UNIVERSITE PARIS-SUD XI

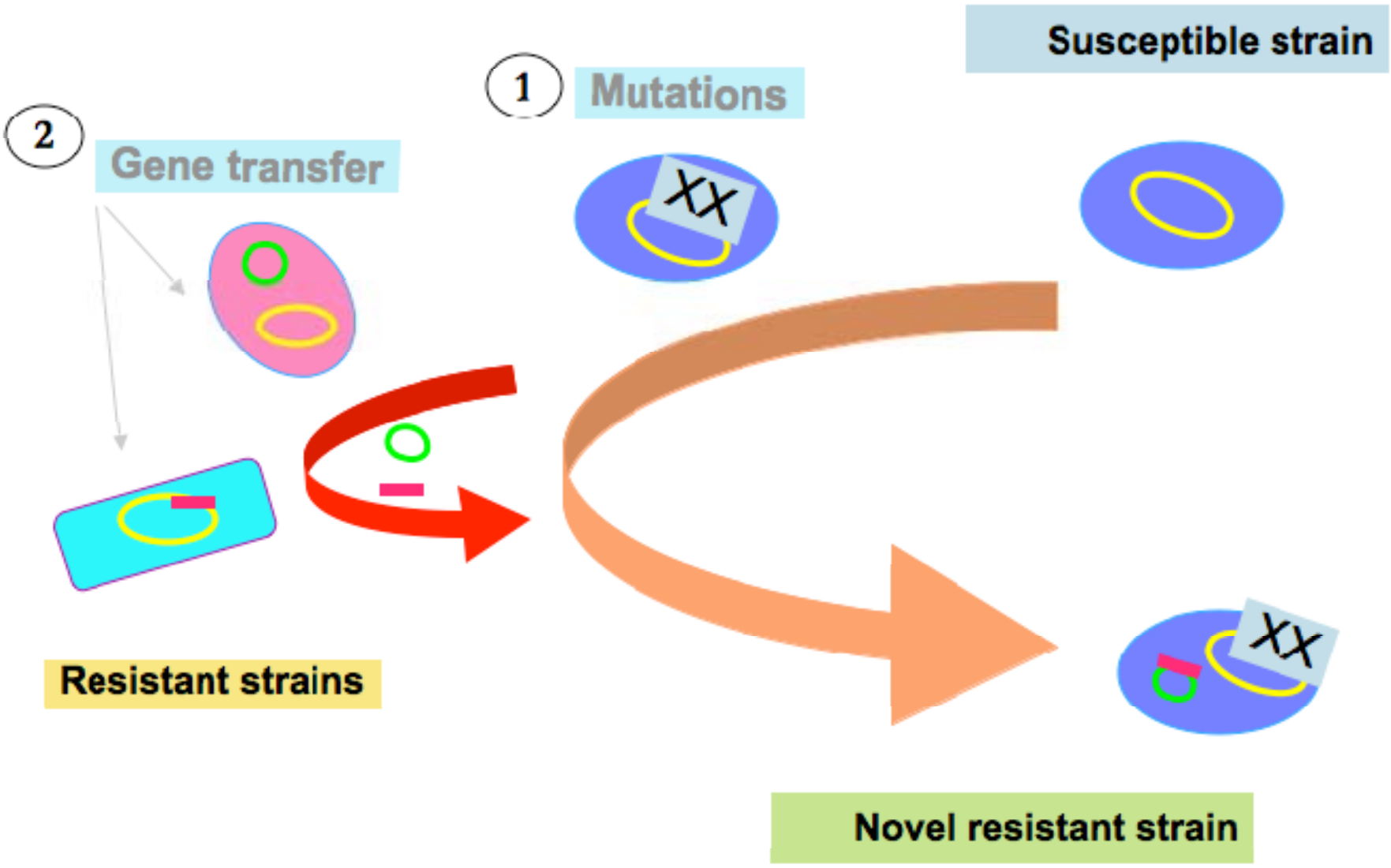
ASSISTANCE PUBLIQUE HÔPITAUX DE PARIS

Génétique; résistances croisées, résistances associées chez les bacilles à Gram négatif

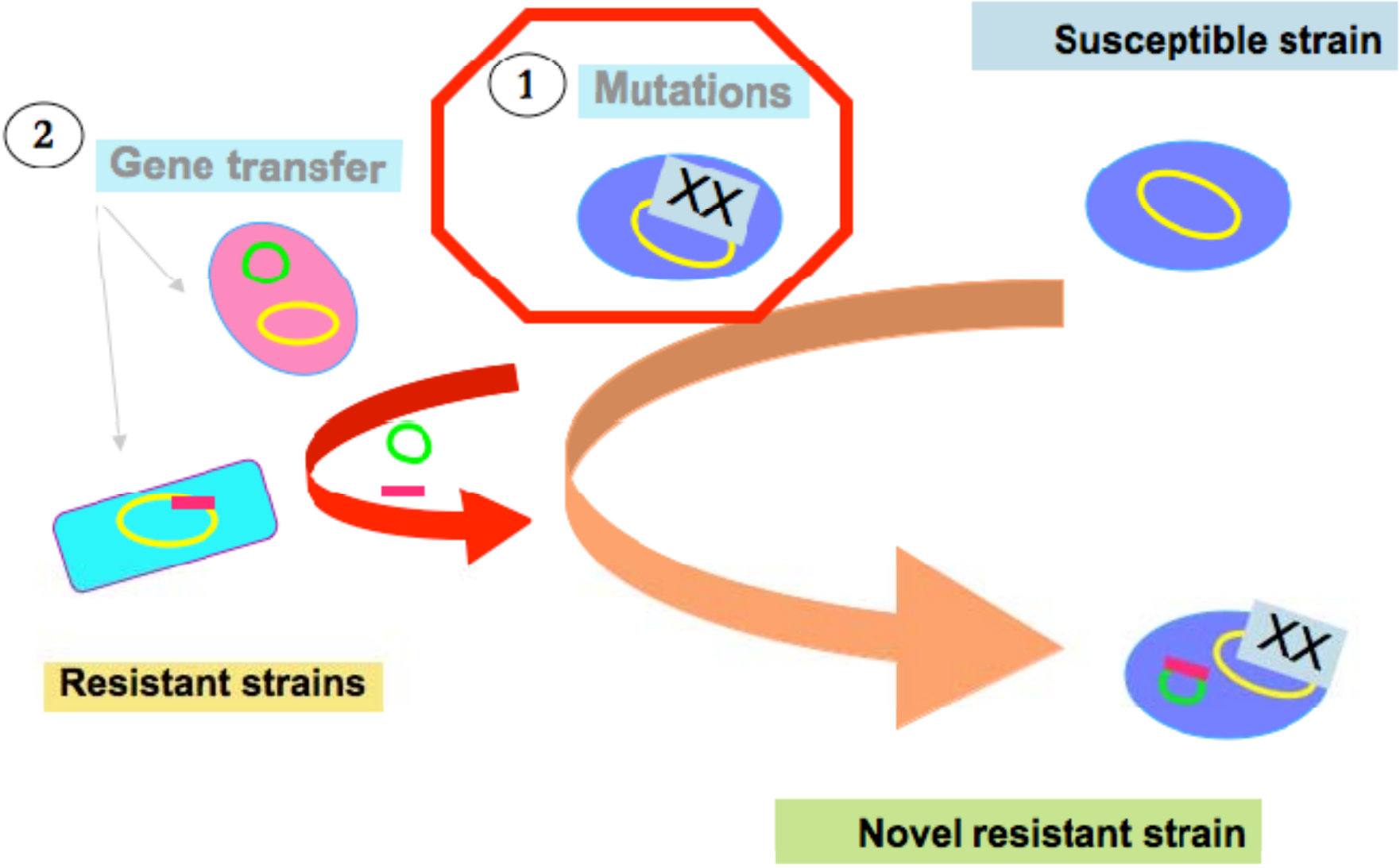
Pr. P. Nordmann

CHU de Bicêtre

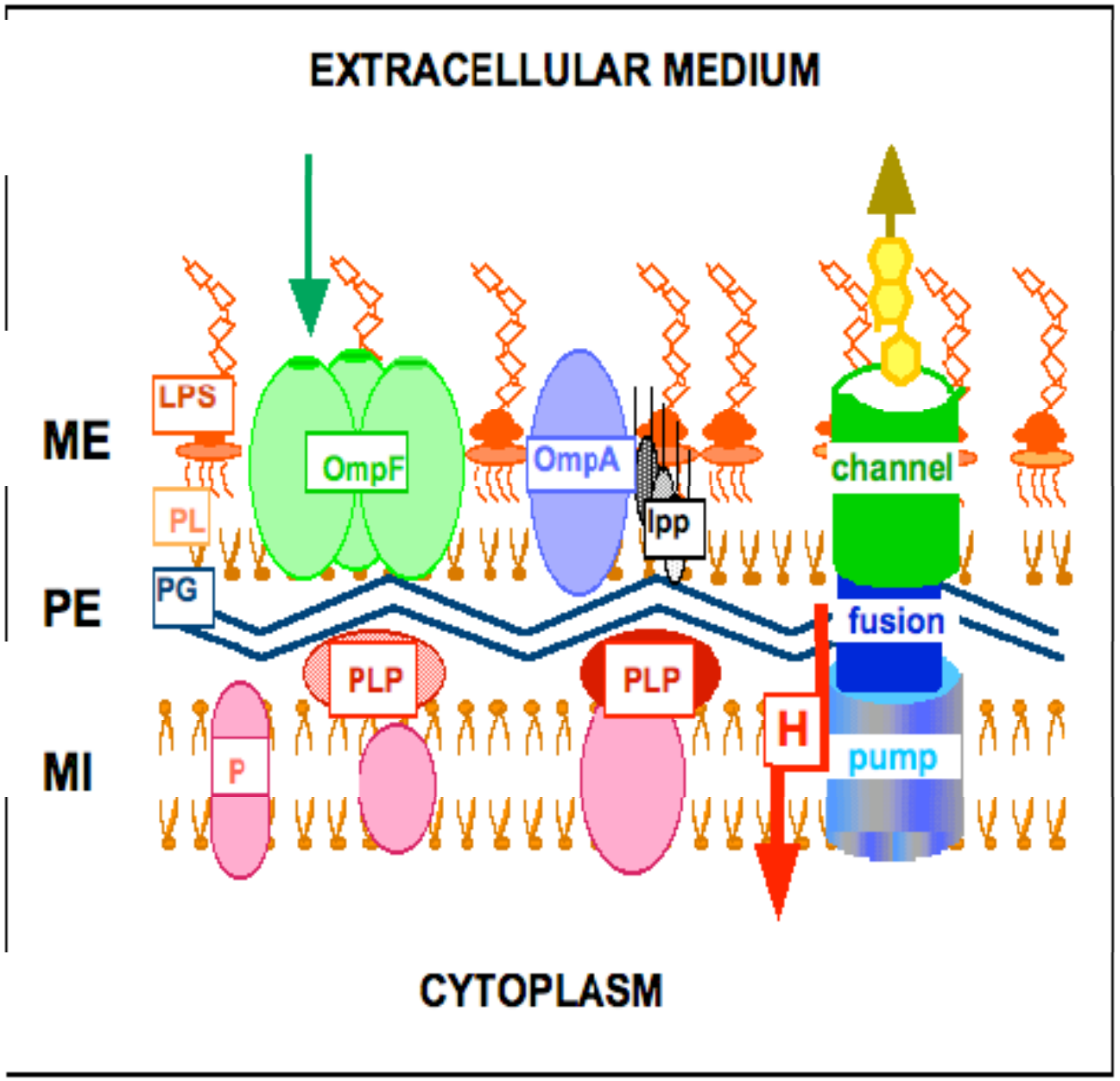
Genetics of Resistance

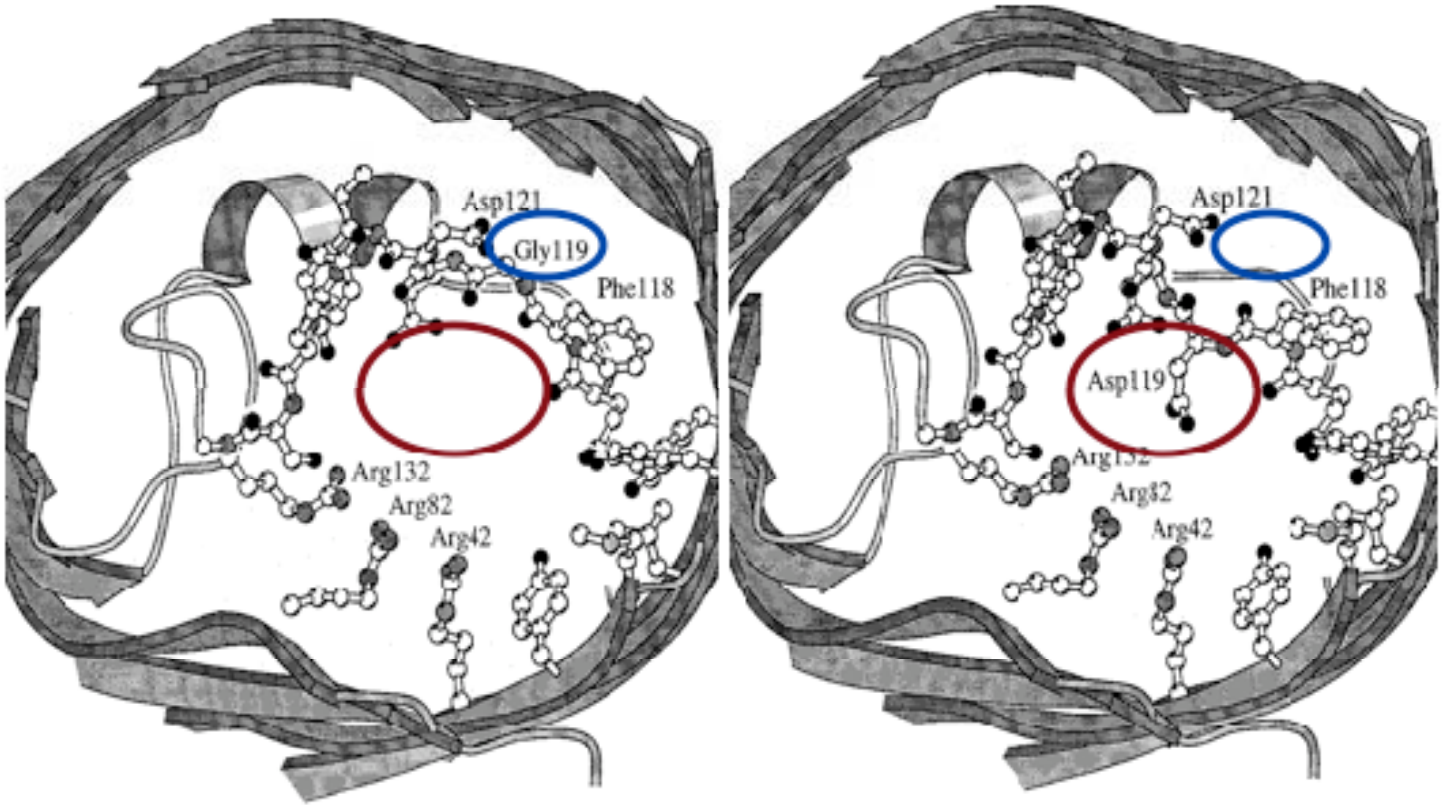


Cross Resistance



Mutations as a source of cross-resistance



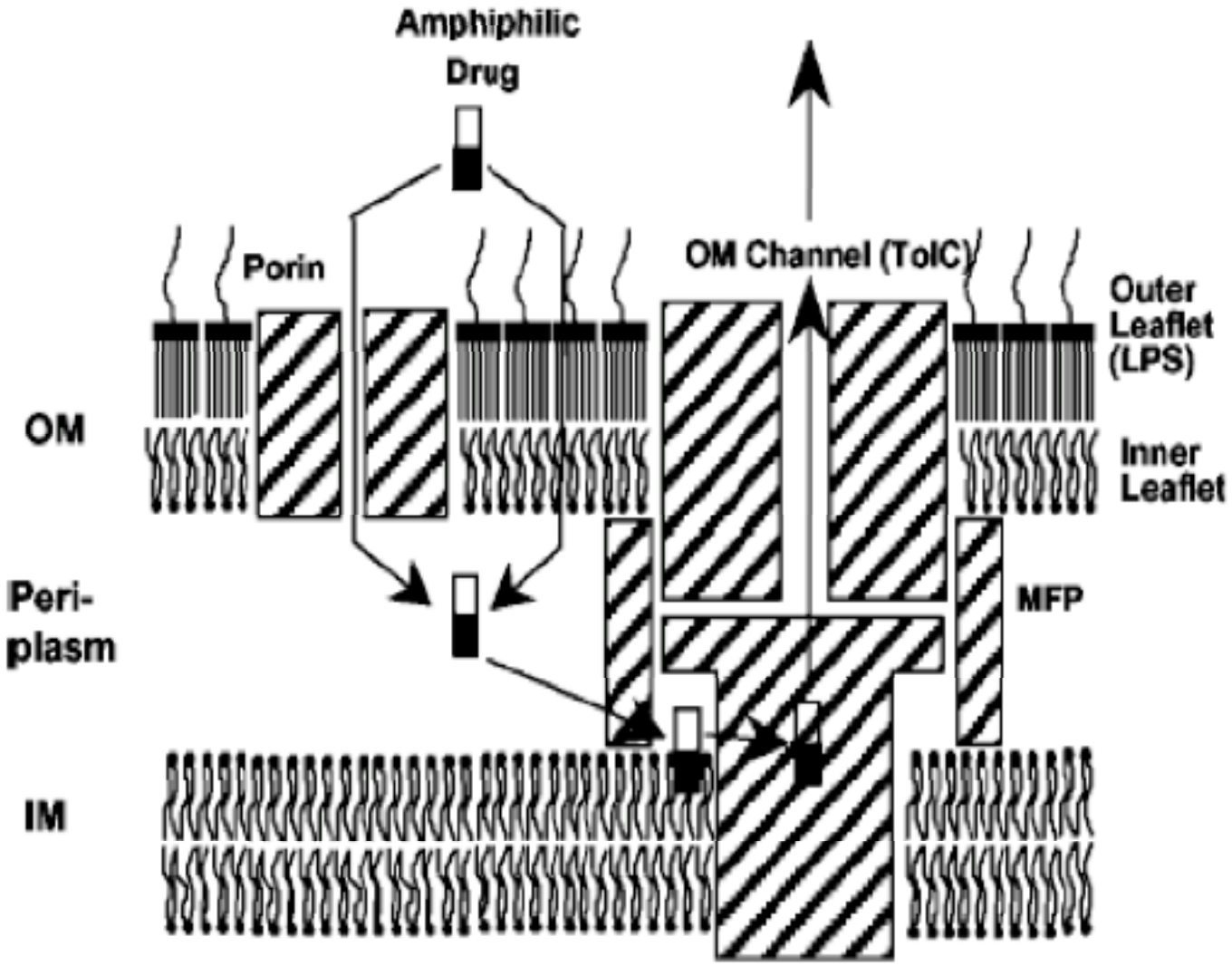


Wild-type porin **Mutated porin**

J Infect Dis. 1985 Mar;151(3):501-7.

Cross-resistance to nalidixic acid, trimethoprim, and chloramphenicol associated with alterations in outer membrane proteins of *Klebsiella*, *Enterobacter*, and *Serratia*.

Gutmann L, Williamson R, Moreau N, Kitzis MD, Collatz E, Acar JF, Goldstein FW


Amphiphilic
Drug
Porin
OM Channel (TolC)
Outer
Leaflet
(LPS)
OM
Inner
Leaflet
Peri-
plasm
MFP
IM

Efflux pump

RND efflux systems in *P. aeruginosa*

System	Operon	Antibiotics as substrates
MexAB-OprM	*mexAB-oprM*	FQ, ß-lactam, Tmp, Cmp, Tet, Nov, Ery...
MexXY (OprM)	*mexXY*	FQ, AG, Fep, Cpo, Cbo, Tet, Ery...
MexCD-OprJ	*mexCD-oprJ*	FQ, Cpo ,Fep, Tmp, Cmp, Tet, Ery...
MexEF-OprN	*mexEF-oprN*	FQ, Tmp, Cmp, Tet *
MexHI-OpmD	*mexGHI-opmD*	FQ, Tet
MexJK (OprM)	*mexJK*	Tet, Ery
MexMN (OprM)	*mexMN*	Cmp
MexPQ-OpmE	*mexPQ*	FQ, Ery
MexVW (OprM)	*mexVW*	FQ, Cmp, Tet, Ery
CzcBA-OpmN	*opmN-czcBA*	? *
TriABC-OpmH	*triABC*	?
YegMNO-OpmB	*yegMNO-opmB*	?

FQ: (fluoro)quinolones; ß-lactam (except imipenem); Tmp: trimethoprime; Cmp: chloramphenicol; Tet: tetracycline; Nov: novobiocin; Ery: erythromycin; AG: aminoglycosides; Fep: cefepime; Cpo: cefpirome; Cbo: ceftobiprole; * imipenem resistance due to porin OprD down-regulation.

Courtesy P. Plésiat

Wild-type phenotype

Courtesy P. Plésiat

MexAB-OprM mutant

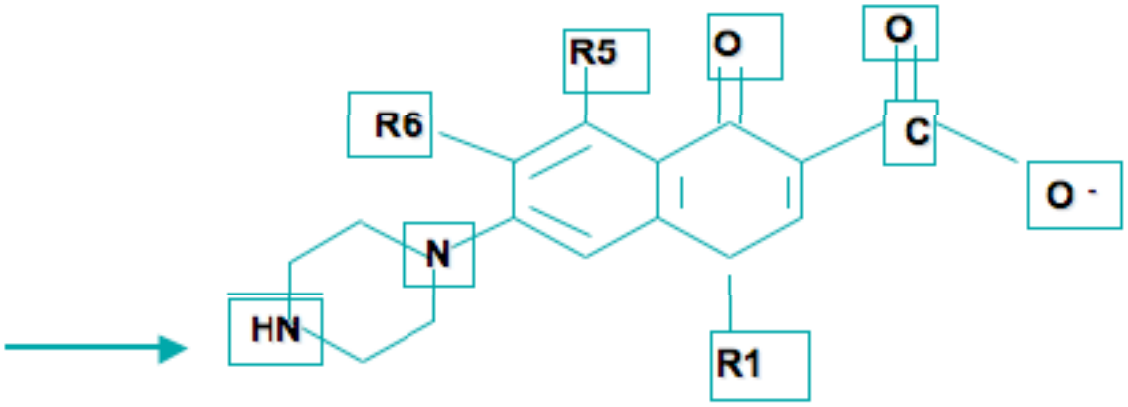
Courtesy P. Plésiat

Enzymatic inactivation of fluoroquinolones:

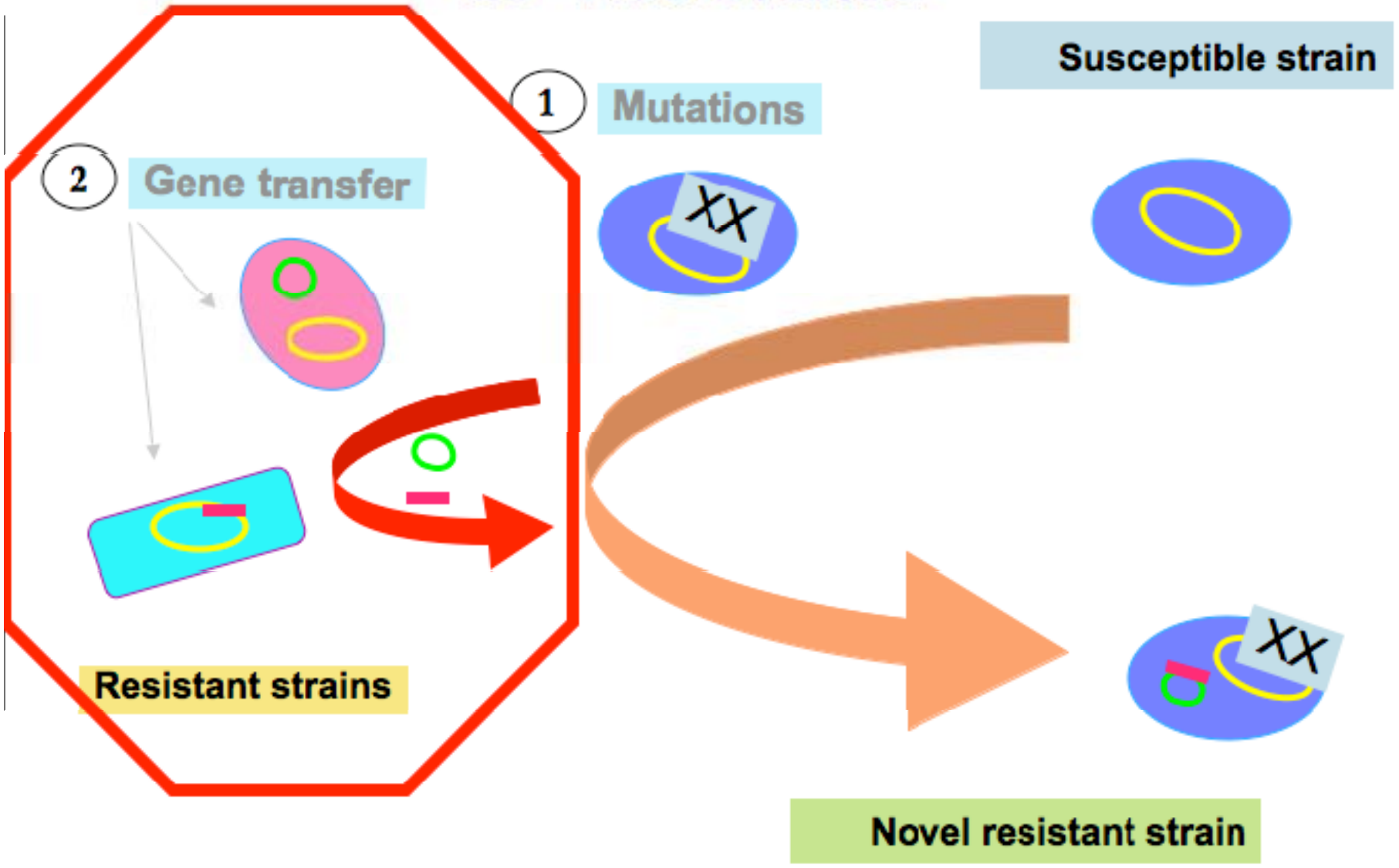
Bifunctional acetyltransferase : **AAC(6')-Ib-cr**

- **Two substitutions: Trp102Arg and Asp179Tyr**
- ***N*-acetylation of fluoroquinolones**
- **Confers reduced susceptibility to aminoglycosides and fluoroquinolones (MIC of ciprofloxacin increased 3 fold)**

(Robicsek *et al.* Nat Med 2006)



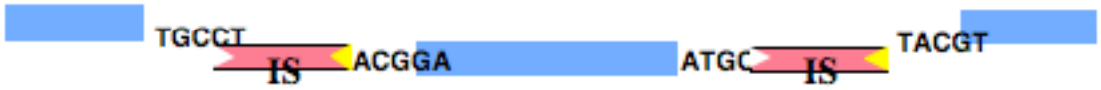
Co-Resistance



Co-Resistance

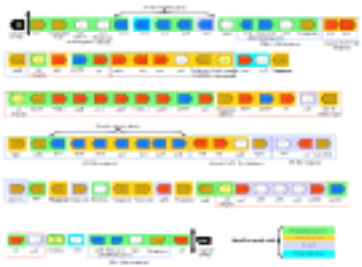
- **Plasmid**

- **Transposon**

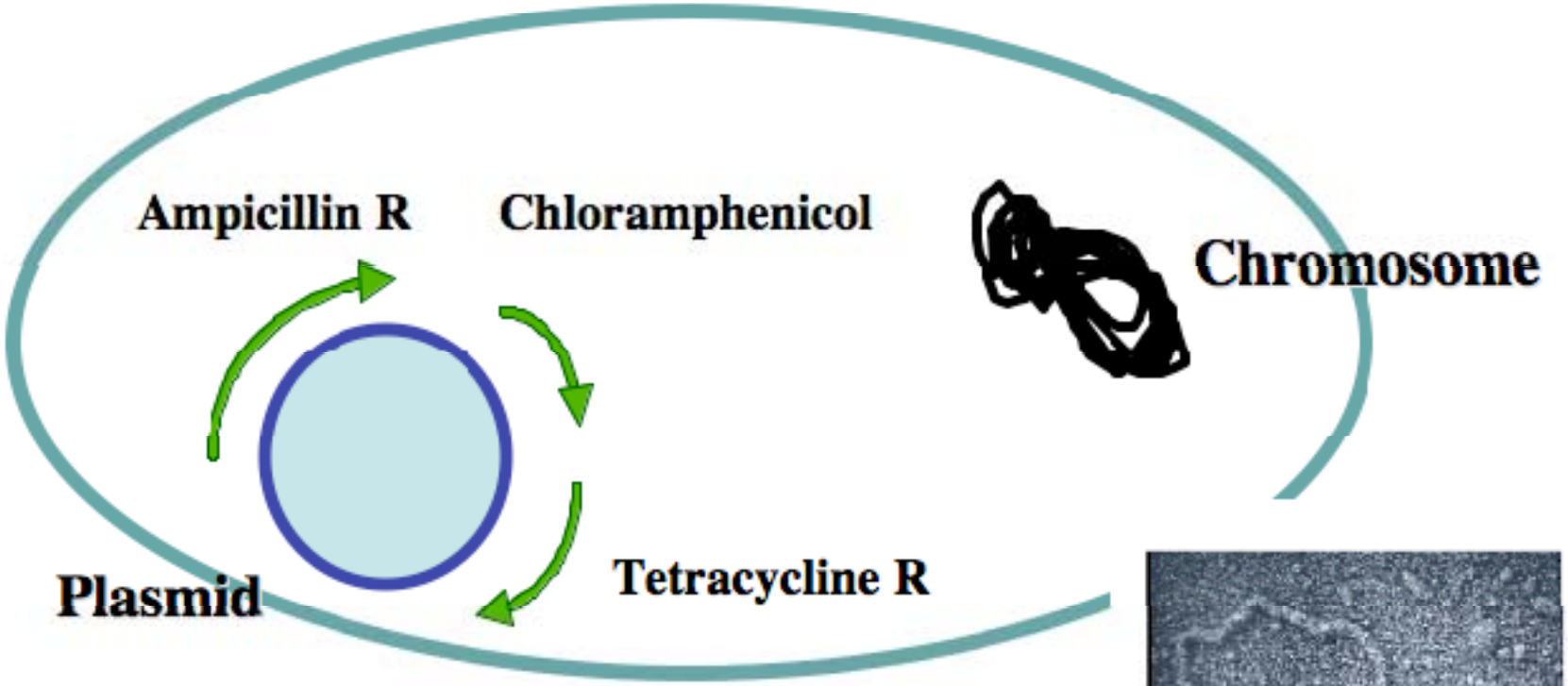


- **Integron**

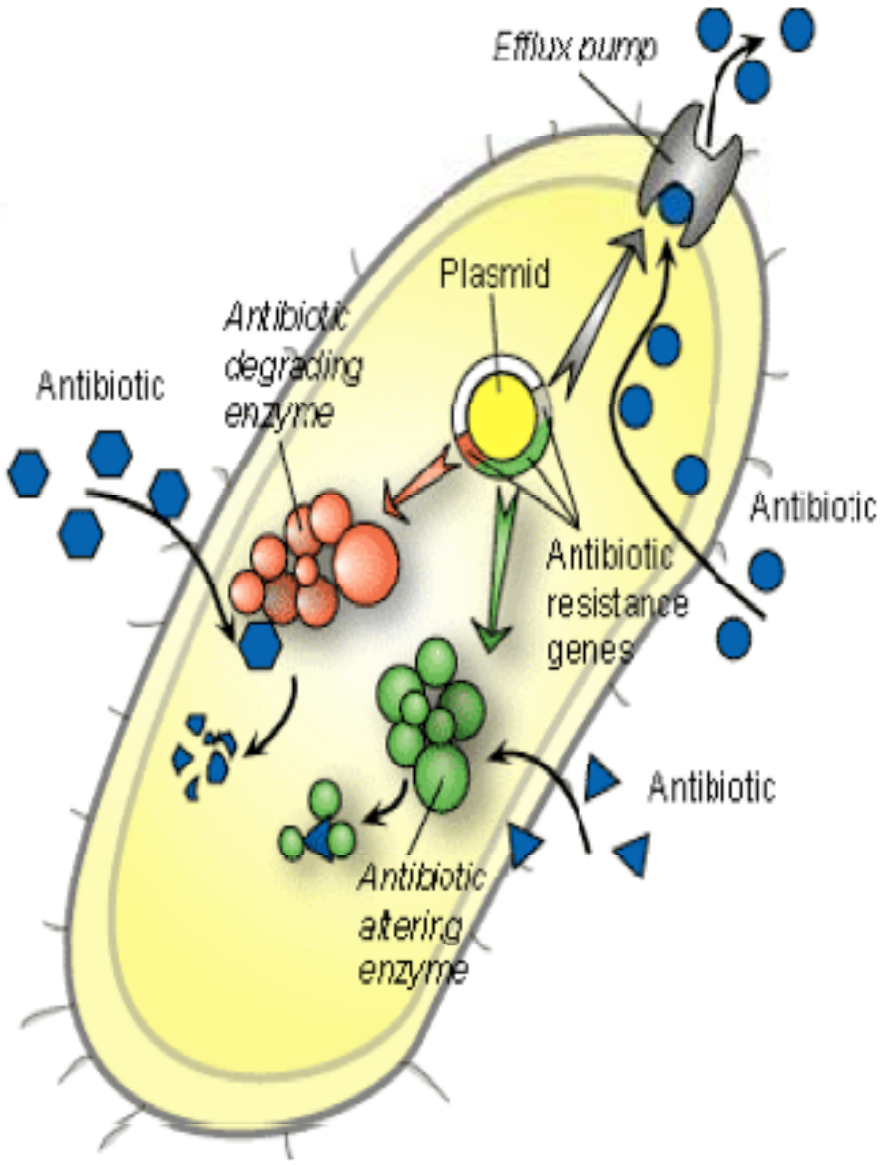
- **Resistance island**



Plasmid



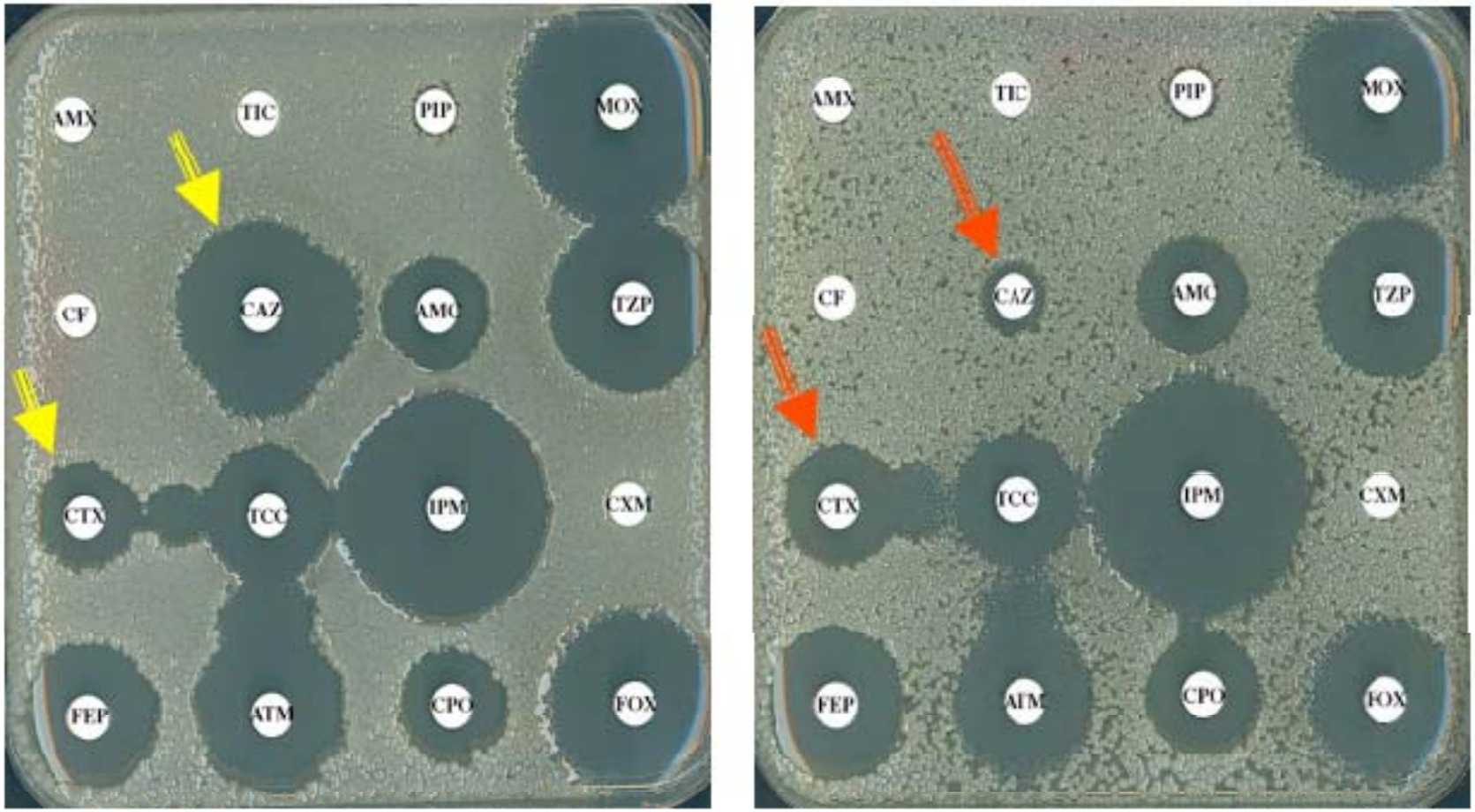
- Interspecies transfer - 5- > 500 kb
- Conjugative, non conjugative mobilisable....


Efflux pump
Plasmid
Antibiotic degrading enzyme
Antibiotic
Antibiotic
Antibiotic resistance genes
Antibiotic
Antibiotic altering enzyme

Biochemical mechanisms of antibiotic resistance and their genetic determinants

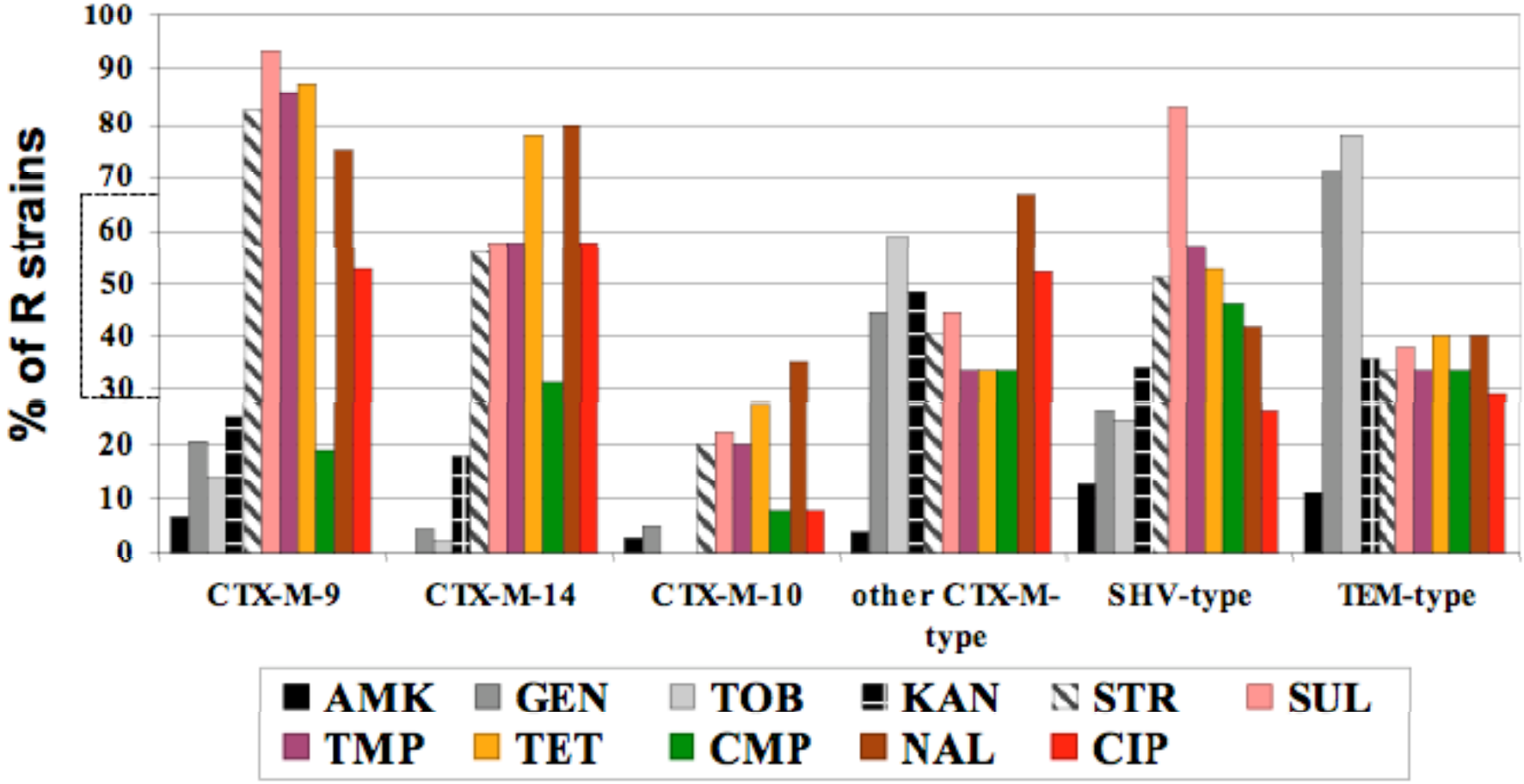
		Genetic determinants	
Mechanism	*Examples*	*Mutation*	*Gene acquisition*
Reduced permeability	Aminoglycosides	+	+
Pro-drug not activated	Isoniazid	+	
Active efflux	Tetracycline		+
	Fluoroquinolones	+	
Alteration of drug target	Erythromycin		+
	Fluoroquinolones	+	+
	Rifampicin	+	+
	Tetracycline		+
Inactivation of drug	Aminoglycosides		+
	Chloramphenicol		+
	β-lactams	+	+
'By-pass' inhibited step	Sulfonamides		+
	Trimethoprim		+
Immunity protein	Bleomycin		+
Amplification of target	Trimethoprim	+	+
	Sulfonamides	+	+
Sequestration of drug	β-lactams	+	+

CTX-Ms: hydrolyse du cefotaxime… et de la ceftazidime



CTX-M-3 ***E. coli*** **CTX-M-15**

Co-resistance of the CTX-M producers



Morosini et al. AAC, 2006

Multidrug resistance among *Klebsiella* spp. collected in an European survey of *K. pneumoniae* isolates from ICUs

Resistance	ESβL Positive (n=110)	ESβL Negative (n=323)
Ceftazidime	75	25
Gentamicin	72	10
Amikacin	61	4
Ciprofloxacin	31	4

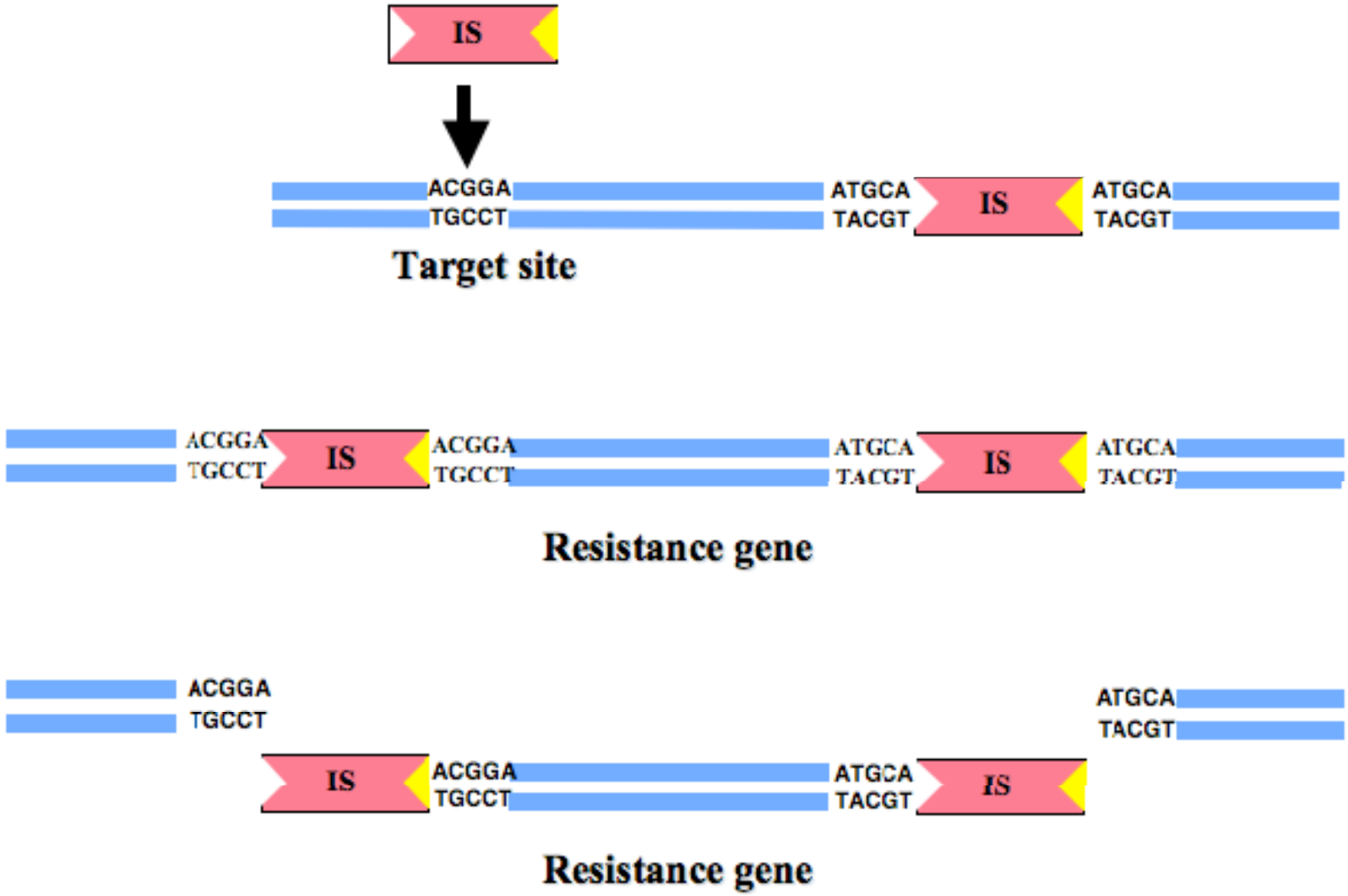
CID, 2003,36: S11-23

Co-resistances

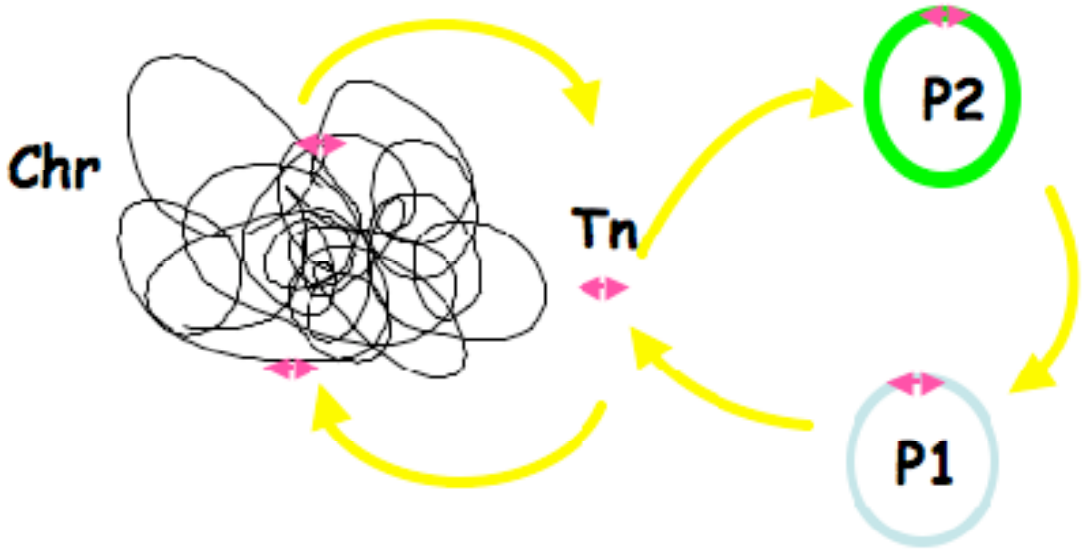
ESBL - Bicetre - *E.coli* - 2007 (n=71)

	% résistance
Gentamicin	39
Tobramycin	51
Amikacin	27
Netilmicin	47
Tetracycline	58
Trimethoprim/Sulfamethoxazole	63
Nalidixic acid	79
Ciprofloxacin	72
Colistin	0
Fosfomycin	0
Ertapenem/Imipenem	0
Tigecycline	0

Transposons



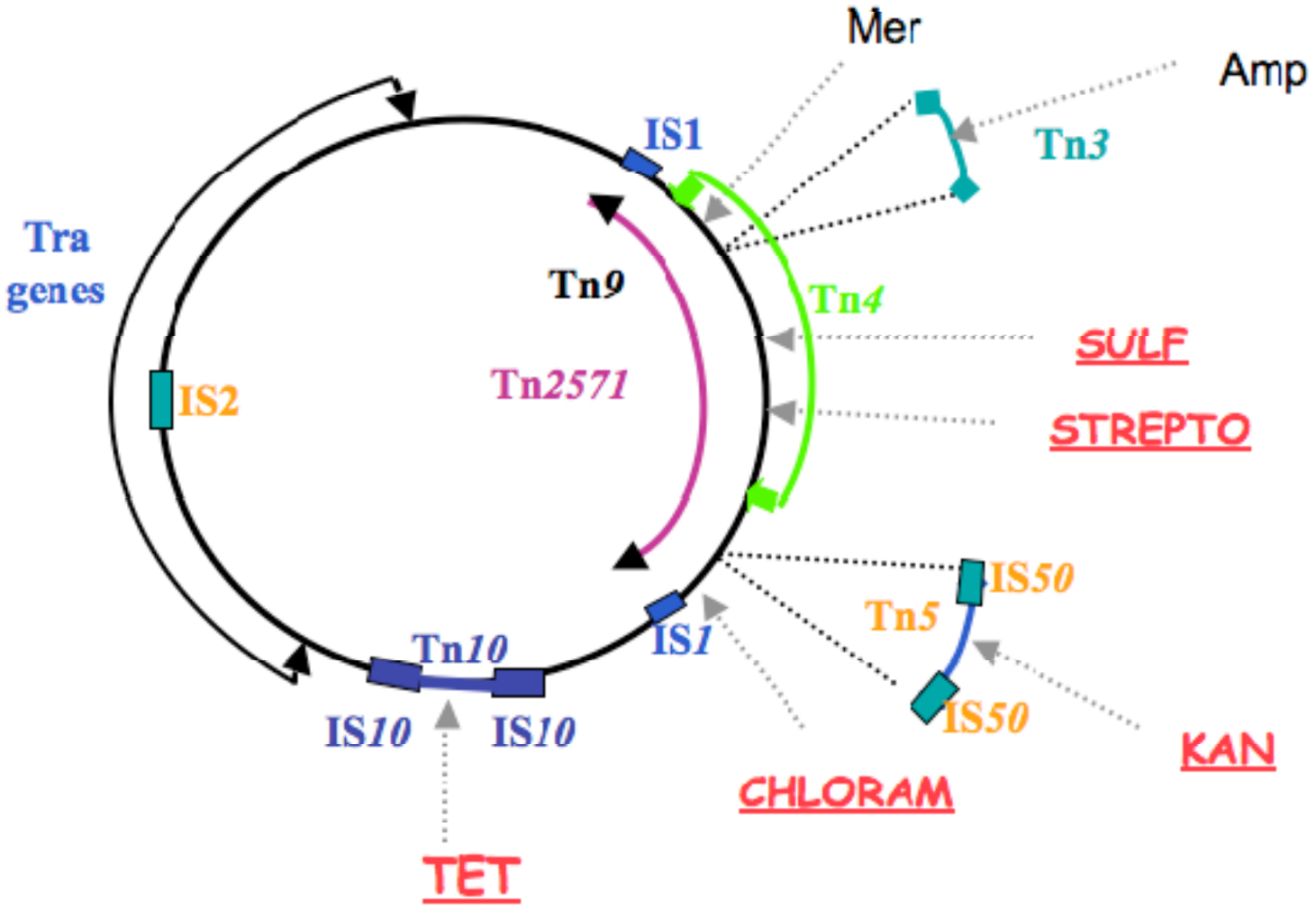
Transposons



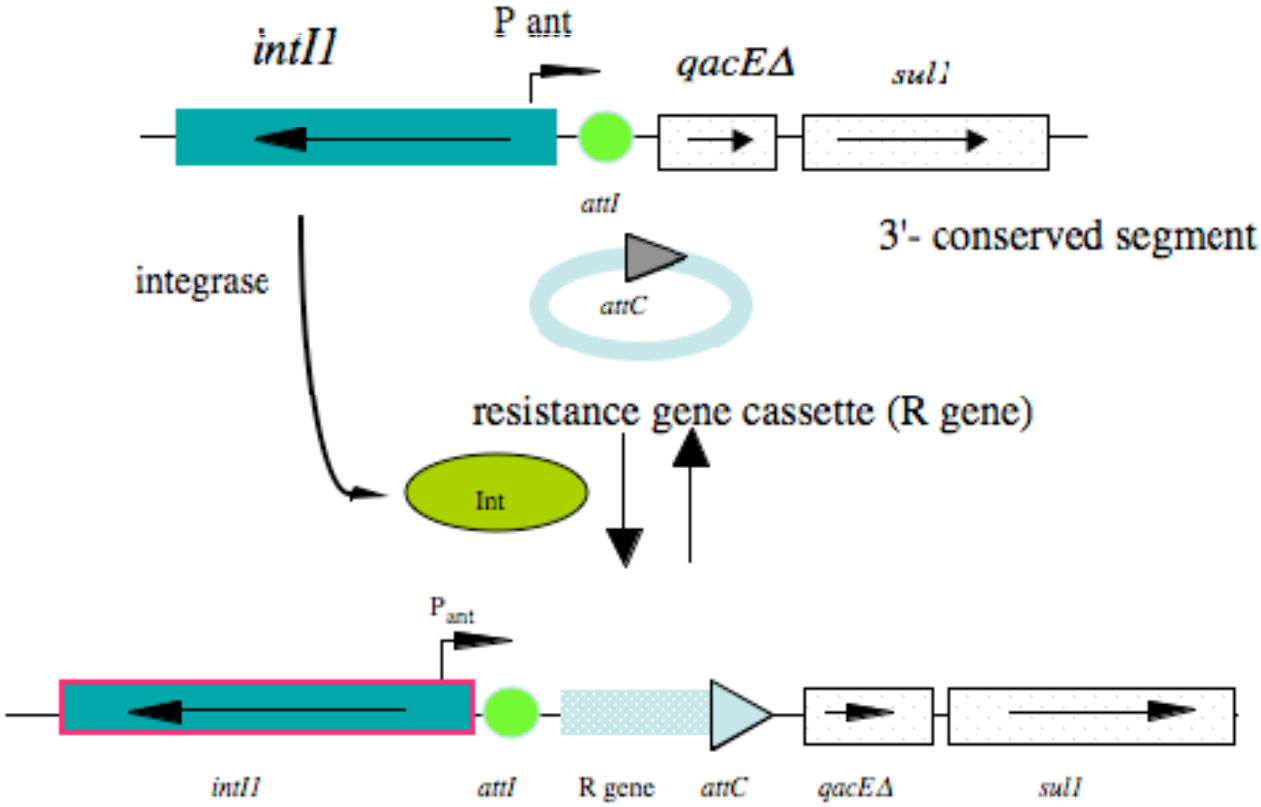
Resistance genes

- Aminoglycosides
- ß-lactamines
- Sulfamides
- Rifampicine
- Cyclines
- Chloramphenicol
- Trimethoprim

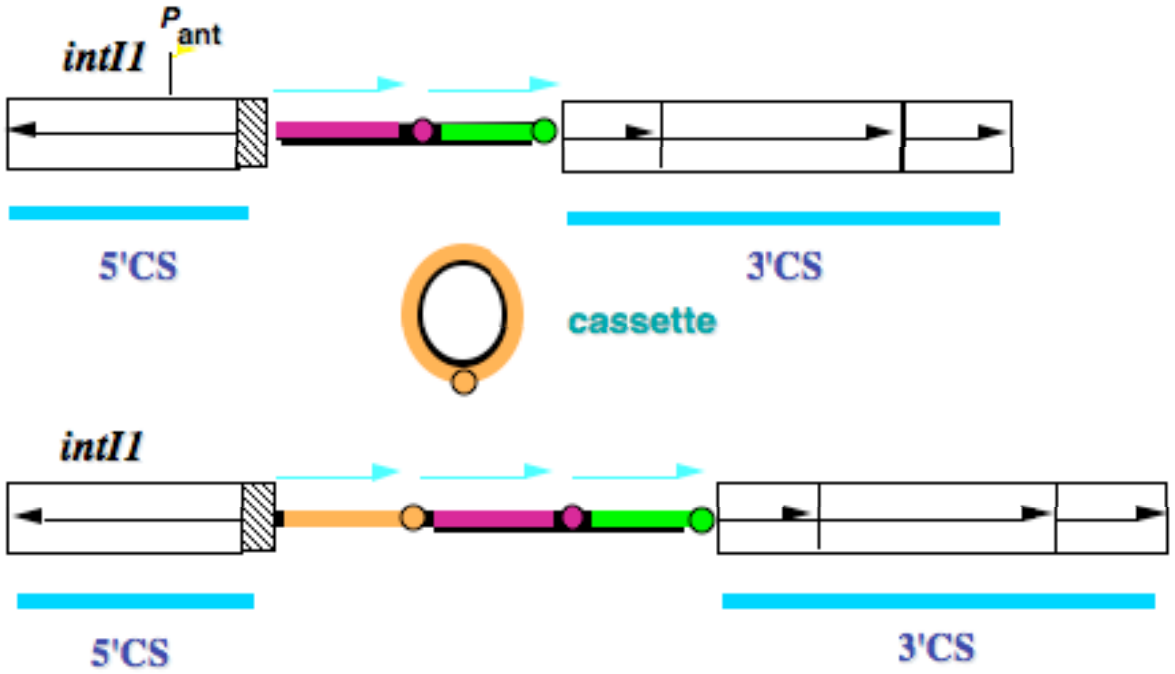
R1 plasmid (100-kbp): an example of resistance accumulation mediated by transposable elements, and especially composite transposons



Integrons

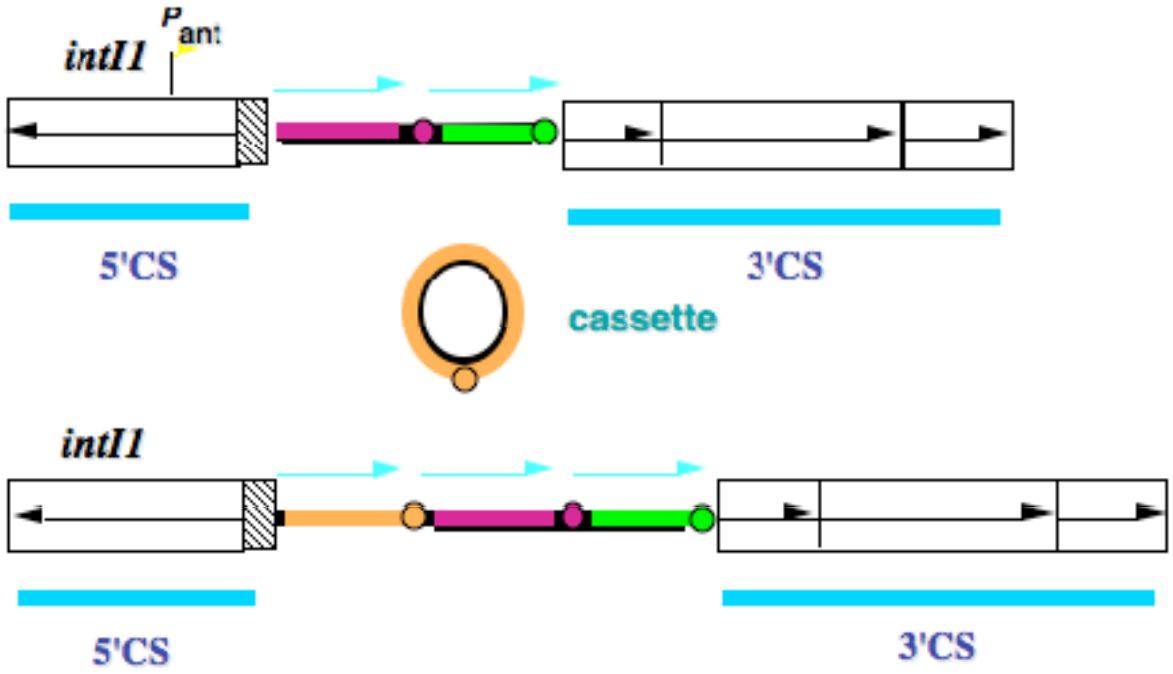

intI1
P ant
qacEΔ
sul1
attI
3'- conserved segment
integrase
attC
resistance gene cassette (R gene)
Int
P ant
intI1
attI
R gene
attC
qacEΔ
sul1

Integrons



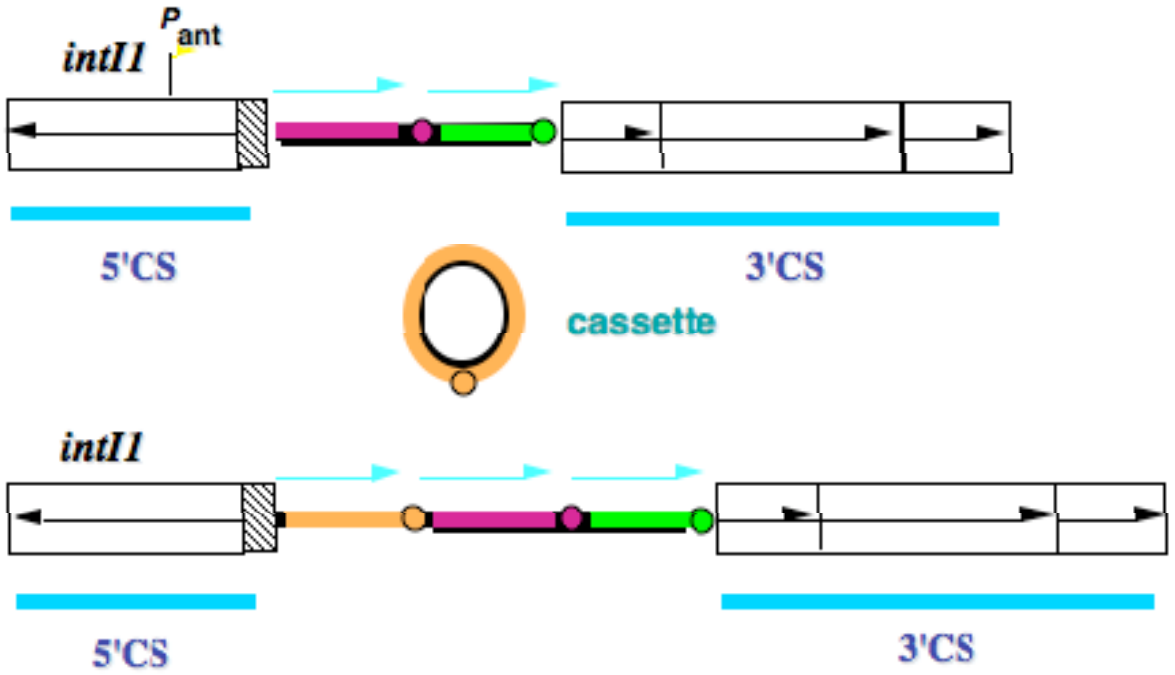
- Consequences: co-resistance

Integrons



- Consequences : co-resistance ; co-expression

Integrons



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

bla_{VEB-1} as a gene cassette identified in In53 in *E. coli*

In53

Naas et al., J Bact 2001

Integrons

- Extensively found in Gram clinical isolates
- Over 80 different antibiotic-resistance-gene cassettes :

β-Lactam resistance	
bla_{CEP-1}	Extended-spectrum β-lactamase
bla_{GES-1}	Extended-spectrum β-lactamase
bla_{GES-2}	Extended-spectrum β-lactamase
bla_{IBC-1}	Extended-spectrum β-lactamase
bla_{IBC-2}	Extended-spectrum β-lactamase
bla_{IMP-2}	Carbapenemase
bla_{IMP-3}	Carbapenemase
bla_{IMP-4}	Carbapenemase
bla_{IMP-6}	Carbapenemase
bla_{IMP-7}	Carbapenemase
bla_{VEB-1}	Extended-spectrum β-lactamase
bla_{VIM-1}	Carbapenemase
bla_{VIM-2}	Carbapenemase
bla_{VIM-3}	Carbapenemase
oxa-10/aadA1	Extended-spectrum β-lactamase/adenyltransferase
oxa-11	Extended-spectrum β-lactamase
oxa-13	Extended-spectrum β-lactamase
oxa-15	Extended-spectrum β-lactamase
oxa-16	Extended-spectrum β-lactamase
oxa-17	Extended-spectrum β-lactamase
oxa-18	Extended-spectrum β-lactamase
oxa-19	Extended-spectrum β-lactamase
oxa-28	Extended-spectrum β-lactamase
oxa-31	Extended-spectrum β-lactamase
oxa-32	Extended-spectrum β-lactamase

Aminoglycoside resistance	
aadA4	Adenyltransferase
aadA5	Adenyltransferase
aadA6	Adenyltransferase
aadA8	Adenyltransferase
aadA10	Adenyltransferase
aac(3)-Ib/aac(6')-Ib	Acetyltransferase
aac29a	Acetyltransferase
aac29b	Acetyltransferase
aacA1b/orfG	Acetyltransferase
ant(3")-Ii-aac(6')-IId	Adenyltransferase/ acetyltransferase
aphA15	*Phosphotransferase*
Chloramphenicol resistance	
cmlA4	
cmlA5	Efflux pump
Rifampicin resistance	
arr-2	ADP-ribosylation
Trimethoprim resistance	
dfr13	Dihydrofolate reductase
dfr17	Dihydrofolate reductase
dfrA17	Dihydrofolate reductase
Quaternary ammonium compound resistance	
qacF'	Efflux pump
qacI	Efflux pump

adpated from Fluit & Schmitz, CMI 2004

JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2001, p. 3724–3726 Vol. 39, No. 10
0095-1137/01/$04.00+0 DOI: 10.1128/JCM.39.10.3724–3726.2001

Increased Prevalence of Class I Integrons in *Escherichia coli*, *Klebsiella* Species, and *Enterobacter* Species Isolates over a 7-Year Period in a German University Hospital

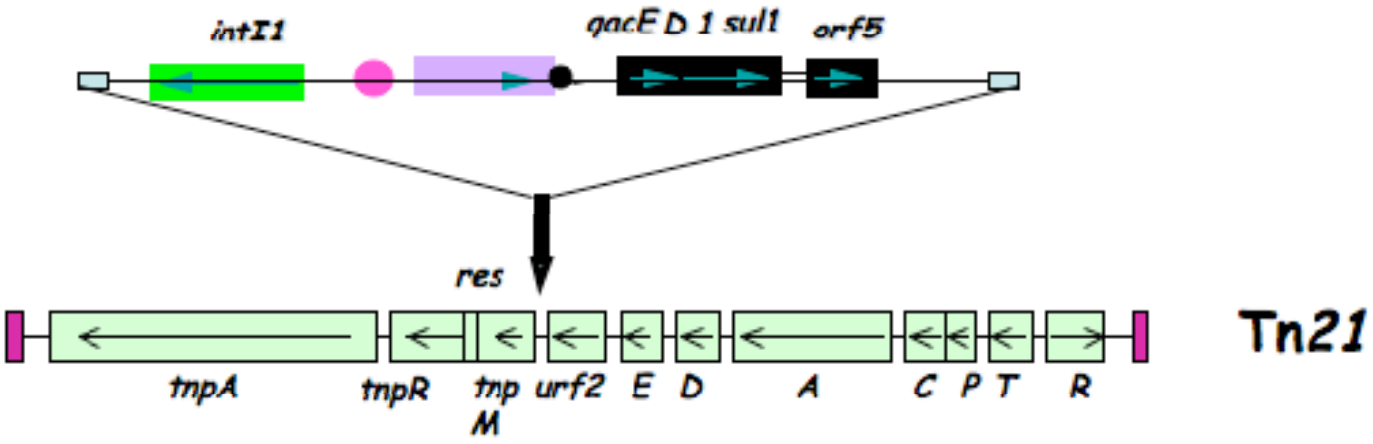
FRANZ-JOSEF SCHMITZ,[1,2]* DIETER HAFNER,[3] ROLAND GEISEL,[1] PATRICK FOLLMANN,[1] CHRISTIAN KIRSCHKE,[1] JAN VERHOEF,[2] KARL KÖHRER,[1] AND AD C. FLUIT[2]

Institute for Medical Microbiology and Virology[1] and Institute of Pharmacology and Clinical Pharmacology,[3] Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, and Eijkman-Winkler Institute for Medical Microbiology, University Medical Center Utrecht, Utrecht, The Netherlands[2]

Received 28 December 2000/Returned for modification 31 May 2001/Accepted 18 July 2001

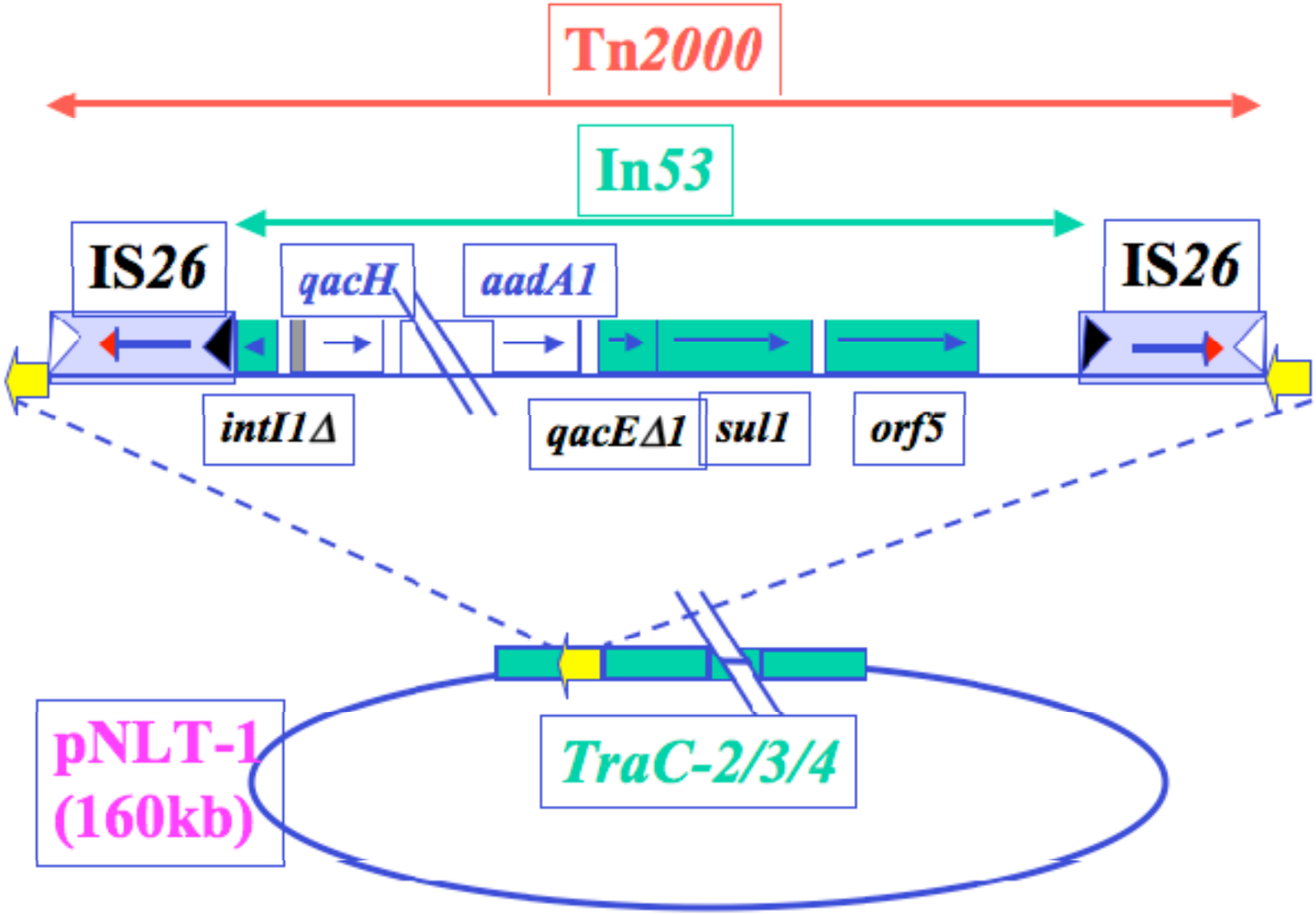
The prevalence of integrons in five enterobacterial species was analyzed in 900 blood culture isolates from 1993, 1996, and 1999. Remarkably, the prevalence increased from 4.7% in 1993 to 9.7% in 1996 and finally to 17.4% in 1999 ($P < 0.01$). Within 7 years the combined percentage of P1 strong promoters and P1 weak plus P2 active promoters with high transcription efficacies has increased from 23.1 to 33.3 and finally 60% ($P < 0.05$).

Integrons are carried by transposons

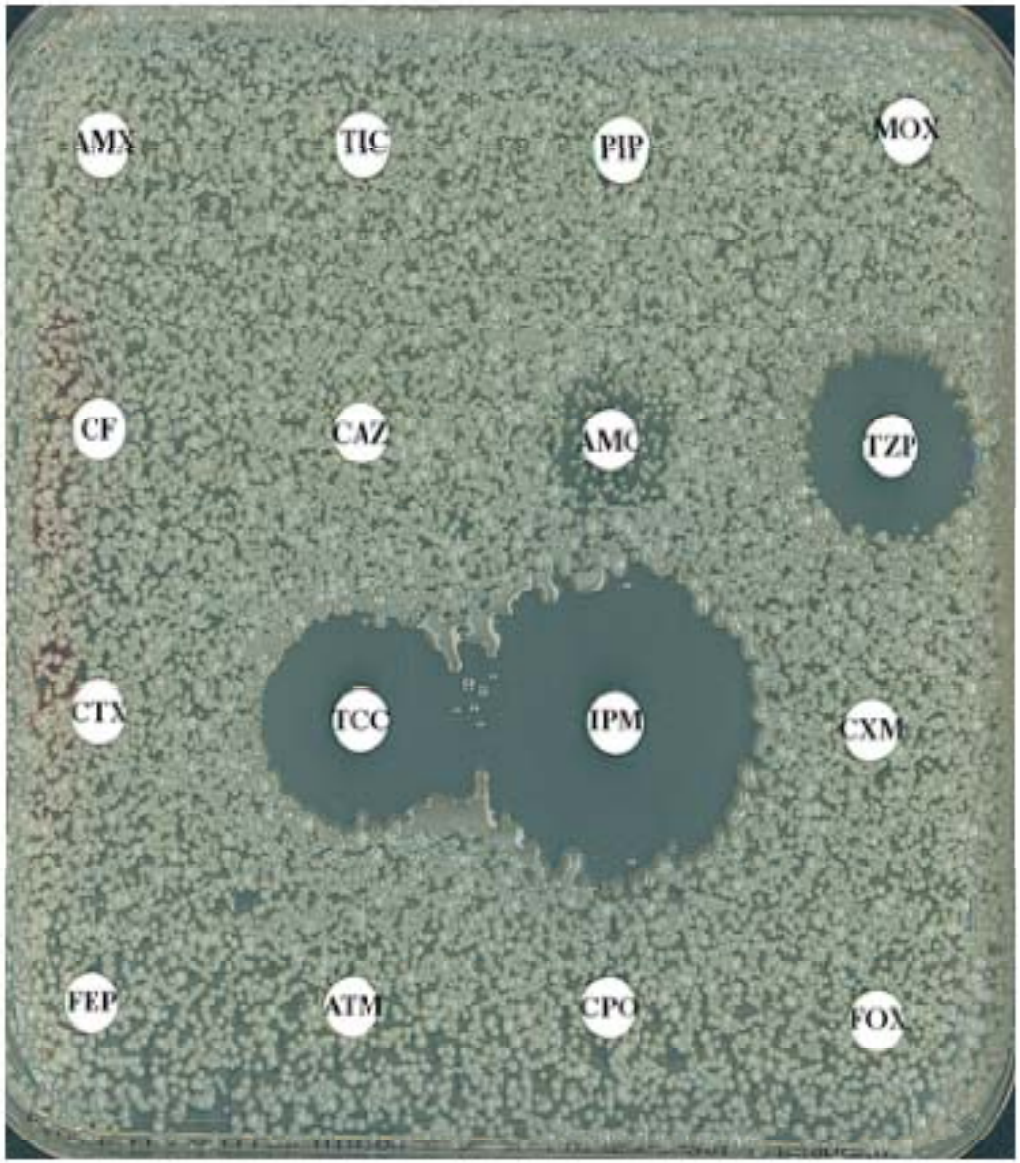


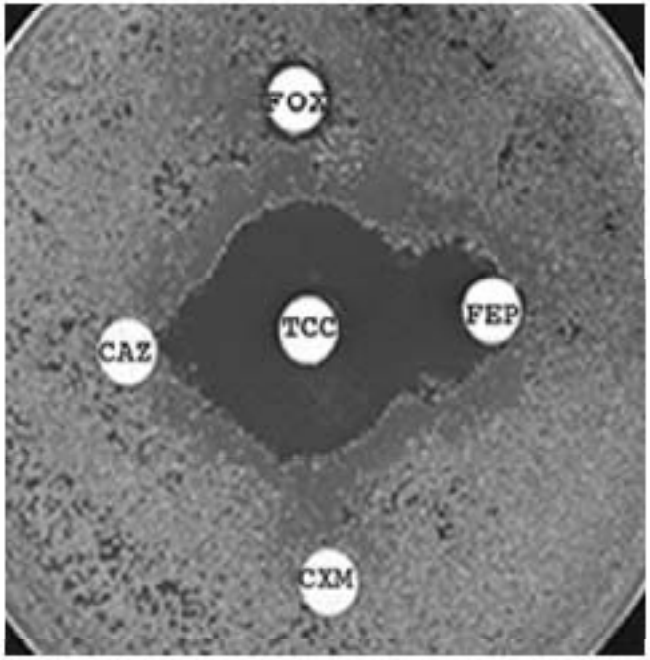
The integron is not self-mobile, it is mobilized by the transposon

Integrons, transposons, plasmids



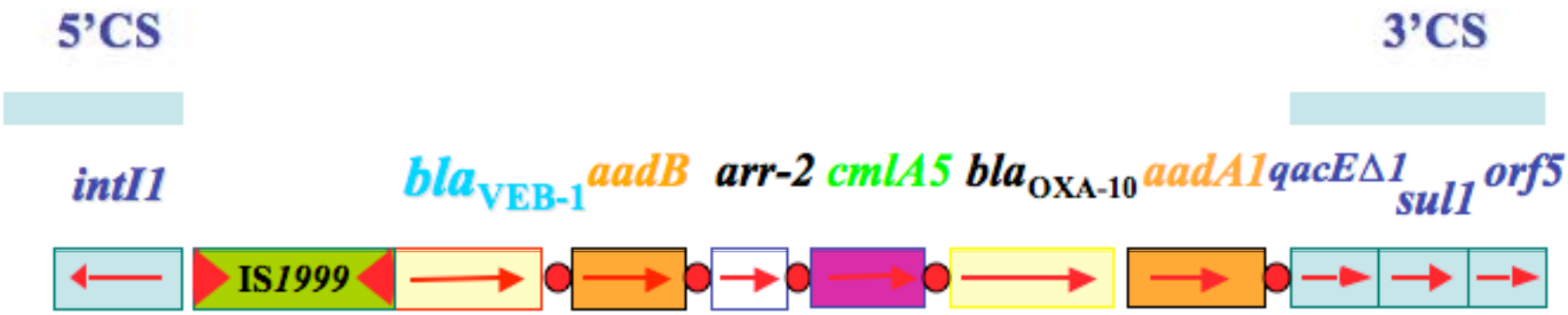
Acinetobacter baumannii: VEB-1





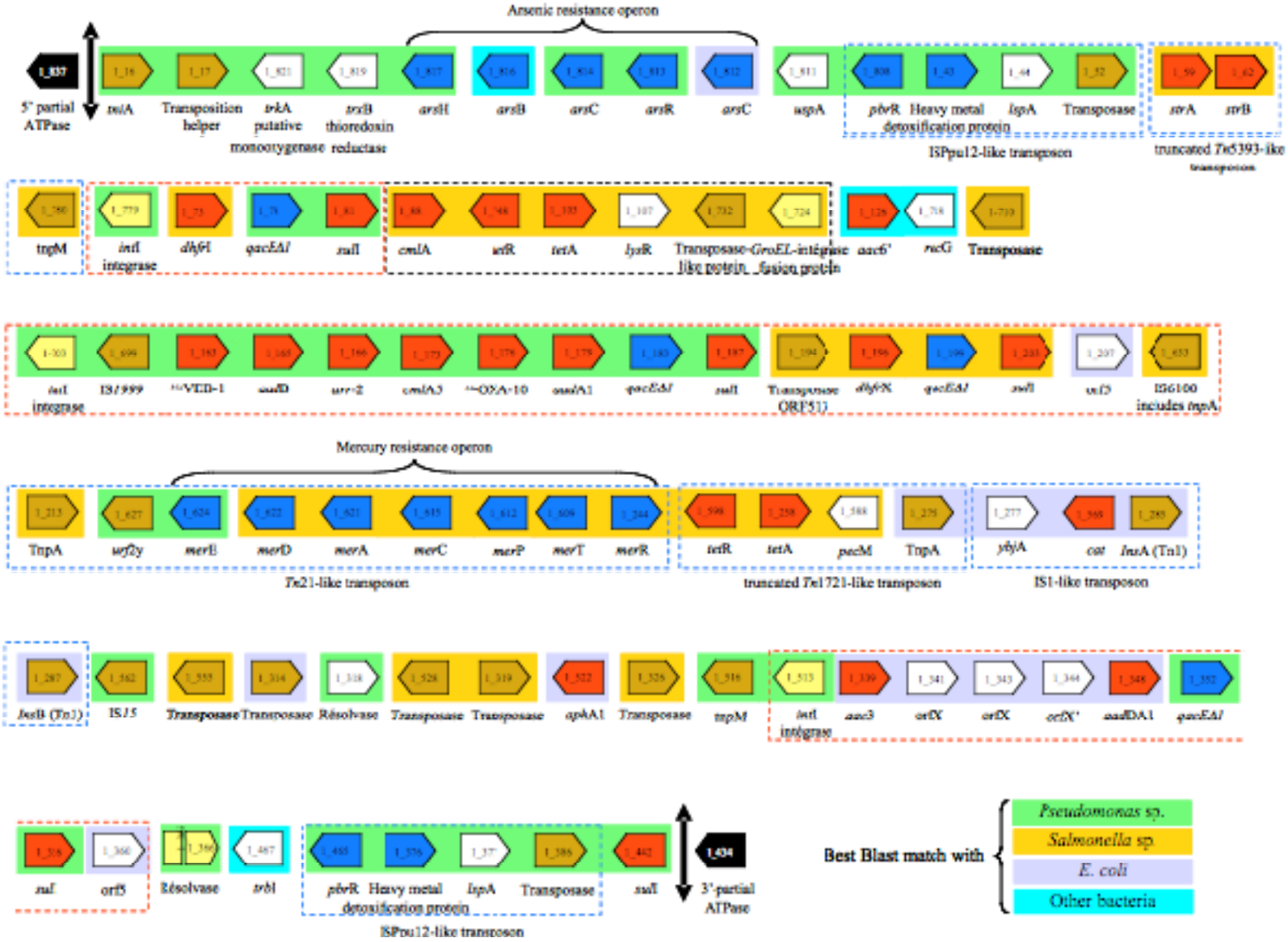
Poirel, Nordmann J. Clin. Microbiol. 2003

Structure of the bla_{VEB-1}-containing integron identified in the epidemic clone of *A. baumannii* in France



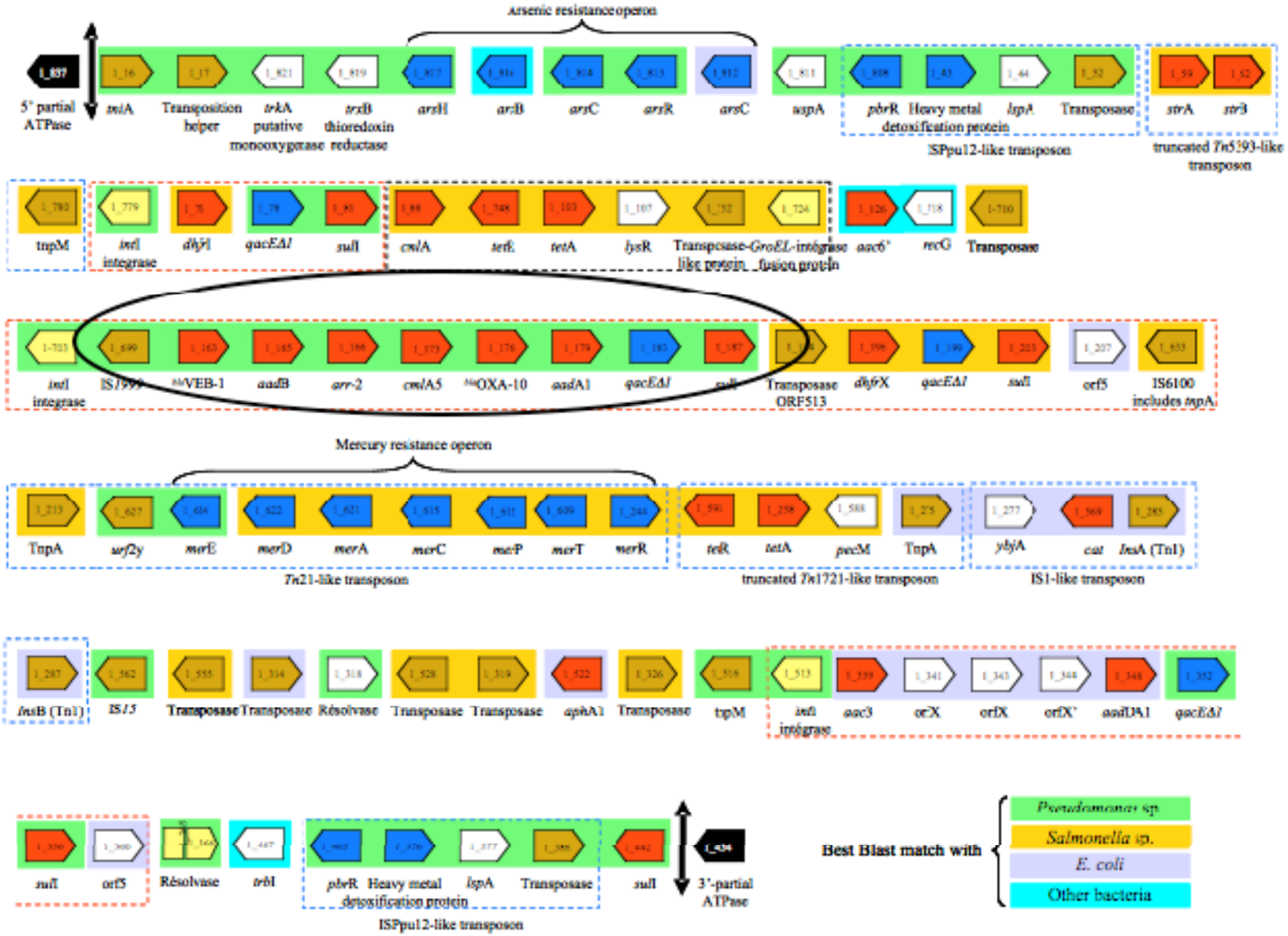
Poirel *et al.*, JCM 2003;41:3542

An 86-kb resistance island in *A. baumannii*



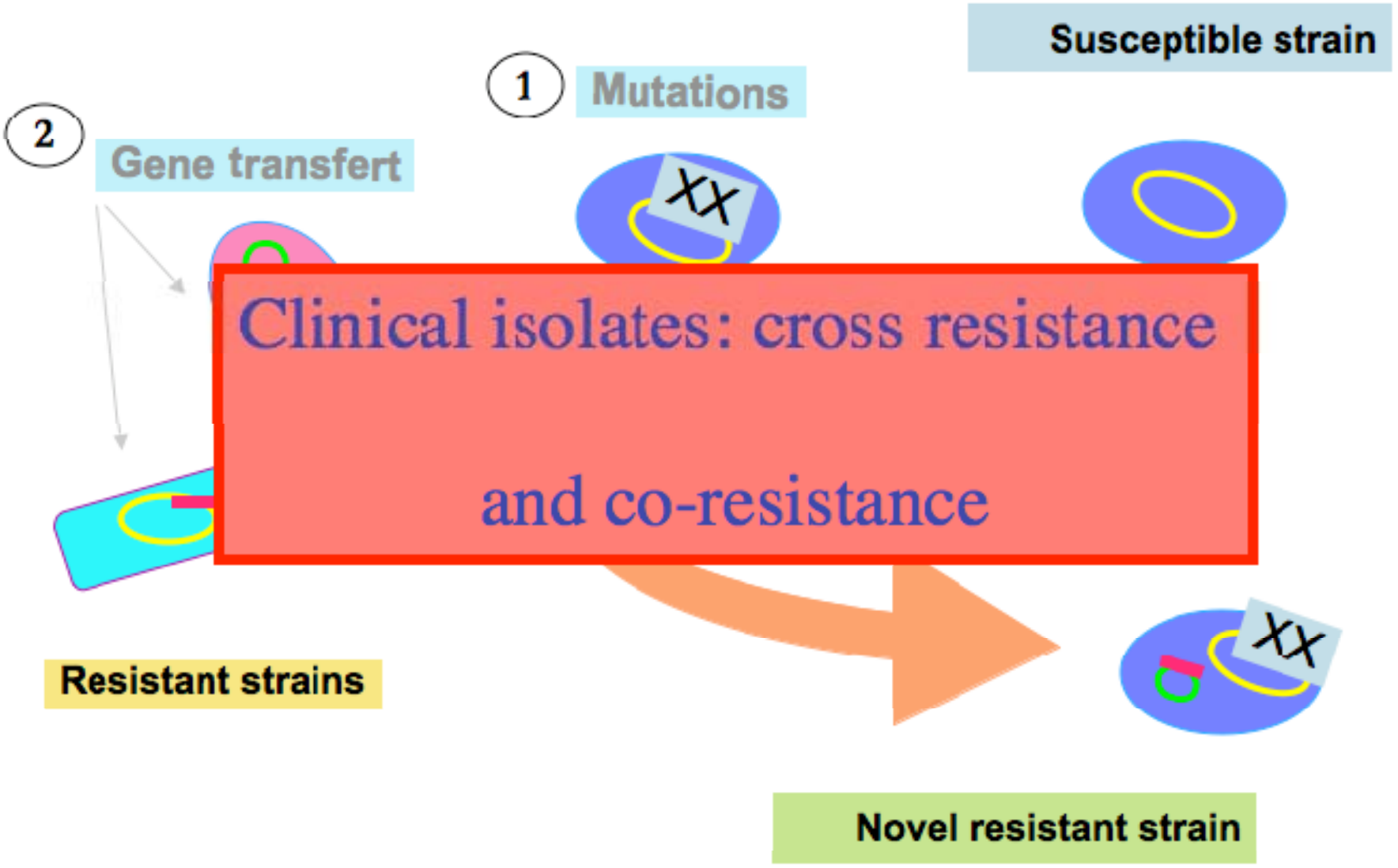
Fournier et al., Plos Gen 2006

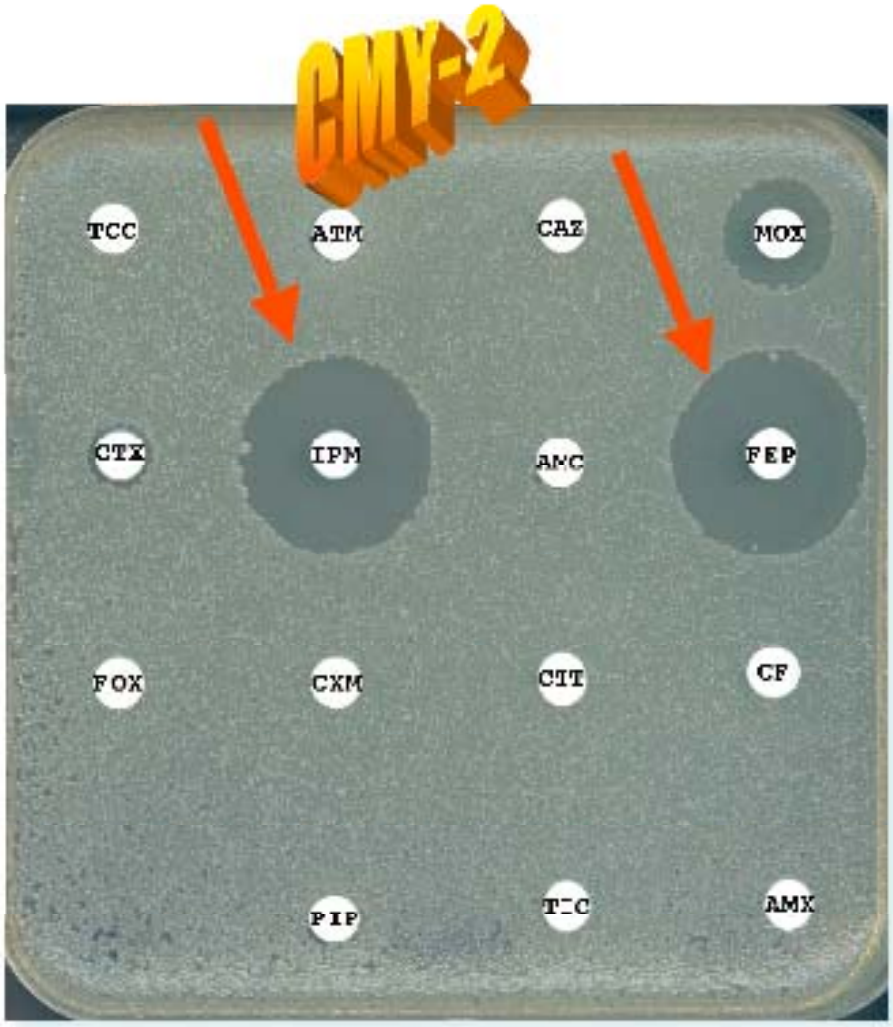
An 86-kb resistance island in *A. baumannii*



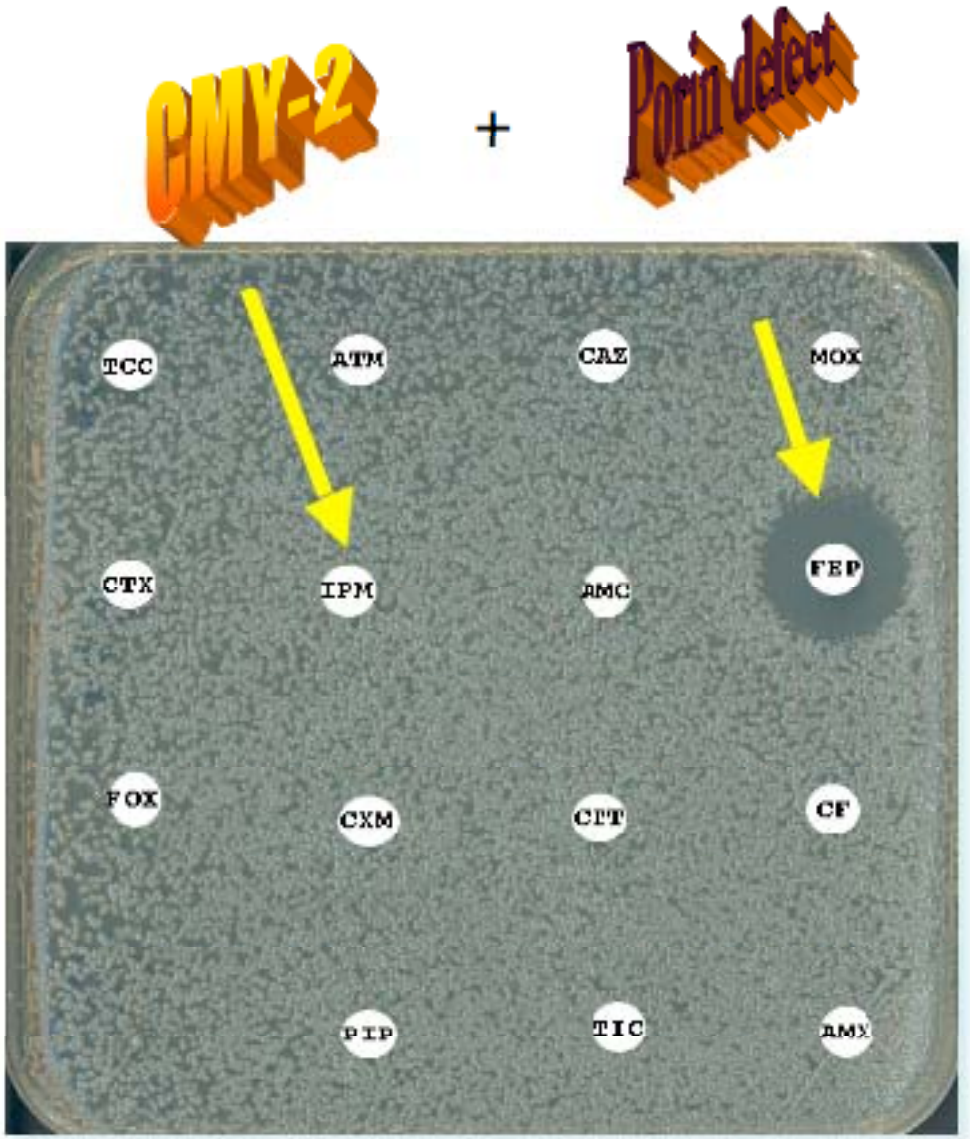
Fournier et al., Plos Gen 2006

Genetics of Resistance





E. coli ND1



E. coli ND 2

Superbacteria NDM-1

Experții avertizează că un nou tip de superbacterie rezistentă la antibiotice ar putea face victime în întreaga lume. Rezistența superbacteriei la antibiotice este dată de enzima New Delhi metallo-beta-lactamase-1.

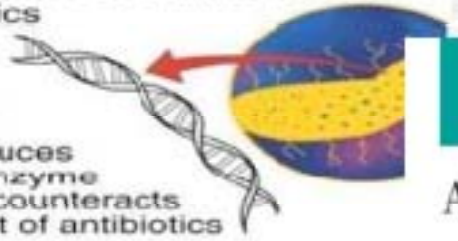
NDM-1: Bacteria poate deveni rezistentă la gama de antibiotice carbapenem, folosită doar în cazuri de urgență.

Origine: Este răspândită în India, Pakistan și Bangladesh. Acum, NDM-1 a ajuns și în SUA, Canada, Australia și Olanda.

Gazde: Enzima NDM-1 a fost găsită în două tipuri de bacterii: ***E.coli*** - ce atacă intestinele - și ***Klebsiella*** - ce atacă plămânii.

Superbug gene

Discovered in pneumonia and E.coli bugs resistant to last-line antibiotics



NDM-1

- Produces an enzyme that counteracts effect of antibiotics
- Found in India, Pakistan

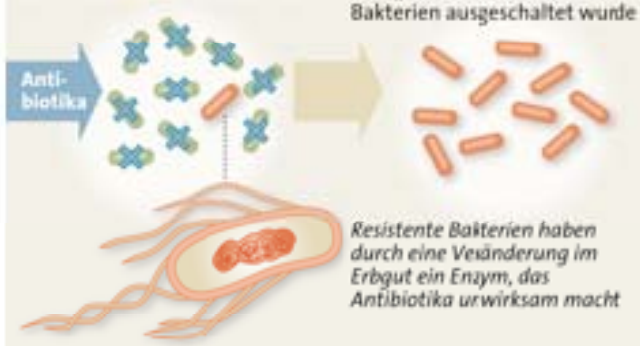
Gefährliche „Superbakterien"

„Normale" Bakterien, werden von Antiobiotika abgetötet

Resistente Bakterien, Antibiotika wirken nicht

Beispiel Patient erhält Antibiotika gegen eine Infektion mit dem Bakterium Escherichia coli

Resistente Keime können sich ungehindert ausbreiten, auch weil „Konkurrenz" durch andere Bakterien ausgeschaltet wurde



Resistente Bakterien haben durch eine Veränderung im Erbgut ein Enzym, das Antibiotika unwirksam macht

Gründe für Entwicklung resistenter Keime

- Häufiger, oft unnötiger Einsatz von Antibiotika (z.B. ge
- Zu kurze Antibiotika-Einnahme durch Patienten
- Besonders gefährliche resistente Keime entstehen in S

Neue Keime

- Entstehung in Indien/Pakistan
- Durch Gen NDM-1 sind auch Peneme unwirksam (Antibiotika, die bisher bei mehr

Grafik: © APA, Quelle: AI

LE FIGARO santé

Alerte aux nouvelles bactéries ultrarésistantes

Venues d'Inde, elles survivent à presque tous les antibiotiques et circulent déjà dans plusieurs pays.

Les bactéries ultrarésistantes dans les pas de l'homme

THE Sun

Daily Mail — 24 HOURS A DAY —

theguardian

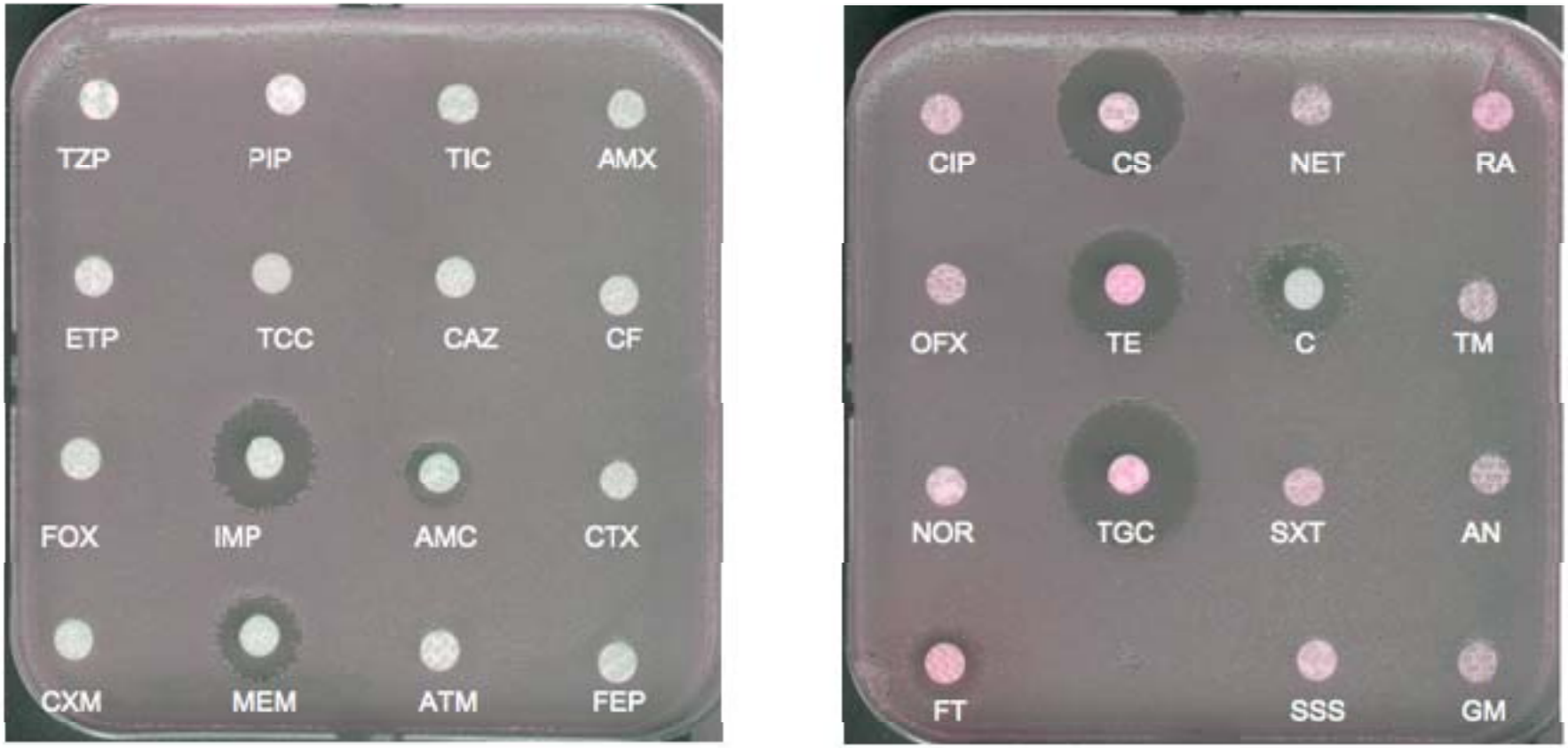
THE HINDU

Spread of NDM-1 from India/Pakistan to the UK

bla$_{NDM-1}$-positive bacterial clones

Courtesy Neil Woodford

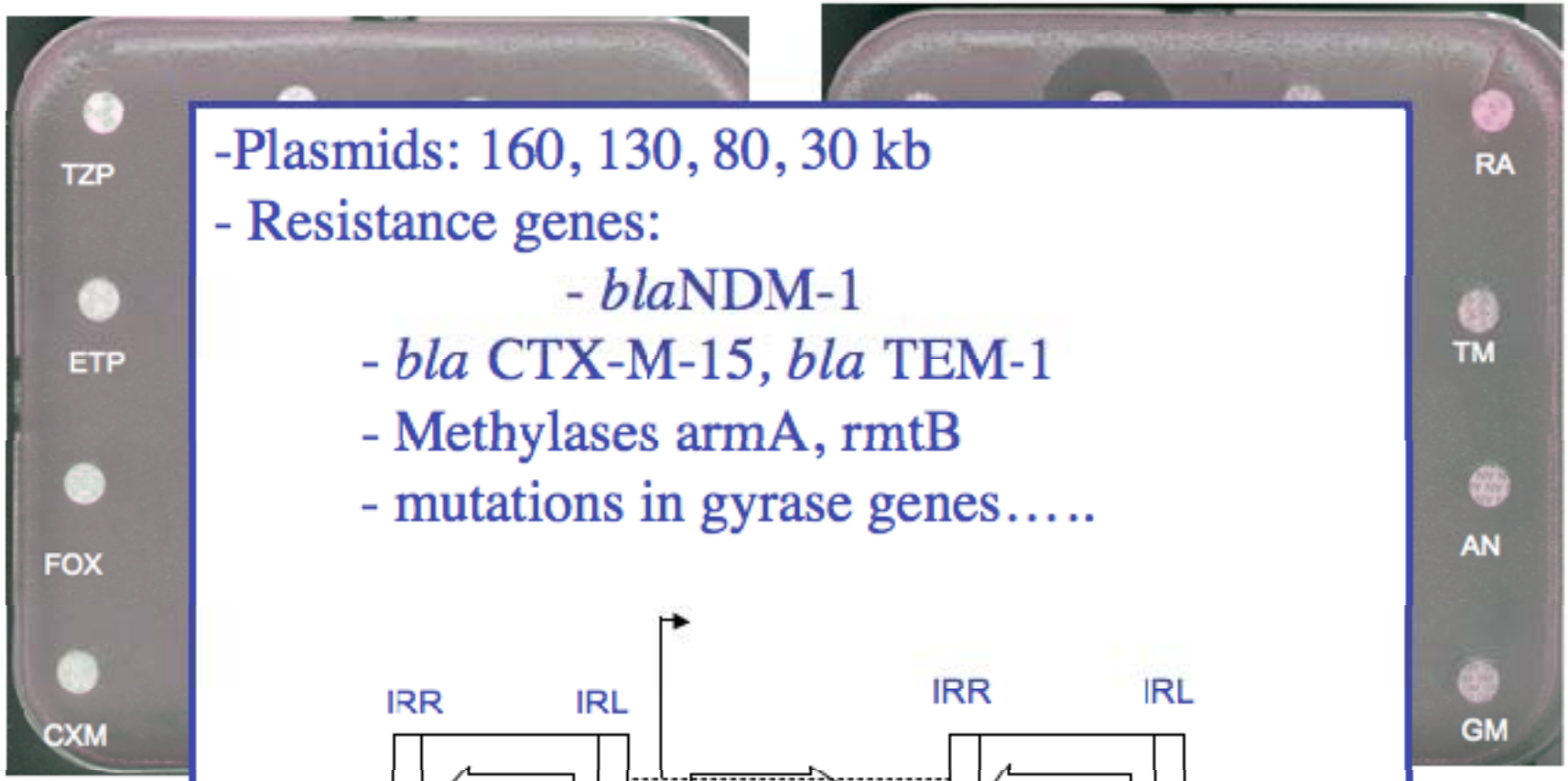
E. coli NDM-1, Australia



Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P

Emergence of metallo-b-lactamase NDM-1-metallo-ß-lactamase producing multidrug resistant Escherchia coli in Australia. Antimicrob Agents Chemother, 2010, 54, 4914-6

E. coli NDM-1, Australia

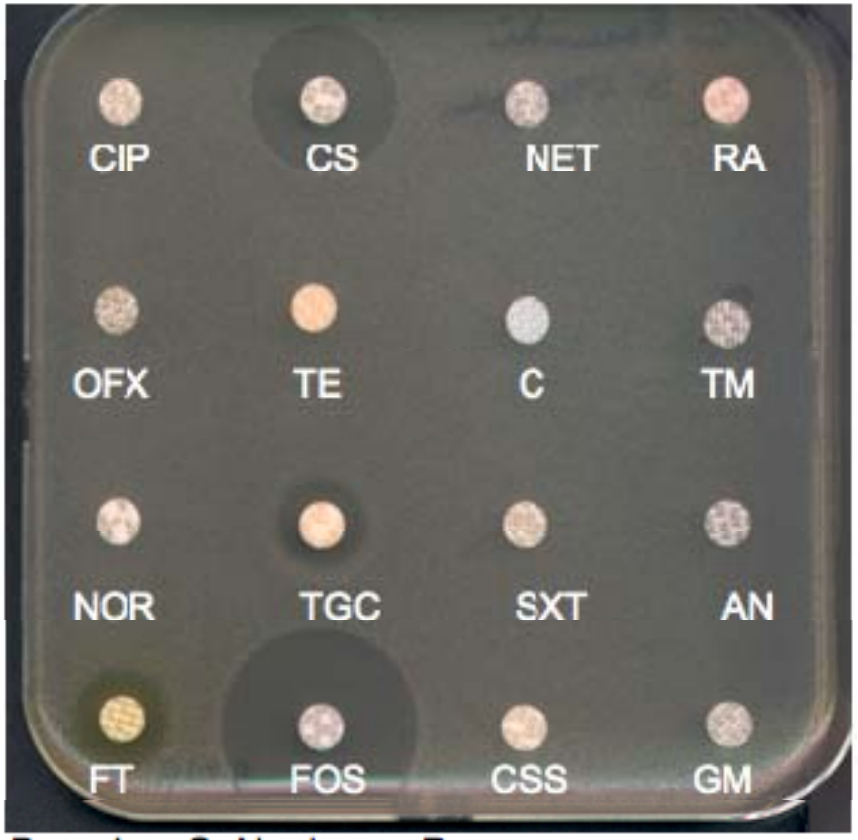


-Plasmids: 160, 130, 80, 30 kb

- Resistance genes:
  - *bla*NDM-1
  - *bla* CTX-M-15, *bla* TEM-1
  - Methylases armA, rmtB
  - mutations in gyrase genes…..

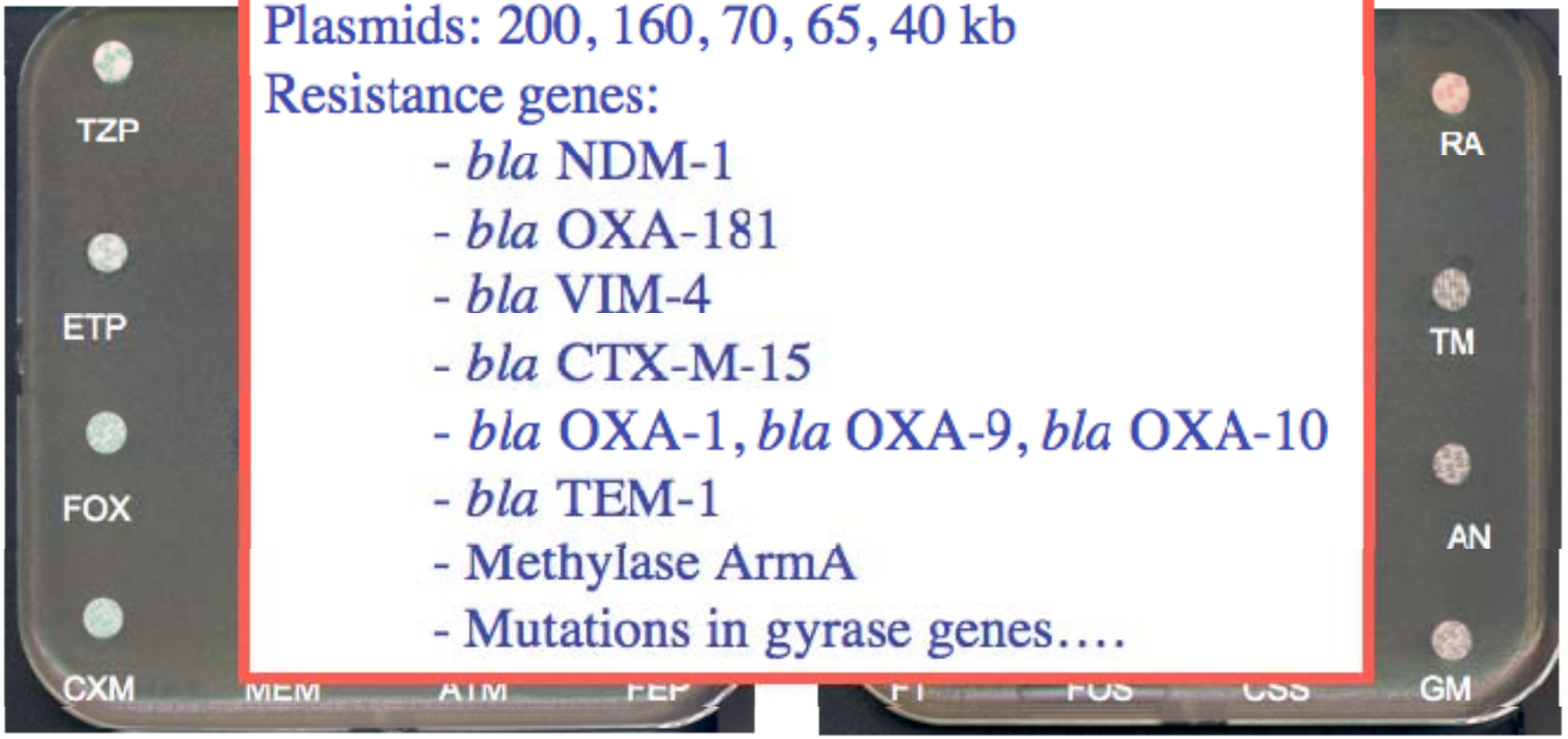
Poirel L, Lagrut
Emergence of
tant
Escherchia coli in Australia. Antimicrob Agents Chemother, 2010, 54, 4914-6

C. freundii NDM-1, France



Poirel L, Ros L, Carricajo A, Berthelot P, Pozetto B, , Bernabeu S,,Nordmann P
Extremely drug-resistant C. freundii in a patient returning from India and producing NDM-1 and other Carbapenemases. Antimicrob. Agents Chemother, 2010, in press

C. freundii NDM-1, France



Plasmids: 200, 160, 70, 65, 40 kb

Resistance genes:

- *bla* NDM-1
- *bla* OXA-181
- *bla* VIM-4
- *bla* CTX-M-15
- *bla* OXA-1, *bla* OXA-9, *bla* OXA-10
- *bla* TEM-1
- Methylase ArmA
- Mutations in gyrase genes….

Poirel L, Ros L, Carricajo A, Berthelot P, Pozetto B, , Bernabeu S,,Nordmann P
Extremely drug-resistant C. freundii in a patient returning from India and producing NDM-1 and other Carbapenemases. Antimicrob. Agents Chemother, 2010, in press

Conclusion

Class of pieces	Genes and sequences		Function
Operative	Resistance genes	*mecA*	PBP2a (meth icillin resista nce in *S. aureus*)
	Regulatory genes	*mecR*	*mecA* regulation expression
Translocative	Integrases	*int*	*attC* recognition in class 1 integrons
	Resolvases	TnpR	*res* sequence recognition in Tn *3*-like transposons
	Invertases	*inv*	Specific recombination sequence
	Transposases	ORF513	CTX-M and AmpC capture (?)
Dispersive	Conjugation genes	*oriT*	Plasmid conjugation
	Incompa tibility genes	*inc*	Plasmid interference
	Integration sequences	*res*	Recombination in Tn3 -like transposons
	Insertion sequences (IS)	IS10	Recombination and integration (*tet* genes)

F. Baquero. Nature Rev Microbiol 2004; 2:510-17