The Genetic Basis for a *Neisseria gonorrhoeae* Clinical Isolate That Contains *mtrR*$_{-79}$ Mutation But Is Highly Susceptible to Antibiotics Effluxed by the Mtr Pump System

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Introduction

- *Neisseria gonorrhoeae* (NG) causes the sexually transmitted disease gonorrhea
- NG possesses multiple drug efflux systems (including MtrCDE, FarAB-MtrE, MacAB-MtrE, MATE, and NorM) which play an important role in evading antibiotics in the treatment for gonorrhea and in helping this pathogen to evade innate antimicrobial defenses during infection[1]
- Expression of the MtrCDE efflux system is highly regulated by both *cis*- and *trans*-acting regulatory elements
- Mutations of *mtrR*$_{-79}$ (a single T base pair [bp] deletion) and *mtr*$_{120}$ (a C–T point mutation) in the promoter region between *mtrR* and *mtr*CDE are common and contribute to overexpression of the MtrCDE efflux pump, resulting in increased efflux[2] to multiple antibiotics, especially macrolides

Methods

- **DNA amplification and sequencing**: The promoter regions of *mtrR* and *mtrCDE* were amplified by polymerase chain reaction before Sanger sequencing to identify mutations
- **NG spot transformation:** 20µL of NG6 inoculum from a 24h plate was spotted onto a GC plate (2mM $MgCl_2$) and mixed with 1µg/10–20µL NG58 genomic DNA before ≥4h incubation (37°C; 5% CO_2). The transformation mix was selected on GC plates with various ciprofloxacin concentrations
- **Susceptibility testing:** We used a modified fastidious broth microdilution method with 2X bacterial inoculum and a minimum inhibitory concentration (MIC) read ~48h after incubation at 37°C (5% CO_2)

Results

- Clinical isolate NG6, similar to the FA1090 strain, is highly susceptible to antibiotics (**Table 1**). *Mtr* promoter region sequence analysis revealed a single-bp deletion mutation, *mtrR*$_{-79}$, in NG6, but not in FA1090, which can confer elevated efflux.[2] NG58B, a clinical isolate with elevated efflux, also contained the *mtrR*$_{-79}$ mutation (**Figure 1**)

- We identified a new *cis*-acting control element in ***mtrC* that can lead to deletion of GC pair(s), resulting in a frameshift mutation to produce truncated MtrC and switch off the MtrCDE pump**
- Our results indicate that genotyping of the promoter region between *mtrR* and *mtrCDE* is insufficient to predict increased efflux phenotype
- Our results provide direct evidence that *N. gonorrhoeae* isolates with elevated efflux can genetically revert to low efflux via this *cis*-acting control element in *mtrC*

Figure 1. A single nucleotide deletion (*mtrR*$_{-79}$) in the *mtr* promoter

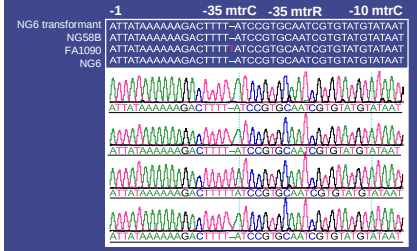
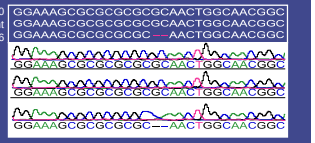
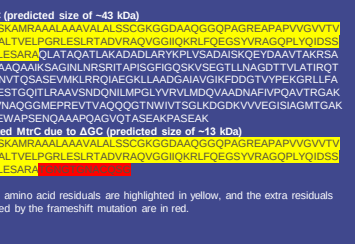


Figure 2. A GC deletion in *mtrC* resulting in a frameshift mutation





Identical amino acid residuals are highlighted in yellow, and the extra residuals introduced by the frameshift mutation are in red.

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Results

Table 1. Antimicrobial susceptibility of NG isolates and transformants

N. gonorrhoeae	Mutation in		Broth microdilution MIC* (µg/mL) at 48h					
	mtrR$_p$	*mtrC*	CIP	AZI	TET	PEN	SPT	CRO
FA1090	wt	wt	0.004	0.03	0.125	NT	NT	NT
NG58B† (donor)	*mtrR*$_{-79}$	wt	8	0.25	1–2	0.03–0.06 (4)‡	8–16	0.002–0.008 (0.06)‡
NG6 (recipient)	*mtrR*$_{-79}$	A117 frameshift	0.002	0.03	0.5	0.125	16	0.002
NG6 transformant #1	*mtrR*$_{-79}$	wt	0.004	0.25	2	0.5–1	16	0.004
NG6 transformant #7	*mtrR*$_{-79}$	wt	0.004	0.25	2	0.5–1	16	0.004

*MIC values are from two independent experiments, which showed significant (≥4-fold) increase in the transformants, relative to those from recipient (shown in blue font); †NG58B contains quinolone target mutations GyrA S91F D95G and ParC D86N, NG6 transformants #1 and #7 do not contain these mutations; ‡The measured MIC values were well below published values[3] (in parentheses), probably due to poor growth of NG58B in the broth medium. AZI, azithromycin; CIP, ciprofloxacin; CRO, ceftriaxone; PEN, penicillin; NT, not tested; SPT, spectinomycin; TET, tetracycline; wt, wild type

Table 2. Mutations in *mtr* promoter and *mtrC* contribute to efflux phenotype in NG

Mutation in		Predicted efflux phenotype
mtr$_p$	*mtrC*	
wt	wt (GCGCGCGCGCGC)	Low
mtrR$_{-79}$ or *mtr*$_{120}$	wt (GCGCGCGCGCGC)	Elevated
mtrR$_{-79}$ or *mtr*$_{120}$	Frameshift mutation	Low
wt	Frameshift mutation	Low

- PCR product analysis of *mtrCDE* from FA1090 and NG6 revealed no size differences between these two isolates, ruling out *mtrCDE* inactivation in NG6 from large insertion/deletion
- DNA sequence analysis of *mtrCDE* from NG6 identified a loss-of-function mutation ΔGC from a six-GC repeat (GCGCGCGCGCGC) in *mtrC*, causing an MtrC A117 frameshift mutation predicted to produce a truncated MtrC protein (**Figure 2**) and give the low efflux phenotype in NG6
- The six-GC repeat occurs four times in the FA1090 genome: 1327197 to 1327208 (*mtrC*); 1483171 to 1483182; 1758066 to 1758077; and 2039716 to 2039727
- Natural transformation of NG6 with wild-type *mtrC* and selection with ciprofloxacin generated transformants that corrected the ΔGC mutation (**Figure 2**), but still retained the *mtrR*$_{-79}$ mutation (**Figure 1**) and restored the increased efflux phenotype (**Table 1** and **Table 2**)
- A survey of the National Center for Biotechnology Information nucleotide collection database found that 5% (3/56) of NG genomes had frameshift mutations (ΔGC or ΔGCGC) in *mtrC*

References

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Disclosures

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