

## Research articles

# MULTIDRUG-RESISTANT *NEISSERIA GONORRHOEAE* WITH REDUCED CEFOTAXIME SUSCEPTIBILITY IS INCREASINGLY COMMON IN MEN WHO HAVE SEX WITH MEN, AMSTERDAM, THE NETHERLANDS

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Antimicrobial resistance is an increasing problem in *Neisseria gonorrhoeae* (NG) treatment. Presently, third-generation parenteral cephalosporins, like ceftriaxone and cefotaxime, are the first option. Resistance to oral, but not to parenteral, third-generation cephalosporins has been reported previously. We analysed the microbial susceptibility (as minimum inhibitory concentration - MIC) of NG cultures obtained from high-risk visitors of the largest Dutch outpatient clinic for sexually transmitted infections (STI) in Amsterdam, the Netherlands. Among 1,596 visitors, we identified 102 patients with at least one NG isolate with reduced susceptibility to cefotaxime ( $0.125 \mu\text{g/ml} < \text{MIC} \leq 0.5 \mu\text{g/ml}$ ). The percentage of NG isolates with reduced susceptibility to cefotaxime rose from 4.8% in 2006 to 12.1% in 2008 ( $\chi^2 17.5$ ,  $p < 0.001$ ). With multivariate logistic regression, being a man who has sex with men (MSM) was significantly associated with reduced susceptibility to cefotaxime ( $p < 0.001$ ). Compared to susceptible NG isolates, those with decreased susceptibility to cefotaxime were more often resistant also to penicillin (16.5% vs. 43.3%), tetracycline (21.5% vs. 68.9%) and ciprofloxacin (44.4% vs. 90.0%, all  $p < 0.001$ ). The increased prevalence of NG strains with reduced susceptibility to cefotaxime among MSM may herald resistance to third-generation parenteral cephalosporins. A considerable proportion of these strains show resistance to multiple antibiotics which could limit future NG treatment options.

### Introduction

Gonorrhoea is a highly contagious sexually transmitted infection caused by *Neisseria gonorrhoeae* (NG). In the majority of cases NG urogenital infections in males cause symptoms like discharge or urethritis whereas anal and pharyngeal NG infections and urogenital NG infections in females are asymptomatic in a large proportion of cases. Uncomplicated urogenital NG infections can lead to salpingitis in females and epididymitis in males, conditions that are associated with infertility. In some cases localised NG

infections can lead to haematogenic dissemination causing severe complications like sepsis, meningitis and endocarditis [1].

Previously we reported a rise in the proportion of fluoroquinolone-resistant *N. gonorrhoeae* (FRNG) isolates among NG isolates obtained from men who have sex with men (MSM) visiting the Amsterdam clinic for sexually transmitted infections (STI) from 0.2% in 2000 to 10.5% in 2003 and among those obtained from men who have sex with women from 0.7% to 3.2%, respectively [2]. A year later, in 2004, a prevalence of FRNG up to 15% was found among heterosexual visitors of STI clinics [3], and in 2008 the proportion of FRNG has risen to 45% among STI clinic visitors throughout the Netherlands [4].

Similar increases in circulating FRNG isolates among STI visitors were documented earlier in the United Kingdom around 2000 [5], and during the 1990s throughout Asia [6]. As soon as the prevalence of antibiotic-resistant strains of a circulating pathogen in a patient population exceeds 5%, both the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) recommend to stop using this antibiotic for treatment of patients infected with this pathogen [1,6]. Therefore since 2004 fluoroquinolones have no longer been recommended as first line treatment for NG in the Netherlands and in many other countries. In the national clinical guidelines issued by the Dutch Dermatological and Venereological Society (Nederlandse Vereniging voor Dermatologie en Venereologie, NVDV) and the Dutch General Practitioners Society (Nederlands Huisartsen Genootschap, NHG), both parenteral third-generation cephalosporins - ceftriaxone and cefotaxime - are the first line treatment option for patients infected with NG [7]. Before fluoroquinolones were abandoned as the first treatment option, penicillin and tetracycline had already been discontinued as preferred treatment option for NG infections due

to unacceptable high prevalence of circulating NG strains resistant to these antibiotics [6].

In collaboration with the Municipal Health Service Public Health Laboratory, we have been closely monitoring the resistance to antibiotics of NG isolates found in the visitors to the STI clinic in Amsterdam. In addition to penicillin (both chromosomal and plasmid mediated resistance), tetracycline and ciprofloxacin, since 2004 we have also monitored the resistance to cefotaxime.

In this article we report an alarming increase in the proportion of multidrug-resistant NG strains with reduced susceptibility to cefotaxime among isolates obtained from visitors frequenting the Amsterdam STI outpatient clinic in 2008 compared to 2006-2007. These NG strains with reduced susceptibility to cefotaxime are found for the larger part among MSM with high-risk behaviour for other STI's. Evolution of true resistance to third-generation cephalosporins would seriously hamper effective control of NG infections.

## Methods

### Time frame and study population

The Amsterdam STI outpatient clinic is the largest setting of its kind in the Netherlands, with nearly 28,000 new consultations in 2008 [8]. Upon arrival at the clinic, visitors are prioritised based on a short questionnaire to estimate the risk for having an STI. The prioritising system is described in more detail elsewhere by Heijman et al. [9]. In short, all visitors that are either referred by a healthcare professional, have a sex partner with an STI, had STI-related complaints, or are MSM, are considered high-risk patients and get a full STI check-up including, for the largest part, the collection of swabs for NG cultivation from the pharynx, urethra, cervix and/or rectum depending on the sex technique practiced in the previous six months. Those with negative answers to the questionnaire, are considered low-risk visitors. From these no NG isolates are available since only a nucleic acid amplification test is used to perform NG diagnostics in this group.

All demographic and clinical characteristics used in this study were recorded in an electronic patient database as described earlier [10]. Patients diagnosed with an NG infection (urogenital, anal or pharyngeal) were treated with 500 mg ceftriaxone i.m. according to the national guidelines of the Dutch Dermatological and Venereological Society (NVDV) [7]. In case symptoms persisted one week after treatment, visitors were requested to return to the clinic and additional swabs for NG cultivation were obtained to see if the treatment had been successful ("test for cure"). Moreover, all visitors were screened for *C. trachomatis* infections (including lymphogranuloma venereum in MSM), syphilis, hepatitis B and upon consent HIV, as described elsewhere [10]. All data and samples for this study were collected as part of the routine clinical procedure; therefore no Ethical Committee approval was needed. Care was provided in accordance with the Helsinki Declaration of 1975, as revised in 1983 [11].

All NG isolates with available MIC information collected between October 2006 and December 2008 from high-risk patients were included in the analysis. We examined whether there was a trend in the proportion of NG isolates with reduced susceptibility per quarter. From patients with more than one isolate with a MIC value, the isolate with the highest MIC value was used in the analysis, for all antibiotics tested. In a sub-analysis we examined whether susceptibility to cefotaxim was associated with the anatomical

site from which the isolate was originating. Statistical analysis was performed using SPSS version 15.0 (SPSS, Inc., Chicago, IL, US) and Stata version 9.2 (Stata Corporation, College Station, TX, US). We examined the association between reduced susceptibility to cefotaxime and age, sexual orientation, nationality, previous and current STI diagnoses, including HIV status and result of *Treponema pallidum* haemagglutination (TPHA) test. Factors associated with reduced susceptibility in univariate analysis at  $p < 0.20$ , were included in a logistic regression model. Factors that were not significantly associated with the outcome were one by one omitted from that model (level of significance set at  $p = 0.05$ ).

### *N. gonorrhoeae* susceptibility testing

All swabs for NG cultivation were swiped on selective feeder plates (GC-LECT; Becton Dickinson, Franklin Lakes, NJ, United States) as described previously [2]. In short, the plates were incubated immediately at 37 °C in CO<sub>2</sub> enriched atmosphere before and after transportation in "candle jars" to the laboratory. After 40-48 hrs the plates were inspected for colony formation. Determination of NG isolates was based on Gram-staining, oxidase-, sugar fermentation-, and aminopeptidase reactions and hybridisation with a DNA probe (Accuprobe, Biomerieux). In cultured NG isolates, the minimum inhibitory concentration (MIC) of penicillin, tetracycline, ciprofloxacin, and cefotaxime was measured using E-tests (AB Biodisk, Solna, Sweden). Moreover, plasmid-mediated penicillin resistance was tested with the help of a beta-lactamase test.

For MIC validation and quality control, the public health laboratory of the Municipal Health Service Amsterdam participated in the European Surveillance of STI (ESSTI) NG isolate panel exchange collaboration programme in 2008. This panel included WHO strains K and L, which both display a reduced susceptibility to third-generation cephalosporins due to a pen A mosaic allele (K) or an A501 mutation in the penA gene (L) [12]. These strains had an MIC for cefotaxime of 0.5 and 0.25 µg/ml, respectively.

Before 2006, ceftriaxone was not available in the Netherlands in acceptable dosages for treating patients with NG infections. Therefore, cefotaxime was the nationally recommended first treatment option and, consequently, the Dutch health authorities (Rijksinstituut voor Volksgezondheid en Milieu, RIVM) provided cefotaxime tests to NG reference laboratories throughout the country for the monitoring of parenteral third-generation cephalosporin susceptibility. From 2006 onwards, the 500 mg ceftriaxone i.m. dosage became available and was then recommended as the first treatment option for NG infections, but the government continued to provide the cefotaxime susceptibility test. Therefore during the study period we treated NG infections with ceftriaxone while testing susceptibility for cefotaxime. Since structural homologies between both molecules are high, we consider cefotaxime susceptibility an appropriate marker for susceptibility to all third-generation cephalosporins. This was confirmed by our finding that genetically well-described WHO reference strains with diminished susceptibility to cefixime and ceftriaxone had also increased MICs for cefotaxime, whereas all other ESSTI control strains were fully susceptible. According to the guidelines and recommendations of the Clinical and Laboratory Standards Institute (CLSI) the following MIC cut-off values were used to define antibiotic susceptibility [13]:

- For cefotaxime: susceptible  $\leq 0.125$  µg/ml; 0.125 µg/ml < reduced susceptibility  $\leq 0.5$  µg/ml; resistant  $> 0.5$  µg/ml.

- For penicillin: susceptible  $\leq 0.06$  µg/ml;  $0.06$  µg/ml < reduced susceptibility <  $2.0$  µg/ml; resistant  $\geq 2.0$  µg/ml.
- For tetracycline: susceptible  $\leq 0.25$ ;  $0.25$  µg/ml < reduced susceptibility <  $2.0$  µg/ml; resistant  $\geq 2.0$  µg/ml.
- For ciprofloxacin: susceptible  $\leq 0.06$  µg/ml;  $0.06$  µg/ml < reduced susceptibility <  $1.0$  µg/ml; resistant  $\geq 1.0$  µg/ml.

## Results

From October 2006 until December 2008, gonorrhoea was diagnosed in 1,821 high-risk patients (out of the total number of 35,411 high-risk patients who visited the clinic in this period, which gives 5.1% gonorrhoea prevalence in this group) and in 115 low-risk patients (out of 25,304 low-risk patients in total, which gives 0.5% gonorrhoea prevalence in this group). In 225 of the high-risk patients NG isolates were not available, in most cases because the gonorrhoea diagnosis was based on a nucleic acid amplification test or because the gonorrhoea culture did not grow. From the remaining 1,596 patients a total of 1,883 NG isolates obtained from various locations were available for which MIC testing was performed. We compared the demographic and clinical characteristics of the high-risk patients with MIC information versus those without MIC information. There was no significant difference between the two groups regarding concurrent syphilis (i.e. stage 1, 2 or early latent syphilis) or lymphogranuloma venereum infection at the time of consultation, past syphilis infection (i.e. TPHA seropositivity), HIV seropositivity and age distribution. However,

the proportion of MSM was significantly higher in the group without MIC information ( $p < 0.001$ ,  $\chi^2$  test).

Among the 1,596 patients with MIC data, we identified 102 with at least one NG isolate with reduced susceptibility to cefotaxime ( $0.125$  µg/ml < MIC <  $0.5$  µg/ml, Table 1). No NG isolates resistant to cefotaxime were identified and isolates obtained from the remaining 1,494 patients were all susceptible to cefotaxime (MIC  $\leq 0.125$  µg/ml). Between October 2006 and December 2008 an important and significant rise in both absolute and relative terms was observed in the number of patients with NG isolates with reduced cefotaxime susceptibility: from 8 (4.8%) in the fourth quarter of 2006 to 23 (12.1%) in the fourth quarter of 2008 ( $\chi^2$  for trend = 17.5,  $p < 0.0001$ , Figure).

## Demographic and clinical characteristics of patients with reduced cefotaxime susceptibility

The following patient characteristics were significantly associated with having an NG isolate with reduced susceptibility to cefotaxime (Table 1): age >35 years ( $p = 0.004$ ), MSM ( $p < 0.001$ ), a concurrent lymphogranuloma venereum infection at the time of consultation ( $p = 0.04$ ), positive HIV serology, either as a new diagnosis or known HIV seropositivity ( $p = 0.023$ ), positive TPHA serology ( $p = 0.01$ , for all comparisons a  $\chi^2$  test was used). In a multivariate logistic regression model, only being MSM was significantly associated with reduced susceptibility to cefotaxime (OR=2.9, 95% CI 1.4-5.8,  $p < 0.001$ , adjusted for age).

TABLE 1

Demographic and clinical characteristics of 1,596 patients with at least one *Neisseria gonorrhoeae* isolate; STI outpatient clinic, Amsterdam, the Netherlands, 2006-2008

Patient characteristics	cefotaxime MIC ≤ 0.125 µg/mL (n= 1,494)	cefotaxime MIC > 0.125 µg/mL, (n=102)	OR (95%CI)	Overall p value <sup>1</sup>
Age				
≤35 years	847 (56.7%)	43 (42.2%)	1 (ref)	0.004
>35 years	647 (43.3%)	59 (57.8%)	1.8 (1.2-2.7)	
Sexual preference				
Men who have sex with women (exclusively)	317 (21.2%)	9 (8.8%)	1 (ref)	<0.001
Women who have sex with men	192 (12.9%)	3 (2.9%)	0.6 (0.1-2.1)	
Men who have sex with men (and/or women)	985 (65.9%)	90 (88.2%)	3.2 (1.6-6.5)	
HIV serology				
Positive, new diagnosis	39 (2.6%)	7 (6.9%)	3.1 (1.3-7.3)	0.023
Known positive	337 (22.6%)	30 (29.4%)	1.5 (1.0-2.4)	
Negative	952 (63.7%)	55 (53.9%)	1 (ref)	
Not tested	166 (11.1%)	10 (9.8%)	1.0 (0.5-2.1)	
Concurrent infectious syphilis <sup>2</sup>				
No infectious syphilis	1,441 (96.5%)	101 (99%)	1 (ref)	0.165
Infectious syphilis	53 (3.5%)	1 (1.0%)	0.3 (0.04-1.96)	
Syphilis serology <sup>3</sup>				
TPHA-negative	1,145 (76.9%)	67 (65.7%)	1 (ref)	0.011
TPHA-positive	345 (23.2%)	35 (34.3%)	1.7 (1.1-2.7)	
Concurrent lymphogranuloma venereum				
No lymphogranuloma venereum	1,466 (98.1%)	97 (95.1%)	1 (ref)	0.04
Lymphogranuloma venereum	28 (1.9%)	5 (4.9%)	2.7 (1.02-7.1)	

Data are number of patients (% of total).

<sup>1</sup>p values were calculated with  $\chi^2$  test.

<sup>2</sup>Infectious syphilis infections are stage 1, 2 or early latent stages diagnosed at the date of visit.

<sup>3</sup>Data on TPHA (Treponema pallidum haemagglutination) test missing for n=4.

### NG isolates from MSM

In total 1,231 isolates with MIC information were obtained from 1,075 MSM patients (Table 2). Of these, 1,134 isolates from 985 patients showed good susceptibility to cefotaxime ( $\text{MIC} \leq 0.125 \mu\text{g/ml}$ ) and 97 isolates from 90 patients showed decreased cefotaxime susceptibility ( $\text{MIC} > 0.125 \mu\text{g/ml}$ ). A considerable number of these isolates originated from rectal location (respectively 534 [47.1%] and 48 [49.5%]). Site of infection was not significantly associated with reduced susceptibility ( $p=0.61$ ).

We analysed the susceptibility to antibiotics other than cefotaxime of all 1,231 NG isolates from 1,075 MSM patients (Table 3). Isolates with decreased susceptibility to cefotaxime showed significantly more often resistance to penicillin (43.3% vs. 16.5%), tetracycline (68.9% vs. 21.5%) and ciprofloxacin (90.0% vs. 44.4%, for all antibiotics separately  $p < 0.001$ ) compared to NG strains susceptible to cefotaxime.

Moreover, multiple resistance to the additionally tested antibiotics (penicillin, tetracycline and ciprofloxacin) was significantly more frequent among isolates with decreased susceptibility to cefotaxime compared to those susceptible (77.5% and 22.9% for at least two of the three additionally tested antibiotics, respectively, and 30.3% and 4.7% for all three additionally tested antibiotics, both comparisons  $p < 0.001$ ).

### Discussion

We report on an alarming significant increase of NG isolates with reduced susceptibility for cefotaxime, a parenteral third-generation cephalosporin, among STI clinic visitors in Amsterdam, the Netherlands from 2006 to 2008. Following the resistance

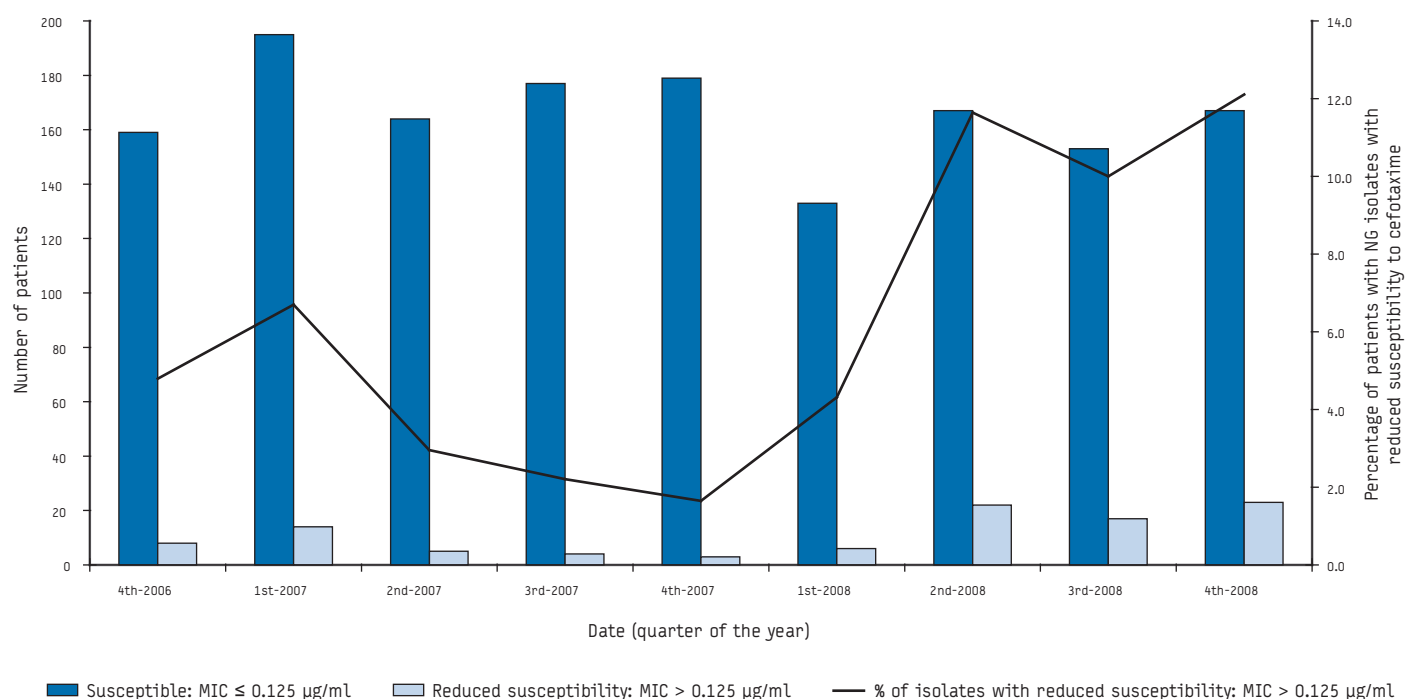
to sulfanilamide in the 1940s, penicillin and tetracycline in the 1980s and, lastly, fluoroquinolones in the early 1990s, third-generation cephalosporins have nowadays become the first option of treatment in most countries [1,6].

*N. gonorrhoeae* strains with reduced susceptibility to oral third generation cephalosporins have been described in Japan [14,15], Sweden [16], Australia [17] and Greece [18]. Mosaic patterns of the *penA* gene, encoding penicillin binding protein 2, partly originating from the pharyngeal commensal species *N. cinerea* and *N. perflava* have been reported in such strains [14,16]. In addition, alterations in *mtrR*, resulting in increased expression of efflux pumps, *porB1b*, resulting in altered permeability of the porin *por1B*, and *ponA*, leading to decreased affinity of penicillin binding protein 1 to beta-lactam antibiotics have been reported [16]. In contrast to susceptible strains, these strains cannot always be eradicated using two 200 mg doses of oral cefixime [19]. For this reason, the European Committee on Antimicrobial Susceptibility Testing (EUCAST), recommends to use only a susceptible/resistant breakpoint for all cephalosporins at  $> 0.12$  [20]. According to EUCAST guidelines, all 102 strains with an  $\text{MIC} > 0.125 \mu\text{g/ml}$  found in our study would have been considered resistant against third-generation cephalosporins.

It is feasible that a decrease in cefotaxime susceptibility among circulating NG strains will necessitate the use of larger doses of third-generation cephalosporins for effective elimination of gonorrhoea in infected individuals, a phenomenon already experienced in the treatment of gonorrhoea patients with penicillin in the past. For uncomplicated gonorrhoea infections, the CDC recommends 125 mg ceftriaxone i.m. [21] and the International

### FIGURE

Numbers and percentages of patients with *Neisseria gonorrhoeae* isolates susceptible and with reduced susceptibility to cefotaxime, by quarter of the year, 2006-2008, STI outpatient clinic, Amsterdam, the Netherlands



Union against sexually transmitted infections (IUSTI)/WHO guidelines a dose of 250 mg ceftriaxone i.m. [22]. Possibly because in the Netherlands we already use higher than recommended doses of 500 mg ceftriaxone i.m., we have not experienced treatment failure in patients treated with ceftriaxone yet (although we do not systematically test all patients treated for gonorrhoea in our clinic to see if the treatment has been successful – “test for cure”). Finally, it is likely that decreasing susceptibility to third-generation cephalosporins will lead to the loss of this class of antimicrobials for the treatment of gonorrhoea [23].

In the present paper, we show that the number of *N. gonorrhoeae* strains with reduced susceptibility to cefotaxime is sharply increasing. The increase is mainly found among MSM patients and is associated with high-risk behaviour as indicated by increased prevalence of other STIs. This differs from the recently published outbreak in Greece, in which 17 patients were infected with NG strains of reduced susceptibility to cephalosporins after casual male-to-female sexual contacts [18].

#### ***N. gonorrhoeae* with reduced cefotaxime susceptibility found mainly among MSM**

A sharp increase in the percentage of NG isolates with decreased susceptibility to cefotaxime has been observed since the last quarter of 2007 and the rising trend was significant for the whole study period. During the last three quarters of 2008 the prevalence of NG

isolates with reduced susceptibility to cefotaxime was continuously above 10% which indicates sustained circulation of these strains.

Patients bearing NG strains with reduced cefotaxime susceptibility (MIC >0.125 µg/ml) were significantly more often 35 years old or older, MSM, HIV-positive, and had concurrent STI, compared to those with cefotaxime-susceptible NG strains. Similar characteristics (MSM, >=35 years, multiple concurrent and previously documented STI, especially HIV) were also identified in patients with emerging STIs like lymphogranuloma venereum and sexually acquired hepatitis C [24,25].

The present finding of NG strains with reduced susceptibility to cefotaxime and multiple resistances to other antibiotics circulating in this MSM core group once again underlines the importance of tailored and intensified STI care for high-risk MSM patients focused on multiple concurrent chronic and incident STI infections. This is important for the individual patient but also for the population at large because emerging STIs circulating within a core group can easily spread to the population at large as experienced with ciprofloxacin-resistant NG strains in the Netherlands.

The incidence of NG isolates with reduced susceptibility to cefotaxime was highly associated with MSM patients, also in multivariate analysis. For this reason we focused the second part of our analysis on the characteristics of NG strains collected from MSM patients only. We did not find an association between antibiotic susceptibility to cefotaxime and the various collection sites. Almost half of the NG strains originated from rectal swabs, both among NG strains susceptible to cefotaxime (47.1%) and among those with reduced cefotaxime susceptibility (49.5%) (Table 2). Rectal gonorrhoea infections are an increasing problem among MSM as reported earlier, and should always be considered since many of these infections are asymptomatic [26].

#### **Multidrug-resistance common in NG strains with reduced susceptibility for cefotaxime**

Among the NG isolates with reduced susceptibility to cefotaxime obtained from MSM patients, a considerable number was found resistant to multiple antibiotics such as penicillin, tetracycline and ciprofloxacin. This implies that if the trend of reduced susceptibility to cefotaxime progresses towards resistance to all third-generation

**TABLE 2**

**Collection site of 1,231 isolates obtained from 1,075 men who have sex with men; STI outpatient clinic, Amsterdam, the Netherlands 2006-2008**

Site	cefotaxime MIC ≤ 0.125 µg/ml (n=1,134)	cefotaxime MIC > 0.125 µg/ml (n=97)	Overall p value <sup>1</sup>
Urethra	469 (41.4%)	41 (42.3%)	0.61
Rectum	534 (47.1%)	48 (49.5%)	
Pharynx	131 (11.6%)	8 (8.2%)	

Data are the number of isolates (% of total) including 1,134 isolates from 985 patients with a cefotaxime MIC value ≤ 0.125 µg/ml and 97 isolates from 90 patients with a cefotaxime MIC value > 0.125 µg/ml.  
1p value based on chi<sup>2</sup> test.

**TABLE 3**

**Other antibiotic resistance characteristics of 1,231 isolates obtained from 1,075 men who have sex with men; STI outpatient clinic, Amsterdam, the Netherlands, 2006-2008**

Antibiotic resistance	cefotaxime MIC ≤ 0.125 µg/ml, (n= 985)	cefotaxime MIC > 0.125 µg/ml, (n=90)	Overall p value <sup>1</sup>
Penicillin resistance <sup>2</sup>	162 (16.5%)	39 (43.3%)	<0.001
Tetracycline resistance <sup>3</sup>	211 (21.5%)	62 (68.9%)	<0.001
Ciprofloxacin resistance <sup>4</sup>	437 (44.4%)	81 (90.0%)	<0.001
Resistance to at least two of the following: penicillin, tetracycline and ciprofloxacin <sup>5</sup>	224 (22.9%)	69 (77.5%)	<0.001
Resistance to all three antibiotics: penicillin, tetracycline and ciprofloxacin <sup>5</sup>	46 (4.7%)	27 (30.3%)	<0.001

Data are the number of isolates (% of total).

1p values are based on chi<sup>2</sup> test.

2For penicillin either chromosomal or plasmid-mediated resistance, missing data on penicillin resistance (excluded from analysis) n=4.

3Missing data on tetracycline resistance (excluded) n=2.

4Missing data on ciprofloxacin resistance (excluded) n=2.

5Missing data (excluded) n=6.



cephalosporins, a switch to previously recommended antibiotics for gonorrhoea will not be an option. It has been suggested that spectinomycin, which is structurally unrelated to third-generation cephalosporins, should be used as the primary therapy for gonorrhoea but this drug is not available everywhere, at least not in the Netherlands [14]. Other treatment options are gentamycin, carbapenems and dual antibiotic therapy [6].

Although we only included high-risk patients in our analysis and excluded the low-risk group we do not think that this could have led to significant bias since the prevalence of NG among low-risk visitors was only 0.5%. Moreover, we compared the selection of patients diagnosed with gonorrhoea with available MIC values to those without MIC data. Being MSM was the only characteristic overrepresented in the group without MIC data. Since the compositions of the two groups were similar, the group with MIC info can be considered representative of the whole.

At present we are working on the molecular typing of the NG isolates with decreased susceptibility to ceftriaxone to investigate if the cefixime-associated mosaic patterns of the *penA* gene are also associated with our findings. The increased prevalence of NG strains with reduced susceptibility to cefotaxime among MSM may herald the evolution towards third-generation cephalosporin-resistant NG strains and this trend needs to be closely monitored. Gonorrhoea can still be treated effectively with third-generation parenteral cephalosporins, however, previous experience has demonstrated that the use of increased doses of antimicrobials only postpones the development of resistance, but does not prevent the eventual demise of the drug.

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