Quinolone Resistance and Microbial Ingenuity

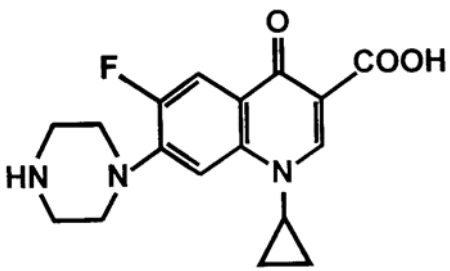
David C. Hooper, M.D.
Division of Infectious Diseases
Infection Control Unit
Massachusetts General Hospital
Harvard Medical School

GSK Chair of Infectious Diseases
Resistance Workshop – Brussels, March 28th, 2007

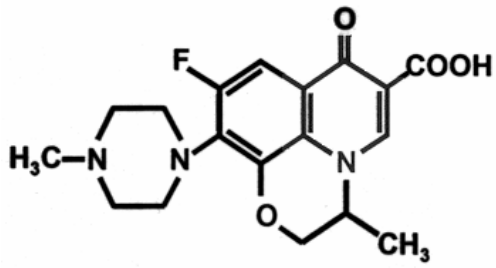
Fluoroquinolones Available in the United States

- Norfloxacin (Noroxin) 1986 (PO)
- Ciprofloxacin (Cipro) 1987 (PO), 1990 (IV)
- Ofloxacin (Floxin) 1990 (PO), 1992 (IV)
- Levofloxacin (Levaquin) 1996 (IV & PO)
- Gatifloxacin (Tequin) 1999 (IV & PO)
- Moxifloxacin (Avelox) 1999 (PO), 2001 (IV)
- Gemifloxacin (Factive) 2003 (PO)

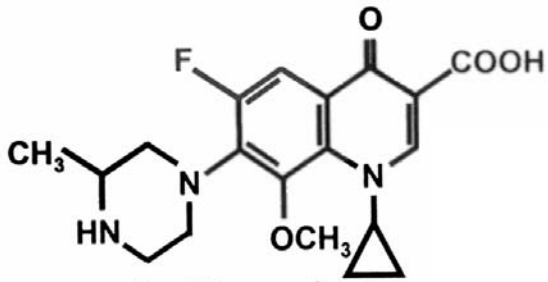
Fluoroquinolone Structures



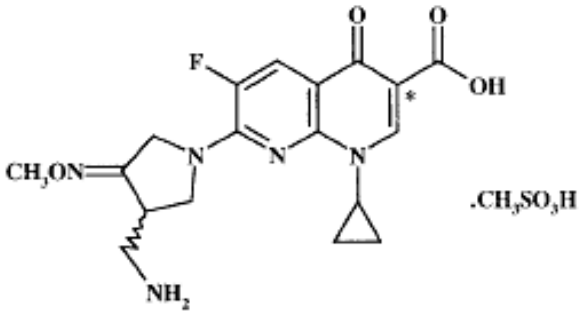
Ciprofloxacin



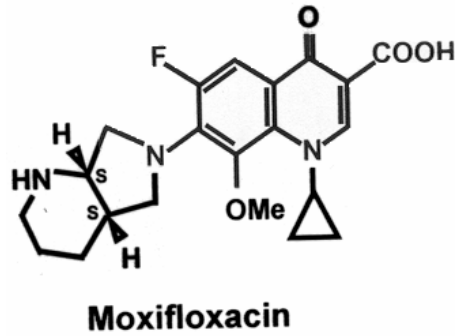
Levofloxacin



Gatifloxacin



Gemifloxacin

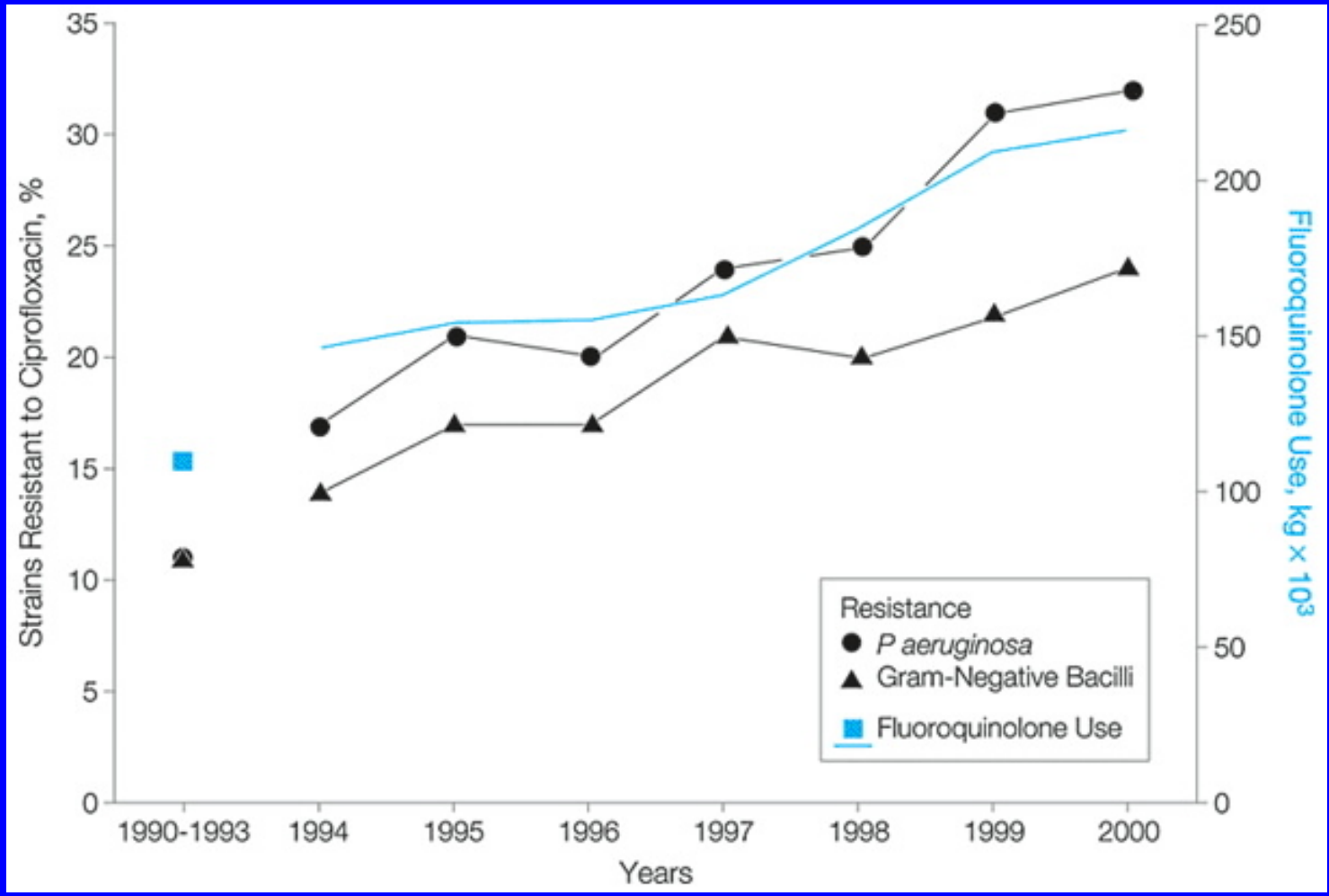


Moxifloxacin

Clinical Uses of Fluoroquinolones

- Urinary Tract Infections
- Prostatitis
- Sexually Transmitted Diseases
- Gastroenteritis
- Intraabdominal Infections
- Respiratory Tract Infections
- Bone & Joint Infections
- Skin & Soft Tissue Infections
- Other Broad Uses in Hospitalized Patients

Increasing Quinolone Resistance Associated with Increasing Use



Neuhauser MM et al. JAMA 2003; 289:885-8

Prevalence of Bacterial Resistance to Fluoroquinolones

Staphylococci (MRSA, MRSE)	60-95%
Pseudomonas aeruginosa	24-44%
Klebsiella pneumoniae	12-20%
Enterobacter spp.	10-12%
Escherichia coli	3-50%
Campylobacter jejuni	3-70%

Factors Contributing to Fluoroquinolone Resistance

Resistant Pathogen	Risk Factors
Staphylococci (MRSA, MRCNS)	Quinolone Use, Co-selection, Nosocomial Spread
Pseudomonas aeruginosa	Quinolone Use Nosocomial Spread

Factors Contributing to Fluoroquinolone Resistance

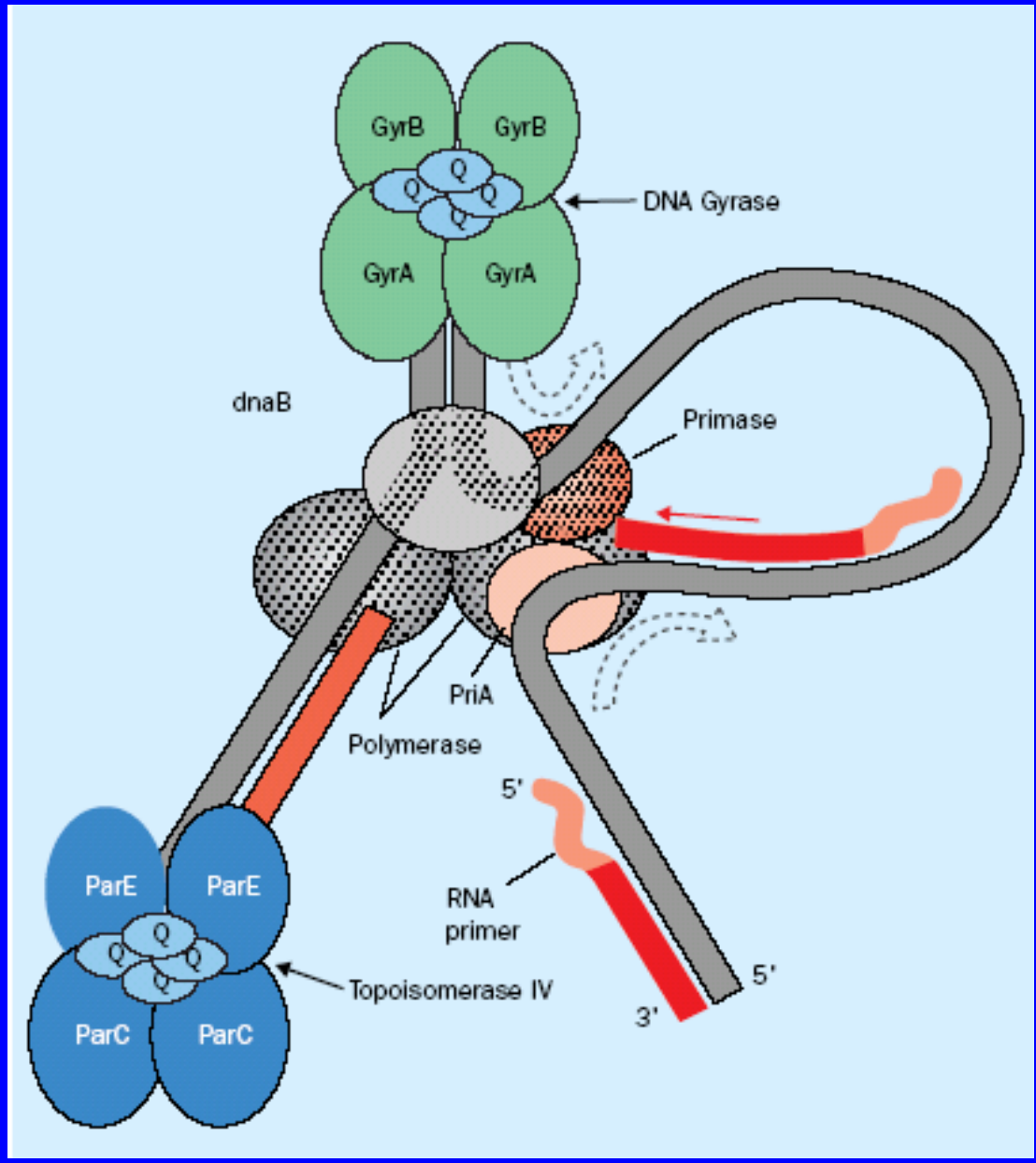
Resistant Pathogen	Risk Factors
Neisseria gonorrheae	Community Spread
	?Quinolone Use
Campylobacter jejuni	Quinolone Use
	Foreign Travel
	Animal Use
Escherichia coli	Quinolone Use
	Urinary abnormalities
	Catheter Use
	?Animal Use

Mechanisms of Resistance to Fluoroquinolones

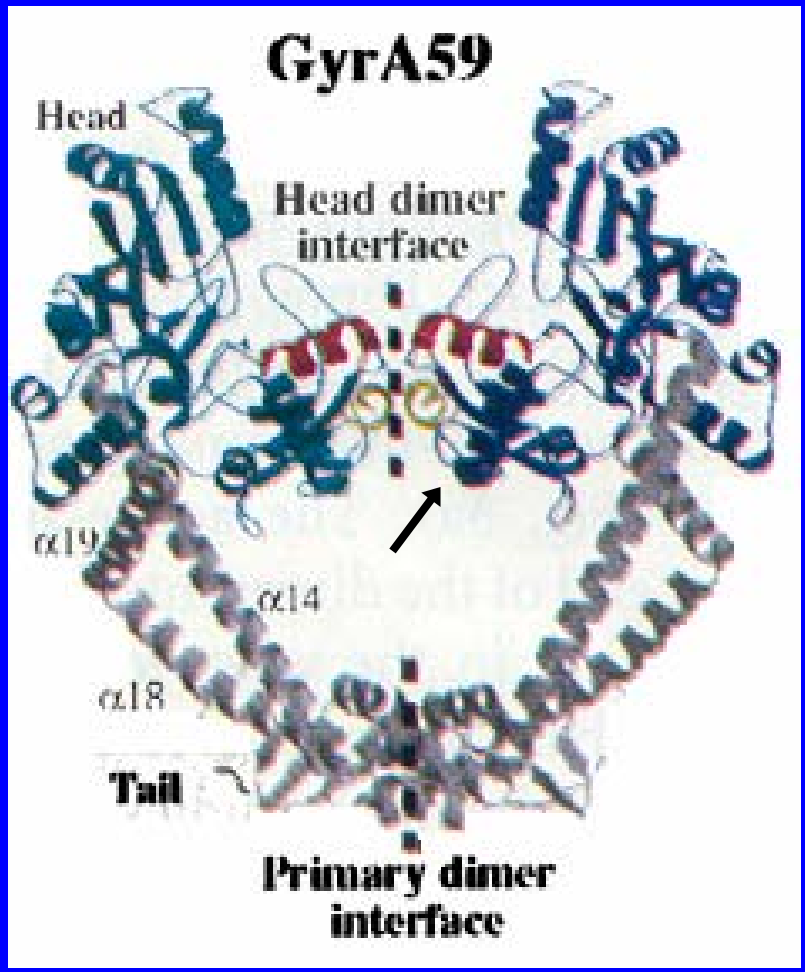
- Chromosomal mutations
  - Alterations in DNA gyrase and/or topoisomerase IV
  - Active drug efflux (MDR pumps) +/- reduced porin diffusion channels
- Plasmid-mediated resistance
  - Enteric gram-negative bacteria; target protection mechanism by Qnr proteins
  - Drug modification

Bacterial Type II Topoisomerases Quinolone Target Enzymes

Enzyme	Subunits	Activities
DNA Gyrase (Topoisomerase II)	2 GyrA 2 GyrB	DNA Supercoiling (DNA Relaxation) (DNA Decatenation)
Topoisomerase IV	2 ParC (GrlA) 2 ParE (GrlB)	DNA Decatenation (DNA Relaxation)


GyrB
GyrB
Q
Q
Q
Q
DNA Gyrase
GyrA
GyrA
dnaB
Primase
PriA
Polymerase
5'
RNA
primer
5'
3'
ParE
ParE
Q
Q
Q
Q
Topoisomerase IV
ParC
ParC

Structure of DNA Gyrase A Subunit



Cabral JHM *et al*. Nature 1997; 388:903-6

Alterations in Quinolone Binding to Mutant Gyrase-DNA Complexes

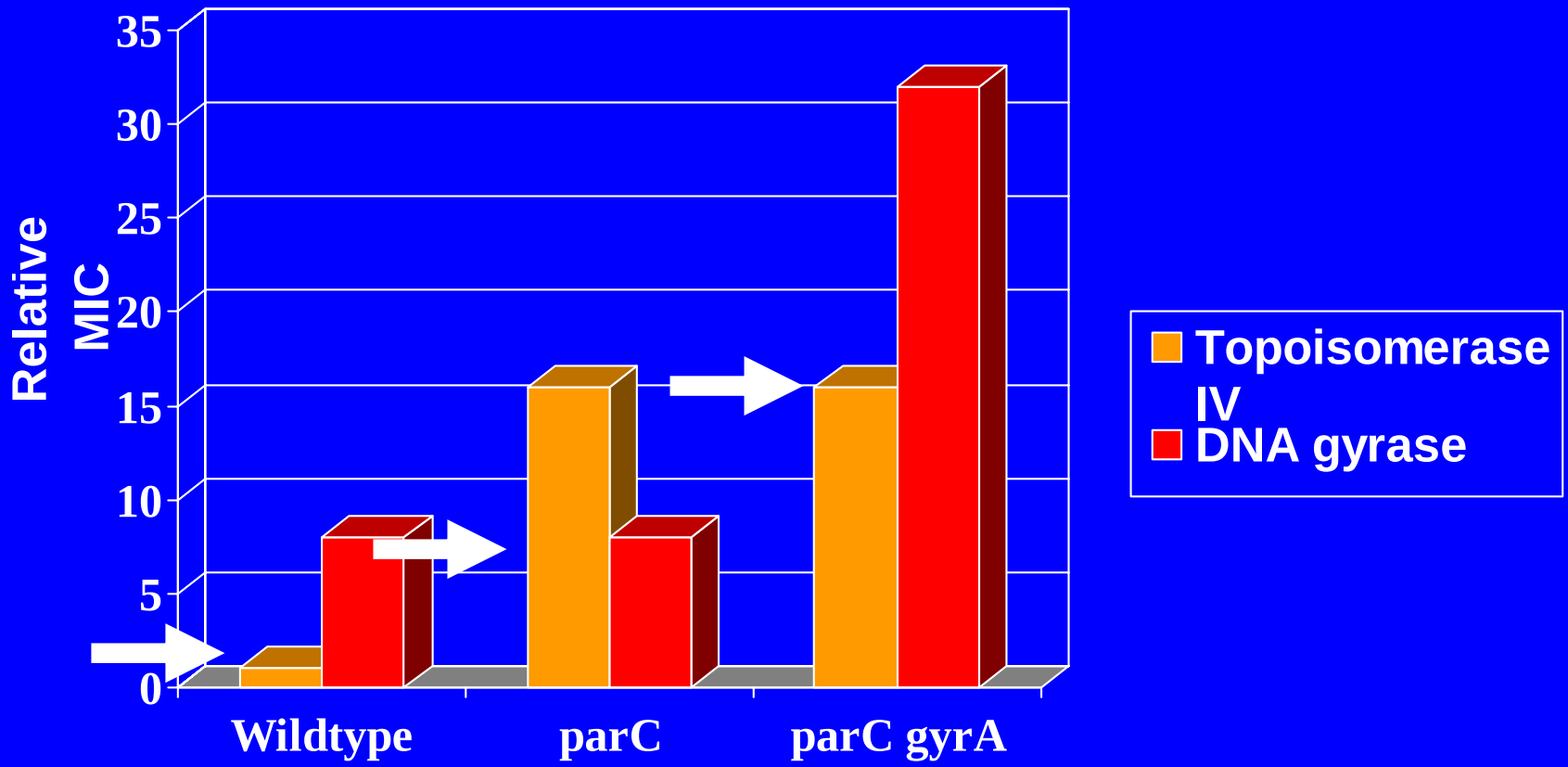
Complex	Norfloxacin Bound (nM)
DNA	0
$GyrA_2GyrB_2$	0.5
$GyrA_2GyrB_2$-DNA	11.9
$GyrA(S83W)_2GyrB_2$	0.4
$GyrA(S83W)_2GyrB_2$-DNA	0.2 (60x ↓)

Willmott, C.J. and A. Maxwell. 1993. AAC 37:126

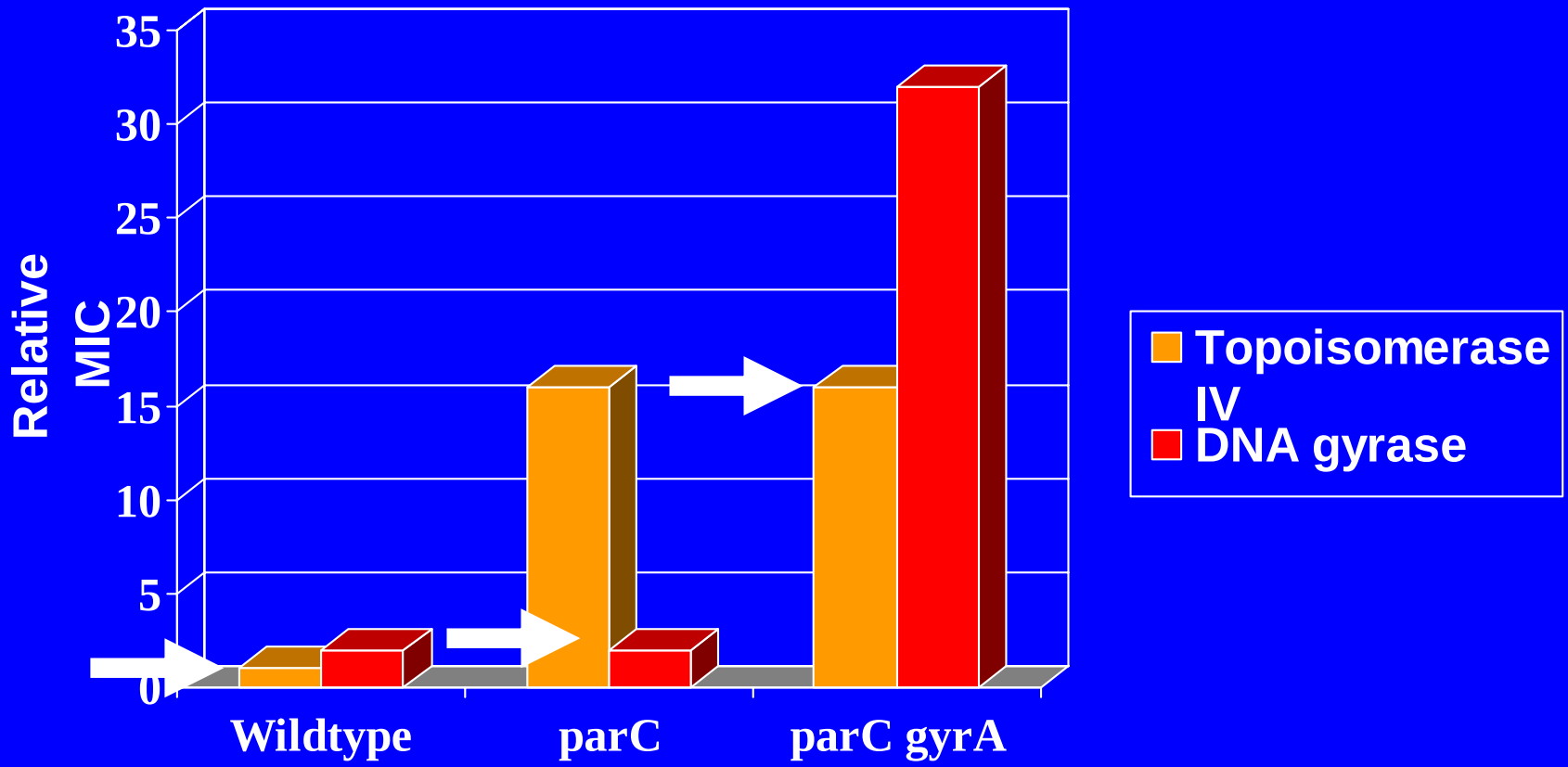
Bacterial Type II Topoisomerases Quinolone Target Enzymes

Enzyme	Subunits	Activities
DNA Gyrase (Topoisomerase II)	2 GyrA 2 GyrB	DNA Supercoiling (DNA Relaxation) (DNA Decatenation)
Topoisomerase IV	2 ParC (GrlA) 2 ParE (GrlB)	DNA Decatenation (DNA Relaxation)

Stepwise Increases in Quinolone Resistance: Role of Differing Sensitivities of Enzyme Targets



Stepwise Increases in Quinolone Resistance: Role of Differing Sensitivities of Enzyme Targets



Activity of Gemifloxacin and Ciprofloxacin Against Topoisomerase IV and Gyrase

Enzyme	IC_{50} (μg/ml)			
	Gemifloxacin		Ciprofloxacin	
Topoisomerase IV				
Wildtype	0.25	200x	2.5-5.0	100x
ParC (Ser80Phe)	50		250	
Gyrase		~1x		2-4x
Wildtype	0.31		10	

Ince D *et al*. Antimicrob Agents Chemother. 2003; 47:274-82

Drug Target Differences and Frequency of Mutant Selection

Drug	Selecting drug concn[a]	Frequency of selection of mutants
Gemifloxacin	1 (0.016)	1.5×10^{-5}–2.4×10^{-5}
	2 (0.032)	7.4×10^{-11}–1.1×10^{-10}
	4 (0.064)	$<7.4 \times 10^{-12}$
Ciprofloxacin	2 (0.5)	2.8×10^{-6}–1.5×10^{-5}
	4 (1.0)	3.0×10^{-8}–6.1×10^{-8}

[a] Drug concentrations are given as factors of the MICs, and the numbers in parentheses are the MICs in micrograms per milliliter.

Ince D *et al*. Antimicrob Agents Chemother. 2003; 47:274-82

Mechanisms of Resistance to Fluoroquinolones

- Chromosomal mutations
  - Alterations in DNA gyrase and/or topoisomerase IV
  - Active drug efflux (MDR pumps) +/- reduced porin diffusion channels
- Plasmid-mediated resistance
  - Enteric gram-negative bacteria; target protection mechanism by Qnr proteins
  - Drug modification

Classes of Bacterial Efflux Systems

- Major Facilatator Superfamily (MFS)

  14-TMS QacA/B, EmrB

  12-TMS Bmr, Blt, NorA, VMAT1/2

- Small Multidrug Resistance Family (SMR)

  Smr, EmrE, QacE/QacΔ1

- Resistance/Nodulation/Cell Division Family

  AcrAB-TolC, MexAB-OprM, MtrCDE

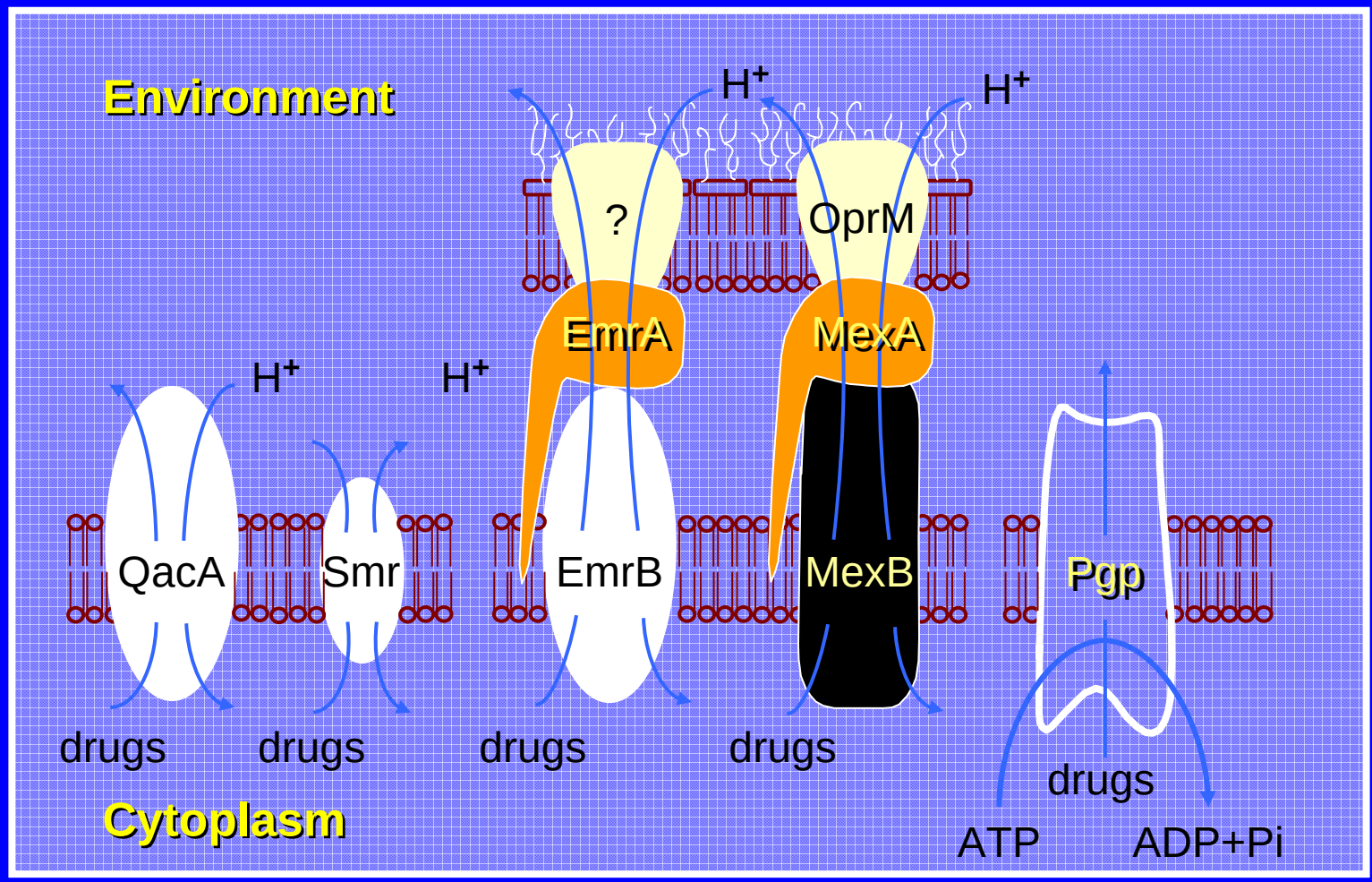
- MATE Family

  NorM

- ABC Multidrug Transporters

  LmrA

Multidrug Efflux Pumps



Lewis K *et al. ASM News*.1997;63:605-610.

Antibiotic Substrates for MDR Efflux Pumps

- Quinolones
  - Nalidixic acid, hydrophilic fluoroquinolones
- Tetracyclines
- Chloramphenicol
- β-Lactams
  - Hydrophobic cephalosporins and others
- Rifampin
- Erythromycin

Efflux Pumps Involved in Quinolone Resistance: Gram-Negative Bacteria

- *E. coli*
  - AcrAB/TolC
  - AcrEF/?
  - MdfA
  - YdhE
- *P. aeruginosa*
  - MexAB/OprM
  - MexCD/OprJ
  - MexEF/OprN
  - MexXY/OprM
- *N. gonorrhoeae*
  - MtrCDE
- *V. parahemolyticus*
  - NorM
- *S. maltophilia*
  - SmeABC
- *A. baumannii*
  - AdeABC
  - AbeM
- *B. cepacia*
  - CeoAB/OpcM

Contributions of Efflux to Resistance in Clinical Isolates

- *Pseudomonas aeruginosa* MexAB-OprM
  - 835 consecutive clinical isolates
  - 21 pairs (pre- and post-therapy) with acquired resistance to anti-pseudomonal β-lactams
    - same strains within pairs
    - 10/21 (48%) ↑ AmpC β-lactamase
    - 11/21 (52%) ↑ OprM
      - 8/11 *mexR* insertions, deletions, or frameshifts
      - 2/11 *mexR* missense mutations

Ziha-Zarifi I. et al. Antimicrob Agents Chemother. 1999; 43:287-91

Quinolone Resistance Mechanisms in *P. aeruginosa* Isolates from Patients with Cystic Fibrosis

Resistance Property	Property/Total (%)	
gyrA mutation(s)	11/20 (55)	
parC mutation	0/20 (0)	
mexR mutation	0/20 (0)	
nfxB mutation	16/20 (80)	6 *gyrA*$^+$
↑ OprJ	8/20 (40)	
↑ OprN	6/20 (30)	

Jalal S *et al*. 2000. Antimicrob Agents Chemother. 44:710-2.

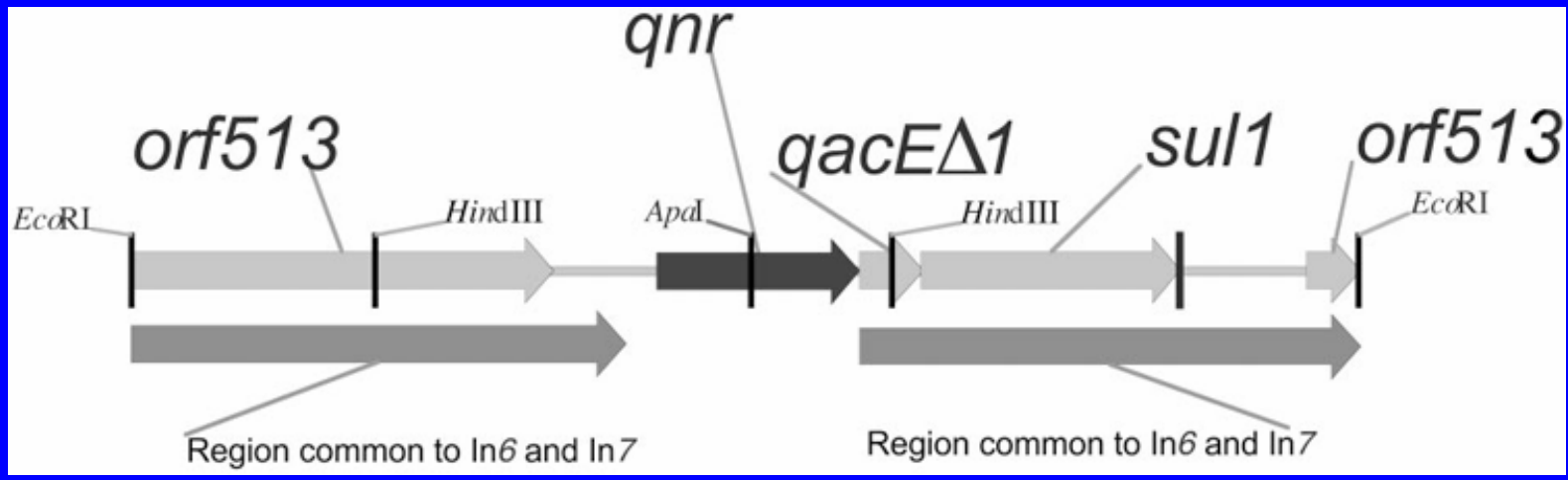
Clinical Importance of MDR Pumps: Physiologic Effects

- Overexpression contributes to resistance
- Baseline expression contributes to intrinisic reduced susceptibility of wildtype bacteria
- Regulated expression and multiplicity of pumps implies that expression may vary under different conditions (*in vivo* vs. *in vitro*) contributing to changes in therapeutic index not reflected in laboratory testing

Mechanisms of Resistance to Fluoroquinolones

- Chromosomal mutations
  - Alterations in DNA gyrase and/or topoisomerase IV
  - Active drug efflux (MDR pumps) +/- reduced porin diffusion channels
- Plasmid-mediated resistance
  - Enteric gram-negative bacteria; target protection mechanism by Qnr proteins
  - Drug modification

Cloning and Sequencing of *qnr*



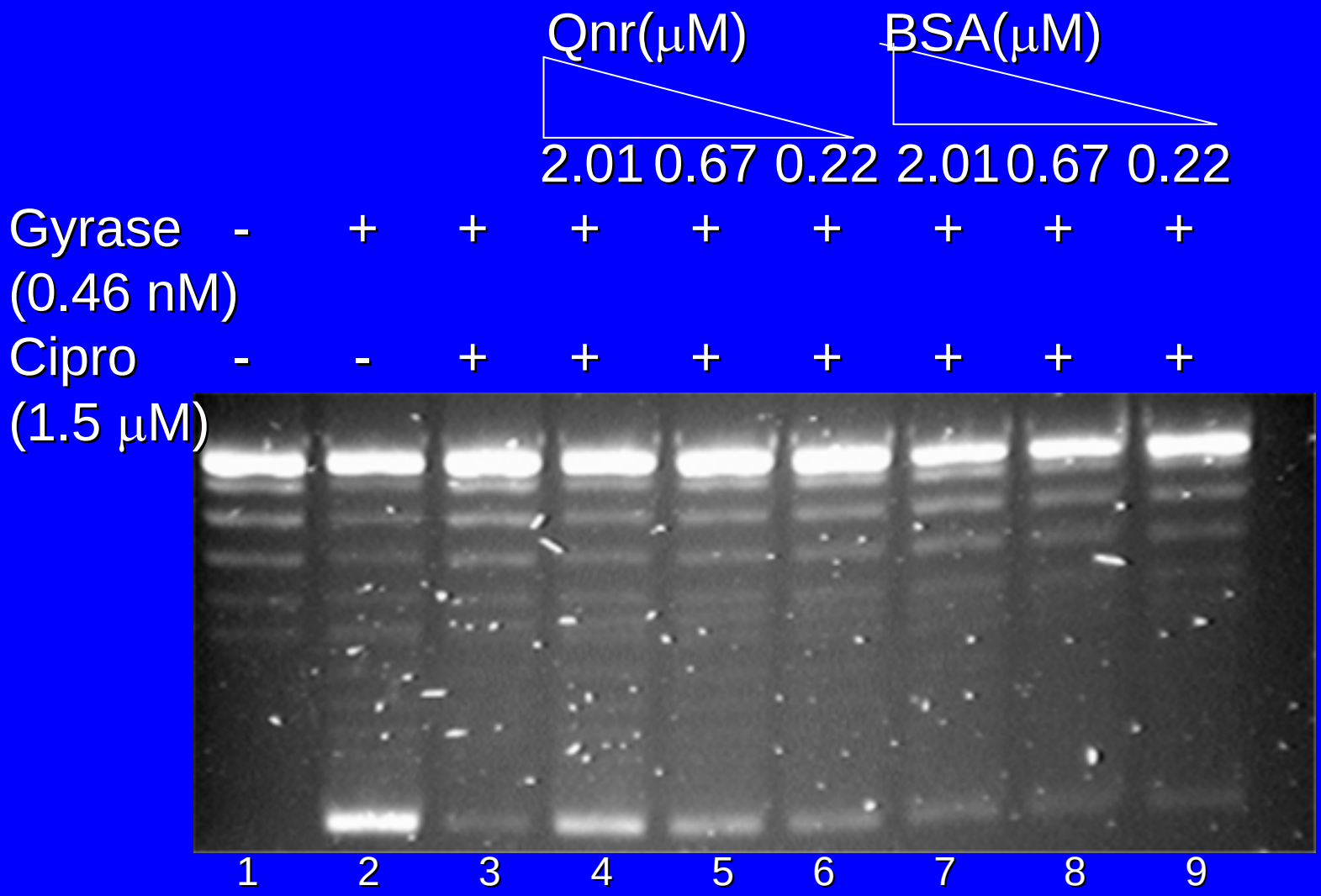
Orf 513 - postulated to be a recombinase
qacEΔ1- encodes for a truncated version of a gene for resistance to quaternary ammonium compounds
Sul1- encodes for sulfonamide resistance

Qnr Belongs to the Pentapeptide Repeat Protein Family

- two pentapeptide repeat domains of 11 and 28 repeat copies, separated by a single glycine (G)
- repeat consensus sequence is A/C D/N L/F X X
- 90% of aa are included in the pentapeptide repeats

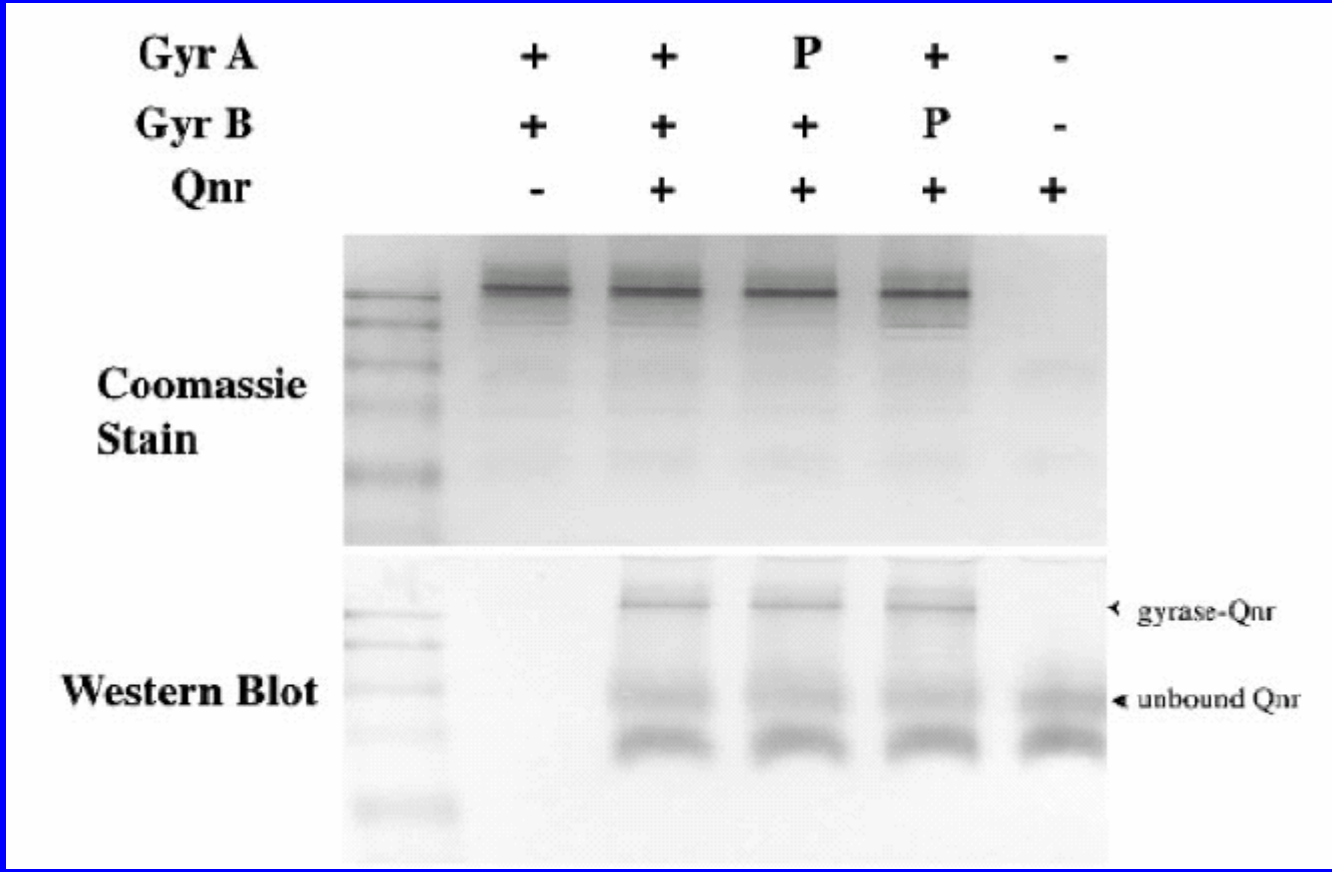
M	**D**	I	I	D		**C**	R	**L**	S	L	76	**A**	**N**	**L**	S	G	151
K	V	**F**	Q	Q		**A**	**N**	**F**	S	G		**A**	S	**L**	M	G	
E	**D**	**F**	S	R		**A**	**N**	C	F	G		S	**D**	**L**	S	R	
Q	**D**	**L**	S	D		I	E	**F**	R	E		G	T	**F**	S	R	
S	R	**F**	R	R	25	**C**	**D**	**L**	K	G		D	C	W	Q	Q	
C	R	**F**	Y	Q		**A**	**N**	**F**	S	R	101	V	**N**	**L**	R	G	176
C	**D**	**F**	S	H		**A**	R	**F**	Y	N		**C**	**D**	**L**	T	F	
C	Q	**L**	Q	D		Q	V	S	H	K		**A**	**D**	**L**	D	G	
A	S	**F**	E	D		M	Y	**F**	C	S		L	**D**	P	R	R	
C	S	**F**	I	E	50	**A**	Y	I	S	G		V	**N**	**L**	E	G	
S	G	A	V	E		**C**	**N**	**L**	A	Y	126	V	K	I	C	A	201
G						T	**N**	**L**	S	G		W	Q	Q	E	Q	
C	H	**F**	S	Y		Q	C	**L**	E	K		L	L	E	P	L	
A	**D**	**L**	R	D		**C**	E	**L**	F	E		G	V	I	V	L	
A	S	**F**	K	A		N	**N**	W	S	N		P	D				218

QnrA Reverses DNA Gyrase Inhibition



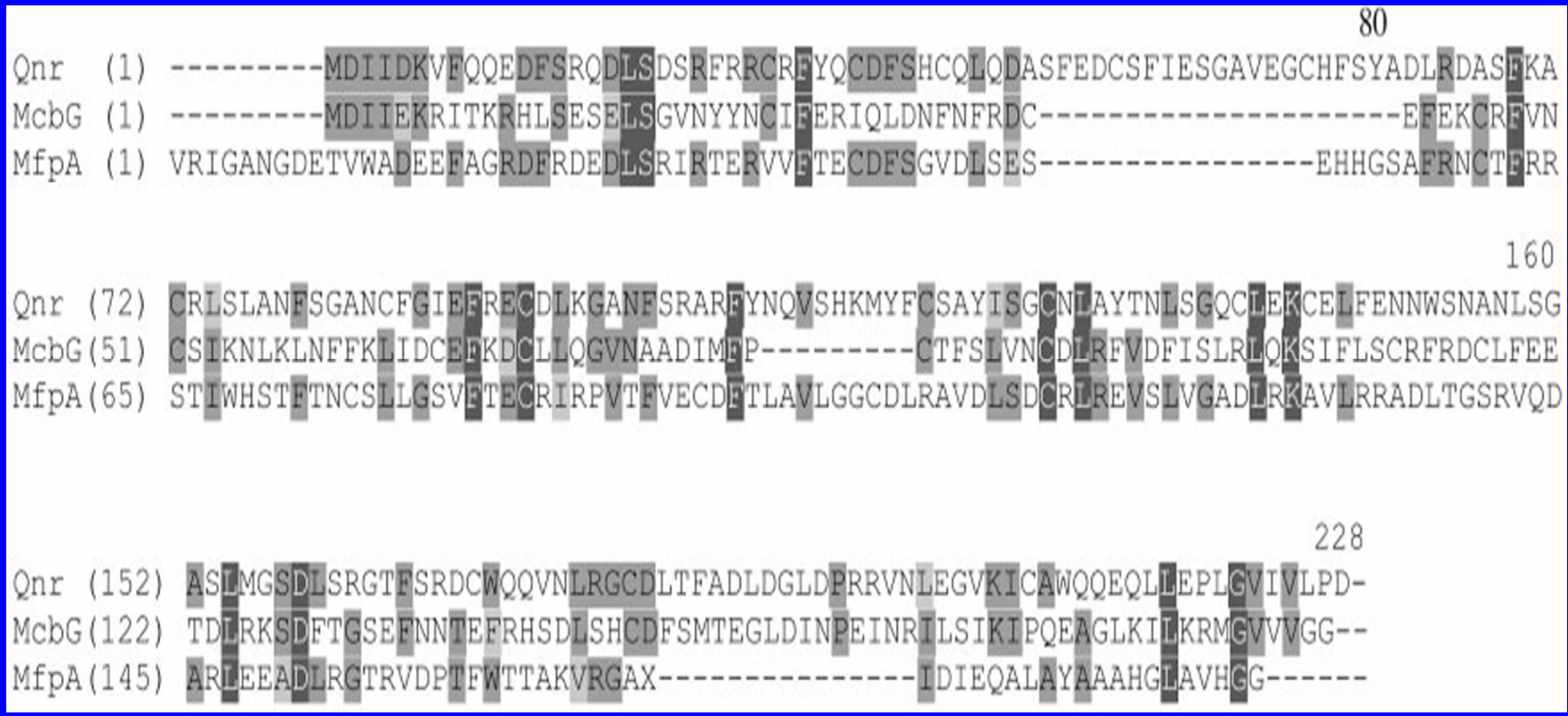
Tran J *et al.* Antimicrob Agents Chemother. 2005; 49:118-25

QnrA Binds to DNA Gyrase

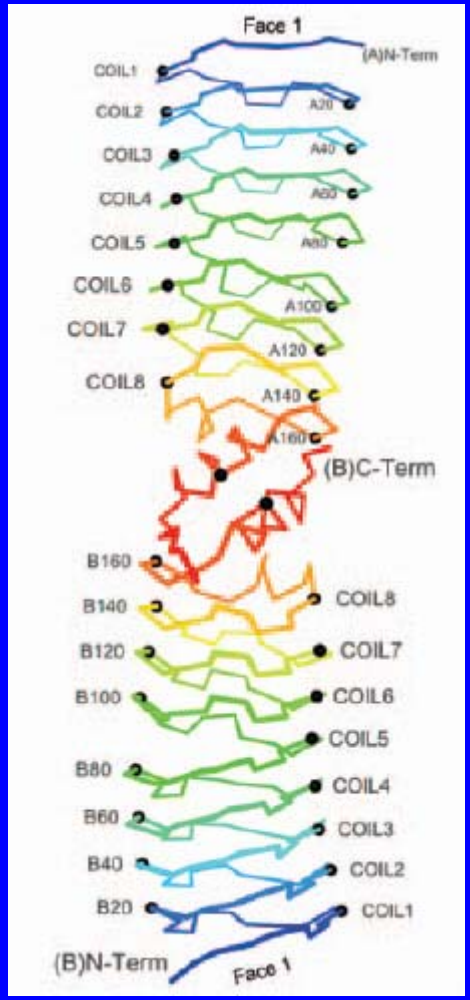


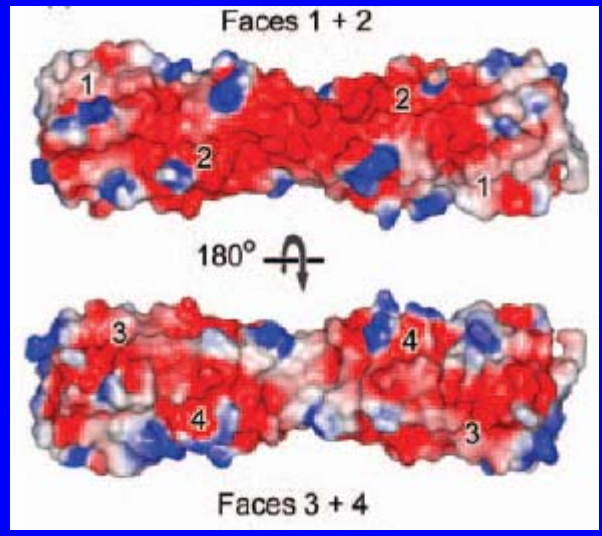
Tran J et al. Antimicrob Agents Chemother 2005; 49:118-125

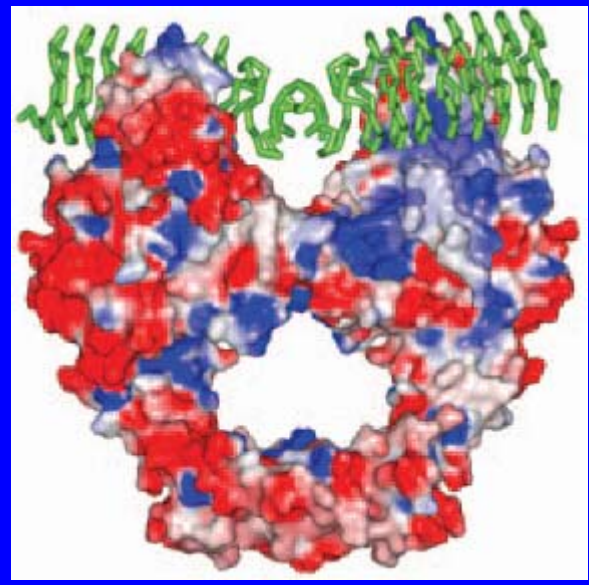
Alignment of Qnr, McbG, and MfpA



Structure of Mfp Protein

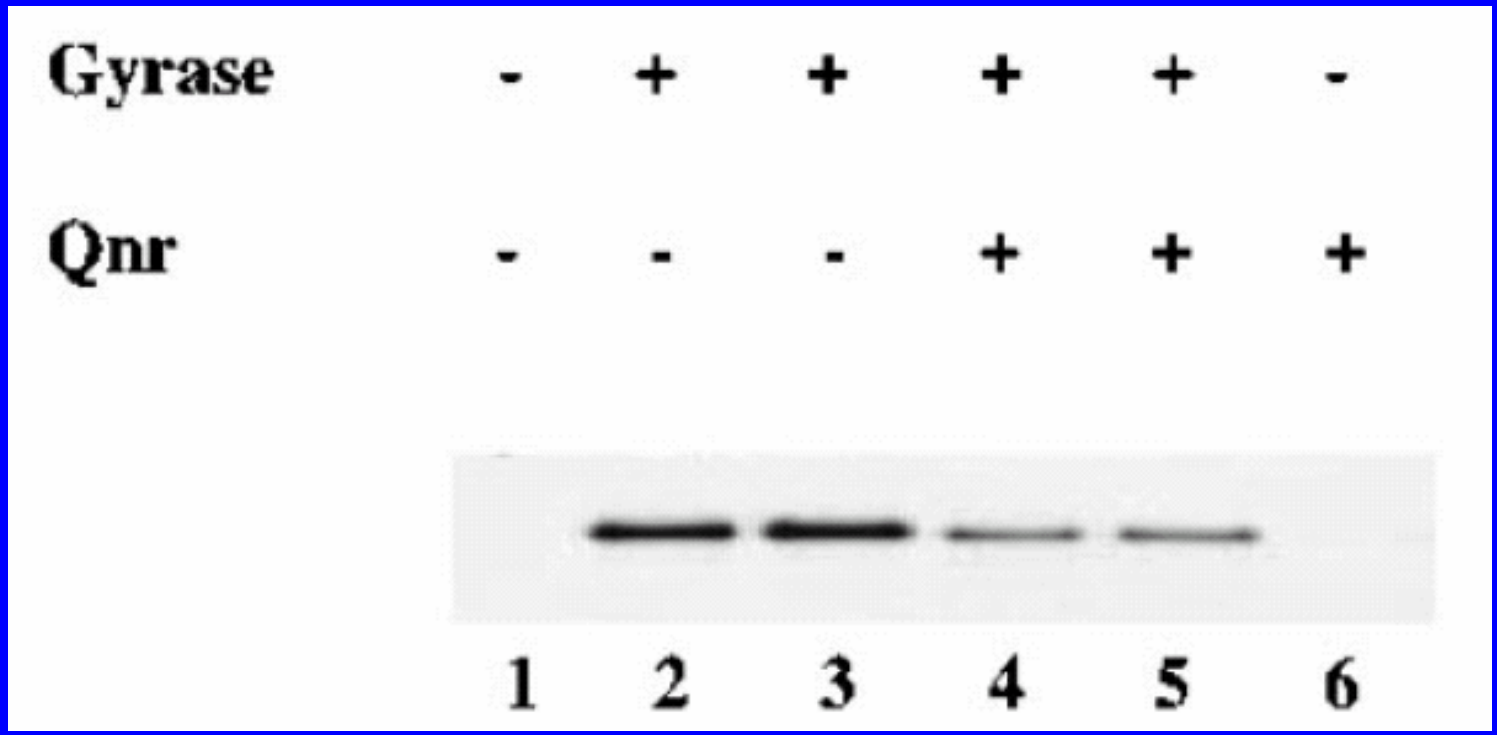






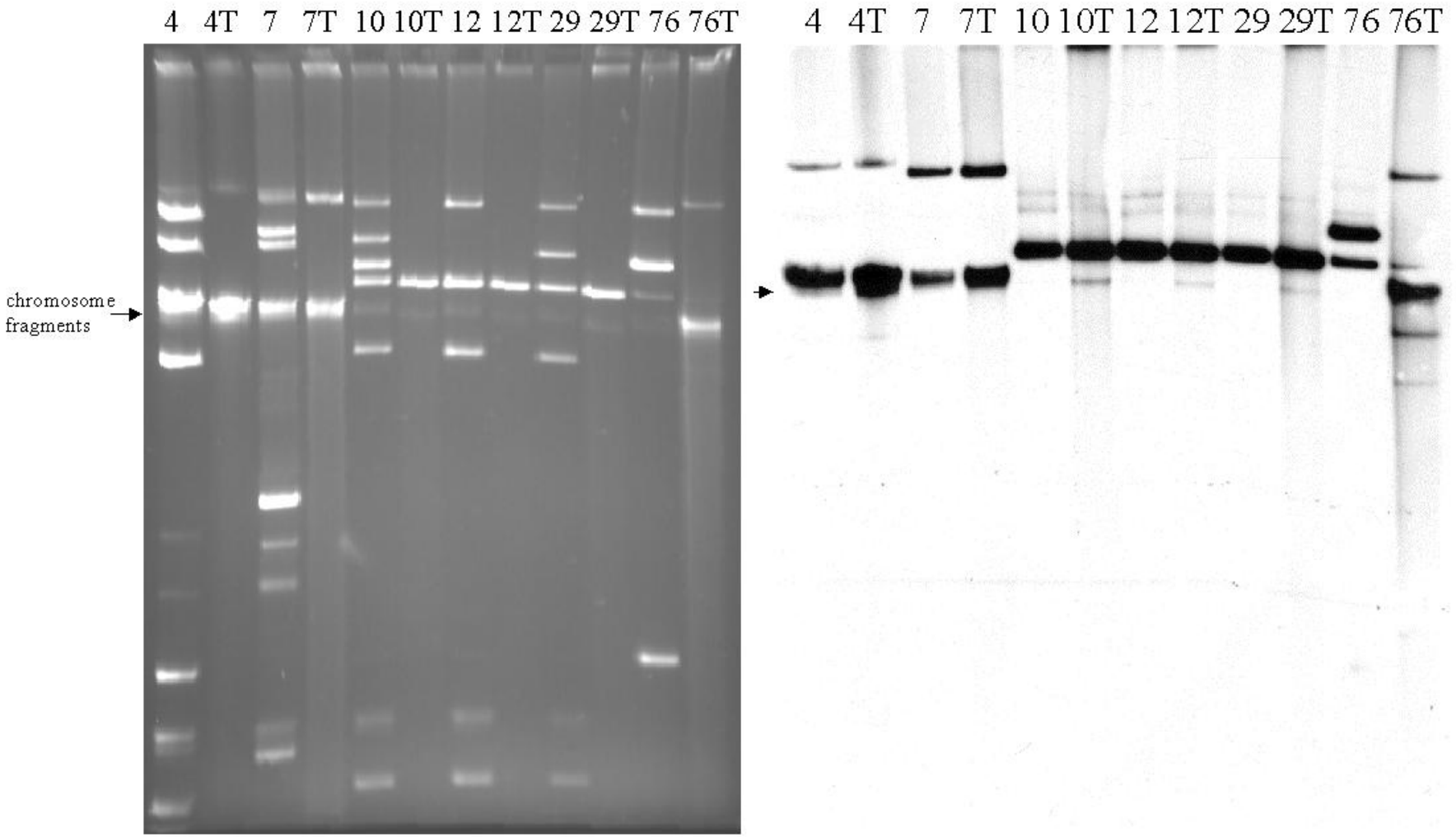
Hegde SS et al. Science 2005; 308:1480

QnrA Reduces Gyrase Binding to DNA



Tran J et al. Antimicrob Agents Chemother 2005; 49:118-125

Plasmid-Encoded *qnr* in Clinical Isolates



Wang M *et al*. Antimicrob Agents Chemother. 2003; 47:2242-8

Phenotypes of *qnr* Donors and Transconjugants

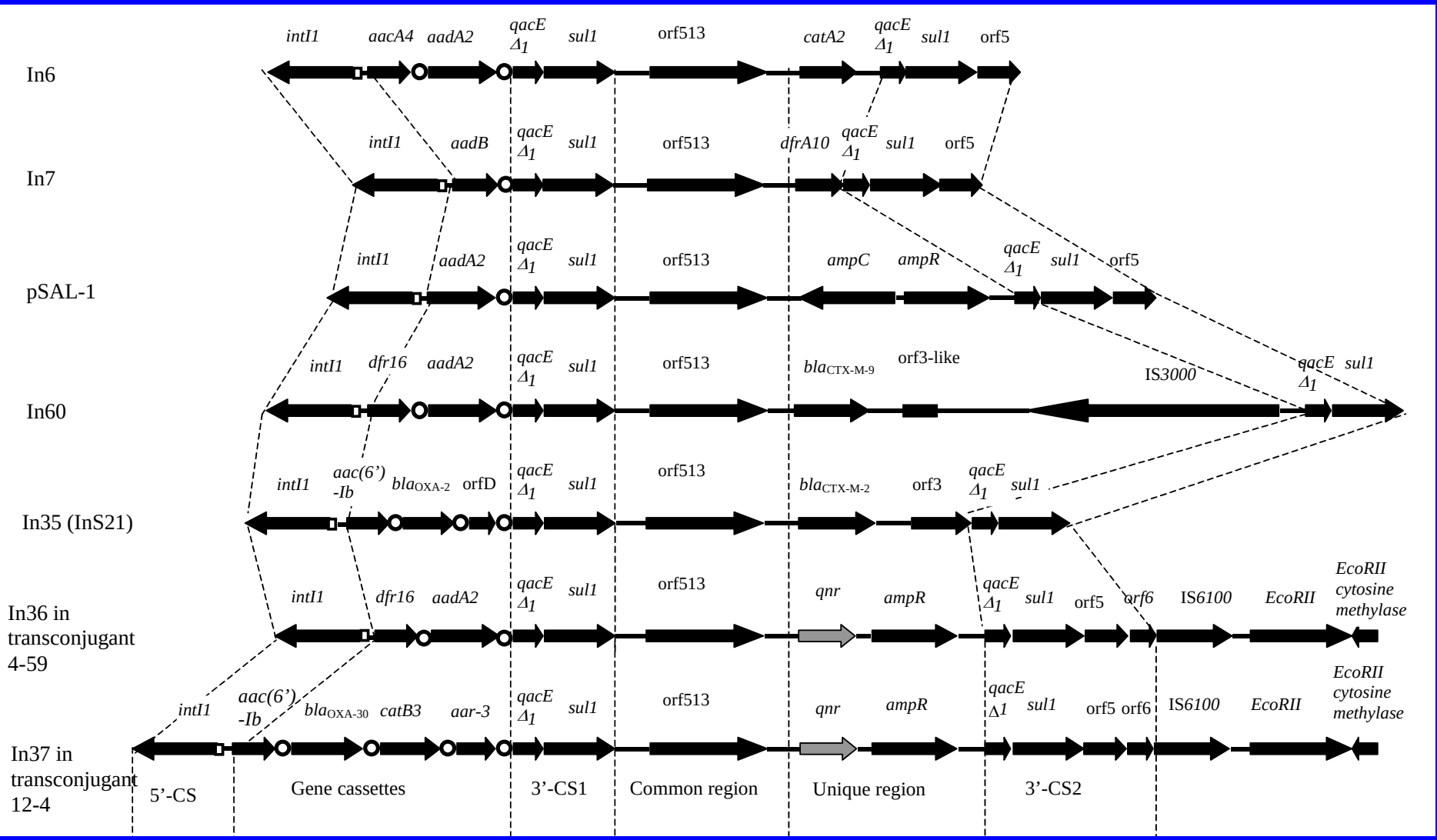
Strain		MIC (μg/ml)						
		CIP	CTX	TET	CHL	GEN	SUL	TMP
Donor	4	64	≥512	≥512	≥512	≥512	≥512	≥128
	7	128	≥512	≥512	≥512	≥512	≥512	≥128
	10	128	≥512	256	64	≥512	≥512	≥128
	12	128	≥512	256	64	≤1	≥512	≥128
Recipient	J53Az^R	0.008	≤0.03	1	4	≤0.25	16	0.125
Transconjug't	4-3	0.25	32	32	512	8	≥512	≥128
	7-24	0.125	≤1	1	≥512	2	≥512	≥128
	10-2	1	≤0.03	64	4	≤0.25	≥512	0.125
	12-4	2	32	64	4	≤0.25	≥512	0.125

Wang M *et al*. Antimicrob Agents Chemother. 2003; in press

QnrA Promotes Selection of Higher-Level Quinolone Resistance

Selection	*E coli* strain	
	J53	J53 pMG252
Ciprofloxacin 0·25 μg/mL	$<1{\cdot}6\times10^{-8}$	$3{\cdot}5\times10^{-6}$
Nalidixic acid 50 μg/mL	$<1{\cdot}6\times10^{-8}$	$3{\cdot}8\times10^{-6}$
Streptomycin 50 μg/mL	$<1{\cdot}6\times10^{-8}$	$1{\cdot}2\times10^{-4}$
Rifampicin 100 μg/mL	$1{\cdot}3\times10^{-8}$	$2{\cdot}4\times10^{-8}$
Valine 40 μg/mL	$4{\cdot}9\times10^{-8}$	$<2{\cdot}0\times10^{-8}$
Methionine positive	$1{\cdot}6\times10^{-8}$	$<2{\cdot}0\times10^{-8}$
Proline positive	$3{\cdot}3\times10^{-8}$	$5{\cdot}9\times10^{-8}$

Martínez-Martínez L et al. Lancet 1998; 351:797-9



Wang M *et al*. Antimicrob Agents Chemother. 2003; 47:2242-8

Occurrence of Integron-Carrying Enteric Bacteria in ICUs

Variable	No. (%) of ICU Patients	
	Medical (n = 277)	Neurosurgical (n = 180)
Total colonized	19 (7)	12 (7)
Acquired colonization	14 (5)	9 (5)
Time to acquisition (d)	10 ± 10	12 ± 10
Acquisition rate (per 1000 patient-days)	10	8

Nijssen S et al. Clin Infect Dis. 2005; 41:1-9.

Resistance Profiles of Integron-Carrying Enteric Bacteria

Antimicrobial	Percent Resistant	
	Integron (-) (n = 120)	Integron (+) (n = 54)
Piperacillin	24	94*
Ceftazidime	26	33
Cefotaxime	29	44*
Meropenem	0	0
Gentamicin	2	94*
Ciprofloxacin	3	33*

Nijssen S et al. Clin Infect Dis. 2005; 41:1-9.

Prevalence of Plasmid-Mediated Quinolone Resistance

- Shanghai, China
  - *E. coli* - *qnrA* gene detected in 6 (7.7%) of 78 ciprofloxacin- and multidrug-resistant strains
- United States
  - *K. pneumoniae* - *qnrA* gene detected in 8 (11%) of 72 strains with MICs of ciprofloxacin ≥ 2 μg/ml and ceftazidime ≥ 16 μg/ml
    - These 8 positive strains were isolated from 6 states (AL, AZ, DE, KY (2), NY(2), TN)
  - *E. coli* - *qnrA* was not found in any of the 38 strains

Wang M et al. Antimicrob Agents Chemother. 2003; 47:2442
Wang M et al. Antimicrob Agents Chemother. 2004; 48:1295

Prevalence of Plasmid-Mediated Quinolone Resistance

- United States
  - *Enterobacter* spp. – *qnrA* in 12 (17%) of 72 strains with ciprofloxacin MIC of 0.25 to > 8 μg/ml and ceftazidime MIC of >16 μg/ml.
    - 11 (24%) of 46 quinolone-resistant strains positive
    - 1 (4%) of 26 quinolone-susceptible strains positive
    - Found in 5 states (NY - 2, AZ - 3, AL -2, WV - 2, CA - 3)
  - *K. pneumoniae* – *qnrA* in 2 (10%) of 20 quinolone-susceptible (both from NY)
  - *Proteus* spp. – *qnrA* in 0 of 6 strains

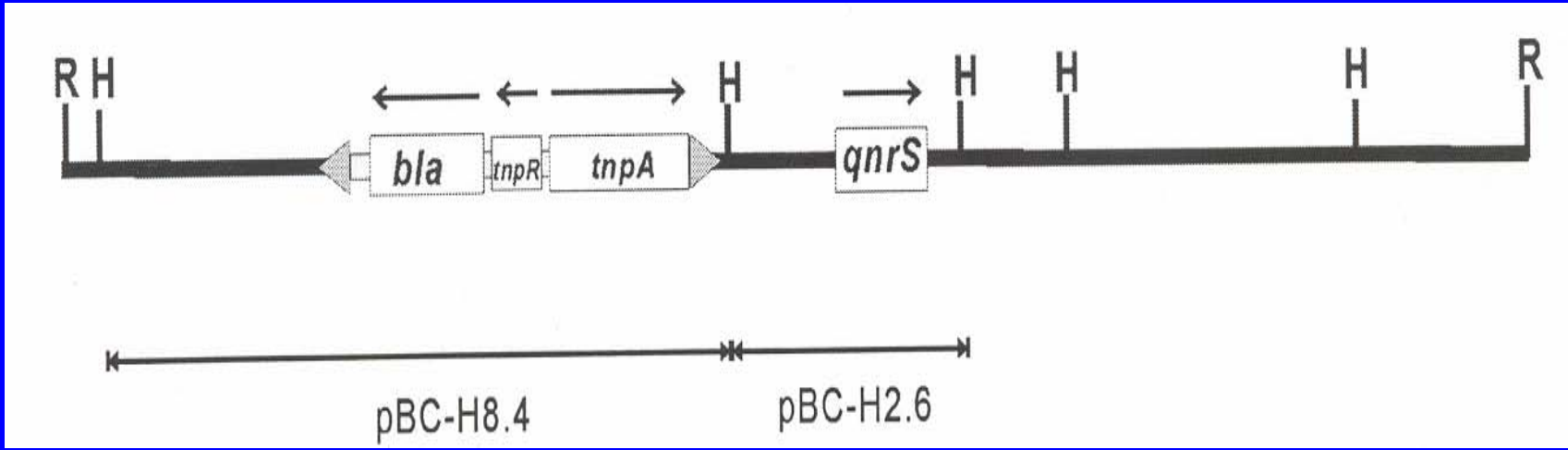
Robicsek A et al. Antimcrob Agents Chemother. 2005; 49:3001

Prevalence of Plasmid-Mediated Quinolone Resistance

- Egypt
  - *Providencia stuartii* – transferable resistance
- Thailand
  - Enterobacteriaceae – 11 *qnrA* of 23 with VEB-1 β-lactamase
- Hong Kong
  - *Salmonella enterica* serotype Enteritidis – *qnrA3* on 4 different plasmids
- Japan
  - *Shigella flexneri – qnrS* (59% identity with *qnrA*)

Hata M et al. Antimicrob Agents Chemother. 2005; 49:801
Poirel L et al. Antimicrob Agents Chemother 2005; 49:3091
Cheung TKM et al. J Antimicrob Chemother 2005; 56:586

Location of *qnrS*



Hata M *et al*. Antimicrob Agents Chemother. 2005; 49:801-3

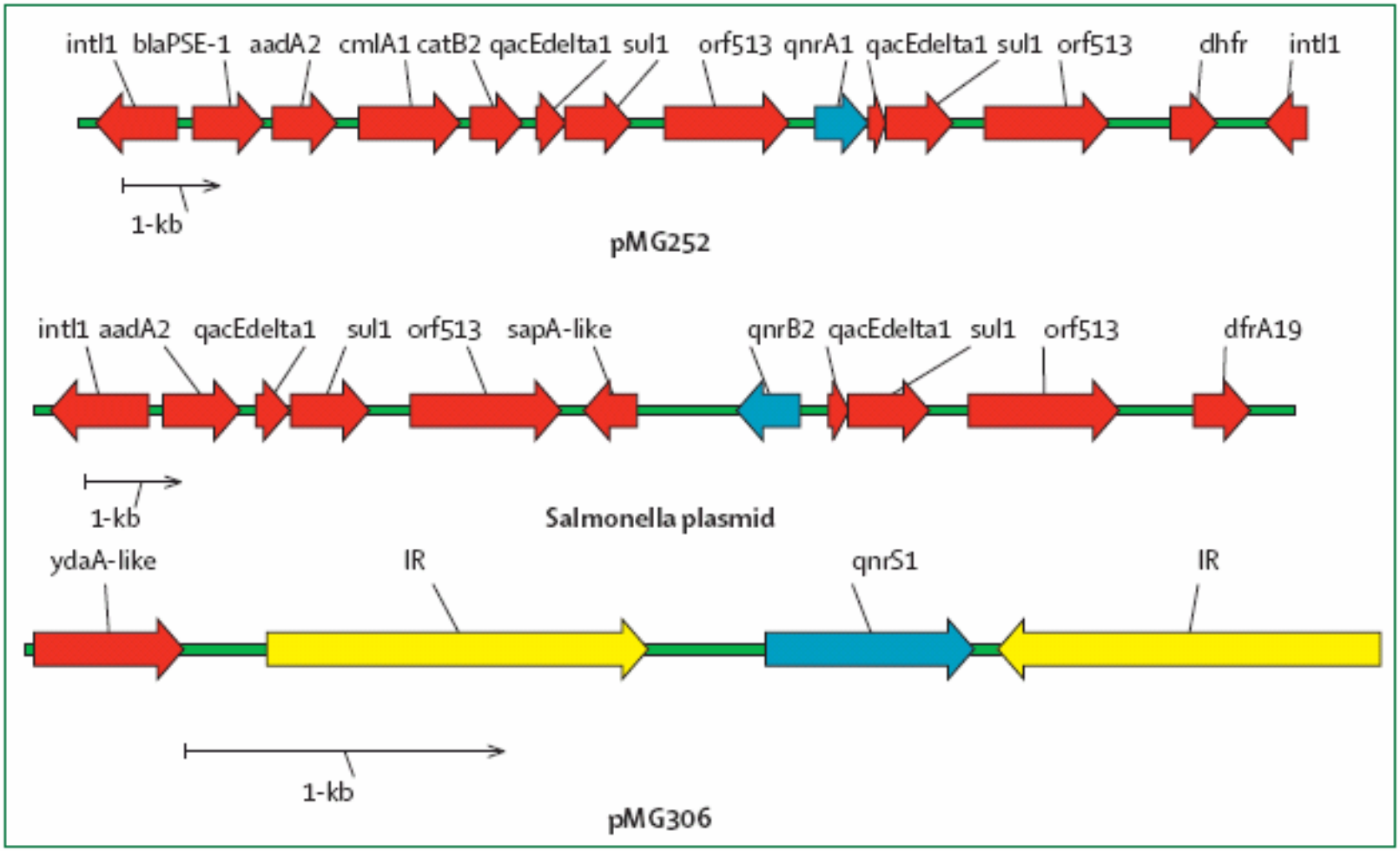
QnrB

- Found first in *K. pneumoniae* clinical isolate from India on MDR plasmid with CTX-M-15 β-lactamase gene
  - Additional 3 of 8 Indian *K. pneumoniae* positive
- 40% amino acid identity with QnrA
- United States
  - 8 of 61 (13%) *K. pneumoniae* isolates positive
  - 11 of 42 (26%) *Enterobacter* spp. isolates positive

Variants of QnrB

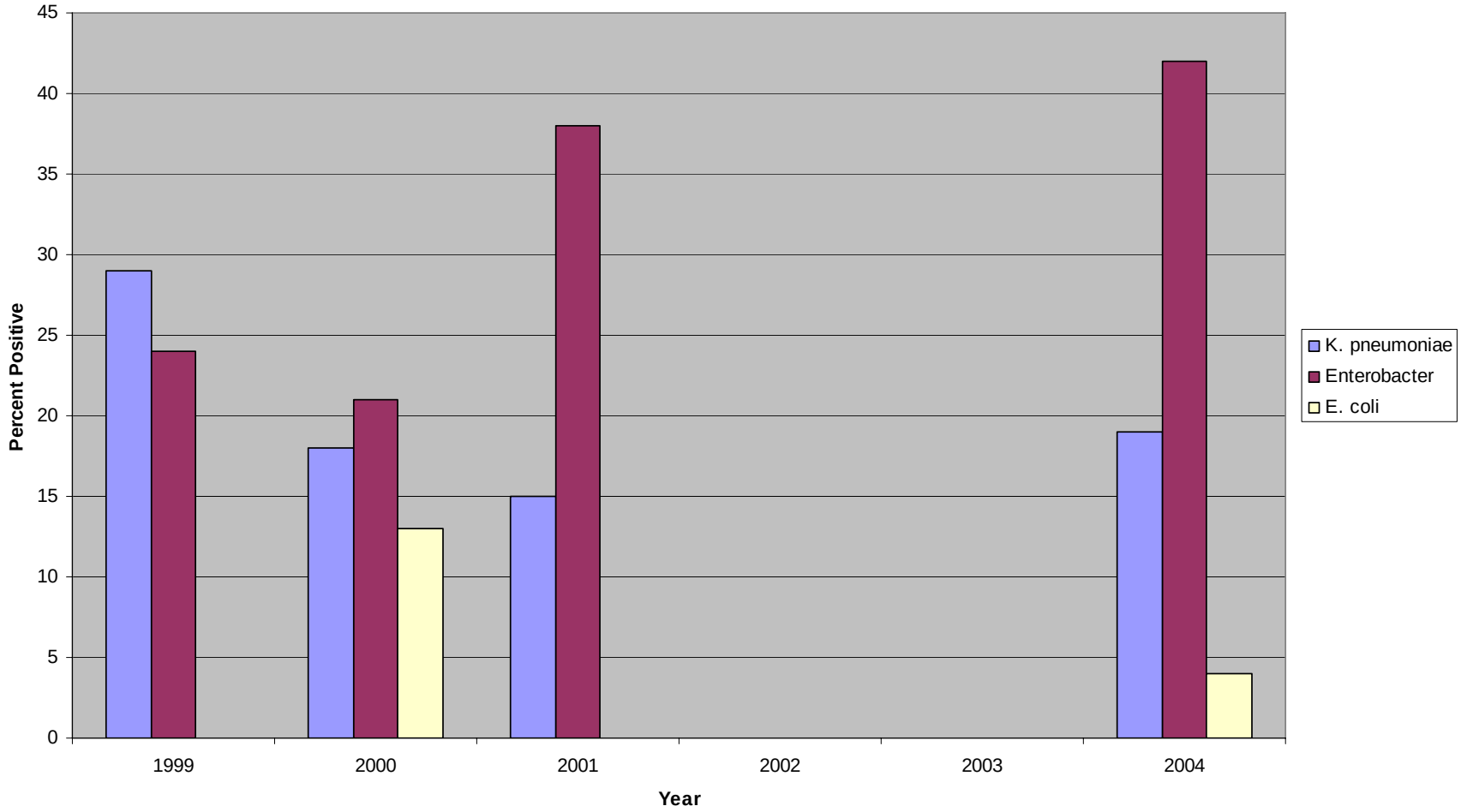
```
qnrB1           MTPLLYKKTGTNMALALVGEKIDRNRFTGEKIENSTFFNCDFSGADLSGTEFIGCQFYDR 60
qnrB3           MTPLLYKKTGTNMALALVGEKIDRNRFTGEKIENSTFFNCDFSGADLSGTEFIGCQFYDR 60
qnrB2           ------------MALALVGEKINRNRFTGEKIENSTFFNCDFSGADLSGTEFIGCQFYDR 48
qnrB5           MTPLLYKNTGIDMTLALVGEKIDRNRFTGEKVENSTFFNCDFSGADLSGTEFIGCQFYDR 60
qnrB4           ------------------------------------------------------------

qnrB1           ESQKGCNFSRAMLKDAIFKSCDLSMADFRNSSALGIEIRHCRAQGADFRGASFMNMITTR 120
qnrB3           ESQKGCKFSRAMLKDAIFKSCDLSMADFRNSSALGIEIRHCRAQGADFRGASFMNMITTR 120
qnrB2           ESQKGCNFSRAMLKDAIFKSCDLSMADFRNASALGIEIRHCRAQGADFRGASFMNMITTR 108
qnrB5           ESQKGCNFSRAMLKDAIFKSCDLSMADFRNVSALGIEIRHCRAQGADFRGASFMNMITTR 120
qnrB4           -------------------SCDLSMADFRNINALGIEIRHCRAQGSDFRGASFMNMITTR 41

qnrB1           TWFCSAYITNTNLSYANFSKVVLEKCELWENRWIGAQVLGATFSGSDLSGGEFSTFDWRA 180
qnrB3           TWFCSAYITNTNLSYANFSKVVLEKCELWENRWMGAQVLGATFSGSDLSGGEFSTFDWRA 180
qnrB2           TWFCSAYITNTNLSYANFSKVVLEKCELWENRWMGAQVLGATFSGSDLSGGEFSTFDWRA 168
qnrB5           TWFCSAYITNTNLSYANFSKVVLEKCELWENRWMGTQVLGATFSGSDLSGGEFSTFDWRA 180
qnrB4           TWFCSAYITNTNLSYANFSKVVLEKCELWENRWMGTQVLGATFSGSDLSGGEFSSFDWRA 101

qnrB1           ANFTHCDLTNSELGDLDIRGVDLQGVKLDNYQASLLMERLGIAVIG 226
qnrB3           ANFTHCDLTNSELGDLDIRGVDLQGVKLDNYQASLLMERLGIAVIG 226
qnrB2           ANFTHCDLTNSELGDLDIRRVDLQGVKLDNYQASLLMERLGIAIIG 214
qnrB5           ANFTHCDLTNSELGDLDIRGVDLQGVKLDSYQASLLMERLGIAIIG 226
qnrB4           ANVTHCDLTNSELGDLDIR------------------------- 120
```

Plasmid-Encoded Quinolone Resistance: Qnr Genes



Robicsek A et al. Lancet Infect Dis 2006; 6:629-40

Progression of Qnr Prevalence



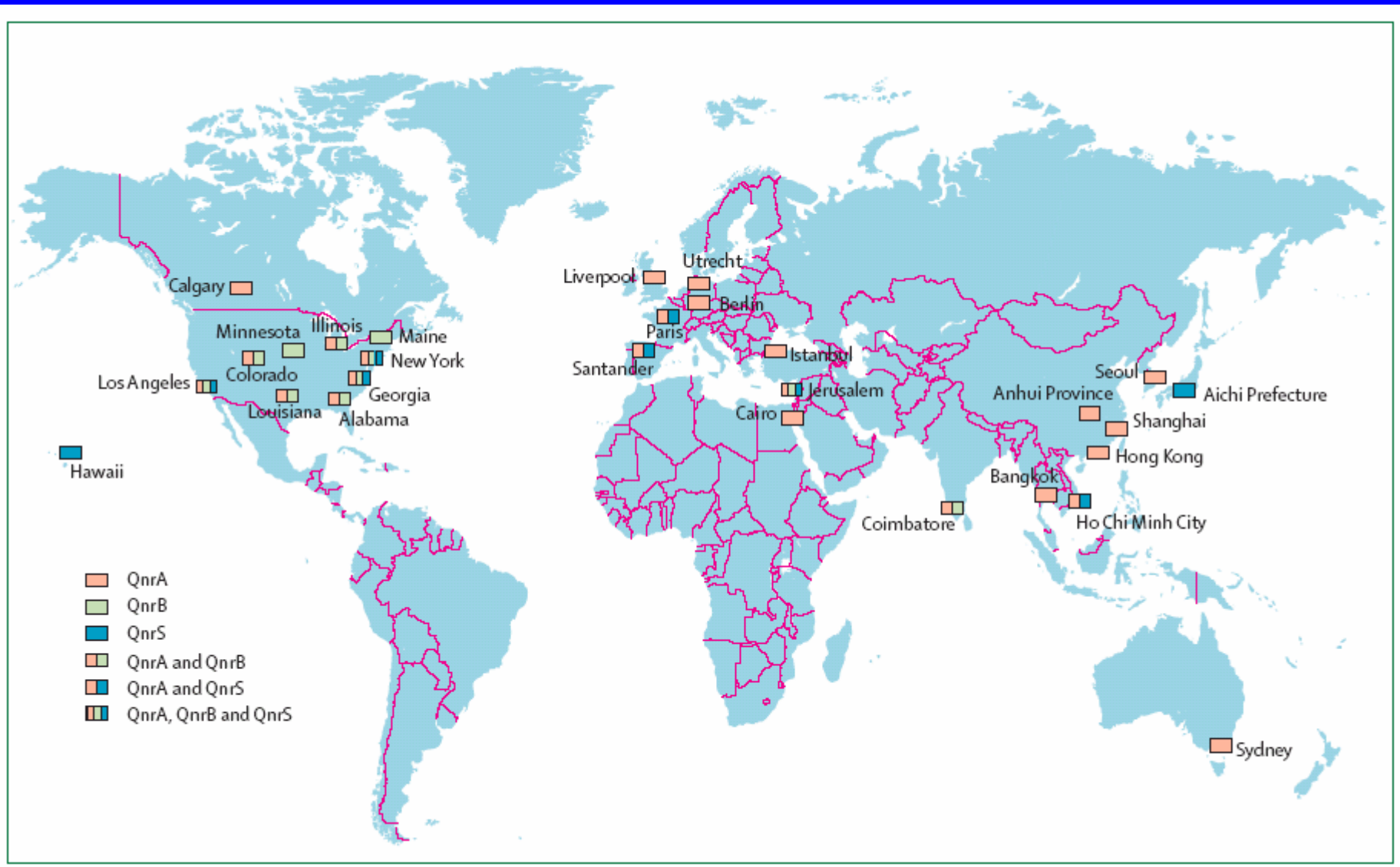
A Robicsek et al. Antimicrob Agents Chemother 2006; in press

Other Sources of *qnr* Genes

- Homologues identified from genome sequences
  - *Photobacterium profundum*
    - 66% related to QnrA
    - Cloned gene confers 80x increase MIC of ciprofloxacin
  - *Vibrio vulnificus*
    - 60% related to QnrA
    - Cloned gene confers 64x increase MIC of ciprofloxacin
  - *Vibrio parahaemolyticus*
    - 58% related to QnrA
    - Cloned gene confers 16x increase in MIC of ciprofloxacin
    - Amino acid 115 affects activity; Tyr > Cys
  - *Shewanella algae*
    - Related to QnrA
    - Cloned gene confers 16x increase in MIC of ciprofloxacin

Poirel L et al. J Antimicrob Chemother. 2005; 56:1118
Saga T et al. Antimicrob Agents Chemother. 2005; 49:2144

Worldwide Distribution of Qnr Quinolone Resistance Genes



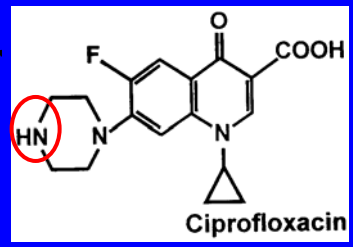
Robicsek A et al. Lancet Infect Dis 2006; 6:629-40

Mechanisms of Resistance to Fluoroquinolones

- Chromosomal mutations
  - Alterations in DNA gyrase and/or topoisomerase IV
  - Active drug efflux (MDR pumps) +/- reduced porin diffusion channels
- Plasmid-mediated resistance
  - Enteric gram-negative bacteria; target protection mechanism by Qnr proteins
  - Drug modification

The Newest Mechanism of Plasmid-Mediated Quinolone Resistance

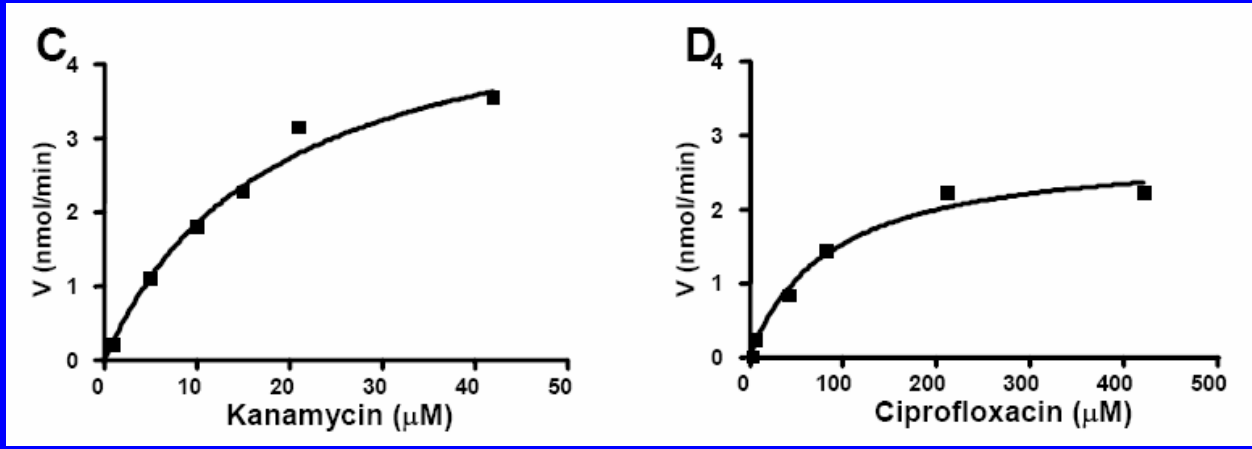
- Specific modification of some quinolones (ciprofloxacin, norfloxacin)
- Mutant of a common aminoglycoside acetyltransferase enzyme, Aac(6')Ib
  - Mutations Trp102Arg and Asp179Tyr = Aac(6')Ib-cr
  - Acetylates ciprofloxacin at piperazinyl *N*
  - Slight decrease in kanamycin acetylation

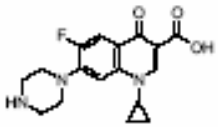
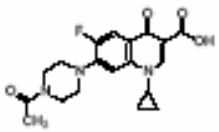
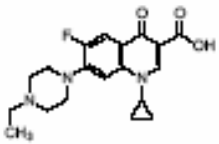
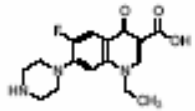
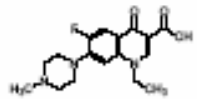
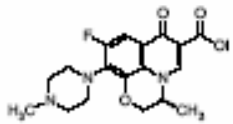
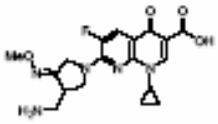


- Low-level resistance (4-fold)
- Promotes selection of high-level resistance with quinolone exposure
- *aac(6')-Ib*-cr located on plasmids with and without *qnr* genes

Robicsek A et al. Nature Medicine 2006; 12;83-88

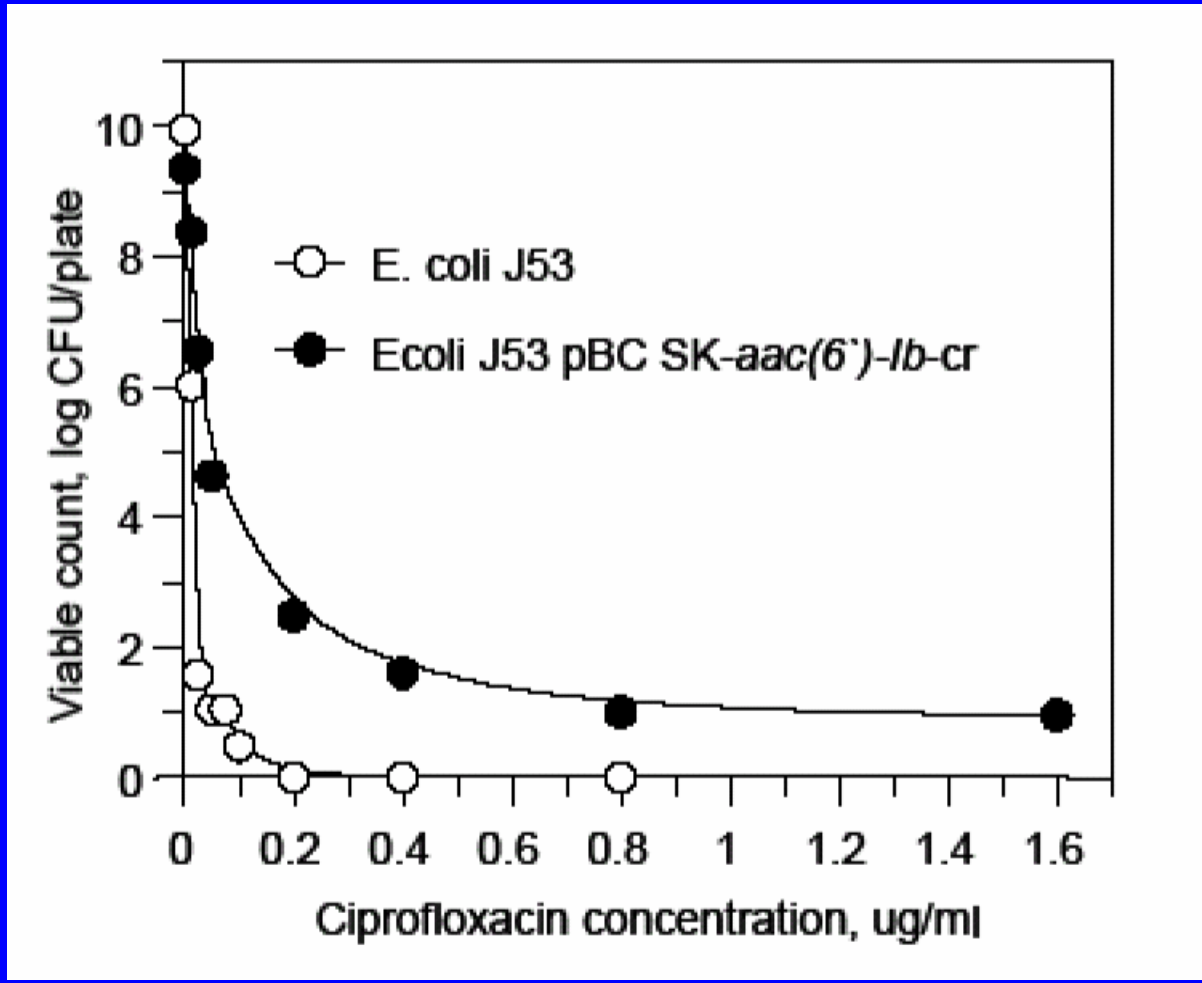
Activity of Aac(6')-Ib-cr



Strain	Ciprofloxacin	*N*-acetyl ciprofloxacin	Enrofloxacin	Norfloxacin	Pefloxacin	Levofloxacin	Gemifloxacin
DH10B pBAD24	0.02	0.08	0.02	0.156	0.08	0.08	0.005
DH10B pBAD24-*aac(6`)-Ib*-cr	0.04-0.08	0.08	0.02	0.625	0.08	0.08	0.005
Chemical structure							

Robicsek A et al. Nature Medicine 2006; 12;83-88

Effect of *aac(6')-Ib-cr* on Selection of Quinolone Resistant Mutants



Robicsek A et al. Nature Medicine 2006; 12;83-88

Epidemiology of *aac(6')-Ib-cr*

Shanghai, China

- 78 ciprofloxacin-resistant clinical *E. coli*
- *aac(6')-Ib*-cr located on 4 of 6 plasmids with *qnrA* genes
- *aac(6')-Ib*-cr >6-fold more prevalent than *qnrA*
  - 51% *aac(6')-Ib*-cr vs 7.7% *qnrA*
  - 84% of strains with *aac(6')-Ib* had the cr variant (all with both mutations)

Robicsek A et al. Nature Medicine 2006; 12;83-88

Epidemiology of *aac(6')-Ib-cr*

United States

- 313 *Enterobacteriaceae* 1999-2004
  - MICs: ciprofloxacin ≥ 0.25 μg/ml, ceftazidime ≥ 16 μg/ml
- *aac(6')-Ib*-cr widely distributed independently of *qnrA* and *qnrB*
- *aac(6')-Ib*-cr similarly present on ciprofloxacin-resistant and –susceptible isolates
- *aac(6')-Ib*-cr
  - slightly more prevalent than *qnrA* alone (14% vs 11%)
  - slightly more prevalent than *qnrB* alone (14% vs 12%)
- 28% of strains with *aac(6')-Ib* had the cr variant

Park CH et al. ICAAC 2006; Abstract C2-92
Antimicrob Agents Chemother 2006; in press

Epidemiology of *aac(6')-Ib-cr*

United Kingdom

- 44 *E. coli* with CTX-M β-lactamases
- *aac(6')-Ib* present in 25 of 27 group 1 CTX-M isolates
- cr variant found in 10 of 10 sequenced isolates
- Co-transfer of $bla_{\text{CTX-M-15}}$ and *aac(6')-Ib-cr* on a single plasmid conferred 8-fold increase in MIC of ciprofloxacin (0.03 → 0.25 μg/ml)
- *qnr* absent from *aac(6')-Ib*-cr isolates

Karisik E et al. Abstract C2-100

Epidemiology of *aac(6')-Ib-cr*

Spain

- Survey of aminoglycoside-modifying enzymes
- Incubation of cell extracts with series of quinolones (ciprofloxacin, levofloxacin, ofloxacin, moxifloxacin, garenoxacin, grepafloxacin) to assess effect on MICs
- Reduction in activity of garenoxacin and grepafloxacin (MICs 0.001-0.03 $\rightarrow \geq$ 16μg/ml) with extracts with Aac(6') activity from *E. faecalis, E. faecium, and E. coli*

Del Campo R et al. Abstract LB

Unanswered Questions

- What is the full extent of plasmid-encoded quinolone resistance and its mechanisms?
  - How many pentapeptide-repeat proteins mediate quinolone resistance in Nature?
  - Normal function of Qnr proteins?
  - How many drug modification mechanisms?
  - Other mechanisms (efflux pumps, bypass targets, mutators)?
- How are *qnr* genes mobilized on plasmids or to and from chromosomes?
- Does the presence of plasmid-encoded genes promote quinolone resistance selection in populations of patients?
- Do similar mechanisms exist in Gram-positive bacteria?

Contributors:

Que Chi Truong-Bolduc

Ari Robicsek

Yoshikuni Onodera

Chi Hye Park

Herin Oh

Dilek Ince

Minggui Wang

John Tran

Jacob Strahilevitz

Jian-Lin Yu

Bénédicte Fournier

Collaborators:

George Jacoby

Dan Sahm

Steve Projan

Paul Dunman

Leo Grinius

Pierre Vaudaux

Carmello Bisognano

Karl Drlica

Xilin Zhao

Tao Liu