**Supplementary Table 1. Characteristics of urethritis cases, for evaluable and for non-evaluable cases, STI clinic Amsterdam, May 2018 - November 2019.**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Total (N=2505)** | **Non-** **evaluable (N=1863)** | **Evaluable (N=642)** | **P6** |
| **Age in years, mean (SD)** |  | 31.2 (10.4) | 30.7 (10.1) | 32.7 (11.1) | <0.001 |
|  |  |  |  |  |  |
| **Age in years, n (%)** | **<25** | 748 (30%) | 572 (31%) | 176 (27%) | 0.003 |
|  | **25-34** | 1036 (41%) | 789 (42%) | 247 (38%) |  |
|  | **35-44** | 394 (16%) | 285 (15%) | 109 (17%) |  |
|  | **≥45** | 327 (13%) | 217 (12%) | 110 (17%) |  |
|  |  |  |  |  |  |
| **Sexual risk group, n (%)** | **MSW** | 1175 (47%) | 912 (49%) | 263 (41%) | 0.002 |
|  | **MSM** | 1330 (53%) | 951 (51%) | 379 (59%) |  |
|  |  |  |  |  |  |
| **Country of origin, n (%)** | **Netherlands** | 1022 (41%) | 726 (39%) | 296 (46%) | 0.042 |
|  | **Turkey, Morocco, North Africa** | 206 (8%) | 158 (8%) | 48 (7%) |  |
|  | **Suriname** | 386 (15%) | 296 (16%) | 90 (14%) |  |
|  | **Europe outside the Netherlands** | 359 (14%) | 273 (15%) | 86 (13%) |  |
|  | **Other** | 532 (21%) | 410 (22%) | 122 (19%) |  |
|  |  |  |  |  |  |
| **Educational level1, n (%)** | **Low** | 205 (9%) | 149 (9%) | 56 (9%) | 0.349 |
|  | **Medium** | 623 (28%) | 471 (28%) | 152 (25%) |  |
|  | **High** | 1428 (63%) | 1037 (63%) | 391 (65%) |  |
|  |  |  |  |  |  |
| **Urethritis symptoms past 2 years, n (%)** | **No** | 1816 (72%) | 1370 (74%) | 446 (69%) | 0.037 |
|  | **Yes** | 689 (28%) | 493 (26%) | 196 (31%) |  |
|  |  |  |  |  |  |
| **Antibiotic use previous 3 months, n (%)** | **None** | 2059 (82%) | 1534 (82%) | 525 (82%) | 0.220 |
|  | **Currently** | 22 (1%) | 19 (1%) | 3 (0%) |  |
|  | **Last 7 days** | 33 (1%) | 22 (1%) | 11 (2%) |  |
|  | **Last 14 days** | 42 (2%) | 35 (2%) | 7 (1%) |  |
|  | **Last 21 days** | 27 (1%) | 20 (1%) | 7 (1%) |  |
|  | **Last 30 days** | 81 (3%) | 52 (3%) | 29 (5%) |  |
|  | **Last 90 days** | 241 (10%) | 181 (10%) | 60 (9%) |  |
|  |  |  |  |  |  |
| **HIV status, n (%)** | **Negative** | 1687 (67%) | 1262 (68%) | 425 (66%) | 0.223 |
|  | **Positive** | 313 (12%) | 219 (12%) | 94 (15%) |  |
|  | **Unknown** | 505 (20%) | 382 (21%) | 123 (19%) |  |
|  |  |  |  |  |  |
| **No. of sex partners in last 6 months2, n (%)** | **0 or 1** | 240 (10%) | 186 (10%) | 54 (8%) | 0.302 |
|  | **2-4** | 994 (40%) | 753 (41%) | 241 (38%) |  |
|  | **5-9** | 629 (25%) | 456 (25%) | 173 (27%) |  |
|  | **≥10** | 631 (25%) | 459 (25%) | 172 (27%) |  |
|  |  |  |  |  |  |
| **NG3, n (%)** | **Negative** | 1841 (74%) | 1373 (74%) | 468 (73%) | 0.547 |
|  | **Positive** | 648 (26%) | 377 (26%) | 174 (27%) |  |
|  |  |  |  |  |  |
| **CT4, n (%)** | **Negative** | 1763 (71%) | 1316 (71%) | 447 (70%) | 0.280 |
|  | **Positive** | 726 (29%) | 531 (29%) | 195 (30%) |  |
|  |  |  |  |  |  |
| **MG5, n (%)** | **Negative** | 1766 (77%) | 1269 (77%) | 497 (77%) | 0.963 |
|  | **Positive** | 522 (23%) | 377 (23%) | 145 (23%) |  |
|  |  |  |  |  |  |
| **MG-MRAM genotype, n (%)** | **WT** | 112 (21%) | 84 (22%) | 28 (19%) | 0.718 |
|  | **MRAM** | 327 (63%) | 233 (62%) | 94 (65%) |  |
|  | **Unknown** | 83 (16%) | 60 (16%) | 23 (16%) |  |

1) data missing for 249 patients; 2) data missing for 11 patients; 3) data missing for 16 patients; 4) data missing for 16 patients; 5) data missing for 217 patients; 6) GEE univariable logistic regression was used to determine significance. STI: sexually transmitted infection; Generalized estimating equation (GEE) SD: standard deviation; MSM: Men who have sex with men; MSW: Men who have sex with women only; NG: *Neisseria gonorrhoeae*; CT: *Chlamydia trachomatis*; MG: *Mycoplasma genitalium*; WT: wildtype; MRAM: macrolide resistance-associated mutations. Univariable logistic regression using GEE was used to determine differences between groups.

**Supplementary Table 2. Clinical improvement (complaints gone versus still complaints) by characteristics of 642 evaluable cases with urethritis, STI clinic Amsterdam, May 2018 - November 2019. Univariable and multivariable logistic regression with GEE was used to determine risk factors for no improvement.**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Number of cases still having complaints as a fraction of evaluable cases** | **P** | **OR** | **95% CI** | **aOR1** | | **95% CI** | |
| **Total** |  | 305/642 (48%) |  |  |  | |  | |  | |
| **Age in years, n (%)** | **<25** | 96/176 (55%) | <0.001 | 1 |  | |  | |  | |
|  | **25-34** | 137/247 (55%) |  | 1.02 | 0.69-1.52 | |  | |  | |
|  | **35-44** | 41/109 (38%) |  | 0.50 | 0.31-0.82 | |  | |  | |
|  | **≥45** | 31/110 (28%) |  | 0.33 | 0.20-0.54 | |  | |  | |
| **Age per 10 years, mean (SD)** |  | Mean age 35 (12) in cases without clinical complaints vs. mean age 30 (10) in cases still having clinical complaints | <0.001 | 0.69 | 0.59-0.80 | | 0.79 | | 0.67-0.93 | |
| **Sexual risk group, n (%)** | **MSW** | 160/263 (61%) | <0.001 | 1 |  | |  | |  | |
|  | **MSM** | 145/379 (38%) |  | 0.39 | 0.28-0.54 | |  | |  | |
| **Country of origin, n (%)** | **Netherlands** | 117/296 (40%) | <0.001 | 1 |  | | 1 | |  | |
|  | **Turkey, Morocco, North Africa** | 25/48 (52%) |  | 1.68 | 0.91-3.07 | | 1.50 | | 0.76-2.93 | |
|  | **Suriname** | 62/90 (69%) |  | 3.46 | 2.06-5.82 | | 3.19 | | 1.85-5.50 | |
|  | **Europe, outside the Netherlands** | 32/86 (37%) |  | 0.91 | 0.55-1.51 | | 0.91 | | 0.53-1.57 | |
|  | **Other** | 69/122 (57%) |  | 1.99 | 1.28-3.08 | | 2.01 | | 1.24-3.27 | |
| **Educational level2, n (%)** | **Low** | 25/56 (45%) | 0.085 | 1 |  | |  | |  | |
|  | **Medium** | 84/152 (55%) |  | 1.49 | 0.79-2.82 | |  | |  | |
|  | **High** | 173/391 (44%) |  | 0.97 | 0.54-1.73 | |  | |  | |
| **Urethritis symptoms past** | **No** | 218/446 (49%) | 0.272 | 1 |  | |  | |  | |
| **2 years, n (%)** | **Yes** | 87/196 (44%) |  | 0.82 | 0.58-1.17 | |  | |  | |
| **Antibiotic use previous, n (%)** | **None** | 247/525 (47%) | 0.887 | 1 |  | |  | |  | |
|  | **Last 7 days** | 7/14 (50%) |  | 1.11 | 0.39-3.14 | |  | |  | |
|  | **Last 14-21 days** | 6/14 (43%) |  | 0.83 | 0.28-2.49 | |  | |  | |
|  | **Last 30 days** | 13/29 (45%) |  | 1.00 | 0.48-2.07 | |  | |  | |
|  | **Last 90 days** | 32/60 (53%) |  | 1.30 | 0.77-2.21 | |  | |  | |
| **Text message response in** | **14** | 168/395 (43%) | 0.001 | 1 |  | | 1 | |  | |
| **days, n (%)** | **15-28** | 137/247 (55%) |  | 1.68 | 1.22-2.31 | | 1.91 | | 1.34-2.72 | |
| **HIV, n (%)** | **Negative** | 203/425 (48%) | 0.167 | 1 |  | |  | |  | |
|  | **Positive** | 37/94 (39%) |  | 0.73 | 0.46-1.16 | |  | |  | |
|  | **Unknown** | 65/123 (53%) |  | 1.25 | 0.83-1.87 | |  | |  | |
| **No. of sex partners in last** | **0 or 1** | 30/54 (56%) | 0.002 | 1 |  | |  | |  | |
| **6 months3, n (%)** | **2-4** | 134/241 (56%) |  | 0.99 | 0.55-1.78 | |  | |  | |
|  | **5-9** | 72/173 (42%) |  | 0.58 | 0.31-1.06 | |  | |  | |
|  | **≥10** | 67/172 (39%) |  | 0.50 | 0.28-0.93 | |  | |  | |
| **Received azithromycin, n (%)** | **No** | 58/192 (30%) | <0.001 | 1 |  | |  | |  | |
|  | **Yes** | 247/450 (66%) |  | 2.78 | 1.93-4.02 | |  | |  | |
| **Received doxycycline, n (%)** | **No** | 280/586 (48%) | 0.572 | 1 |  | |  | |  | |
|  | **Yes** | 25/56 (45%) |  | 0.85 | 0.48-1.50 | |  | |  | |
| **Received ceftriaxone, n (%)** | **No** | 253/467 (54%) | <0.001 | 1 |  | |  | |  | |
|  | **Yes** | 52/175 (30%) |  | 0.36 | 0.25-0.53 | |  | |  | |
| **NG, n (%)** | **Negative** | 261/468 (56%) | <0.001 | 1 |  | | 1 | |  | |
|  | **Positive** | 44/174 (25%) |  | 0.27 | 0.18-0.40 | | 0.29 | | 0.19-0.44 | |
| **CT, n (%)** | **Negative** | 197/447 (44%) | 0.007 | 1 |  | | 1 | |  | |
|  | **Positive** | 108/195 (55%) |  | 1.59 | 1.14-2.22 | | 1.30 | | 0.87-1.94 | |
| **MG, n (%)** | **Negative** | 219/497 (44%) | 0.001 | 1 |  | |  | |  | |
|  | **Positive** | 86/145 (59%) |  | 1.87 | 1.28-2.73 | |  | |  | |
| **MG genotype, n (%)** | **Negative for MG** | 219/497 (44%) | <0.001 | 1 |  | | 1 | |  | |
|  | **WT** | 12/28 (43%) |  | 0.91 | 0.42-1.99 | | 0.70 | | 0.31-1.54 | |
|  | **MRAM** | 65/94 (69%) |  | 2.88 | 1.79-4.63 | | 3.65 | | 2.12-6.28 | |
|  | **Unknown** | 9/23 (39%) |  | 0.89 | 0.39-2.06 | | 0.86 | | 0.36-2.10 | |

1: 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. 2: data missing from 43 patients. 3: data missing from 2 patients. In the final model included 642 patients. STI: sexually transmitted infection; GEE: Generalized estimating equation; SD: standard deviation; OR: odds ratio; aOR: adjusted odds ratio; MSM: Men who have sex with men; MSW: Men who have sex with women only; NG: *Neisseria gonorrhoeae*; CT: *Chlamydia trachomatis*; MG: *Mycoplasma genitalium*; WT: wildtype; MRAM: macrolide resistance-associated mutations.

**Supplementary Table 3. Clinical improvement by characteristics of 468 evaluable cases with NGU (i.e. all 174 NG cases excluded), STI clinic Amsterdam, May 2018 - November 2019. Univariable and multivariable logistic regression with GEE was used to determine risk factors for no improvement. Variables forced into multivariable analysis were CT, MG genotype and age.**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Number of cases without clinical improvement as a fraction of evaluable cases** | **P** | **OR** | **95% CI** | **aOR1** | **95% CI** |
| **Total** |  | 112/468 (24%) |  |  |  |  |  |
|  |  |  |  |  |  |  |  |
| **Age in years, n (%)** | **<25** | 39/149 (26%) | 0.620 | 1 |  |  |  |
|  | **25-34** | 47/196 (24%) |  | 0.84 | 0.51-1.38 |  |  |
|  | **35-44** | 16/62 (26%) |  | 0.98 | 0.50-1.94 |  |  |
|  | **≥45** | 10/61 (16%) |  | 0.61 | 0.28-1.33 |  |  |
|  |  |  |  |  |  |  |  |
| **Age per 10 years, mean (SD)** |  | Mean age 30 (9) in patients without vs. 31 (10) in patients with clinical improvement | 0.328 | 0.90 | 0.72-1.12 | 0.85 | 0.67-1.09 |
|  |  |  |  |  |  |  |  |
| **Sexual risk group, n (%)** | **MSW** | 63/240 (26%) | 0.248 | 1 |  |  |  |
|  | **MSM** | 49/228 (21%) |  | 0.77 | 0.50-1.20 |  |  |
|  |  |  |  |  |  |  |  |
| **Country of origin, n (%)** | **Netherlands** | 44/211 (21%) | <0.001 | 1 |  | 1 |  |
|  | **Turkey, Morocco, North Africa** | 4/40 (10%) |  | 0.43 | 0.14-1.27 | 0.41 | 0.14-1.17 |
|  | **Suriname** | 34/71 (48%) |  | 3.38 | 1.87-6.11 | 3.32 | 1.77-6.23 |
|  | **Europe, outside the Netherlands** | 10/58 (17%) |  | 0.81 | 0.38-1.74 | 0.69 | 0.32-1.50 |
|  | **Other** | 20/88 (23%) |  | 1.11 | 0.60-2.06 | 0.96 | 0.50-1.86 |
|  |  |  |  |  |  |  |  |
| **Educational level2, n (%)** | **Low** | 7/35 (20%) | 0.265 | 1 |  |  |  |
|  | **Medium** | 36/115 (31%) |  | 1.59 | 0.63-4.04 |  |  |
|  | **High** | 62/286 (22%) |  | 1.07 | 0.44-2.59 |  |  |
|  |  |  |  |  |  |  |  |
| **Urethritis symptoms past** | **No** | 77/339 (23%) | 0.394 | 1 |  |  |  |
| **2 years, n (%)** | **Yes** | 35/129 (27%) |  | 1.23 | 0.76-2.01 |  |  |
|  |  |  |  |  |  |  |  |
| **Antibiotic use previous n (%)** | **None** | 93/379 (25%) | 0.819 | 1 |  |  |  |
|  | **Last 7 days** | 2/12 (17%) |  | 0.68 | 0.18-2.63 |  |  |
|  | **Last 14-21 days** | 1/9 (11%) |  | 0.47 | 0.09-2.54 |  |  |
|  | **Last 30 days** | 5/22 (23%) |  | 1.25 | 0.50-3.12 |  |  |
|  | **Last 90 days** | 11/46 (24%) |  | 0.96 | 0.46-1.99 |  |  |
|  |  |  |  |  |  |  |  |
| **Text message Response in** | **14** | 59/288 (20%) | 0.044 | 1 |  | 1 |  |
| **days, n (%)** | **15-28** | 53/180 (29%) |  | 1.58 | 1.01-2.46 | 1.69 | 1.06-2.69 |
|  |  |  |  |  |  |  |  |
| **HIV, n (%)** | **Negative** | 83/316 (26%) | 0.184 | 1 |  |  |  |
|  | **Positive** | 8/45 (18%) |  | 0.50 | 0.20-1.24 |  |  |
|  | **Unknown** | 21/107 (20%) |  | 0.72 | 0.43-1.20 |  |  |
|  |  |  |  |  |  |  |  |
| **No. of sex partners in last** | **0 or 1** | 10/48 (21%) | 0.789 | 1 |  |  |  |
| **6 months3, n (%)** | **2-4** | 49/190 (26%) |  | 1.34 | 0.67-2.69 |  |  |
|  | **5-9** | 30/127 (24%) |  | 1.03 | 0.49-2.16 |  |  |
|  | **≥10** | 23/101 (23%) |  | 1.12 | 0.51-2.45 |  |  |
|  |  |  |  |  |  |  |  |
| **Received azithromycin4, n (%)** | **No** | 9/36 (25%) | 0.876 | 1 |  |  |  |
|  | **Yes** | 103/432 (24%) |  | 0.94 | 0.43-2.06 |  |  |
|  |  |  |  |  |  |  |  |
| **Received doxycycline4, n (%)** | **No** | 103/424 (24%) | 0.571 | 1 |  |  |  |
|  | **Yes** | 9/44 (20%) |  | 0.80 | 0.37-1.72 |  |  |
|  |  |  |  |  |  |  |  |
| **Received ceftriaxone, n (%)** | **No** | 109/446 (24%) | 0.214 | 1 |  |  |  |
|  | **Yes** | 3/22 (14%) |  | 0.42 | 0.10-1.66 |  |  |
|  |  |  |  |  |  |  |  |
| **CT, n (%)** | **Negative** | 81/295 (27%) | 0.017 | 1 |  | 1 |  |
|  | **Positive** | 31/173 (18%) |  | 0.58 | 0.37-0.91 | 0.61 | 0.36-1.03 |
|  |  |  |  |  |  |  |  |
| **MG, n (%)** | **Negative** | 72/363 (20%) | 0.003 | 1 |  |  |  |
|  | **Positive** | 40/105 (24%) |  | 2.20 | 1.32-3.68 |  |  |
|  |  |  |  |  |  |  |  |
| **MG genotype, n (%)** | **Negative for MG** | 72/363 (20%) | <0.001 | 1 |  | 1 |  |
|  | **WT** | 4/26 (15%) |  | 0.51 | 0.09-2.78 | 0.63 | 0.19-2.07 |
|  | **MRAM** | 32/67 (48%) |  | 3.44 | 2.04-5.80 | 3.45 | 1.83-6.49 |
|  | **Unknown** | 4/12 (33%) |  | 1.98 | 0.65-6.06 | 1.81 | 0.46-7.09 |

1: All variables with p<0.20 were considered for the multivariable model; in the final model the following variables were included: MG genotype, CT, age, country of origin, response in days. 2: data missing from 30 cases. 3: data missing from 2 cases, 4: logistic regression without GEE. In the final model included 468 cases. STI: sexually transmitted infection; GEE: Generalized estimating equation; SD: standard deviation; NGU: non-gonococcal urethritis; MSM: Men who have sex with men; MSW: Men who have sex with women; NG: *Neisseria gonorrhoeae*; CT: *Chlamydia trachomatis*; MG: *Mycoplasma genitalium*; WT: wildtype; MRAM: macrolide resistance-associated mutations.

**Supplementary Table 4. Infection status (single, dual or triple) and *Mycoplasma genitalium* infection status and genotype of urethritis cases by country of origin, STI clinic Amsterdam, May 2018 - November 2019.**

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| NG | **CT** | **MG** | **Total, N (%)** | **Netherlands, n (%)** | **Turkey, Morocco, North Africa, n (%)** | **Suriname, n (%)** | **Europe, outside the Netherlands, n (%)** | **Other, n (%)** |
| - | **-** | **-** | 208 (32%) | 85 (29%) | 20 (42%) | 29 (32%) | 33 (38%) | 41 (34%) |
| + | **-** | **-** | 120 (19%) | 58 (20%) | 7 (15%) | 10 (11%) | 19 (22%) | 26 (21%) |
| - | **+** | **-** | 155 (24%) | 82 (28%) | 14 (29%) | 25 (28%) | 12 (14%) | 22 (18%) |
| - | **-** | **+** | 87 (14%) | 34 (11%) | 5 (10%) | 15 (17%) | 12 (14%) | 21 (17%) |
| + | **+** | **-** | 14 (2%) | 11 (4%) | 1 (2%) | 1 (1%) | 0 | 1 (1%) |
| + | **-** | **+** | 32 (5%) | 13 (4%) | 0 | 3 (3%) | 9 (10%) | 7 (6%) |
| - | **+** | **+** | 18 (3%) | 10 (3%) | 1 (2%) | 2 (2%) | 1 (1%) | 4 (3%) |
| + | **+** | **+** | 8 (1%) | 3 (1%) | 0 | 5 (6%) | 0 | 0 |
|  | **Total1** |  | 642 | 296 (100%) | 48 (100%) | 90 (100%) | 86 (100%) | 122 (100%) |
| No MG | | | 497 (77%) | 236 (80%) | 42 (88%) | 65 (72%) | 64 (74%) | 90 (74%) |
| MG WT | | | 28 (4%) | 12 (4%) | 2 (4%) | 3 (3%) | 3 (3%) | 8 (7%) |
| MG MRAM | | | 94 (15%) | 38 (13%) | 3 (6%) | 15 (17%) | 18 (21%) | 20 (16%) |
| MG, type unknown | | | 23 (4%) | 10 (3%) | 1 (2%) | 7 (8%) | 1 (1%) | 4 (3%) |
|  | **Total2** |  | 642 (100%) | 296 (100%) | 48 (100%) | 90 (100%) | 86 (100%) | 122 (100%) |

1: Chi-square test was used to determine overall significance between infection status by different country of origins, p=0.004. 2: Chi-square test was used to determine overall significance between *Mycoplasma genitalium* infection status and genotype by different country of origins, p=0.229. STI: Sexually transmitted infection; NG: *Neisseria gonorrhoeae*; CT: *Chlamydia trachomatis*; MG: *Mycoplasma genitalium*.

**Figure legends**

**Supplementary Figure 1. Flow chart of clinical improvement for urethritis cases by STI infection status, STI clinic Amsterdam, May 2018 and November 2019. STI: sexually transmitted infection; NGU: non-gonococcal urethritis; NG: *Neisseria gonorrhoeae*; CT: *Chlamydia trachomatis*; MG: *Mycoplasma genitalium*, WT: wildtype, MRAM: macrolide resistance-associated mutation.**