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

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Objectives: Antimicrobial resistance in Neisseria gonorrhoeae is a major public health concern globally. We report the first verified treatment failure of gonorrhoea with 2 g of azithromycin (extended-release formulation) in Japan and characteristics of the corresponding N. gonorrhoeae isolates.

Methods: Pre- and post-treatment isolates (n = 4) were investigated by Etest for antimicrobial susceptibility. The isolates were examined for molecular epidemiology by multilocus sequence typing (MLST), N. gonorrhoeae multi-antigen sequence typing (NG-MAST) and multiple-locus variable-number tandem repeat analysis (MLVA), and for the presence of azithromycin resistance determinants (23S rRNA gene mutations, erm genes and mtrR mutations).

Results: All isolates were resistant to azithromycin (MIC 4 mg/L) and ciprofloxacin, but remained susceptible to cefixime, ceftriaxone and spectinomycin. All isolates were assigned to MLST ST1901 and NG-MAST ST1407 and three of four isolates possessed MLVA profile 8-3-21-16-1. All isolates contained the previously described C2599T mutation (N. gonorrhoeae numbering) in all four 23S rRNA alleles and the previously described single-nucleotide (A) deletion in the mtrR promoter region.

Conclusions: This verified treatment failure occurred in a patient infected with an MLST ST1901/NG-MAST ST1407 strain of N. gonorrhoeae. While this international strain commonly shows resistance or decreased susceptibility to multiple antimicrobials, including extended-spectrum cephalosporins, the strain reported here remained fully susceptible to the latter antimicrobials. Hence, two subtypes of azithromycin-resistant gonococcal MLST ST1901/NG-MAST ST1407 appear to have evolved and to be circulating in Japan. Azithromycin should not be recommended as a single antimicrobial for first-line empirical treatment of gonorrhoea.

Keywords: N. gonorrhoeae, N. gonorrhoeae multi-antigen sequence typing, NG-MAST, antimicrobial resistance, 23S rRNA, test of cure

Introduction

Neisseria gonorrhoeae infections are major public health concerns worldwide. In 2008, the WHO estimated there were 106 million gonorrhoea cases among adults globally, making it the most prevalent bacterial sexually transmitted infection.1 In Japan, based on a sentinel surveillance system (~1000 sentinel sites) for gonorrhoea, the number of reported cases peaked (21 921 cases) in 2002, but declined to 10 247 cases in 2011. Resistance in N. gonorrhoeae to previously recommended first-line antimicrobials for treatment of gonorrhoea is prevalent worldwide.2–4 Although attempts have been made to establish surveillance of antimicrobial resistance in N. gonorrhoeae in Japan, this has proved difficult due to the low number of isolates obtained for study.3 Dual antimicrobial therapies have been introduced in the USA6 and Europe,7 recommending ceftriaxone (one dose of 250–500 mg intramuscularly) together with azithromycin (one dose of 1–2 g orally) for treatment of uncomplicated gonorrhoea. Furthermore, in the USA one dose of 2 g of azithromycin is recommended if the patient has severe cephalosporin allergy8 and, despite not being recommended, in several countries, including Japan, azithromycin (one dose of 1–2 g) as single antimicrobial
therapy is occasionally used, due to its wide availability and ease of administration.

Here we report the first verified treatment failure of gonorrhea with 2 g of azithromycin [extended-release formulation (azithromycin-ER)] in Japan and the phenotypic and genetic characteristics of the corresponding N. gonorrhoeae isolates.

Methods

The work was performed at the Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan.

Case report

In early 2013, an asymptomatic woman in her late teens presented to a sexually transmitted infection clinic in Osaka city. She sought care because 3 days earlier her male sex partner had been diagnosed with N. gonorrhoeae urethritis at another private clinic (no further information regarding this case was available). During the visit she was treated with 2 g of azithromycin-ER orally, which is formulated as sustained-release microspheres. Pharyngeal and vaginal culture specimens were also taken, which were confirmed as N. gonorrhoeae positive in 2 days (referred to as FC-195 and FC-196, respectively). On the follow-up visit (Day 9), vaginal and pharyngeal culture specimens were taken for test of cure and the patient also received 1 g of ceftriaxone intravenously due to azithromycin resistance (see the Results section) in the isolates cultured on Day 1. The vaginal and pharyngeal test of cure specimens were both confirmed 2 days later as still culture-positive for N. gonorrhoeae (isolates designated FC-200 and FC-201, respectively). The patient denied any sexual contacts from the time of receiving azithromycin treatment to test of cure. On Day 22, the patient returned for a test of cure following the ceftriaxone treatment and at this visit vaginal and pharyngeal culture specimens were negative for N. gonorrhoeae.

Characterization of isolates

The four clinical isolates were characterized as described below. For comparison, seven additional azithromycin-resistant gonococcal isolates of N. gonorrhoeae multi-antigen sequence type (NG-MAST) ST1407 collected in the same geographical region in 2011 and 2012 were analysed.

Antimicrobial susceptibility testing

The MIC (mg/L) of azithromycin was determined using the Etest method (bioMérieux, AB, Solna, Sweden) according to the manufacturer’s instructions. MIC values were interpreted in accordance with EUCAST clinical breakpoint criteria (V4.0; www.eucast.org/clinical_breakpoints/; see Table 1).

Genetic characterization

For molecular epidemiology, all isolates were characterized by multilocus sequence typing (MLST), NG-MAST and multiple-locus variable-number tandem repeat analysis (MLVA), as previously described. The methylase-encoding ermA, ermB, ermC and ermF resistance genes were detected with PCR, as previously described. The mtrR gene, which is located in the promoter region, and the penA gene were sequenced as previously described.

Results

The phenotypic and genetic characterization of all four N. gonorrhoeae pre- and post-treatment isolates (AZM-TF isolates) is summarized in Table 1, which also includes seven additional azithromycin-resistant NG-MAST ST1407 isolates from the same geographical region (Kyoto/Osaka) for comparison.

Antimicrobial susceptibility testing

All AZM-TF isolates were resistant to azithromycin (MIC ≥4 mg/L) and ciprofloxacin (MIC ≥32 mg/L), but were susceptible to cefixime (MIC 0.032–0.064 mg/L), ceftriaxone (MIC 0.016–0.032 mg/L) and spectinomycin (MIC 4–8 mg/L). The additional azithromycin-resistant isolates had similar antibigrams; however, the MICs of cefixime (0.125–0.25 mg/L) and ceftriaxone (0.032–0.125 mg/L) for these isolates were 2- to 8-fold higher (Table 1).

Genetic characterization

All AZM-TF isolates were assigned to MLST ST1901 and NG-MAST ST1407. Using MLVA, all isolates, with the exception of FC-196, possessed an identical number of repeat units (8-3-21-16-1) in loci VNTR04-03, VNTR04-10, VNTR07-02, VNTR15-02 and VNTR16-01, respectively. The pre-treatment pharyngeal isolate FC-196 had a closely related MLVA profile, 10-3-21-16-1, i.e. a single-locus variant with slight differences in the VNTR04-03 locus. The additional azithromycin-resistant NG-MAST ST1407 isolates were all assigned as MLST ST1901, with one exception (IT-027: ST10241). These isolates had four different MLVA profiles, which differed from those of the AZM-TF isolates with at least two loci (Table 1).

All AZM-TF isolates contained the previously described C2599T mutation in all four alleles of the 23S rRNA gene (Table 1), but did not have any A2143G mutation. Among the additional seven azithromycin-resistant isolates, HI-015 also possessed the C2599T mutation in all four alleles, but the others had the C2599T mutation in two or fewer alleles. All isolates analysed here, except FC-107, also contained the previously described single-nucleotide (A) deletion in the mtrR promoter region; however, no isolates contained the ermA, ermB, ermC or ermF genes.

With regard to the main cephalosporin resistance determinant, the AZM-TF isolates and HI-015 possessed the penA XXXIV allele and the remaining six isolates had a penA XXXIV variant with an additional P551S or A501V mutation (Table 1). These latter alleles are known to result in higher MICs of cephalosporins, in accordance with the results of this study.

Discussion

This is the first reported case of failure of gonorrhea treatment with azithromycin in Japan, which was strictly verified in accordance with WHO criteria, i.e. a detailed clinical history was recorded, reinfection was ruled out, the pre-treatment and post-treatment isolates were mainly phenotypically and genetically indistinguishable by highly discriminatory molecular epidemiological typing methods, and the isolates were resistant to azithromycin and contained genetic resistance determinants causing the azithromycin resistance. The clinical failure occurred after using a 2 g dose of azithromycin-ER (an extended-release formulation).
Table 1. Antimicrobial susceptibility and molecular characteristics of *N. gonorrhoeae* isolates with low-level resistance to azithromycin in Japan

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<th>Isolate</th>
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<th>PEN</th>
<th>CFM</th>
<th>CIP</th>
<th>MLST</th>
<th>NG-MAST</th>
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<th>04-10</th>
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AZM, azithromycin; CRO, ceftriaxone; SPT, spectinomycin; PEN, penicillin G; CFM, cefixime; CIP, ciprofloxacin; CDS, coding sequence; WT, wild-type.

FC-195/FC-196 and FC-200/FC-201 are pre- and post-treatment isolates, respectively, from the first verified treatment failure with 2 g of azithromycin in Japan.

*Susceptibility (S) and resistance (R) according to EUCAST breakpoints (www.eucast.org): azithromycin, S ≤ 0.25 mg/L and R > 0.5 mg/L; ceftriaxone, S ≤ 0.125 mg/L and R > 0.125 mg/L; spectinomycin, S ≤ 64 mg/L and R > 64 mg/L; penicillin, S ≤ 0.064 mg/L and R > 1 mg/L; cefixime, S ≤ 0.125 mg/L and R > 0.125 mg/L; and ciprofloxacin, S ≤ 0.032 mg/L and R > 0.064 mg/L.*

A C2599T mutation in 23S rRNA alleles results in decreased target affinity and increased MICs of azithromycin.\(^{10}\)

Two different sequences of the promoter region of the *mtrR* gene were identified, i.e. the previously described deletion of one nucleotide (A)\(^{8,12}\) and a new sequence, `GGTACAAAGTCTTTTTTATAATCCGCCCCTCAT` (accession number AB914770), in which underlined nucleotides differ from those in the wild-type sequence.
formulation using microspheres that results in an extended duration of effect and less severe adverse effects), which is the highest dose of azithromycin used for treatment of gonorrhoea. All four AZM-TF isolates belonged to MLST ST1901 and NG-MAST ST1407, and all isolates, with the exception of one, were also assigned to an identical MLVA profile. The single-locus MLVA variant of the remaining isolate most likely only represents the intra-strain variability and instability of the VNTR04-03 locus. The C2599T mutation was identified in all four alleles of the 23S rRNA gene, which has been associated with low-level resistance to azithromycin, in all AZM-TF isolates and the HI-015 isolate (azithromycin MIC 4–8 mg/L). The additional examined azithromycin-resistant isolates with this mutation in two or fewer alleles had an azithromycin MIC of only 1 mg/L. All these results are in accordance with previous publications.

As mentioned earlier, the treatment failure was caused by N. gonorrhoeae MLST ST1901 and NG-MAST ST1407, which is, together with its evolved genetic subtypes, a multidrug-resistant gonococcal clone accounting for a high proportion of the decreased susceptibility and resistance to extended-spectrum cephalosporins in many settings, including the Kyoto/Osaka area, worldwide. Interestingly, the azithromycin-resistant gonococcal MLST ST1901 and NG-MAST ST1407 strain causing the treatment failure in Osaka was fully susceptible to extended-spectrum cephalosporins (e.g. cefixime MICs were 0.032–0.064 mg/L) and instead had substantially higher MICs of azithromycin compared with conventional ST1901/ST1407 isolates. In the ongoing gonococcal surveillance in the Kyoto/Osaka area, among 413 isolates collected from April 2010 to March 2013, 12 additional isolates showing azithromycin resistance have been identified. Of these 12 isolates, 7 were assigned to NG-MAST ST1407, but all 7 had decreased susceptibility to cefixime (MICs 0.125–0.25 mg/L) (Table 1). Furthermore, three azithromycin-resistant NG-MAST ST1407 isolates were found in the Tokyo area in 2011. Two of these three isolates had an MIC of 16 mg/L and possessed the C2599T mutation in all four 23S rRNA alleles. Both of these two isolates also showed decreased susceptibility to cefixime (MIC 0.125 mg/L), in accordance with conventional gonococcal NG-MAST ST1407 isolates. Accordingly, two subtypes of azithromycin-resistant gonococcal NG-MAST ST1407 appear to have evolved and to be circulating in Japan.

Azithromycin-ER has been approved for gonorrhoea treatment since 2009 in Japan. This drug has high activity against many Gram-positive and Gram-negative bacteria, high tissue penetration (making it effective for intracellular pathogens), a single-dose oral regimen, less severe adverse effects compared with conventional azithromycin (immediate-release formulation (azithromycin-IR)) and ease of administration, increasing compliance. According to pharmacokinetic/pharmacodynamic investigations, a 2 g dose of azithromycin-ER results in 3- to 4-fold higher AUC in serum than a 500 mg dose of azithromycin-IR. As the efficacy of azithromycin is best correlated with the parameter AUC/MIC, azithromycin-ER (in a single dose of 2 g) might be a more effective option than azithromycin-IR (in a single dose of 2 g) for treatment of gonorrhoea. However, appropriate comparisons between a 2 g dose of each formulation, taking into account selection of resistance, are crucial. Gonococcal strains with high-level resistance to azithromycin, due to an A2143G mutation in three or four of the 23S rRNA alleles, have been isolated in several countries worldwide, though not yet in Japan. Nevertheless, data from the gonococcal antimicrobial resistance surveillance in the Kyoto/Osaka area showed that 3.2% of the gonococcal isolates from April 2010 to March 2013 were resistant to azithromycin. Previously, levels of azithromycin-resistant N. gonorrhoeae isolates between 0.4% and 6.6% have been reported in Japan. However, additional data, including clinical trial data, are needed for the determination of an evidence-based resistance breakpoint for azithromycin-ER, as illustrated in the present study, which shows that N. gonorrhoeae strains with low-level azithromycin resistance can cause gonorrhoea treatment failures. Given the occurrence of azithromycin resistance in many countries globally already and the rapid selection of azithromycin resistance when it is widely used (including at doses of 2 g using an extended-release formulation), azithromycin should not be recommended as monotherapy for first-line empirical treatment of gonorrhoea.

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Transparency declarations
None to declare.

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