AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

# Ceftriaxone-Resistant *Neisseria gonorrhoeae* Isolates (2010 to 2014) in France Characterized by Using Whole-Genome Sequencing

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**Two extended-spectrum cephalosporin-resistant *Neisseria gonorrhoeae* isolates were discovered among 6,340 (0.03%) French isolates between 2010 and 2014. One isolate corresponded to the F89 multidrug-resistant *N. gonorrhoeae* isolate harboring a *penA* mosaic; whole-genome sequencing highlighted an additional R251H substitution in the *ftsX* gene recently involved in cephalosporin resistance. The other, ceftriaxone-resistant isolate (MIC, 0.25 mg/liter) harbored the PBP2 pattern XXXVI plus a P551S substitution and belonged to sequence type ST1579 (multilocus sequence typing [MLST]).**

Gonorrhea is currently a major public health problem, given the worldwide emergence of isolates resistant to extended-spectrum cephalosporins (ESCs) (1). Since 2010, five different high-level ESC-resistant *Neisseria gonorrhoeae* strains have been described: H041, GU140106, and FC428 in Japan (2–4); F89 in France and Spain (5, 6); and A8806 in Australia (2, 7). The isolates A8806, GU140106, and FC428 belong to the same multilocus sequence typing (MLST)-determined type ST7363 as H041, whereas the F89 isolate belongs to the sequence type ST1901 (5). In 2013, 4.7% and 0.4% of European isolates were resistant to cefixime (MIC, >0.125 mg/liter) and ceftriaxone (MIC, >0.125 mg/liter), respectively (8). This study was conducted to monitor the emergence of ESC-resistant *N. gonorrhoeae* isolates in France from 2010 to 2014. Whole-genome sequencing was used to characterize 4 *N. gonorrhoeae* isolates having the highest MICs of ceftriaxone (MIC, ≥0.125 mg/liter).

From January 2010 to December 2014, a total of 6,340 *N. gonorrhoeae* isolates were collected from the Renago network in France and sent to the French National Reference Center for gonococci. MICs were determined by the Etest method (bioMérieux, France). Statistical analysis was performed using the chi-square test with R. During this period, the percentage of *N. gonorrhoeae* isolates resistant to cefixime (MIC, >0.125 mg/liter) or having a MIC of ceftriaxone above the epidemiological cutoff value (Ecoff) (>0.032 mg/liter) was 1.39% (88/6,340) or 3.47% (220/6,340), respectively. From 2011 to 2012, the proportion of cefixime-resistant isolates significantly increased up to 3.28% (36/1,099) ($P < 0.0001$) and then decreased significantly to 1.24% (14/1,131) ($P = 0.0019$) (Table 1). There is a decreasing trend of ESC-resistant *N. gonorrhoeae* isolates in France since 2013 and elsewhere in Europe since 2012 (8). This decline was confirmed in 2014 in contrast with the new increase observed in Europe in 2013 (9). This stabilization could be a result of (i) national recommendations to clinicians in favor of the use of ceftriaxone (500-mg single-dose administration), instead of cefixime at 400 mg (10), and (ii) European recommendations for the application of dual therapy combining ceftriaxone and azithromycin (11).

Only 2 isolates (0.03%) exhibited resistance to ceftriaxone (MIC, >0.125 mg/liter), and 2 isolates had MICs of ceftriaxone at 0.125 mg/liter. To explain the ESC resistance, these four *N. gonorrhoeae* isolates were investigated by whole-genome sequencing. The WHO A and WHO B isolates were sequenced to serve as a control. DNA was extracted using the Wizard genomic DNA purification kit (Promega, Madison, WI), according to the manufacturer's protocol. A DNA library was constructed according to the Rapid Library preparation manual of GS Junior (Roche Diagnostics, Meylan, France). Amplification was performed with the GS Junior Lib-L library, and sequencing was carried out using the GS Junior XL+ kit in the 454-GS Junior instrument. Sequence analysis and assembly were performed by the software GS Junior Sequencer version 3.0, GS Run Browser version 3.0, and GS *De novo* assembler version 3.0. The Genoscope server annotated the genome on the MicroScope platform (8). Single nucleotide polymorphism analysis of *N. gonorrhoeae* isolates was done by comparison to the FA1090 *N. gonorrhoeae* genome (Table 2).

These 4 isolates were clonally related to sequence type ST1901 by multilocus sequence typing or to ST1579 and, by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST), belonged to ST1407, ST225, ST3378, and ST6711. Unsurprisingly, the ESC resistance resulted from the *penA* mosaic alleles (either XXXIV or XXXVI pattern) associated with other determinants of resistance: (i) an adenine deletion in the *mtrR* promoter (deletion A) facilitating an overexpression of the efflux pump MtrCDE; (ii) a *ponA1* variant created by an L421P substitution in the *ponA* gene (PBP1); (iii) a *penB* variant with a G101K/A102D or G101K/A102N change in the PorBIb protein favoring the decrease of antibiotic

**Received** 18 July 2016 **Returned for modification** 17 August 2016
**Accepted** 1 September 2016

**Accepted manuscript posted online** 6 September 2016

**Citation** de Curraize C, Kumanski S, Micaëlo M, Fournet N, La Ruche G, Meunier F, Amarsy R, Jacquier H, Cambau E, Goubard A, Bercot B. 2016. Ceftriaxone-resistant *Neisseria gonorrhoeae* isolates (2010 to 2014) in France characterized by using whole-genome sequencing. Antimicrob Agents Chemother 60:6962–6964. doi:10.1128/AAC.01568-16.

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**TABLE 1** Evolution of *N. gonorrhoeae* isolates with cefixime resistance and ceftriaxone MICs above Ecoff from 2010 to 2014 in France (Renago network)

| Yr | No. of isolates (%) | | | |
|---|---|---|---|---|
| | Total | CFM$^{r}$[a] | CRO$^{sd}$[b] | CRO$^{r}$[b] |
| 2010 | 1,399 | 8 (0.57) | 83 (5.93) | 2 (0.14) |
| 2011 | 1,521 | 10 (0.66) | 43 (2.83) | 0 |
| 2012 | 1,099 | 36 (3.28) | 44 (4.00) | 0 |
| 2013 | 1,190 | 20 (1.68) | 32 (2.69) | 0 |
| 2014 | 1,131 | 14 (1.24) | 18 (1.59) | 0 |
| Total | 6,340 | 88 (1.39) | 220 (3.47) | 2 (0.03) |

[a] Resistance to cefixime (CFM$^{r}$) was defined by a MIC of >0.125 mg/liter in accordance with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.
[b] Decreased-susceptibility ceftriaxone (CRO$^{sd}$) isolates had MICs above the Ecoff of *N. gonorrhoeae* (MIC, >0.032 mg/liter).

entry in the bacterium; or (iv) a PilQ alteration, either pattern VI or VII, including an 180QAATPAKQ insertion in all PilQ types (Table 2).

The *penA* XXXIV pattern was classically described in the successful NG-MAST ST1407 clone spreading worldwide (12–15) and observed in the mosaic *penA* of the F89 strain. In the ESC-resistant *N. gonorrhoeae* F89 isolate, the major contribution of the A501P substitution in the *penA* mosaic to the increase of the MIC of ceftriaxone was demonstrated (5). Interestingly, the whole-genome sequencing highlighted in the F89 isolate an additional mutation in the FtsX protein at position 251 (R251H), which was not described in the publication by Unemo et al. (5). This mutation in a gene encoding a subunit of an ABC transporter has been recently involved in cephalosporin resistance, through *in vitro* mutant experiments after 86 subcultures under ceftriaxone selection pressure (16). We suggest that this additional mutation in the FtsX protein could favor the elevation of the MIC of ceftriaxone especially when it is combined with an A501P substitution. The R251H substitution was also found in *N. gonorrhoeae* M1 and M2 isolates and in a multidrug-resistant *N. gonorrhoeae* isolate, NCCP11945, found in South Korea in 2008 (17). Further studies are necessary to demonstrate the implication of this amino acid substitution in the resistance.

The second *N. gonorrhoeae* isolate resistant to ceftriaxone, M3 (MIC, 0.25 mg/liter), had the *penA* XXXVI allele but included a P551S substitution. This P551S substitution is known to be responsible for a significant increase in MIC of ceftriaxone and for a decrease in acylation by penicillin (17). This PBP2 amino acid alteration associated with those described in PilQ, PonA, and MtrR and its promoter (Table 2) could explain the elevation of the MIC of ceftriaxone.

For isolate M2, a truncated PilQ protein was found; azithromycin resistance remain unexplained as neither 23S rRNA alteration nor overexpression of the efflux pump MtrCDE was found by

**TABLE 2** Phenotypic and molecular characterization of *N. gonorrhoeae* isolates with decreased susceptibility to extended-spectrum cephalosporins[e]

| Characteristic | Data for patient sample: | | | | | |
|---|---|---|---|---|---|---|
| | M1 | M2 | M3 | F89 | WHO A | WHO B |
| Yr of isolation | 2013 | 2010 | 2010 | 2010 | | |
| MIC of drug (mg/liter) | | | | | | |
| Penicillin[a] | 2 | 4 | 0.38 | 1 | 0.006 | 0.125 |
| Cefixime | 0.064 | 0.25 | 0.5 | 4 | <0.016 | <0.016 |
| Ceftriaxone | 0.125 | 0.125 | 0.25 | 2 | <0.016 | <0.016 |
| Tetracycline | 4 | 6 | 3 | 2 | 0.25 | 1 |
| Ciprofloxacin | >32 | >32 | 0.008 | >32 | 0.002 | 0.008 |
| Azithromycin | 0.25 | 1.5 | 0.25 | 1 | 0.016 | 0.064 |
| Spectinomycin | 6 | 8 | 6 | 16 | 96 | 8 |
| Molecular characterization (pattern, mutation, or type) | | | | | | |
| PBP2 (*penA*) | XXXVI + P551L | XXXIV | XXXVI | XXXIV + A501P | WT | XIV |
| *mtrR* promoter | Deletion A | Deletion A | Deletion A | Deletion A | WT | WT |
| MtrR protein | H105Y | H105Y | H105Y | H105Y | A39T | D79N, T86A, H105Y |
| PBP1 (*ponA*) | L421P | L421P | L421P | L421P | WT | WT |
| Porin PorB1b (*penB*) | G101K/A102D | G101K/A102N | G101K/A102D | G101K/A102N | WT | A102S |
| PilQ (*pilQ*) | VII | Truncated[d] | VI | VII | VI | VII |
| FtsX | R251H | R251H | WT | R251H | WT | WT |
| QRDR mutation (GyrA) | S91F/D95G | S91F/D95G | WT | S91F/D95G | WT | WT |
| QRDR mutation (ParC) | S87R | S87R | WT | S87R | WT | WT |
| NG-MAST | ST225 | ST3378 | ST6711 | ST1407 | ST1752 | New[b] |
| MLST | ST1901 | ST1901 | ST1579 | ST1901 | ST10316 | New[c] |

[a] β-Lactamase was not detected by nitrocefin chromogenic test for all isolates.
[b] This sequence type was not previously assigned and was determined by its *porB* allele number 3622 and *tbpB* allele number 24 (99% homologous).
[c] This sequence type was not previously assigned and was determined by its allele numbers for seven genes, *pgm* (223), *pdhC* (153), *gdh* (188), *aroE* (67), *fumC* (111), *adk* (39), and *abcZ* (129).
[d] PilQ is truncated at its 3′ end at the 349th amino acid.
[e] Abbreviations: WT, wild type; QRDR, quinolone resistance-determining region. Wild-type sequence was determined by comparison with the genome of the *N. gonorrhoeae* FA1090 isolate (accession number NC_002946.2).

whole-genome sequencing (18). For all ciprofloxacin-resistant *N. gonorrhoeae* isolates, mutations in the quinolone resistance-determining region (QRDR) of GyrA and ParC explained the resistance.

In France, the proportion of *N. gonorrhoeae* isolates with resistance to ESCs remains low. The F89 strain has not disseminated widely, and the main clone circulating in France belongs to the sequence type ST1901 (NG-MAST 1407). Whole-genome sequencing is a rapid and useful tool to investigate ESC-resistant *N. gonorrhoeae* isolates in new clones. Nevertheless, phenotypic tests remain crucial to surveillance of multidrug-resistant *N. gonorrhoeae* isolates in the absence of commercial molecular tests available for resistance identification.

**Accession number(s).** The sequences were registered on project PRJEB13093 (ERP014629) of the European Bioinformatics Institute (EMBL-EBI).

## ACKNOWLEDGMENTS

The LABGeM (CEA/IG/Genoscope and CNRS UMR8030) and the France Génomique National Infrastructure (funded as part of the Investissement d'avenir program managed by the Agence Nationale pour la Recherche, contract ANR-10-INBS-09) are acknowledged for support within the MicroScope annotation platform. This work was supported by an annual grant awarded by the French Institute for Public Health to the French National Reference Center for Gonococci.

We thank all the biologists who provided isolates to the French National Reference Center for Gonococci and the Renago network.

We have no conflicts of interest to declare.

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