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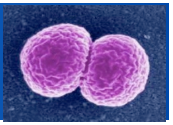
Molecular epidemiology of quinolone resistance for *Neisseria gonorrhoeae* in France



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Introduction

Fluoroquinolones were the reference treatment for gonorrhoea in the world until the 2000s, when resistance to quinolones emerged worldwide (1).

Acquired resistance to quinolones in *Neisseria gonorrhoeae* (NG) is mainly related to mutations in the Quinolone-Resistance Determining Regions (QRDR) of the GyrA subunit of DNA gyrase and the ParC subunit of Topoisomerase IV (2).

In France, resistance rate to quinolones was 43 % in 2012 (3) but the molecular characterisation of the QRDR in NG has not been yet done.

Objectives

The aim of the study was to characterize the molecular patterns of quinolone resistance among multidrug-resistant NG isolates and low-level quinolone-resistant NG from France.

Materials and methods

- In 2011, 85 clinical isolates of NG with a reduced susceptibility to 3rd generation cephalosporins (C3G) (MICs to ceftriaxone or to cefixime ≥ 0.032 mg/l) were isolated in the French National Center and investigated for resistance to quinolones. In addition, 20 clinical isolates with low level of resistance to ciprofloxacin (MIC ranging from > 0.06 to 4 mg/L) were also investigated.
- The QRDR of g*yrA* and *parC* genes was amplified by PCR from the DNA of all isolates. Mutations were detected by using a pyrosequencing-based method (PyroMark ™ Q96 ID;Qiagen).
- The QRDR of *gyrB* and *parE* genes QRDR was characterized by PCR sequencing.
- Detection of Plasmid-Mediated Quinolone Resistance (PMQR):
  - AAC(6')-Ib-cr encoding gene was sought using conventional PCR (4)
  - *qnr genes (qnrA, qnrB, qnrS, qnrC, qnrD)* and *qepA* genes were screened using a real-time PCR method (5) at the « Plate-forme Régionale de Biologie Innovante » in CHU Reims.
- Molecular epidemiology typing was performed by the reference Multi-Antigen Sequence Typing (NG-MAST) method (6). The genes *tpbB* and *porB* were amplified and sequenced. Each strain ST was determined using the NG-MAST database (http://www.ng-mast.net/).

Results and discussion

Mechanism of resistance to quinolones:

- ✓ All the 85 isolates with reduced 3rd generation cephalosporin susceptibility were resistant to ciprofloxacin (MICs ranging from 1.5 to ≥ 32mg/L).
- ✓ Resistance was associated to at least two mutations in the QRDRs of GyrA and ParC. The increase of MIC was correlated to the number of mutations in the QRDRs (Tables 1 and 2)
- ✓ Mutations were observed at the following codons:
  - for GyrA in codons 91 and 95 (S91F; D95N; D95A; D95G)
  - for ParC in codons 86, 87 and 88 (D86N; S87N; S87R; S88P).
- ✓ A predominant genotype (80%) was observed: S91F and D95G in GyrA and S87R in ParC.
- ✓ No mutation in GyrB was found. No PMQR genes were detected.
- ✓ Two strains harboured a P439S mutation in ParE, but showed different MICs of ciprofloxacin (1.5 and 32 mg/L).

Table 1: Correlation of MICs and mutations in QRDRs of GyrA and ParC for 85 NG isolates with reduced susceptibility to C3G

	Amino acids in QRDR						MICs to Ciprofloxacine (mg/L)	Number (%)
	GyrA		ParC					
Wild Type	S91	D95	D86	S87	S88	E91		
Genotype 1	F	G		R			≥ 32	68 (80%)
Genotype 2	F	N		R	P		≥ 32	10 (11.8%)
Genotype 3	F	A	N				24 - 32	3 (3.5%)
Genotype 4	F	A					1.5 - 8	2 (2.3%)
Genotype 5	F	G					32	2 (2.3%)

Table 2: Correlation of MICs and mutations in QRDRs of GyrA and ParC for 20 isolates with a low level of resistance to ciprofloxacin

	Amino acids in QRDR						MICs to Ciprofloxacine (mg/L)	Number (%)
	GyrA		ParC					
Wild Type	S91	D95	D86	S87	S88	E91		
No mutations							0.004-0.016	6 (30%)
Genotype 1		N					0,094	1 (5%)
Genotype 2	F			N			0.19	1 (5%)
Genotype 3	F	A					0.25-1.5	8 (40%)
Genotype 4	F	N					0.38	1 (5%)
Genotype 5	F	G	N				1	1 (5%)
Genotype 6	F	A		N			4	2 (10%)

- **Analysis of NG-MAST**

Genotyping analysis by NG-MAST found 17 different STs. The ST1407, known as a multiresistant clone currently circulating in Europe (7), was predominant (34.7%) followed by the ST3168 (11%) previously described in isolates from India.

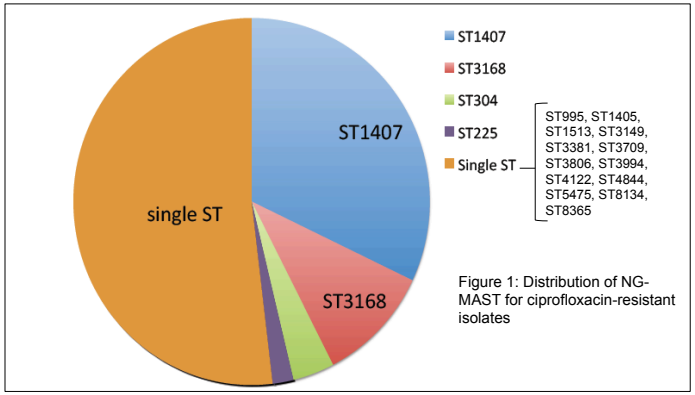

Figure 1: Distribution of NG-MAST for ciprofloxacin-resistant isolates

Conclusions

Our study shows that mutations in *gyrA* and *parC* remain the main quinolone resistance mechanism.

The major genotype (GyrA, Ser91Phe and Asp95Gly – ParC, Ser87Arg) characterized here is also the most common genotype reported in literature.

According to the NG-MAST results, quinolone resistance of NG in France seems not only related to the dissemination of two clones, ST1407 and ST3168, but also to unique isolates selected under treatment.

References

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