

Antibacterial susceptibility testing and implications for resistance surveillance and treatment

Problems with low level resistance against quinolones

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REFERENCE MODEL: Enterobacteria

Conceptual approach also valid for other organisms
(Nonfermenters, Grampositives,...)

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PROBLEMS FOR DEFINITION OF LOW LEVEL RESISTANCE

1. Identification of the [susceptible] population lacking [acquired] mechanisms of resistance
2. Insufficient information for defining clinical breakpoints, ...particularly from patients!

BREAKPOINTS FOR FLUORQUINOLONES AGAINST ENTEROBACTERIA

	EUCAST	CLSI
Ciprofloxacin	0.5/1	1-4
Levofloxacin	1/2	2-8
Norfloxacin	0.5/1	4-16

MECHANISMS OF LOW LEVEL RESISTANCE TO QUINOLONES IN ENTEROBACTERIA

1. Target alteration
2. Decreased permeability
3. (Over-) Expression of active efflux
4. Target protection
5. Production of inactivating enzyme
6. Other mechanisms (poorly characterized and... to be discovered!)

MUTATIONS IN QRDR AND QUINOLONE ACTIVITY

GyrA	ParC	CIPRO	NAL
Ser83...Asp87	Ser80...Glu84	<0.12	4
Phe83/Tyr83		0.25-4	>128
Phe83Gly87		4-16	>128
Phe83Asn87	Ile/Arg80	16->256	>1024
	Gly/Lys84		

PORIN LOSS AND QUINOLONE ACTIVITY IN *K. pneumoniae*

Strain	GyrA	OMPs	NORFLO
LB3	Tyr83	K36	2
LB4	Tyr83	-	4
CSUB10S	Phe83	K36	4
CSUB10R	Phe83	-	16



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Proportional MICs of quinolones for *Salmonella* Typhimurium with LPS defects

Agent	Hydrophobicity	Smooth	<i>rfaG</i>	<i>rfaF</i>	<i>rfaE</i>
Ciprofloxacin	0.02	1	1	1	1
Pefloxacin	1.32	1	0.5	0.5	0.5
Flumequin	13.0	1	0.13	0.13	0.06

E. coli null mutations in efflux systems MIC decreases

Strain	AMP	ERM	CIP	RIF	STR	CLO	BEZ
W3110	12.5	50	0.01	5	1.95	6.25	62.5
Def-TolC	2	32	4	2	0	8	32
Def-AcrAB	2	32	4	2	0	8	32
Def-[AcreF, AcrD, EmrD, EmrE, MefA,...]	0	0	0	0	0	0	0

Activity of antimicrobials agents against *K. pneumoniae* 52145R and derived mutants

Agent	52145R	52145R $\Delta acrB$	52145R $\Delta acrR$
Nalidixic acid	4	0.5	8
Ciprofloxacin	0.06	0.008	0.12
Imipenem	0.125	0.125	0.125

QUINOLONE RESISTANCE: MULTIFACTORIAL

STRAIN	NORFLO	GyrA	OMPs	Active Efflux
LB3	2	Tyr83	YES	NO
LB4	4	Tyr83	NO	YES
CSUB10S	4	Phe83	YES	NO
CSUB10R	16	Phe83	NO	YES

Porin and active efflux expression among *K. pneumoniae* strains producing or not ESBL

	ESBL			ESBL	
	Pos	Neg		Pos	Neg
Por (+)	22	20	Efflux (-)	24	18
Por (-)	10	0	Efflux (+)	8	3

E. coli AG100

EFFECT OF AcrAB AND GyrA

<i>gyrA</i>	AcrAB	CIP
wt	wt	<0.015/<0.015
D87G	wt	0.25/0.06
D87G	↑↑	0.5/0.06
S83L/D87G	↑↑	2/0.125



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PLASMID-MEDIATED QUINOLONE RESISTANCE

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QNR PROTEINS

QnrA, QnrB, QnrC, QnrD, QnrS

[Chromosomal variants in both GNeg and GPos]

Pentapeptide Repeat Proteins

Enterobacteria/*Vibrionaceae*

[*Shewanella*, *Stenotrophomonas*,... *Enterococcus*,...]

Protect DNA-gyrase and Topo IV from quinolone attack

GUEST COMMENTARY

qnr Gene Nomenclature[∇]

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<http://www.lahey.org/qnrStudies>

Table 1. MICs (mg/L) of eight quinolones against *E. coli* and *K. pneumoniae* strains with or without plasmid pMG252

Strain (relevant phenotype/genotype)	CIP	CLX	L VX	NAL	NOR	PEF	SPX	TVA
<i>E. coli</i> J53 (F ⁻ <i>met pro ompC⁺ ompF⁺ gyrA⁺ gyrB⁺</i>) ⁴	0.008	0.004	0.03	4	0.125	0.06	0.015	0.015
<i>E. coli</i> J53 pMG252	0.25	0.125	0.5	32	1	2	1	1
<i>E. coli</i> KL16 (Hfr <i>thi-1 relA ompC⁺ ompF⁺ gyrA⁺ gyrB⁺</i>) ⁴	0.008	0.004	0.03	4	0.125	0.125	0.015	0.015
<i>E. coli</i> KL16 pMG252	0.125	0.03	0.25	16	0.5	0.5	0.25	0.25
<i>E. coli</i> KF130 (KL16 <i>gyrA</i>) ⁵	0.25	0.015	0.5	≥256	1	2	0.25	0.5
<i>E. coli</i> KF130 pMG252	1	0.06	2	≥256	4	8	4	4
<i>E. coli</i> N-24 [KL16 <i>nal-24 (gyrB)</i>] ⁶	0.03	0.004	0.06	32	0.125	0.25	0.015	0.015
<i>E. coli</i> N-24 pMG252	0.25	0.06	0.5	32	1	1	1	1
<i>E. coli</i> N-31 [KL16 <i>nal-31 (gyrB)</i>] ⁶	≤0.002	≤0.002	0.004	64	0.015	0.015	0.008	0.004
<i>E. coli</i> N-31 pMG252	0.06	0.125	0.25	64	0.5	1	1	1
<i>E. coli</i> WT-4 (<i>parC</i>) ⁷	≤0.002	≤0.002	0.015	2	0.015	0.015	0.015	0.015
<i>E. coli</i> WT-4 pMG252	0.03	0.06	0.25	16	0.5	0.5	1	0.5
<i>E. coli</i> JF568 (<i>aroA ilv metB his purE cyc xyl lacY rpsL tsx ompC⁺ ompF⁺</i>) ⁸	0.015	0.008	0.03	1	0.06	0.125	0.03	0.03
<i>E. coli</i> JF568 pMG252	0.25	0.125	0.5	32	1	2	1	2
<i>E. coli</i> JF703 (JF568 <i>ompF254</i>) ⁸	0.03	0.008	0.06	4	0.125	0.125	0.03	0.03
<i>E. coli</i> JF703 pMG252	0.5	0.125	1	64	2	4	2	2
<i>E. coli</i> JF694 (JF568 <i>ompC264 ompF254 rmpA1</i>) ⁹	0.03	0.008	0.06	4	0.25	0.125	0.03	0.06
<i>E. coli</i> JF694 pMG252	0.5	0.125	1	64	4	4	2	2
<i>E. coli</i> AG100 [<i>argE thi rpsL xyl mtl supE D (gal-uvrB) marR⁺</i>] ⁹	0.008	0.004	0.03	4	0.03	0.03	0.015	0.03
<i>E. coli</i> AG100 pMG252	0.25	0.06	0.5	32	1	1	1	1
<i>E. coli</i> AG102 (AG100 <i>marR1</i>) ⁹	0.015	0.008	0.03	16	0.25	0.25	0.06	0.06
<i>E. coli</i> AG102 pMG252	0.5	0.125	1	64	4	4	2	2
<i>K. pneumoniae</i> C3 (O1+K66) ¹⁰	0.004	0.002	0.008	1	0.03	0.03	0.015	0.015
<i>K. pneumoniae</i> C3 pMG252	0.125	0.03	0.06	4	0.5	0.25	0.5	0.25
<i>K. pneumoniae</i> KT793 (O-K-) ¹¹	0.004	0.001	0.008	1	0.06	0.03	0.015	0.004
<i>K. pneumoniae</i> KT793 pMG252	0.125	0.06	0.125	4	0.5	0.5	0.5	0.125
<i>K. pneumoniae</i> KT707 (C3 <i>ompK35</i>) ¹¹	0.004	0.002	0.015	1	0.03	0.03	0.008	0.008
<i>K. pneumoniae</i> KT707 pMG252	0.125	0.03	0.125	4	0.25	0.5	0.25	0.25
<i>K. pneumoniae</i> KT5001 (KT793 <i>ompK36</i>) ¹⁰	0.004	0.002	0.03	2	0.03	0.03	0.008	0.015
<i>K. pneumoniae</i> KT5001 pMG252	0.125	0.03	0.125	4	0.25	0.5	0.25	0.25
<i>K. pneumoniae</i> KT5003 (C3 <i>ompK35 ompK36</i>) ¹²	0.015	0.004	0.03	2	0.125	0.06	0.008	0.015
<i>K. pneumoniae</i> KT5003 pMG252	0.25	0.03	0.25	16	1	0.5	0.25	0.125

CIP, ciprofloxacin; CLX, clinafloxacin; LVX, levofloxacin; NAL, nalidixic acid; NOR, norfloxacin; PEF, pefloxacin; SPX, sparfloxacin; TVA, trovafloxacin.



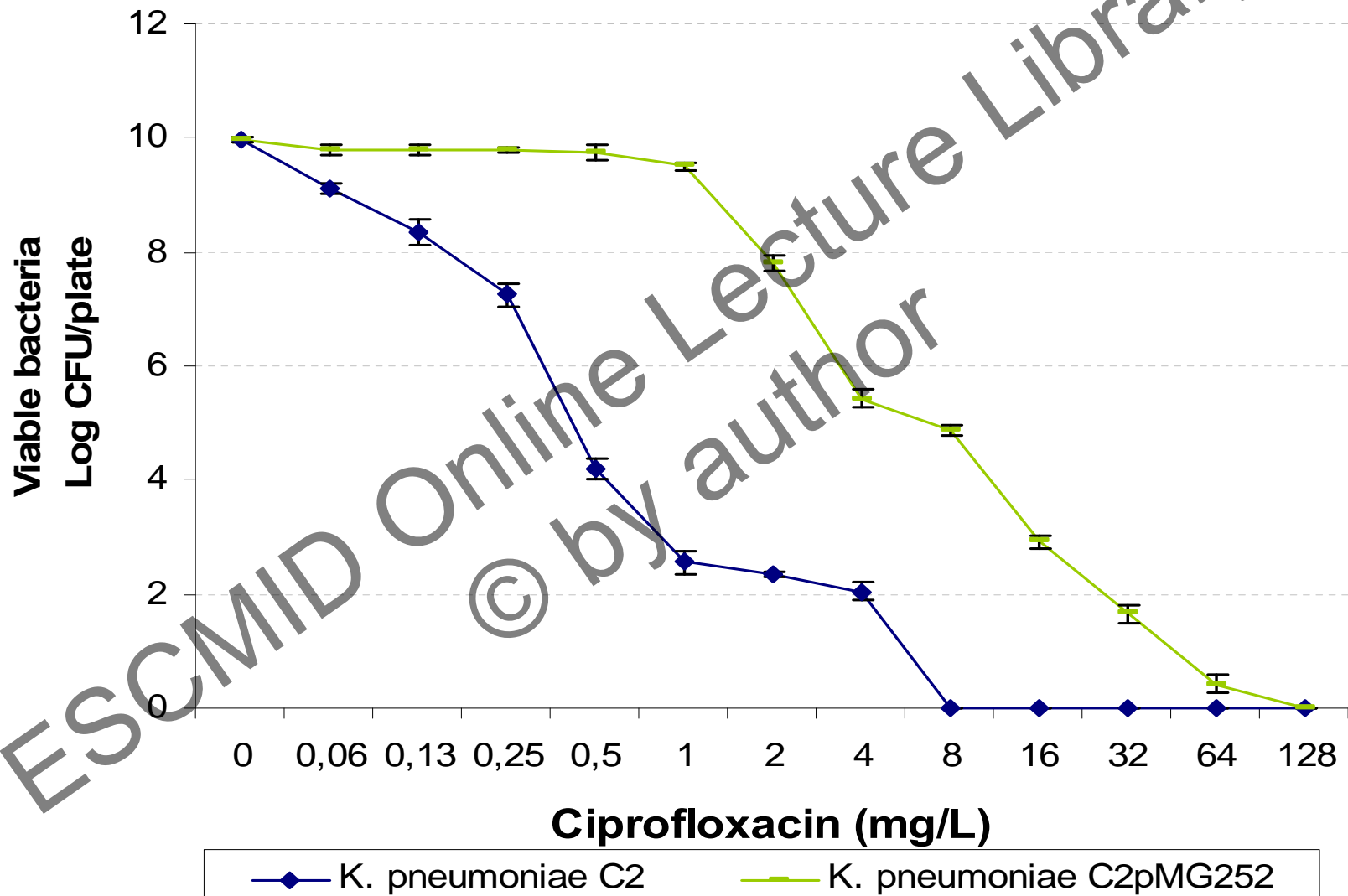
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Effect of *qnrA1* in CIP MPC



MICs OF QUINOLONES AND OTHER AGENTS AGAINST MUTANTS

SELECTED WITH CIPROFLOXACIN FROM *E. coli* J53 (QnrA1)

Strain	Parent	Step 1*	Step 2†	Step 3‡
Ciprofloxacin	0.25	2	2	4
Clinafloxacin	0.06	0.25	0.25	0.5
Levofloxacin	0.5	2	4	4
Nalidixic acid	32	128	256	256
Norfloxacin	1	8	8	16
Pefloxacin mesilate	4	16	16	32
Sparfloxacin	1	4	8	8
Trovafloxacin	2	8	8	8
Cefoxitin	256	512	512	512
Chloramphenicol	256	512	512	1024
Tetracycline	2	2	4	8

*Selected from parent J53 pMG252 on 0.25 µg/mL ciprofloxacin.

†Selected from step 1 mutant on 1 µg/mL ciprofloxacin at a frequency of 1×10^{-7} .

‡Selected from step 2 mutant on 2 µg/mL ciprofloxacin at a frequency of 3×10^{-7} .

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*}

The presence of *qnrA1* in *E. coli* from a patient with a UTI (treated with norfloxacin) precedes the emergence of changes in Ser83Leu and Asp87Asn, (GyrA gyrase subunit) and Ser80Ile (ParC topoisomerase IV subunit)

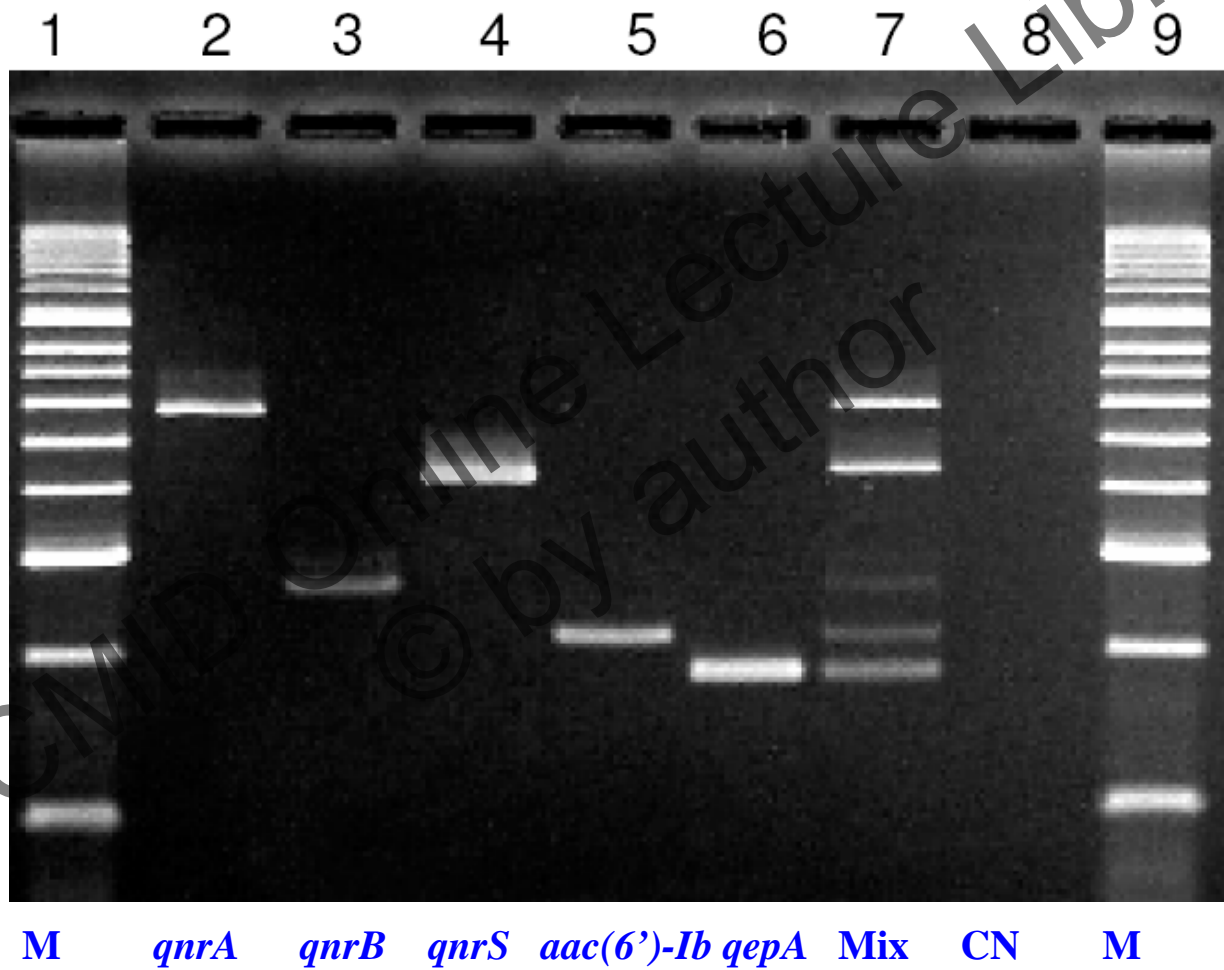
MICs of ciprofloxacin increased from 0.5 to >32 mg/l

Abstract: P586.

A. Amin, D. Wareham

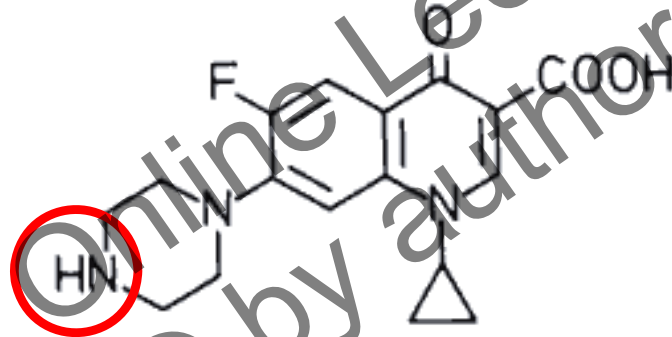
Ability of ciprofloxacin 1µg discs and British Society for Antimicrobial Chemotherapy (BSAC) zone diameter breakpoints to detect plasmid-mediated quinolone resistance determinants in urinary enterobacterial isolates

The BSAC zone diameter breakpoint for 1µg ciprofloxacin discs is adequate to detect isolates with PMQR genes, although a very small proportion of such isolates may be missed



ENZYMATIC INACTIVATION: AAC(6')-Ib-cr

Two substitutions at the *aac(6')-Ib* gene: Trp102Arg and Asp179Tyr
N-acetylation at the amino radical of the piperacynil group
(It does not compromise activity against aminoglycosides)



It affects ciprofloxacin, norfloxacin,... (but not other quinolones)

Moderate increase in MIC values

It favors the emergence of more resistant mutants

Plasmid-Mediated Active efflux

- QepA (*qepA1* y *qepA2*)
- OqxAB (Chromosome of *K. pneumoniae*!)
- Moderate increase in MIC values

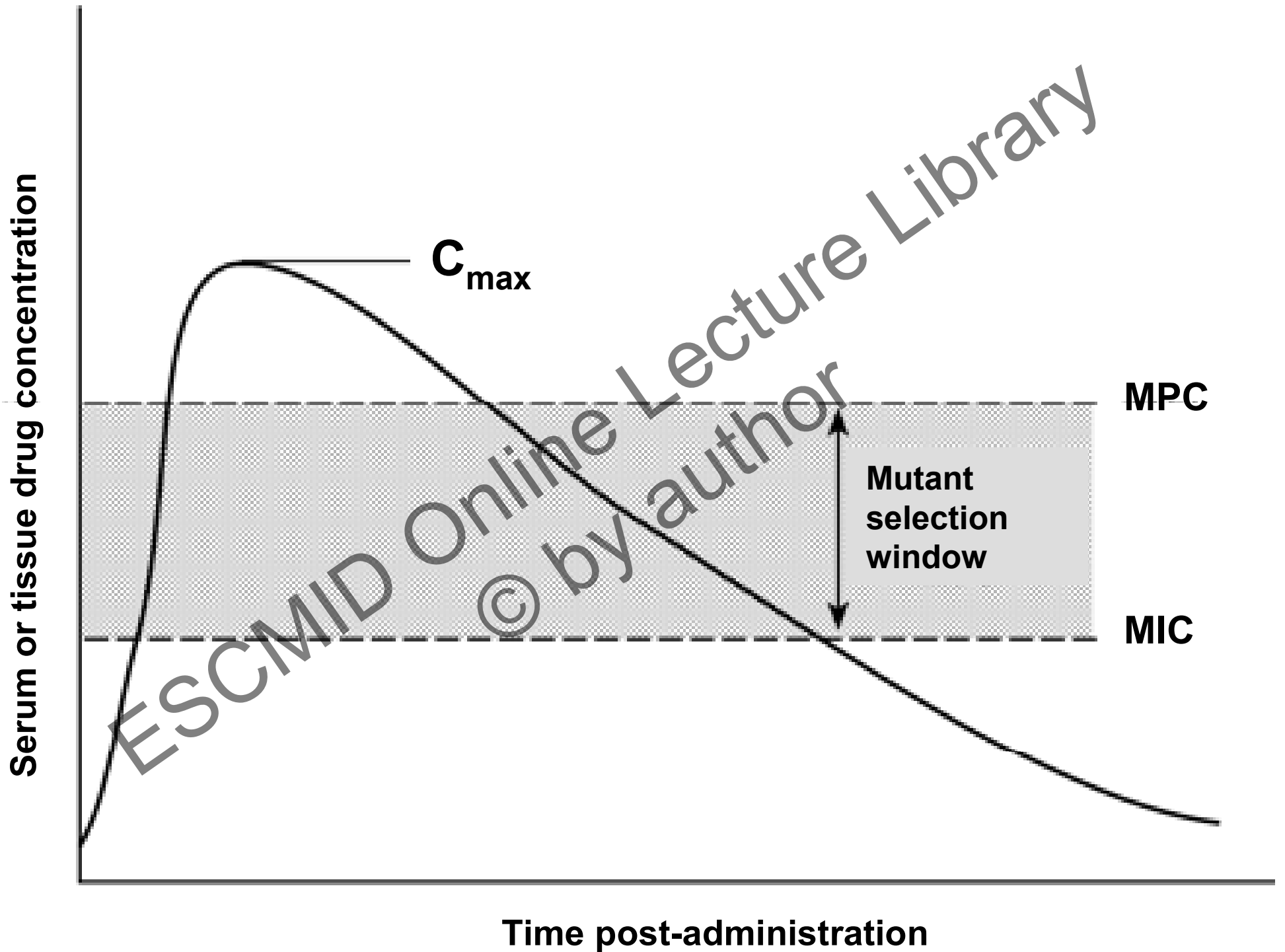


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PK/PD parameters of fluorquinolones related to good *in vivo* activity

(AUC)/MIC: $\geq 25-30$ (immunocompetent)

(AUC)/MIC: $\geq 100-125$ (immunocompromised)

C_{max}/MIC: > 8

CLINICAL RESPONSE TO OFLOXACIN IN PATIENTS (n=150) WITH UNCOMPLICATED TYPHOID FEVER

	TIME FOR FEVER CLEARANCE	RETREATMEN T REQUIRED
NAL-R	156 (30-366)	33.3%
NAL-S	84 (12-378)	0.8%

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EFFECT OF THREE QUINOLONES ON SURVIVAL AND LUNG BACTERIAL CLEARANCE IN MICE

(*K. pneumoniae*/QnrA1)

	<i>Kp C2</i>			<i>Kp C2 QnrA1</i>		
	CMI	%Superv	CFU/g	CMI	%Superv	CFU/g
Control		27	9,2±2,1		14	9,7±2,5
Ciprofloxacin	0.5	100	3,5±1,0	4	53	7,7±2,7
Levofloxacin	0.5	100	3,4±0,5	2	53	7,6±3,8

Abstract: P1567.

J. Domínguez-Herrera, et al.

Effect of *qnrA*, *qnrB* and *qnrS* on the *in vivo* activity of fluoroquinolones

The presence of the *qnrA*, *qnrB* or *qnrS* genes in *E. coli* strains reduces the therapeutic efficacy of ciprofloxacin and levofloxacin in an experimental pneumonia model in mice

LOW LEVEL RESISTANCE

CONCLUSIONS

- **Caused by multiple mechanism encoded by chromosomal or plasmid genes. Phenotypic methods are not reliable for detecting many of these mechanisms**
- **LLR increases the level of resistance due to high-level resistance mechanisms, and ensures bacterial viability to allow acquisition of additional resistance mechanisms**
- **Overexpression of LLR or coexpression of several LLR mechanisms may translate into clinical resistance, as defined by usual breakpoints**
- **PK/PD data indicate that even the moderate changes in MIC caused by LLR affect the clinical efficacy of quinolones**



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