

# Influence of chromosomal mutations in genes encoding MtrCDE efflux, PorB1b porin, and PBP1 on susceptibility towards multiple antibiotic classes in clinical isolates of *Neisseria gonorrhoeae*.

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## Introduction & Purpose

Antibiotic resistance in *Neisseria gonorrhoeae* is steadily rising, reducing treatment options. Common resistance mechanisms include increased efflux through the MtrCDE efflux pump, decreased permeability through the PorB1b porin (*porB*), and reduced affinity of PBP1 (*ponA*) for  $\beta$ -lactam antibiotics (Unemo & Shafer, 2014).

These three mechanisms all result from chromosomal mutations, most commonly a deletion in the promoter of the *mtrR* gene, leading to overexpression of MtrCDE, and substitutions at specific sites in the *porB* and *ponA* genes, respectively.

The aim of this study was to investigate the relationship between these mutations and antibiotic resistance in a collection of 50 clinical isolates.

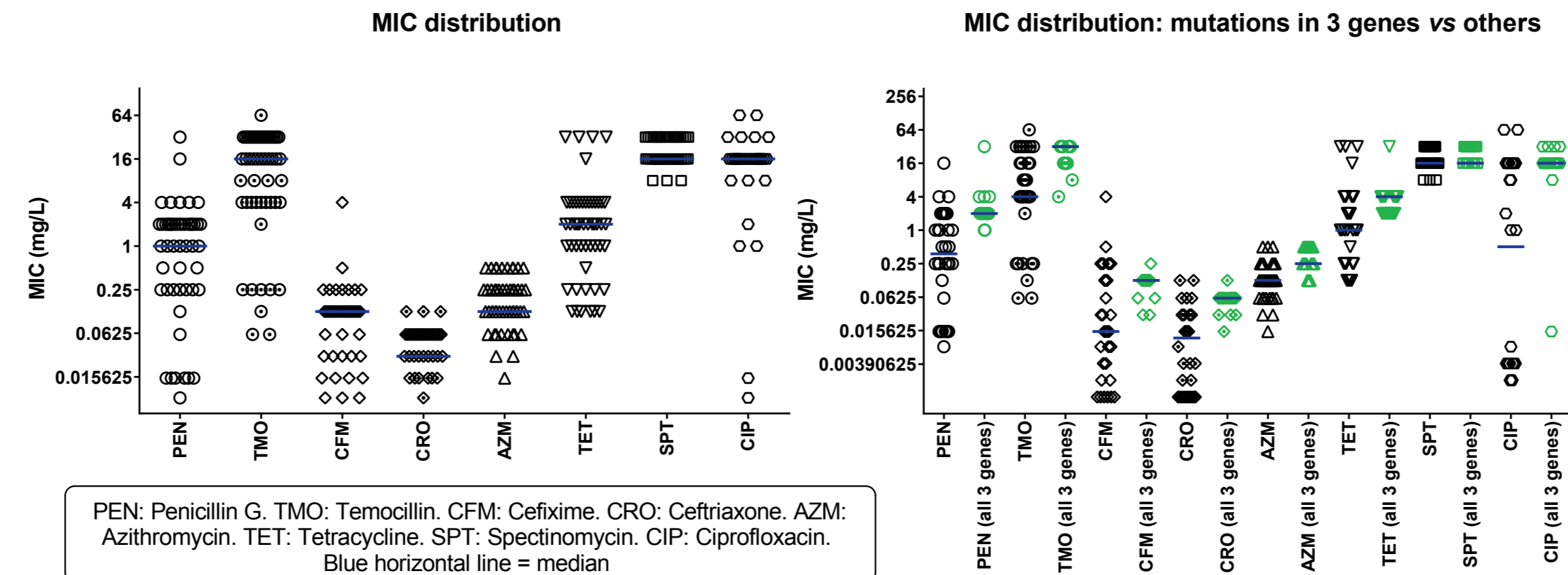
## Materials & Methods

- 50 clinical isolates from the national reference centre of Belgium.
- Minimum inhibitory concentrations (MICs) determined by agar dilution using GC agar plates with increasing concentrations of 8 antibiotics.
- Presence of mutations in the *mtrR*, *porB* and *ponA* genes determined by PCR.

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## Results



- all strains are susceptible to CRO and SPT.
- 1/4 are intermediate to AZM but 2/3 are co-resistant to PEN, TET, CIP.
- Isolates with mutations in *mtrR* have higher MICs to all antibiotics tested, except CFM and SPT (Table).
- Isolates with mutations in *porB* or *ponA* have higher MICs to all antibiotics except SPT (Table).
- Isolates with mutations in all 3 genes have higher MICs to all antibiotics except SPT (right Figure and Table).

## Conclusions

- Mutations in *mtrR* seem to increase MICs of many but not all antibiotics among those studied, highlighting the interest of further studying the substrate specificity of this transporter.
- The fact that mutations in porin or PBP are also found in isolates with elevated MICs to non- $\beta$ -lactams like AZM and CIP is probably simply attributable to the concomitant presence of other mechanisms reducing antibiotic activity in these isolates. This warrants the interest of studying these mechanisms, like mutations in macrolide or fluoroquinolone target, in this collection.

## REFERENCE

Unemo, Shafer. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st Century: Past, evolution, and future, Clin. Microbiol. Rev. 27 (2014) 587–613.  
[doi:10.1128/CMR.00010-14](https://doi.org/10.1128/CMR.00010-14).

This poster will be made available after the meeting at  
<http://www.facm.ucl.ac.be/posters.htm>

Antibiotics	PEN	TMO	CFM	CRO	AZM	TET	SPT	CIP
<b>Mutations</b>								
S EUCAST Bkpt (mg/L)	0.06	-	0.125	0.125	0.25	0.5	64	0.03
% S	16	nd	82	100	80	24	100	32
<b><i>mtrR</i> promoter deletion</b>								
median MIC w/o mutation (27)	0.5 (0.008-16)	6 (0.06-32)	0.225 (0.001-0.5)	0.015 (0.001-0.125)	0.125 (0.015-0.5)	1 (0.125-32)	16 (8-32)	1 (0.002-64)
median MIC with mutation (23)	2 (0.25-32)	16 (4-64)	0.125 (0.004-4)	0.06 (0.001-0.125)	0.25 (0.125-0.5)	2 (0.25-32)	16 (16-32)	16 (0.004-32)
p-value for MIC difference	0.0017	0.0058	ns	0.0005	0.0014	0.01	ns	0.0055
<b><i>porB</i> G120 substitution*</b>								
Median MIC w/o mutation (12)	0.1875 (0.008-4)	3 (0.06-16)	0.004 (0.001-0.06)	0.01 (0.001-0.125)	0.1 (0.015-0.25)	0.25 (0.125-32)	16 (8-32)	0.04 (0.002-8)
median MIC with mutation (36)	2 (0.5-32)	32 (4-64)	0.125 (0.008-4)	0.06 (0.004-0.125)	0.25 (0.03-0.5)	2 (0.5-32)	32 (8-32)	16 (0.004-64)
p-value for MIC difference	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0013	ns	<0.0001
<b><i>porB</i> A121 substitution</b>								
median MIC w/o mutation (18)	0.0375 (0.008-4)	0.25 (0.06-16)	0.002 (0.001-0.06)	0.01 (0.001-0.125)	0.125 (0.015-0.25)	0.125 (0.125-32)	16 (8-32)	0.004 (0.002-1)
median MIC with mutation (32)	2 (0.125-32)	16 (2-64)	0.125 (0.004-4)	0.06 (0.002-0.125)	0.25 (0.03-0.5)	2 (0.5-32)	32 (8-32)	16 (0.004-64)
p-value for MIC difference	0.0101	<0.0001	0.0409	<0.0001	0.0012	<0.0001	0.0335	<0.0001
<b><i>ponA</i> L421P substitution</b>								
median MIC w/o mutation (14)	0.06 (0.008-4)	0.25 (0.06-16)	0.02 (0.001-0.06)	0.001 (0.001-0.125)	0.06 (0.015-0.25)	0.25 (0.125-32)	16 (8-32)	0.04 (0.004-8)
median MIC with mutation (36)	2 (0.25-32)	16 (4-32)	0.125 (0.004-0.5)	0.06 (0.001-0.125)	0.25 (0.03-0.5)	2 (0.25-32)	16 (8-32)	16 (0.004-64)
p-value for MIC difference	<0.0001	<0.0001	<0.0001	<0.0001	0.0113	<0.0001	ns	<0.0001
<b>Mutations in the 3 genes</b>								
median MIC w/o mutation (31)	0.375 (0.008-16)	4 (0.06-64)	0.015 (0.001-4)	0.012 (0.001-0.125)	0.125 (0.015-0.5)	1 (0.125-32)	16 (8-32)	0.5 (0.002-64)
median MIC with mutation (19)	2 (1-32)	32 (4-32)	0.125 (0.03-0.25)	0.06 (0.015-0.125)	0.25 (0.125-0.5)	4 (2-32)	16 (16-32)	16 (0.015-32)
p-value for MIC difference	<0.0001	0.002	0.0288	<0.0001	<0.0001	0.0014	ns	0.0116

Statistical analyses using Wilcoxon Rank-Sum Test \* No data for 2 isolates