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ORIGINAL ARTICLE

Prevalence of *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium* and *Trichomonas vaginalis* including relevant resistance-associated mutations in a single center in the Netherlands

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Abstract

Purpose In this study, we report the prevalence of *Chlamydia trachomatis* (CT), *Neisseria gonorrhoeae* (NG), *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) amongst clinical specimens of patients suspected for sexually transmitted infections received at our laboratory and in addition report the prevalence of resistance-associated mutations (RAM) for ciprofloxacin in NG and azithromycin and moxifloxacin in MG.

Methods All specimens received from December 2018 to May 2019 were tested for the four pathogens. In addition, the presence of RAM associated with resistance to ciprofloxacin in NG and to azithromycin and moxifloxacin in MG was determined by different real-time PCR assays on all NG- and MG-positive specimens.

Results CT was detected most often (267/2613, 10.2%), followed by MG (106/2592, 4.1%), NG (41/2613, 1.6%) and TV (10/2592, 0.4%) amongst all specimens. The prevalence of ciprofloxacin RAM in NG was 21.2%, and the prevalence of RAM in MG was 40.6% for azithromycin and 8.1% for moxifloxacin. Nearly all specimens containing moxifloxacin-resistant MG also contained azithromycin-resistant MG.

Conclusion CT is found most often in our population followed by MG and NG. By using molecular assays to detect RAM supplementary to pathogen identification of NG and MG, optimal therapy can be advised.

Keywords Antimicrobial resistance · Fluoroquinolone · Macrolide

Introduction

Chlamydia trachomatis (CT), *Neisseria gonorrhoeae* (NG), *Mycoplasma genitalium* (MG) and *Trichomonas vaginalis* (TV) are pathogens responsible for a variety of sexually transmitted infections (STIs) which can be symptomatic as well as asymptomatic. In the Netherlands, first-line STI screening for high-risk groups consists of, amongst others, nucleic acid amplification testing (NAAT) for CT and NG. All other patients are tested for CT only, unless specific symptoms warrant more extensive diagnostics. [1] However, since available NAAT assays almost exclusively include both CT and NG, NG is usually tested as well. Testing for MG and TV is only advised when specific symptoms are presented and/or in case of persistent symptoms and after testing negative for both CT and NG. The prevalence of these 4 STI pathogens in the Netherlands varies amongst different studies performed, depending on population, region and settings. In most studies, CT was identified predominantly, followed by MG. [2–4]

Management of NG and MG infections can be challenging. In the Dutch situation, NG is treated primarily with ceftriaxone. [1] Although treatment failure using ceftriaxone has been reported in Europe, no resistant isolates

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have been found in the Netherlands so far. [5] Alternatively, ciprofloxacin might be used as option for oral treatment, but since resistance rates in the Netherlands are increasing from < 30% (2017) up to 50% (2019), susceptibility testing should be performed prior to treatment. [1, 5, 6] Dutch guidelines advise azithromycin as first-choice antibiotic to treat MG infection. [1] Azithromycin resistance rates as determined by evaluating the presence of resistance-associated mutations (RAM) are reported to be 20–44% in the Netherlands, and show an increase over the years. [7–9] In case of treatment failure, moxifloxacin is advised. [1] No data on the prevalence of MG moxifloxacin RAM is available for the Dutch situation, although the finding of a multi-resistant MG indicates the presence of moxifloxacin-resistant MG in the Netherlands. [10]

The objective of our study was to report the prevalence of the STI pathogens CT, NG, MG and TV amongst specimens received for STI diagnostics at our laboratory in the middle of the Netherlands and to determine the prevalence of RAM for ciprofloxacin in NG and azithromycin and moxifloxacin in MG.

Methods

All consecutively received ano- and urogenital and throat specimens for STI diagnostics at the laboratory for microbiology of the Meander Medical Center (a tertiary care center in the middle of the Netherlands), during the period of December 2018–May 2019 were included in the study and tested for CT, NG, MG and TV. Specimens originated from both primary care and hospital care; no specimens from STI clinics were received. Of all specimens, only data as available upon test application could be used: gender, age, sample material and applicant (Table 1).

Detection of CT, NG, MG and TV

CT and NG were tested using the CE-IVD RealTi*m*e CT/NG assay (Abbott, Des Plaines, IL, USA) and were performed on the Abbott *m*2000system. Besides adding 5 μl internal control as included in the CE-IVD RealAccurate® TVMGres PCR kit (PathoFinder B.V., Maastricht, Netherlands) prior to extraction, the CT/NG assay was performed according to the manufacturer's instructions.

Table 1 Overview of the specimens included in this study

	CT/NG						TV/MG					
	M			F			M			F		
	N	CT (%)	NG (%)	*N*	CT (%)	NG (%)	*N*	TV (%)	MG (%)	*N*	TV (%)	MG (%)
Specimen type												
Genital swab	91	10 (11.0)	5 (5.5)	1607	154 (9.6)	12 (0.7)	90	1 (1.1)	10 (11.1)	1593	6 (0.4)	61 (3.8)
Anal/rectal swab	36	1 (2.8)	3 (8.3)	25	2 (8.0)	0 (-)	38	0 (-)	2 (5.3)	25	0 (-)	0 (-)
Urine	643	85 (13.2)	20 (3.1)	67	5 (7.5)	0 (-)	638	1 (0.2)	27 (4.2)	65	1 (1.5)	3 (4.6)
Throat swab	40	1 (2.5)	1 (2.5)	39	4 (10.3)	0 (-)	40	0 (-)	0 (-)	39	0 (-)	0 (-)
Unknown	6	0 (-)	0 (-)	59	5 (8.5)	0 (-)	5	0 (-)	0 (-)	59	1 (1.7)	3 (5.1)
Age category												
0–10	0	0 (-)	0 (-)	7	0 (-)	0 (-)	0	0 (-)	0 (-)	7	0 (-)	0 (-)
11–20	96	18 (18.8)	5 (5.2)	326	56 (17.2)	6 (1.8)	96	1 (1.0)	2 (2.1)	324	0 (-)	6 (1.9)
21–30	353	60 (17.0)	11 (3.1)	689	80 (11.6)	5 (0.7)	351	0 (-)	13 (3.7)	680	6 (0.9)	28 (4.1)
31–40	161	9 (5.6)	5 (3.1)	395	15 (3.8)	1 (0.3)	161	1 (1.0)	16 (9.9)	390	1 (0.3)	24 (6.2)
41–50	107	8 (7.5)	0 (-)	250	14 (5.6)	0 (-)	106	0 (-)	5 (4.7)	250	1 (0.4)	8 (3.2)
51–60	69	1 (1.4)	7 (10.1)	105	3 (2.9)	0 (-)	69	0 (-)	3 (4.3)	105	0 (-)	1 (1.0)
61–70	27	1 (3.7)	1 (3.7)	20	2 (10)	0 (-)	25	0 (-)	0 (-)	20	0 (-)	0 (-)
> 70	3	0 (-)	0 (-)	5	0 (-)	0 (-)	3	0 (-)	0 (-)	5	0 (-)	0 (-)
Applicant												
General practitioner	771	93 (12.1)	25 (3.2)	1432	155 (10.8)	10 (0.7)	767	2 (0.3)	37 (4.8)	1419	6 (0.4)	57 (4.0)
Outpatient clinic	24	1 (4.2)	3 (12.5)	320	12 (3.8)	1 (0.3)	23	0 (-)	0 (-)	317	1 (0.3)	7 (2.2)
Hospital	11	3 (27.3)	0 (-)	25	1 (4.0)	0 (-)	11	0 (-)	2 (18.2)	25	1 (4.0)	1 (4.0)
Other	10	0 (-)	1 (10)	20	2 (10)	1 (5.0)	10	0 (-)	0 (-)	20	0 (-)	2 (10)
Total	816	97 (11.9)	29 (3.6)	1797	170 (9.5)	12 (0.7)	811	2 (0.2)	39 (4.8)	1781	8 (0.4)	67 (3.8)

Remnant eluate of the *m*2000 extraction was tested using the RealAccurate® TVMGres PCR assay, that detects and differentiates MG and TV. [11, 12] Real-time PCR was performed according to the manufacturer's instructions using an ABI7500 system (Applied Biosystems, Foster City, CA, USA).

Detection of RAM

The presence of fluoroquinolone RAM in NG was tested using remnant eluate of the *m*2000 extraction and the RUO NG-FQ^{res} qPCR assay (NYtor B.V., Nijmegen, the Netherlands), that simultaneously detects NG and ciprofloxacin RAM. [13] Prevalence of this RAM was determined amongst all specimens that tested positive for NG with the Abbott CT/NG assay, but could only be determined when NG (targeting adenylate kinase, *adk*) tested positive in this assay.

Detection of azithromycin and moxifloxacin RAM in MG was tested by 2 different real-time PCR assays. Azithromycin RAM were determined by the RealAccurate® TVMGres PCR assay. For the detection of moxifloxacin RAM in MG, the RUO MG-FQres qPCR assay (NYtor B.V.) was used. Both assays combine detection of MG (targeting MgPA) and respective RAM. Presence of RAM in the assays could only be determined when MG tested positive as well.

For details of all procedures as described above, refer to online resource 1.

Nucleotide sequence analysis

Sanger sequencing was performed for all MG-positive, RAM-containing specimens to determine the prevalence of the various RAM of both azithromycin and moxifloxacin in MG. Procedures of this sequencing analysis are described in online resource 2.

Results and discussion

Detection of CT, NG, MG and TV

A total of 2615 specimens obtained from 2354 unique patients were included in this study. A single specimen was received from the majority of patients (92%), 2 and 3 or more specimens were received from 5.9% and 2.1% of the patients, respectively. Characteristics of specimens included in this study as well as percentage of different pathogens per specimen identified per category (specimen, age distribution, applicant) are shown in Table 1.

Conclusive results were available for 2613 specimens when tested for CT and NG (inhibition rate 0.1%), and 2592 for TV and MG (inhibition rate 0.9%). Of the conclusive specimens, CT was detected most often (10.2%), followed by MG (4.1%), NG (1.6%) and TV (0.4%), corresponding to 10.9%, 4.4%, 1.6% and 0.4% in patients, respectively. Twenty-nine co-infections were found amongst all specimens, comprising 13 CT/NG, 7 CT/MG, 5 CT/TV, 2 NG/MG and 2 TV/MG. The distribution of pathogens identified is comparable with earlier similar Dutch studies, although a lower prevalence of CT was found at that time: 5–7% in studies performed from 2013 to 2015. [2, 3] Retrospective analysis of our local data showed a CT prevalence comparable with the earlier studies and an increase in detecting CT from 7.4% (2013) to 10.2% (2019) amongst all tested specimens (unpublished data). The increasing detection of CT is in line with national data obtained from sexual health centers in the Netherlands in 2018. [5]

Since in the Netherlands TV and MG are not part of the regular STI screenings and it is recommended to only test when specific symptoms are present or in case of ongoing complaints, no surveillance on the prevalence of these pathogens is available. The results of this study contribute to an overview of the prevalence of these pathogens. TV and MG were detected in 0.4% and 4.1% of all the specimens included in the study, respectively. The prevalence of MG might be underestimated, since (adapted) real-time PCR assays targeting the MgPA gene were used, [14] while transcription-mediated amplification assays showed a superior sensitivity resulting in a higher proportion of MG positives. [15, 16]

Detection of RAM

The NG-FQ^{res} qPCR assay successfully identified 33 of the 41 NG-positive specimens (Table 2). Six eluates were no longer available and in 2 NG *adk* identification remained negative. The latter can be explained by a difference in sensitivity of the used assays. The Abbott CT/NG assay targets the *opa* multicopy gene [17], whereas the NG-FQ^{res} qPCR assay targets adenylate kinase (*adk*).

Ciprofloxacin RAM were identified in 21.2% of the tested specimens, which is relatively low compared with the 50% as found by the Dutch "gonococcal resistance against antibiotics surveillance" (GRAS) project (2019) using culture. [5] This difference cannot be explained by the method used, as earlier studies have shown that molecular detection of RAM responsible for a gyrase S81F mutation has high agreement with results found by susceptibility testing by culture. [18] This is also confirmed by a recent study to evaluate the NG-FQ^{res} qPCR assay. [13] The difference in ciprofloxacin resistance is most likely explained by the population and region tested. The GRAS project mostly includes patients visiting sexual health centers, in contrast to our study in which the main applicant is the general practitioner (Table 1). Antibiotic treatment of NG infections with ciprofloxacin might thus be an option in our population, but only when therapy-guiding diagnostics are used.

Table 2 Prevalence of RAM found in specimens included in the study

	Tested	Total (%)	Men (%)	Women (%)
NG ciprofloxacin RAM	33 (M: 22, F: 11)	7 (21.2)	5 (22.7)	2 (18.2)
MG azithromycin RAM	106 (M: 39, F: 67)	43 (40.6)	15 (38.5)	28 (41.8)
MG moxifloxacin RAM	86 (M: 31, F: 55)	7 (8.6)	4 (12.9)	3 (5.5)

Azithromycin RAM in MG were detected in parallel with MG detection and tested positive for 43 (40.6%) of the specimens (Table 2). This is slightly higher than found in previous Dutch studies, ranging from 20.9 to 34% [7–9], but is obviously depending on years of study, included patients and region in which is tested. Nucleotide sequence analysis showed clear results for 38 specimens. As shown in Table 3, A2058G and A2059G were mainly identified, which is in line with earlier studies [7, 9].

The MG-FQ^{res} qPCR assay showed a positive MG result by detecting MgPA in 86 (81.1%) of the MG-positive specimens (Table 2). Seven samples (8.1%) tested positive for the fluoroquinolone RAM. As shown in Table 3, RAM S83I, D87N and D87Y were confirmed by sequence analysis. In six of the specimens, corresponding to the same number of patients, a multidrug-resistant MG was present, since both azithromycin RAM and moxifloxacin RAM were detected. To the best of our knowledge, this is the first study from the Netherlands to evaluate the prevalence of both azithromycin and moxifloxacin resistance amongst MG-positive patients. Considering the outcome that multidrug-resistant MG isolates are not rare, it seems highly advisable to routinely perform subsequent resistance testing for at least azithromycin as recommended earlier [19].

Conclusion

This study shows that CT is identified most often as pathogen causing STIs, but TV and especially MG should not be forgotten in the diagnostic algorithm and be tested when initially no CT/NG is identified in symptomatic patients. Moreover, by using molecular assays to detect RAM in NG and MG, optimal treatment can be advised.

Table 3 Distribution of azithromycin RAM ($n = 38$) and moxifloxacin ($n = 7$) in MG-positive specimens identified in this study as determined by sanger sequencing

		N (%)
Azithromycin[a]	A2058G	15 (41.5)[c]
	A2058T	7 (17.1)
	A2059G	16 (39.0)[d]
Moxifloxacin[b]	S83I	3 (42.9)
	D87N	3 (42.9)[c]
	D87Y	1 (14.2)

[a] According to *E. coli* numbering

[b] According to *M. genitalium* numbering

[c] Of which 1 combined with WT

[d] Of which 2 combined with WT

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Code availability Not applicable.

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Data availability Data is available upon request. Material is available when stored and upon request.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval The medical research involving human subjects act (WMO) does not apply to this study as concluded by the Medical Research Ethics Committees United (MEC-U, Nieuwegein, the Netherlands).

Informed consent Not applicable. A patient and corresponding diagnostic data was included only if the patient had not specifically indicated that sample material and data could not be used for other purposes than diagnosis of disease, as regulated by law and stated in “Human tissue and medical research: code of conduct for responsible use” (2011).

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