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Simultaneous Detection of *Neisseria gonorrhoeae* and Fluoroquinolone Resistance Mutations to Enable Rapid Prescription of Oral Antibiotics

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Background: Absence of rapid antimicrobial resistance testing of *Neisseria gonorrhoeae* (*Ng*) hinders personalized antibiotic treatment. To enable rapid ciprofloxacin prescription, a real-time polymerase chain reaction (PCR) for simultaneous detection of *Ng* and fluoroquinolone resistance–associated *gyrA*-S91F mutation was evaluated.

Methods: Analytical NG^{RES} quantitative PCR kit (NYtor BV) performance was assessed on 50 *Ng* transcription-mediated amplification (TMA)–negative and 100 *Ng* TMA-positive samples. To assess clinical use, 200 samples were prospectively analyzed, in parallel to routine diagnostic tests. Also, 50 urine, 50 anal, 50 pharyngeal, and 50 vaginal *Ng* TMA-positive samples were retrospectively analyzed. To assess if patients carried strains with different ciprofloxacin sensitivity at different anatomical locations, 50 urine/anal or vaginal/anal sample pairs collected during a single visit were analyzed.

Results: The NG^{RES} quantitative PCR kit showed 97% sensitivity and 100% specificity for *Ng* detection and 92% sensitivity and 99% specificity for *gyrA*-S91F detection. Relative to TMA results, 85% *Ng* detection sensitivity and 99% specificity were found. Regarding the 200 prospectively analyzed clinical samples, 13 were *Ng* positive, of which 10 were also tested for antibiotic susceptibility by culture. The kit showed concordance for *GyrA*-S91F detection in 9 of 10 samples. *Ng* was detected in 96% and 94% of vaginal and urine TMA-positive samples, in 84% of anal samples and only in 22% of pharyngeal samples. Discordant ciprofloxacin sensitivity was found for 2 of 26 characterized urine/anal sample pairs.

Conclusion: The NG^{RES} quantitative polymerase chain reaction (qPCR) kit can be implemented in diagnostic testing for vaginal, urine, and anal *Ng* TMA-positive samples to enable rapid prescription of oral ciprofloxacin.

Gonorrhea, the sexually transmitted infection (STI) caused by *Neisseria gonorrhoeae* (*Ng*), is one of the most common bacterial STIs worldwide.[1] *N. gonorrhoeae* infection is treated with antimicrobial therapy; however, antimicrobial-resistant *Ng* is a major global public health concern.[2] Treatment recommendations are based on antimicrobial resistance surveillance data and change according to the prevalence of resistant *Ng* strains. Because of high genomic plasticity of *Ng* and the exposure to antibiotics without susceptibility testing before treatment, *Ng* antimicrobial resistance develops continuously.[3] The World Health Organization regularly updates their guidelines for the treatment of *Ng* infection to reduce the spread of multidrug-resistant gonococcal strains. Guidelines of 2016 included the recommendation of single-dose intramuscular ceftriaxone and oral azithromycin as first-choice treatment.[4] However, azithromycin- or ceftriaxone-resistant *Ng* strains have been reported all over the world.[2,5–8] Because alternative antimicrobial treatment options are scarce, the current treatment needs to change toward a more personalized approach.[9]

In the past, fluoroquinolones such as ciprofloxacin were effectively used as treatment of *Ng* infection. Ciprofloxacin treatment had several advantages over other antimicrobials such as oral administration and relatively few adverse effects. However, fluoroquinolones were eliminated as first-choice treatment once the World Health Organization–defined threshold for the prevalence of fluoroquinolone resistant *Ng* strains was reached.[10] Nevertheless, still large proportions of *Ng* strains remain susceptible to ciprofloxacin: 70% in the United States, more than 70% in Australia and 53% in Europe.[11–13] In the Netherlands, 65% of all *Ng* strains collected in 2018 were ciprofloxacin sensitive.[14] Because ciprofloxacin is highly efficacious in susceptible *Ng* infections of any anatomic site, the British Association for Sexual Health and HIV guidelines still suggest the use of ciprofloxacin when susceptibility is known before treatment.[15] Rapid detection of susceptibility status of clinical *Ng* isolates could therefore reintroduce treatment with oral ciprofloxacin and provide tailor-made therapy.

The mechanism of ciprofloxacin resistance in *Ng* is well defined by the presence of mutations in serine codon 91 of the DNA gyrase subunit A (*gyrA)* gene.[16,17] Previous studies have demonstrated the potency of targeting the *gyrA* gene by using a real-time PCR assay to assess ciprofloxacin susceptibility of clinical *Ng* isolates.[18–20] For even faster analysis, multiplex real-time PCR assays were developed to simultaneously detect *Ng* and *gyrA* mutations.[21,22] However, most of these assays are not easily available because of in-house development or because extensive validation for use in routine diagnostics has not been performed. In this study, we extensively assessed the performance of the NG^{RES} qPCR kit, developed by the Dutch company NYtor BV, for simultaneous detection of *Ng* and the ciprofloxacin resistance–associated *gyrA*-S91F mutation for use in diagnostic routine. The NG^{RES} qPCR kit enables rapid ciprofloxacin susceptibility testing and could allow for the prescription of oral ciprofloxacin based on the results of a single qPCR assay.

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METHODS

Sample Selection

NG^{RES} qPCR kit (NYtor BV, Nijmegen, the Netherlands) performance was assessed on clinical samples obtained from patients visiting the STI outpatient clinic of Amsterdam and on samples obtained from general practitioners who requested *Ng* testing. For samples of STI clinic visitors, routine diagnostic tests consisted of a transcription-mediated amplification (TMA) test on the Aptima Combo 2 CT/NG assay (Hologic, Marlborough, MA). *N. gonorrhoeae* culture was performed when gram-negative diplococci were present in a gram-stained smear or with material collected at a return visit when the TMA test showed an *Ng*-positive result. For *Ng* culture, the clinical sample was inoculated on a plate with BBL GC-Lect Agar (Becton, Dickinson and Company, Franklin Lakes, NJ). A pure *Ng* colony was picked for growth on a GC agar plate, enriched with 1% IsoVitaleX (BioTRADING Benelux BV, Mijdrecht, the Netherlands), and ciprofloxacin minimum inhibitory concentration (MIC) values were routinely determined with an e-test according to the manufacturer's instructions (bioMérieux SA, Marcy l'Étoile, France). According to the European Committee on Antimicrobial Susceptibility Testing, an MIC ≤0.03 μg/mL is considered as sensitive and an MIC ≥0.06 μg/mL as resistant to ciprofloxacin. Samples obtained from general practitioners were only tested with the TMA according to routine diagnostics because cultures could not be performed on samples from TMA collection tubes and additional culture material was unavailable.

Analytical performance of the NG^{RES} qPCR kit was assessed with 100 *Ng* TMA-positive and 50 *Ng* TMA-negative samples from STI clinic visitors that were randomly selected and retrospectively analyzed. Samples were run at the Public Health Laboratory in Amsterdam on the Rotorgene (Qiagen, Holden, Germany) qPCR platform. The same samples were also run on the CFX96 (Bio-Rad, Hercules, CA) by NYtor BV, and results were compared. Successful cultures were performed with additional material from these 100 *Ng*-positive patients so MIC values were available.

To compare results from the NG^{RES} qPCR kit and routine diagnostic tests, 100 urine and 100 anal samples were selected from male visitors of the STI clinic and prospectively analyzed with the kit in parallel to routine diagnostic tests and culture. Results from the Rotorgene qPCR platform were compared with TMA and culture-based results.

To assess NG^{RES} qPCR kit performance on samples from different anatomical locations, 200 *Ng* TMA-positive samples (50 urinal, 50 anal, 50 vaginal, 50 pharyngeal) were retrospectively analyzed with the kit on the Rotorgene qPCR platform. All samples were obtained from general practitioners and collected in TMA collection tubes. Because additional culture material was unavailable, MIC values were unknown.

Lastly, we determined the fraction of patients that carried *Ng* strains with different ciprofloxacin sensitivity at different anatomical locations. From 41 male patients, urine and anal samples collected during a single visit were selected and paired as well as vaginal and anal samples from 9 female patients. All resulting 50 sample pairs were analyzed with the NG^{RES} qPCR kit, and pairwise comparisons were made.

Sample Preparation

Previously collected samples had been stored in Aptima Combo 2 collection buffer (Hologic) at −20°C according to routine storage. Retrospectively analyzed samples were taken from storage, thawed, and vortexed. DNA was extracted from 200 μL of each sample by isopropanol precipitation after lysis with NucliSENS easyMAG Lysis buffer (bioMérieux SA) enriched with glycogen (40 μg/mL). The pellet was washed with 70% ethanol twice, dissolved in 50 μL Tris-HCl at pH 8.0, and stored at −20°C until use in amplification experiments. During each DNA extraction experiment, a negative control was included by adding 200 μL Aptima Combo 2 buffer to the lysis buffer.

NG^{RES} qPCR Kit

The NG^{RES} qPCR kit, developed by NYtor BV, contained a primer/probe mix designed for specific detection of the *Ng adenylate kinase (adk)* gene and ciprofloxacin resistance–associated *gyrA* single-nucleotide polymorphism S91F. During analytical validation, an additional mutation A92P in the *gyrA* probe binding region was detected, which prevented the original probe from binding. Subsequently, NYtor BV provided an adapted NG^{RES} qPCR kit, which effectuated probe binding even in the presence of the *gyrA*-A92P mutation. The adapted kit was then used for further clinical validation. Positive signals were detected in the FAM (*adk*) and VIC (*gyrA*-S91F) channels. The corresponding internal control was detected in the Cy5 channel, and the positive control generated signals in both the FAM and VIC channels.

A qPCR mix was prepared by mixing 10 μL master mix solution and 5 μL primer/probe/internal control solution (provided in NG^{RES} qPCR kit), and 5 μL DNA extract was added. Polymerase chain reactions were performed on the CFX96 and/or the Rotorgene with the following program: 1 cycle 3 minutes 95°C and 45 cycles 15 seconds 95°C and 60 seconds 60°C. Raw data were manually checked and analyzed with Bio-Rad CFX Manager software version 3.1 or Rotorgene software version 1.7.0.75, respectively. According to diagnostic routine guidelines of the Public Health Laboratory, detection of *Ng* and/or *gyrA*-S91F was confirmed in case of well-defined sigmoidal curves and Ct values <36 for both samples and controls. Samples that showed sigmoidal curves with Ct values >36 and <40 or badly shaped sigmoidal curves were repeated, and a Ct value <40 was then determined positive.

RESULTS

Concordance Between Results of CFX96 and Rotorgene qPCR Platforms

To assess the analytical performance of the NG^{RES} qPCR kit, 100 *Ng* TMA-positive and 50 *Ng* TMA-negative samples were randomly selected for retrospective analysis. One *Ng*-positive sample could not be traced back and was excluded from analysis. For all 99 *Ng* TMA-positive samples, ciprofloxacin MICs were determined on routine cultures of additional material from the same patient. All 149 samples were analyzed on both the CFX96 and the Rotorgene qPCR platforms. One sample was inhibited on both qPCR platforms. The NG^{RES} qPCR kit detected *Ng* in 96 (98%) of 98 and 94 (96%) of 98 *Ng* TMA-positive samples on the CFX96 and Rotorgene qPCR platform, respectively. Regarding the *Ng* TMA-negative samples, 50 (100%) of 50 were also negative on both platforms (Table 1).

Ciprofloxacin sensitivity of the *Ng* strains in the samples was determined for all 100 *Ng* TMA-positive samples on routine cultures of additional material from the same patient. Of all clinical samples that contained a ciprofloxacin-sensitive *Ng* strain and that were *Ng* positive with the NG^{RES} qPCR kit (CFX96: n = 53, Rotorgene: n = 51), 52 (98%) of 53 and 51 (100%) of 51 were also *gyrA*-S91F negative, so ciprofloxacin sensitive, on the CFX96 or Rotorgene qPCR platform. One *Ng* sample was *gyrA*-S91F positive on the CFX96 but negative on the Rotorgene (Table 1). Cultured urethra material of the same patient was subsequently tested as ciprofloxacin sensitive, indicating *gyrA*-S91F absence. Of all 43 clinical samples that contained a resistant *Ng* strain and were *Ng* positive with the NG^{RES} qPCR kit, *gyrA*-S91F was detected in 39 (91%)

TABLE 1. Comparison of Results From Diagnostic Tests and the NG^{RES} qPCR Kit

	Results Diagnostic Tests		
	Ng TMA Pos Culture CIP Sensitive* (n = 53†)	*Ng* TMA Pos Culture CIP Resistant* (n = 45)	*Ng* TMA Neg (n = 50)
CFX96			
Ng pos/S91F neg	52	4‡,§	0
Ng pos/S91F pos	1¶	39	0
Ng neg	0	2	50
Rotorgene			
Ng pos/S91F neg	51	3‡,§	0
Ng pos/S91F pos	0	40	0
Ng neg	2	2	50

*CIP sensitive, MIC ≤0.03 μg/mL; CIP resistant, MIC ≥0.06 μg/mL.
†Of the 100 *Ng* TMA-positive samples, one sample could not be traced back, and one sample was inhibited on both qPCR platforms.
‡Including 2 samples with A92P mutation in probe binding region (see text).
§Including samples that showed results in accordance with culture-based results after repetition (see text).
¶Resistance was not confirmed in cultured strain (see text).
CIP indicates ciprofloxacin; neg, negative; pos, positive.

of 43 and 40 (93%) of 43 samples on the CFX96 or Rotorgene qPCR platform, respectively. The *gyrA*-S91F mutation was thus not detected in 4 samples on the CFX96 and in the same 3 samples on the Rotorgene (Table 1). For the 4 samples that had shown discordant genotype results, DNA was again extracted from fresh cultures and analyzed by NYtor BV on the CFX96 platform. The *gyrA*-S91F mutation was detected in 2 of 4 samples after repetition, which was in accordance with the culture-based results. For the other 2 samples, the NG^{RES} qPCR kit was still unable to detect the *gyrA*-S91F mutation, although these samples contained ciprofloxacin-resistant *Ng* strains with MIC values ≥0.06 μg/mL. The *gyrA* genes from the 4 discordant samples were also sequenced, and it seemed that the 2 samples that remained discordant after repetition carried an additional mutation, as described hereinafter.

GyrA-A92P Mutation Prevented Probe Binding

For 2 samples, no *gyrA*-S91F mutation was detected by using the NG^{RES} qPCR kit on both qPCR platforms (Table 1), although the MIC values obtained from concomitant cultures were >32.0, which indicates ciprofloxacin resistance. These samples were collected from the anus and vagina of the same patient. The *gyrA* genes of these samples were partly sequenced, and an additional *gyrA*-A92P mutation was found, which prevented probe binding during the qPCR reaction (Fig. 1). NYtor BV provided an adapted NG^{RES} qPCR kit, which effectuated probe binding even in the presence of a *gyrA*-A92P mutation. The adapted kit was then used for further clinical validation.

Comparison of Results From NG^{RES} qPCR Kit and Routine Diagnostic Tests

NG^{RES} qPCR kit performance was compared with routine diagnostic test results with 100 urine and 100 anal samples. These

Figure 1. Sequences of the *gyrA* probe region without mutation (WT), with single S91F or with both S91F and A92P mutations. WT indicates wild type.

were selected from male visitors of the STI clinic and prospectively analyzed with the kit in parallel to routine diagnostic tests, which included culture and MIC assessment. Regarding the total number of samples, 186 (99%) of 187 were negative and 11 (85%) of 13 were positive with the kit compared with TMA results (Table 2). For 3 of 13 *Ng*-positive samples, cultures were not available so the ciprofloxacin MIC values could not be compared with the *gyrA* genotype. For the other 10 samples, ciprofloxacin sensitivity was assessed on routine cultures of additional material from the same patient. The kit detected *gyrA*-S91F in 5 (100%) of 5 samples that contained a ciprofloxacin-resistant *Ng* strain, and no mutation was detected in 4 (80%) of 5 samples that contained a ciprofloxacin-sensitive *Ng* strain. For one sample, the kit detected the *gyrA*-S91F mutation, indicating ciprofloxacin resistance, although the *Ng* strain had been assessed as ciprofloxacin sensitive on concomitant culture (Table 2).

High NG^{RES} qPCR Kit Sensitivity in Urine and Vaginal Samples

To assess NG^{RES} qPCR kit performance on samples from various anatomical locations, 200 *Ng* TMA-positive samples were retrospectively analyzed with the kit. Samples were collected from urine (n = 50), vagina (n = 50), anus (n = 50), or pharynx (n = 50). *Ng* was detected in 48 (96%) of 50 urine samples, 47 (94%) of 50 vaginal samples, and 42 (84%) of 50 anal samples. Only 11 (22%) of 50 pharyngeal samples were *Ng* positive with the kit. The *gyrA*-S91F mutation was detected in 50 (34%) of 148 samples that were *Ng* positive (Table 3). Additional material from these patients was not available for culture so *gyrA*-S91F detection could not be validated.

Patients Mostly Carried Similar *Ng* Strains at 2 Anatomical Locations

To determine the fraction of patients that carried *Ng* strains with discordant ciprofloxacin sensitivity at different anatomical locations, urine/anal or vaginal/anal samples that were collected during a single clinic visit were selected from 41 men and 9 women. All 50 sample pairs were analyzed with the NG^{RES} qPCR kit. For 15 of 41 men, the kit could not detect *Ng* in the anal sample so these sample pairs could not be further characterized. For the other 26 men, 14 men (54%) carried *gyrA*-S91F mutant *Ng* strains and 10 men (38%) carried *gyrA* wild-type *Ng* strains in the urine and anus. In the other 2 men, strains with different *gyrA* types were found in the urine and anus: 1 man carried a *gyrA*-S91F mutant strain in the urine and a *gyrA* wild-type strain in the anus, and 1

TABLE 2. Comparison of Results From NG^{RES} qPCR Kit and Routine Diagnostic Tests: TMA Test and Culture-Based Antibiotic Susceptibility Testing

	Results TMA/*Ng* Culture			
	***Ng* TMA Neg (n = 187)**	***Ng* TMA Pos (n = 13*)**	***Ng* TMA Pos Culture CIP Sensitive† (n = 5*)**	***Ng* TMA Pos Culture CIP Resistant† (n = 5*)**
Results NG^{RES} qPCR kit				
Ng pos	1	11	—	—
Ng neg	186	2	—	—
Ng pos/S91F neg	—	—	4	0
Ng pos/S91F pos	—	—	1	5

*From 3 of 13 *Ng*-positive sample, cultures were not available so ciprofloxacin sensitivity could not be compared with the detected *gyrA* genotype.
†CIP sensitive, MIC ≤0.03 μg/mL; CIP resistant, MIC ≥0.06 μg/mL.
CIP indicates ciprofloxacin; neg, negative; pos, positive.

man carried a *gyrA*-S91F mutant strain in the anus and a *gyrA* wild-type strain in the urine.

For 2 of 9 women, the kit could not detect *Ng* in the anal sample so these sample pairs could not be further characterized. The other 7 women carried *gyrA* wild-type *Ng* strains in both the vagina and the anus. Regarding ciprofloxacin susceptibility, 10 (38%) of 26 men carried only ciprofloxacin-sensitive *Ng* strains in contrast to 7 (100%) of 7 women.

DISCUSSION

We assessed the performance of the NG^{RES} qPCR kit for simultaneous detection of *Ng* and the ciprofloxacin resistance–associated *gyrA*-S91F mutation. Use of this kit may allow for the prescription of oral ciprofloxacin based on the results of a single qPCR assay, which takes 4 hours including DNA extraction from the clinical sample. Implementation of the kit in diagnostic screening would increase the turnaround time with about half a day. However, this facilitates more rapid antibiotic sensitivity analysis compared with the current susceptibility testing based on bacterial cultures, which require at least 2 days. A detailed cost-benefit analysis for implementation is hard to perform because both costs and benefits, such as the use of an oral instead of an injectable antibiotic, are very much depending on the local situation.

The NG^{RES} qPCR kit showed good performance compared with our current daily routine tests, which include the highly sensitive TMA test for *Ng* detection and culture for antimicrobial sensitivity testing. Only 5 samples showed discordant results between culture-based determination of ciprofloxacin sensitivity and detection of the *gyrA*-S91F mutation with the NG^{RES} qPCR kit (Tables 1, 2). These samples either showed concordant results after repeated culture, DNA extraction, and analysis, or the discrepancy was caused by an additional *gyrA*-A92P mutation, which prevented probe binding in the initial, nonoptimized kit format. Assessing the type of errors in the discrepant results is important to determine its clinical impact. When typing is impossible, treatment recommendation remains ceftriaxone. False-positive *gyrA*-S91F detection would result in not using ciprofloxacin, which is not of clinical concern. However, false-negative *gyrA*-S91F detection would result in using ciprofloxacin to treat-resistant infections, which certainly is of clinical concern. In our study, only 2 samples were false negative for *gyrA*-S91F (Table 1). For these samples, the Ct value for *Ng* detection was high (>36), which indicates a low bacterial load. When using the NG^{RES} qPCR kit in clinical practice, samples with such high Ct values should be repeated or assessed as nontypable. Because only 2 samples gave false-negative results, we are confident that the kit performance is sufficient and will increase if a certain cutoff Ct value is established by further validation in clinical practice.

TABLE 3. NG^{RES} qPCR Kit Analysis Results of 200 *Ng* TMA-Positive Samples Originating From Different Anatomical Locations

	NG^{RES} qPCR Kit Results	
	***Ng* Positive**	***gyrA* Mutant***
Urine (n = 50)	48 (96%)	16 (33%)
Vagina (n = 50)	47 (94%)	9 (19%)
Rectum (n = 50)	42 (84%)	18 (43%)
Pharynx (n = 50)	11 (22%)	7 (64%)
Total (n = 200)	148 (74%)	50 (34%)

*Ciprofloxacin sensitivity as detected with the NG^{RES} qPCR kit because cultures were not available.

SpeeDx recently developed the ResistancePlus assay for dual detection of *Ng* and *gyrA*-S91F mutation, which is already approved and commercially available.[22] Ebeyan et al[22] also reported 2 samples that gave discordant results when comparing culture and genotyping with this ResistancePlus assay. The results of one sample became concordant after repetition and one sample remained discrepant. A possible explanation for the discrepancies observed by Ebeyan et al and these in our study could be that patients are infected with multiple *Ng* strains at the same anatomical location, and that strains with discrepant ciprofloxacin sensitivity were taken for culturing and for genotyping with the NG^{RES} qPCR kit. Such mixed infections have been reported to occur in high-risk populations, albeit at low prevalence.[23,24] When implementing the kit in clinical practice, it should be further validated to what extent mixed infections influence the genotyping results.

When assessing the performance of the NG^{RES} qPCR kit on samples from different anatomical locations, the NG^{RES} qPCR kit showed excellent results for urine and vaginal samples. *N. gonorrhoeae* detection sensitivity was lower in anal samples, but for pharyngeal samples, the sensitivity was clearly insufficient, suggesting a low bacterial load in throat swabs as described previously.[25,26] Compared with the previously published real-time PCR assay developed by Hemarajata et al,[18] the NG^{RES} qPCR kit demonstrated higher ability to characterize the genotype. Compared with the assay developed by Ellis et al,[20] higher genotyping sensitivity was found for urine and rectal samples but lower sensitivity for pharyngeal samples. The ResistancePlus assay reported by Ebeyan et al[22] showed better *gyrA*-S91F detection sensitivity and specificity, especially in pharyngeal samples. However, anal samples were missing in the validation of this assay, which seemed difficult to characterize in our study and could have an effect on the proportion of characterized samples. Also, initial *Ng* screening was not done with the Aptima Combo 2 but with other commercial assays, so direct comparison is not possible. Recently, Allan-Blitz et al[27] showed that the ResistancePlus assay was able to genotype some of the samples that were nontypable in the method described by Ellis et al. Comparing the NG^{RES} qPCR kit with the ResistancePlus assay would be valuable. Although the NG^{RES} qPCR kit could not

characterize a small proportion of the anal samples, by analyzing urine/anal and vaginal/anal sample pairs that were obtained from one patient during a single visit, we showed that only 2 of 33 patients carried *Ng* strains that differed in ciprofloxacin sensitivity, whereas the other 31 patients had strains with similar ciprofloxacin sensitivity. This indicated that analysis of only the urine or vaginal sample without the anal sample would be suitable for guided ciprofloxacin treatment for most cases.

When assessing the performance of the kit on samples from different anatomical locations, 148 of 200 samples could be characterized. In 66% of these samples, a *gyrA* wild-type *Ng* strain was detected, which is in accordance with the previously published fraction of 65% of ciprofloxacin-sensitive strains in the Netherlands.[14] The presence of this significant fraction of ciprofloxacin-sensitive *Ng* strains shows that ciprofloxacin can still be used for treatment of *Ng* detection if rapid ciprofloxacin sensitivity detection is part of routine diagnostics. We suggest to implement the NG^{RES} qPCR kit in addition to a highly sensitive assay. In our setting, we used the TMA test, which proved to be very suitable for first screening of *Ng*-suspected samples, followed by analysis of *Ng* TMA-positive samples with the NG^{RES} qPCR kit. Based on the genotype determined by the NG^{RES} qPCR kit, physicians could be advised if treatment with ciprofloxacin is indicated. Effectiveness of the assay should be carefully validated by comparing results with current diagnostic test results, and ciprofloxacin use could be monitored by a test-of-cure study.

Results of the analysis of sample pairs from 26 men and 7 women showed that 38% of the men and 100% of the women carried *gyrA* wild-type *Ng* strains. Although the number of men included in the study was much higher than the number of women, this result suggests that men are more often carrying resistant *Ng* strains. It has been reported that pharyngeal *Ng* infections are particularly important in transmission networks of men who have sex with men and that *Neisseria* species could exchange resistance determinants at the pharyngeal site.[28] Because 80% of the STI clinic visitors are men who have sex with men, this could explain the high percentage of men carrying ciprofloxacin-resistant strains. However, further research on transmission networks of ciprofloxacin-resistant strains among general populations is needed.

In conclusion, our results show that the NG^{RES} qPCR kit is suitable for concomitant detection of *Ng* and *gyrA*-S91F, especially in urine and vaginal clinical samples, to enable rapid prescription of ciprofloxacin.

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