Ciprofloxacin

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Abstract

Document holding some notes from the ciprofloxacin literature I've read.

1 Known mutations

Table 1 shows the ciprofloxacin resistance mutations I have seen in the literature along with some notes.

2 Experimental questions

- **How does ciprofloxacin affect cellular motility?** E. coli exposed to 1-10×MIC filamented [6] – will surely affect motility once cells start to deviate from rod shape. In Bob Austin's paper [2] 99.5% of cells filament at low concentrations (as low as 0.125×MIC. The paper [8] states that cells did not lose their motility for sub-inhibitory concentrations but no quantitative measurements were made. It would be interesting to know if experimentally (i) wildtype wavespeed was affected in different, uniform sub-inhibitory concentrations; (ii) the form of this dependence (gradual or cut-off); (iii) to see if some of the resistance-evolved strains behave the same, or have different wavespeeds in the presence / absence of ciprofloxacin. This behaviour could be important in understanding the results of the experiments.
- **How does ciprofloxacin affect mutation rate for mutated strains?** Bartek has measured a 10-fold increase for 0.5 MIC concentrations, for the wildtype. In [4] they state that from the studied strains, the spontaneous mutation rate varied by 2 orders of magnitude between strains. SOS response can apparently increase bp-mutation rate by 4 orders of magnitude [2]. It would be interesting to know if an evolved strain still had roughly the same base mutation rate as the wildtype, and how it varied when we increased ciprofloxacin beyond the wildtype MIC. Currently I implement a simple power-law: $\gamma_{[a]} = \gamma_{WT} * \left[1 + \left(\frac{X[a]}{MIC_{WT}}\right)\right]$, choose X and Y arbitrarily, and put some hard upper limit (200 times WT mutation rate, say).
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3 Notes for specific papers

Reference [5].
Construct 28 strains containing different combinations of 5 different mutations (gyrA x 2, parC, marR, acrR) and measures the fitness and MIC for each of them.

Reference [3].

- Quinolones can be mutagenic and can induce the SOS response. "In this work, we show that preventing induction of the SOS response by interfering with the activity of the protease LexA renders pathogenic Escherichia coli unable to evolve resistance in vivo to ciprofloxacin".
- "Given the low mutation rate, only three post-exposure ciprofloxacin-resistant lexA(S119A) mutants were isolated (from more than 10^{11} bacteria plated, overall), but all three acquired resistance by deletion of the Ser83 codon and not by substitution mutation". Resistance in the post-exposure mutants was acquired strictly through deletion of either Ser83 or Ala84, and not through substitution.
- Ciprofloxacin kills cells: "On solid media, we found that 40 ng/ml ciprofloxacin killed 99% of the cells within 24 h of plating, while the remaining 1% of the population persisted for several weeks."

- Ciprofloxacin induces resistance by a factor of 10^4: "We observed a pre-exposure mutation rate of $9.0(\pm 9.5) \times 10^{-10}$ mutants/viable cell/d, and a post-exposure mutation rate of $1.8(\pm 0.69) \times 10^{-5}$ mutants/viable cell/d.

Reference [2].

- Multi-nucleated filaments appear at low ciprofloxacin concentrations. SOS response can increase mutation rate from 10^{-9} to 10^{-5} per base pair per generation.
- In their experiment, 87% of clones had a single gyrA (S83L) mutation.
- Deletion(s) of 1 or 11 nt in the marR gene encoding the MarR efflux pump regulator were identified in 37% of the sequenced clones. E. coli bacteria in the presence of 0.125 of the minimal inhibitory antibiotic concentration (MIC=40ng/mL) of cipro: time-lapse imaging revealed that 99.5% of the bacteria formed long filaments (up to 200 μm) and expanded their length exponentially.

Reference [4].

- **54 E. coli strains of from patients with UTI analyzed**. Most resistant strains carried two mutations in gyrA and one mutation in parC. In addition, many resistant strains had mutations in parE, marOR, and/or acrR. Measure MIC of all strains (for 7 antibiotics in total). See Table 3 for mutations present in all 54 strains.
- The spontaneous mutation rate in these clinical strains varied by 2 orders of magnitude. A high mutation rate correlated strongly with a clinical resistance phenotype. This correlation suggests that an increased general mutation rate may play a significant role in the development of high-level resistance to fluoroquinolones by increasing the rate of accumulation of rare new mutations.
- gyrA: 26/54 strains carried mutations at both S83 and D87.
- gyrB: No resistance mutation was found in gyrB.
- parC: With the exception of one double mutant (strain C1204, with the S80I and E84G mutations), each mutated strain had a single parC alteration. All 25 strains with parC mutations also carried one or two gyrA mutations.
- marR: Mutations in the marOR sequence were identified in 11 strains.
- acrR: Mutations expected to alter the AcrR protein sequence were found in 33 of the 54 clinical strains.
- parE: "Putative fluoroquinolone resistance mutations in parE (grlB) have been found in other bacterial species. These are (with E. coli numbering), D420N (53), P439S (51, 55) P439Q (21), L440F (8), N458D (15), and E460K (8)".
- 21 of the 53 clinical isolates had a relative mutation rate at least 10-fold higher than that of Nu14. One mutant had a mutation rate over 100-fold greater than that of Nu14.

Reference [7].

- Acquisition of gyrA mutation has the effect of reducing virulence of uropathogenic E. coli.
- "Two types of mutants are predominantly found among clinical isolates: low-level resistant isolates (CIP MIC<2 mg/liter) frequently carrying a single gyrA mutation, which generates a substitution of serine 83 to leucine (S83L), and high-level resistant isolates (CIP MIC>4 mg/liter) carrying two gyrA mutations in addition to mutations affecting serine 80 (S80) and glutamic acid 84 (Glu84) in parC."

Reference [6].

- E. coli cells exposed to 1 and 10 times the MIC began to elongate after 30 min of exposure to the antimicrobial agent and went on to form filaments after 90 min. These filaments (mean length, 27.6 ± 16 μm) were still motile after 120 min of exposure to the MIC.
- Cells exposed to 100 times the MIC of ciprofloxacin were not filamentous, although their mean length (6.7 ± 3.9 μm) was greater than those for the control culture and the culture exposed to 0.1 times the MIC.

References

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Gene	Mutation	Notes	Ref.
gyrA	S83L		[5]
	D87N		[5]
	S83Δ	Deletion, not substitution	[3]
	D87Y	Found in 4/54 strains [4]	[4]
	D87G	3/54 strains	[4]
	D87H	1/54 strain	[4]
	A196E		[4]
parC	S80I	(FO) (22/54 strains in [4])	[5]
	S80R	1/54 strain in [4])	[5], [4]
	E84K	2/54 strains	[5], [4]
	E84G	1/54 strains	[4]
parE	I444F		[4]
	S458T		[4]
	D475E		[4]
	I529L		[4]
	L445H		[1]
marR	Δ		[5]
marOR	Q110 stop; K62R (x3);	K62R not thought to confer resistance	[4]
	{Δ*G* at aa 52, A52R, G/S103N}		
	L78M (x2); C47G; D76G; V79I		
	+17 nt (duplication) (aa 26–31)		
	Δ5 nt (-68 to 72)		
acrR	Δ		[5]
acrR	F24L; T5N; M201R (x2);		[4]
	S25P; P155L; N69H		
	K146T; V125I; A212S; I62F (x2)		
	T213I (x8); N214T (x4); E215A (x17);	Claim: mutations 213 to 215 do NOT confer resistance	
	Found in various combinations:		
	{T213I, N214T, E215A}		
	{E215A, S25P}; {E215A, N69H}		
	{A212S, T213I, N214T, E215A}		
	{C→A at nt -25, T213I, N214T, E215A}		
	{K146T, IS5 at aa 73}; {I16F, V125I}		
	{T213I, N214T, P155L}		
	{E215A, T→A at nt -10}		
	4-nt duplication (GATT) at aa 111–12		
	Insertion of IS1 at aa 9		
	{Δ*A* at aa 80, T213I}		
	+2 nt(GC) at aa 206		
	{I62F, E215A}; {A212S, T213I }		

Table 1: Mutations encountered whilst reading about ciprofloxacin. The Δ is the notation used in [5] to signify any sort of deletion / insertion that affects these genes. Table 3 in [4] more clearly shows all observed marR and acrR mutations in 54 E. coli strains isolated from patients. The notation {...} indicates that all are co-present in one strain.