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Macrolide-Resistant *Mycoplasma genitalium* Impairs Clinical Improvement of Male Urethritis After Empirical Treatment

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Background: *Mycoplasma genitalium* (MG) is associated with urethritis in men and could play a role in clinical outcome. We examined clinical improvement of symptoms in men receiving empirical treatment for urethritis and correlated the outcome with *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), MG, and MG macrolide resistance-associated mutations (MRAM) status.

Methods: At the sexually transmitted infection clinic in Amsterdam, the Netherlands, empirical treatment for gonococcal urethritis is 1 g ceftriaxone and for nongonococcal urethritis 1 g azithromycin. In 2018 to 2019, we tested urine samples of men with urethritis for CT, NG, and MG using transcription-mediated amplification assays. *Mycoplasma genitalium*–positive samples were tested for MRAM using quantitative polymerase chain reaction. Two weeks after receiving therapy, men were sent a text message inquiring after clinical improvement.

Results: We evaluated 2505 cases of urethritis. The positivity rates of NG, CT, and MG were 26% (648 of 2489), 29% (726 of 2489), and 23% (522 of 2288), respectively. In 768 of 2288 of the cases (34%), no causative agent was detected. Most cases were infected with a single pathogen: NG, 417 of 2288 (18%); CT, 486 of 2288 (21%); and MG, 320 of 2288 (14%). The prevalence of MRAM among MG-positives was 74% (327 of 439). For 642 (25.6%) cases, we could evaluate clinical improvement after treatment of whom 127 (20%) indicated no improvement; 9% (15 of 174) in NG cases, 18% (35 of 195) in CT cases, 14% (4 of 28) in MG wild-type cases, and 40% (38 of 94) in MG-MRAM cases. Clinical improvement in MG-MRAM cases was significantly lower compared with all other groups ($P < 0.001$).

Conclusions: Presence of MG-MRAM is associated with lack of clinical improvement in azithromycin-treated nongonococcal urethritis.

Acute urethritis in men is mostly caused by sexually transmitted infection (STI) *Chlamydia trachomatis* (CT) or *Neisseria gonorrhoeae* (NG).[1] Distinction between gonococcal urethritis and nongonococcal urethritis (NGU) can be readily made by examination of Gram stains of discharge.[2] Empirical treatment is often preferred over delay of treatment awaiting definite molecular test results. Empirical treatment in cases of gonococcal urethritis is ceftriaxone with or without azithromycin.[3–5]

In most clinical guidelines, identifying a pathogen in NGU is based on molecular testing.[3,4,6,7] Recommendations on what pathogens to test vary somewhat, depending on local prevalence. All guidelines recommend testing for CT. The association of *Mycoplasma genitalium* (MG) with NGU was first described in the 1980s,[8] and in the last 2 decades only after molecular tests became available, its role has become more apparent.[1,9–11] Some guidelines, such as the Australian,[6] have rapidly incorporated testing for MG. In contrast to Australian[6] and European guidelines,[7] the Centers for Disease Control and Prevention[3] and Dutch[4] guidelines currently do not recommend standard screening for MG in urethritis, but only in persisting or recurrent NGU.

Different treatment recommendations exist also for treatment of NGU, in part as a consequence of different testing guidelines for MG. Dutch guidelines[4] still recommend the macrolide azithromycin (1 g single dose), whereas Australian[6] and European guidelines[7] recommend doxycycline (100 mg twice daily for 7 days). The Centers for Disease Control and Prevention changed its recommendation from single-dose azithromycin to a course of doxycycline in their recently updated guidelines.[3] According to the Australian guidelines, patients with MG urethritis should be additionally treated with azithromycin (1 g stat then 500 mg daily for 3 days) or moxifloxacin (400 mg daily 7 days) after doxycycline resulting in a 10- to 14-day treatment course.[6] The European guidelines recommend treatment with azithromycin (500 mg stat, then 250 mg for 4 days) in case of MG urethritis instead of treatment with doxycycline.[7]

According to a recent study performed at the STI clinics in Amsterdam and The Hague in 2018, the prevalence of MG among men with urogenital symptoms at the STI clinic in Amsterdam is 29%, whereas it was only 6% in 2014.[12,13] Among the MG positive samples in the recent study, MG macrolide resistance-associated mutations (MRAM) was detected in 66%.[13]

A review showed that there is a correlation between MG infection and persistent or recurrent NGU.[14] Read et al.[15] observed that

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recurrent or persistent urethral symptoms were more common in patients without microbiological cure (34 of 44 [77%]; 95% confidence interval [CI], 62%–89%) compared with those who were cured (10 of 63 [16%]; 95% CI, 8%–28%; $P < 0.001$) after azithromycin treatment. Treatment failure was thus associated with MG-MRAM. This—in combination with the high prevalence of MG and rapid increase of antibiotic resistance[16]—may indicate that current Dutch treatment guidelines for NGU need to be updated and take MG presence and MRAM into account. This study was designed to evaluate the clinical outcome of empirical treatment for both gonococcal urethritis and NGU and whether MG infection and MRAM are of influence.

METHODS

Setting of the Clinic

The study was performed at the STI clinic in Amsterdam, the Netherlands. The clinic is part of the Public Health Service, which means it is free of charge for members of the public at risk for STI, with or without referral. Diagnostics and treatment, including empirical treatment, are done according to Dutch guidelines.[4] Testing in cases of male urethritis according to those guidelines includes CT and NG, but not MG. Testing for MG is currently only considered optional in cases of (persisting) urethritis. We performed this study to assess if testing for MG would benefit our patient population.

Cases Included

From May 2018 to November 2019, we included all men with urethritis at the STI outpatient clinic in Amsterdam. Men received standard care and treatment and could be included for different episodes. Urethritis was defined as presence of >10 leucocytes per high-power field in Gram stains of urethral discharge. Symptoms were dysuria, discharge, or urethral discomfort. Additional presence of intracellular gram-negative diplococci defined a presumptive diagnosis of gonococcal urethritis.

Treatment

Empirical treatment for gonococcal urethritis was 1 g ceftriaxone given intramuscularly and for NGU 1 g azithromycin per os, according to local and national guidelines.[4] When the use of macrolides was contraindicated in a man with NGU, doxycycline 100 mg twice daily for 1 week was prescribed. Patients were asked to return to the clinic when molecular diagnostics showed NG and patients had not been given ceftriaxone previously, when rectal CT was detected and additional treatment with doxycycline needed, and when syphilis was detected and additional benzathine penicillin was needed.

Molecular Detection of STIs

First-void urine was collected and routinely tested for NG and CT (Aptima Combo 2; Hologic Inc, San Diego, CA). Samples with equivocal results were retested using the Aptima CT single assay (Hologic Inc) and for NG with a quantitative polymerase chain reaction targeting *opa* genes.[17] Urine samples were tested for MG using transcription-mediated amplification (TMA) assays (Aptima, Hologic Inc). From all samples that tested positive for MG in the MG-TMA assay, DNA was extracted with isopropanol precipitation and subsequently tested for macrolide resistance using the MG-MRAM quantitative polymerase chain reaction to detect wild-type (MG-WT) or any mutations (MRAM) in the *23S rRNA* gene at nucleotide positions 2058 and 2059 (*Escherichia coli* numbering).[18] *Mycoplasma genitalium* and MRAM testing were done for study purposes only, and results were disclosed neither to the men nor to the healthcare professionals. A subset of samples was tested for *Trichomonas vaginalis* (TV) using TMA assays (Aptima, Hologic Inc).

Text Message

Men diagnosed with urethritis at initial consultation were informed about and asked to respond to an online survey sent to them by text message 2 weeks later. The text message was sent in Dutch and English. The question was, "How are the physical complaints that caused you to visit our clinic?" The answer options provided were as follows: "I did not have physical complaints," "The physical complaints are gone," "I'm doing better," "The same, not improved," and "I'm doing worse."

Statistical Analysis

Only men with an initial consultation for urethritis were included in the analysis. Sociodemographic data, sexual behavior, the presence of clinical symptoms, recent antibiotic treatment, and antibiotics prescribed for the current case were extracted from electronic patient files. The variable "Education" was categorized into low (no education, primary school, lower secondary vocational education and intermediate secondary general education), mid (higher secondary general education, senior secondary vocational education, and preuniversity secondary education), and high (higher professional or university education). In the analysis, the answers "The physical complaints are gone" and "I'm doing better" were combined into one category, "improvement"; the answers "The same, not improved" and "I'm doing worse" were combined into one category, "no improvement." We performed an additional analysis with a more stricter definition of clinical improvement: (1) the physical complaints are gone and (2) still having physical complaints (combining the other 3 answers in one category). The following men with urethritis were considered evaluable for clinical improvement analysis: men who indicated to have symptoms at the time of the consultation; who were tested for NG, CT, and MG; who were informed about the text message; who responded within 28 days after treatment; and of whom text message data were recorded. Treatments that were considered in analysis were treatments given up to 2 days before the completion of the online questionnaire regarding clinical improvement; several patients have received additional treatment after that time point. Univariable logistic regression analysis using generalized estimating equation was performed to compare the characteristics and infections of evaluable and nonevaluable cases and to compare cases indicating improvement to those reporting no improvement. Factors associated with clinical improvement were examined using generalized estimating equation logistic regression analysis. Variables included a priori in multivariable analysis were NG, CT, MG genotype, and age; variables with $P < 0.20$ in univariable analysis were considered in multivariable analysis, but only retained if $P < 0.05$. Significance was assessed 2-sided for all variables, applying a cutoff value of $P < 0.05$. Data were analyzed using Stata Intercooled 15 (StataCorp LLC, College Station, TX).

Ethics Statement

Men of the STI outpatient clinic Amsterdam were informed of the "opt-out" system regarding research on remnants of patient material. All data were pseudonymized before analysis. The study protocol was evaluated by the Medical Ethics Committee of the Academic Medical Center in Amsterdam (W18.013#18.024) and deemed not to require a full review of the board; signed informed consent was not deemed to be required.

RESULTS

Characteristics and Infection Status of All Urethritis Cases

We registered 2505 cases of urethritis in 2095 men in the study period (Fig. 1 and Supplementary Table 1, http://links.lww.

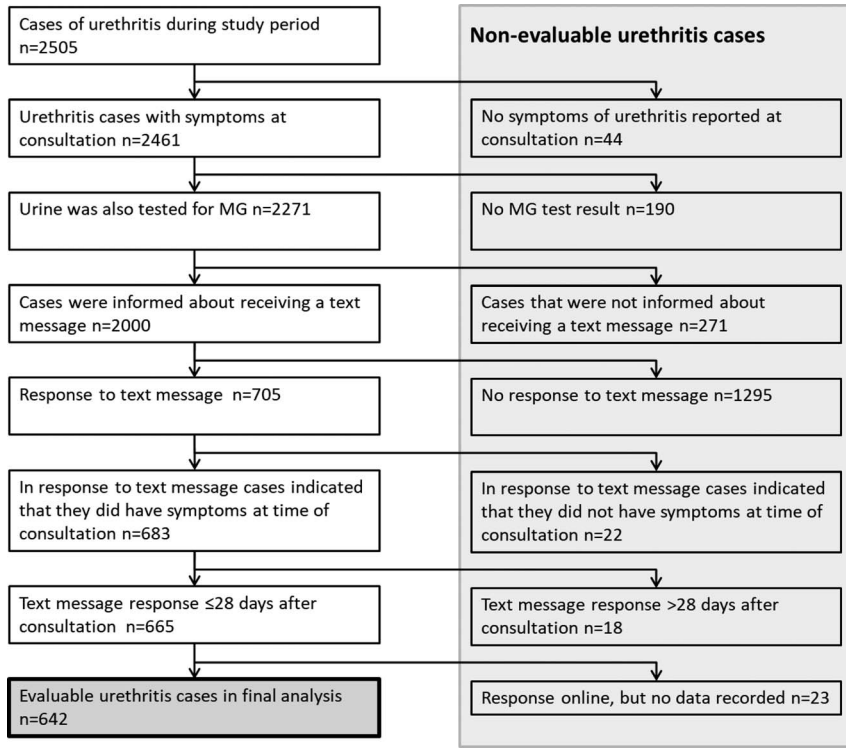

Figure 1. Flowchart of men included in the study. In the study period 2505 urethritis cases in 2095 men were observed. In the final analysis, data of 642 cases of 593 men were included, who were tested for *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG) and responded within 28 days to the text message. N, number of urethritis cases.

com/OLQ/A781). Mean (SD) age was 31.2 (10.4) years. The majority of cases were men who have sex with men (53%), most were of Dutch origin (41%) and had a high education level (63%), and 12% of the cases were HIV positive. *Neisseria gonorrhoeae*, CT, and MG test results were available from 2288 urethritis cases. In 768 (34%) cases, none of the 3 microorganisms were detected (Table 1). *Neisseria gonorrhoeae*, CT and MG were detected in, respectively, 26%, 29%, and 23% of urethritis cases. *Mycoplasma genitalium* MRAM genotyping was successful in 439 of 522 MG positives (84%). Macrolide resistance-associated mutation was detected in 74% (327 of 439) of the successful MG-MRAM genotyped cases. Single infections were detected in 18% for NG, 21% for CT, and 14% for MG. Dual infections occurred in 4% for each pathogen combination and a triple infection in 1% of the urethritis cases (Table 1).

No Differences in Infection Rates Between Evaluable and Nonevaluable Cases

In 642 (25.6%) cases, clinical improvement after empirical treatment could be evaluated (Fig. 1). Evaluable cases were older (mean age, 32.7 vs. 30.7 years; $P < 0.001$), were more often men who have sex with men ($P = 0.002$), were more often of Dutch origin ($P = 0.042$), and reported less often to have had urethritis symptoms in the past 2 years ($P = 0.037$) compared with nonevaluable cases (Supplementary Table 1, http://links.lww.

TABLE 1. Infection Status (Single, Dual, or Triple) of Urethritis Cases, by Evaluation Status and Reported Improvement, STI Clinic in Amsterdam, May 2018 to November 2019

NG	CT	MG	Total, n (%)	Evaluable*, n (%)	No Improvement in Evaluable Cases†, n (%)
−	−	−	768 (34)	208 (27)	48 (23)
+	−	−	417 (18)	120 (29)	8 (7)
−	+	−	486 (21)	155 (32)	24 (15)
−	−	+	320 (14)	87 (27)	33 (38)
+	+	−	95 (4)	14 (15)	1 (7)
+	−	+	93 (4)	32 (34)	3 (9)
−	+	+	81 (4)	18 (22)	7 (39)
+	+	+	28 (1)	8 (29)	3 (38)
	Total		2288	642 (28)	127 (20)

*χ^2 Test was used to determine overall significance between evaluable and nonevaluable cases, $P = 0.028$.
†χ^2 Test was used to determine overall significance between cases reporting improvement and no improvement, $P < 0.001$.
CT indicates *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; NG, *Neisseria gonorrhoeae*; STI, sexually transmitted infection.

com/OLQ/A781). Infection rates did not differ between evaluable and nonevaluable cases (Supplementary Table 1, http://links.lww.com/OLQ/A781).

Reported Clinical Improvement of Urethritis After Empirical Treatment

One hundred twenty-seven of the 642 evaluable cases (20%) indicated that their symptoms had not improved (Table 2). Patients infected with MG-MRAM had an OR of 3.38 (95% CI, 2.11–5.40) for no improvement 2 weeks after therapy, compared with patients not infected with MG. This association remained after adjusting for CT and NG coinfection, age, ethnicity, and text-message response delay (not immediately responding to the text message but on days 15–28; adjusted odds ratio [aOR], 3.58; 95% CI, 2.05–6.24). Treatments with ceftriaxone and azithromycin were considered in the multivariable model but were both not significant. In the analysis in which we used a more stricter definition of clinical improvement, similar results were found (Supplementary Table 2, http://links.lww.com/OLQ/A781), except for ethnicity and CT infections, but these factors did not have a major effect on the association between MG-MRAM and clinical improvement. We performed a separate analysis for all NGU cases in which patients with MG-MRAM had a similar OR of 3.44 (95% CI, 2.04–5.80) for no improvement compared with patients with NGU not infected by MG (Supplementary Table 3, http://links.lww.com/OLQ/A781).

We additionally analyzed improvement considering single, dual, and triple infections (Fig. 2). Few patients with single NG infection, or with dual infections of NG + CT or NG + MG reported no clinical improvement (7%–9%; Table 1 and Supplementary Fig. 1, http://links.lww.com/OLQ/A782). Patients infected with single MG, dual CT + MG, or triple NG + CT + MG infection most often reported no improvement of symptoms (37%–39%). In a subanalysis on all cases with an MG infection, cases with MG-MRAM had an OR of 4.00 (95% CI, 1.27–12.58) for no improvement 2 weeks after therapy compared with cases with MG-WT. After adjusting for CT and NG coinfection, age, and text-message response delay, the aOR for patients with MG-MRAM was 5.86 (95% CI, 1.88–18.3) compared with MG-WT.

Men of Surinamese origin significantly more often reported no clinical improvement (42% [38 of 90]) compared with men of Dutch origin (17% [49 of 296]; $P < 0.001$). Surinamese men had an OR of 3.66 (95% CI, 2.14–6.26) of no clinical improvement compared with men of Dutch origin, and this remained similar after adjusting for CT and NG coinfection, age, and text-message response delay (aOR, 3.38; 95% CI, 1.91–6.00). We subdivided the infection status and MG genotype by country of origin (Supplementary Table 4, http://links.lww.com/OLQ/A781) and found a significant difference in infection status between the different counties of origin. Surinamese men have relatively less often NG infections, but more MG infections and also more coinfections. This is not significantly different from other ethnicities, however. In a subset of 55 of 90 Surinamese men, we found that only one man was infected with TV, not explaining the high number of men reporting no clinical improvement.

Antibiotic Treatment and Clinical Improvement

The majority of patients received only antibiotic treatment during their initial consultation (575 of 642 [90%]). Patients who reattended the clinic after empirical treatment did this with an median of 7 days (range, 2–14 days). Patients were asked to reattend the clinic in case of additional diagnostic findings on specimens taken at entry visit. Fifty-five patients received additional treatment at reattendance: 18 patients received ceftriaxone, 10 patients received azithromycin, 26 patients received doxycycline, and 1 patient received benzathine benzylpenicillin. Treatments that were considered in analysis were treatments given up to 2 days before the completion of the online questionnaire regarding clinical improvement. From all evaluable cases with an NG monoinfection, 98 of 120 (82%) had been treated with ceftriaxone alone, and 7% (7 of 98) indicated that their symptoms had not improved (Table 3). From the evaluable cases with a CT monoinfection, 131 of 155 (85%) were treated with azithromycin alone, and 17% (22 of 131) indicated no improvement. Evaluable cases with an MG monoinfection were treated with azithromycin alone in 81 of 87 of the cases (93%), and 40% (32 of 81) indicated that their symptoms had not improved (Table 3). Cases that were infected with MG-MRAM (monoinfection and coinfection) and that were treated with azithromycin indicated no improvement in 46% (33 of 71), whereas cases infected with MG-WT who were treated with azithromycin indicated no improvement in 15% (4 of 26; $P = 0.005$).

DISCUSSION

We evaluated patient-reported clinical outcome of empirical treatment in men with gonococcal and nongonococcal acute urethritis. Overall, the majority of NGU cases indicated clinical improvement after a single-dose azithromycin. Nevertheless, half of NGU cases testing positive for MG-MRAM did not report clinical improvement. The use of a single-dose azithromycin might therefore be continued for NGU cases without MG-MRAM. In gonococcal urethritis, empirically treated with ceftriaxone only, presence of MG did not affect clinical outcome.

Patients with NGU received empirical treatment with a single dose of oral azithromycin, according to current Dutch guidelines.[4] Known advantages of this treatment compared with longer treatment regimens are compliance and tolerability.[19] We now show for the first time that the presence of MG-MRAM significantly influences clinical outcome of empirical treatment for NGU and that 40% of the cases with MG-MRAM have persisting symptoms. Previously, Bachmann et al.[20] reported a similar trend with more often persistent symptoms after treatment (26%) in MG-MRAM infected men in the United States, but this was not statistically significantly different compared with men with MG-WT infection or MG-negative men. In their study, the proportions of men with no improvement for MG-WT and without MG infection (13% and 17%, respectively) were comparable to those in our study (14% and 16%, respectively). In contrast, a meta-analysis found that 83% (76 of 92; 95% CI, 73%–90%) of individuals with MG-MRAM failed treatment with azithromycin.[21] In the meta-analysis, treatment failure was defined as a positive test of cure. It could well be that antimicrobial treatment failure and symptom persistence differ considerably in case of MG-MRAM infections.

Interestingly, 46% of NGU patients with a single MG-MRAM infection indicated that their symptoms had not improved after treatment with azithromycin. Although this is higher compared with MG-WT (15%), this still means that half of the patients infected with MG-MRAM reported clinical improvement. This might mean that mutations do not cause full resistance to azithromycin, but that bacteria become less sensitive. Another explanation for clinical improvement despite MRAM is the anti-inflammatory effect of macrolides, which may also account for a reduction of symptoms.[22] Another option is that the human body is in itself able to eliminate the bacterium to a certain extent. Research showed that the majority of women (74%–93%) may clear MG infection within 12 months.[23,24] It is to be expected that men can also spontaneously clear MG infection. Lastly, antibiotic treatment might have also been effective to other causative bacteria we did not test for, such as *Mycoplasma penetrans* and *Haemophilus* species.[25,26]

TABLE 2. Clinical Improvement by Characteristics of 642 Evaluable Cases With Urethritis, STI Clinic in Amsterdam, May 2018 to November 2019

	No. Cases Without Clinical Improvement as a Fraction of Evaluable Cases	*P*	OR	95% CI	aOR*	95% CI
Total	127/642 (20%)					
Age in years, n (%)						
<25	43/176 (24%)	0.065	1			
25–34	53/247 (21%)		0.79	0.50–1.27		
35–44	19/109 (17%)		0.72	0.39–1.32		
≥45	12/110 (11%)		0.39	0.19–0.78		
Age per 10 y, mean (SD)	Mean age of 33 (11) y in cases with clinical improvement vs. mean age of 30 (10) y in cases without clinical improvement	0.012	0.77	0.63–0.93	0.83	0.66–1.05
Sexual risk group, n (%)						
MSW	66/263 (25%)	0.008	1			
MSM	61/379 (16%)		0.58	0.39–0.87		
Country of origin, n (%)						
The Netherlands	49/296 (17%)	<0.001	1		1	
Turkey, Morocco, North Africa	5/48 (10%)		0.60	0.22–1.60	0.51	0.19–1.36
Suriname	38/90 (42%)		3.66	2.14–6.26	3.38	1.91–6.00
Europe, outside the Netherlands	12/86 (14%)		0.86	0.43–1.71	0.73	0.36–1.47
Other	23/122 (19%)		1.20	0.68–2.09	1.02	0.56–1.87
Educational level†, n (%)						
Low	11/56 (20%)	0.280	1			
Medium	39/152 (26%)		1.20	0.57–2.50		
High	68/391 (17%)		0.82	0.41–1.64		
Urethritis symptoms past 2 y, n (%)						
No	87/446 (20%)	0.893	1			
Yes	40/196 (20%)		1.03	0.66–1.60		
Antibiotic use previous, n (%)						
None	103/525 (20%)	0.591	1			
Last 7 d	2/14 (14%)		0.72	0.17–3.00		
Last 14–21 d	1/14 (7%)		0.39	0.08–1.91		
Last 30 d	6/29 (21%)		1.32	0.61–2.87		
Last 90 d	15/60 (25%)		1.30	0.69–2.44		
Text message response in days, n (%)						
14	68/395 (17%)	0.048	1		1	
15–28	59/247 (24%)		1.48	1.00–2.18	1.60	1.04–2.44
HIV, n (%)						
Negative	93/425 (22%)	0.104	1			
Positive	11/94 (12%)		0.47	0.23–0.95		
Unknown	23/123 (19%)		0.84	0.51–1.38		
No. sex partners in the last 6 mo‡, n (%)						
0 or 1	10/54 (19%)	0.397	1			
2–4	56/241 (23%)		1.33	0.66–2.69		
5–9	32/173 (18%)		0.93	0.44–1.95		
≥10	29/172 (17%)		0.90	0.43–1.92		
Received azithromycin, n (%)						
No	22/192 (11%)	0.001	1			
Yes	105/450 (23%)		2.52	1.44–4.39		
Received doxycycline, n (%)						
No	118/586 (20%)	0.152	1			
Yes	9/56 (16%)		0.55	0.25–1.24		
Received ceftriaxone, n (%)						
No	110/467 (24%)	<0.001	1			
Yes	17/175 (10%)		0.36	0.21–0.63		
NG, n (%)						
Negative	112/468 (24%)	<0.001	1		1	
Positive	15/174 (9%)		0.32	0.18–0.56	0.28	0.15–0.52
CT, n (%)						
Negative	92/447 (21%)	0.517	1		1	
Positive	35/195 (18%)		0.87	0.56–1.33	0.67	0.40–1.11
MG, n (%)						
Negative	81/497 (16%)	<0.001	1			
Positive	46/145 (32%)		2.20	1.41–3.45		

Continued next page

TABLE 2. *(Continued)*

	No. Cases Without Clinical Improvement as a Fraction of Evaluable Cases	*P*	OR	95% CI	aOR*	95% CI
MG genotype, n (%)						
Negative for MG	81/497 (16%)	<0.001	1		1	
WT	4/28 (14%)		0.59	0.12–2.90	0.58	0.17–2.05
MRAM	38/94 (40%)		3.38	2.11–5.40	3.58	2.05–6.24
Unknown	4/23 (17%)		1.16	0.44–3.07	1.10	0.35–3.50

Univariable and multivariable logistic regression with GEE was used to determine risk factors for no improvement.

*All variables with $P < 0.20$ were considered for the multivariable model; in the final model, the following variables were included: MG genotype, CT, NG, age, country of origin, response in days.

†Data missing from 43 patients.

‡Data missing from 2 patients. In the final model, 642 patients were included.

aOR indicates adjusted odds ratio; GEE, generalized estimating equation; MG, *Mycoplasma genitalium*; MRAM, macrolide resistance-associated mutations; MSM, men who have sex with men; MSW, men who have sex with women only; NG, *Neisseria gonorrhoeae*; OR, odds ratio; STI, sexually transmitted infection; WT, wild-type.

We also looked into the treatment success of patients with gonococcal urethritis, in combination with coinfections with CT and MG. Few cases infected with NG + MG reported no improvement (9%), in spite of the fact that, in most of these cases, treatment consisted of ceftriaxone only, whereas ceftriaxone is unlikely to affect MG directly. This suggests that MG infection in urethritis is of less clinical importance in the presence of NG. Although asymptomatic MG infections are common,[13] it is striking that, in this symptomatic patient group, clinical treatment failure was so limited, considering the amount of MG coinfections found in the gonococcal urethritis cases in this study (19% [121 of 633]).

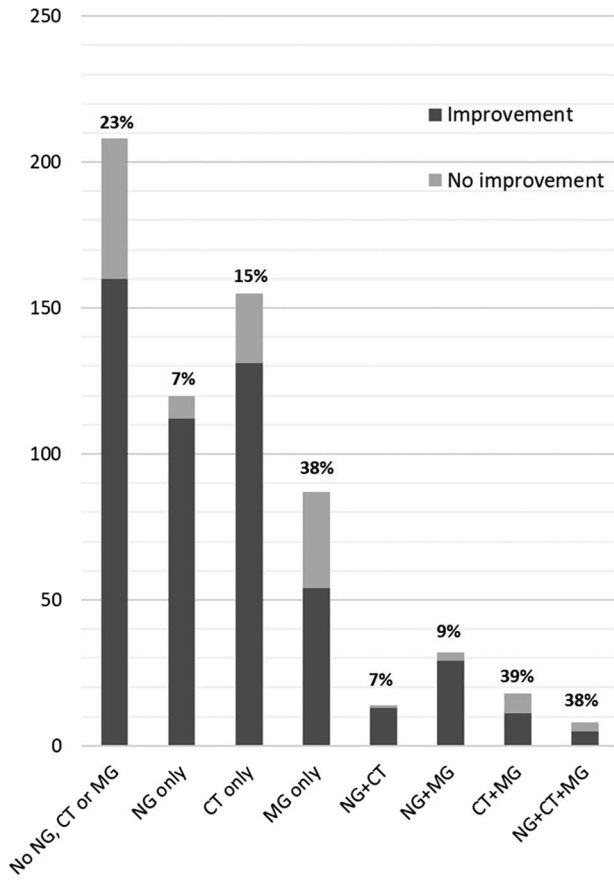

Figure 2. Reported improvement of evaluable cases, by infection status (single, dual, or triple), STI clinic in Amsterdam, May 2018 to November 2019. Percentages above the bars indicate the proportion of evaluable cases without improvement. Overall χ^2 test, $P < 0.001$. STI, sexually transmitted infection; NG, *Neisseria gonorrhoeae*; CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*.

In 34% of urethritis cases in our study, no NG, CT, or MG was detected. This finding is consistent with data from other urethritis studies. Although an array of other possible pathogens such as TV, herpes simplex virus, and adenovirus,[1] *Haemophilus influenzae* and *M. penetrans* are associated with urethritis,[26] a large proportion of NGU cases remain without microbial etiology.[1] Another consistent finding throughout urethritis research is the suboptimal clinical response to antimicrobial treatment.[1,14,15,20,27,28] Whether or not these findings are related, their consistency is remarkable and suggests that we should consider other, nonmicrobial causes for urethral inflammation.

We found a surprisingly high proportion of men of Surinamese origin who reported no clinical improvement, and Surinamese ethnicity was independently associated with no clinical improvement. Surinamese men had NG infections relatively less often, but they had more MG infections and also more coinfections (Supplementary Table 4, http://links.lww.com/OLQ/A781). Moreover, the proportion of MG-MRAM was relatively high. However, these differences were not statistically significant compared with other ethnicities. We hypothesized that this might be due to infection with TV, which is relatively common among people of Surinamese origin.[12] In the Netherlands, prevalence of TV is relatively low (between 0.4% and 0.5%) and therefore not routinely tested for.[12,29] In the study from van der Veer et al., half of the patients with a TV infection were of Surinamese or Dutch Antillean origin. In a subset of 55 of 90 Surinamese men, we found that only one man was infected with TV, so this cannot explain the large proportion of Surinamese men reporting no clinical improvement. Another explanation might be ethnic differences in the immune response. Previously, it was shown that there is a difference in immune responses between European and African populations.[30] Dutch men are predominantly of European descent, whereas many Surinamese men have a more mixed, including African and Southeast Asian, background. Further research is needed to understand why Surinamese men report clinical improvement less often.

Only 35% (705 of 2000) of the men who were eligible responded to the text message, which raises the question whether results might have been biased because men with persisting symptoms may have been more likely to respond than men without. Although the response rates to our text message was much lower than expected, we decided to extend the study instead of intensifying requests for response, which would have certainly induced

TABLE 3. Treatment Given and Reported Improvement of Evaluable Urethritis Cases, by Infection Status (None, Single, Dual, or Triple), STI Clinic in Amsterdam, May 2018 to November 2019

Treatment	Infections							
	No NG, CT or MG, n (%)	NG Only, n (%)	CT Only, n (%)	MG Only, n (%)	NG + CT, n (%)	NG + MG, n (%)	CT + MG, n (%)	NG + CT + MG, n (%)	Total, n (%)
Azithromycin	39/175 (22)	0/1 (0)	22/131 (17)	32/81 (40)	0/1 (0)	0/2 (0)	5/13 (38)	0/1 (0)	98/405 (24)
Ceftriaxone	1/3 (33)	7/98 (7)	0	0	1/3 (33)	2/25 (8)	0	2/4 (50)	13/133 (10)
Doxycycline	4/13 (31)	0/1 (0)	1/6 (17)	0/1 (0)	0/2 (0)	0	1/1 (100)	0	6/24 (25)
Azithromycin + benzathine penicillin	0	0	0/1 (0)	0	0	0	0	0	0/1 (0)
Azithromycin + ceftriaxone	1/8 (13)	0/3 (0)	0/1 (0)	1/2 (50)	0/6 (0)	1/2 (50)	0	1/2 (50)	4/24 (17)
Azithromycin + doxycycline	1/4 (25)	0	1/10 (10)	0	0	0	1/2 (50)	0	3/16 (19)
Ceftriaxone + benzathine penicillin	0	0/1 (0)	0	0	0	0	0	0	0/1 (0)
Ceftriaxone + doxycycline	0/2 (0)	0/5 (0)	0/1 (0)	0/1 (0)	0/2 (0)	0/2 (0)	0	0	0/13 (0)
Azithromycin + benzathine penicillin + ceftriaxone	0/1 (0)	0	0	0	0	0	0	0	0/1 (0)
Azithromycin + ceftriaxone + doxycycline	0	0	0/1 (0)	0/1 (0)	0	0	0/1 (0)	0	0/3 (0)
No treatment	2/2 (100)	1/11 (9)	0/4 (0)	0/1 (0)	0	0/1 (0)	0/1 (0)	0/1 (0)	3/21 (14)
Total	48/208 (23)	8/120 (7)	24/155 (15)	33/87 (38)	1/14 (7)	3/32 (9)	7/18 (39)	3/8 (38)	127/642 (20)

*Treatments listed here are treatments given up to 2 days before the completion of the online questionnaire regarding clinical improvement; several patients have received additional treatment after that time point. Number of cases without clinical improvement are given as a fraction of evaluable cases.

CT indicates *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; NG, *Neisseria gonorrhoeae*; STI, sexually transmitted infection.

bias. We think the chance of a bias due to the low response rate is low, because there were no differences in infection rates between evaluable and nonevaluable cases (Table 1 and Supplementary Table 1, http://links.lww.com/OLQ/A781). We included a large number of cases and used a silent study design without additional study visits and treatment interventions. To our knowledge, this is the only study analyzing the effect of MG and MG-MRAM on clinical improvement after empirical treatment with this design. Because of this real-life design, we were unable to evaluate urine or discharge for microbiological cure or signs of persistent urethritis. However, we believe that symptoms experienced after treatment by a patient are as important and valid as microbiological cure in the evaluation of treatment efficacy. A recent study from Australia showed that even with extended courses of resistance-guided therapy, 100% microbiological cure could not be achieved.[27]

To conclude, although a majority of NGU cases reported clinical improvement after empirical treatment with a single-dose azithromycin, the improvement was significantly lower in NGU cases with MG-MRAM. Presence of MG did not hamper clinical improvement in gonococcal urethritis treated with a single-dose ceftriaxone. Therefore, we recommend testing for and treating MG-MRAM especially in patients with NGU. Resistance-guided treatment might be considered as treatment approach for NGU.[27,28]

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