Ceftriaxone remains a first-line treatment for patients infected by *Neisseria gonorrhoeae* in most settings. We investigated the possible spread of a ceftriaxone-resistant FC428 *N. gonorrhoeae* clone in Japan after recent isolation of similar strains in Denmark (GK124) and Canada (47707). We report 2 instances of the FC428 clone in Australia in heterosexual men traveling from Asia. Our bioinformatic analyses included core single-nucleotide variation phylogeny and in silico molecular typing; phylogenetic analysis showed close genetic relatedness among all 5 isolates. Results showed multilocus sequence type 1903; *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) 233; and harboring of mosaic penA allele encoding alterations A311V and T483S (penA-60.001), associated with ceftriaxone resistance. Our results provide further evidence of international transmission of ceftriaxone-resistant *N. gonorrhoeae*. We recommend increasing awareness of international spread of this drug-resistant strain, strengthening surveillance to include identifying treatment failures and contacts, and strengthening international sharing of data.

Ceftriaxone is among the last remaining recommended therapies for treating *Neisseria gonorrhoeae* infections and is used in many countries around the world as part of a dual therapy with azithromycin. Cephalosporin resistance in *N. gonorrhoeae* has been associated with modifications of the penA gene, which encodes penicillin-binding protein 2 (PBP2), a target for β-lactam antimicrobial drugs (1). During 2009–2015, several ceftriaxone-resistant (MIC 0.5–4 mg/L) *N. gonorrhoeae* strains were reported: in 2009, H041 in Japan (2); in 2010, F89 in France (3); in 2011, F89 in Spain (4); in 2013, A8806 in Australia (5); in 2014, GU140106 in Japan (6); and in 2015, FC428 and FC460 in Japan (7). However, until 2017, all of these strains were considered to have occurred sporadically because, except for limited transmission of F89 among persons in France and Spain during 2010–2011, there had been no reports of sustained transmission of these strains identified nationally or internationally. In 2017, this changed, substantiated by independent reports from Canada (8) and Denmark (9) of gonococcal isolates that had substantive similarity to the previously described FC428 strain in Japan.

The first reported case of the FC428 ceftriaxone-resistant *N. gonorrhoeae* strain was in Japan during January 2015 in a heterosexual man in his twenties who had urethritis (7). The FC428 isolate was resistant to ceftriaxone (MIC 0.5 mg/L), cefixime (MIC 1 mg/L), and ciprofloxacin (MIC ≥32 mg/L); susceptible to spectinomycin (MIC 8 mg/L) and azithromycin (MIC 0.25 mg/L); and, unlike all previously described ceftriaxone-resistant strains, a penicillinase-producing *N. gonorrhoeae* (PPNG; MIC ≥32 mg/L) bacterium. The patient was treated successfully with a single dose of spectinomycin 2 g intramuscularly (IM); however, a second isolate with an identical susceptibility profile (FC460) was subsequently cultured from the same patient 3 months later, suggesting reinfection by a separate contact.

In Canada, during January 2017, a gonococcal isolate (47707) (8) of similar susceptibility to the first reported case
isolates by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Melbourne, Victoria, Australia; bioMérieux, Brisbane, Queensland, Australia). We determined antimicrobial susceptibilities of N. gonorrhoeae (MIC ≥2.0 mg/L); tetracycline resistance (MIC ≥2.0 mg/L); ciprofloxacin resistance (MIC ≥1.0 mg/L); and spectinomycin resistance (MIC ≥128.0 mg/L). Because the Clinical and Laboratory Standards Institute does not have an azithromycin breakpoint, and ceftriaxone breakpoints only state susceptibility (≤0.25 mg/L), we used the European Committee on Antimicrobial Susceptibility Testing (13) breakpoints for ceftriaxone resistance (MIC>0.12 mg/L) and azithromycin resistance (MIC>0.5 mg/L). β-lactamase production was analyzed by using nitrocefin (Thermo-Fisher Scientific, Melbourne, Victoria, Australia). We subcultured isolates on GC agar base with Vitox Supplement (Thermo-Fisher Scientific) and incubated for 24 h at 35°C in a 5% CO₂ atmosphere with or without antimicrobial drugs and stored in Tryptone (Thermo-Fisher Scientific) soya broth with 10% glycerol at –80°C.

**Methods**

We confirmed N. gonorrhoeae isolates by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Melbourne, Victoria, Australia; bioMérieux, Brisbane, Queensland, Australia). We determined antimicrobial susceptibilities of N. gonorrhoeae to ceftriaxone, penicillin, tetracycline, azithromycin, gentamicin, and ciprofloxacin by using Etest (bio-Mérieux) and spectinomycin by using the agar dilution method (11). We interpreted MIC on the basis of interpretive criteria from the Clinical and Laboratory and Standards Institute (12); penicillin resistance (MIC ≥2.0 mg/L); tetracycline resistance (MIC ≥2.0 mg/L); ciprofloxacin resistance (MIC ≥1.0 mg/L); and spectinomycin resistance (MIC ≥128.0 mg/L). Because the Clinical and Laboratory Standards Institute does not have an azithromycin breakpoint, and ceftriaxone breakpoints only state susceptibility (≤0.25 mg/L), we used the European Committee on Antimicrobial Susceptibility Testing (13) breakpoints for ceftriaxone resistance (MIC>0.12 mg/L) and azithromycin resistance (MIC>0.5 mg/L). β-lactamase production was analyzed by using nitrocefin (Thermo-Fisher Scientific) and incubated for 24 h at 35°C in a 5% CO₂ atmosphere with or without antimicrobial drugs and stored in Tryptone (Thermo-Fisher Scientific) soya broth with 10% glycerol at –80°C.

**Genomic Analyses**

We put each isolate from Japan and Australia through DNA extraction, library preparation, and sequencing (Illumina, San Diego, CA, USA). From the strains from Japan, FC428 and FC460, we extracted DNA samples with the DNeasy Blood & Tissue Kit (QIAGEN, Tokyo, Japan). We created multiplexed libraries with Nextera XT DNA sample prep kit (Illumina) and generated paired-end 300-bp indexed reads on the Illumina MiSeq platform (Illumina) yielding 6,121,575 reads/genome and genome coverage of 845× for FC428 and 1,272,909 reads/genome and genome coverage of 845× for FC460.

**Table 1. Phenotypic and molecular characterization of ceftriaxone-resistant Neisseria gonorrhoeae**

<table>
<thead>
<tr>
<th>Isolate ID</th>
<th>Year</th>
<th>Country (ref)</th>
<th>MIC, mg/L</th>
<th>MLST</th>
<th>porB tetB</th>
<th>NG-MAST</th>
<th>penA</th>
<th>NG-STAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC428</td>
<td>2015</td>
<td>Japan (7)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>FC460</td>
<td>2015</td>
<td>Japan (7)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>GK124</td>
<td>2017</td>
<td>Japan (7)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>A7846</td>
<td>2017</td>
<td>Japan (7)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>A7536</td>
<td>2017</td>
<td>Japan (7)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>F89</td>
<td>2010</td>
<td>France (3,10)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>A8806</td>
<td>2013</td>
<td>Japan (5,10)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
<tr>
<td>H041</td>
<td>2009</td>
<td>Japan (2)</td>
<td>CEFSMPETCIPAZMGENPCNNNG-STAR</td>
<td>1903</td>
<td>21</td>
<td>60.001</td>
<td>233</td>
<td></td>
</tr>
</tbody>
</table>

*AU, Australia; AZM, azithromycin; β-lac, β-lactamase; CEF, ceftriaxone; CFM, cefixime; CIP, ciprofloxacin; DEN, Denmark; GEN, gentamicin; MLST, multilocus sequence type; NG-MAST, Neisseria gonorrhoeae multi-antigen sequence type; NG-STAR, Neisseria gonorrhoeae sequence type for antimicrobial resistance; NA, not available; PCN, penicillin; PPNG, penicillinase-producing N. gonorrhoeae; ref, reference; SPX, spectinomycin; TET, tetracycline; +, positive; –, negative.
To analyze the strains from Australia, A7536 and A7846, we extracted DNA on the QIAsymphony SP (QIAGEN) by using the DSP DNA Mini Kit (QIAGEN). We prepared the libraries according to manufacturer instructions for the Nextera XT library preparation kit (Illumina) and sequenced on the NextSeq 500 (Illumina) by using the NextSeq 500 Mid Output V2 kit (Illumina). Sequencing generated 6,763,774 reads and genome coverage of 361× for A7536 and 3,672,072 reads and genome coverage of 202× for A7846.

We then provided sequencing data to the Canadian National Microbiology Laboratory, where bioinformatic analyses were performed as previously described (14). Quality reads were assembled by using SPAdes (15) (http://bioinf.spbau.ru/spades) and annotated with Prokka (16) (https://github.com/tseemann/prokka), and produced an average of 86 contigs per isolate, an average contig length of 26,276 nt, and an average N50 length of 68,884 nt. Quality metrics for whole-genome sequencing (WGS) are shown in online Technical Appendix Table 1 (https://wwwnc.cdc.gov/EID/article/24/4/17-1873-Techapp1.pdf). A core single-nucleotide variation (SNV) phylogeny was created by mapping reads to FA1090 (GenBank accession no. NC_002946.2) by using a custom Galaxy SNVPhyl workflow (17). Repetitive and highly recombinant regions with >2 SNVs per 500 nt were removed from the analysis. The percentage of valid and included positions in the core genome was 97.6%; 567 sites were used to generate the phylogeny. We used a meta-alignment of informative core SNV positions to create a maximum-likelihood phylogenetic tree for A7536, A7846, FC428, FC460, and 47707 (Figure). The H041, F89, and A8806 ceftriaxone-resistant strains (available in the World Health Organization [WHO] reference panel as WHO-X, WHO-Y, and WHO-Z, respectively) (10) were included for comparison. WGS read data for A7536, A7846, FC428, and FC460 are available under BioProject PRJNA416507, and previously reported 47707 was submitted under BioProject PRJNA415047 (8).

We implemented *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) (18), multilocus sequence typing (MLST) (19), and *N. gonorrhoeae* sequence typing for antimicrobial resistance (NG-STAR) (20) by using gene sequences extracted in silico from WGS data. We submitted the sequences to the NG-MAST (http://www.ng-mast.net/), Neisseria MLST (http://pubmlst.org/neisseria/), and NG-STAR (https://ngstar.canada.ca) databases to determine respective sequence types. Sequence data for the GK124 strain (9) were not available for these analyses; however, a summary of the documented susceptibility and MLST and NG-MAST data is provided (Table 1).

**Results**

**Case Histories and Isolate Details**

The first documented case-patient in Australia was a man in his forties who was visiting from the Philippines. He went to a sexual health clinic in Adelaide in April 2017 reporting urethral discharge and dysuria. He reported recent heterosexual contact with multiple female sex workers in Cambodia and the Philippines; it was unclear where the infection was acquired. An *N. gonorrhoeae* isolate (A7846) of similar susceptibility to FC428 (showing the characteristic ceftriaxone resistance and PPNG; Table 1) was cultured. The patient was treated with a 1-time dose combination therapy of ceftriaxone (500 mg IM) and azithromycin.

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**Figure.** Core single-nucleotide variation (SNV) phylogenetic tree of ceftriaxone-resistant *Neisseria gonorrhoeae* isolates. The maximum-likelihood phylogenetic tree is rooted on the reference genome of *N. gonorrhoeae* FA1090 (GenBank accession no. NC_002946.2). Isolates are indicated by country and year. Strains F89, A8806, and H041 (World Health Organization [WHO] reference panel WHO-Y, WHO-Z, and WHO-X, respectively) are previously reported ceftriaxone-resistant reference strains (10). Scale bar indicates estimated evolutionary divergence between isolates on the basis of average genetic distance between strains (estimated number of substitutions in the sample/total number of high-quality SNVs). MLST, multilocus sequence type; NG-MAST, *Neisseria gonorrhoeae* multiantigen sequence type; NG-STAR, *Neisseria gonorrhoeae* sequence type for antimicrobial resistance; PenA, penicillin-binding protein 2.
(1 g orally). A test result 7 days after treatment was negative for \textit{N. gonorrhoeae}.

A second case-patient in Australia was a man visiting from China. He was in his early 40s and described symptoms of urethral discharge and dysuria to a general practitioner in Sydney in August 2017. He reported heterosexual contact in China, but none in Australia. An isolate (A7536) of similar susceptibility to FC428 (ceftriaxone-resistant and PPNG; Table 1) was cultured. The patient was treated with a 1-time dose combination therapy of ceftriaxone (500 mg IM) and azithromycin (1 g orally); he returned to China shortly thereafter. Attending physicians advised him to return to follow up for test of cure and to trace contacts, but follow-up was not confirmed.

Core SNV phylogenetic analysis results (Figure) showed a close genetic relatedness among the FC428, FC460, 47707, A7536, and A7846 isolates. These isolates were distinct from the other previously described F89, A8806, and H041 ceftriaxone-resistant strains; the 2 groups of isolates were separated from each other by an average of 292 core SNVs. We detected no SNVs in the 2 isolates from Japan (FC428, FC460 collected from the same patient 3 months apart). For other isolates, 12 SNVs separated FC428 from both 47707 and A7536; 17 SNVs separated FC428 and A7846 (47707, A7536, and A7846 shared 8 identical SNVs); 8 SNVs separated 47707 and A7536; 5 SNVs separated 47707 and A7846; and 11 SNVs separated A7536 and A7846 (online Technical Appendix Table 2).

Molecular typing of FC428, FC460, 47707, A7536, and A7846 from the WGS showed an identical MLST of ST1903, which was also reported for FC428 from both 47707 and A7536; 17 SNVs separated FC428 and A7846 (47707, A7536, and A7846 shared 8 identical SNVs); 8 SNVs separated 47707 and A7536; 5 SNVs separated 47707 and A7846; and 11 SNVs separated A7536 and A7846 (online Technical Appendix Table 2).

The recent reports of the \textit{N. gonorrhoeae} FC428 clonal strain in Denmark, Canada, and now Australia provide new evidence that there is sustained international transmission of a ceftriaxone-resistant \textit{N. gonorrhoeae} strain. This strain appears to have been circulating globally for \geq 2 years. Thus, it is highly likely this strain is prevalent elsewhere, possibly in Asia, but undetected. There are serious gaps in \textit{N. gonorrhoeae} antimicrobial resistance surveillance worldwide (21), and we estimate that samples from as few as 0.1% of the estimated 80 million cases of \textit{N. gonorrhoeae} reported globally each year (22) are tested for antimicrobial resistance. Therefore, there are many opportunities for such strains to avoid detection.

Fortunately, the ceftriaxone MICs of the FC428 clonal strain remain lower than the H041 strain from Japan (MIC 2 mg/L) (2), and further, the FC428 strain does not exhibit resistance to azithromycin (Table 1). Therefore, treatment failure is arguably less likely against ceftriaxone infections than in H041 and F89 infections, particularly when using ceftriaxone and azithromycin dual therapy; treatment failure was not observed in our study. Nevertheless, previous pharmacodynamic analyses indicate that ceftriaxone MICs of 0.5–1.0 mg/L can result in treatment failures with ceftriaxone-resistant \textit{N. gonorrhoeae} (23). As such, a dissemination of the FC428 clone could offset dual therapy guidelines because azithromycin resistance is being increasingly reported (24, 25).

### Table 2. PenA types identified in ceftriaxone-resistant \textit{Neisseria gonorrhoeae} strains*

<table>
<thead>
<tr>
<th>PenA type</th>
<th>Strain ID</th>
<th>Amino acid position in PenA protein (2, 18)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>M32091</td>
<td>MCAKDDVNYGEDQQAADRAVAGTDLNERIQPSPR.SRGAEFEITLRNRPVLQIFESRENPPATFAVAAHGAPPKII.A</td>
</tr>
<tr>
<td>37</td>
<td>H041</td>
<td>E.ASHAGEE.VEKQVMPSV.TTDTL.ATQ.TMTPK.DVSV.K..VEKVIA.KKEAS1.LVY...N.ST.VQVNV</td>
</tr>
<tr>
<td>42</td>
<td>F89</td>
<td>E.ASHAGEE.VEKQ.MTS.V.ATDFTLSATQ.TMTPK.DV...S.QKEVKVIA.KKEA..PLVY...N.S........</td>
</tr>
<tr>
<td>60</td>
<td>FC428/FC460/A7536/A7846/47707</td>
<td>VMTS.V.PTDTL.ATQ.TMTPK.DV...S.QKEVKVIA.KKEAS1.LVY...N.ST.VQVNV</td>
</tr>
<tr>
<td>64</td>
<td>A8806</td>
<td>E.ASHAGEE.....VMTS.V.PTDTL.ATQ.TMTPK.DV...S.QKEVKVIA.KKEAS1.LVY...N.ST.VQVNV</td>
</tr>
</tbody>
</table>

*Arrows indicate key amino acid positions associated with high-level \beta-lactam resistance. PenA, penicillin-binding protein 2.
The cases of *N. gonorrhoeae* described here and the circumstances under which these analyses took place are also a timely reminder of the need for international collaboration in addressing the overall *N. gonorrhoeae* problem and highlight the benefits of rapid access to genomic data by using electronic communications. In fact, in the absence of WGS data, it would have been very difficult to identify the links between these isolates. Not only have we been able to use these tools to readily identify the problem but we also arguably achieved identification in a sufficiently timely manner as to enable countries to put in place interventions that can limit further the spread of this strain, including intensifying follow-up and contact tracing.

Differences in extraction and sequencing procedures among the 3 countries could introduce variations in DNA concentrations that might affect the quality of the sequencing, such as number of reads and depth of coverage. This limitation was minimized because downstream processing of the data, such as assembly and reference mapping software algorithms, standardizes input data before detailed analyses of the genomes are conducted. Laboratory and epidemiologic findings are critical for surveillance that closely tracks the dissemination and emergence of epidemic antimicrobial-resistant strains and for rapid recognition and implementation of control measures to limit the expansion of clones through sexual networks. We recommend that health departments in all countries be made aware of this spreading resistant strain and strengthen antimicrobial-resistance monitoring, including treatment failure identification, adequate follow-up and contact tracing of cases, and STI prevention programs.

In conclusion, international collaboration based on WGS typing methods revealed the dissemination of a ceftriaxone-resistant *N. gonorrhoeae* in Japan, Canada, and Australia. Sustained transmission spanning 2 years suggests unidentified cases are likely present in other locations. These findings warrant the intensification of surveillance strategies and establishment of collaborations with other countries to monitor spread and inform national and global policies and actions.

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D.W. reports research funding from SpeeDx Pty Ltd.

The study was approved by the South Eastern Sydney Local Health District Human Research Ethics Committee (HREC) and The University of Queensland HREC.

**About the Author**

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**References**


Neisseria gonorrhoeae, the causative pathogen of gonorrhoeae, has been designated an urgent antimicrobial drug resistance threat by the Centers for Disease Control and Prevention. Since the introduction of antimicrobial drugs in the first half of the 20th century, N. gonorrhoeae has successively developed resistance to each antimicrobial agent recommended for gonorrhoeae treatment. In the United States, the prevalence of resistance in N. gonorrhoeae often varies by sex of partner and by geographic region. Prevalence is often greater in isolates from gay, bisexual, and other men who have sex with men than those from men who have sex only with women, and prevalence is often highest in the West and lowest in the South. Resistant strains, in particular penicillinnase-producing N. gonorrhoeae, fluoroquinolone-resistant N. gonorrhoeae, and gonococcal strains with reduced cephalosporin susceptibility, seemed to emerge initially in the West (Hawaii and the West Coast) before spreading eastward across the country. These geographic patterns seem to support the idea that importation of resistant strains from other regions of the world, such as eastern Asia, is a primary factor of the emergence of resistant gonococci in the United States. Whereas antimicrobial drug prescribing patterns have been clearly associated with the emergence of resistance in other bacterial pathogens, the degree to which domestic antimicrobial use and subsequent selection pressure contributes to the emergence of gonococcal antimicrobial resistance in the United States is unclear. Using an ecologic approach, we sought to investigate the potential geographic and temporal association between antimicrobial drug susceptibility among US N. gonorrhoeae isolates and domestic outpatient antimicrobial drug prescribing rates in the United States during 2005–2013.

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