Pseudomonas aeruginosa; ß-lactamases…

and other enzymes

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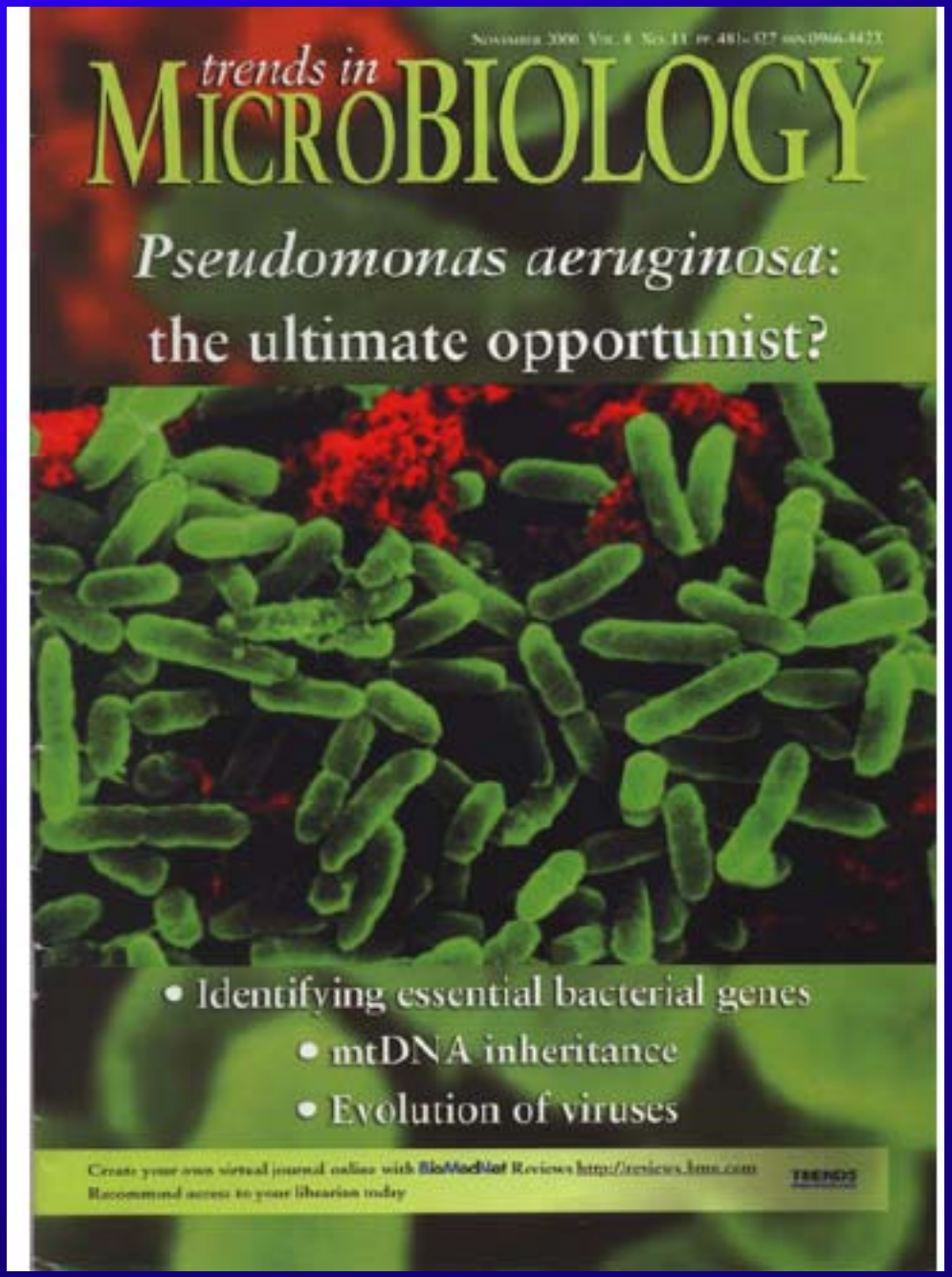
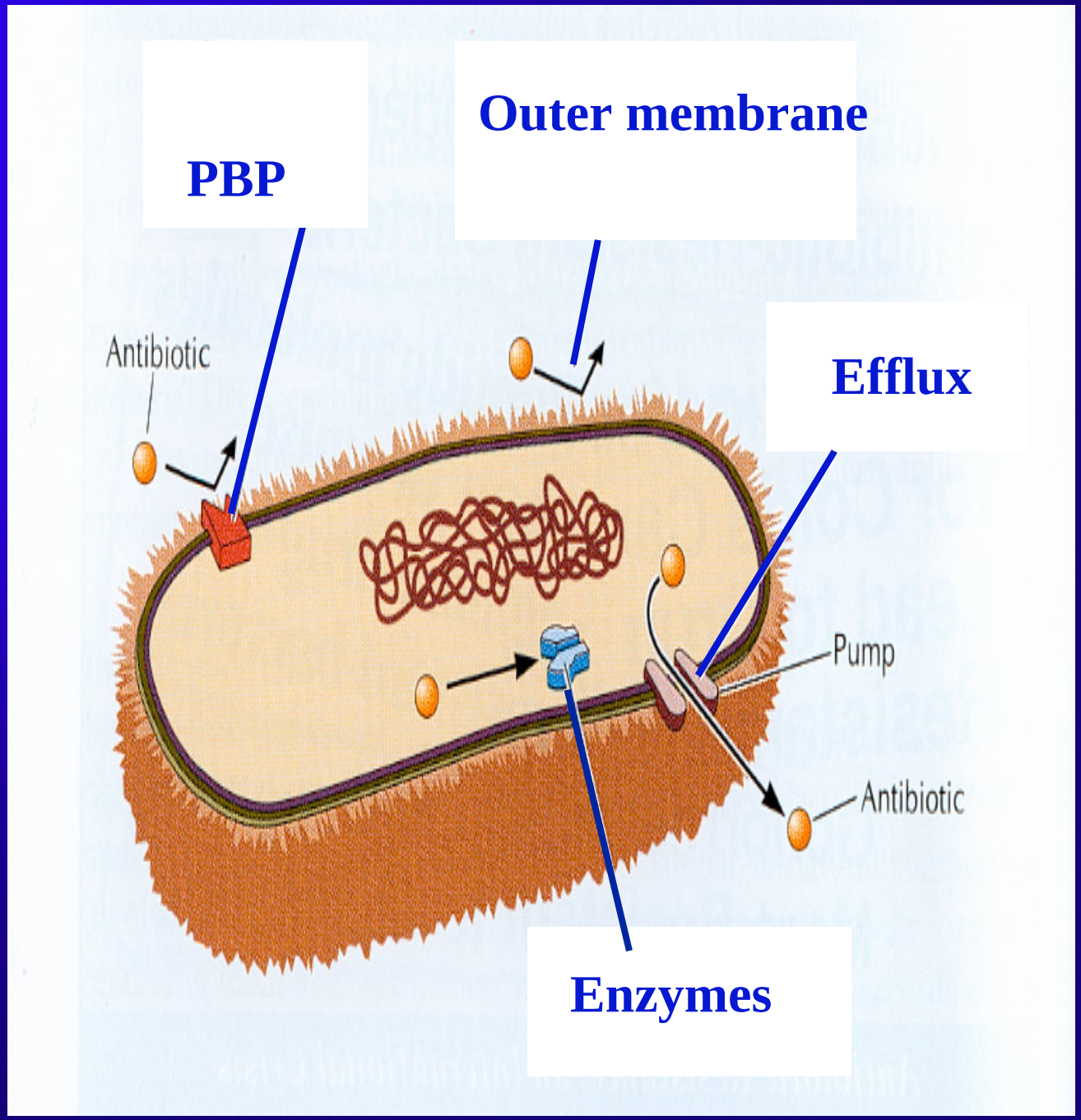
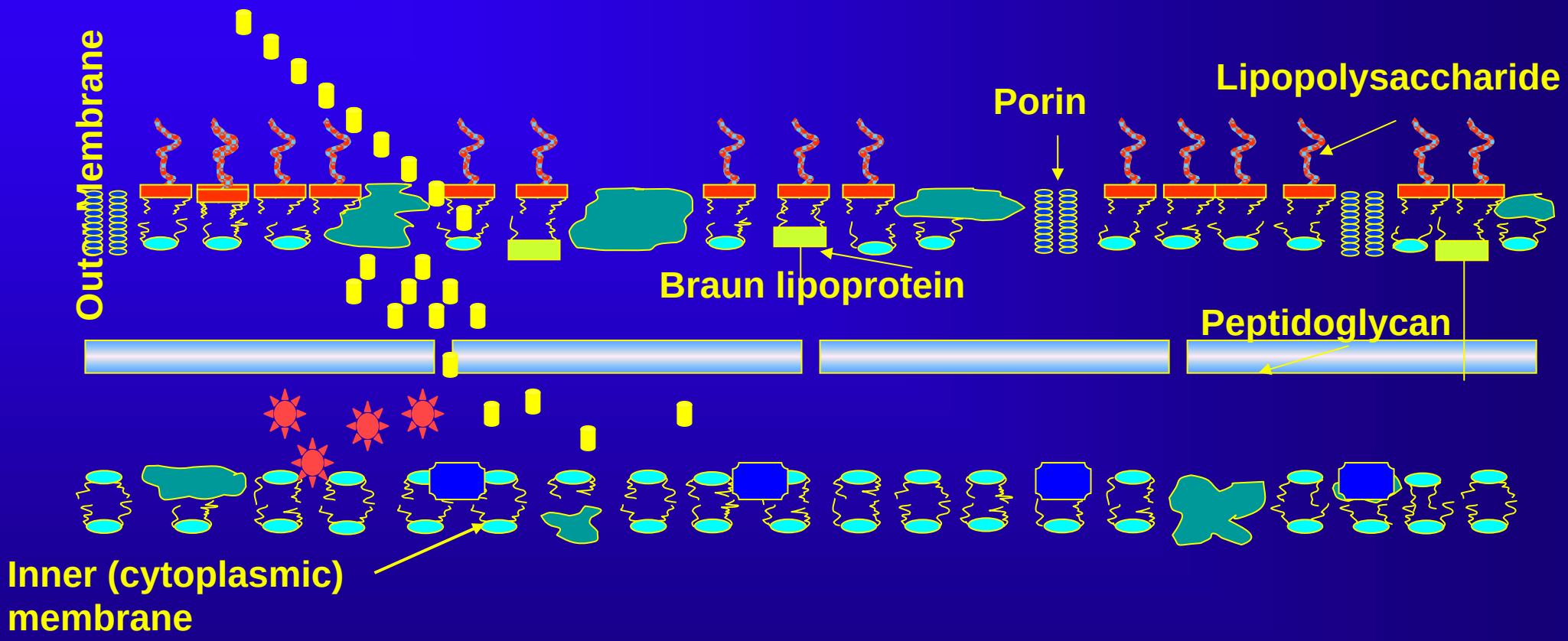

trends in
MICROBIOLOGY
Pseudomonas aeruginosa:
the ultimate opportunist?
• Identifying essential bacterial genes
• mtDNA inheritance
• Evolution of viruses

- Outermembrane permeability (100-fold less than that of *E. coli*)
- Efflux : at least twelve systems...
- Naturally-occurring cephalosporinase and oxacillinase



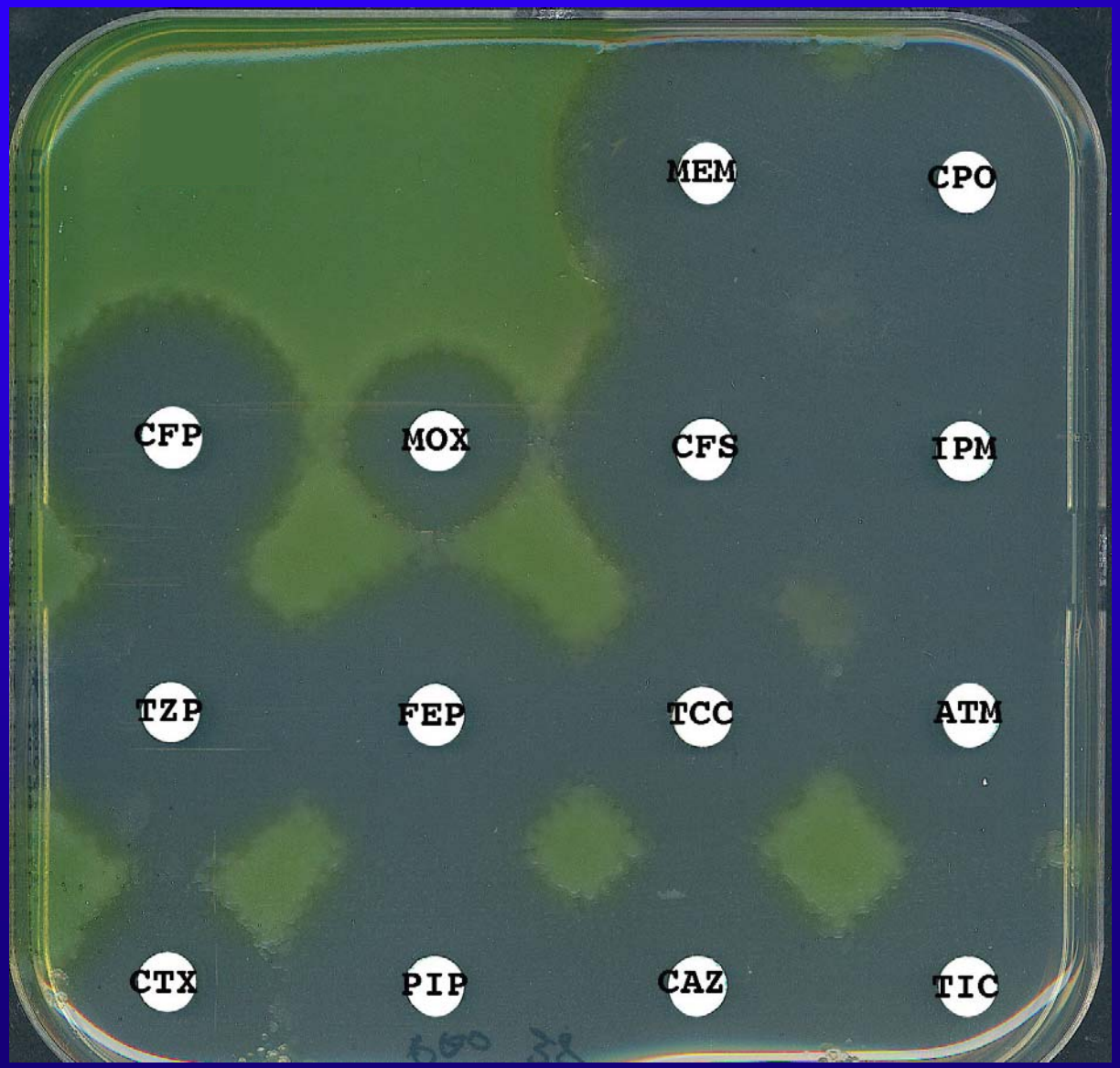
Gram-negatives



Cytoplasm

ß-Lactam resistance = +++ ß-lactamases

Wild-type *P. aeruginosa*



Natural resistance :

- aminopénicillines ; aminopénicilline/a. clav.
- C1G, C2G
- C3G (cefotaxime, ceftriaxone)
- ertapénème

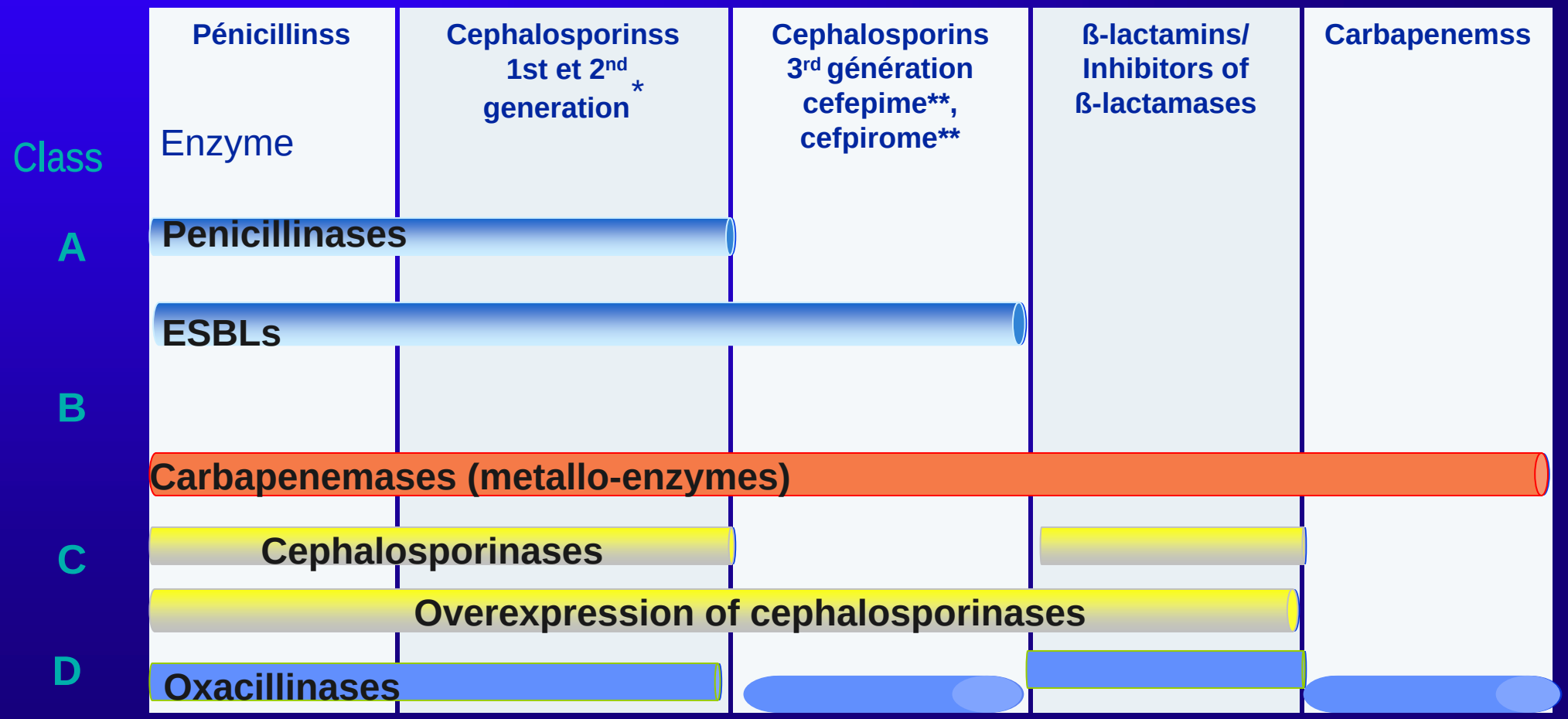
Natural susceptibility:

- carboxypenicillins (ticarcillin)
- ureidopenicillins (piperacillin)
- cephalosporins (ceftazidime, cefsulodin, cefepime, cefpirome)
- monobactam (aztreonam)
- carbapenems (imipenem, meropenem)

Acquired resistance to ß-lactams; ß-lactamases

- Overexpression of cephalosporinase
- Restricted-spectrum penicillinases
- Expanded-spectrum ß-lactamases (ESBLs)
- Carbapenemases (metallo-ß-lactamases)
- Expanded-spectrum oxacillinases

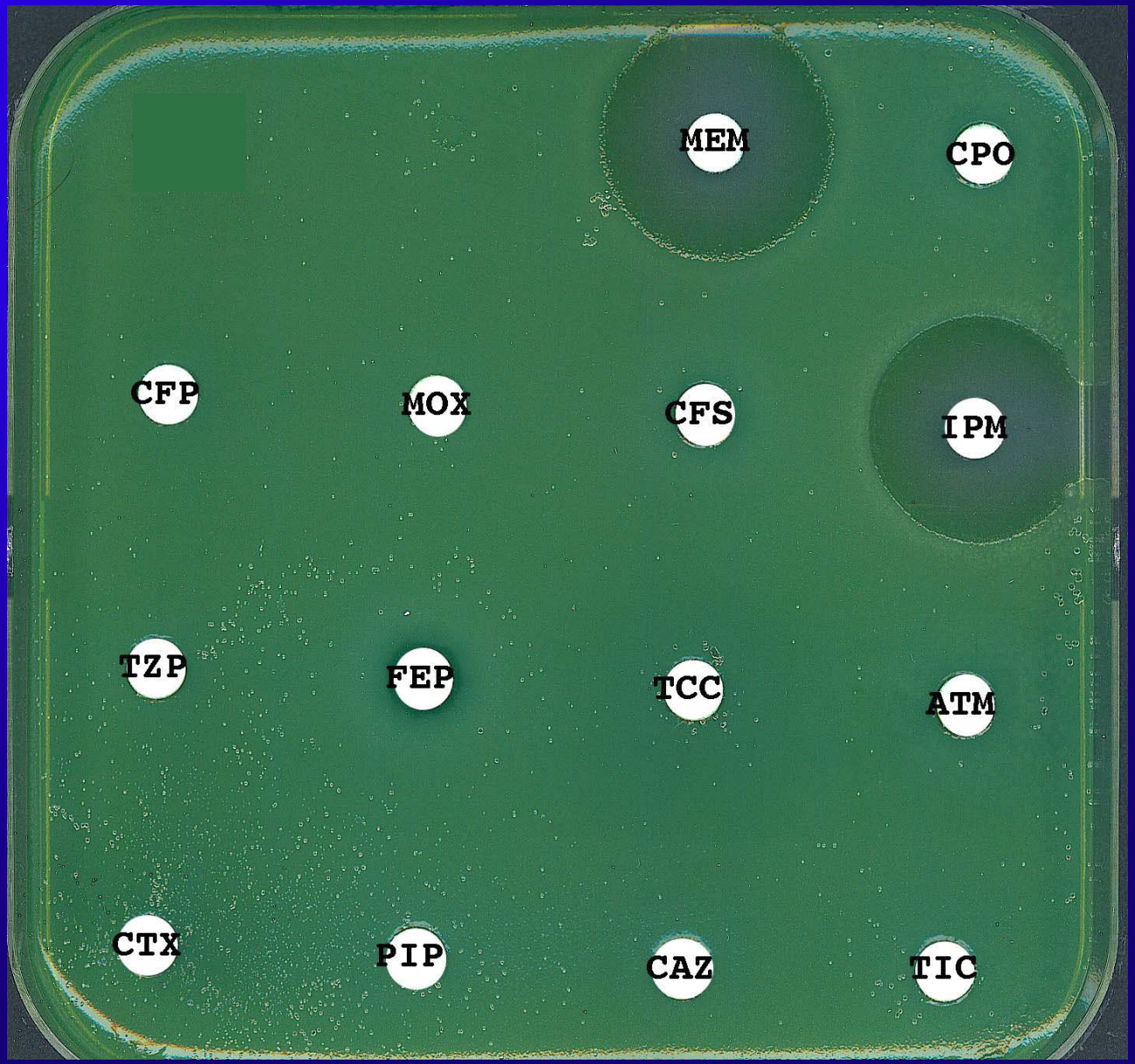
In-vitro spectrum of ß-lactamases



* Cephamycins excluded for ESBLs

** Cefepime, cefpirome excluded for overexpressed cephalosporinase

Overexpressed cephalosporinases



- Resistance to all β-lactams except to carbapenems

- 9-12 % of the strains

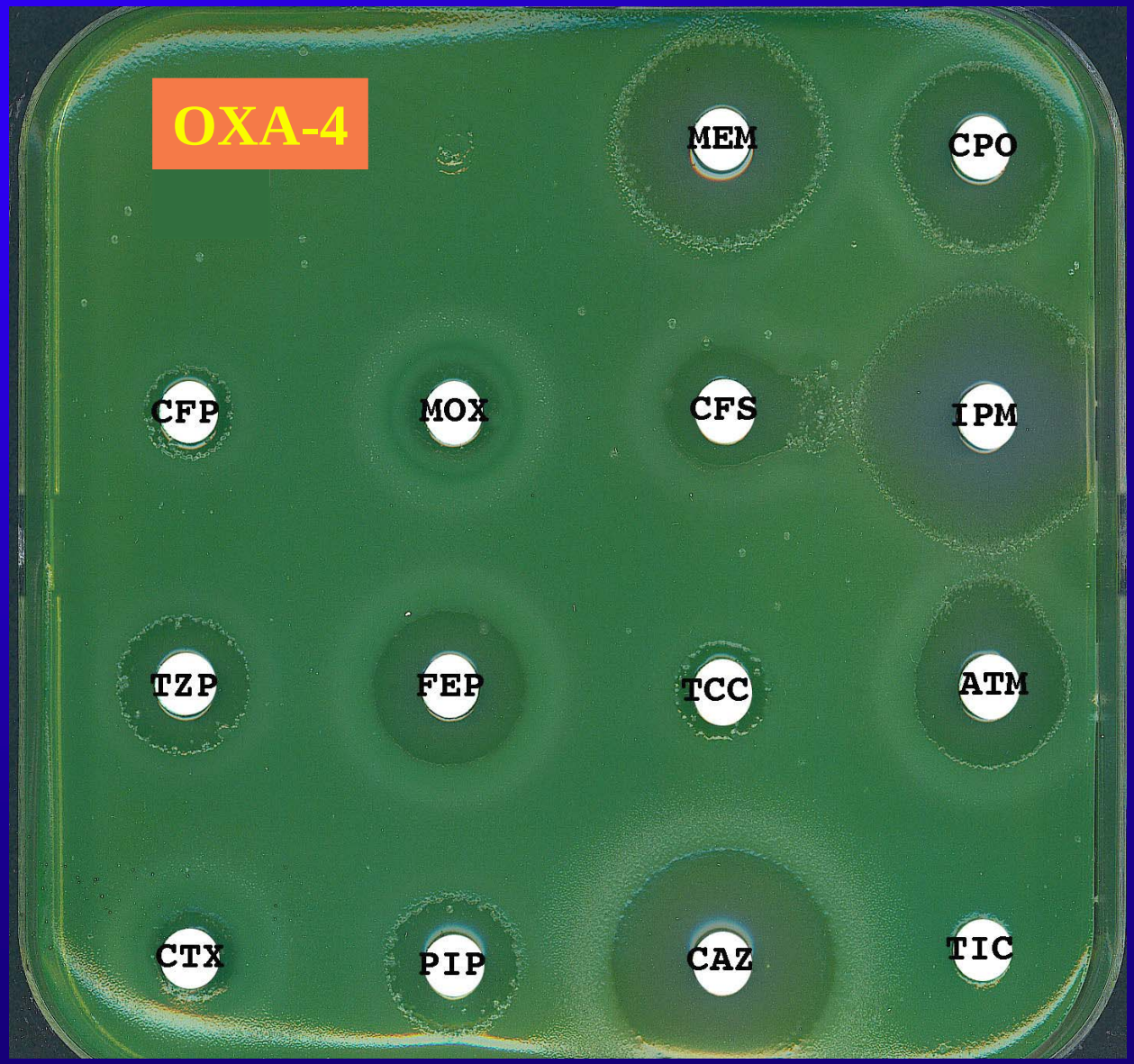
Narrow-spectrum penicillinases

- **Carbenicillinases (PSE), oxacillinases (OXA), TEM**

➡ **resistance to : ticarcillin, piperacillin, cefsulodin**
➡ **intermediated susceptibility to : cefepime, cefpirome, aztreonam**
➡ **susceptibility to : ceftazidime, imipenem,meropenem**

- **10 % of** *P. aeruginosa* **strains (90 % with PSE-1).**

- **Non-inhibitory activity of inhibitors against oxacillinases and PSE-1**


OXA-4
MEM
CPO
CFP
MOX
CFS
IPM
TZP
FEP
TCC
ATM
CTX
PIP
CAZ
TIC

Extended-spectrum ß-lactamases; ESBL (Ambler class A)

- **TEM, SHV, VEB, PER, GES, BEL**

➡ **ressitance to all ß-lactams except to carbapenems**

➡ **inhibition by clavulanic acid, tazobactam, sulbactam**

- **An exception : GES-2**

➡ **Additional resistance to carbapenems**

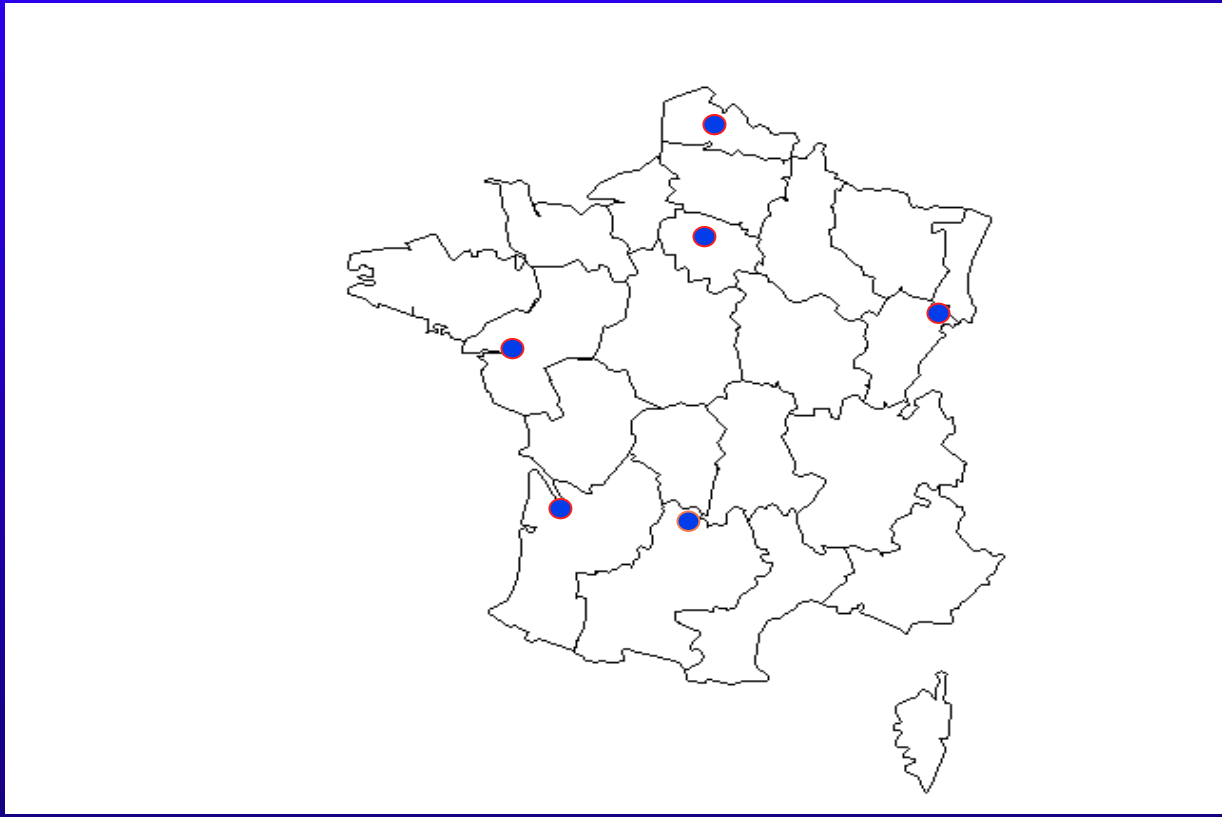
PER-1
MEM
CPO
CFP
MOX
CFS
IPM
TZP
FEP
TCC
ATM
CAZ
CTX
PIP
TIC

VEB-1
MEM
CPO
CFP
MOX
CFS
IPM
TZP
FEP
TCC
ATM
CAZ
CTX
PIP
TIC

Clavulanic-acid inhibited ESBLs in *P. aeruginosa*

ESBLs in *P. aeruginosa*

Distribution in France



Detection

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2005, p. 3743–3748 Vol. 49, No. 9
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.9.3743–3748.2005
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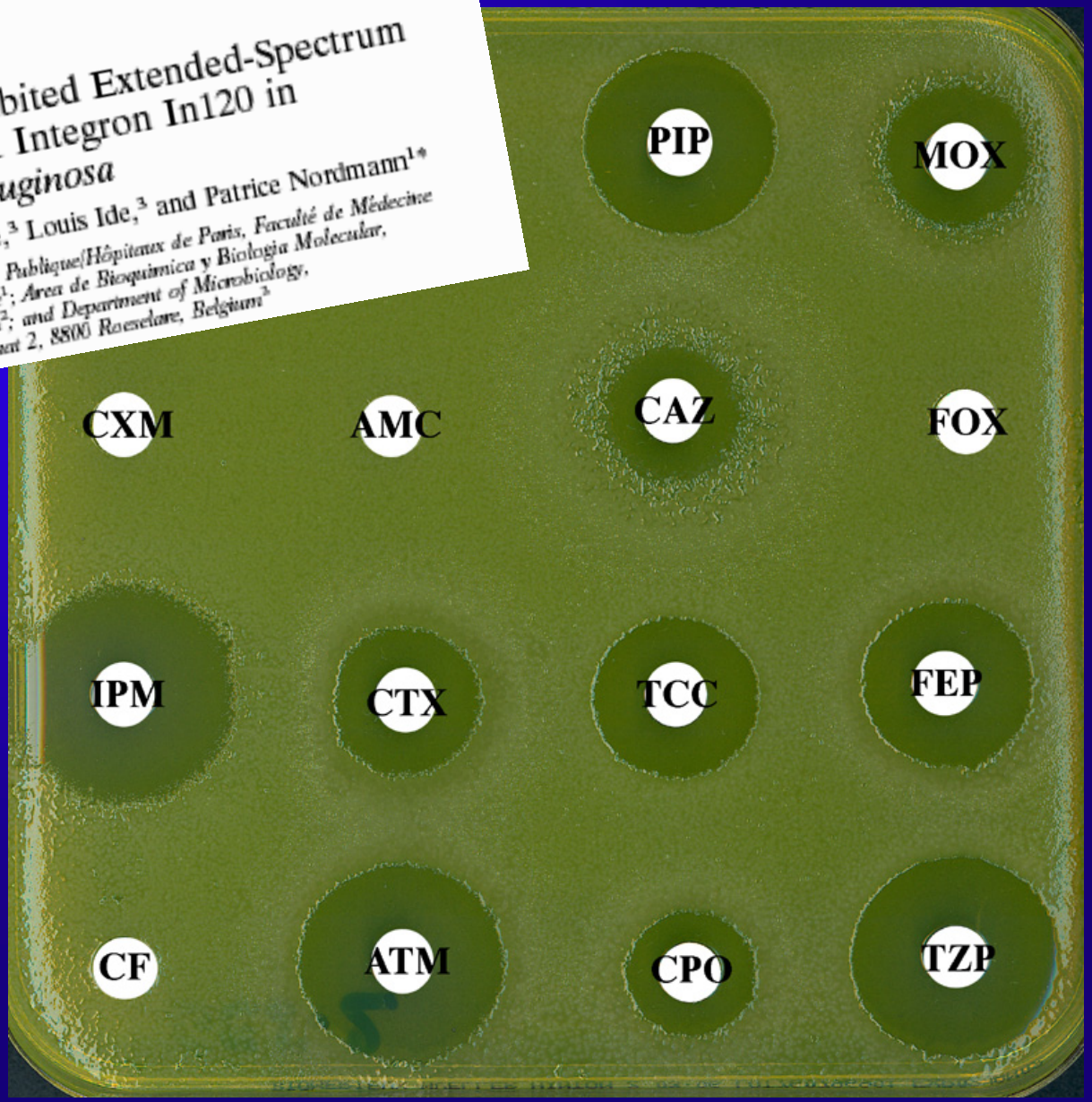
BEL-1, a Novel Clavulanic Acid-Inhibited Extended-Spectrum β-Lactamase, and the Class 1 Integron In120 in *Pseudomonas aeruginosa*

Laurent Poirel,[1] Laura Brinas,[1,2] Annemie Verlinde,[3] Louis Ide,[3] and Patrice Nordmann[1]*
Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris XI, Le Kremlin-Bicêtre, France[1]; Area de Bioquimica y Biologia Molecular, Universidad de La Rioja, 26006 Logrono, Spain[2]; and Department of Microbiology, Heilig Hartziekenhuis Roeselare, Wilgenstraat 2, 8800 Roeselare, Belgium[3]



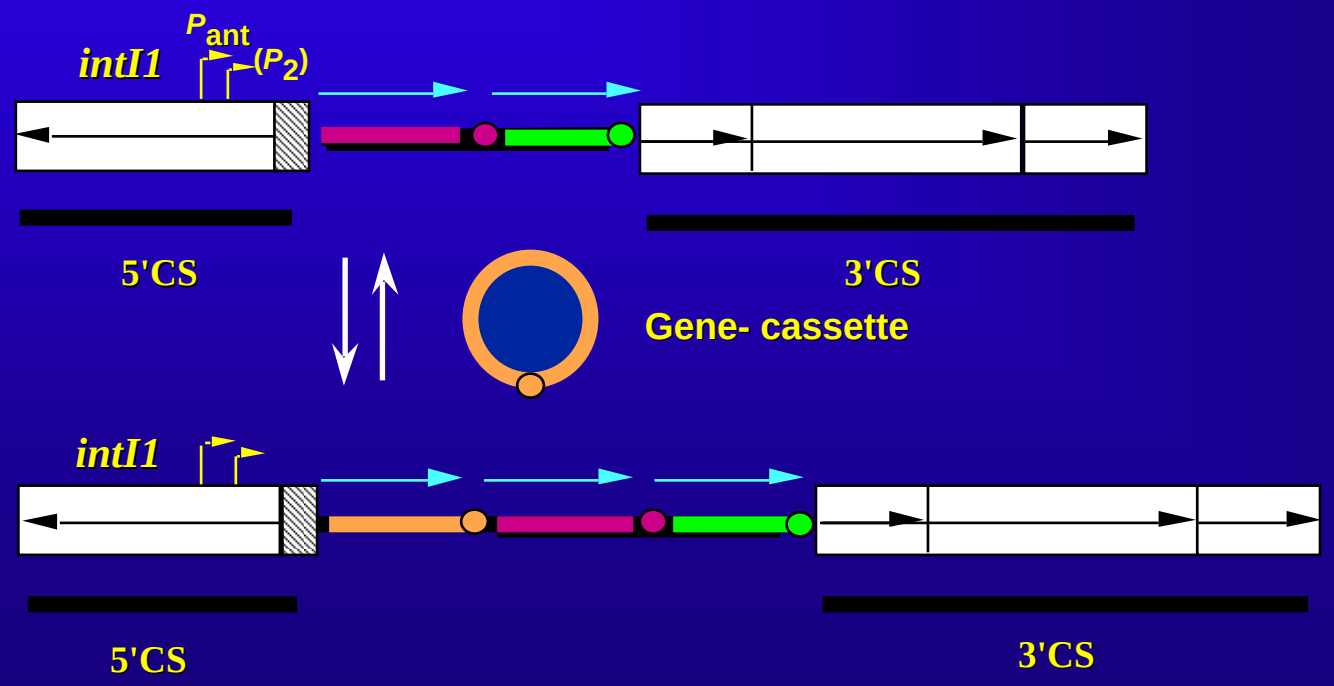
P. aeruginosa: GES-2

GES-1/GES-2: Gly170Asn

170

ESBLs and *P. aeruginosa*

- ESBL genes associated with insertion sequence and transposons (SHV, PER-1) ,with class 1 integron and specific sequences (Re); VEB, GES, BEL-1



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

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2005, p. 1708–1713 Vol. 49, No. 5
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.5.1708–1713.2005
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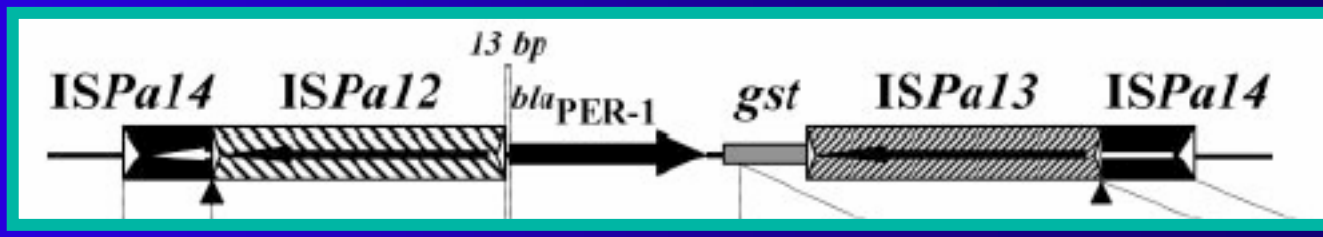
Genetic Environment and Expression of the Extended-Spectrum β-Lactamase bla_{PER-1} Gene in Gram-Negative Bacteria

Laurent Poirel,[1] Ludovic Cabanne,[1] Haluk Vahaboglu,[2] and Patrice Nordmann[1]*



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2004, p. 3284–3290 Vol. 48, No. 9
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.9.3284–3290.2004
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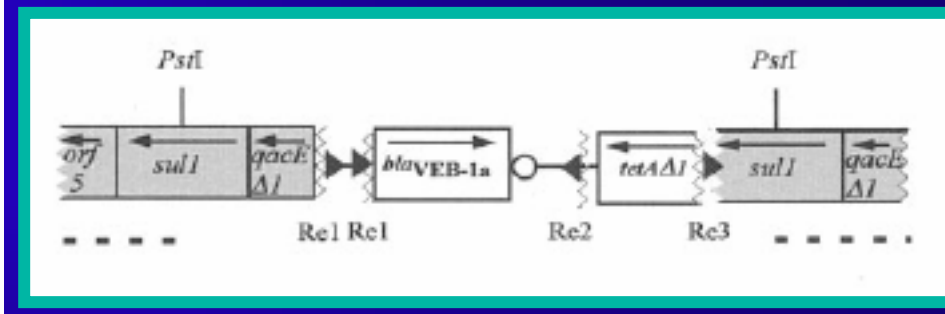
Functional and Structural Characterization of the Genetic Environment of an Extended-Spectrum β-Lactamase bla_{VEB} Gene from a *Pseudomonas aeruginosa* Isolate Obtained in India

Daniel Aubert,[1] Delphine Girlich,[1] Thierry Naas,[1] Shanta Nagarajan,[2] and Patrice Nordmann[1]*



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2006, p. 001 Vol. 50, No. 5
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.5.000–000.2006
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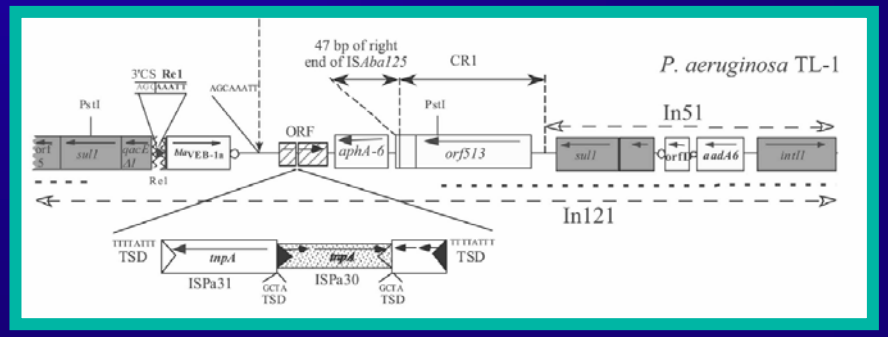
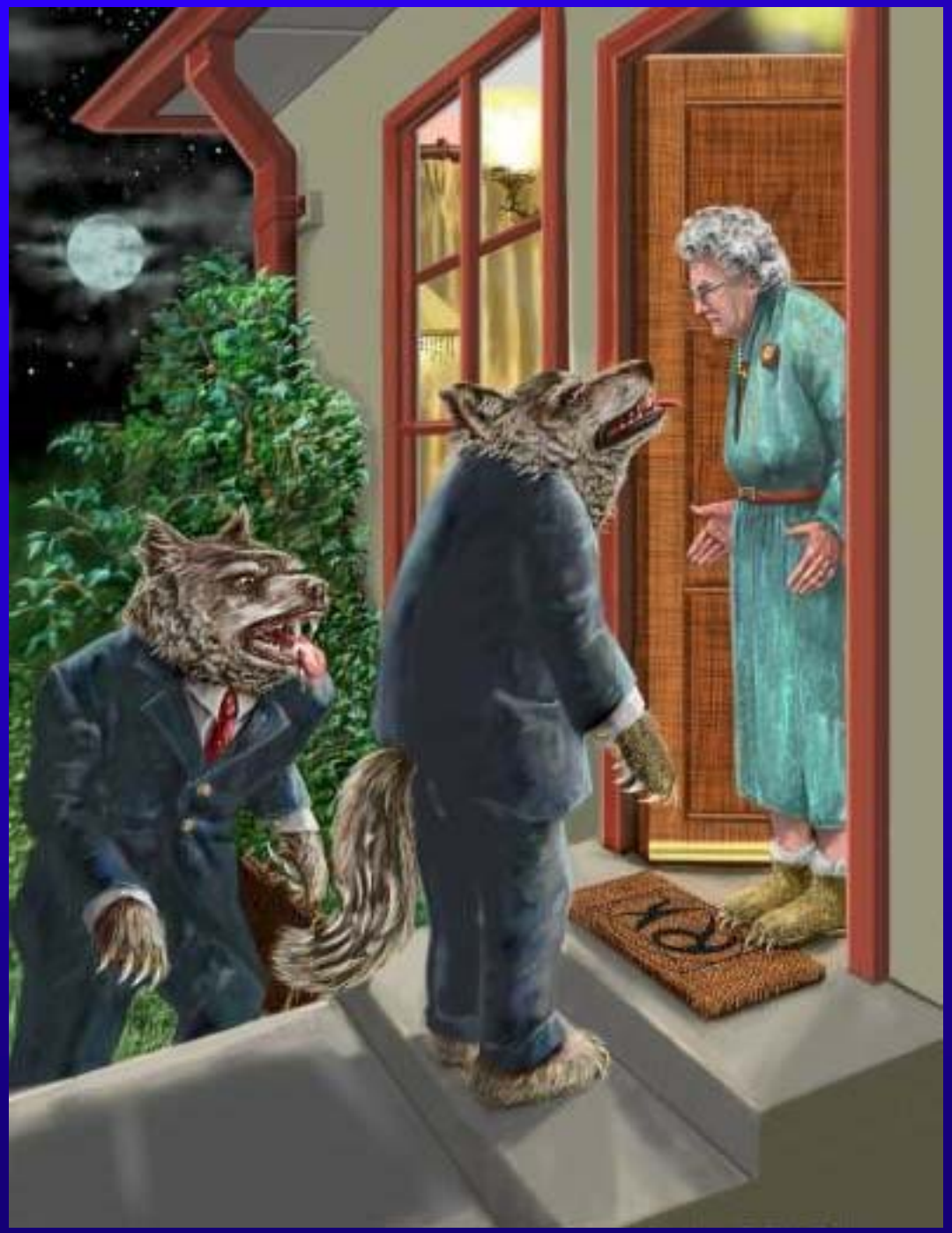
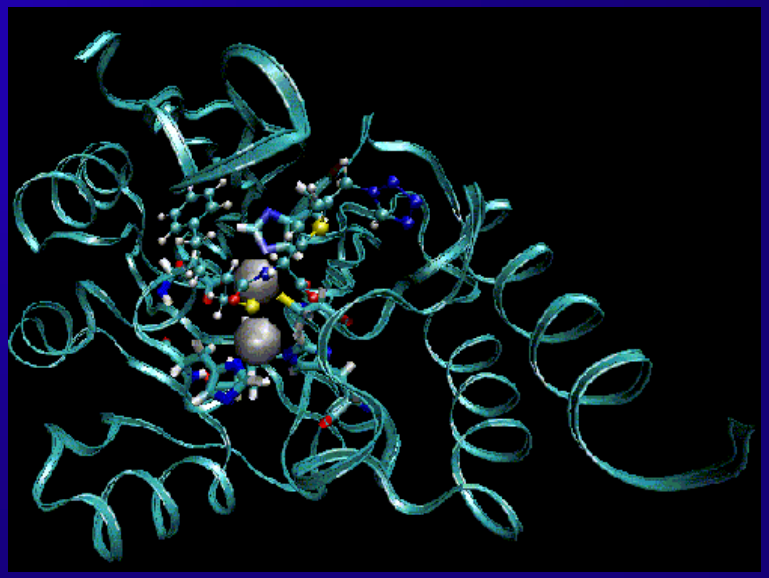
Complex Genetic Structures with Repeated Elements, a *sul*-Type Class 1 Integron, and the bla_{VEB} Extended-Spectrum β-Lactamase Gene

Thierry Naas,[1]* Daniel Aubert,[1] Thierry Lambert,[2] and Patrice Nordmann[1]

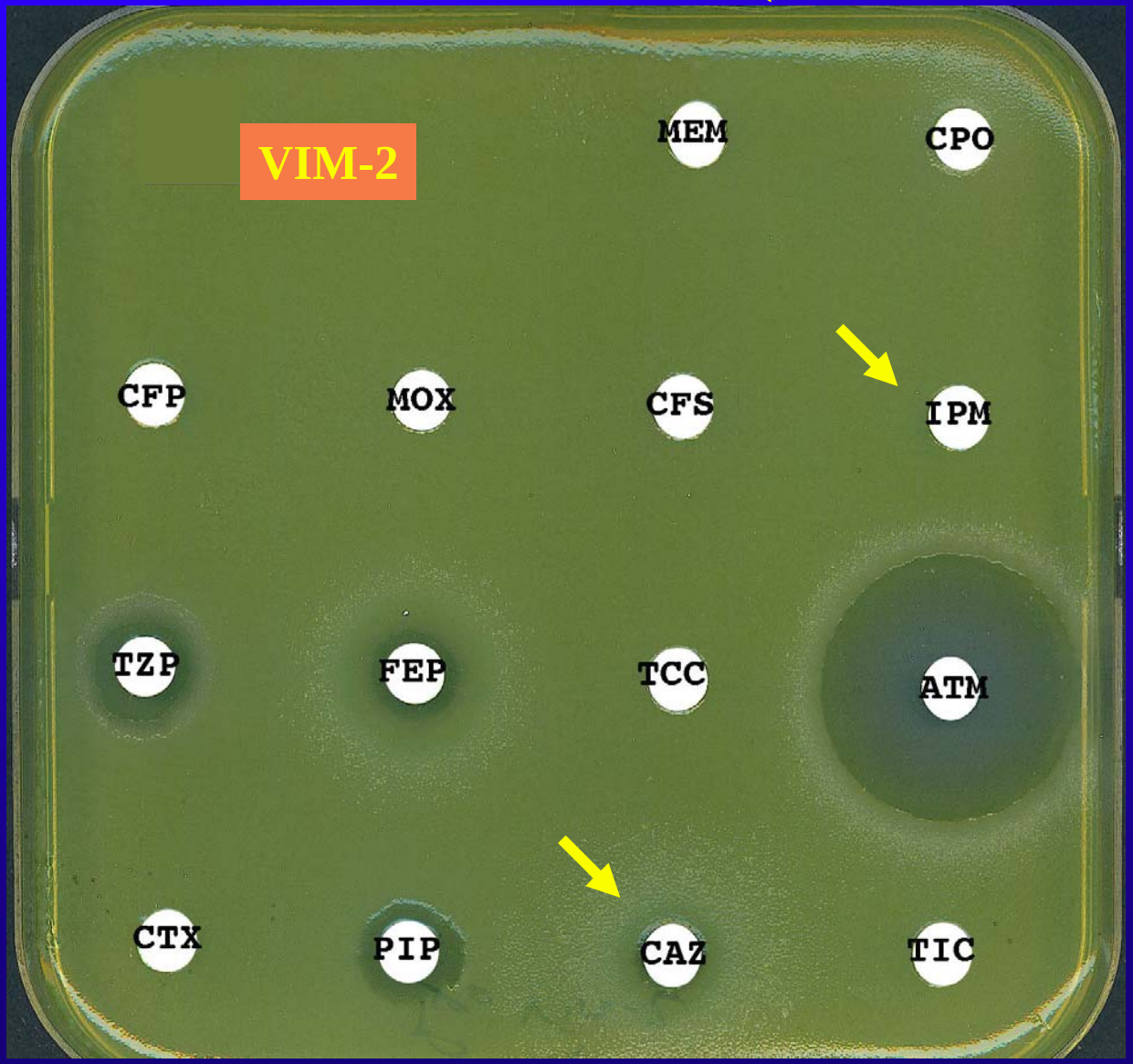




Metallo-ß-lactamases

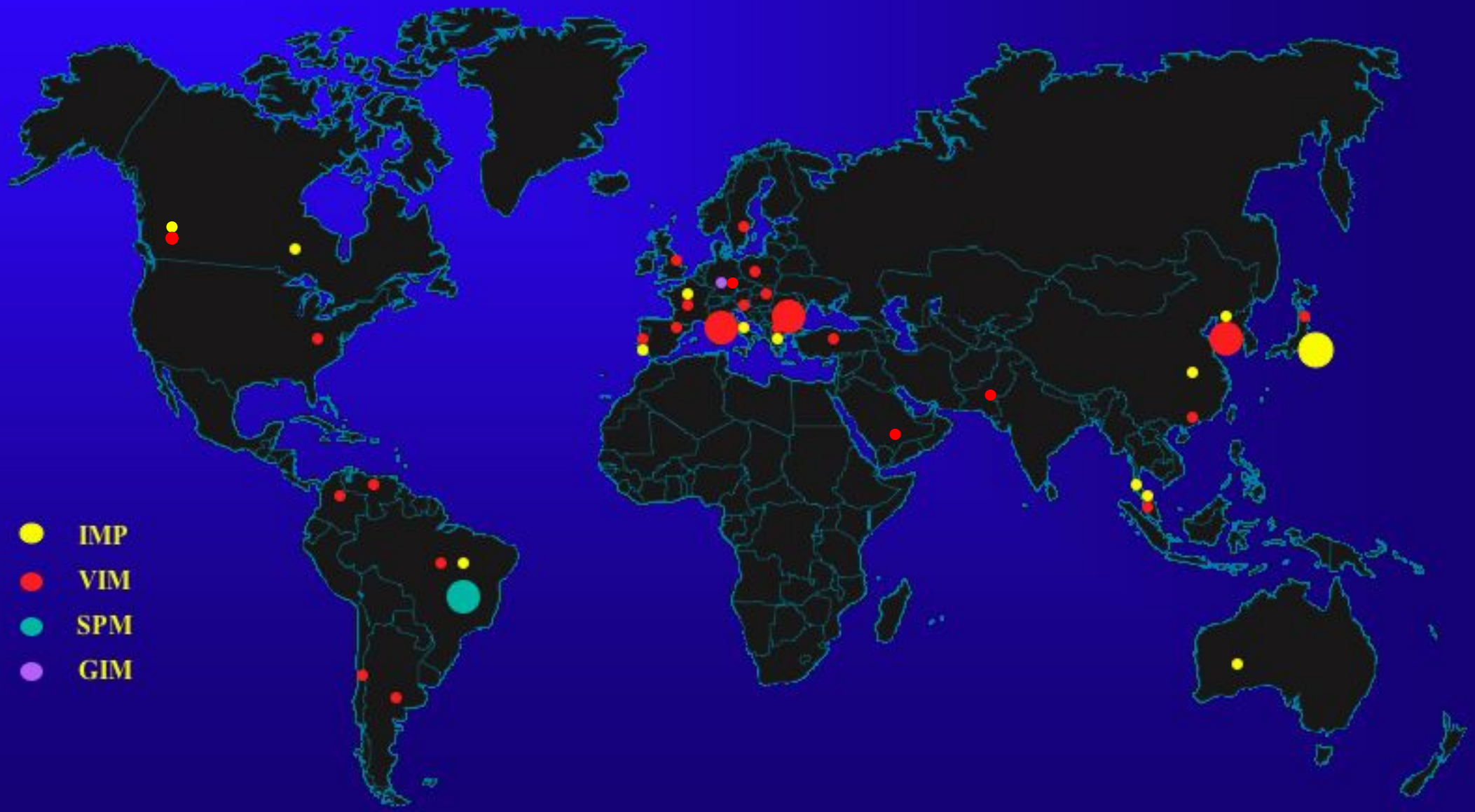


Metallo-ß-lactamases: carbapenemases (Ambler class B)

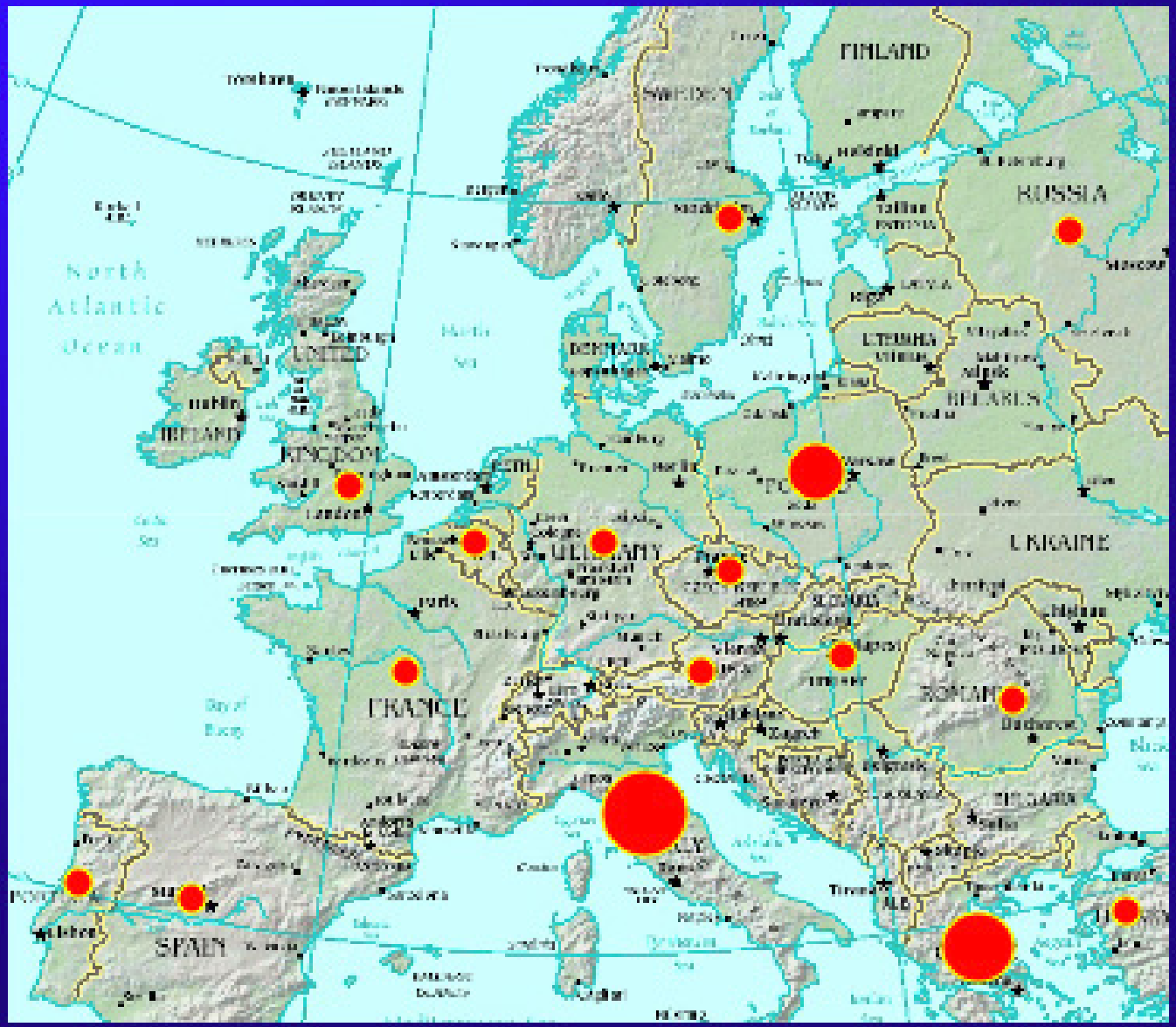


- Resistance to all ß-lactamins except to aztreonam
- Genetic background; plasmids, transposons, integrons…
- Associated resistance; aminoglycosides ++

Ambler class B carbapenemases in *P. aeruginosa*



Metallo-ß-lactamases in Europe



Metallo-ß-lactamases

Distribution in France

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2000, p. 891–897 Vol. 44, No. 4
0066-4804/00/$04.00+0
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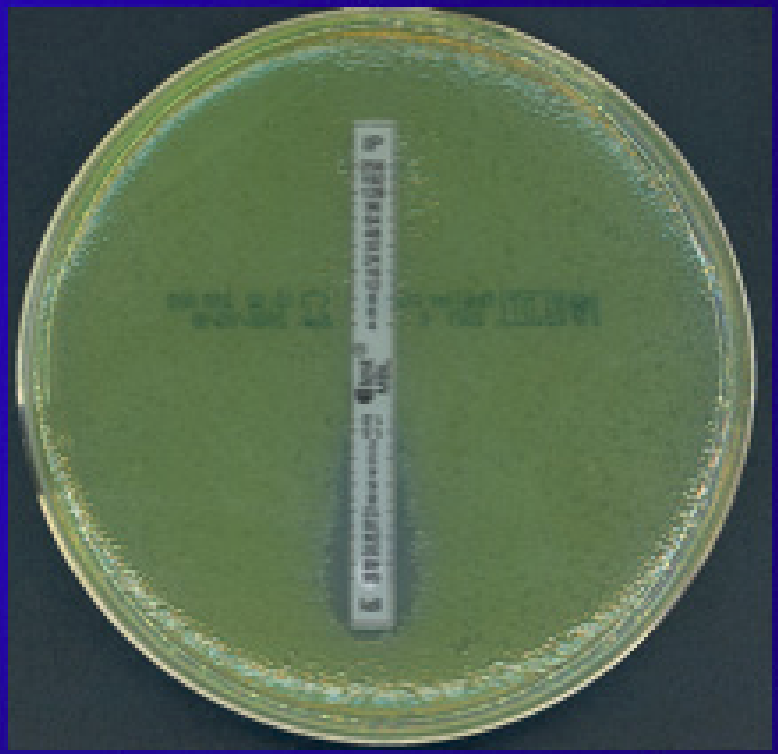
Characterization of VIM-2, a Carbapenem-Hydrolyzing Metallo-β-Lactamase and Its Plasmid- and Integron-Borne Gene from a *Pseudomonas aeruginosa* Clinical Isolate in France

LAURENT POIREL,[1] THIERRY NAAS,[1] DELPHINE NICOLAS,[1] LOUIS COLLET,[2] SAMUEL BELLAIS,[1] JEAN-DIDIER CAVALLO,[3] AND PATRICE NORDMANN[1]*

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre,[1] Laboratoire de Biologie, Institut Paoli-Calmettes, 13273 Marseille,[3] and Laboratoire de Biologie Médicale, Hôpital d'Instruction des Armées Bégin, 94160 Saint Mandé,[2] France

Detection



Heterogeneous expression of metallo-enzymes

(Senda *et al*. Antimicrob. Agents Chemother. 1996 ; 40, 349-353)

	MICs (μg/ml)			
	IMP	**MPM**	**CAZ**	**AZT**
P. aeruginosa				
MNA 1456	**>128**	**>128**	**>128**	**>128**
MNA 14102	**>128**	**>128**	**>128**	**64**
MNA 14115	**32**	**64**	**>128**	**16**
MNA 1560	**2**	**16**	**>128**	**32**

Metallo-β-Lactamases: epidemiology

Country	Year(s)	Species	Isolates	Prevalence
Japan	92-94	*P. aeruginosa*	3700 (17 hosp.)	**0.4%** (IMP)
Japan	96-97	*P. aeruginosa* *S. marcescens*	2533 3222 Nationwide	**1.3%** (IMP) **4.4%** (IMP)
Japan	98 & 00	*P. aeruginosa* *Enterobacteriaceae*	200 800 Regional	**0.7%** (IMP)
Japan	98-02	*Gram-negatives*	801 (3 hosp.)	**1.1%** (IMP)
Japan	02	*P. aeruginosa*	594 Nationwide	**1.9%** (IMP/VIM)
Greece	98-00	*Pseudomonas* spp.	233 (1 hosp.)	**0.9%** (VIM)
Italy	01	*P. aeruginosa*	506 (1 hosp.)	**0.8%** (VIM)

Senda *et al*., Antimicrob. Agents Chemother. 1996; 40:349
Kurokawa *et al*., Lancet 1999; 354:955
Yamasaki *et al*., J. Antimicrob. Chemother. 2003; 51:631

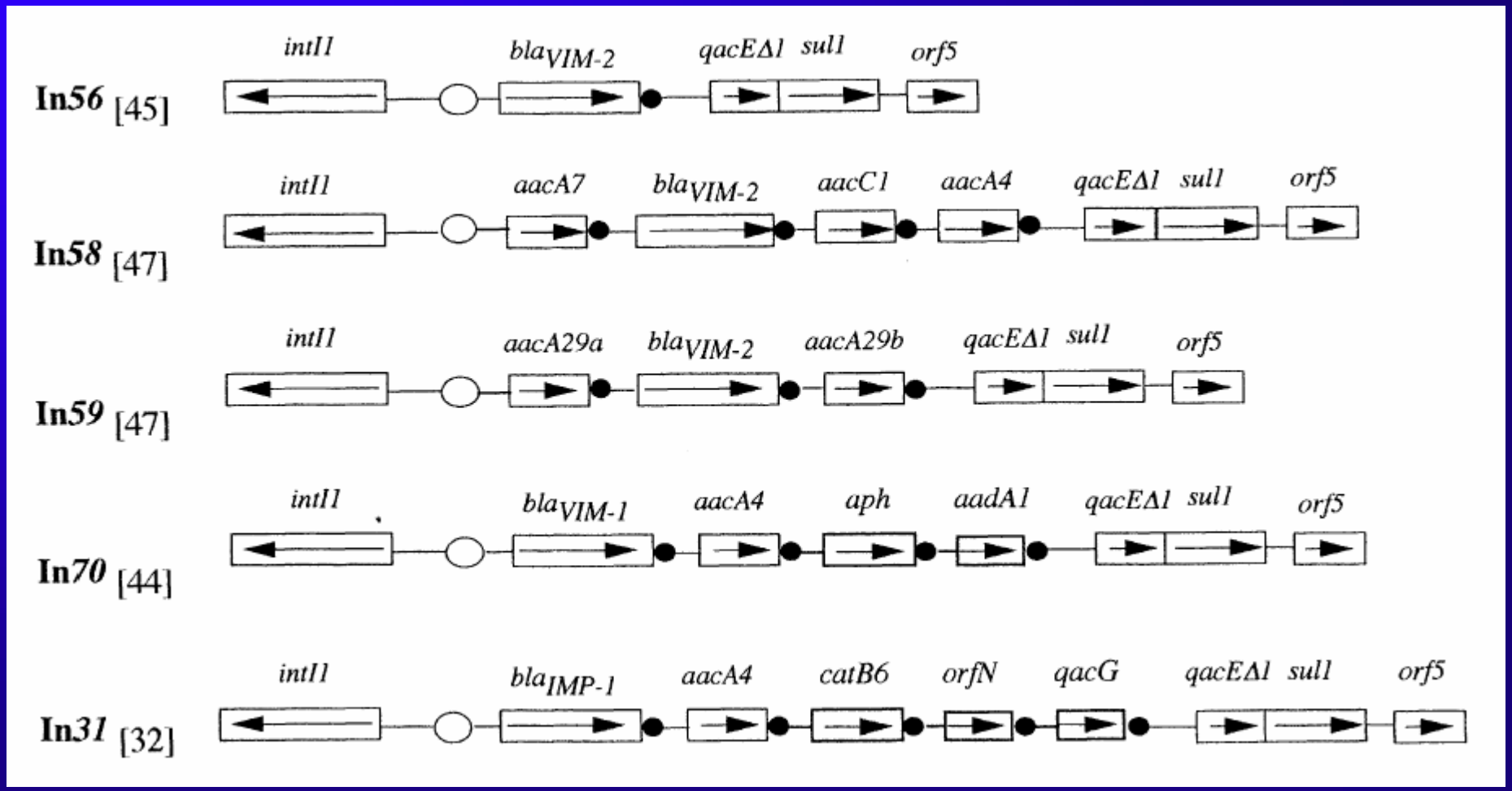
Tsakris *et al*., Clin. Microbiol. Infect. 2003; 9:846
Luzzaro *et al*., Diagn. Microbiol. Infect. Dis. 2004; 48:13
Jones *et al*., Diagn. Microbiol. Infect. Dis. 2004; 49: 289
Kimura *et al*., J. Clin, Microbiol. 2005; 43: 458

Metallo-β-Lactamases: Impact on resistance

Country	Year(s)	Species	Phenotype	Prevalence
Japan	92-94	*P. aeruginosa*	IMI-R CAZ-R	**11%** (IMP)
Japan	91-96	*P. aeruginosa* *Enterobacteriaceae*	CAZ-R	**25%** (IMP) **32%** (IMP)
Japan	01-02	*P. aeruginosa* *Other GNNFs* *Enterobacteriaceae*	CAZ-R PER/SUL-R	**62%** (IMP) **11%** (VIM)
Korea	00-01	*Pseudomonas* spp. *Acinetobacter* spp.	IMI-R/I	**11%** (VIM) **14%** (VIM/IMP)
Greece	01	*P. aeruginosa*	IMI-R/I	**62%** (VIM)
Italy	99-02	*P. aeruginosa*	IMI-R	**39%** (VIM/IMP)

Senda *et al*., Antimicrob. Agents Chemother. 1996; 40:349
Hirakata *et al*., Antimicrob. Agents Chemother. 1998; 42:2006
Shibata *et al*., J.Clin.Microbiol. 2003; 41:5407
Lee *et al*., Emerg. Infect. Dis.. 2003; 9:868
Giakkoupi *et al*., J.Clin.Microbiol. 2003; 41:822
Toleman *et al*., J. Antimicrob. Chemother. 2005; 55:61

Associated resistance….



Nordmann P, Poirel L. **Emerging carbapenemases in Gram-negative aerobes**. Clin Microbiol Infect. 2002 .
Walsh TR, Toleman MA, Poirel L, Nordmann P. **Metallo ß-lactamases; the quiet before the storm**. Clin Microbol Rev 2005

Extended-spectrum oxacillinases (Ambler class D)

ß-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-31	512	512	256	256	2	256	32	

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

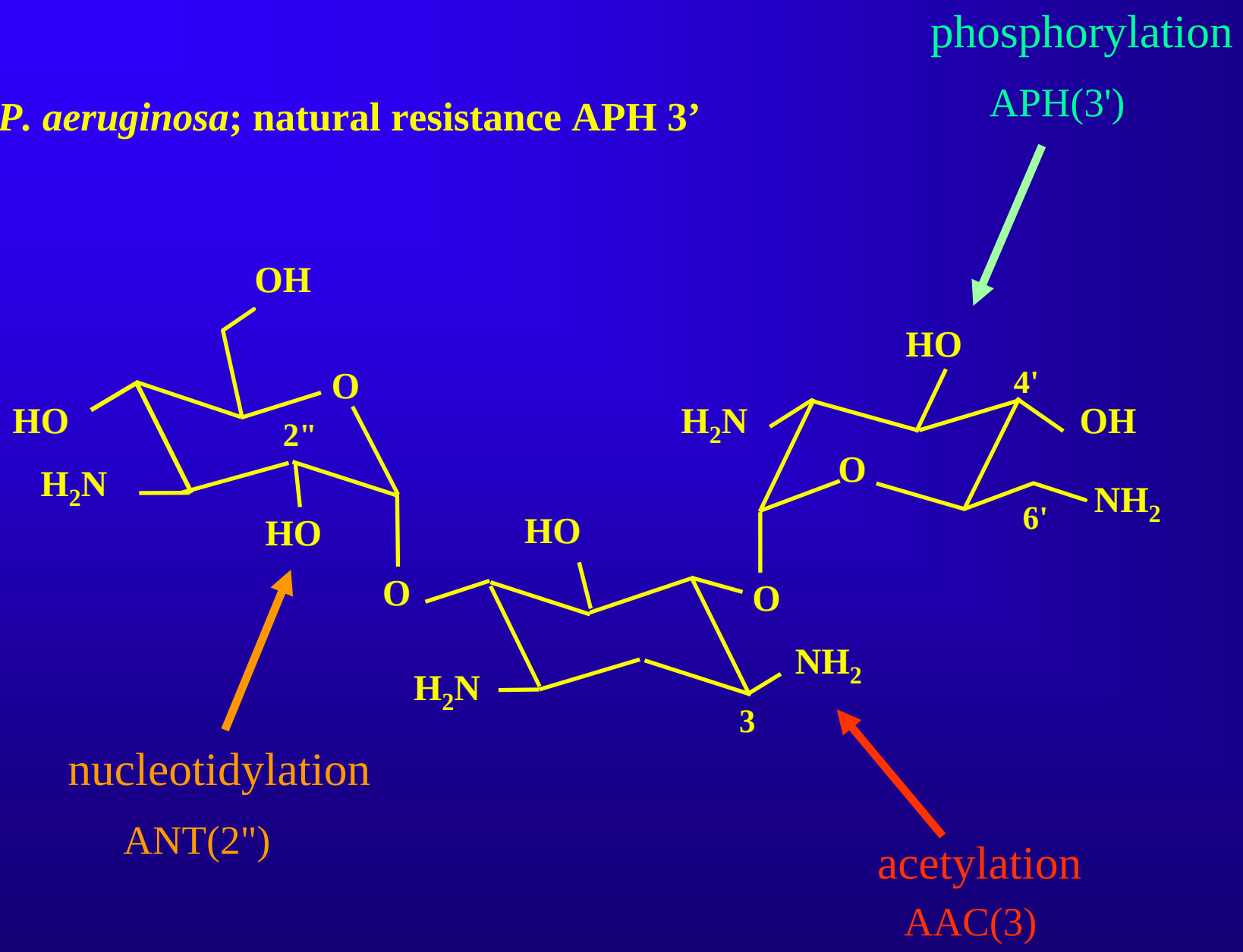
Detection ? Epidemiology ?

Diversity of oxacillinase resistance patterns

Aminoglycoside resistance

- Efflux
- Outer membrane permeability defect
- Modification enzymes
- Target alteration

***P. aeruginosa*; natural resistance APH 3'**



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2001, p. 546–552 Vol. 45, No. 2
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.2.546–552.2001
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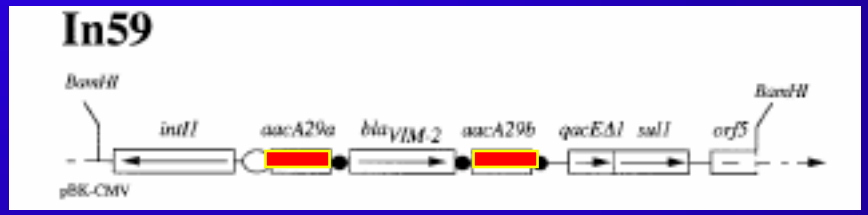
Characterization of Class 1 Integrons from *Pseudomonas aeruginosa* That Contain the bla_{VIM-2} Carbapenem-Hydrolyzing β-Lactamase Gene and of Two Novel Aminoglycoside Resistance Gene Cassettes

LAURENT POIREL,[1] THIERRY LAMBERT,[2] SALIH TÜRKOGLÜ,[1,3] ESTHEL RONCO,[4]
JEAN-LOUIS GAILLARD,[4] AND PATRICE NORDMANN[1]*



ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2003, p. 1577–1583 Vol. 47, No. 5
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.5.1577–1583.2003
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Aminoglycoside Resistance Resulting from Tight Drug Binding to an Altered Aminoglycoside Acetyltransferase

Sophie Magnet,[1] Terry-Ann Smith,[2] Renjian Zheng,[1] Patrice Nordmann,[3] and John S. Blanchard[1]*

Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461[1]; Cornell University, Ithaca, New York 14853[2]; and Service de Bactériologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre, France[3]

Mechanisms of disease

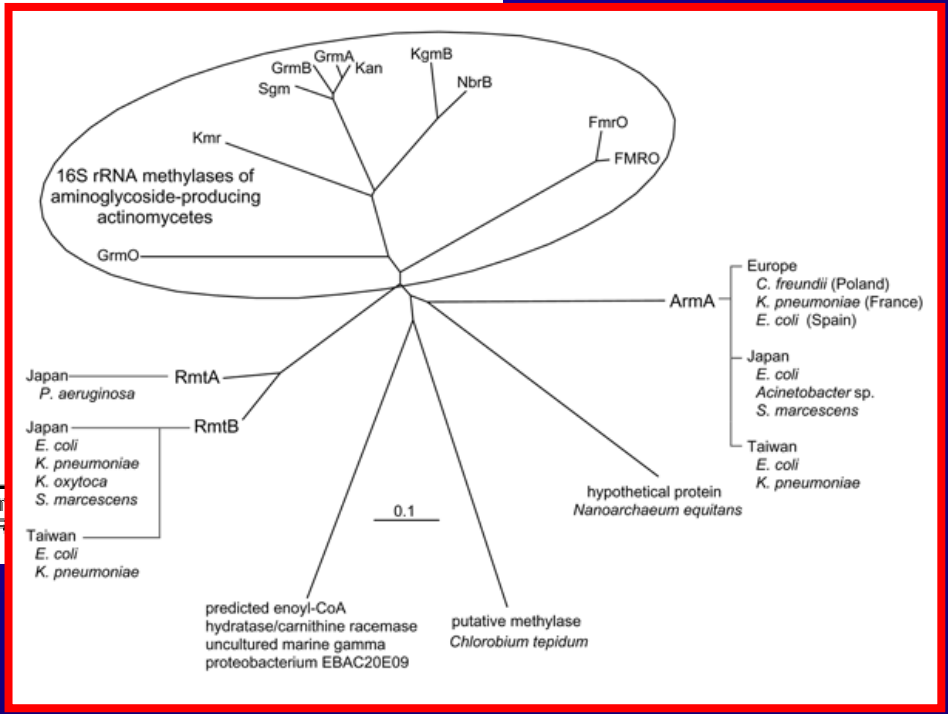
THE LANCET • Vol 362 • December 6, 2003 • www.thelancet.com

Acquisition of 16S rRNA methylase gene in *Pseudomonas aeruginosa*

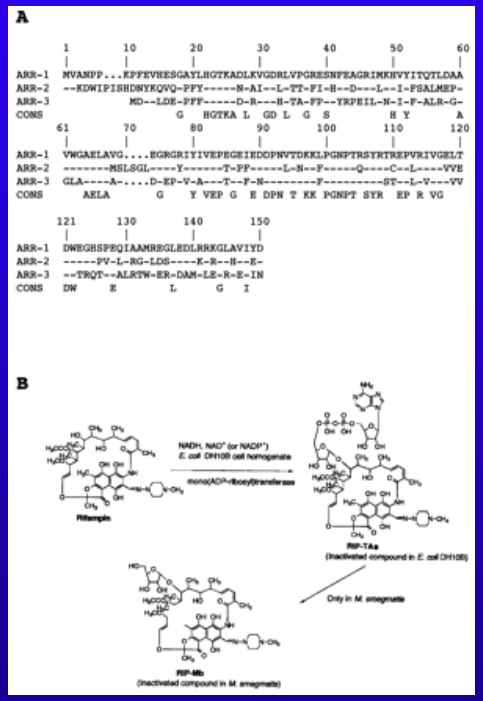
Keiko Yokoyama, Yohei Doi, Kunikazu Yamane, Hiroshi Kurokawa, Naohiro Shibata, Keigo Shibayama, Tetsuya Yagi, Haru Kato, Yoshichika Arakawa

	P aeruginosa AR-2	*E coli* XL1-blue			*P aeruginosa* PAO1		*P aeruginosa*	
		pBCH9	pBCH9-13	pBC-SK+	pTORmtA	pTO001	Transconjugant	105*
4,6-substituted deoxystreptamine antimicrobials								
Kanamycin groups								
Arbekacin	>1024	>1024	>1024	0·5	>1024			
Amikacin	>1024	>1024	>1024	1	>1024			
Kanamycin	>1024	>1024	>1024	2	>1024			
Tobramycin	>1024	>1024	512	1	>1024			
Gentamicin groups								
Gentamicin	>1024	>1024	1024	0·5	>1024			
Sisomicin	>1024	512	>1024	0·5	>1024			
Isepamicin	>1024	>1024	>1024	0·5	>1024			
4,5-substituted deoxystreptamine antimicrobials								
Neomycin	>1024	4	>1024	4	512			
Other aminoglycosides								
Streptomycin	128	4	2	4	32			
Hygromycin B	1024	64	2	32	512			
Others								
Ceftazidime	2	0·5	0·25	0·25	ND			
Imipenem	1	0·25	0·25	0·125	ND			
Ciprofloxacin	0·25	0·125	0·125	0·125	ND			

ND=not determined. pBCH9, pBCH9-13, pBC-SK+, pTORmtA, and pTO001 are the plasmids harboured by each tr
expression vector. pBCH9=pBC-SK+ + 8 kb insert fragment. pBCH9-13=pBC-SK+ + 1–2 kb insert fragment. pTOR
conjugation study.



Novel mechanism of resistance to rifampicin



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Characterization of In53, a Class 1 Plasmid- and Composite Transposon-Located Integron of *Escherichia coli* Which Carries an Unusual Array of Gene Cassettes

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Nosocomial Spread of the Integron-Located *veb-1*–Like Cassette Encoding an Extended-Spectrum β-Lactamase in *Pseudomonas aeruginosa* in Thailand

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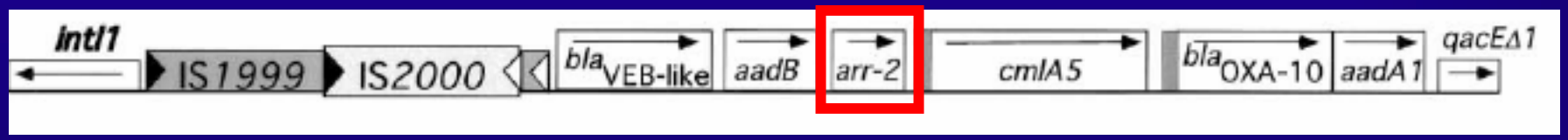
CID 2002:34 (1 March) • 603



Conclusion

- **Emerging mechanisms of resistance; ESBL, metallo-ß-lactamases, novel aminoglycoside resistance genes…**
- **Novel genetic elements for their mobility**
- **Environmental reservoir of resistance genes ?**
- **Increased rate of multidrug-resistant *P. aeruginosa* worldwide**

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Molecular Characterization of an Epidemic Clone of Panantibiotic-Resistant *Pseudomonas aeruginosa*

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