Neisseria gonorrhoeae Treatment Failure and Susceptibility to Cefixime in Toronto, Canada

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Because of Neisseria gonorrhoeae resistance to all prior first-line antimicrobial agents, cephalosporin therapy with adjuvant azithromycin or doxycycline is recommended for treatment of gonorrhea.1-3 Cefixime is the only oral cephalosporin recommended for gonorrhea treatment, critical to the success of expedited partner therapy. An increase in the minimum inhibitory concentration (MIC) of Neisseria gonorrhoeae to cefixime, and to a lesser extent, an intramuscularly administered cephalosporin, ceftriaxone, has been identified in cultured isolates worldwide.4-8 The World Health Organization has sounded alarms for the threat of untreatable gonorrhea.9

The US Centers for Disease Control and Prevention (CDC) recently revised recommendations for treatment of uncomplicated urogenital, anorectal, and pharyngeal gonorrhea.4 Ceftriaxone, 250 mg, intramuscularly combined with either azithromycin, 1 g, orally or doxycycline, 100 mg, orally twice a day for 7 days is the sole preferred treatment regimen, with oral cephalosporins no longer recommended as front-line therapy. If cefixime is used as an alternative therapy for gonorrhea, it is to be followed with a test of cure in 1 week. Use of cefixime

Importance Although cephalosporins are the cornerstone of treatment of Neisseria gonorrhoeae infections, cefixime is the only oral antimicrobial option. Increased minimum inhibitory concentrations (MICs) to cefixime have been identified worldwide and have been associated with reports of clinical failure.

Objective To assess the risk of clinical treatment failure of Neisseria gonorrhoeae infections associated with the use of cefixime.

Design, Setting, and Population A retrospective cohort study of culture-positive Neisseria gonorrhoeae infections at a single sexual health clinic in Toronto, Canada, that routinely performs test of cure. The cohort comprised Neisseria gonorrhoeae culture-positive individuals identified between May 1, 2010, and April 30, 2011, treated with cefixime as recommended by Public Health Agency of Canada guidelines.

Main Outcome Measures Cefixime treatment failure, defined as the repeat isolation of Neisseria gonorrhoeae at the test-of-cure visit identical to the pretreatment isolate by molecular typing and explicit denial of reexposure.

Results There were 291 Neisseria gonorrhoeae culture-positive individuals identified. Of 133 who returned for test of cure, 13 were culture positive; 9 patients were determined to have experienced cefixime treatment failure, involving urethral (n = 4), pharyngeal (n = 2), and rectal (n = 3) sites. The overall rate of clinical treatment failure among those who had a test of cure was 6.77% (95% CI, 3.14%-12.45%; 9/133). The rate of clinical failure associated with a cefixime MIC of 0.12 μg/mL or greater was 25.0% (95% CI, 10.69%-44.87%; 7/28) compared with 1.90% (95% CI, 0.23%-6.71%; 2/105) of infections with cefixime MICs less than 0.12 μg/mL, with a relative risk of 13.13 (95% CI, 2.88-59.72; P < .001).

Conclusion and Relevance The rate of clinical failure following treatment of Neisseria gonorrhoeae infections with cefixime was relatively high at a Toronto clinic and was associated with elevated MICs.


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See also p 185 and Patient Page.
as the backbone for expedited therapy is relegated to heterosexual partners only.∗

Pharmacokinetic and pharmacodynamic data using the recommended single 400-mg oral dose of cefixime suggest that N gonorrhoeae with an MIC of 0.12 μg/mL or greater may not be treated successfully with cefixime therapy.10,11 Reports from Asia and Europe indicate cefixime treatment failures of N gonorrhoeae infections in urethral and pharyngeal sites due to isolates with cefixime MICs of 0.12 μg/mL or greater.12-17 However, clinical studies on cefixime efficacy given increasing MICs are lacking.

To determine whether N gonorrhoeae strains with reduced susceptibilities to cefixime are associated with clinical failures, we carried out a retrospective cohort study of N gonorrhoeae infections in a clinical setting advocating routine test of cure.

**METHODS**

Clinical failures of the treatment of N gonorrhoeae infection with cefixime were identified via a retrospective cohort study of patients attending a single clinic in Ontario from May 1, 2010, to April 30, 2011. This clinic routinely uses culture-based methods for detecting N gonorrhoeae and requests a test of cure (ie, repeat testing 2-4 weeks after completing therapy) for all N gonorrhoeae infections. In addition, there is a policy of observing the single-dose therapy and explicitly asking at the test-of-cure visit whether the patient had sexual reexposure since the original treatment. Treatment at this clinic was guided by the Public Health Agency of Canada Sexually Transmitted Guidelines; oral cefixime, 400 mg, was the only front-line therapy recommended for suspected or confirmed N gonorrhoeae infections during the study period.2

A medical record review was performed for each case of culture-confirmed N gonorrhoeae infection. Information collected included basic demographics such as age and sex, sexual orientation, reason for testing (screening, symptomatic, or contact of a case), co-infections, sites of infection, initial treatment, and details of test of cure and associated management. Information about race and human immunodeficiency virus infection status was not available. Cefixime treatment failures were defined as the isolation by culture of N gonorrhoeae at the test-of-cure visit that was identical to the original isolate as determined by molecular typing and explicit denial of reexposure since the initial culture based on record review.

**Laboratory Testing**

All patient isolates of N gonorrhoeae were identified at the Public Health Ontario laboratories, which provide primary testing for dedicated sexually transmitted infection clinics throughout the province of Ontario, Canada. Culture confirmation of N gonorrhoeae isolates was performed by biochemical testing and target probe confirmation using an AccuProbe (Gen-Probe). Susceptibility testing was performed by agar dilution in accordance with Clinical Laboratories Standards Institute (CLSI) guidelines.18 Reduced susceptibility to cefixime was defined as an MIC of 0.12 μg/mL or greater based on pharmacokinetic/pharmacodynamic data and reports of cefixime failure in Japan and Europe.10,13,19

Molecular analysis of N gonorrhoeae isolates with reduced susceptibility to cefixime included the genes of penicillin binding proteins PBP1 (ponA) and PBP2 (ponA), outer membrane porin PIB (porB), and the efflux system regulated by MtrR and the mtrR gene promoter.20-28 Mosaic PBP2s are novel gene cassettes that suggest an admixture of genetic material from both wild-type N gonorrhoeae and other commensal Neisseria species. Several specific amino acid substitutions in the resulting mosaic PBP2s have been associated with reduced susceptibility of N gonorrhoeae to the cephalosporins.29 Full details of the methods and sequences were previously described.9 To assess clonality, N gonorrhoeae multiantigen sequence typing (NG-MAST) was performed on all isolates by sequencing internal fragments of 2 highly polymorphic loci, porA/B and tbpB, and trimmed sequences were uploaded to the NG-MAST website for sequence type (ST) assignment.27

**Statistical Analyses**

Sample sizes were determined by the study period and were verified using methods described by Kelsey et al.28 Assumptions in sample size calculations included baseline reported rates of clinical failure with cefixime of 2%,29 a 20% rate of isolates with decreased susceptibility to cefixime as assessed from baseline data from the study clinic, a 10-fold increase in the rate of clinical failure postulated with decreased susceptibility to cefixime, power greater than 80%, and a 2-sided significance level of greater than .05.

The overall rate of clinical failure of gonorrhea associated with use of cefixime in the clinic was determined among those who had a test of cure using the exact binomial test. The rate of clinical failure associated with reduced susceptibility to cefixime was determined by the Fisher exact test. Individual cases with missing information about potential reexposure were excluded as cases of clinical failure but were retained in the denominator. Sensitivity analyses were also calculated with all cases of culture-confirmed N gonorrhoeae infections as the denominator, regardless of test of cure. All statistical tests were 2 sided, and P < .05 was considered statistically significant. All analyses were conducted using Stata version 12 (StataCorp).

**Ethics**

As approved by the University of Toronto research ethics board, individual patient consent was not requested. The underlying rationale was that no personal health information was extracted as part of this historical cohort study, and previous studies at this clinic that involved contacting patients appeared to lead to increased anxiety on the part of those contacted.
Weighing risks and benefits, individual patient consent was not pursued. Individual patient-level data were deidentified, and linkage was provided by using the specimen number of the first isolate collected from each individual.

**RESULTS**

A total of 291 patients who were culture positive for *N gonorrhoeae* received care at the study clinic in Toronto, Canada, from May 1, 2010, to April 30, 2011 (Figure). One hundred thirty-three patients returned for the test-of-cure visit. There were no significant differences between those who did and did not have test of cure in terms of age, sex, sexual orientation, and site of infection. All 7 individuals with confirmed pharyngeal infection did return for test of cure (Table 1).

Thirteen of those with test of cure were culture positive for *N gonorrhoeae* at repeat testing. Nine of 13 patients met the case definition for treatment failure. These patients had infections involving the urethra (n=4), pharynx (n=2), and rectum (n=3). Clinical failure occurred among 4 of 76 urethral infections (5.26%) compared with 2 of 7 pharyngeal infections (28.6%) and 3 of 39 rectal infections (7.69%). Seven of the 9 isolates had a cefixime MIC of at least 0.12 μg/mL, and the remaining 2 isolates had MICs of less than 0.12 μg/mL (Table 2 and Table 3).

In the 4 excluded cases, there was no explicit denial in their medical record of sexual reexposure during the time period between isolation of the original culture and the test of cure. Of these, 3 individuals had *N gonorrhoeae* isolated from the urethra and 1 individual had *N gonorrhoeae* isolated from the rectum. The initial and subsequent isolates from each patient had a cefixime MIC of 0.12 μg/mL or greater. The sequence type of the *N gonorrhoeae* isolated from the follow-up cultures was the same as the initial isolate among the excluded cases.

The overall rate of clinical treatment failure of *N gonorrhoeae* infections due to strains with reduced susceptibility to cefixime were identified in the study clinic from May 1, 2010, to April 30, 2011.

### Table 1. Characteristics of *Neisseria gonorrhoeae* Culture-Positive Patients Who Received Care at the Study Clinic

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Returned for Test-of-Cure Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Yes (n = 133)</td>
</tr>
<tr>
<td>Age, mean (SD), y</td>
<td>35.5 (11.16)</td>
</tr>
<tr>
<td>Male sex, No. (%)</td>
<td>129 (97.0)</td>
</tr>
<tr>
<td>Men who have had male-to-male sexual contact, No. (%)</td>
<td>120 (90.2)</td>
</tr>
<tr>
<td>Site of initial isolate, No. (No. with cefixime MIC ≥0.12 μg/mL)</td>
<td>76 (16)</td>
</tr>
<tr>
<td>Urethral</td>
<td>7 (2)</td>
</tr>
<tr>
<td>Pharyngeal</td>
<td>39 (8)</td>
</tr>
<tr>
<td>Rectal</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cervical</td>
<td>11 (2)</td>
</tr>
</tbody>
</table>

Table 1. Characteristics of *Neisseria gonorrhoeae* Culture-Positive Patients Who Received Care at the Study Clinic

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### Table 2. Characteristics of Patients With *Neisseria gonorrhoeae* Infection Who Experienced Treatment Failure With Cefixime

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Site of <em>N gonorrhoeae</em> Culture</th>
<th>Treatment</th>
<th>Possible Concomitant <em>Chlamydia trachomatis</em></th>
<th>MIC, μg/mL</th>
<th>STa</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/M/26</td>
<td>Urethra</td>
<td>Cefixime, 400 mg, orally</td>
<td>Doxycycline, 100 mg, orally twice daily for 7 d</td>
<td>Cefixime 0.12</td>
<td>Doxycycline 2 (resistant)</td>
</tr>
<tr>
<td>14 Recurrence urethral discharge</td>
<td>Urethra</td>
<td>Cefixime, 400 mg, orally</td>
<td>None</td>
<td>Cefixime 0.06</td>
<td>4269</td>
</tr>
<tr>
<td>21 Ongoing urethral discharge</td>
<td>Urethra</td>
<td>Ceftriaxone, 250 mg, intramuscular</td>
<td>None</td>
<td>Cefixime 0.06</td>
<td>4269</td>
</tr>
<tr>
<td>2/M/40</td>
<td>Urethra</td>
<td>Cefixime, 400 mg, orally</td>
<td>Doxycycline, 100 mg, orally twice daily for 7 d</td>
<td>Cefixime 0.12</td>
<td>Doxycycline 1 (intermediate)</td>
</tr>
<tr>
<td>29 Test of cure</td>
<td>Urethra</td>
<td>Cefixime, 800 mg, orally</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>5643</td>
</tr>
<tr>
<td>3/M/38</td>
<td>Rectum</td>
<td>Cefixime, 400 mg, orally</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>4985</td>
</tr>
<tr>
<td>14 Test of cure</td>
<td>Rectum</td>
<td>None</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>4985</td>
</tr>
<tr>
<td>22 Test of cure</td>
<td>Rectum</td>
<td>Cefixime, 800 mg, orally</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>4985</td>
</tr>
<tr>
<td>44 Test of cure</td>
<td>Rectum</td>
<td>Ceftriaxone, 250 mg, intramuscular</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>4985</td>
</tr>
<tr>
<td>4/M/24</td>
<td>Urethra</td>
<td>Cefixime, 400 mg, orally</td>
<td>Doxycycline, 100 mg, orally twice daily for 7 d</td>
<td>Cefixime 0.12</td>
<td>Doxycycline 2 (resistant)</td>
</tr>
<tr>
<td>14 Recurrence urethral discharge</td>
<td>Urethra</td>
<td>Cefixime, 800 mg, orally</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>5372</td>
</tr>
<tr>
<td>5/F/29</td>
<td>Pharynx</td>
<td>Cefixime, 400 mg, orally</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>1407</td>
</tr>
<tr>
<td>12 Test of cure</td>
<td>Pharynx</td>
<td>Cefixime, 800 mg, orally</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>1407</td>
</tr>
<tr>
<td>6/M/26</td>
<td>Rectum</td>
<td>Cefixime, 800 mg, orally</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>5643</td>
</tr>
<tr>
<td>13 Test of cure</td>
<td>Rectum</td>
<td>Cefixime, 400 mg, orally</td>
<td>Doxycycline, 100 mg, orally twice daily for 7 d</td>
<td>Cefixime 0.12</td>
<td>Doxycycline 1 (intermediate)</td>
</tr>
<tr>
<td>35 Test of cure</td>
<td>Rectum</td>
<td>Ceftriaxone, 250 mg, intramuscular</td>
<td>None</td>
<td>Cefixime 0.06</td>
<td>-</td>
</tr>
<tr>
<td>7/M/24</td>
<td>Pharynx</td>
<td>Cefixime, 800 mg, orally</td>
<td>Azithromycin, 1 g, orally, single dose</td>
<td>Cefixime 0.12</td>
<td>Azithromycin ≤0.25 (susceptible)</td>
</tr>
<tr>
<td>17 Test of cure</td>
<td>Pharynx</td>
<td>Ceftriaxone, 250 mg, intramuscular</td>
<td>None</td>
<td>Cefixime 0.12</td>
<td>1407</td>
</tr>
<tr>
<td>8/M/51</td>
<td>Rectum</td>
<td>Cefixime, 400 mg, orally</td>
<td>None</td>
<td>Cefixime ≤0.03</td>
<td>495</td>
</tr>
<tr>
<td>15 Test of cure</td>
<td>Rectum</td>
<td>None</td>
<td>None</td>
<td>Cefixime ≤0.03</td>
<td>495</td>
</tr>
<tr>
<td>21 Test of cure</td>
<td>Rectum</td>
<td>Ceftriaxone, 250 mg, intramuscular</td>
<td>None</td>
<td>Cefixime ≤0.03</td>
<td>495</td>
</tr>
<tr>
<td>9/M/36</td>
<td>Urethra</td>
<td>Cefixime, 400 mg, orally</td>
<td>Doxycycline, 100 mg, orally twice daily for 7 d</td>
<td>Cefixime 0.06</td>
<td>Doxycycline 2 (resistant)</td>
</tr>
<tr>
<td>23 Test of cure</td>
<td>Urethra</td>
<td>Ceftriaxone, 250 mg, intramuscular</td>
<td>None</td>
<td>Cefixime 0.06</td>
<td>4985</td>
</tr>
</tbody>
</table>

Abbreviations: F, female; M, male; MIC, minimal inhibitory concentration; ST, sequence type.

aDetermined by *N gonorrhoeae* multiantigen sequence typing (NG-MAST), whereby matching ST designations indicate clonality by this method.
with a cefixime MIC of 0.12 μg/mL or greater was 25.0% (7/28; 95% CI, 10.69%-44.87%), where the denominator represents all isolates with test of cure and a cefixime MIC of ≥0.12 μg/mL compared with 1.90% (2/105; 95% CI, 0.23%-6.71%) of isolates with a cefixime MIC less than 0.12 μg/mL. The associated relative risk of clinical failure associated with isolates with a cefixime MIC of 0.12 μg/mL or greater compared with lower MICs was 13.13 (95% CI, 2.88-59.72; P < .001). An analysis assuming that no clinical failures occurred among those who failed to return for test of cure demonstrated a failure rate of 11.86% among all individuals in this study harboring isolates demonstrating cefixime MICs of equal or greater than 0.12 μg/mL (7/59; 95% CI, 4.21%-22.93%) compared with 0.86% among those with isolates demonstrating a cefixime MIC of less than 0.12 μg/mL (2/232; 95% CI, 0.01%-3.08%). The relative risk was 13.76 (95% CI, 2.93-64.53; P < .001).

The clonality of pretreatment and posttreatment isolates for each clinical failure was confirmed by NG-MAST (Table 2 and Table 3). However, there were different clones seen between individuals; among the 9 cases of clinical failure, 6 sequence types were found with slight variation between them. All isolates associated with clinical failure and decreased susceptibility to cefixime shared the identical sequence type for \( \text{tpbp} \) (allele 110) and 4 of 5 \( \text{porB} \) sequences displayed a high degree of genetic identity with only 2 nucleotides of difference between them. The remaining \( \text{porB} \) type (\( \text{porB} \) allele 3257) harbored a 27-nucleotide insertion with respect to allele 3024.

Sequencing of the known targets related to reduced susceptibility to third-generation cephalosporins in all isolates associated with clinical failure showed a mosaic PBP2 pattern (Table 3). The mosaic PBP2 type XXXIV, described in a previous surveillance study in Ontario, was found in all but 1 of the isolates associated with clinical failure in our study and contained the 3 mutations associated with reduced susceptibility to cefixime in \( N \) gonorrhoeae (G545S, I312M, and V316T) but remained wild-type for the positions associated with reduced susceptibility to ceftriaxone (G542 and

Table 3. Additional Microbiological Characteristics of \( N \) gonorrhoeae Isolates From Cefixime Clinical Failures

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Visit Day</th>
<th>Cefixime, μg/mL</th>
<th>Ceftriaxone, μg/mL</th>
<th>Penicillin, μg/mL</th>
<th>Doxycycline, μg/mL</th>
<th>Azithromycin, μg/mL</th>
<th>Molecular Markers of Cephalosporin Resistance</th>
<th>NG-MAST</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.12</td>
<td>0.06</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>Mosaic XXXIV G120K, A121N</td>
<td>2623</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.12</td>
<td>0.06</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>Mosaic XXXIV No mutations at 120, 121</td>
<td>3424</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0.12</td>
<td>0.06</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>Mosaic XXXIV G120K, A121N</td>
<td>3024</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0.12</td>
<td>0.12</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>Mosaic XXXIV G120K, A121N, IS(^c)</td>
<td>3257</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0.12</td>
<td>0.06</td>
<td>2</td>
<td>2</td>
<td>0.5</td>
<td>Mosaic XXXIV G120K, A121N</td>
<td>908</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0.12</td>
<td>0.06</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>Mosaic XXXIV No mutations at 120, 121</td>
<td>3424</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0.12</td>
<td>0.06</td>
<td>1</td>
<td>2</td>
<td>0.25</td>
<td>Mosaic XXXIV G120K, A121N</td>
<td>908</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0.12</td>
<td>0.06</td>
<td>1</td>
<td>2</td>
<td>0.25</td>
<td>Mosaic XXXIV G120K, A121N</td>
<td>908</td>
</tr>
<tr>
<td>9</td>
<td>0</td>
<td>0.06</td>
<td>0.06</td>
<td>1</td>
<td>2</td>
<td>0.5</td>
<td>Mosaic XXXIV G120K, A121N</td>
<td>3024</td>
</tr>
</tbody>
</table>

Abbreviations: IS, insertion sequence; MIC, minimal inhibitory concentration; NG-MAST, \( N \) gonorrhoeae multiantigen sequence typing; PBP2, penicillin binding protein 2; ST, sequence type.

\(^a\)The PBP2 mosaic pattern XXXIV contains the 3 mutations associated with reduced susceptibility to cefixime in \( N \) gonorrhoeae (G545S, I312M, and V316T). In contrast, the PBP2 mosaic pattern XXXVI has no known mutations associated with reduced susceptibility to the cephalosporins.

\(^b\)The amino acid substitutions G120K and A121N in the \( \text{porB} \) gene, \( \text{porB} \), have been associated with high-level cephalosporin resistance. The substitution pattern in \( \text{porB} \), G120K and A121D, has been identified among both cephalosporin-susceptible and -resistant strains.

\(^c\)The \( \text{tpbp} \) gene encodes for the gonococcal outer membrane porin and is one of the 2 loci used for typing NG-MAST.

\(^d\)The \( \text{tpbp} \) gene encodes for the \( \beta \)-subunit of the transferrin-binding protein and is one of the 2 loci used for typing by NG-MAST.

\(^e\)Presence of IS2 in the pretreatment and posttreatment isolates further supports the clonality of strains.
All isolates associated with clinical failure with a cefixime MIC of at least 0.12 μg/mL were wild-type for MtrR and the \textit{mtrR} gene promoter. The clinical isolates associated with patient No. 8 herein had a cefixime MIC of 0.03 μg/mL or less and PB2 mosaic pattern XXXVI with no known mutations associated with reduced susceptibility to the cefalosporins (Table 2 and Table 3). The isolates cultured from patient No. 8 also shared the same \textit{tbpB} sequence type 110 as those isolates with reduced susceptibility to cefixime from patients No. 1 through 7, suggesting a related clone with a moderate elevation in cefixime MIC of 0.06 μg/mL. The amino acid substitutions, G120K and A121N, in the porin gene, \textit{porB}, have been associated with high-level cefalosporin resistance.\textsuperscript{20} The substitution pattern in \textit{porB} associated with patient No. 8, G120K and A121D, has been identified among both cefalosporin-susceptible and cephalosporin-resistant strains.\textsuperscript{31}

**COMMENT**

This study presents the first series of clinical failures of gonorrhea associated with the use of cefixime in North America, identified by the concurrent strategies of routine test-of-cure and culture-based testing for \textit{N} \textit{gonorrhoeae}. Clinical treatment failures of gonorrhea occurred in 6.77% (95% CI, 3.14%-12.45%) of all of those treated with cefixime and who had a test of cure at the clinic. This exceeds the 95% efficacy threshold established by the both the CDC and the World Health Organization for acceptable empirical therapy for gonorrhea.\textsuperscript{32,33} A more conservative estimate of the clinical failure rate in this study, assuming that there were no clinical failures that occurred among those without test of cure, was 3.09% (9/291; 95% CI, 1.01%-5.08%). Baseline characteristics were similar among those with and without test of cure. Individuals infected with isolates of \textit{N} \textit{gonorrhoeae} with a cefixime MIC of 0.12 μg/mL or greater had a clinical failure rate of 25%, compared with 1.90% in those with isolates with an MIC of less than 0.12 μg/mL.

A rise in cephalosporin MICs among \textit{N} \textit{gonorrhoeae} was identified in parts of Asia as early as the late 1990s, followed by similar reports from other regions of the world over the next 2 decades.\textsuperscript{34} Increasing resistance of \textit{N} \textit{gonorrhoeae} to the cephalosporins in the United States and Canada has evolved since 2000. The CDC’s Gonococcal Isolate Surveillance Project (GISP) analyzed isolates from 2000-2010; isolates with cefixime MICs 0.25 μg/mL or greater increased from 0.2% to 1.4% and isolates with ceftriaxone MICs 0.12 μg/mL or greater increased from 0.1% to 0.3%.\textsuperscript{7} In Canada, as part of the National \textit{N} \textit{gonorrhoeae} Surveillance Program that includes all Canadian isolates of \textit{N} \textit{gonorrhoeae} resistant to at least 1 antibiotic, a right shift in the modal MICs of both ceftriaxone and cefixime was observed between 2001 and 2010, from 0.016 to 0.12 μg/mL for cefixime and from 0.016 to 0.063 μg/mL for ceftriaxone.\textsuperscript{35}

Soon after \textit{N} \textit{gonorrhoeae} with decreased susceptibility to the cephalosporins emerged in Asia, treatment failures were reported from Japan in patients with urethritis treated with oral cefdinir.\textsuperscript{12,19,36} Since then, there have been case reports from the United Kingdom, Austria, France, and Norway and reports of failures of cefixime to treat gonococcal urethritis with MICs of 0.19 μg/mL or greater.\textsuperscript{15,17}

The routine identification of \textit{N} \textit{gonorrhoeae} treatment failures due to reduced susceptibility is problematic because of the increasing use of NAAT (nucleic acid amplification test), which is not able to provide antibiotic susceptibility results. In 2007, a web-based survey of US public health laboratories showed that only 4.9% of all testing for \textit{N} \textit{gonorrhoeae} was performed by culture.\textsuperscript{37} Furthermore, generally no test of cure is recommended for uncomplicated gonorrhea in the absence of recurrent symptoms, unless prescribing alternative therapies.\textsuperscript{4}

We were able to overcome many of these limitations with our study population. The clinic routinely uses culture-based methods for detecting \textit{N} \textit{gonorrhoeae} and requests a test of cure. Given that the cephalosporins are the last commercially available class of antibiotics for the treatment of gonorrhea, this study demonstrates the feasibility and yield of such a preemptive approach to the identification of clinical failures.

No threshold for resistance to cefixime (or ceftriaxone) in \textit{N} \textit{gonorrhoeae} has yet been defined in North America, hindering the preemptive identification of those at risk of clinical failure. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) suggests that a cefixime MIC 0.25 μg/mL or greater represents resistance,\textsuperscript{38} whereas the Clinical Laboratories Standards Institute has set only a single “susceptible” breakpoint of 0.25 μg/mL or less.\textsuperscript{18} A resistance threshold equal to or greater than 0.12 μg/mL for cefixime in the treatment of \textit{N} \textit{gonorrhoeae} infection is supported by other studies. Deguchi et al,\textsuperscript{11} using a regimen of 2 doses of 200 mg each of cefixime 6 hours apart, saw consistent therapeutic success (45/45 cases) in male patients treated for gonococcal urethritis with isolates that had MICs 0.06 μg/mL or less but reported 5 failures in 11 patients infected with isolates that had MICs of 0.125 μg/mL. Chisholm et al\textsuperscript{10} estimated periods for which free drug concentrations of cefixime exceeded the MIC after a 400-mg single-dose regimen and found that for isolates with cefixime MICs 0.06 μg/mL or less, the time above the MIC was 22 hours or longer. However, durations became markedly shorter at higher MICs, decreasing to 18.8 hours, 15.3 hours, and 11.7 hours at MICs of 0.125, 0.25, and 0.5 μg/mL, respectively, suboptimal for effective treatment within their model. Even increasing the cefixime dosage may not be a solution to finding an effective oral therapy. We found that 2 of the patients in our study experienced treatment failure with a single dose of 800 mg of cefixime given as ini-
tial therapy (patients No. 6 and No. 7) (Table 2).

Of note, there is a high degree of genetic homology among the isolates of *N gonorrhoeae* associated with clinical failure when treated with cefixime. These related clones of *N gonorrhoeae* are not unique to North America. The 2 reported cases of clinical failures associated with reduced susceptibility to cefixime identified in Norway are identical by NG-MAST to 2 cases in our series (ST 1407). The recently described high-level cefixime- and ceftriaxone-resistant strains identified in France and Spain demonstrated the same NG-MAST (ST 1407) and shared the same penA mosaic pattern (XXXIV) as all of the clinical failures associated with reduced susceptibility to cefixime in our study. The only identified difference between F89 and the isolates with reduced susceptibility to cefixime identified in our series accounting for the high-level cephalosporin resistance is an additional alteration at A501P within the gene encoding PBP2. Similarly, the 2 cases identified in England share the same *tbpB* allele (sequence type 110). The genetic homology of the strains in Ontario and Europe suggests the possibility of the emergence of a clonal complex of *N gonorrhoeae* with a greater propensity to treatment failure.

Two limitations of our study can be described. First, it may be noted that these findings may have limited generalizability. The study was conducted at an urban clinic in Toronto, Canada, that primarily serves individuals who have had male-to-male sexual contact, and has a high rate of isolates of *N gonorrhoeae* with elevated cefixime MICs (20.2% of unique patient isolates had an MIC ≥0.12 μg/mL). As with initial increases in resistance of *N gonorrhoeae* to the fluoroquinolones, such men may represent a sentinel population for cephalosporin-resistant *N gonorrhoeae*.

The markedly increased rate of clinical failure associated with isolates with cefixime MICs 0.12 μg/mL or greater has broader potential generalizability.

Isolates of *N gonorrhoeae* with elevated cefixime MICs have been identified worldwide among women, heterosexual men, and men who have had male-to-male sexual contact. In the current study, 1 clinical failure involved a urethral infection in a heterosexual man, and a second clinical failure involved a woman. Both of these clinical failures were associated with elevated cefixime MICs. Similarly, 3 of the 5 European clinical failures of *N gonorrhoeae* associated with elevated cefixime MICs occurred in heterosexual men. Although the CDC’s GISP has identified higher rates of *N gonorrhoeae* with decreased susceptibility to cephalosporins among men who have had male-to-male sexual contact, elevated cefixime MICs are not limited to this group. Second, it may be noted that only 6 of 9 clinical failures identified in this study received cefixime plus azithromycin or doxycycline combination therapy as recommended by the CDC and this may confound the actual rate of clinical failure if these recommendations had been followed. While this may be true, there are a paucity of data to support combination therapy for the treatment of *N gonorrhoeae*. The studies quoted in the 2010 CDC guidelines focus on the effect of combination therapy for the treatment of pharyngeal infection, an anatomical compartment for which limited tissue concentrations of cephalosporins can be attained. Further studies are needed to determine whether combination therapy may act synergistically to overcome elevated MICs in nonpharyngeal compartments.

The World Health Organization recommends the discontinuation of empirical use of an antibiotic once 5% of locally acquired isolates of *N gonorrhoeae* demonstrate resistance. Data from the GISP in 2009 demonstrate that more than 5% of the isolates in Detroit, Honolulu, Las Vegas, Minneapolis, Portland, San Diego, and Seattle have an MIC of 0.12 μg/mL or greater. If indeed the more appropriate resistance breakpoint is 0.12 μg/mL or greater, then we may well have already reached that threshold, thereby eliminating the 1 oral therapy for *N gonorrhoeae* that remains. In light of the increases in cefixime MICs among isolates of *N gonorrhoeae* across North America, this study offers preliminary clinical data to support the recent CDC recommendations that cefixime is no longer optimal first-line therapy for the successful treatment of gonorrhea. As elevated MICs to ceftriaxone are also emerging, albeit at 1 to 2 MIC dilutions less than the cefixime MIC, proactive strategies for the identification of clinical failures of *N gonorrhoeae* to this last commercially available agent are recommended.

In summary, we identified a relatively high rate of clinical failure in a clinic in Toronto after treatment of *N gonorrhoeae* infections with cefixime, which was associated with elevated MICs.

**Author Contributions:** Dr Allen had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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