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 (1g 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 (2g 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...
mutations were detected in the described [4]. None of these genes were detected. No
(ereA, ereB, mefA/E, mphA
ermF
) and other genes involved in macrolide resistance
using methods previously
to the NG isolate via the on line NG-MAST data base
database, the sequence type ST6360 was assigned
the isolate was identified as sequence type ST7363.
cus sequence typing method previously described [6],
the reference method of multi-antigen sequence typing
ance to azithromycin.
mutation could contribute to the high level of resist-
MtrCDE efflux pump [4], we found one A to C mutation
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'TTGCACGGATACAAAGTCTTTTTTATAAT3' (nucleo-
tides in bold indicate the -35 and -10 boxes). This mutation could contribute to the high level of resist-
ance 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 multilo-
cus 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 bind-
ing site of azithromycin (NG numbering). In this report,
our isolate harbour the C2599T mutation in the four
rrl alleles. However, our isolate was more resistant
to azithromycin that those previously described with
these mutations (MIC ranging from 2 to 16 mg/L) [9],
arguing that some additional mechanisms of resist-
ance 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 treat-
ment with 1 g azithromycin monotherapy. We do not
know if the patient had acquired a high-level azithro-
mycin-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 acquisi-
tion of the observed mutations.
Azithromycin is not recommended as monotherapy for
gonorrohea [15,16], but it is recommended as co-treat-
ment 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 ure-
thritis 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 bet-
ter survey multidrug resistant NG isolates. This finding
highlights the importance of enhancing and strength-
ening 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 col-
lected clinical information. BB and EC wrote the first draft of
the paper and all co-authors were involved in finalising the
paper.