Sources and Spread of *qnr* genes: worldwide impact

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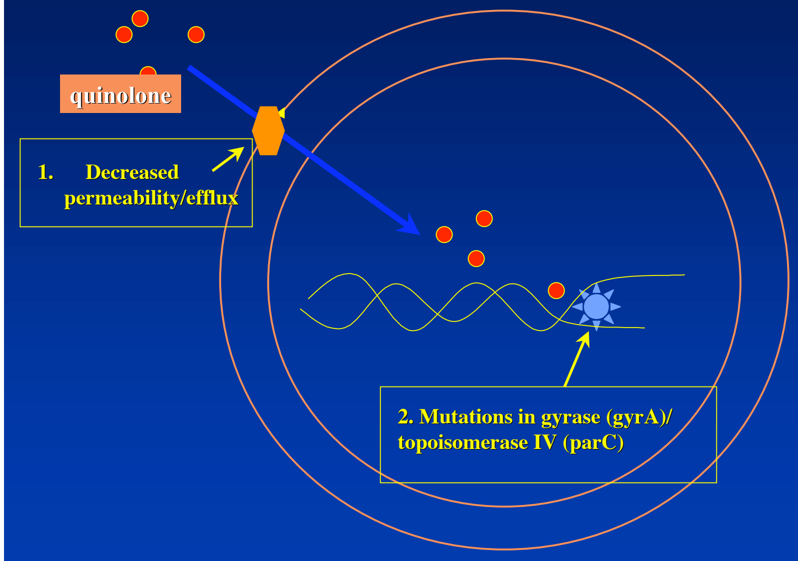
Chromosome-encoded mechanisms of resistance to quinolones in *Enterobacteriaceae*



quinolone

1. Decreased permeability/efflux

2. Mutations in gyrase (gyrA)/ topoisomerase IV (parC)

Plasmid-mediated resistance to quinolones: the « founding fathers »

THE LANCET • Vol 351 • March 14, 1998

Early report

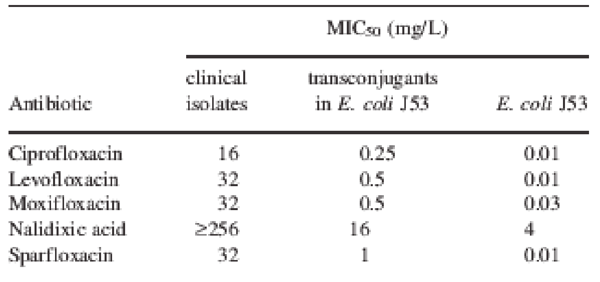
Quinolone resistance from a transferable plasmid

Luis Martínez-Martínez, Alvaro Pascual, George A Jacoby

1998 : Pentapeptide protein Qnr (renamed later QnrA) from a *K. pneumoniae* isolate from USA

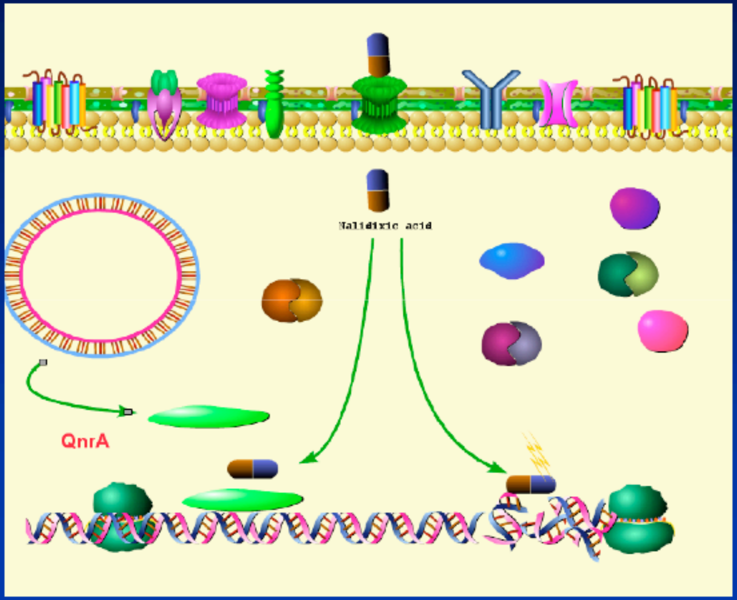
- **Confers increased MIC value of nalidixic acid (4 to 32 mg/l in *E. coli*)**
- **Transferable with a plasmid-mediated cephalosporinase**

MICs of quinolones and of fluoroquinolones



Antibiotic	MIC$_{50}$ (mg/L) clinical isolates	 transconjugants in *E. coli* J53	 *E. coli* J53
Ciprofloxacin	16	0.25	0.01
Levofloxacin	32	0.5	0.01
Moxifloxacin	32	0.5	0.03
Nalidixic acid	≥256	16	4
Sparfloxacin	32	1	0.01

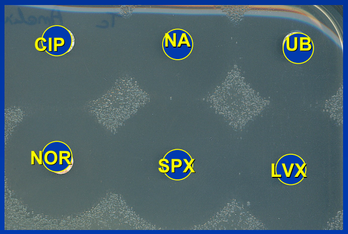
Mechanism of Action



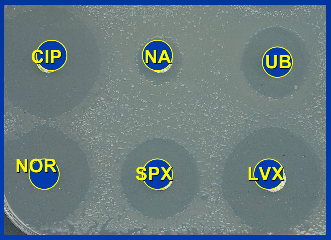
Plasmid-mediated resistance to quinolones; the other determinants

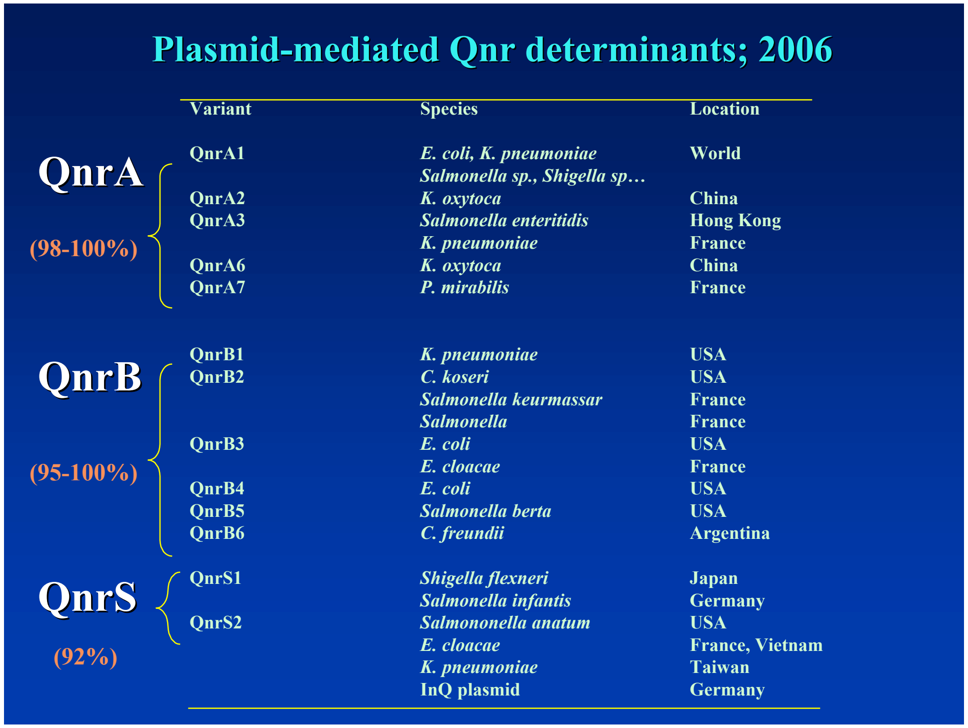
- 2004 : QnrB (40 % amino acid identity with QnrA) identified in enterobacterial isolates from USA and India (Jacoby et al. AAC 2006)

- 2005 : QnrS (60 % amino acid identity with QnrA) identified in:
  - A single *Shigella flexneri* isolate from Japan (Hata et al. AAC 2005)
  - A single *E. cloacae* isolate from Vietnam (Poirel et al., CMI 2006)



QnrS

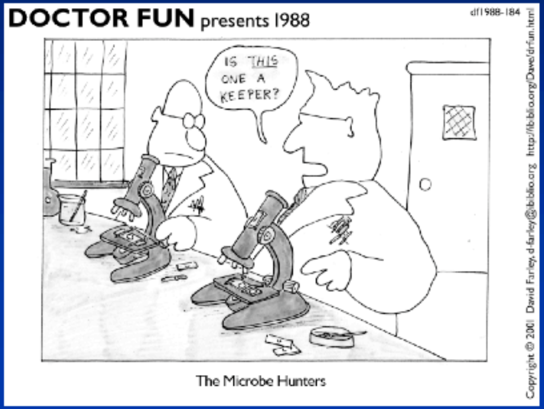




Plasmid-mediated Qnr determinants; 2006

	Variant	Species	Location
QnrA (98-100%)	QnrA1	*E. coli, K. pneumoniae* *Salmonella sp., Shigella sp…*	World
	QnrA2	*K. oxytoca*	China
	QnrA3	*Salmonella enteritidis*	Hong Kong
		K. pneumoniae	France
	QnrA6	*K. oxytoca*	China
	QnrA7	*P. mirabilis*	France
QnrB (95-100%)	QnrB1	*K. pneumoniae*	USA
	QnrB2	*C. koseri*	USA
		Salmonella keurmassar	France
		Salmonella	France
	QnrB3	*E. coli*	USA
		E. cloacae	France
	QnrB4	*E. coli*	USA
	QnrB5	*Salmonella berta*	USA
	QnrB6	*C. freundii*	Argentina
QnrS (92%)	QnrS1	*Shigella flexneri*	Japan
		Salmonella infantis	Germany
	QnrS2	*Salmononella anatum*	USA
		E. cloacae	France, Vietnam
		K. pneumoniae	Taiwan
		InQ plasmid	Germany

…where do Qnr determinants come from?

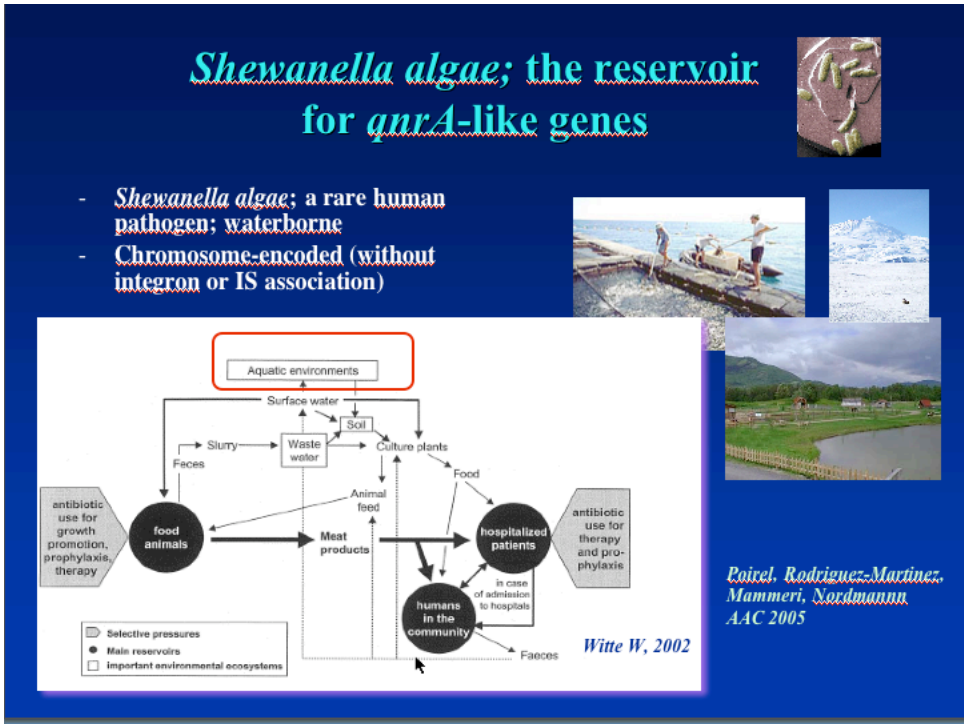


The Microbe Hunters

Search for the reservoirs of *qnrA, B, S* genes

- Species screened: 72 species
  Pseudomonacaceae,
  Enterobacteriacae,
  Xanthomonadaceae,
  Aeromonadaceae,
  Vibrionaceace,
  Shewanellaceae

- PCR amplifications
  Southern
  Hybridizations; I-*Ceu*-I



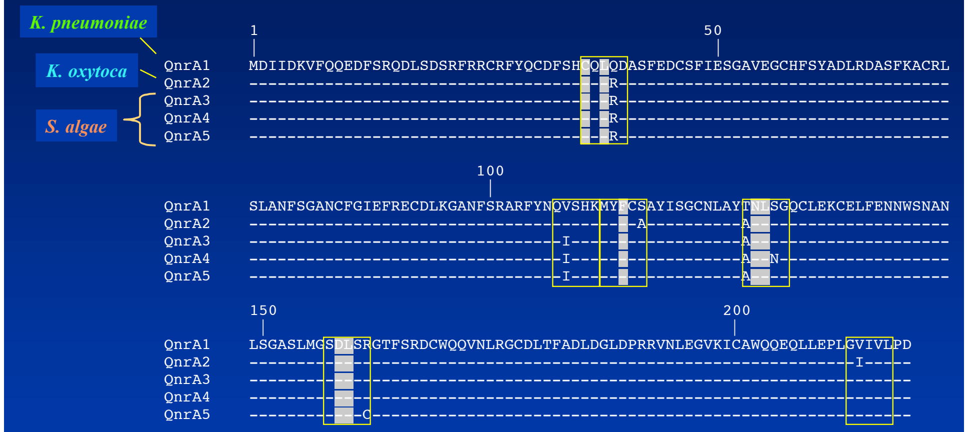
Shewanella algae; the reservoir for *qnrA*-like genes

- *Shewanella algae*; a rare human pathogen; waterborne
- Chromosome-encoded (without integron or IS association)

Witte W, 2002

Poirel, Rodriguez-Martinez, Mammeri, Nordmannn AAC 2005

QnrA-like determinants

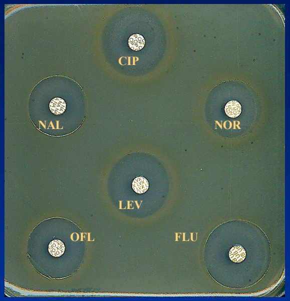


```
         1                                                50
         |                                                |
QnrA1    MDIIDKVFQQEDFSRQDLSDSRFRRCRFYQCDFSHCQLQDASFEDCSFIESGAVEGCHFSYADLRDASFKACRL
QnrA2    --------------------------------------R-----------------------------------
QnrA3    --------------------------------------R-----------------------------------
QnrA4    --------------------------------------R-----------------------------------
QnrA5    --------------------------------------R-----------------------------------

                                 100
                                  |
QnrA1    SLANFSGANCFGIEFRECDLKGANFSRARFYNQVSHKMYFCSAYISGCNLAYTNLSGQCLEKCELFENNWSNAN
QnrA2    -----------------------------------------A----------A---------------------
QnrA3    ---------------------------------I------------------A---------------------
QnrA4    ---------------------------------I------------------A--N------------------
QnrA5    ---------------------------------I------------------A---------------------

         150                                               200
          |                                                 |
QnrA1    LSGASLMGSDLSRGTFSRDCWQQVNLRGCDLTFADLDGLDPRRVNLEGVKICAWQQEQLLEPLGVIVLPD
QnrA2    ----------------------------------------------------------------I-----
QnrA3    ----------------------------------------------------------------------
QnrA4    ----------------------------------------------------------------------
QnrA5    ------------C---------------------------------------------------------
```

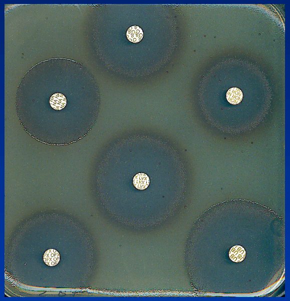
QnrA3 from *S. algae* identified recently as plasmid-encoded in *Salmonella* in China and in *K . pneumoniae* in France

Susceptibility levels of the natural QnrA producer

***S. algae* (*qnrA*-like +)**



***S. putrefaciens* (*qnrA*-like -)**



	S. algae 106454	*E. coli* TOP10 (pSA-1)	*E. coli* TOP10
Nalidixic acid	4	2	0.75
Flumequine	0.5	1	0.25
Oxolinic acid	0.25	0.5	0.25
Sparfloxacin	0.75	0.06	0.03
Norfloxacin	1.5	0.25	0.03
Ofloxacin	0.75	0.12	0.01
Ciprofloxacin	0.38	0.04	0.003

Journal of Antimicrobial Chemotherapy (2005) **56**, 1118–1121
doi:10.1093/jac/dki371
Advance Access publication 14 October 2005

JAC

Vibrionaceae as a possible source of Qnr-like quinolone resistance determinants

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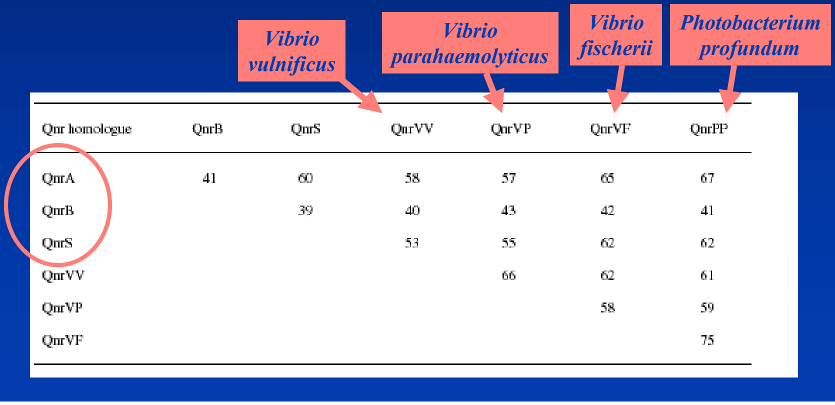
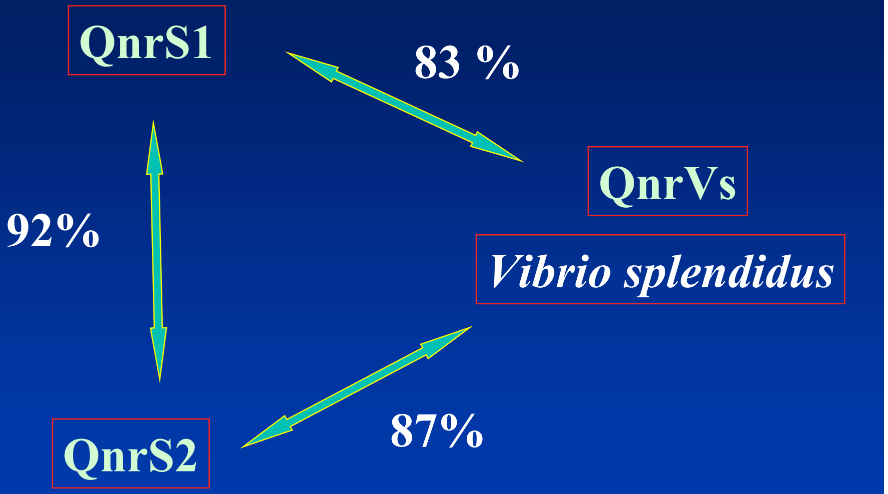


Qnr homologue	QnrB	QnrS	QnrVV	QnrVP	QnrVF	QnrPP
QnrA	41	60	58	57	65	67
QnrB		39	40	43	42	41
QnrS			53	55	62	62
QnrVV				66	62	61
QnrVP					58	59
QnrVF						75

…where do Qnr B and QnrS come from?

…the origin of QnrS ?



QnrS1

83 %

QnrVs

Vibrio splendidus

92%

QnrS2

87%

Epidemiology

Spread of QnrA

USA: 72 *K. pneumoniae* isolates + 38 *E. coli* isolates (CIP ^{R}, CAZ ^{R})
8 *K. pneumoniae* QnrA (+) (prevalence of 11.1 %)
(Wang et al. AAC 2004) USA: 313 ceftazidime-resistant Enterobacteriaceae
(*E. coli* , *K. pneumoniae, Enterobacter* spp:QnrB or QnrA in 4% of *E. coli*, 20% of *K. pneumoniae* and 31% of *Enterobacter* sp. (Robicsek et al. AAC 2006)

China: 78 *E. coli* isolates (CIP ^{R}) : 6 QnrA (+) (prevalence 7.7 %)
(Wang et al. AAC 2003)

France: 297 *E. coli* isolates (Nal ^{R}) : 1 QnrA (+) (prevalence 0.34 %)
(Mammeri et al. AAC 2005); 185 *E. coli* isolates (Nal ^{R} 2005) and 186 ESBL (+) :2.2% and 0.5% were carriers, respectively (Poirel et al. AAC 2006, in press);1,468 enterobacterial isolates (NalR or ESBLs, 2002-2005; 3.3% of ESBLs (+) only (Cambau et al. CMI 2006)

Canada : 110 *E. coli* isolates (Nal ^{R} , ESBL (+) : 1 QnrA (+) (prevalence 0.9 %)
(Poirel et al. AAC 2006)

Spain: 100 *E. coli* isolates (Nal ^{R}) 2004-2005: 2 QnrA (+) (prevalence 2 %)
(Cano et al. ICAAC 2005)

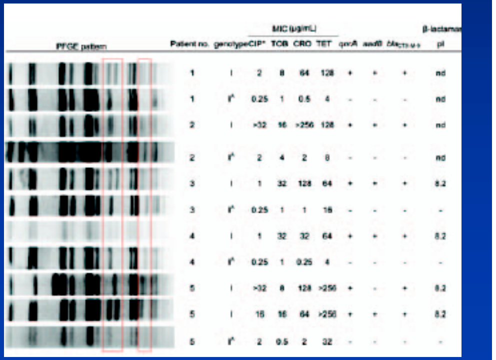
UK: 47 enterobacterial isolates CIP ^{R}, CTX ^{R} : 15 QnrA (+) (prevalence 32 %)
and 12 clonally distinct (Corkill et al. JAC 2005)

Enterobacter cloacae Outbreak and Emergence of Quinolone Resistance Gene in Dutch Hospital

Armand Paauw,* Ad C. Fluit,* Jan Verhoef,* and Maurine A. Leverstein-van Hall*

Emerging Infectious Diseases • www.cdc.gov/eid • Vol. 12, No. 5, May 2006

An outbreak of *Enterobacter cloacae* infections with variable susceptibility to fluoroquinolones occurred in the University Medical Center Utrecht in the Netherlands in 2002. Our investigation showed that a *qnrA*1 gene was present in 78 (94%) of 83 outbreak isolates and that a *qnrA1*-encoding plasmid transferred to other strains of the same species and other species. The earliest isolate carrying this same plasmid was isolated in 1999. *qnrA1* was located in a complex integron consisting of the *intI1*, *aadB*, *qacE∆1*, *sul1*, *orf513*, *qnrA1*, *ampR*, *qacE∆1*, and *sul1* genes that were not described previously. On the same plasmid, 2 other class 1 integrons were present. One was a new integron associated with the $bla_{CTX-M-9}$ extended-spectrum β-lactamase.



PFGE pattern	Patient no.	genotype	MIC (µg/mL)				*qnrA*	*aadB*	$bla_{CTX-M-9}$	β-lactama
			CIP*	TOB	CRO	TET				pI
	1	I	2	8	64	128	+	+	+	nd
	1	I^A	0.25	1	0.5	4	-	-	-	nd
	2	I	>32	16	>256	128	+	+	+	nd
	2	I^A	2	4	2	8	-	-	-	nd
	3	I	1	32	128	64	+	+	+	8.2
	3	I^A	0.25	1	1	16	-	-	-	-
	4	I	1	32	32	64	+	+	+	8.2
	4	I^A	0.25	1	0.25	4	-	-	-	-
	5	I	>32	8	128	>256	+	-	+	8.2
	5	I	16	16	64	>256	+	+	+	8.2
	5	I^A	2	0.5	2	32	-	-	-	-

Spread of QnrB and QnrS

USA: 313 ceftazidime-resistant Enterobacteriaceae (*E. coli* , *K. pneumoniae, Enterobacter* spp:QnrB or QnrA in 4% of *E. coli*, 20% of *K. pneumoniae* and 31% of *Enterobacter* sp. *(Robicsek Jacoby Hooper AAC 2006*); 435 non-Typhi salmonella; 8 isolates :QnrB; 3 isolates QnrS *(Gay,Robicsek, Hooper CID, 2006)*

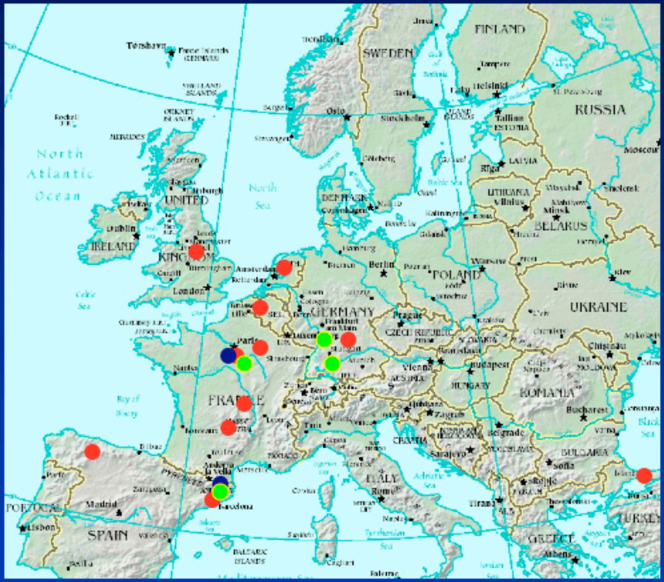
Vietnam: 28 gentamicin-resistant *K. pneumoniae*, 32 ESBL (+) *E. coli* or *Citrobacter* sp.; 86% of *K. pneumoniae* and 25% of ESBL (+) carry either QnrA or QnrS *(Schultz. et al. ICAAC 2005)*

France: 185 *E. coli* isolates (Nal^{R} 2005) and 186 ESBL (+) :2.2% and 1.6% of the strains carry QnrS, respectively (*Poirel, Leviandier, Nordmann. AAC 2006, in press)*

Worldwide distribution of Qnr determinants

Qnr determinants spread in Europe

- 🟥 QnrA
- 🟦 QnrB
- 🟩 QnrS



Impact on fluoroquinolone resistance association of mechanisms (i)

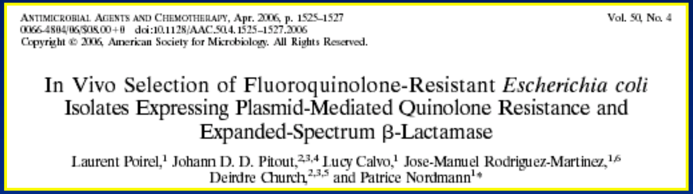
Chromosomal-encoded mutations in *gyrA* and *parC*genes in *E. cloacae*

E. cloacae isolates	GyrA		ParC	
	83	87	80	84
Wild strain	Ser(TCC)	Asp (GAG)	Ser (AGC)	Glu (ACC)
S1	Phe (TTC)	-	-	-
S2	Ile (ATC)	-	Ile (ATC)	-
S4	Val (GTC)	-	-	-
S5	Phe (TTC)	-	-	-
S6	Tyr (TAC)	-	-	-
S7	Tyr (TAC)	-	-	-
A1	Phe(TTC)	-	Arg (AGA)	-

=> All *qnrA* (+) or *qnrS* (+) *E. cloacae* isolates harbored at least one mutation in *gyrA* or *parC*

Poire L, Leviandier C, Nordmann P, AAC, in press

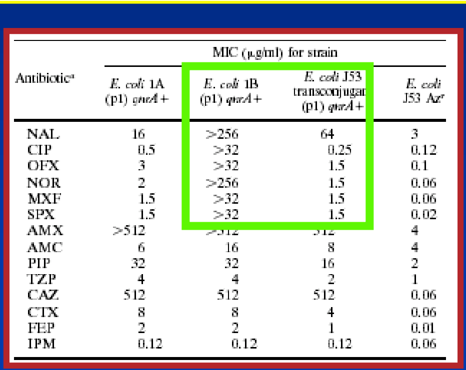
Impact on fluroquinolone resistance (ii)



Antimicrobial Agents and Chemotherapy, Apr. 2006, p. 1525–1527 Vol. 50, No. 4
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.4.1525–1527.2006

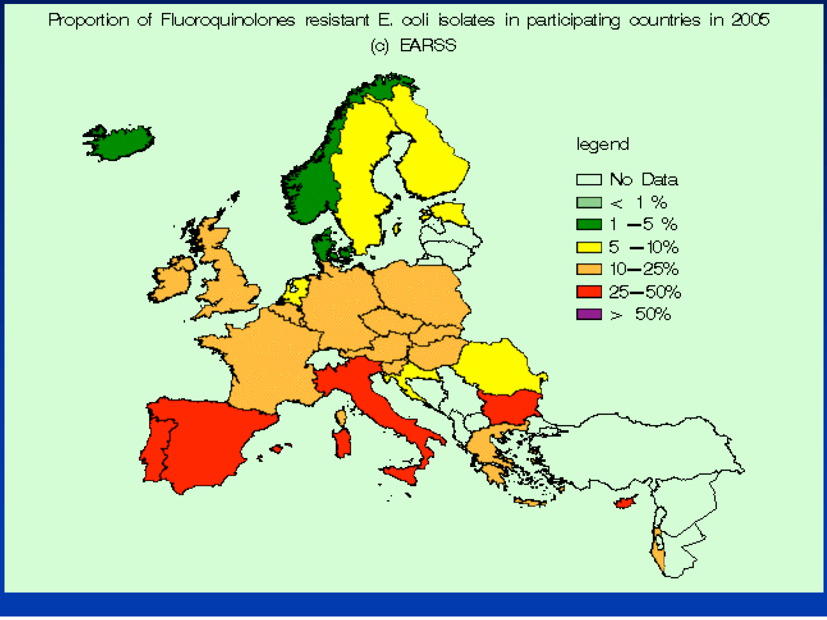
In Vivo Selection of Fluoroquinolone-Resistant *Escherichia coli* Isolates Expressing Plasmid-Mediated Quinolone Resistance and Expanded-Spectrum β-Lactamase

Laurent Poirel,[1] Johann D. D. Pitout,[2,3,4] Lucy Calvo,[1] Jose-Manuel Rodriguez-Martinez,[1,6]
Deirdre Church,[2,3,5] and Patrice Nordmann[1*]



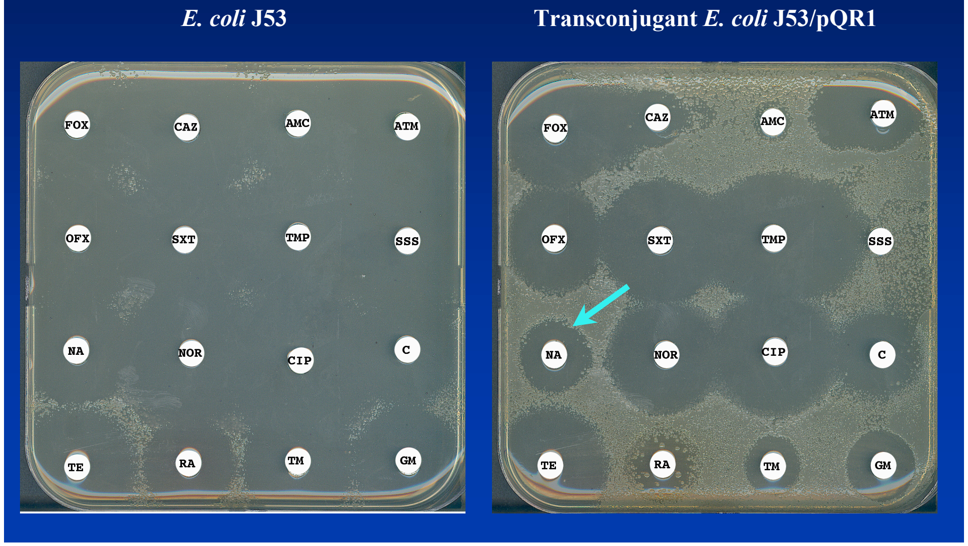
Antibiotic[a]	MIC (μg/ml) for strain			
	E. coli 1A (p1) *qnrA*+	*E. coli* 1B (p1) *qnrA*+	*E. coli* J53 transconjugan (p1) *qnrA*+	*E. coli* J53 Az^r
NAL	16	>256	64	3
CIP	0.5	>32	0.25	0.12
OFX	3	>32	1.5	0.1
NOR	2	>256	1.5	0.06
MXF	1.5	>32	1.5	0.06
SPX	1.5	>32	1.5	0.02
AMX	>512	>512	512	4
AMC	6	16	8	4
PIP	32	32	16	2
TZP	4	4	2	1
CAZ	512	512	512	0.06
CTX	8	8	4	0.06
FEP	2	2	1	0.01
IPM	0.12	0.12	0.12	0.06

Fluoroquinolone resistance in *E. coli* in Europe


Proportion of Fluoroquinolones resistant E. coli isolates in participating countries in 2005
(c) EARSS

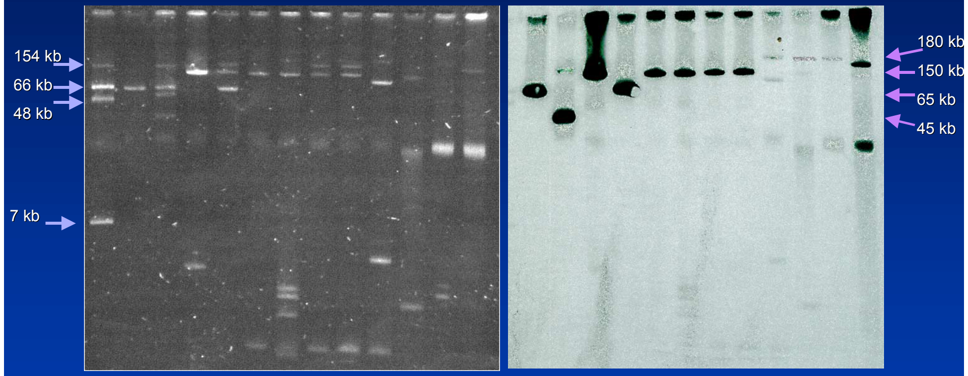
Genetics
as source of *qnr* spread

Transfer of quinolone resistance marker


E. coli J53

Transconjugant *E. coli* J53/pQR1

Plasmids



Plasmids
***qnrS* (+)**

Plasmids
***qnrA* (+)**

Poirel, Leviandier, Nordmann, 2006, AAC, in press

Consequences of plasmid location of *qnr* genes

-Associated resistance genes in particularly with those coding for:

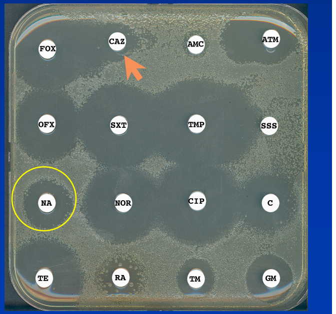
- clavulanic-acid inhibited expanded-spectrum ß-lactamases

+ QnrA: CTX-M-1, CTX-M-9, CTX-M-14, SHV-2a, SHV-5, SHV-7, SHV-12, VEB-1, PER-1
+ QnrB: CTX-M-15, SHV-12, SHV-30
+ QnrS: CTX-M-1, CTX-M-2,SHV-2, SHV-12, TEM-52

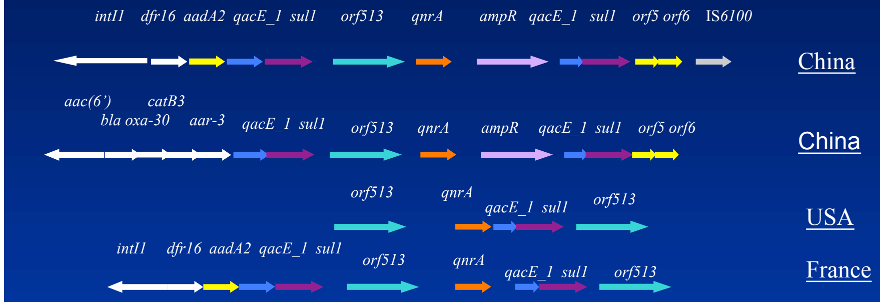
- plasmid-mediated cephalosporinases

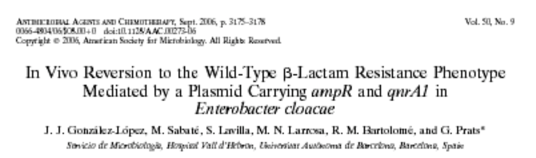
+ QnrA: FOX-5, DHA-1

- Transfer among enterobacterial species



QnrA environment




ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2006, p. 3175–3178 Vol. 50, No. 9
0066-4804/06/$08.00+0 doi:10.1128/AAC.00273-06
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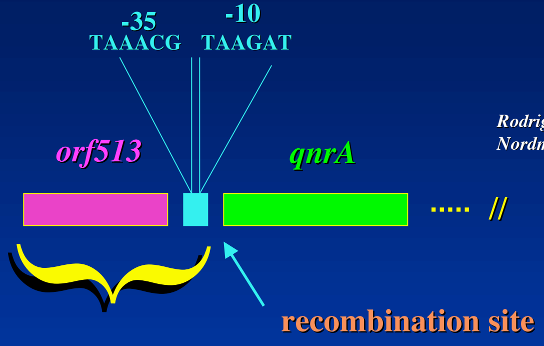
In Vivo Reversion to the Wild-Type β-Lactam Resistance Phenotype Mediated by a Plasmid Carrying *ampR* and *qnrA1* in *Enterobacter cloacae*

J. J. González-López, M. Sabaté, S. Lavilla, M. N. Larrosa, R. M. Bartolomé, and G. Prats*
Servicio de Microbiología, Hospital Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain

IS*CR1* enhances the expression of *qnrA*



-35
TAAACG

-10
TAAGAT

orf513

qnrA

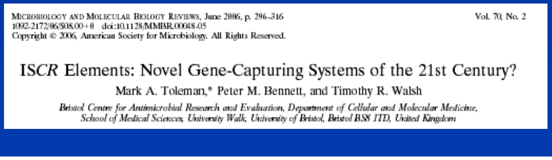
..... //

recombination site

Rodriguez-Martinez, Poirel, Nordmann AAC 2006

CR1= IS*CR1*

IS*CR1* = A IS*91*-like sequence

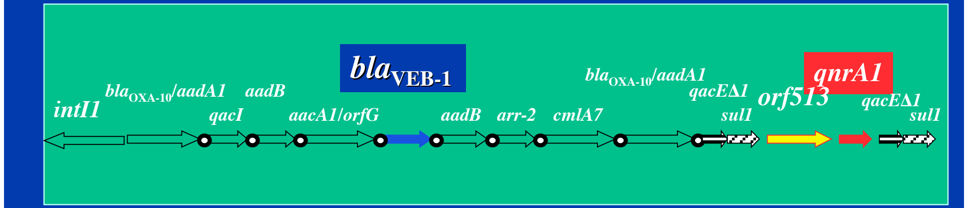


Microbiology and Molecular Biology Reviews, June 2006, p. 296–316 Vol. 70, No. 2
1092-2172/06/$08.00+0 doi:10.1128/MMBR.00048-05

IS*CR* Elements: Novel Gene-Capturing Systems of the 21st Century?

Mark A. Toleman,* Peter M. Bennett, and Timothy R. Walsh
Bristol Centre for Antimicrobial Research and Evaluation, Department of Cellular and Molecular Medicine, School of Medical Sciences, University Walk, University of Bristol, Bristol BS8 1TD, United Kingdom

Association of *bla*VEB-1 and *qnr*A genes in a same *sul*1-type class 1 integron



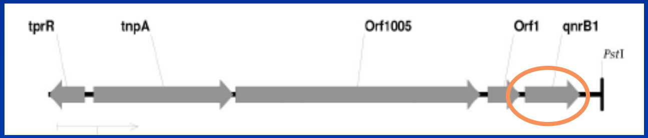
Poirel, Pitout, Nordmann AAC 2006

Genetic environment of *qnrB gene* (i)

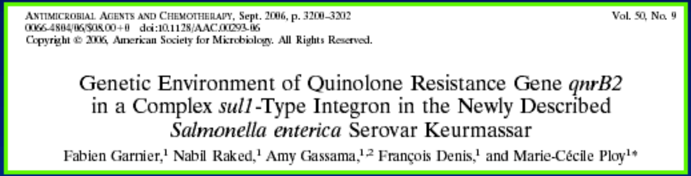
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2006, p. 1178–1182 Vol. 50, No. 4
0066-4804/06/$08.00+0 doi:10.1128/AAC.50.4.1178–1182.2006

qnrB, Another Plasmid-Mediated Gene for Quinolone Resistance

George A. Jacoby,[1*] Kelley E. Walsh,[1] Debra M. Mills,[1] Victoria J. Walker,[1] Herin Oh,[2]
Ari Robicsek,[2] and David C. Hooper[2]
Lahey Clinic, Burlington,[1] and Massachusetts General Hospital, Boston,[2] Massachusetts



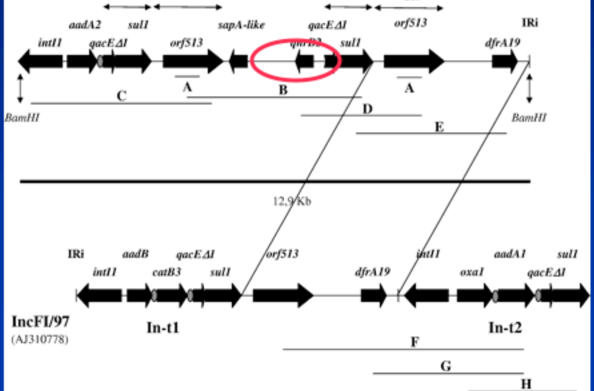
Genetic environment of *qnrB* (ii)



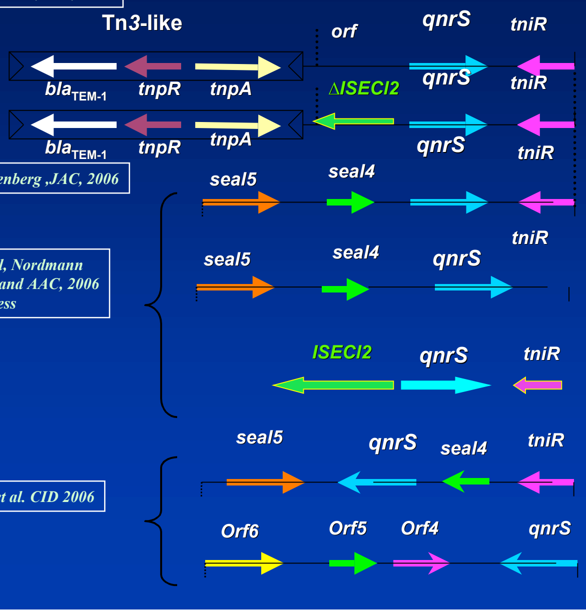
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2006, p. 3200–3202 Vol. 50, No. 9
0066-4804/06/$08.00+0 doi:10.1128/AAC.00293-06

Genetic Environment of Quinolone Resistance Gene *qnrB2* in a Complex *sul1*-Type Integron in the Newly Described *Salmonella enterica* Serovar Keurmassar

Fabien Garnier,[1] Nabil Raked,[1] Amy Gassama,[1,2] François Denis,[1] and Marie-Cécile Ploy[1*]



Genetic environment of the *qnrS*



Hata et al. ,AAC, 2005

Shigella flexneri (Japan)

S. enterica (Germany)

Kehrenberg ,JAC, 2006

Enterobacter sp (France)

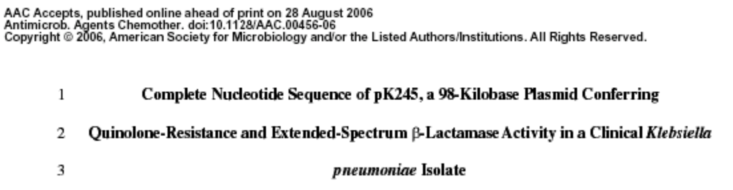
Poirel, Nordmann
CMI and AAC, 2006
In press

Enterobacter sp
Serratia sp (France)

Enterobacter sp
(France, Vietnam)

Gay et al. CID 2006

S. bovismorbificans
(USA)
S. Anatum (USA)



Antimicrob. Agents Chemother. doi:10.1128/AAC.00456-06

Complete Nucleotide Sequence of pK245, a 98-Kilobase Plasmid Conferring Quinolone-Resistance and Extended-Spectrum β-Lactamase Activity in a Clinical *Klebsiella pneumoniae* Isolate

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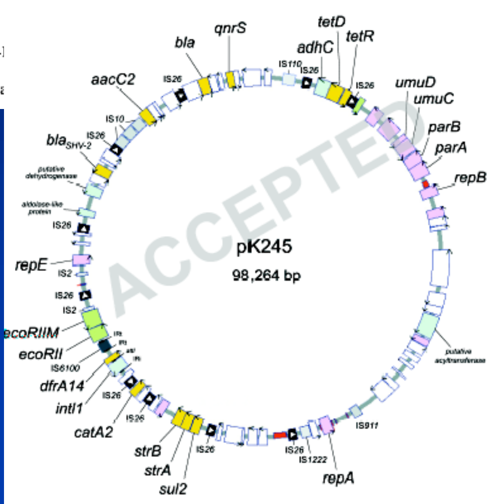
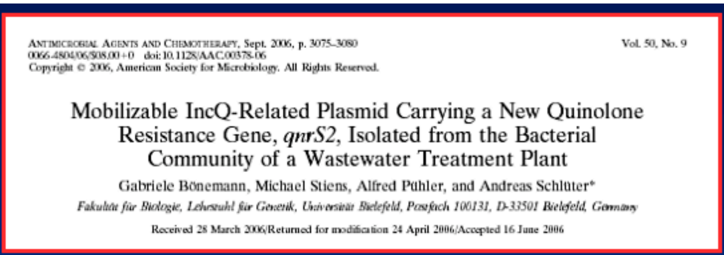
Shiau,[4] Jing-Jou Yan,[5] Ih-Jen Su,[4] Shih-Feng Tsa





Antimicrobial Agents and Chemotherapy, Sept. 2006, p. 3075–3080 Vol. 50, No. 9
0066-4804/06/$08.00+0 doi:10.1128/AAC.00378-06

Mobilizable IncQ-Related Plasmid Carrying a New Quinolone Resistance Gene, *qnrS2*, Isolated from the Bacterial Community of a Wastewater Treatment Plant

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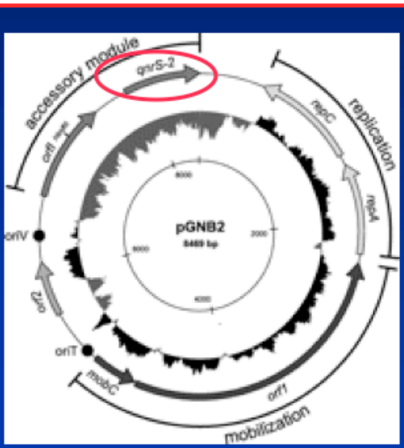
Received 28 March 2006/Returned for modification 24 April 2006/Accepted 16 June 2006



Conclusion; the known (i)

- Qnr determinants are widely distributed worldwide. Community-acquired and nosocomial *Enterobacteriaceae*

- Source; *Shewanella algae* for qnrA-like genes

- Association with ESBL genes to a given extend

- Variability of the genetic vehicles of their spread

- Resistance to nalidixic acid and background for fluoroquinolone resistance

Conclusion; the unknown (ii)

- How are the *qnr* genes mobilized to plasmids and from chromosomes ? Different mobilization mechanisms according to the *qnr* type ?
- Is plasmid-mediated quinolone resistance a novel event ?
- Spread to *H. influenzae* or *N. gonorrheae* ?
- Which is the reservoir of QnrB and QnrS determinants ?
- What is the normal function of Qnr determinants ?
- Which is the impact of ß-lactam/quinolone treatment for selection/mobilization of those genes ? Which is the impact of quinolones in animals or in the environment for promoting transfer of those *qnr* genes ?

Acknowledgments

L. Poirel, Ph D

A. Liard, Graduate st.

H. Mammeri, PharmD, PhD
C. Leviandier, Pharm D

JM Rodriguez-Martinez PhD
(Sevilla, Spain)
M. Van De Loo, MD