

# Multidrug-resistant *Neisseria gonorrhoeae* failing treatment with ceftriaxone and doxycycline in France, November 2017

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**We report a multidrug-resistant *Neisseria gonorrhoeae* urogenital and pharyngeal infection with ceftriaxone resistance and intermediate resistance to azithromycin in a heterosexual woman in her 20s in France. Treatment with ceftriaxone plus doxycycline failed for the pharyngeal localisation. Whole-genome sequencing of isolate F90 identified MLST<sub>1903</sub>, NG-MAST ST<sub>3435</sub>, NG-STAR<sub>233</sub>, and relevant resistance determinants. F90 showed phenotypic and genotypic similarities to an internationally spreading multidrug-resistant and ceftriaxone-resistant clone detected in Japan and subsequently in Australia, Canada and Denmark.**

The emergence of ceftriaxone resistance in *Neisseria gonorrhoeae* (NG) has caused public health concern worldwide [1-3] and it is a grave concern that the first global failure to treat pharyngeal gonorrhoea with dual antimicrobial therapy was reported in 2016 in the United Kingdom (UK) [4].

We report a multidrug-resistant (MDR) NG pharyngeal infection with ceftriaxone resistance and intermediate resistance to azithromycin that failed dual therapy with ceftriaxone and doxycycline. This treatment targeted an infection due to *Chlamydiae trachomatis*; the choice of the clinician was based on recent finding on the efficacy of doxycycline compared to that of azithromycin in urogenital infections [5].

## Case description

In November 2017, a heterosexual woman in her early 20s attended a local Hospital in Paris due to a vaginal discharge that had persisted for 3 days. She had only had unprotected sexual relations (oral and intercourse) with one regular French male partner who had urethritis symptoms in the 6 months prior and had received antimicrobial treatment at another clinic. Neither she nor her male partner had travelled abroad or had a known history of sexually transmitted infections. She was treated empirically with ceftriaxone (250 mg intramuscularly) and doxycycline (100 mg orally twice a day, for 7 days). During the same visit, vaginal and pharyngeal swabs were sampled for detection of NG and *Chlamydia trachomatis* using nucleic acid amplification test (NAAT), RealTime CT/NG assay on the m2000 System, (Abbott Diagnosis, Abbott Molecular Inc., Des Plaines, IL, USA), and NG culture. NAAT and culture detected NG in both vaginal and pharyngeal samples, but all samples were negative for *C. trachomatis*. HIV, hepatitis B and C viruses, syphilis serological tests were also negative. At test of cure 4 weeks later, she had no symptoms or signs of gonorrhoea and was not given any additional treatment. However, it was found that only the vaginal swab was negative for NG, as the NAAT and culture taken from the pharyngeal swab remained positive, indicating that only the urogenital infection had been cured. Reinfection was excluded because the patient denied any sexual relations between the first visit and the test of cure. The patient and her male partner were

requested to return for follow-up visit, but they are currently lost for follow-up.

## Microbiological investigation

The NG test of cure isolate (referred to as F90) was obtained on PolyVitek chocolate agar incubated at  $+36\pm 1^\circ\text{C}$  in a 5%  $\text{CO}_2$ -enriched atmosphere for 24 hours. F90 was investigated at the Associated laboratory for gonococci of the French National Reference Centre for bacterial STI, Paris, France, where Gram-stained microscopy and matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) (Vitek MS, bioMérieux, Marcy l'Etoile, France) were used for NG species verification. Minimum inhibitory concentrations (MIC) of nine antimicrobials were determined by Etest (bioMérieux, Marcy l'Etoile, France) and interpreted using breakpoints from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [6].

F90 displayed resistance to ceftriaxone (MIC=0.5 mg/L), cefixime (MIC=1 mg/L), tetracycline (MIC=4 mg/L), ciprofloxacin (MIC $>$ 32 mg/L), and rifampicin (MIC $>$ 32 mg/L). Furthermore, F90 showed intermediate resistance to azithromycin (MIC=0.5 mg/L), however, it was susceptible to spectinomycin (MIC=8 mg/L) and had low MIC for ertapenem (MIC=0.004 mg/L) and gentamicin (MIC=8 mg/L), for which EUCAST does not state resistance breakpoints.

## Molecular investigation

Whole-genome sequencing (WGS) was performed in the Associated laboratory for gonococci of the French National Reference Centre for bacterial STI, Paris, France. DNA extraction was conducted using Wizard Genomic DNA Purification kit (Promega), as previously described [7]. Multiplexed DNA libraries were prepared with the Nextera XT construction protocol (Illumina, San Diego, CA, USA). Paired-end, 1,547,414 150-bp indexed reads with an average depth of 151 were obtained on a MiSeq platform (Illumina). De novo assembly was performed using SPAdes 3.11.1 software [8]. QUAST software [9] revealed that the assembly provided 106 contigs with an average length of 20,394 nucleotides, a N50 of 47,830 nucleotides. The contigs covered 93.7% of the genome of NG reference strain FA1090. The genome annotation was performed using the MicroScope platform (<http://www.genoscope.cns.fr/agc/microscope>) [10]. The whole nucleotide sequence of the *penA* gene of F90 is available on GenBank under accession number MH172152.

Using the WGS data, NG antimicrobial resistance determinants were determined *in silico* with the NG FA1090 genome as reference. Sequence types (ST) were also determined *in silico* from the WGS data using the NG multi-antigen sequence typing (NG-MAST) online database (<http://www.ng-mast.net>) [11], the multilocus sequence typing (MLST) from the PubMLST database [12], and the NG Sequence Typing for Antimicrobial Resistance (NG-STAR) [13].

F90 was assigned as MLST<sub>1903</sub>, NG-MAST ST<sub>3435</sub>, and NG-STAR<sub>233</sub>. Regarding ceftriaxone resistance determinants, the isolate harboured a mosaic *penA*-60.001 allele, sharing a 100% homology with the *penA*<sub>FC428</sub> of the Japanese ceftriaxone-resistant FC428 strain [14]. The *penA*<sub>FC428</sub> gene encodes a mosaic penicillin-binding protein 2 (PBP2) including the amino acid alterations A311V, I312M, V316T, T483S, and G545S associated with resistance to extended-spectrum cephalosporins [15]. Furthermore, F90 contained the adenine deletion in the inverted repeat sequence of the *mtrR* promoter, resulting in an overexpression of the MtrCDE efflux pump, and G120K and A121D amino acid substitutions in PorB1b, which further increase ESC MICs and contribute to the MDR phenotype of F90. The quinolone resistance determining regions (QRDRs) carried S91F and D95A substitutions in GyrA (subcomponent of DNA gyrase) and a S87R substitution in ParC (subcomponent of Topoisomerase IV), which explained the high-level resistance to ciprofloxacin. No *tetM* gene was detected but the *rpsJ* gene contained a mutation conferring the V57M amino acid alteration in the S10 ribosomal protein, which contributes to low-level chromosomally-mediated resistance to tetracycline.

## Discussion

In 2010, the first European high-level ceftriaxone-resistant gonococcal isolate was detected in France and characterised in detail [16]. The isolate (F89) harboured a mosaic *penA* XXXIV allele with an additional A501P alteration in PBP2 and belonged to the internationally spread MDR NG-MAST ST1407 clone [17]. Since 2010, F89 has only been reported in Spain [18] and does not appear to have spread further, which is likely due to a decreased biological fitness of F89 [19]. From 2012 to 2014, the proportion of NG isolates with resistance to cefixime decreased nearly three-fold from 3.3% to 1.2% in France [7].

Here, we report a new MDR NG strain (F90) with ceftriaxone resistance and intermediate resistance to azithromycin that lead to treatment failure of pharyngeal gonorrhoea with ceftriaxone and doxycycline. F90 differs from the French F89 isolate detected in 2010 [16], however, it is similar to a ceftriaxone-resistant clone initially described in Japan in 2016 (FC428 strain; similar antibiogram and an identical mosaic *penA*-60.001 allele, MLST<sub>1903</sub>, and NG-STAR<sub>233</sub>) [14]. The FC428 strain appears to have spread internationally and has been subsequently detected in Australia, Canada and Denmark [20]. In early-2018 the first global strain with ceftriaxone resistance combined with high-level azithromycin resistance was identified in the UK [21], followed by two similar cases in Australia [22].

Unfortunately, the patient presented in this article and her partner are currently lost for further follow-up which is of concern as it creates opportunities for spread of this ceftriaxone-resistant strain.

## Conclusion

An MDR gonococcal strain with ceftriaxone resistance and intermediate resistance to azithromycin was found in France in late-2017. The strain might belong to an internationally spread ceftriaxone-resistant clone, which threatens the recommended dual therapy of gonorrhoea and poses a public health threat. Increased awareness of the spread of ceftriaxone-resistant strains, enhanced antimicrobial resistance surveillance, improved implementation of the recommended dual antimicrobial therapy, partner notification and treatment, and TOC are imperative on an international level. Our treatment failure also illustrates the substantial difficulties in treating pharyngeal gonorrhoea and enhanced focus on detection and treatment is, therefore, crucial. Ultimately, novel therapeutic antimicrobials for gonorrhoea are essential.

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## Conflict of interest

None declared.

## References

1. Wi T, Lahra MM, Ndowa F, Bala M, Dillon JR, Ramon-Pardo P, et al. Antimicrobial resistance in *Neisseria gonorrhoeae*: Global surveillance and a call for international collaborative action. *PLoS Med*. 2017;14(7):e1002344. <https://doi.org/10.1371/journal.pmed.1002344> PMID: 28686231
2. World Health Organization (WHO). Global action plan to control the spread and impact of antimicrobial resistance in *Neisseria gonorrhoeae*. Geneva: WHO; 2012. Available from: <http://www.who.int/reproductivehealth/publications/rtis/9789241503501/en/>
3. European Centre for Disease Prevention and Control (ECDC). Response plan to control and manage the threat of multidrug-resistant gonorrhoea in Europe. Stockholm: ECDC; 2012. Available from: <https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/1206-ECDC-MDRgonorrhoea-response-plan.pdf>
4. Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, et al. Failure of dual antimicrobial therapy in treatment of gonorrhoea. *N Engl J Med*. 2016;374(25):2504-6. <https://doi.org/10.1056/NEJMc1512757> PMID: 27332921
5. Geisler WM, Uniyal A, Lee JY, Lensing SY, Johnson S, Perry RC, et al. Azithromycin versus Doxycycline for Urogenital Chlamydia trachomatis Infection. *N Engl J Med*. 2015;373(26):2512-21. <https://doi.org/10.1056/NEJMoa1502599> PMID: 26699167
6. European Committee on Antimicrobial Susceptibility Testing (Eucast). Breakpoint tables for interpretation of MICs and zone diameters Version 7.1, 2017. Europe: Eucast; 2017. Available from: [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint\\_tables/v\\_7.1\\_Breakpoint\\_Tables.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf)
7. de Curraize C, Kumanski S, Micaëlo M, Fournet N, La Ruche G, Meunier F, et al. Ceftriaxone-resistant *Neisseria gonorrhoeae* isolates (2010 to 2014) in France characterized by using whole-genome sequencing. *Antimicrob Agents Chemother*. 2016;60(11):6962-4. <https://doi.org/10.1128/AAC.01568-16> PMID: 27600036
8. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol*. 2012;19(5):455-77. <https://doi.org/10.1089/cmb.2012.0021> PMID: 22506599
9. Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics*. 2013;29(8):1072-5. <https://doi.org/10.1093/bioinformatics/btt086> PMID: 23422339
10. Vallet D, Calteau A, Cruveiller S, Gachet M, Lajus A, Josso A, et al. MicroScope in 2017: an expanding and evolving integrated resource for community expertise of microbial genomes. *Nucleic Acids Res*. 2017;45(D1):D517-28. <https://doi.org/10.1093/nar/gkw1101> PMID: 27899624
11. Unemo M, Dillon J-AR. Review and international recommendation of methods for typing *Neisseria gonorrhoeae* isolates and their implications for improved knowledge of gonococcal epidemiology, treatment, and biology. *Clin Microbiol Rev*. 2011;24(3):447-58. <https://doi.org/10.1128/CMR.00040-10> PMID: 21734242
12. Bennett JS, Jolley KA, Sparling PF, Saunders NJ, Hart CA, Feavers IM, et al. Species status of *Neisseria gonorrhoeae*: evolutionary and epidemiological inferences from multilocus sequence typing. *BMC Biol*. 2007;5(1):35. <https://doi.org/10.1186/1741-7007-5-35> PMID: 17825091
13. Demczuk W, Sidhu S, Unemo M, Whiley DM, Allen VG, Dillon JR, et al. *Neisseria gonorrhoeae* sequence typing for antimicrobial resistance, a novel antimicrobial resistance multilocus typing scheme for tracking global dissemination of *N. gonorrhoeae* strains. *J Clin Microbiol*. 2017;55(5):1454-68. <https://doi.org/10.1128/JCM.00100-17> PMID: 28228492
14. Nakayama S, Shimuta K, Furubayashi K, Kawahata T, Unemo M, Ohnishi M. New ceftriaxone- and multidrug-resistant *Neisseria gonorrhoeae* strain with a novel mosaic penA gene isolated in Japan. *Antimicrob Agents Chemother*. 2016;60(7):4339-41. <https://doi.org/10.1128/AAC.00504-16> PMID: 27067334
15. Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev*. 2014;27(3):587-613. <https://doi.org/10.1128/CMR.00010-14> PMID: 24982323
16. Unemo M, Golparian D, Nicholas R, Ohnishi M, Galloway A, Sednaoui P. High-level cefixime- and ceftriaxone-resistant *Neisseria gonorrhoeae* in France: novel penA mosaic allele in a successful international clone causes treatment failure. *Antimicrob Agents Chemother*. 2012;56(3):1273-80. <https://doi.org/10.1128/AAC.05760-11> PMID: 22155830
17. Harris SR, Cole MJ, Spiteri G, Sánchez-Busó L, Golparian D, Jacobsson S, et al. Euro-GASP study group. Public health surveillance of multidrug-resistant clones of *Neisseria gonorrhoeae* in Europe: a genomic survey. *Lancet Infect Dis*. 2018;S1473-3099(18)30225-1. (Forthcoming). PMID: 29776807
18. Càmarà J, Serra J, Ayats J, Bastida T, Carnicer-Pont D, Andreu A, et al. Molecular characterization of two high-level ceftriaxone-resistant *Neisseria gonorrhoeae* isolates detected in Catalonia, Spain. *J Antimicrob Chemother*. 2012;67(8):1858-60. <https://doi.org/10.1093/jac/dks162> PMID: 22566592
19. Vincent LR, Kerr SR, Tan Y, Tomberg J, Raterman EL, Dunning Hotopp JC, et al. In vivo-selected compensatory mutations restore the fitness cost of mosaic penA alleles that confer ceftriaxone resistance in *Neisseria gonorrhoeae*. *MBio*. 2018;9(2):e01905-17. <https://doi.org/10.1128/mBio.01905-17> PMID: 29615507
20. Lahra MM, Martin I, Demczuk W, Jennison AV, Lee K-I, Nakayama S-I, et al. Cooperative recognition of internationally disseminated ceftriaxone-resistant *Neisseria gonorrhoeae* strain. *Emerg Infect Dis*. 2018;24(4):735-43. <https://doi.org/10.3201/eid2404.171873> PMID: 29553335
21. British Government. Multi-drug resistant gonorrhoea in England: 2018. March 2018. England: British Government; 2018. Available from: <https://www.gov.uk/government/publications/multi-drug-resistant-gonorrhoea-in-england-2018>
22. Australian Government Department of Health. Multi-drug resistant gonorrhoea. April 2018. Australia: Department of Health; 2018. Available from: <http://www.health.gov.au/internet/main/publishing.nsf/Content/mr-yr18-dept-dept004.htm>

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