High-level azithromycin-resistant *Neisseria gonorrhoeae* clinical isolate in France, March 2014

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We report the first case in France of a high-level azithromycin-resistant *Neisseria gonorrhoeae* (minimum inhibitory concentration (MIC)=96 mg/L) assigned to MLST7363 (NG-MAST ST6360), also resistant to ciprofloxacin and tetracycline but susceptible to ceftriaxone. The patient was a 51 year-old heterosexual man who returned following 1g azithromycin monotherapy. Mechanisms of azithromycin resistance were a C2599T mutation in the four copies of the *rrl* gene and a novel mutation in the promoter of the *mtrR* gene.

The surveillance of *Neisseria gonorrhoeae* (NG) susceptibility to antibiotics in France is based on a voluntary sentinel network of laboratories [1]. In March 2014, the first NG strain showing a high level of azithromycin resistance was isolated from the urine of a 51 year-old man living in the south of France. He had a history of a *Chlamydia trachomatis* infection 20 years ago. The patient was heterosexual and declared having sex with only two regular female partners (last sexual contact four and ten days before symptoms).

Case description

At the first visit, the patient presented with mild symptoms of urethritis. He was in good general state of health and his HIV test was negative. No microbiological analyses were performed and the patient was treated empirically with a single dose of azithromycin (1 g orally) for suspected *C. trachomatis* infection. The patient presented again with persisting symptoms 48 hours later. At that time, he was treated empirically with a single dose of spectinomycin (2 g intramuscularly) and bacteriological examination was performed with direct microscopic examination and culture. Culture results indicated the presence of an azithromycin-resistant NG isolate. The absence of *C. trachomatis* infection was determined retrospectively by negative nucleic acid amplification test. At a third visit, one week later the patient was successfully cured and free of clinical symptoms.

In France, the first-line treatment of uncomplicated urogenital gonorrhoea is ceftriaxone (500 mg intramuscularly), whereas spectinomycin is used in cases of contraindication to beta-lactams. For our patient, the clinician chose spectinomycin because of his own prescribing practices. Infection with the azithromycin-resistant-strain was presumably acquired in France since neither the patient nor his sexual contacts had travelled in the past six months or had other known sexual contacts. In addition, they had not received any antibiotic treatment in the preceding six months.

Microbiological investigation

The isolate was sent to the French National Reference Centre for gonococci (Paris, France), which confirmed the identification of NG by conventional biochemical criteria and by mass spectrometer laser Maldi-Tof (MicroFlex platform, Bruker Daltonik, Bremen, Germany). The antimicrobial susceptibility profile of the isolate was determined via gradient minimum inhibitory concentrations (MIC) (Etest bioMérieux, Marcy-l'Etoile, France). The isolate was resistant to azithromycin at a high level (MIC = 96 mg/L), resistant to ciprofloxacin (MIC > 32 mg/L) and to tetracycline (MIC = 2 mg/L) and had intermediate susceptibility to penicillin (MIC = 0.25 mg/L) without production of beta-lactamase enzyme. It was susceptible to cefixime (MIC = 0.016 mg/L), to ceftriaxone (MIC = 0.032 mg/L), to imipenem (MIC = 0.19 mg/L), to ertapenem (MIC = 0.023 mg/L), to gentamicin (MIC = 8 mg/L) and to spectinomycin (MIC = 8 mg/L) according to interpretive criteria from the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [2].

Molecular investigation

Investigation of the mechanism of azithromycin resistance was further performed at the Associated Laboratory for the French National Reference Centre for gonococci (Paris, France). To fully determine the role of mutations in the peptidyltransferase loop of domain V

of the *rrl* gene encoding the 23S rRNA, all four alleles were amplified individually by PCR as described previously [3]. All four copies of the *rrl* gene harboured the substitution C2599T in the NG numbering system, which corresponds to C2611T in *Escherichia coli* numbering. This mutation can explain in part, but not fully, the high level of resistance to azithromycin.

The isolate was also screened by PCR for acquisition of rRNA methylase genes (*ermA*, *ermB*, *ermC* and *ermF*) and other genes involved in macrolide resistance (*ereA*, *ereB*, *mefA/E*, *mphA*) using methods previously described [4]. None of these genes were detected. No mutations were detected in the *rplD* and *rplV* genes encoding the ribosomal proteins L4 and L22, known to be involved in azithromycin resistance [4].

Interestingly, when screening the *mtrR* promoter region for mutations associated with upregulation of the MtrCDE efflux pump [4], we found one A to C mutation (underlined) resulting in a novel promoter of the *mtrR* gene: 5'TTGCACGGATACAAAGTCTTTTATAAT3' (nucleotides in bold indicate the -35 and -10 boxes). This mutation could contribute to the high level of resistance to azithromycin.

Molecular epidemiology typing was performed using the reference method of multi-antigen sequence typing (NG-MAST) [5]. Comparing the sequences determined for the *tpbB* and *porB* genes to those of the NG-MAST database, the sequence type ST6360 was assigned to the NG isolate via the on line NG-MAST data base (<http://www.ng-mast.net>). Moreover, using the multilocus sequence typing method previously described [6], the isolate was identified as sequence type ST7363.

Discussion

Sporadic infections with highly azithromycin-resistant *N. gonorrhoeae* isolates have been reported since 2001 in Argentina [7], in Scotland (2004) [8], in Wales (2007) [9], in Italy (2007) [10], in the United States (2011) [11] and in Sweden in 2013 [12]. Here we described the first isolate identified and presumably acquired in France. In previous publications [7,9,11,12], high-level azithromycin resistance was shown to result from the accumulation of mutations in the four *rrl* alleles at positions 2143 (G instead of A) or 2599 (T instead of C) in the domain V of the 23S rRNA involved in the binding site of azithromycin (NG numbering). In this report, our isolate harboured the C2599T mutation in the four *rrl* alleles. However, our isolate was more resistant to azithromycin than those previously described with these mutations (MIC ranging from 2 to 16 mg/L) [9], arguing that some additional mechanisms of resistance to azithromycin were active in this isolate.

In European countries, the decrease in sensitivity to third generation cephalosporin and to azithromycin has been linked to the spread of clone ST1407 [13]. The ST1407 is the most frequently observed sequence type in Europe. In contrast, it was comparatively uncommon

in France where ST2992 and ST2 were the most frequent sequence types observed in 2010 [13]. Our NG isolate was assigned to MLST7363 (NG-MAST ST6360), never described in France but one of the four most prevalent sequence types found in Japan and associated with decreased susceptibility to cefixime [14]. However it has not been reported in connection with azithromycin resistance. Here, the NG strain was isolated after treatment with 1 g azithromycin monotherapy. We do not know if the patient had acquired a high-level azithromycin-resistant isolate from one of his partners or if an azithromycin-resistant mutant with a higher level of resistance was selected under treatment. However, this latter hypothesis is unlikely since the delay of 48 h between the antibiotic treatment and taking of the bacteriological sample seems too short for the acquisition of the observed mutations.

Azithromycin is not recommended as monotherapy for gonorrhoea [15,16], but it is recommended as co-treatment in combination with ceftriaxone at a dose of either 1 g or 2 g [15]. In addition, azithromycin is commonly administered empirically in monotherapy to treat urethritis that is presumed to be due to *C. trachomatis* or *Mycoplasma genitalium*. This practice can pose a risk of selecting azithromycin-resistant NG isolates [17,18].

Conclusion

In conclusion, the discovery of the first high-level azithromycin-resistant NG isolate in France argues for antimicrobial resistance surveillance to be continued and reinforces the need for cultivating bacteria to better survey multidrug resistant NG isolates. This finding highlights the importance of enhancing and strengthening measures to ensure adequate treatment for the patient and prevent further spread of azithromycin-resistant NG isolate.

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Conflict of interest

None declared.

Authors' contributions

BB, EC AG, GL and PS designed and initiated a surveillance of azithromycin resistance for all NG isolates collected in the sentinel network of laboratories. AB and FM performed and analysed all the laboratory work. AB, GL, AG and PS collected clinical information. BB and EC wrote the first draft of the paper and all co-authors were involved in finalising the paper.

References

1. La Ruche G, Goubard A, Bercot B, Cambau E, Semaille C, Sednaoui P. Gonococcal infections and emergence of gonococcal decreased susceptibility to cephalosporins in France, 2001 to 2012. *Euro Surveill*. 2014;19(34):20885. <http://dx.doi.org/10.2807/1560-7917.ES2014.19.34.20885> PMID:25188611
2. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 4.0. EUCAST; 2014. Available from: <http://www.eucast.org/clinical-breakpoints/>
3. Ng LK, Martin I, Liu G, Bryden L. Mutation in 23S rRNA associated with macrolide resistance in *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother*. 2002;46(9):3020-5. <http://dx.doi.org/10.1128/AAC.46.9.3020-3025.2002> PMID:12183262
4. Allen VG, Farrell DJ, Rebbapragada A, Tan J, Tijet N, Perusini SJ, et al. Molecular analysis of antimicrobial resistance mechanisms in *Neisseria gonorrhoeae* isolates from Ontario, Canada. *Antimicrob Agents Chemother*. 2011;55(2):703-12. <http://dx.doi.org/10.1128/AAC.00788-10> PMID:21098249
5. Unemo M, Dillon JA. 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. <http://dx.doi.org/10.1128/CMR.00040-10> PMID:21734242
6. 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. <http://dx.doi.org/10.1186/1741-7007-5-35> PMID:17825091
7. Galarza PG, Alcalá B, Salcedo C, Canigia LF, Buscemi L, Pagano I, et al. Emergence of high level azithromycin-resistant *Neisseria gonorrhoeae* strain isolated in Argentina. *Sex Transm Dis*. 2009;36(12):787-8. <http://dx.doi.org/10.1097/OLQ.0b013e3181b61bb1> PMID:19734823
8. Palmer HM, Young H, Winter A, Dave J. Emergence and spread of azithromycin-resistant *Neisseria gonorrhoeae* in Scotland. *J Antimicrob Chemother*. 2008;62(3):490-4. <http://dx.doi.org/10.1093/jac/dkn235> PMID:18552343
9. Chisholm SA, Dave J, Ison CA. High-level azithromycin resistance occurs in *Neisseria gonorrhoeae* as a result of a single point mutation in the 23S rRNA genes. *Antimicrob Agents Chemother*. 2010;54(9):3812-6. <http://dx.doi.org/10.1128/AAC.00309-10> PMID:20585125
10. Starnino S, Stefanelli P; *Neisseria gonorrhoeae* Italian Study Group. Azithromycin-resistant *Neisseria gonorrhoeae* strains recently isolated in Italy. *J Antimicrob Chemother*. 2009;63(6):1200-4. <http://dx.doi.org/10.1093/jac/dkp118> PMID:19357159
11. Katz AR, Komeya AY, Soge OO, Kiaha MI, Lee MV, Wasserman GM, et al. *Neisseria gonorrhoeae* with high-level resistance to azithromycin: case report of the first isolate identified in the United States. *Clin Infect Dis*. 2012;54(6):841-3. <http://dx.doi.org/10.1093/cid/cir929> PMID:22184617
12. Unemo M, Golparian D, Hellmark B. First three *Neisseria gonorrhoeae* isolates with high-level resistance to azithromycin in Sweden: a threat to currently available dual-antimicrobial regimens for treatment of gonorrhea? *Antimicrob Agents Chemother*. 2014;58(1):624-5. <http://dx.doi.org/10.1128/AAC.02093-13> PMID:24189248
13. Chisholm SA, Unemo M, Quaye N, Johansson E, Cole MJ, Ison CA, et al. Molecular epidemiological typing within the European Gonococcal Antimicrobial Resistance Surveillance Programme reveals predominance of a multidrug-resistant clone. *Euro Surveill*. 2013;18(3):20358. PMID:23351652
14. Shimuta K, Unemo M, Nakayama S, Morita-Ishihara T, Dorin M, Kawahata T, et al.; Antibiotic-Resistant Gonorrhea Study Group. Antimicrobial resistance and molecular typing of *Neisseria gonorrhoeae* isolates in Kyoto and Osaka, Japan, 2010 to 2012: intensified surveillance after identification of the first strain (Ho41) with high-level ceftriaxone resistance. *Antimicrob Agents Chemother*. 2013;57(11):5225-32. <http://dx.doi.org/10.1128/AAC.01295-13> PMID:23939890
15. Bignell C, Unemo M. 2012 European guideline on the diagnosis and treatment of gonorrhoea in adults. European STI Guidelines Editorial Board. Copenhagen: Statens Serum Institute; 2012. 24 p. http://www.iusti.org/regions/Europe/pdf/2012/Gonorrhoea_2012.pdf
16. Morita-Ishihara T, Unemo M, Furubayashi K, Kawahata T, Shimuta K, Nakayama S, et al. Treatment failure with 2 g of azithromycin (extended-release formulation) in gonorrhoea in Japan caused by the international multidrug-resistant ST1407 strain of *Neisseria gonorrhoeae*. *J Antimicrob Chemother*. 2014;69(8):2086-90. <http://dx.doi.org/10.1093/jac/dku118> PMID:24777907
17. Lanjouw E, Ossewaarde JM, Stary A, Boag F, van der Meijden WJ. 2010 European guideline for the management of Chlamydia trachomatis infections. *Int J STD AIDS*. 2010;21(11):729-37. <http://dx.doi.org/10.1258/ijsa.2010.010302>. Available from: http://www.iusti.org/regions/europe/pdf/2010/Euro_Guideline_Chlamydia_2010.pdf PMID:21187352
18. Daley G, Russell D, Tabrizi S, McBride J. Mycoplasma genitalium: a review. *Int J STD AIDS*. 2014;25(7):475-87. <http://dx.doi.org/10.1177/0956462413515196> PMID:24517928