Dissecting the Multifaceted Nature of Antibiotic Resistance in Clinical Isolates of *Neisseria gonorrhoeae* by Natural Transformation

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Introduction

- *Neisseria gonorrhoeae* (NG) causes the sexually transmitted disease gonorrhea. Clinical isolates of NG with multidrug resistance mechanisms against different antibiotics introduced for its treatment are increasingly common[1]
- Clinical antibiotic resistance is often the product of multi-resistance determinants, which are difficult to dissect and quantify directly using clinical isolates
- Our study utilized high frequency (up to 10^{-2}) of natural transformation with NG genomic DNA[2] in order to transfer genetic resistance determinants to a susceptible NG isolate

Methods

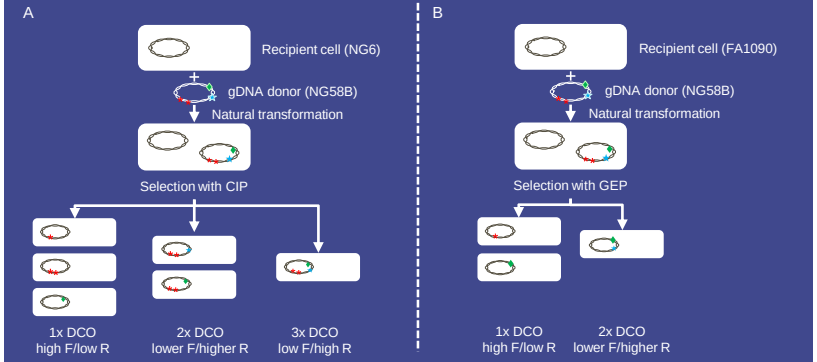
- **NG spot transformation:** Piliated colonies from a 24h plate were used in inoculum preparation. 20µL inoculum was spotted onto a GC plate containing 2mM $MgCl_2$, then 1µg/10–20µL genomic DNA was added to the spot and mixed. After incubation at 37ºC (5% CO_2) for ≥4h, the transformation was selected on GC plates with various concentrations of ciprofloxacin (CIP) or gepotidacin (GEP)
- **DNA amplification and sequencing:** Quinolone resistance-determining regions of *gyrA* and *parC* and the promoter region of *mtr* were polymerase-chain-reaction amplified before Sanger sequencing to identify mutations
- **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 37ºC (5% CO_2)

Results

- Natural transformation was used to transfer genetic determinants from donor DNA of CIP-resistant NG to a CIP-susceptible strain under selection of various concentrations of CIP or GEP (**Figure 1**)
  - A series of transformants containing single or multiple genetic determinants was generated depending on the transformation frequencies and resistance levels

- We were able to dissect genetic determinants ($mtrR_{-79}$, GyrA S91F D95G, and ParC D86N) of *Neisseria gonorrhoeae* to measure their contributions to ciprofloxacin resistance
- We also identified pre-existing genetic determinants ($mtrR_{-79}$, GyrA S91F, and ParC D86N) that minimally influenced gepotidacin susceptibility
- We successfully demonstrated the utility of natural transformation in dissecting the multifaceted nature of antibiotic resistance in *Neisseria gonorrhoeae* clinical isolates

Figure 1. Generation of isogenic *N. gonorrhoeae* strains with varying genetic determinants and susceptibility to (A) ciprofloxacin and (B) gepotidacin



DCO, double crossover; F, frequency; gDNA, genomic DNA; R, resistance

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Results

- Antibiotic susceptibility testing and genetic characterization of selective transformants identified specific genetic determinants that influenced CIP resistance and GEP susceptibility (**Table 1**)
- Regeneration of CIP resistance in recipient cells allowed us to detect potential resistance development pathways (**Figure 2**)

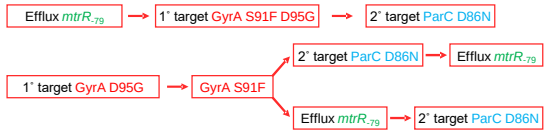
Table 1. Genetic determinants identified in NG transformants and their contributions to (A) CIP resistance and (B) GEP susceptibility

A	Broth MIC (µg/mL) ~48h		Fold increase vs recipient		Mutation in				
NG isolate	CIP	AZI	CIP	AZI	GyrA	ParC	*mtr*	*mtrC*	
NG58B (donor)	8	0.5	4000	32	S91F D95G	D86N	$mtrR_{-79}$	wt	
NG6 (recipient)	0.002	0.016	1	1	wt	wt	$mtrR_{-79}$	A117 frameshift	
NG6-1 (N=8)	0.008	0.125	4	8	wt	wt	$mtrR_{-79}$	wt	
NG6-2 (N=2)	0.031	0.016	16	1	D95G	wt	$mtrR_{-79}$	A117 frameshift	1XDCO
NG6-3 (N=5)	0.25	0.016	128	1	S91F D95G	wt	$mtrR_{-79}$	A117 frameshift	
NG-4 (N=1)	1	0.125	500	8	S91F D95G	wt	$mtrR_{-79}$	wt	
NG-5 (N=5)	4	0.016	2000	1	S91F D95G	D86N	$mtrR_{-79}$	A117 frameshift	2XDCO
NG6-6 (N=2)	8	0.125	4000	8	S91F D95G	D86N	$mtrR_{-79}$	wt	3XDCO

B	Broth MIC (µg/mL) ~48h		Fold increase vs recipient		Mutation in			
NG isolate	GEP	AZI	GEP	AZI	GyrA	ParC	*mtr*	
NG58 (donor)	1	0.5	16	8	S91F D95G	D86N	$mtrR_{-79}$	
FA1090 (recipient)	0.063	0.063	1	1	wt	wt	wt	
FA1090-G1 (N>5)	0.25/0.125	0.25/0.125	2–4	2–4	wt	wt	$mtrR_{-79}$	1XDCO
FA1090-G2 (N=1)	0.125	0.031	2	0.5	S91F	wt	wt	
FA1090-G3 (N=2)	0.25	0.25/0.125	4	2–4	wt	D86N	$mtrR_{-79}$	2XDCO

The CIP resistance phenotype and genotypes of transformants NG6-6 (highlighted in red) are the same as the donor. AZI, azithromycin; N, the number of transformants tested for MIC and a subset (generally ≥2) were genotyped for the indicated genes

Figure 2. Potential CIP resistance development pathways



References

1. Unemo M and Shafer WM. *Clin Microbiol Rev* 2016;27:587–613;
2. Hamilton HL and Dillard JP. *Mol Microbiol* 2006;59:376–85.

Disclosures

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