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Delafloxacin exerts potent anti-gonococcal activity despite mutations that decrease antibiotic susceptibility due to target modification or drug efflux

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Abstract

Background: The emergence of *Neisseria gonorrhoeae* (NG) strains resistant to extended-spectrum cephalosporins represents a major public health problem that requires the development of new drugs for effective treatment. Accordingly, we examined the susceptibility of gonococcal clinical isolates expressing decreased antibiotic susceptibility (including ciprofloxacin; CIP) to a novel fluoroquinolone (delafloxacin; DLX) that like CIP targets DNA gyrase and topoisomerase IV. Due to its unique structural characteristics, DLX has potent antibacterial action against gonococci including against CIP-resistant strains.
Methods: The minimal inhibitory concentration (MIC) of antibiotics against NG strains was performed by agar dilution. Mutations in *mtrR*, *gyrA* and *parC* were determined by DNA sequencing.
Results: We found that NG is highly susceptible to DFX (MIC <0.0005-0.06 µg/ml) even if it expresses significant decreased susceptibility or resistance to other antibiotics (e.g., CIP; MIC 2-8 µg/ml). Loss of the MtrC-MtrD-MtrE, but not the NorM efflux pump, was found to increase susceptibility to several antibiotics by 2-8 fold. This suggests DLX is not a substrate for NorM. A gonococcal strain (FA19) bearing wild-type *gyrA* and *parC* genes was found to be highly susceptible to DLX (MIC <0.0005 µg/ml) and this MIC value was only marginally increased due to mutations that enhance expression of the *mtrCDE*efflux pump operon. Moreover, loss of the MtrC-MtrD-MtrE pump in clinical isolates was found to increase susceptibility to DLX by 8-fold, further suggesting that DLX is a substrate for this pump.
Conclusion: We conclude that DLX, which is now in Phase III clinical trials for treatment of uncomplicated gonorrhea, retains potent anti-gonococcal activity even against antibiotic-resistant strains. Thus, use of DLX for the treatment of uncomplicated gonorrhea is of likely value in an era when multidrug resistant strains of NG continue to emerge

Background

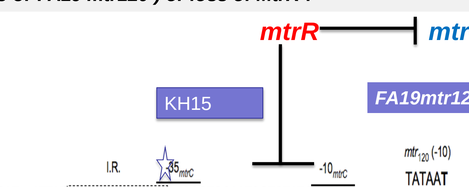
- Gonorrhea is one of the most common and widespread sexually transmitted diseases in the world. The emergence of drug resistant gonococcal strains poses a continuous threat.
- Resistance to penicillins, tetracyclines and fluoroquinolones (FQs) rendered oral therapy ineffective. Current use of iv/im cephalosporins is being challenged by recent reports of strains resistant against extended-spectrum cephalosporins(1). Therefore, novel therapies are urgently sought.
- FQs block the DNA gyrase and topoisomerase IV, necessary for DNA replication. Single amino acid substitutions in the target proteins GyrA and ParC confer resistance. Additionally, increased expression of efflux pumps can decrease bacterial susceptibility to FQs.
- Delafloxacin is a novel FQ with enhanced activity against gonococcal strains including CIP-resistant strains. While it targets the same protein, its physicochemical properties allow enhanced intracellular accumulation (Figure 1).
- To quantify their impact on FQ resistance, target protein sequence and efflux pump expression were characterized in a panel of stains that exhibit different levels of quinolone resistance. Furthermore, strains with deficient efflux pumps were examined for changes in levels of FQ susceptibility (Figure 2)

Methods

GC cultures: material form frozen aliquots was streaked out on chocolate agar plates and grown at 37° C in an atmosphere supplemented with 5% CO2. Fluid cultures were started using single colonies immersed into PPM media supplemented with MgCl2 and factor V.
Strains: CDC1 and CDC13 isolates from China, CDC 19 from Lansing, MI, USA. Genetic derivatives bearing mutations that enhance *mtrCDE* gene expression or inactivate the MtrC-MtrD-MtrE or NorM efflux pumps were from previous studies published by the Shafer laboratory (2-5) **MIC** testing was performed by agar dilution (GC agar)
DNA isolation and sequencing. Single colonies were heat killed and used as DNA templates. PCR of target genes was performed, including upstream promoter regions.

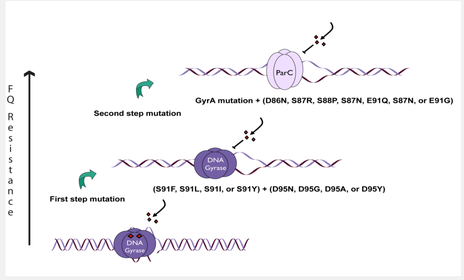
Results

1) Chemical properties of delafloxacin



A distinct charge profile of delafloxacin (polar group instead of positively charged group in moxifloxacin) leads to increased intracellular accumulation

2) Quinolone target gene mutations

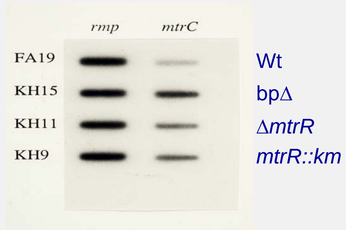


Resistant mutations are confined to the quinolone resistance determining regions of GyrA (AA 91-95), and ParC, respectively. Both, gyrA *and* parC *regions, were sequenced for all strains. (2)*

3) The efflux pump *mtrCDE* operon is negatively regulated by MtrR and expression can be enhanced by promoter mutations (strains KH15 or FA19 *mtr120) or loss of MtrR* :

Mutations in the mtrR repressor or the promoter regions of the mtrCDE operon lead to over-expression (3). KH15 (as FA19 but mtrR 171) has a single bp deletion in the wild-type promoter that abrogates expression of mtrR. FA19 mtr120 has a point mutation that generates a new -10 hexamer that drives transcription of mtrCDE independent of control by MtrR (2)

4) Strains with different levels of *mtrC* expression



*Differential expression , as assessed by RNA slot-blot (Data from Hagman et al. 1995) of mtrC in 4 different strains: **FA19** with wild type mtrCDE sequence and mtrR gene and promoter, **KH15** with an enhancing deletion in the promoter region, **KH11** with a mtrR deletion, and **KH9** with mtrR insertion mutant. Similarly, norM deficient strains were constructed (expression not shown but not impacted by mtrR mutations)*(4). Expression *levels of mtrC in FA19*mtr*120 is similar to that of strain KH15* (2).

5) Correlation of genetic background and MIC data of tested strains

MIC in µg/ml

Strain	gyrA	parC	Ciprofloxacin	Delafloxacin
FA19	WT	WT	**<0.0075**	**<0.00049**
FA19 *mtr120*			**<0.0075**	**0.0098**
KH15 (FA19 *mtrR 171*)			**<0.0075**	**0.0098**
RD1 (KH15 *mtrE::kan*)			**<0.0075**	**<0.00049**

Strains that overproduce mtrCDE efflux pump do slightly affect activity of delafloxacin but do not render it ineffective

CDC-1	S91F	S87N	**2**	**0.06**
CDC-1 *mtrE::kan*			**0.5**	**0.0075**
CDC-1 *norM::kan*			**2**	**0.06**
CDC-13	S91F	S87R	**4**	**0.06**
CDC-13 *mtrE::kan*			**2**	**0.0075**
CDC-13 *norM::kan*			**4**	**0.06**
CDC-19	S91F, D95G	S87R	**4**	**0.06**
CDC-19 *mtrE::kan*			**4**	**0.0075**
CDC-19 *norM::kan*			**4**	**0.06**

Clinical isolates with gyrA/parC mutations (highlighed in red) are well-covered by delafloxacin. Further, delafloxacin does not appear to be a substrate for the NorM efflux pump.

Conclusion

- Drug resistance is a widespread phenomenon and poses continuous challenges on the treatment of gonorrhea.
- Currently, oral therapeutic options are not available, and resistance to current systemic cephalosporin therapy is emerging.
- Delafloxacin is a novel FQ with superior bactericidal effect compared to currently clinically available FQs and is currently being tested in phase III clinical trials for treatment of uncomplicated gonorrhea.
- While it is a potential substrate for Mtr-mediated efflux, it still retains its activity. It does not appear to be a target for NorM
- In conclusion, we show that delafloxacin, which is now in phase III clinical trials for treatment of uncomplicated gonorrhea, retains potent anti-gonococcal activity even against antibiotic-resistant strains.
- Thus, use of delafloxacin for the treatment of uncomplicated gonorrhea is of likely value in an era when multidrug resistant strains of NG continue to emerge.

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