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ESCMID STUDY GROUP FOR ANTIMICROBIAL RESISTANCE SURVEILLANCE
European Society of Clinical Microbiology and Infectious Diseases

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Mechanisms of resistance to quinolones in Gram positive bacteria

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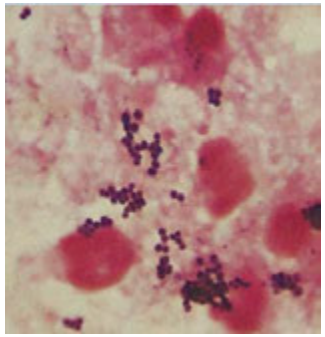


Which Gram-positives?

Cocci

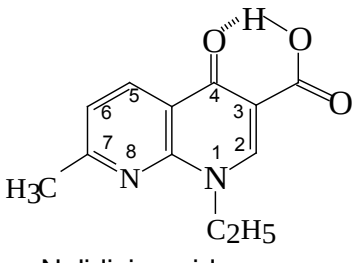
- Streptococci
- Staphylococci
- Enterococci

Bacilli

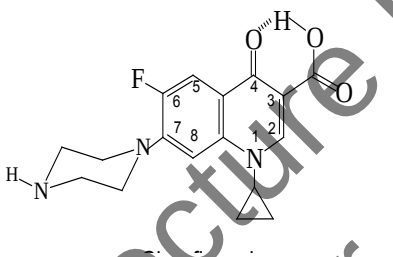
- Listeria
- Clostridium
- Bacillus
- Corynebacterium
- Mycobacterium

Structure activity relationship

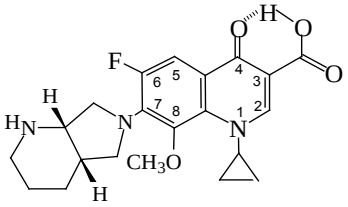
(modal MIC in mg/L)



Nalidixic acid



Ciprofloxacin



Moxifloxacin

E. coli	**4**	**0.001**	**0.06**
S. aureus	**32**	**0.5**	**0.03**
S. pneumoniae	**> 512**	**2**	**0.12**

⇒Gram positive bacteria are naturally less susceptible to classical and to fluoroquinolones than Gram negative bacteria but are more susceptible to new fluoroquinolones

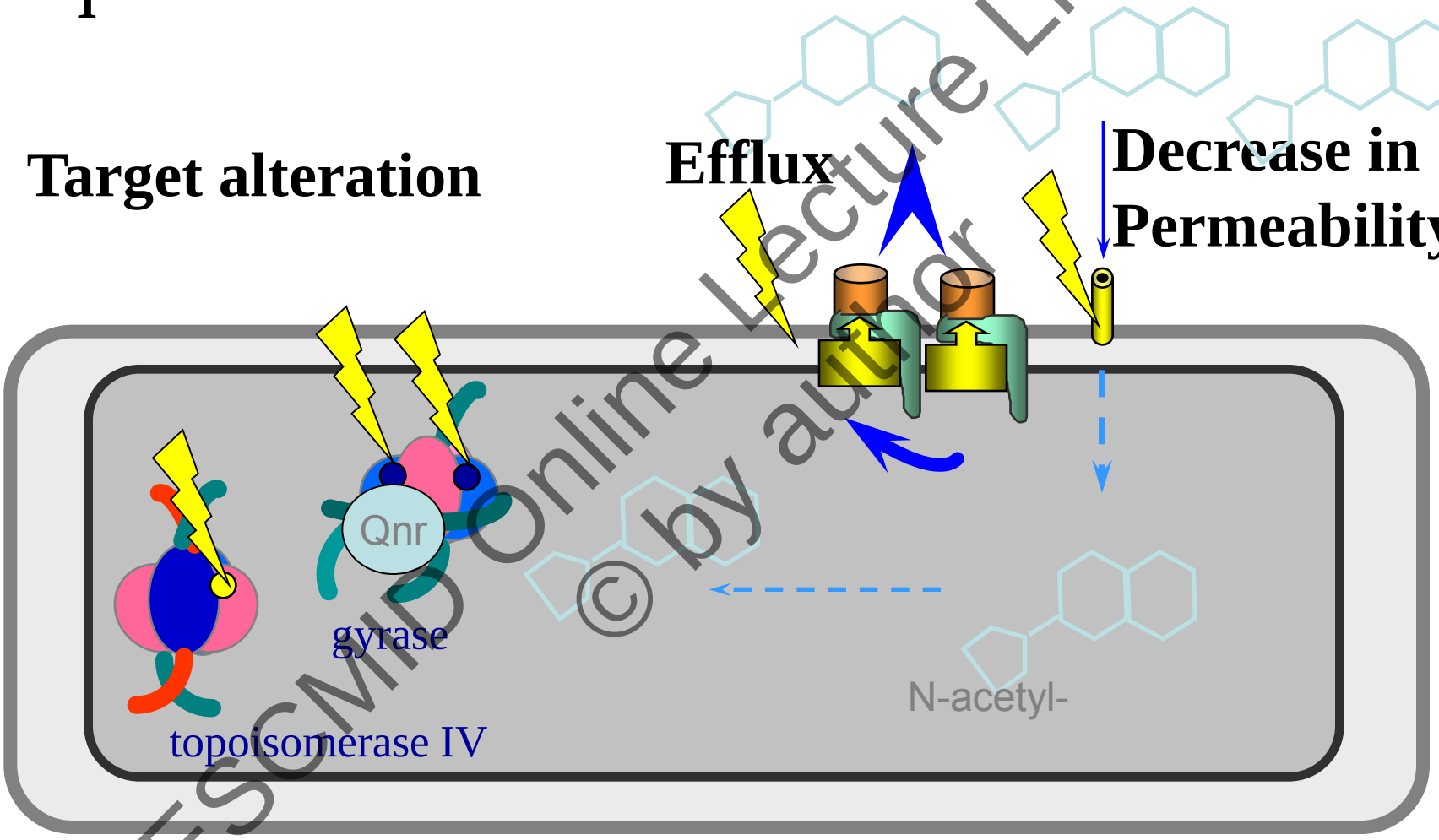
Antibacterial activity of new fluoroquinolones on *Streptococcus pneumoniae*

Quinolones	MIC 50 (mg/L)
Ciprofloxacin	2
Ofloxacin	2
Levofloxacin	1
Sparfloxacin	0,5
Gatifloxacin	0,5
Grepafloxacin	0,5
Trovafloxacin	0,25
Moxifloxacin	0,25
Clinafloxacin	0,12
Sitafloxacin	0,06
Gemifloxacin	0,03
Garénoxacin	0,03

Antibacterial activity of new fluoroquinolones on staphylococci

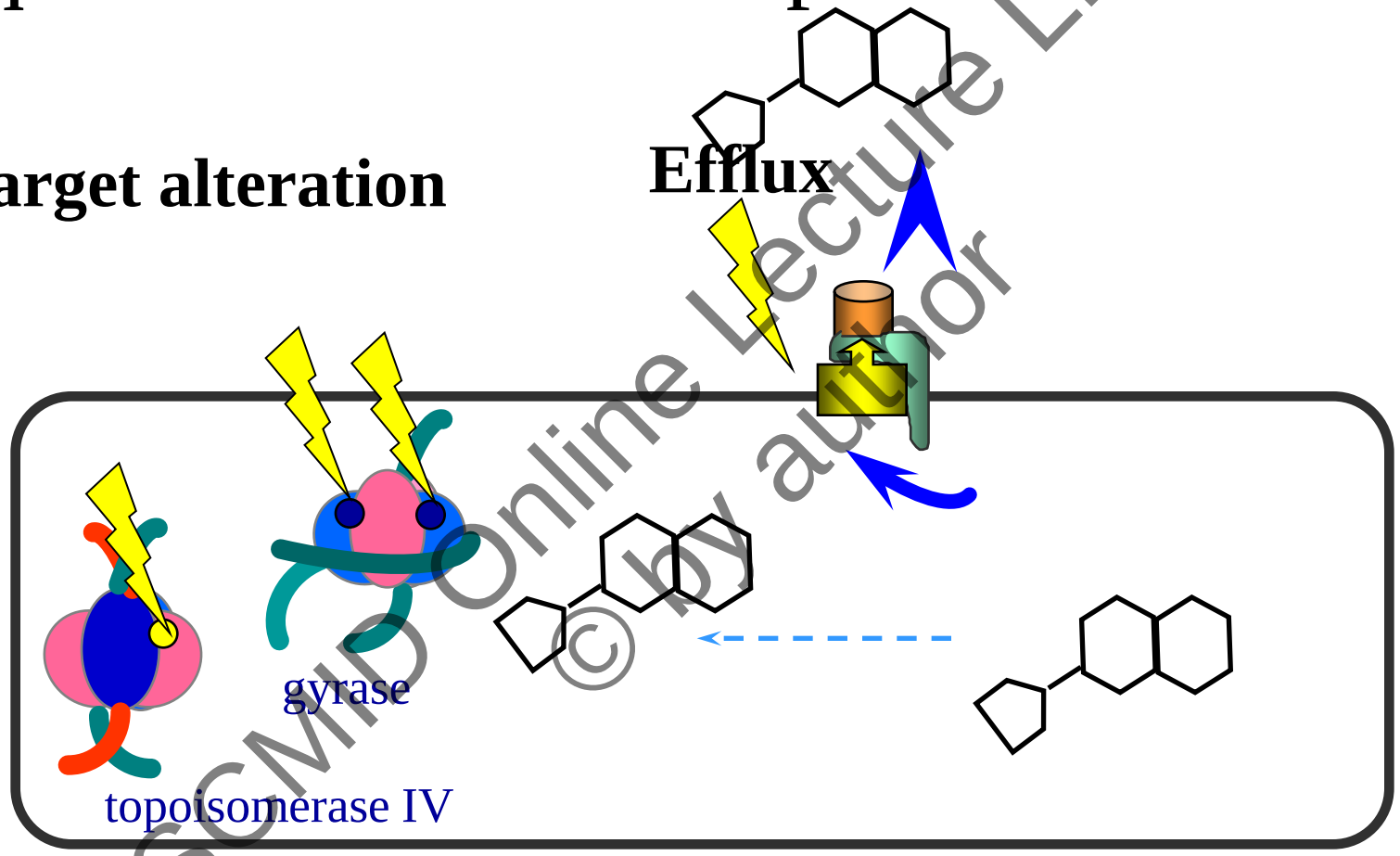
Quinolones	MIC 50 (mg/L)	
	S. aureus	*S. epidermidis*
Ciprofloxacin	0,5	0,25
Ofloxacin	0,5	0,5
Levofloxacin	0,25	0,25
Sparfloxacin	0,12	0,12
Trovafloxacin	0,03	0,03
Grepafloxacin	0,06	0,12
Moxifloxacin	0,06	0,06
Clinafloxacin	0,06	0,12
Gemifloxacin	0,01	0,03
Gatifloxacin	0,12	0,12
Sitafloxacin	0,03	0,03
Garenoxacin	0,03	0,03

Mechanisms of resistance to quinolones



adapted from Peter Heisig

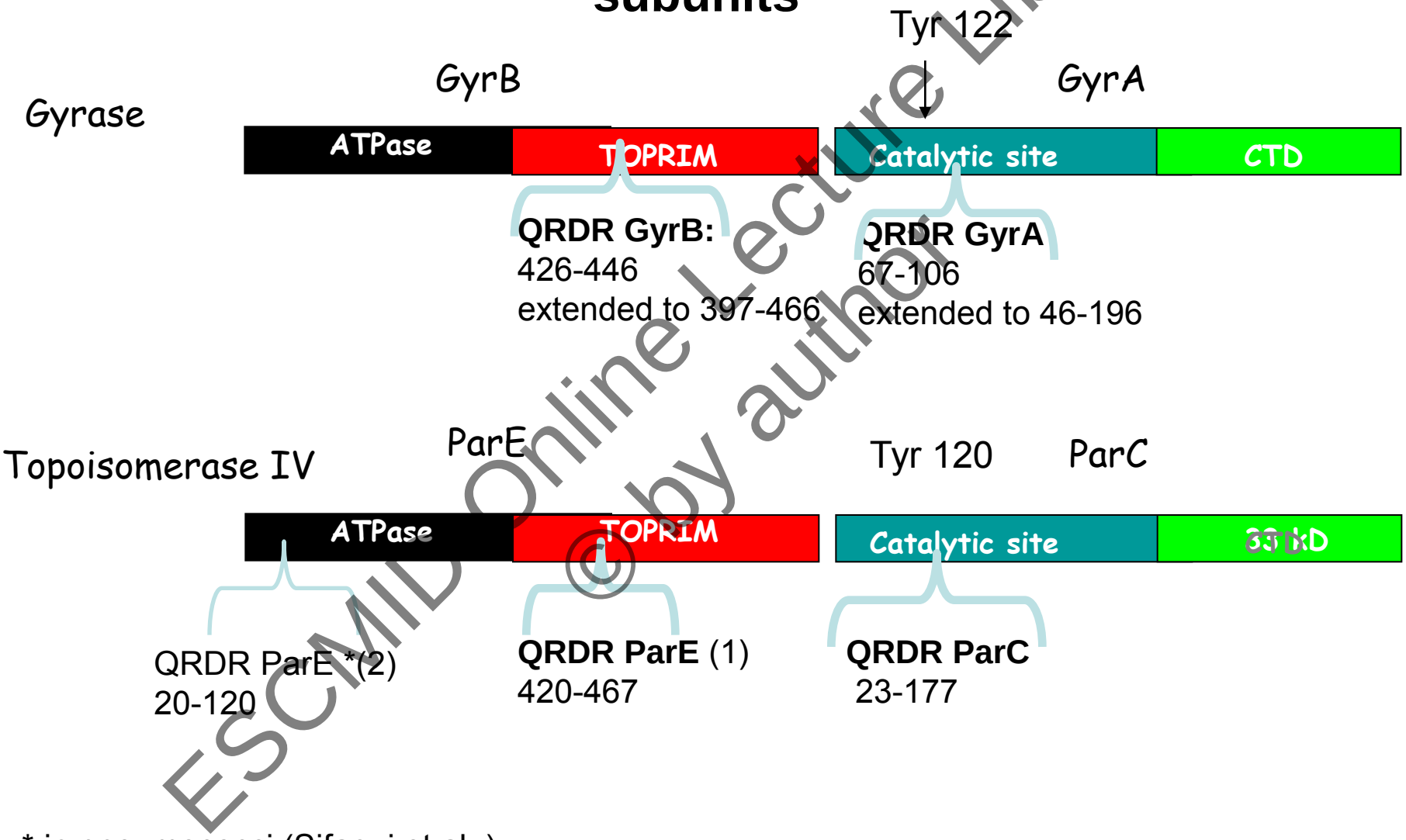
Mechanisms of resistance to quinolones in Gram positive bacteria



(Target protection)

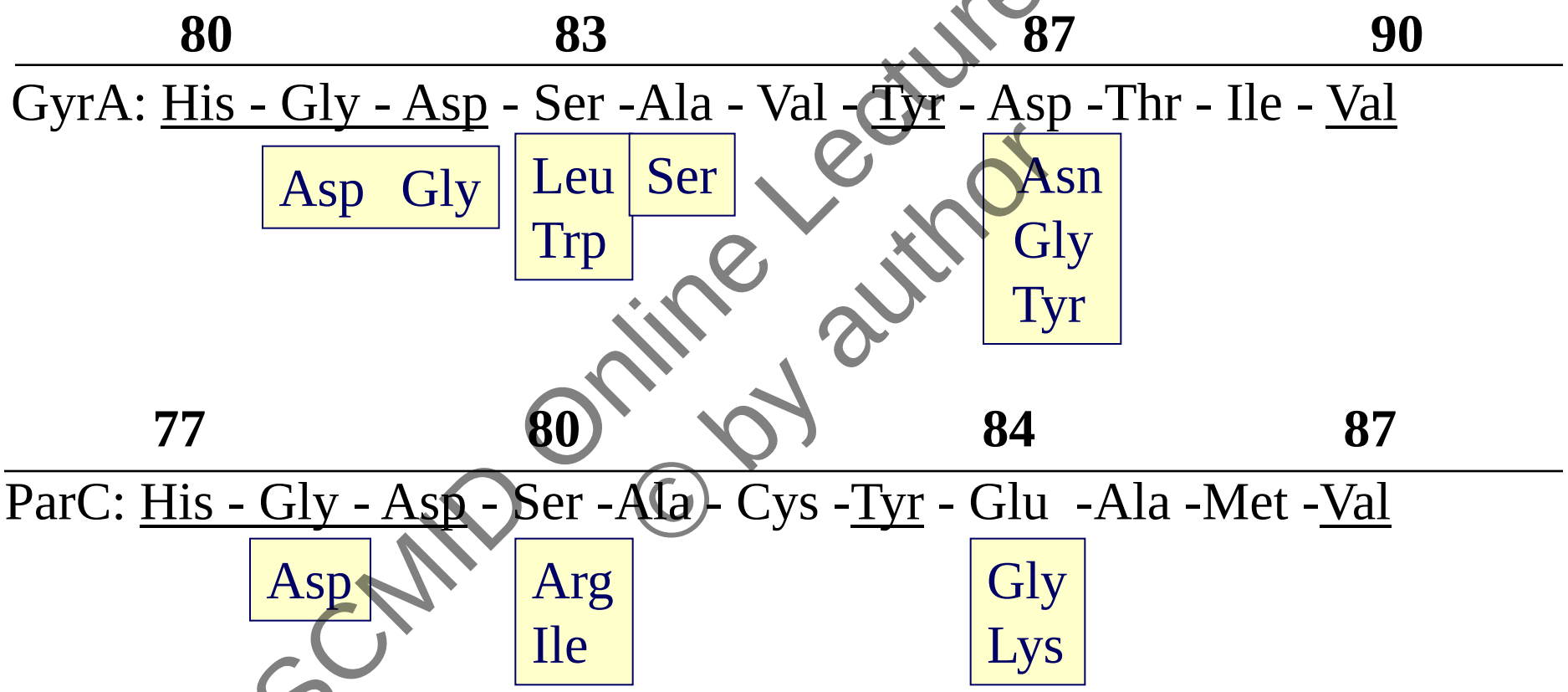
adapted from Peter Heisig

Location of the Quinolone Resistance Determining Regions (QRDR) in gyrase and topoisomerase IV subunits



* in pneumococci (Sifaoui et al.)

Substitutions involved in quinolone resistance
All bacteria – numbering system of *E. coli*



Substitutions involved in quinolone resistance numbering system of *S. aureus*

81 **84** **88** **91**

GyrA: His - Gly - Asp - Ser -Ser - Val - Tyr - Glu -Thr - Ile - Val

Leu Pro Lys Val

77 **80** **84** **87**

ParC: His - Gly - Asp - Ser -Ala - Cys -Tyr - Glu -Ala -Met -Val

Tyr Phe Lys Val

Substitutions involved in quinolone resistance numbering system of *S. pneumoniae*

78 **81** **85** **91**

GyrA: His - Gly - Asp - Ser -Ala - Val - Tyr - Glu -Thr - Ile - Val

Tyr
Phe
Ala

Lys
Gly

76 **79** **83** **86**

ParC: His - Gly - Asp - Ser -Ala - Cys -Tyr - Asp -Ala -Met -Val

Tyr
Phe
Ala

Asn
Tyr
Gly

Two targets for quinolones: which one is the most important?

Primacy of DNA gyrase or topoisomerase IV?

Involvment of the topoisomerase IV as a primary target

Staphylococcus aureus and **ciprofloxacin**

Mutant	MIC Cip (mg/L)	GyrA 83	GyrA 87	ParC 80	ParC 84
Wild-type	0.5	Ser	Glu	Ser	Glu
M1	8			**Tyr**	
	8				**Lys**
M2	128		**Lys**		
M3	> 128	**Leu**			

Numbering system of *E. coli*

Ferrero L. et al. 1995

Involvment of the topoisomerase IV as a primary target

Streptococcus pneumoniae and **ciprofloxacin**

Mutants	**MIC (mg/L)**		**GyrA**		**ParC**	
	Cip	**Spfx**	**83***	**87***	**80***	**84***
Parental strain	1	0.25	Ser	Glu	Ser	Asp
1st step	3	0.25	-	-	-	-
2nd step	8	0.25	-	-	Tyr	-
			-	-	Phe	-
			-	-	-	Tyr
3rd step	64	16	Tyr	-	Tyr	-
			-	Lys	Tyr	-

numbering system of *Escherichia coli*

Pan X. et al. 1996

Primary target depends on the quinolone

Streptococcus pneumoniae and **sparfloxacin**

Mutant	MIC (mg/l)		GyrA		ParC	
	Cip	Spx	83	87	80	84
Wild-type	1	0.25	Ser	Glu	Ser	Asp
M1	1	2	**Phe**			
	1	2	**Tyr**			
M2	32	16				**Asn**
	64	32				**Tyr**

Pan X. et al. 1997

Primary target in pneumococci

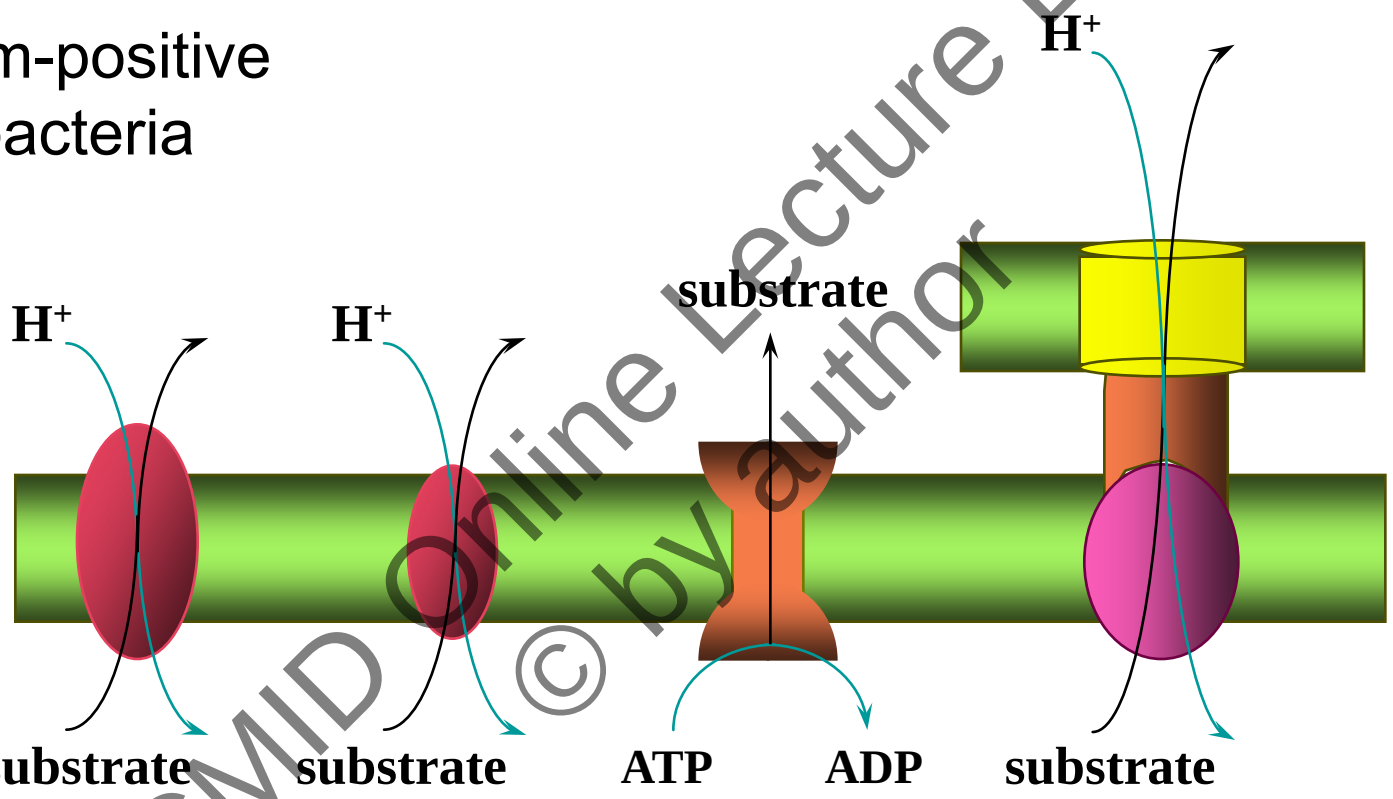
Topoisomerase IV	ADN gyrase
– Ciprofloxacin	– Sparfloxacin
– (lev) ofloxacin	– Gatifloxacin
– Pefloxacin	– Moxifloxacin
– Norfloxacin	– Gemifloxacin
– Trovafloxacin	– Grepafloxacin
Clinafloxacin	
Sitafloxacin	
Besifloxacin	

from L. Mark Fisher

Enhanced efflux
by specific pumps

Gram-negative bacteria

Gram-positive bacteria



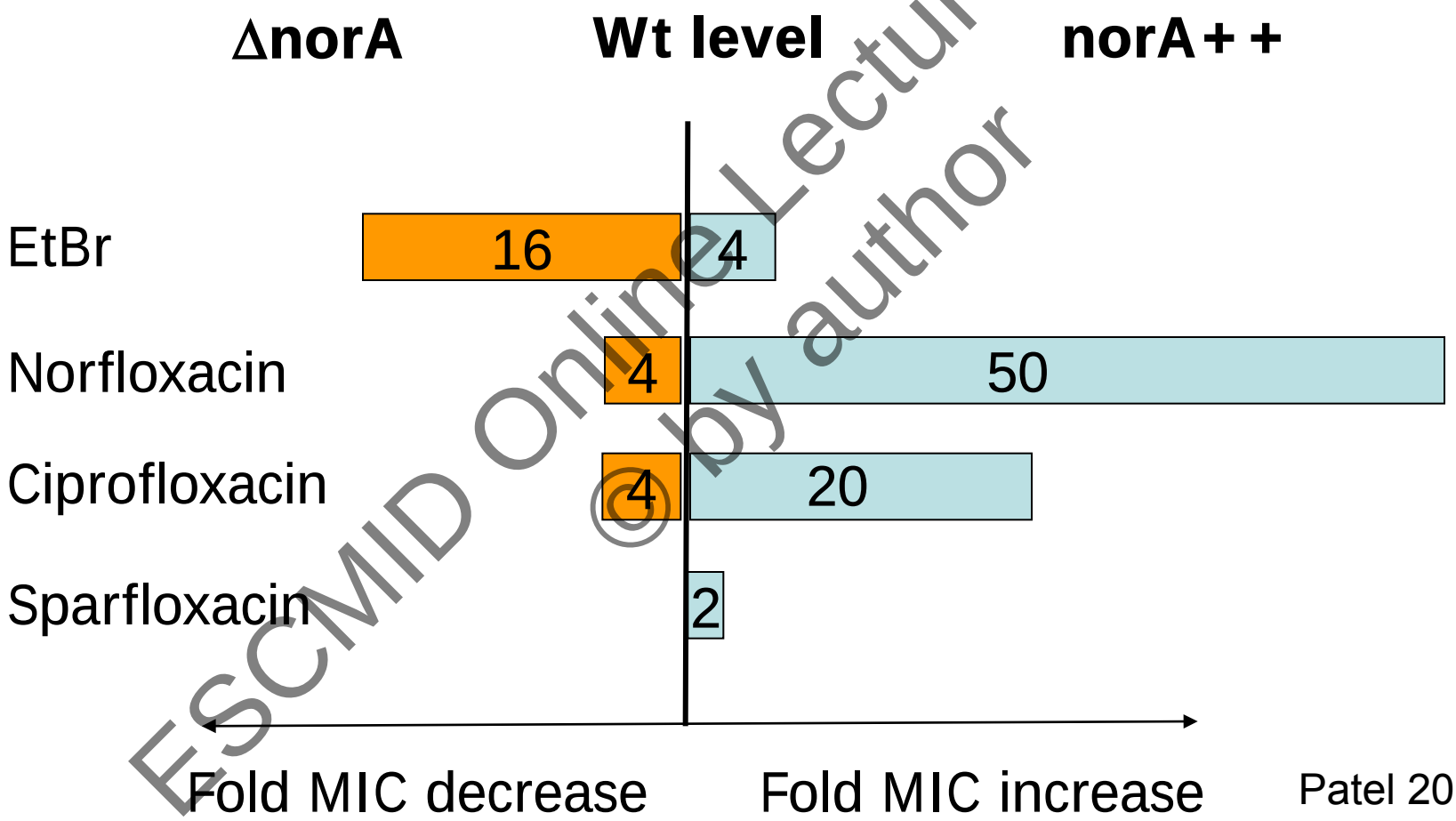
MFS
NorA, PmrA emeA, LfrA

SMR

ABC

RND
Acr, Mex

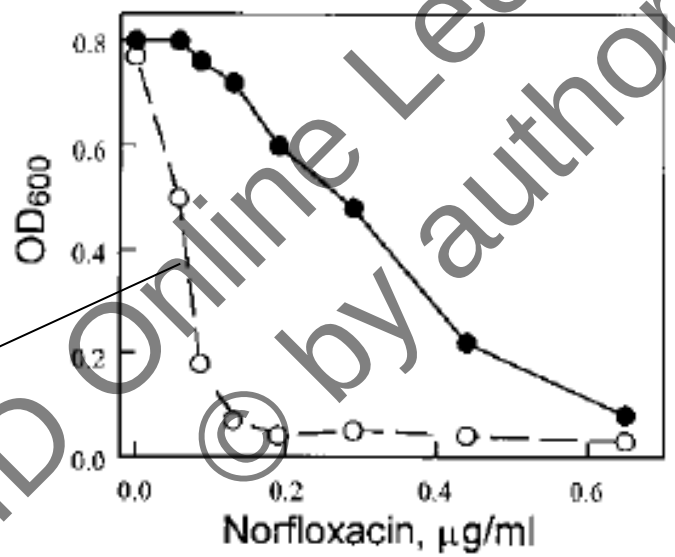
Quinolone resistance conferred by NorA in *Staphylococcus aureus*



Patel 2010
Kumar 2006

EPIs for Gram+ efflux pumps

Reserpine inhibits NorA pump from *S. aureus*



Markham and Neyfakh AAC (1996)

Level of quinolone resistance conferred by each mechanism

Mechanism	Fold increase in MICs	Resistance to other antibiotics
Target alteration (gyrase or topo IV mutation)	x 2 to 30 per **each mutation**	No*
Enhanced efflux	x 2 to 8 (FQ > NFQ)	No**

*Hypersusceptibility to coumarins
** chloramphenicol, ethidium bromide

Mode of acquisition of quinolone resistance

1. Point mutation in chromosomal genes
   - Target genes : *gyrA* TCG => TTG, Ser83 => Leu
   - Regulator genes for efflux pumps

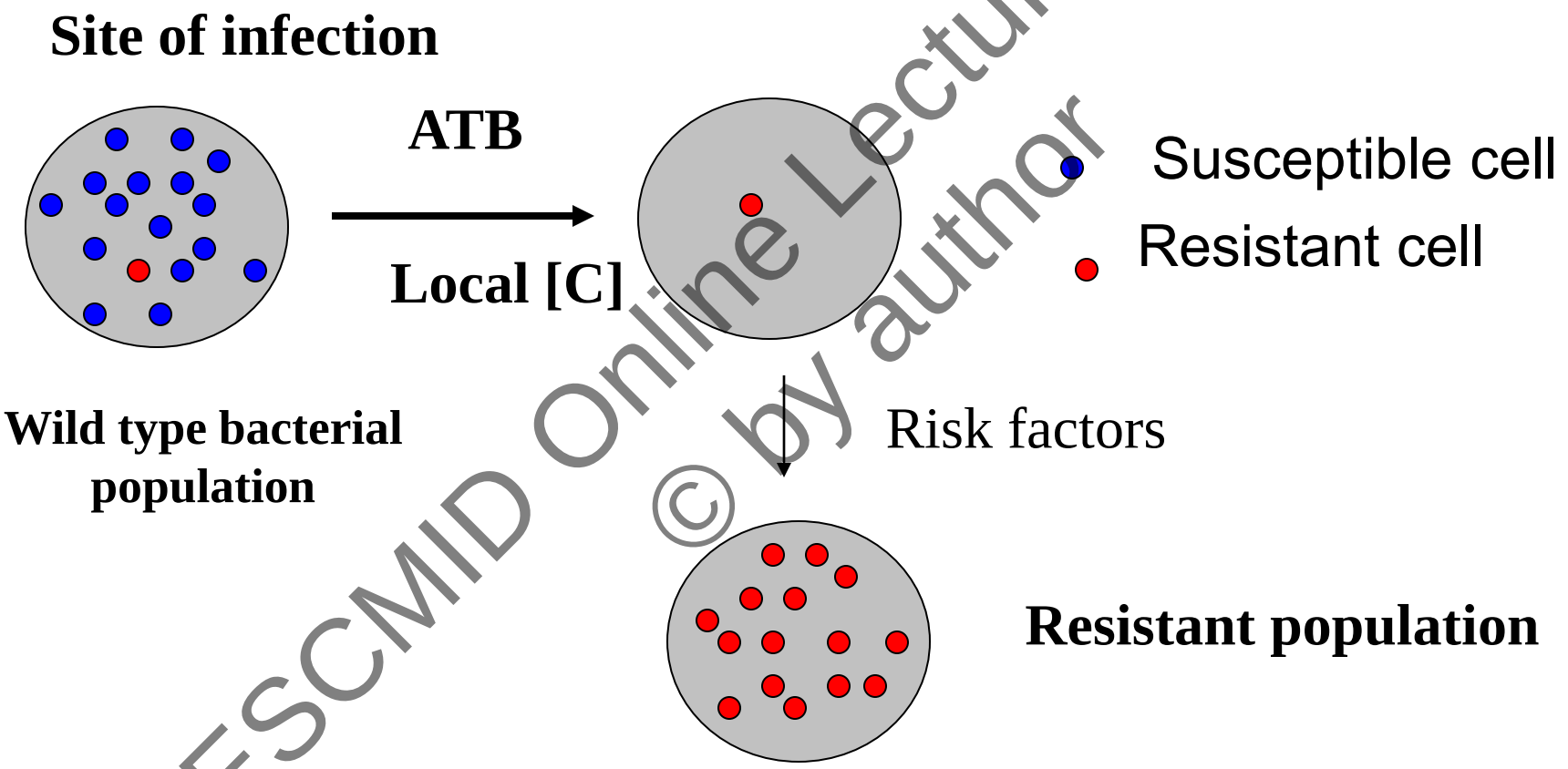
   Acquisition is the result of selection of resistant mutant at a proportion of 1 / 10^5 to 10^{10}

2. Transfer of resistance gene
   - Recombination and natural transformation for the target genes in streptoccoci

   Acquisition is the result of a contact with a quinolone resistant donor strain and subsequent selection of the resistant recipient strain

3. Cross-transmission of a quinolone resistant strain (MRSA, PRP, VRE)

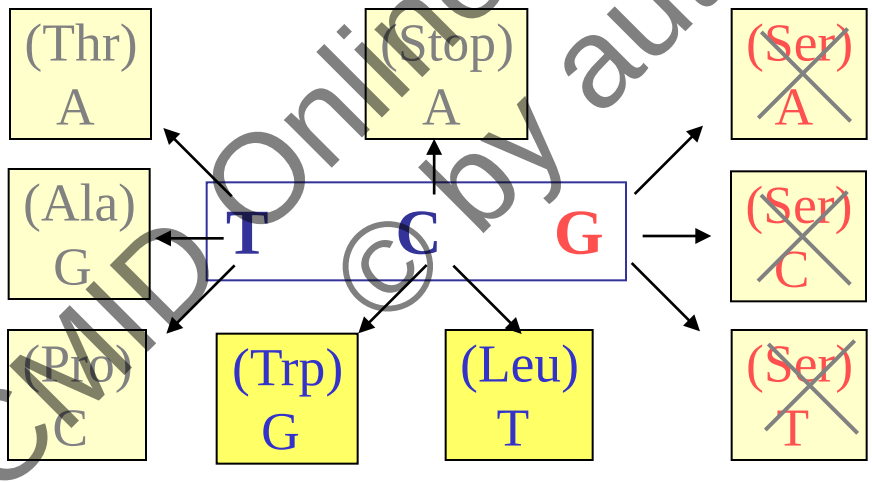
Sheme of in vivo selection of resistant mutants



Mechanisms of acquisition of quinolone resistance in Gram positive bacteria

Chromosomal mutation in genes encoding the targets

Serine-83 in *gyrA*



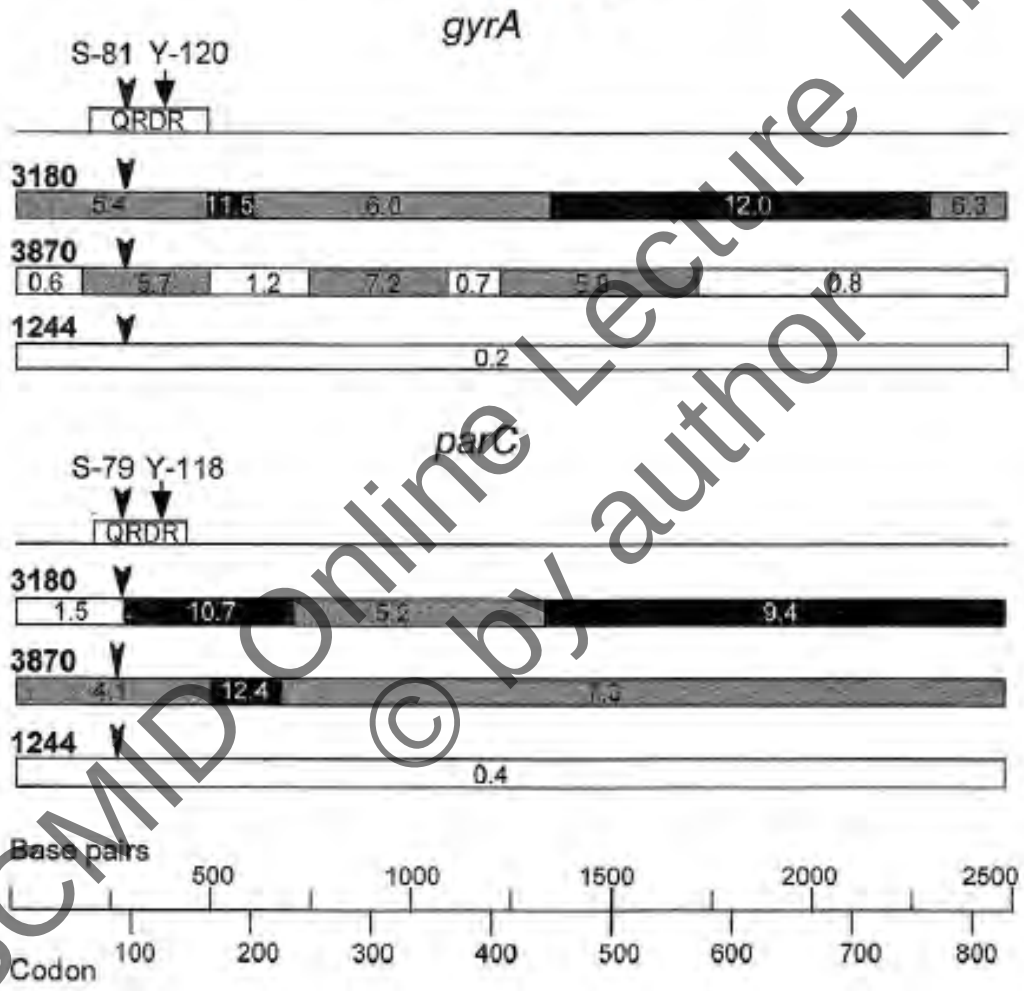
Proportion of mutants：**1/ 10 to 100 millions (10^7-10^8)**

Interspecific transfer conferring FQ resistance

Recipient (WT)	ParC mutant donor	Frequency of transformation	QRDR homology %
S. pneumoniae	*S. pneumoniae*	10^{-2}	100
	S. mitis	5×10^{-3}	91
	S. oralis	5×10^{-3}	95
	S. sanguis	5×10^{-5}	85
	S. constellatus	10^{-8}	81
	S. mutans	None	ND
	E. faecalis	None	<75
S. mitis	*S. pneumoniae*	5×10^{-4}	
S. oralis	*S. pneumoniae*	10^{-6}	

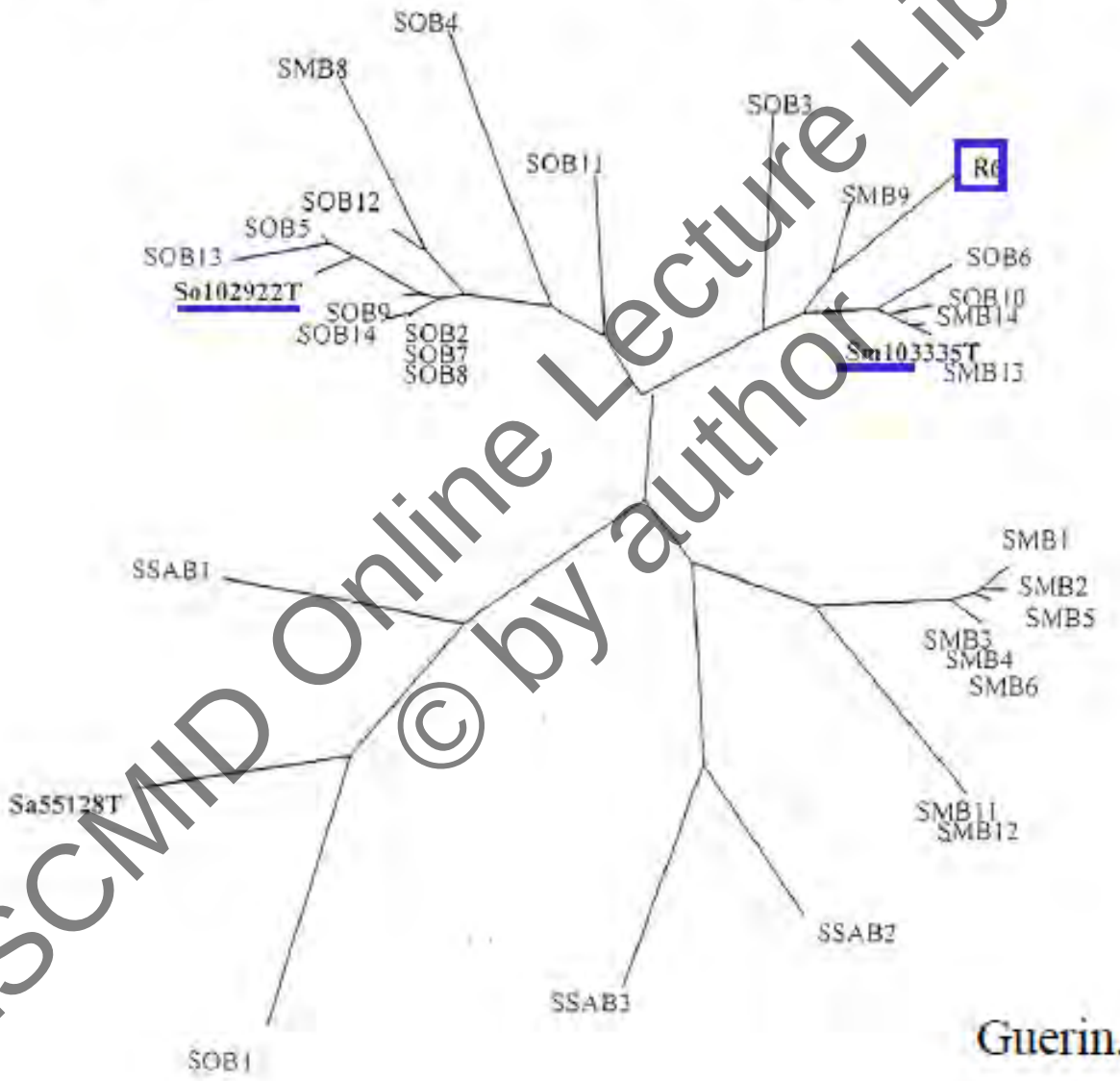
Janoir *et al*, *J. Infect. Dis.* 1999.

Mosaic genes of ParC and GyrA



Ferrandiz *et al*. *AAC* 2000

Phylogenetic tree of the gyrA QRDR



Guerin. 2000

Mechanisms of quinolone-resistance in clinical isolates of *S.aureus*

- 420 Ciprofloxacin-resistant MRSA isolates
- 13 Ciprofloxacin-resistant MSSA isolates
- from 24 European university hospitals
- *parC* and *gyrA* QRDRs were analysed

Schmitz et al. 2002

Amino acid changes in ParC and GyrA of 433 ciprofloxacin-resistant *S. aureus*

ParC	GyrA	(%)
Ser-80 -> Phe	Ser-84 -> Leu	56
Ser-80 -> Phe	Glu-88 -> Lys	21
Ser-80 -> Tyr	Ser-84 -> Leu	6
Ser-80 -> Tyr	Glu-88 -> Lys	6
Glu-84 -> Lys	Ser-84 -> Leu	1
Glu-84 -> Lys	Glu-88 -> Lys	5
Ala-116 -> Glu	Ser-84 -> Lys	1
Ala-116 -> Pro	Glu-88 -> Val	1
Ser-80 -> Phe + Glu-84 -> Val	Ser-84 -> Leu	3

Mechanisms of quinolone-resistance in clinical isolates of *S.aureus*

- No amino acid changes at codon 80, 84 or 116 in ParC
--> Ciprofloxacin-susceptibility

- Amino acid changes in Ser-80, Glu-84 or Ala-116 of parC in combination with Ser-84 or Glu-88 of GyrA
--> Ciprofloxacin-resistance

- Efflux pump is detectable in approximately 30% of ciprofloxacin-resistant isolates

Schmitz et al. 2002

FQ resistance in MRSA

- 100 MRSA isolates from Medical center Tokio (2002) tested against 9 FQ
  - 97% mutations in targets : *grlA (parC)* and/or *gyrA*
  - 15% overexpressed efflux pump NorA
  - all 15 isolates with NorA also had target mutations

NorA always associated with target to confer significant FQ-R

Noguchi et al. 2005

Mechanisms of quinolone resistance in clinical strains of *Streptococcus pneumoniae*

MIC CIP (mg/L)	N strains	No mutation	*parC mutation*	*parC + gyrA mutation*
≤ 1	12	100	-	-
2	14	64	36	-
4	41	49	49	2
8	13	15.5	69	15.5
16	21	-	43	57

Chen D.K. et al. N.E.J.M. 1999

Molecular epidemiology of quinolone resistant strains in Spain

- 75 cip-R *S. pneumoniae* strains isolated in Spain
- 70 strains with *parC* , *gyrA*, *parE* mutations
- 5 strains with mosaic genes in *parC, parE* or/and *gyrA*
- Low level Cip-R: *parC* mutations
- High level Cip-R : *parC* + *parE* or *ParC* + *gyrA* or *parC* +*parE* + *gyrA*

De la Campa A. EID 2004

Mechanisms of quinolone resistance in other streptococci

TABLE 1. Susceptibilities of strains to selected Fqs and mutations in the *parC*, *parE*, *gyrA*, and *gyrB* genes[a]

Strain	MIC (μg/ml)					Amino acid change (codon change)[b]			
	Cp		SPA		CLX, agar dilution	ParC	ParE	GyrA[d]	GyrB
	E test	Agar dilution	E test	Agar dilution					
S. pneumoniae R6	0.5	0.5	0.25	0.25	0.125	—	—	—	—
S. oralis NCTC11427	4	2	0.5	0.5	0.125	—	—	—	ND
S. oralis ATCC10557	2	2	0.5	0.5	0.125	—	—	—	ND
S. mitis NCTC12261	1	1	0.25	0.25	0.06	—	—	ND	—
S. mitis V1[c]	16	4	1	0.25	0.125	—	—	ND	ND
S. mitis V2	16	4	1	0.5	0.125	—	—	ND	ND
S. mitis V3	16	4	1	0.25	0.125	—	—	ND	ND
S. mitis V4	16	4	1	0.5	0.125	—	—	ND	ND
S. sanguis V5	16	4	1	0.5	0.125	—	—	ND	^{425}A→G (GCT→GGT)
S. mitis V6	16	4	2	0.5	0.125	—	—	—	^{425}A→G (GCT→GGT)
S. mitis V10	16	4	1	0.5	0.125	^{79}S→F (TCT→TTT)	—	—	ND
S. mitis V8	>32	16	2	1	0.25	^{79}S→F (TCT→TTT)	—	—	—
S. oralis V9	>32	32	2	1	0.5	^{79}S→Y (TCT→TAT)	—	—	—

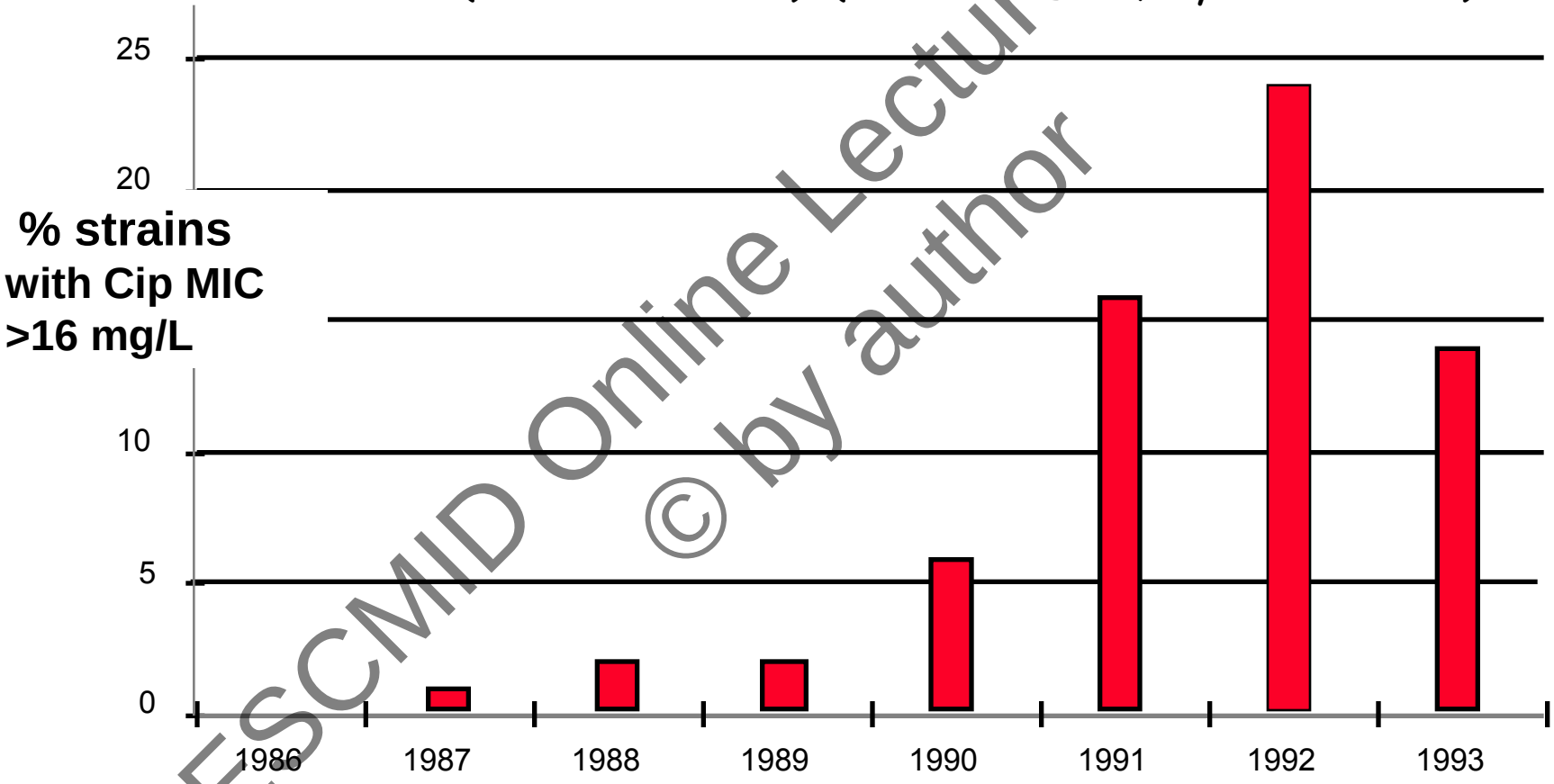
De la Campa A. 1999

Quinolones and *Enterococcus faecalis*

	MIC (mg/L)	
Quinolone	range	MIC 50
Ofloxacin	1-64	4
Ciprofloxacin	0.5-32	2
Levofloxacin	1-32	1
Moxifloxacin	0.25-16	0.25

Quinolone resistance in clinical strains of *Enterococcus faecalis*

In resistant strains: mutations in GyrA (Ser83 et Glu87)
Or/and ParC (Ser80 et Glu84); (Tankovic 1996, Oyamada 2006)



Hospital Henri Mondor, R.Leclercq, personal data

Combined mechanisms are necessary to achieve a high level of quinolone resistance

In Staphylococcus aureus

	MIC (mg/L)				
	Cip	**Levo**	**Spx**	**Grepa**	**Gémi**
. wild type	0,25	0,12	0,06	0,12	0,03
. efflux (norA)	2	0,5	0,06	0,12	0,06
. parC	4	2	0,06	0,5	0,25
. parC + gyrA	16	8	2	16	1
. parC + gyrA + norA	128	64	2	16	2

Combined mechanisms are necessary to achieve a high level of quinolone resistance

In Streptococcus pneumoniae

	MIC (mg/L)					
	Cip	**Levo**	**Spx**	**Grepa**	**Gémi**	**Mox**
. wild type	2	1	0,25	0,25	0,03	0,12
. efflux	8	2	0,25	0,25	0,06	0,12
. parC	8	8	0,5	0,5	0,12	0,25
. parC + gyrA	64	64	32	32	1	4
. parC + gyrA + efflux	128	64	32	32	4	4

Cross resistance between quinolones in *S. pneumoniae*

	≤ 0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64
Ciprofloxacin								55	72	87	92	100
Levofloxacin					1	16	63	67	80	95	100	
Sparfloxacin				3	52	63	64	73	92	95	99	100
Grepafloxacin				19	51	64	38	85	93	100		
Trovafloxacin				19	60	68	77	91	99	100		
Gatifloxacin				4	59	64	75	93	100			
Moxifloxacin			4	56	63	71	88	97	100			
Gemifloxacin	32	61	75	92	95	100						

Chen et al.., N.E.J.M., 1999

Detection of resistance by phenotypic tests

TABLE 3. Three test options for detection of the mechanisms of FQ resistance in *S. pneumoniae*

Mechanism of resistance	FQ testing[a] (interpretive values)								
	Option 1				Option 2			Option 3	
	NOR (R <10 mm)	PEF (R <10 mm)	SPX/CIP	LVX	NOR (R <10 mm)	PEF (R <10 mm)	LVX	NOR (R <10 mm)	LVX
Topoisomerase IV[c]	**R**	**R**	SPX>CIP	S	**R**	**R**	S	**R**[b]	S
Efflux[c]	**R**	**S**	SPX>CIP	S	**R**	**S**	S	**R**	S
Gyrase (GyrA)[c]	S	S	**SPX<CIP**	S	—[d]	—	—	—	—
Topoisomerase IV + gyrase	R	R	—	**I or R**	R	R	**I or R**	R	**I or R**

Varon E et al. 2006

Summary

- Mechanisms of quinolone resistance in Gram positive bacteria are
  - Mutations in the gyrase genes and topoisomerase IV genes
  - Enhancement of efflux pumps
- These mechanisms are often combined in clinical strains, and to reach a high level of resistance
- Acquisition of resistance is generally due to the selection of chromosomal mutant
- In streptococci, it may be due to transfer of genes through natural transformation