

First *Neisseria gonorrhoeae* strain with resistance to cefixime causing gonorrhoea treatment failure in Austria, 2011

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We describe the first cefixime-resistant *Neisseria gonorrhoeae* strain in Austria that caused treatment failure. It follows the first five cases in Europe of cefixime treatment failure, reported in Norway in 2010 and the United Kingdom in 2011. Effective treatment of gonorrhoea is crucial for public health control and, at present, requires substantially enhanced awareness, more frequent test-of-cure, interaction with experts after therapeutic failure, tracing and therapy of contacts, and surveillance of gonococcal antimicrobial resistance and treatment failures worldwide.

We report here the first *Neisseria gonorrhoeae* strain with resistance to cefixime in Austria, which caused a treatment failure with cefixime.

Gonorrhoea is the second most prevalent bacterial sexually transmitted infection (STI) worldwide, and the aetiological agent, *N. gonorrhoeae*, has developed resistance to all antimicrobials used as first-line treatments. In most countries, the currently recommended first-line drugs are the extended-spectrum cephalosporins (ESCs) ceftriaxone (injectable) and cefixime (oral). However, the susceptibility of *N. gonorrhoeae* to both is decreasing worldwide [1,2]. Cefixime standard treatment (400 mg single oral dose) has been preferred in many countries due to its effectiveness, the ease of an oral, single-dose regimen, and because, before 2010, verified treatment failures had only been reported in Japan [3]. However, two cases of clinical failures with cefixime standard treatment were described in 2010 in Norway [4], which were strictly verified using the World Health Organization (WHO) criteria [1], and three cases in 2011 in the United Kingdom [5,6]. Furthermore, the first gonococcal strain with high-level clinical resistance to ceftriaxone (the last remaining option for empirical first-line treatment), i.e. the first extensively drug-resistant gonococcus [1], was recently found in Japan [7]. It is now possible that gonorrhoea will become untreatable in certain circumstances and especially some settings [1,7].

Case report

In early July 2011, an Austrian man-who-has-sex-with-men (MSM) had unprotected sex with one anonymous MSM in a gay sauna in Munich, Germany. Some days later, the Austrian man presented to a urologist in the region of Innsbruck, Austria (day 1), with symptoms of urethritis (urethral discharge and dysuria) that had been present for two days. The patient was administered cefixime at a 400 mg oral dose once a day for seven days. On day 4, a urine sample taken on day 1 was shown to contain *N. gonorrhoeae*- as well as *Chlamydia trachomatis*-specific DNA using the Abbott m200ort RealTime CT/NG PCR (Abbott Molecular Diagnostics). On day 8, he presented to a general practitioner with persisting symptoms, and the same treatment was prescribed for an additional 14 days. On day 22, the patient returned with persisting symptoms to the urologist he had visited initially, and microscopy of a urethral smear displayed urethritis and intracellular Gram-negative diplococci within polymorphonuclear leukocytes. Furthermore, *N. gonorrhoeae* was cultured from an additional urethral sample taken on that day, and *N. gonorrhoeae*- as well as *C. trachomatis*-specific DNA was found in a urine sample using the Abbott PCR. The patient was on the same day given one oral dose of 2 g azithromycin. On day 43, follow-up examination showed that the symptoms and signs had resolved, and a PCR test (urine sample; Abbott PCR) was negative for *N. gonorrhoeae* as well as for *C. trachomatis*. The patient repeatedly denied (on each visit) any sexual activities after recognition of symptoms and, in particular, between first treatment and test-of-cure.

Characterisation of the cefixime-resistant *Neisseria gonorrhoeae* strain

Unfortunately, no pre-treatment *N. gonorrhoeae* isolate was available. The post-treatment strain (cultured on day 22) was however species-confirmed using culture on selective agar medium, rapid oxidase production, presence of Gram-negative diplococci, and two species-verifying assays, an in house sugar utilisation

test and Phadebact GC Monoclonal Test (Bactus AB, Sweden). The results of the characterisation of the strain are summarised in the Table.

The strain was assigned to serovar Bpyut, multilocus sequence typing (MLST) ST1901 and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) ST1407, performed as previously described [7,8]. The strain had minimum inhibitory concentrations (MICs) of five antimicrobial drugs as follows: 1.0 mg/L of cefixime (average of 0.8 mg/L in four Etest determinations), 0.5 mg/L of ceftriaxone (average of 0.3 mg/L in four Etest determinations), 0.25 mg/L of azithromycin, 8 mg/L of spectinomycin, and >32 mg/L of ciprofloxacin (see Table). Accordingly, the strain was resistant to cefixime (>0.12 mg/L), ceftriaxone (>0.12 mg/L), and ciprofloxacin (>32 mg/L), based on the breakpoints stated by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [9]. The strain did not produce any beta-lactamase.

Sequencing of resistance determinants for penicillins and ESCs (*penA*, *mtrR*, *porB1b*, *ponA* and *pilQ* alterations) were performed as previously described [7,8,10]. The strain contained a *penA* mosaic XXXIV allele [7] with a single additional amino acid alteration (T534A) in the gene for penicillin binding protein 2, and additionally the *mtrR* and *penB* resistance determinants, which all together caused the high MICs of the ESCs [7,10,11] (Table). Transformation experiments, performed as previously described [7,12], confirmed that the only new resistance determinant, i.e. the novel *penA* mosaic allele (*penA* mosaic XXXIV allele with an additional T534A alteration), was responsible for the resistance to ESCs.

TABLE

Characteristics of the first *Neisseria gonorrhoeae* strain with resistance to cefixime and causing treatment failure, Austria, 2011

MIC (mg/L)		MLST	NG-MAST	<i>penA</i> allele	<i>mtrR</i>	<i>penB</i>	<i>ponA</i>
IX	TX						
1.0	0.5	ST1901	ST1407	Mosaic (novel) ^a	A-del in promoter ^b	G120K A121N ^c	L421P ^d

IX: cefixime; MIC: minimum inhibitory concentration (Etest was used and only whole MIC dilutions are presented); MLST: multilocus sequence typing; NG-MAST: *Neisseria gonorrhoeae* multiantigen sequence typing; PCR: polymerase chain reaction; ST: sequence type; TX: ceftriaxone.

- ^a Mosaic allele encodes a mosaic penicillin binding protein 2 (PBP2), which causes decreased susceptibility to extended-spectrum cephalosporins.
- ^b Characteristic single nucleotide (A) deletion in the inverted repeat of the promoter region of *mtrR* that causes overexpression of the MtrCDE efflux pump, which results in a further decreased susceptibility to extended-spectrum cephalosporins.
- ^c Alterations of amino acids 120 and 121 in the porin PorB1b that cause a decreased intake of extended-spectrum cephalosporins and, accordingly, a further decreased susceptibility to extended-spectrum cephalosporins.
- ^d Alteration of amino acid 421 in the penicillin-binding protein 1 (PBP1), which results in decreased susceptibility to penicillins.

Discussion

We report here the first *N. gonorrhoeae* strain with resistance to cefixime in Austria, which caused a treatment failure with the internationally recommended first-line treatment cefixime. This treatment failure as well as two of the three previously reported ones in the United Kingdom [5,6] occurred in MSM, and an enhanced focus on prevention and control of gonorrhoea in MSM may be necessary. The present treatment failure was identified in an Austrian patient, however, the infection was contracted in Germany, which suggests that this strain may be present in the MSM community in Germany.

Because no pre-treatment isolate was available, the same situation as for two of the three reported cases of cefixime treatment failures in the United Kingdom [5,6], it was not possible to verify the treatment failure in full accordance to the WHO criteria [1]. However, a detailed clinical history was recorded, reinfection was ruled out as much as is possible (denied on each visit by the patient), the post-treatment isolate was in vitro highly resistant to cefixime, and the strain contained genetic resistance determinants explaining the high cefixime MIC. Furthermore, the high cefixime MIC of the strain makes it most likely that this was a treatment failure. According to Monte Carlo simulations, a 400 mg dose of cefixime results in a median time of free cefixime above MIC ($fT_{>MIC}$) of only 6.8 h (3.8-9.6 h) for the detected MIC of 1.0 mg/L [13]. Furthermore, one day (ca. 24 h) after administration of 400 mg cefixime the concentration of free cefixime is very low (ca. 0.03 mg/L) [13]. Consequently, especially due to the short half life of cefixime (3.4 hours) administration of one 400 mg dose per day for several days does not substantially extend the cefixime $fT_{>MIC}$ unless the MIC of the strain is relatively low [13]. This regimen accordingly does not provide any major benefits, compared with the recommended cefixime single-dose regimen, for the treatment of gonorrhoea, is evidently not able to clear an infection with a gonococcal strain that has an MIC of cefixime of 1.0 mg/L; rather, it may select for higher ESC resistance. This emphasises the importance of adhering to appropriate treatment guidelines (i.e. using a 400 mg cefixime single-dose regimen, and, if failure is confirmed or suspected, another antimicrobial drug), and of involving STI experts when the commonly used recommended treatment fails in a patient. It is now evident that cefixime treatment failures have occurred in several European countries. In many cases they may not be recognised because azithromycin is additionally administered to many of the gonorrhoea patients (due to suspicion of chlamydial infection) [1,13], follow-up examination and test-of-cure are rarely performed [1], or treatment failures are not appropriately verified and reported. The recently updated treatment guidelines in the United States [14] and the United Kingdom [15] recommend cefixime 400 mg only as an alternative treatment (if ceftriaxone is not an option), and this change may need to be considered also in the European [16] and other treatment

guidelines. In all countries, it is crucial to maintain as much capacity as possible to culture and perform antimicrobial resistance (AMR) testing of gonococci, for AMR surveillance purposes but also for adequate verification of treatment failures and, if needed, for informing the antimicrobial treatment of individual patients.

The present treatment failure was caused by a gonococcal strain of ST1407, which is multidrug-resistant and is spreading in many countries worldwide [17,18]. ST1407 or closely related subtypes also caused the recent treatment failures in Norway [4] and the United Kingdom [5].

In conclusion, clinical failures of gonorrhoea treatment with the internationally recommended first-line treatment cefixime have occurred in three European countries. Improved prevention (e.g. condom use) and control of gonorrhoea, enhanced awareness of cefixime treatment failures, more frequent follow-up examination including test-of-cure and appropriate collection of demographic and behavioural data (e.g. sexual orientation), and surveillance of gonococcal AMR and treatment failures (appropriately verified and subsequently reported) are crucial worldwide to mitigate the spread and minimise the impact of ESC-resistant gonococcal strains and, accordingly, to ensure that gonorrhoea remains a treatable infection. Furthermore, adherence to appropriate treatment guidelines and timely evidence-based revision of these guidelines, involvement of STI experts when the commonly used recommended treatment fails in a patient, as well as tracing and therapy also of sexual contacts of the index case are imperative.

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