Detection of fluoroquinolone resistance in *S. pneumoniae*

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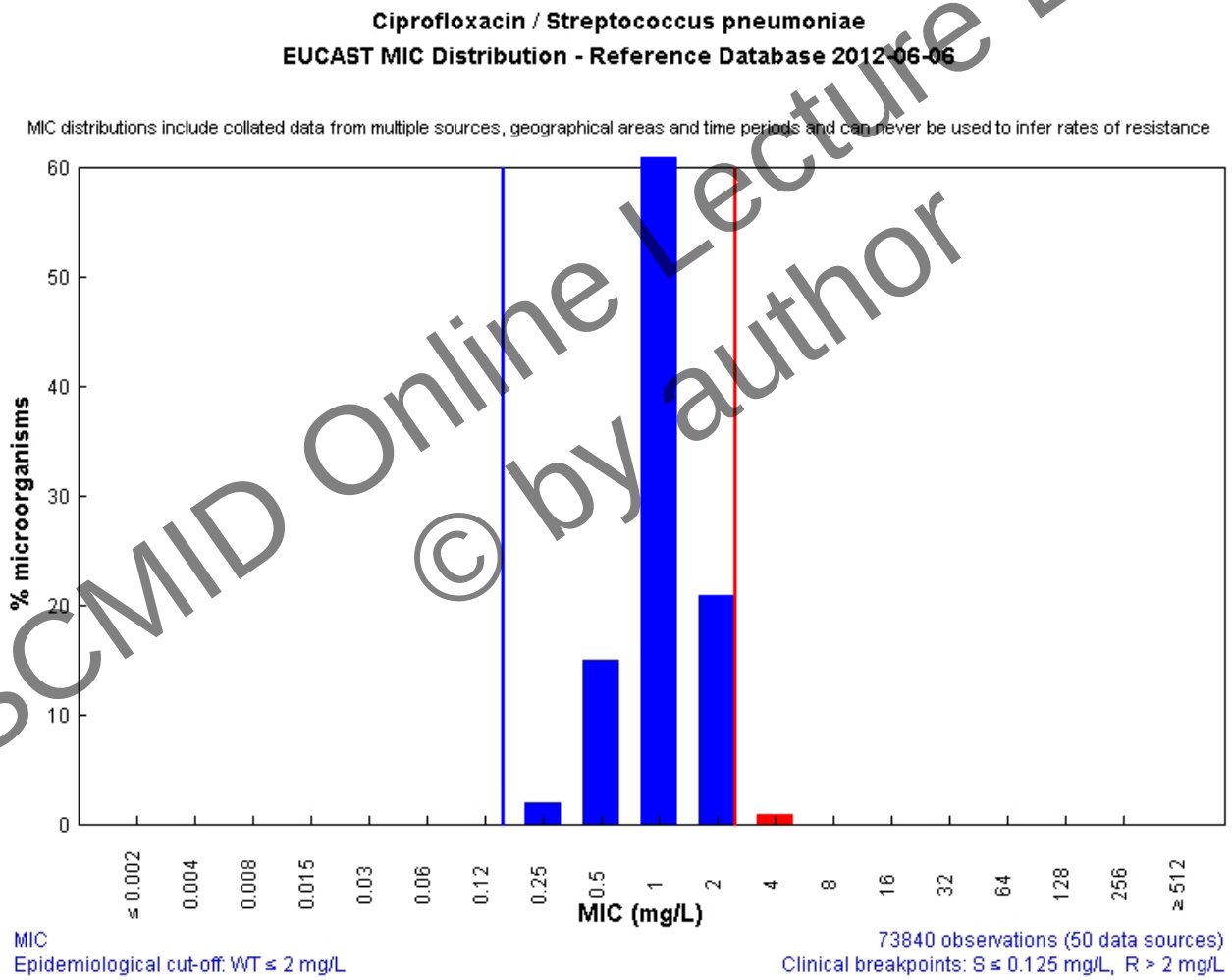
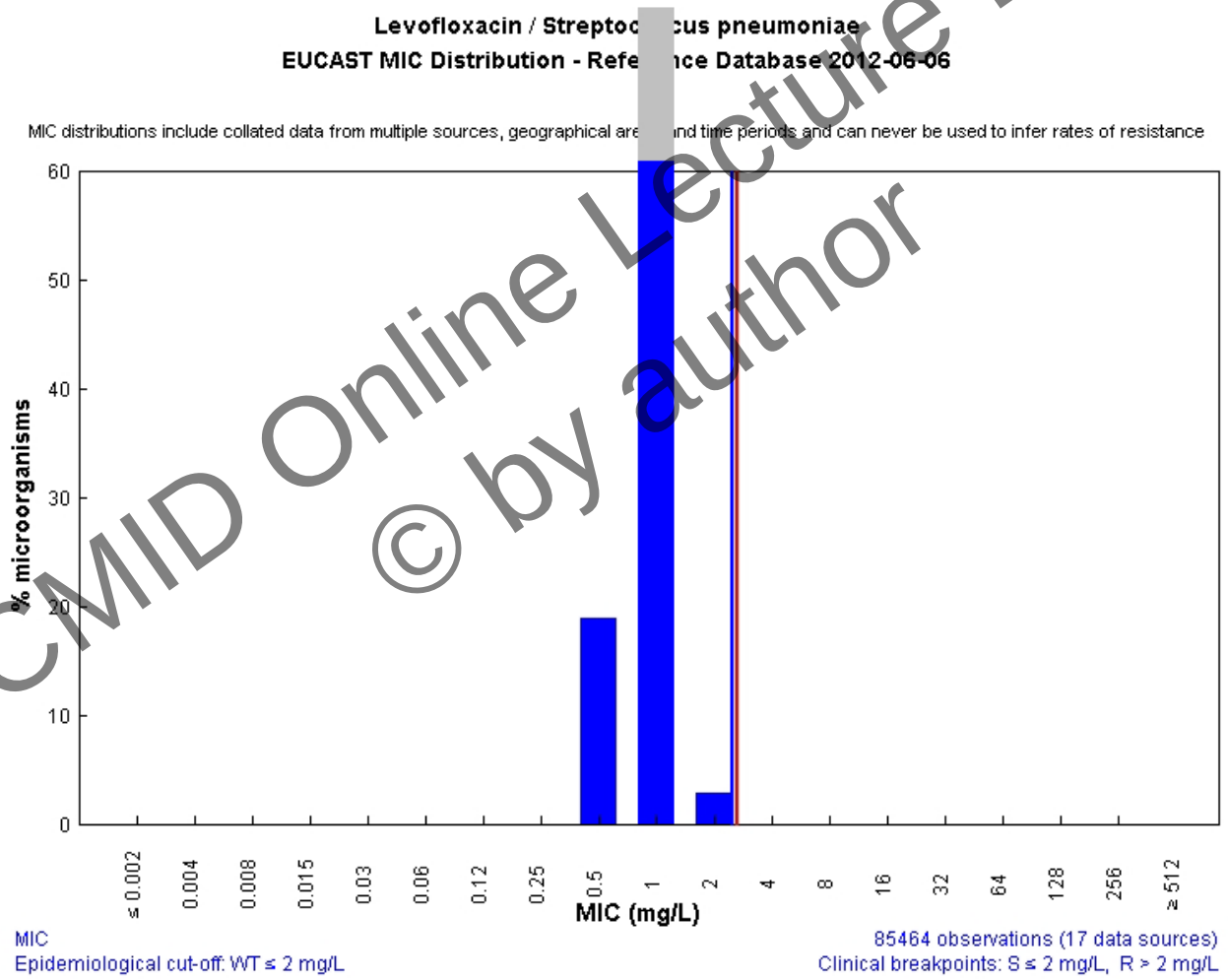
EUCAST and susceptibility testing of *S. pneumoniae* vs fluoroquinolones

Fluoroquinolones[1]	MIC breakpoint (mg/L)		Disk content (µg)	Zone diameter breakpoint (mm)		Notes Numbers for comments on MIC breakpoints Letters for comments on disk diffusion
	S ≤	R >		S ≥	R <	
						1/A. The norfloxacin disk diffusion test can be used to screen for fluoroquinolone resistance. Isolates categorised as susceptible can be reported susceptible to levofloxacin and moxifloxacin and intermediate to ciprofloxacin and ofloxacin. Isolates categorised as resistant should be tested for susceptibility to individual agents.
Ciprofloxacin[2]	0.12	2	**5**	50[A]	18[A]	**2.** Wild type *S. pneumoniae* are not considered susceptible to
Levofloxacin[3]	2	2	**5**	19[A]	19[A]	**3.** The breakpoints for levofloxacin relate to high dose therapy.
Moxifloxacin	0.5	0.5	**5**	22[A]	22[A]	
Nalidixic acid (screen)	NA	NA		NA	NA	
Norfloxacin (screen)	NA	NA	**10**	12[A]	12[A]	
Ofloxacin[4]	0.12	4	**5**	50[A]	15[A]	**4.** Wild type *S. pneumoniae* are not considered susceptible to ofloxacin and are therefore categorised as intermediate.

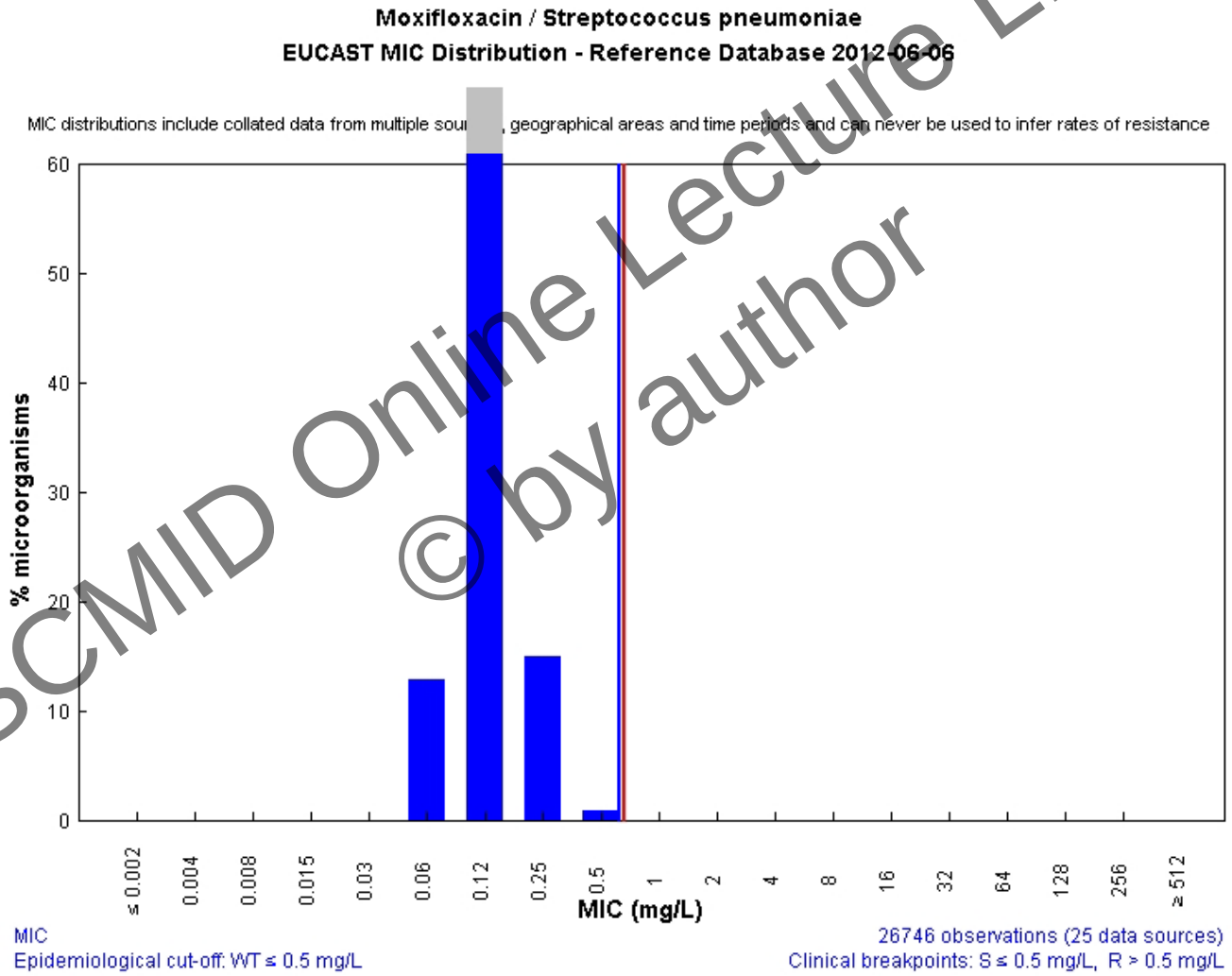
Wild-type distribution for *S. pneumoniae* vs ciprofloxacin



Wild-type distribution for *S. pneumoniae* vs levofloxacin

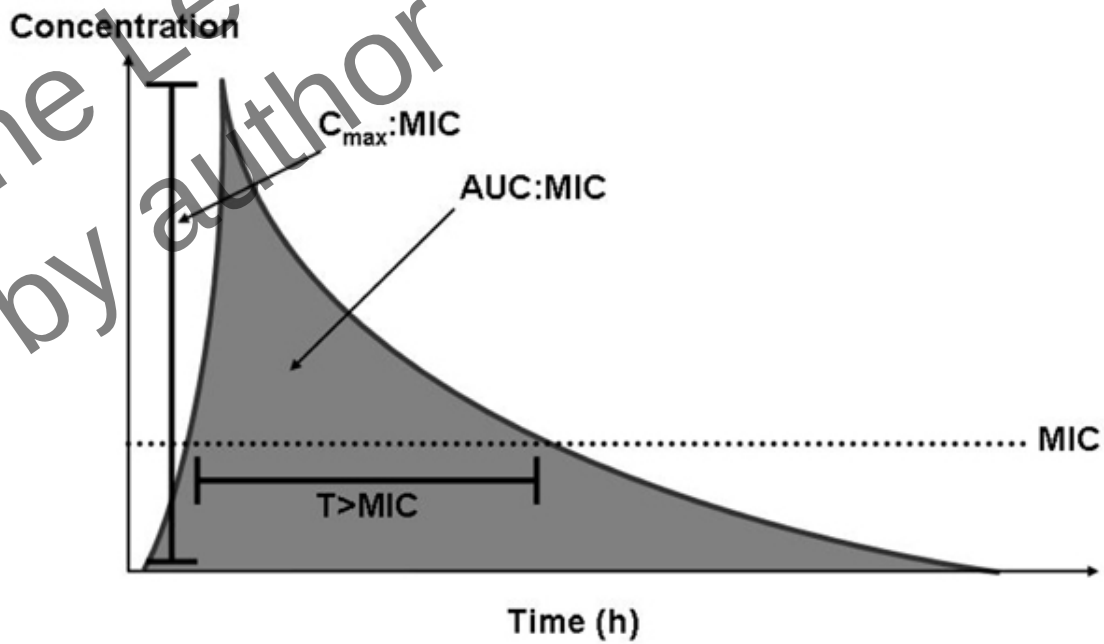


Wild-type distribution for *S. pneumoniae* vs moxifloxacin



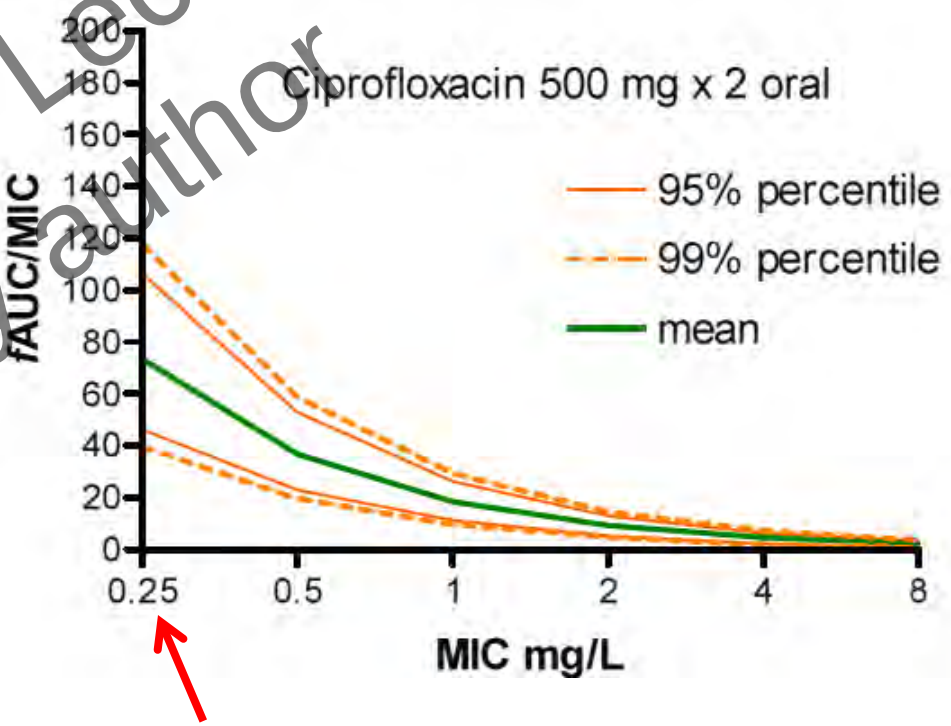
What is the rationale for classifying WT *S. pneumoniae* as intermediate?

- The pharmacodynamic index predicting clinical success when using FQs for treatment is AUC/MIC
- Clinical studies have established a target for gram-positive bacteria of 40, whereas the corresponding value in gram-negative bacteria is 80
- This is based on both clinical and animal studies



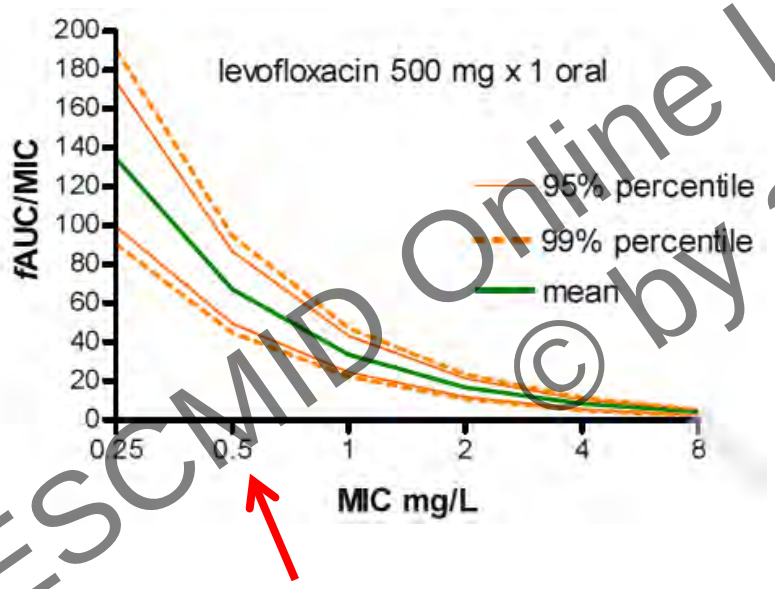
When a PD-target has been defined a Monte Carlo simulation can be conducted

- An MC-simulation is a mathematical modelling system where target attainment can be simulated in 10,000 patients
- Output is usually expressed as percent of the population reaching a particular PD-target
- Normally around 95% of the population should reach the target

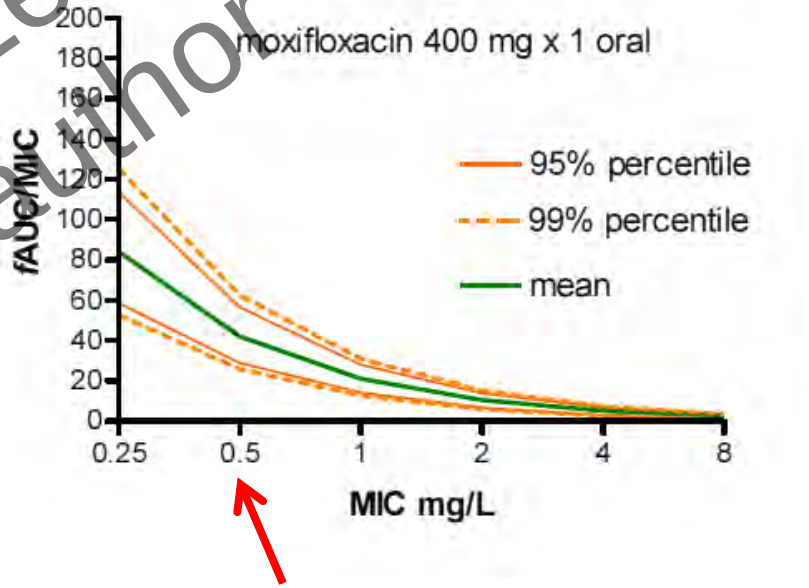


PKPD-breakpoint

MC-simulations for levofloxacin and moxifloxacin



PKPD-breakpoint



PKPD-breakpoint

Preliminary conclusions

- Levofloxacin and moxifloxacin are the two FQs with clinically useful activity against pneumococci (moxifloxacin is superior from a PKPD point of view)
- Ciprofloxacin does not have useful activity against WT pneumococci
- EUCAST clinical breakpoints reflect that target attainments are high with levofloxacin and moxifloxacin

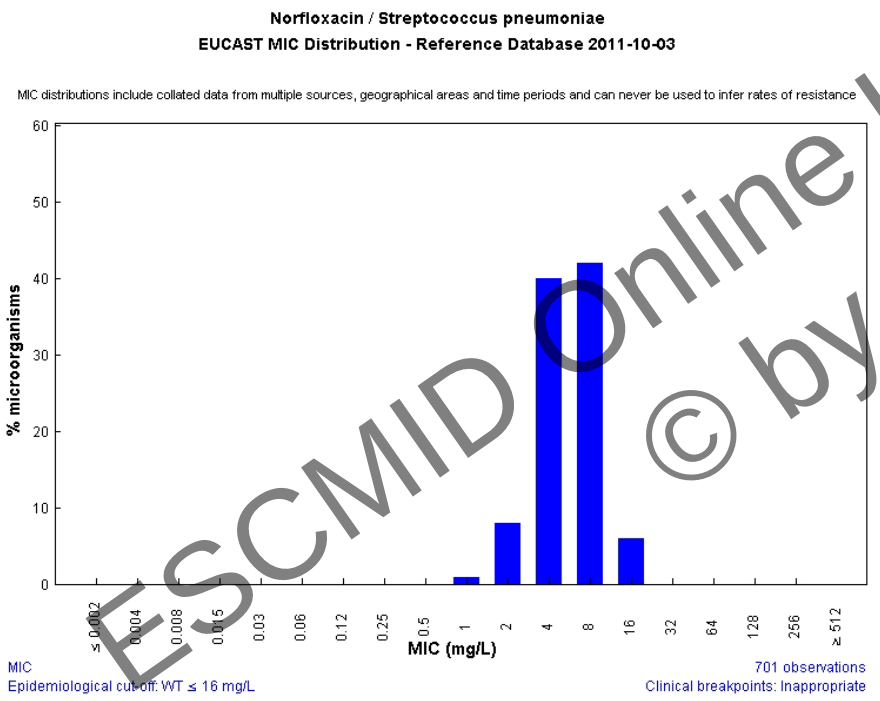
- BUT: which FQs should we test in every routine diagnostics and how should we test them?

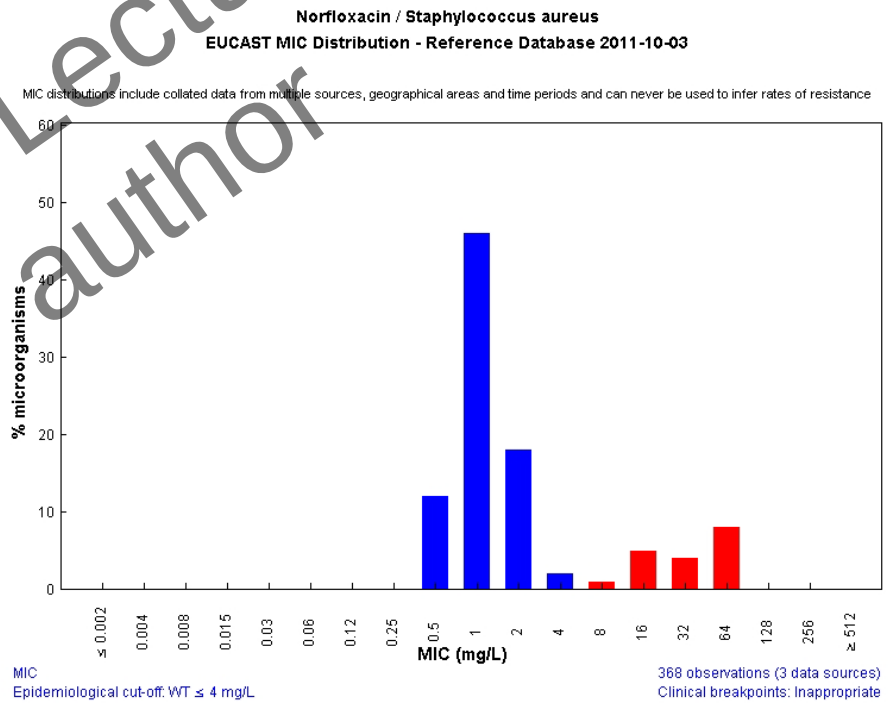
  → In particular: how can we detect FQ-resistance with disk diffusion?

Chromosomal resistance mechanisms to fluoroquinolones

Bacteria	ParC (topo IV)	Gyrase (topo II)	Efflux	Phenotype
Gram-positive	+	-	-	NOR R CIP/LEV R
Gram-positive	-	-	+	NOR R CIP R
Gram-positive	+	+		NOR/CIP/LEV R MOX R
Gram-negative	-	-	+	NAL R CIP/LEV r
Gram-negative	+/-	+(+)	-	NAL R CIP/LEV R

Norfloxacin – a marker of low-grade FQ-resistance in Gram-positive bacteria



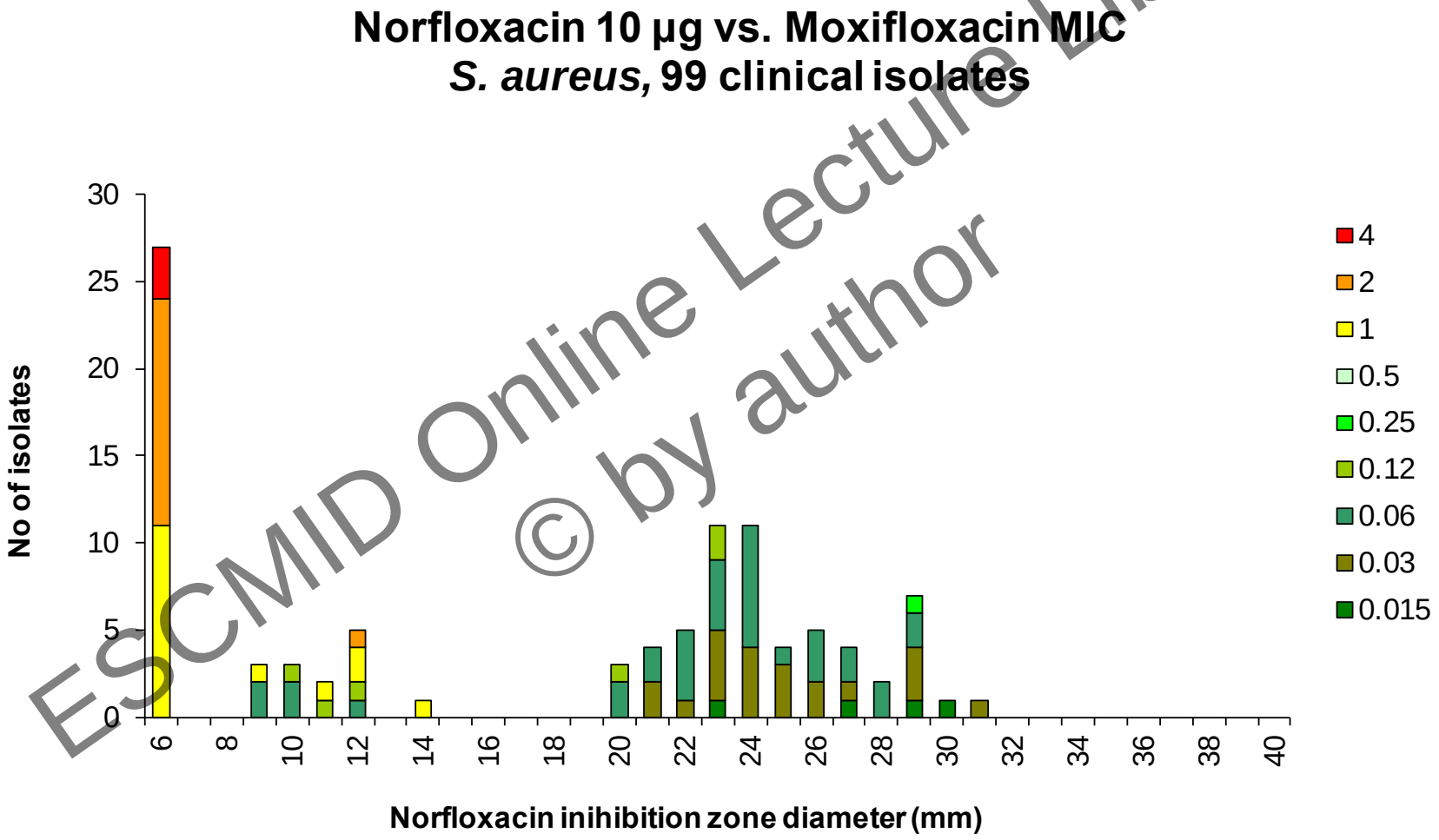


Norfloxacin and *S. pneumoniae*

Strains	Genotype	No. of strains	Mean zone diam[a] (mm) ± SD			
			NOR[b]	CIP	LVX	MXF
WT strains	Wild type	1,151	15 ± 3	24 ± 2	24 ± 2	32 ± 3
LLR mutants	*parC/parE*	46	**6 ± 0**	17 ± 3	20 ± 2	29 ± 3
	parC + efflux	3	**6 ± 0**	**8 ± 3**	17 ± 0	30 ± 1
	Efflux	14	**6 ± 0**	18 ± 2	22 ± 1	29 ± 3
	gyrA	16	15 ± 3	**21 ± 3**	21 ± 2	25 ± 3
HLR mutants	*parC* + *gyrA*	57	6 ± 0	6 ± 1	**7 ± 2**	17 ± 3
	parE + *gyrA*	7	6 ± 0	11 ± 4	**8 ± 2**	20 ± 2

Varon E et al. AAC 2006 Feb;50(2):572-9

Norfloxacin disk screening



Courtesy of EUCAST, JMI Laboratories (US) and Statens Serum Institut (DK).

What are the recommendations for screening and surveillance?

- Test all pneumococci from lower respiratory tract or invasive samples for susceptibility to FQs
- Use the norfloxacin disk test as a robust screening method
  - → Further characterization of norfloxacin-R isolates is necessary for therapeutical purposes
  - → Norfloxacin alone will be sufficient from a surveillance perspective

What is done in EUCAST regarding guidance on testing?

- EUCAST subcommittee for detection of resistance mechanisms
- Background: Guidance on methods of detection and characterisation of resistance mechanisms are required to tie in with the EUCAST MIC breakpoints, the EUCAST disk diffusion method, EUCAST Expert Rules and to meet ECDC requirements for update of the EARS-Net manual
- Remit: To develop practical guidelines for detection of specific antimicrobial resistance mechanisms of clinical and/or epidemiological importance
- One of several tasks: FQ-resistance in pneumococci
- Document to be finalized by December 2012

How common is FQ-resistance in Europe?

- No recent data for the entire Europe
- Spain February 2007-January 2009: 1,349 invasive *S. pneumoniae*, 45 levofloxacin-R strains (3.3%) (Rodriguez-Avial, AAC 2011)
- Belgian Pneumococcal Reference Laboratory 2004-2009 (Simoens et al, AAC 2011)
  - → LEV and MOX-R around 1%
- Resistance generally expected to be <5% in invasive isolates of *S. pneumoniae* in most European countries
  - → Depends on method used for detection (low-level R could be more common)
  - → Pan-European data would be desirable in EARS-Net

Serotypes and sequence types

PFGE Profile

Isolate	Serotype	LEV	PEN	CTX	ERY	CC	GyrA	ParC	Sequence Type
.254	19A	>32	0.5	0.5	>256	0.5	S81F	S79F	
.804	19A	>32	1	1	>256	0.5	S81F	S79F	ST276
.696	19A	>32	1	1	>256	0.5	E85K	S79F	
.1094	19A	>32	0.03	0.03	>256	>256	S81F	S79F	ST1201
.854	7F	>32	0.03	0.06	0.25	0.12	S81F	S79F	ST191
.855	7F	>32	0.03	0.06	0.25	0.12	S81F	S79F	
.100	10A	>32	0.06	0.06	>256	256	S81Y	S79F	ST2639
.1099	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.1201	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.1289	8	>32	0.03	0.03	>256	>256	S81F	S79F	ST63
.648	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.784	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.1067	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.829	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.1204	8	>32	0.03	0.015	>256	>256	S81Y	S79F	
.1095	8	>32	0.03	0.03	>256	>256	S81F	S79 F	
.1416	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.506	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.1261	8	>32	0.03	0.03	>256	>256	S81F	S79F	ST63
.927	8	>32	0.03	0.015	>256	>256	S81F	S79F	
.949	8	>32	0.03	0.015	>256	>256	S81F	S79F	
.952	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.785	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.849	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.816	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.1229	8	>32	0.03	0.015	>256	>256	S81F	S79F	
.1279	8	>32	0.03	0.03	>256	>256	S81Y	S79F	
.1286	8	>32	0.03	0.03	>256	>256	S81Y	S79F	
.101	8	>32	0.03	0.03	>256	>256	S81Y	S79F	
.118	8	>32	0.03	0.03	>256	>256	S81F	S79F	
.370	8	>32	0.03	0.015	>256	>256	E85K	S79F	ST63
.435	8	>32	0.06	0.03	>256	>256	S81F	S79F	
.653	8	>32	0.015	0.03	>256	>256	S81Y	S79F	
.732	8	>32	0.06	0.03	>256	>256	E85K	S79F	
.402	8	>32	0.03	0.015	>256	>256	S81F	S79F	
.219	NT	>32	0.015	0.015	>256	>256	S81F	S79F	ST63
.573	9V	>32	2	2	0.12	0.12	S81F	S79F	ST156
.922	19F	>32	0.25	0.25	>256	>256	S81F	S79F	ST88
.1241	6B	>32	2	2	>256	>256	E85K	S79F	ST1542

Serotype 8 (ST63) most common (not in PCV13) Rodriguez-Avial, AAC 2011)

Final conclusions

- FQ-resistance in pneumococci is a potentially growing problem and should be closely monitored
- Testing ciprofloxacin is an obsolete practice and should be discontinued and replaced by preferably the NOR disk test
- Isolates resistant to NOR should be subjected to MIC-testing vs levofloxacin and moxifloxacin
- The NOR disk test is robust and easy to implement
- The main clone responsible for dissemination of FQ-R pneumococci in Spain is not covered by PCV13