

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 *rml* gene encoding the 23S rRNA, all four alleles were amplified individually by PCR as described previously [3]. All four copies of the *rml* 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'TTGCACGGATACAAAGTCTTTTTATAAT3' (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 *rml* 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 *rml* 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.

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