Direct Detection of penA gene Associated with Ceftriaxone-Resistant Neisseria gonorrhoeae FC428 Strain by Using PCR

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The ceftriaxone-resistant Neisseria gonorrhoeae FC428 clone was first observed in Japan in 2015, and in 2017, it was documented in Denmark, Canada, and Australia. Here, we describe a PCR for direct detection of the penA gene associated with this strain that can be used to enhance surveillance activities.

Ceftriaxone, either monotherapy or in dual therapy with azithromycin, is the mainstay of treatment of patients diagnosed with Neisseria gonorrhoeae infection in most settings (1). Therefore, the identification of any strains exhibiting resistance to ceftriaxone is of considerable public health concern. Until 2017, ceftriaxone-resistant strains of N. gonorrhoeae had been rare and typically sporadic, including 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, there is now evidence of sustained international transmission of FC428, reported during 2017 in Canada (8) and Denmark (9) (1 case each) and in Australia (2 cases) (10). Rapid and timely detection is pivotal to contain further spread of antimicrobial drug-resistant N. gonorrhoeae. Here, we describe a real-time PCR protocol to facilitate enhanced surveillance for the FC428 clone. The study was approved by the University of Queensland Human Research Ethics Committee.

The Study

We designed a real-time PCR to target unique sequences on the penA gene of the FC428 N. gonorrhoeae clone (10).

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Table 1. Primer and probe sequences for PCR to detect Neisseria gonorrhoeae FC428 strain*

| Designation | Oligonucleotide sequence, 5’ → 3’ |
|-------------|---------------------------------|
| Forward primer | CGCAACCCTGTGCCCTT |
| Reverse primer | GGGTATTTGAATGTCTGTGTGGA |
| Probe 1 | Fam-TTCA+T+G+A+CA+G+AAC-iowa Black FQ |
| Probe 2 | Hex-TCA+T+G+G+CA+GA-iowa Black FQ |

*LNA bases are indicated by * preceding the base in the sequence.
positive signals by using probe 1 with cycle threshold (C<sub>t</sub>) values <20 cycles. The A8806 strain provided a late reaction at 37.8 cycles (probe 1), as did 2 commensal Neisseria strains: 1 N. lactamica isolate at 42.8 cycles for (probe 1), and 1 N. meningitidis isolate at 32.6 cycles (probe 2). The Figure shows a sequence alignment of the partial penA sequences from these 3 isolates compared with the FC428 PenA-60.001 allele. The A8806 strain shows considerable sequence homology with PenA-60.001 (including 100% match with the A311V Probe 1 sequence), albeit for 2 mutations in the forward primer designed to limit detection of A8806. We do not consider this a limitation of the assay because there has only been 1 reported case of infection with the A8806 strain. Neither the N. lactamica nor N. meningitidis isolates harbored the A311V alteration.

To compare detection limits, we tested 10-fold dilutions of FC428 DNA by both the FC428 PCR and a previously described in-house N. gonorrhoeae PCR, directed at the gonococcal porA and opa sequences (12). The in-house N. gonorrhoeae PCR had the lowest detection limit at 0.3 genome copies/reaction, whereas the detection limit of the FC428 PCR was 3.0 genome copies/reaction, indicating the FC428 PCR was 1 log less sensitive than the diagnostic method.

We then applied the assay to a convenience panel of N. gonorrhoeae–positive clinical samples (n = 358) submitted to Pathology Queensland Laboratory (Brisbane, Queensland, Australia) during February–September 2017 (Table 2). In brief, these samples comprised remnant nucleic acids from samples that tested positive for N. gonorrhoeae by the Cobas 4800 CT/NG test and were confirmed positive by using the in-house N. gonorrhoeae PCR (12). All samples provided negative results by the FC428 PCR, suggesting that the FC428 strain was not present in Queensland during this period.

![Figure](https://www.cdc.gov/eid/)

**Figure.** Sequence alignment showing the expected 112-bp PCR product for the PCR to detect Neisseria gonorrhoeae FC428 strain. PenA type 60.001 is provided as the reference sequence. Gray indicates the primer targets and the 311 codon within the probe target sequences. The penA sequences from the N. gonorrhoeae A8806, N. meningitidis, and N. lactamica isolates that cross-reacted with the FC428 PCR are also provided. Dots indicate sequence identity.

**Table 2. Neisseria spp. isolates and specimens tested in development of PCR to detect Neisseria gonorrhoeae FC428 strain***

| Isolates/samples | PCR results for FC428 (C<sub>t</sub>) | Probe 1 | Probe 2 |
|------------------|--------------------------------------|---------|---------|
| N. gonorrhoeae, n = 144 | | | |
| N. gonorrhoeae FC428, n = 2 (9)† | Positive (19.8 and 18.17 cycles) | Negative | Negative |
| N. gonorrhoeae H041, n = 1 (1) | Negative | Negative | Negative |
| N. gonorrhoeae A8806, n = 1 (4) | Positive (37.8 cycles) | Negative | Negative |
| N. gonorrhoeae, n = 68‡ | Negative | Negative | Negative |
| Nongonococcal species, n = 111 | | | |
| N. cinerea, n = 4 | Negative | Negative | Negative |
| N. elongata, n = 1 | Negative | Negative | Negative |
| N. flavescens, n = 1 | Negative | Negative | Negative |
| N. lactamica, n = 15 | Negative | Negative | Negative |
| N. lactamica, n = 1 | Negative | Negative | Negative |
| N. meningitidis, n = 55 | Positive (42.8 cycles) | Negative | Negative |
| N. meningitidis, n = 1 | Negative | Positive (32.6 cycles) | Negative |
| N. mucosa, n = 1 | Negative | Negative | Negative |
| N. polysacchareae, n = 4 | Negative | Negative | Negative |
| N. sicca, n = 4 | Negative | Negative | Negative |
| N. subflava, n = 14 | Negative | Negative | Negative |
| N. weaveri, n = 1 | Negative | Negative | Negative |
| Moraxella catarrhalis, n = 7 | Negative | Negative | Negative |
| M. osloensis, n = 2 | Negative | Negative | Negative |
| N. gonorrhoeae NAAT-positive clinical specimens, n = 358 | | | |
| Urogenital, n = 172 | Negative | Negative | Negative |
| Anal swab, n = 81 | Negative | Negative | Negative |
| Throat swab, n = 95 | Negative | Negative | Negative |
| Other, n = 10 | Negative | Negative | Negative |

*CT, cycle threshold; NAAT, nucleic acid amplification test. †Isolates A7846 and A7536. ‡Other local clinical isolates collected in New South Wales, Australia.
Conclusions
Overall, our results suggest that the FC428 PCR is suitable for screening for the FC428 N. gonorrhoeae clone in clinical specimens for which culture is not available. The method could prove to be a strategic tool to enhance surveillance if FC428 continues to spread. We recommend that positive results be confirmed by, for example, DNA sequencing, particularly if the strain is detected in a pharyngeal sample in which other commensal Neisseria species are prevalent.

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