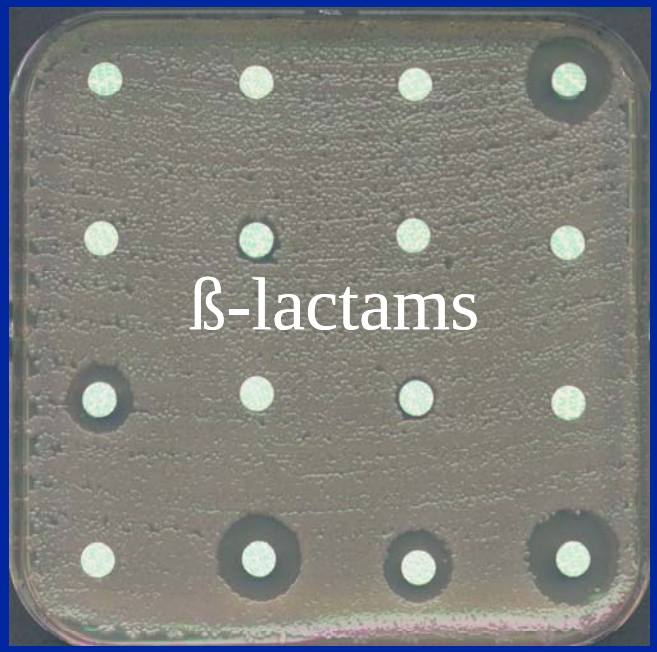


tn3





US patient at Cochin (2005)



K. pneumoniae

CONCLUSIONS

* Multiresistance increasing

* Continuous emergence of new types

* Combination of several types of Bla

e.g. VIM-4 CTX-M-15 CMY-4 TEM-1 (Hammami A.)

Strict use of antibiotics
Detection of BMR.....