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# Seek and you shall find! Prevalence and trends of chlamydial and gonococcal infections in a high-density urban area in Italy around the COVID era: a laboratory-based survey

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## ABSTRACT

**Background** A comprehensive understanding of the epidemiology of *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) infections is essential for developing effective prevention strategies. This retrospective, laboratory-based study assessed the prevalence and trends of CT and NG infections in a densely populated area in northern Italy between 2018 and 2023.

**Methods** We collected data on all samples submitted to the Microbiology of IRCCS Azienda Ospedaliero-Universitaria of Bologna for CT/NG testing. Data were stratified by year, sex, age, testing site and anatomical sampling site.

**Results** A total of 63 569 samples from 46 501 individuals were analysed. CT prevalence was 7.2%, with a peak in those aged 18–25 and a male-to-female ratio of 1.56. Although one-third of tests were from family counselling centres or gynaecologists, the highest CT positivity was found at the STI outpatient clinic (69%) and infectious diseases centres (13%). NG prevalence was 4.8%, with very low rates in family and gynaecology settings. NG showed a striking male predominance (M:F=8.6). For both CT and NG, we observed (1) a rise in case numbers over time, especially in males; (2) a decline during the COVID-19 pandemic followed by a rebound; and (3) a notable proportion of pharyngeal and rectal infections.

**Conclusions** This study highlights key epidemiological trends of CT and NG infections based on population characteristics, healthcare access points and the influence of external factors such as COVID-19 pandemic. These findings underscore the importance of targeted screening and prevention programmes and reinforce the role of laboratory-based surveillance to guide public health interventions.

### WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Chlamydia and gonorrhoea are the most commonly reported bacterial sexually transmitted infections (STIs) in Europe, with high burden in young adults and men who have sex with men (MSM).
⇒ Testing practices vary widely across healthcare providers, and extragenital infections are frequently underdiagnosed.

### WHAT THIS STUDY ADDS

⇒ This study confirms high *Chlamydia trachomatis*/*Neisseria gonorrhoeae* (CT/NG) prevalence in northern Italy, particularly among males and young adults.
⇒ It also highlights significant underuse of extragenital testing outside STI-specialised clinics and documents the impact of the COVID-19 pandemic and HIV pre-exposure prophylaxis rollout on STI trends.

### HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Findings support expanding extragenital screening and STI services beyond specialised clinics to include general practice and gynaecology.
⇒ Policymakers should prioritise STI screening programmes, especially for high-risk populations, and address barriers to access and stigma in broader healthcare settings.

## INTRODUCTION

*Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) are the most common bacterial sexually transmitted infections (STIs) worldwide.[1] Genital infections, often asymptomatic, can cause prostatitis, pelvic inflammatory disease, infertility and adverse pregnancy outcomes if untreated.[2] Both pathogens also increase HIV acquisition and transmission risk.[3]

Extragenital colonisation of the pharynx and rectum, especially after oral or anal intercourse, is frequent in women and men who have sex with men (MSM). These infections are typically asymptomatic, delaying diagnosis and facilitating transmission.[4] Pharyngeal gonorrhoea is particularly important for the emergence of multidrug resistance, as *N. gonorrhoeae* can acquire resistance genes from commensal *Neisseria* in the oropharynx.[5]

Nucleic acid amplification tests (NAATs) are the diagnostic gold standard for both genital and extragenital infections due to high accuracy and rapid turnaround.[6] Their adoption has supported global prevalence studies stratified by sex, age, sexual orientation and site.[7]

Recently, two events have strongly influenced CT/NG epidemiology: (1) the COVID-19 pandemic and (2) the spread of HIV pre-exposure prophylaxis (PrEP). The pandemic reduced access to STI services, initially decreasing CT/NG diagnoses, followed by a rebound.[8] Declines were also linked to reduced sexual activity during restrictions ('sexual distancing').[9]

PrEP, effective for HIV prevention, is widely used in high-risk groups such as MSM, serodiscordant couples and people who use drugs.[10] In Italy, PrEP was introduced in 2016 and became fully reimbursed in April 2023.[10] Concerns remain that PrEP may alter sexual behaviours—less condom use, more partners—potentially increasing other STIs, including CT and NG.[11] However, data on PrEP's impact are inconsistent, reporting increases, decreases or no effect.[11 12]

In this context, epidemiological data are essential to guide STI prevention, identify high-risk groups and monitor service performance. This study evaluated the prevalence and temporal trends of CT and NG infections in Bologna (2018–2023), stratified by year, sex, age, healthcare setting and anatomical site.

## METHODS

### Study design and setting

We conducted a retrospective laboratory-based study of CT and NG infections in Bologna, Northern Italy (population ~1 million) over 2018–2023. All samples submitted for CT/NG testing to the Microbiology Unit of the IRCCS Azienda Ospedaliero-Universitaria, the only public microbiology laboratory in the metropolitan area, were included. Detection was performed with the Alinity M STI NAAT (Abbott Molecular, Illinois, USA), validated for genital and extragenital specimens.[13] Given its high accuracy, confirmatory NG testing was not routinely performed.

### Data collection and study variables

Data were extracted from electronic laboratory records for individuals ≥18 years. Both positive and negative results were included, stratified by year, age, sex, testing site and sample type. Testing sites comprised:

1. STI outpatient clinic (Dermatology Unit, S. Orsola Hospital)—Free access for symptomatic patients or those with exposure/unprotected sex. In Italy, STI clinics have historically been embedded within Dermatology Departments.
2. Infectious disease centres (S. Orsola and Imola Hospitals)—Routine screening of HIV-positive individuals and PrEP users.
3. Gynaecology/obstetrics—routine care, pregnancy screening, infertility visits.
4. Family counselling centres—Mainly for young women, contraception and STI screening.
5. Community health services/general practitioners—General or non-specific urogenital symptoms.

Specimens included urine, endocervical, vaginal, urethral, pharyngeal, anorectal and conjunctival swabs.

### Data analysis

Descriptive statistics calculated absolute and relative frequencies of CT and NG, stratified by year, age (18–25, 26–34, 35–44, >45), sex and site. Only one sample per subject per day was included in subject-level analyses; multiple samples on the same day were considered positive if ≥1 tested positive. Temporal trends were visualised graphically.

Specimen-level analyses evaluated infection rates and testing volumes by sample type, including all submitted specimens. Access-level analyses defined a 'diagnostic access' as the unique combination of subject and sample-acceptance date. An access was classified as positive if ≥1 specimen tested positive. Both analyses were stratified by sex, age and testing setting. Test-of-cures were excluded.

All analyses were performed in STATA/SE V.17.0. To evaluate differences among subgroups of samples or subjects, t-tests were used for continuous variables and Pearson's $\chi^2$ tests for categorical variables. Temporal differences in prevalence across calendar years were assessed using Pearson's $\chi^2$ test of heterogeneity. All tests were two-sided, and $p<0.05$ was considered statistically significant.

### Ethical considerations

Only anonymised data were used. As no patient identifiers or interventions were involved, consent was waived. Ethical approval was deemed unnecessary by the local Ethics Committee (CE-AVEC). Procedures complied with institutional and national standards and the Declaration of Helsinki (1975, revised 2013).

## RESULTS

### Study population and samples

From 2018–2023, 63 569 clinical samples from 46 501 individuals were analysed. Females represented 52% (n=24 527) and were younger than males (mean±SD: 32.2±9.4 vs 35.0±10.7 years; p<0.0001). Most subjects were aged 26–34 (37.7%), followed by 35–44 (25.1%) and 18–25 (23.7%).

The STI outpatient clinic accounted for 48% of the tested subjects, followed by family counselling (18%) and community services (14%). Marked sex differences were observed: males predominated at the STI clinic (70%) and infectious disease centres (92%), while gynaecology/obstetrics and family counselling served almost exclusively females. Community health services showed equal sex distribution.

Subjects at the STI clinic were significantly younger (31.4±9.5 years) than those at other facilities (p<0.0001). Testing increased steadily from ~7150 individuals in 2018 to ~10 365 in 2023, except for declines during the pandemic (5059 in 2020; 7156 in 2021). Full demographic data for CT and NG cases are provided in tables 1 and 2, respectively.

Urine was the most common specimen (n=35 090; 55.2%). Of these, 25 213 (71.9% of urine samples) were collected as single-sample accesses (ie, without a concurrent specimen from the same access). In contrast, only 501 of 10 353 pharyngeal swabs (4.8%) and 447 of 7631 rectal swabs (5.9%) were obtained as single-sample accesses. Endocervical and vaginal swabs accounted for 6266 (9.8%) and 3774 (5.9%), respectively. Pharyngeal (n=10 353) and rectal swabs (n=7631) were mainly collected at the STI clinic and infectious diseases, primarily from males. Nearly half of all specimens came from the STI clinic, while infectious diseases and family counselling contributed 21% and 13%, respectively (online supplemental table 1).

Extragenital samples (pharyngeal and rectal swabs) were collected almost exclusively at the STI clinic and infectious diseases centres, and primarily from male patients (88.8% and 94.1%, respectively). Urine specimens were more evenly distributed across sites and sexes (59% males, 41% females), with 59.2% originating from the STI Clinic. Vaginal and cervical swabs were mostly obtained from gynaecology/obstetrics (19.6% and 32.3%) and family counselling centres (70% and 37.3%, respectively). Complete data on specimen-level volumes are provided in online supplemental table 1.

**Table 1** Absolute and percentage distribution of positive and negative cases of *Chlamydia trachomatis* by year, age, sex and testing location in Bologna, Italy (2018–2023)

| | | 2018 n (%) | | 2019 n (%) | | 2020 n (%) | | 2021 n (%) | | 2022 n (%) | | 2023 n (%) | | 2018–2023 n (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Total |
| Age (years) | 18–25 | 216 (11.8) | 1621 (88.2) | 205 (10.3) | 1789 (89.7) | 143 (11.8) | 1067 (88.2) | 212 (12.5) | 1490 (87.5) | 214 (10.4) | 1840 (89.6) | 187 (8.5) | 2026 (91.6) | 1177 (10.7) | 9833 (89.3) | 11 010 (100.0) |
| | 26–34 | 200 (7.9) | 2334 (92.1) | 234 (7.7) | 2823 (92.3) | 154 (8.3) | 1706 (91.7) | 245 (9.1) | 2458 (90.9) | 248 (7.9) | 2895 (92.1) | 241 (6.1) | 3720 (93.9) | 1322 (7.7) | 15 936 (92.3) | 17 258 (100.0) |
| | 35–44 | 68 (3.8) | 1734 (96.2) | 85 (4.2) | 1950 (95.8) | 47 (3.6) | 1255 (96.4) | 93 (5.2) | 1705 (94.8) | 92 (4.4) | 2012 (95.6) | 120 (4.5) | 2539 (95.5) | 505 (4.3) | 11 195 (95.7) | 11 700 (100.0) |
| | ≥45 | 44 (4.5) | 936 (95.5) | 56 (5.0) | 1060 (95.0) | 34 (5.0) | 653 (95.0) | 53 (5.6) | 900 (94.4) | 48 (3.8) | 1215 (96.2) | 92 (6.0) | 1442 (94.0) | 327 (5.0) | 6206 (94.5) | 6533 (100.0) |
| Sex | Male | 308 (9.7) | 2873 (90.3) | 318 (8.7) | 3357 (91.4) | 225 (10.7) | 1873 (89.3) | 376 (11.3) | 2967 (88.7) | 378 (8.5) | 4079 (91.5) | 435 (8.1) | 4795 (91.9) | 2030 (9.2) | 19 944 (90.8) | 21 974 (100.0) |
| | Female | 220 (5.5) | 3752 (94.5) | 262 (5.8) | 4265 (94.2) | 153 (5.2) | 2808 (94.8) | 227 (6.0) | 3586 (94.0) | 224 (5.5) | 3885 (94.6) | 215 (4.2) | 4932 (95.8) | 1301 (5.3) | 23 226 (94.7) | 24 527 (100.0) |
| STI testing location | Dermatology/STI clinic | 438 (10.0) | 3921 (90.0) | 441 (9.5) | 4184 (90.5) | 264 (12.0) | 1933 (88.0) | 399 (12.7) | 2743 (87.3) | 382 (9.8) | 3517 (90.2) | 369 (9.0) | 3744 (91.0) | 2293 (10.3) | 20 042 (89.7) | 22 335 (100.0) |
| | Infectious diseases centres | 8 (10.7) | 67 (89.3) | 25 (6.4) | 369 (93.6) | 49 (9.9) | 444 (90.1) | 93 (10.1) | 832 (89.9) | 116 (8.0) | 1338 (92.0) | 143 (6.9) | 1942 (93.1) | 434 (8.0) | 4992 (92.0) | 5426 (100.0) |
| | Gynaecology/ obstetrics centres | 15 (2.6) | 572 (97.4) | 22 (3.3) | 636 (96.7) | 14 (3.0) | 448 (97.0) | 23 (4.7) | 469 (95.3) | 20 (2.9) | 682 (97.1) | 25 (2.5) | 989 (97.5) | 119 (3.0) | 3796 (97.0) | 3915 (100.0) |
| | Family counselling centres | 31 (2.7) | 1112 (97.3) | 32 (2.3) | 1342 (97.7) | 28 (2.5) | 1099 (97.5) | 48 (3.1) | 1498 (96.9) | 44 (3.3) | 1272 (96.7) | 49 (2.7) | 1766 (97.3) | 232 (2.8) | 8089 (97.2) | 8321 (100.0) |
| | Community services/other access points | 36 (3.6) | 953 (96.4) | 60 (5.2) | 1091 (94.8) | 23 (3.0) | 757 (97.0) | 40 (3.8) | 1011 (96.2) | 40 (3.4) | 1153 (96.6) | 54 (4.0) | 1286 (96.0) | 253 (3.9) | 6251 (96.1) | 6504 (100.0) |
| Total | | 528 (7.4) | 6625 (92.6) | 580 (7.1) | 7622 (92.9) | 378 (7.5) | 4681 (92.5) | 603 (8.4) | 6553 (91.6) | 602 (7.0) | 7962 (93.0) | 640 (6.2) | 9727 (93.8) | 3331 (7.2) | 43 170 (92.8) | 46 501 (100.0) |

STI, sexually transmitted infection.

**Table 2** Absolute and percentage distribution of positive and negative cases of *Neisseria gonorrhoeae* by year, age, sex and testing location in Bologna, Italy (2018–2023)

| | | 2018 n (%) | | 2019 n (%) | | 2020 n (%) | | 2021 n (%) | | 2022 n (%) | | 2023 n (%) | | 2018–2023 n (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Pos. | Neg. | Total |
| Age (years) | 18–25 | 75 (4.1) | 1762 (95.9) | 91 (4.6) | 1904 (95.4) | 51 (4.2) | 1160 (95.8) | 100 (5.9) | 1602 (94.1) | 138 (6.7) | 1916 (93.3) | 100 (4.5) | 2111 (95.5) | 555 (5.0) | 10 452 (95.0) | 11 010 (100.0) |
| | 26–34 | 104 (4.1) | 2431 (95.9) | 137 (4.5) | 2921 (95.5) | 75 (4.0) | 1785 (96.0) | 155 (5.7) | 2548 (94.3) | 169 (5.4) | 1973 (94.6) | 166 (4.2) | 3794 (95.8) | 806 (4.7) | 16 456 (95.3) | 17 258 (100.0) |
| | 35–44 | 62 (3.4) | 1740 (95.6) | 84 (4.1) | 1951 (95.9) | 39 (3.0) | 1262 (97.0) | 85 (4.7) | 1714 (95.3) | 111 (5.3) | 1993 (94.7) | 124 (4.7) | 2535 (95.3) | 505 (4.3) | 11 194 (95.7) | 11 700 (100.0) |
| | ≥45 | 44 (4.5) | 936 (95.5) | 45 (4.0) | 1071 (96.0) | 29 (4.2) | 658 (5.8) | 65 (6.8) | 888 (93.2) | 91 (7.2) | 1172 (92.8) | 91 (5.9) | 1443 (94.1) | 365 (5.6) | 6168 (94.4) | 6533 (100.0) |
| Sex | Male | 244 (7.7) | 2937 (92.3) | 303 (8.2) | 3374 (91.8) | 172 (8.2) | 1925 (91.8) | 373 (11.2) | 2971 (88.8) | 469 (10.5) | 3988 (89.5) | 438 (8.4) | 4780 (91.6) | 1999 (9.1) | 19 975 (90.9) | 21 974 (100.0) |
| | Female | 41 (1.0) | 3932 (99.0) | 54 (1.2) | 4472 (98.8) | 22 (0.7) | 2939 (99.3) | 32 (0.8) | 3781 (99.2) | 40 (1.0) | 4067 (99.0) | 43 (0.8) | 5104 (99.2) | 232 (1.0) | 24 295 (99.0) | 24 527 (100.0) |
| STI testing location | Dermatology/STI clinic | 259 (5.9) | 4101 (94.1) | 304 (6.6) | 4322 (93.4) | 133 (6.1) | 2063 (93.9) | 289 (9.2) | 2854 (90.8) | 334 (8.6) | 3565 (91.4) | 288 (7.0) | 3823 (93.0) | 1607 (7.2) | 20 728 (92.8) | 22 335 (100.0) |
| | Infectious diseases centres | 9 (12.0) | 66 (88.0) | 40 (10.2) | 354 (89.9) | 50 (10.1) | 443 (90.9) | 103 (11.1) | 822 (88.9) | 151 (10.4) | 1303 (89.6) | 175 (8.4) | 1910 (91.6) | 528 (9.7) | 4898 (90.3) | 5426 (100.0) |
| | Gynaecology/ obstetrics centres | 3 (0.5) | 584 (99.5) | 3 (0.5) | 655 (99.5) | 1 (0.2) | 461 (99.8) | 3 (0.6) | 489 (99.4) | 8 (1.1) | 694 (98.9) | 7 (0.7) | 1007 (99.3) | 25 (0.6) | 3890 (99.4) | 3915 (100.0) |
| | Family counselling centres | 6 (0.5) | 1137 (99.5) | 3 (0.2) | 1371 (99.8) | 4 (0.4) | 1123 (99.6) | 5 (0.3) | 1541 (99.7) | 4 (0.3) | 1312 (99.7) | 1 (0.1) | 1814 (99.9) | 23 (0.3) | 8298 (99.7) | 8321 (100.0) |
| | Community services/ other access points | 8 (0.8) | 981 (99.2) | 7 (0.6) | 1144 (99.4) | 6 (0.8) | 774 (99.2) | 5 (0.5) | 1046 (99.5) | 12 (1.0) | 1181 (99.0) | 10 (0.8) | 1330 (99.2) | 48 (0.7) | 6456 (99.3) | 6504 (100.0) |
| Total | | 285 (4.0) | 6869 (96.0) | 357 (4.4) | 7847 (95.6) | 194 (3.8) | 4865 (99.2) | 405 (5.7) | 6752 (94.3) | 509 (5.9) | 8054 (94.1) | 481 (4.6) | 9883 (95.4) | 2231 (4.8) | 44 270 (95.2) | 46 501 (100.0) |

STI, sexually transmitted infection.

### Prevalence and trends of CT infection

Overall CT prevalence was 7.2% (3331/46 501). Highest positivity was at the STI clinic (10.3%) and infectious diseases centres (8.0%), while prevalence was lower at family counselling (2.8%) and community services (3.9%; see table 1). By age, prevalence peaked at 10.7% in 18–25 years, declining with age (7.7% in 26–34 years, 4.3% in 35–44 years). The largest number of cases occurred in 26–34 years (n=1322). Males had higher prevalence than females (9.2% vs 5.3%; M:F ratio 1.56).

Prevalence ranged from 7.4% in 2018 to 6.2% in 2023, with a pandemic-related drop in 2020 (n=378 cases) and rebound in 2021 (n=603; prevalence 8.4%) (online supplemental Figure 1).

Overall differences in CT prevalence over calendar years were statistically significant (p<0.0001).

The infectious diseases centres showed the largest increase in CT absolute cases, rising from 8 in 2018 to 143 in 2023, primarily driven by expanded testing (75 subjects tested in 2018 vs 2085 in 2023).

In 2023, there was a notable shift in CT positivity towards older age groups (>45 years), along with a rise in the M:F ratio from 1.4 in 2018 to 2.0 in 2023.

Access-level analyses revealed heterogeneous CT positivity across specimen types. The highest proportions of CT-positive accesses were observed for multi-site samplings (≥3 specimens: 803/6701, 12.0%) and for certain extragenital single-site specimens, namely, rectal swabs (78/447, 17.4%) and conjunctival swabs (3/21, 14.3%). In contrast, single-site pharyngeal, endocervical and vaginal samples exhibited low prevalences (13/501, 2.6%; 189/5,973, 3.2%; and 122/3,697, 3.3%, respectively). Urine-only accesses showed a moderate prevalence (1829/25 213, 7.3%) but represented the largest absolute burden of CT-positive accesses (n=1829), thereby contributing substantially to the overall case load and sex distribution. Urine-only positivity was higher in males than in females (8.2% vs 6.4%). Complete access-level prevalence estimates are provided in table 3.

### Prevalence and trends of NG infection

NG prevalence was 4.8% (2231/46 501) (table 2). Nearly all cases were diagnosed at the STI clinic (7.2%) and infectious diseases centres (9.7%); prevalence elsewhere was negligible (≤0.7%). Unlike CT, NG prevalence was consistent across age groups (4.3–5.6%). The M:F ratio was 8.6 (2000 male vs 230 female cases).

NG cases increased from 285 in 2018 to 481 in 2023, with prevalence stable overall (4.0–4.6%, peak 5.7% in 2021). Cases declined during 2020 (n=194) and rebounded in 2021–2022. Infectious diseases centres showed the largest increase (9 cases in 2018 vs 175 in 2023), driven by expanded testing (online supplemental figure 1).

Overall differences in NG prevalence over calendar years were statistically significant (p<0.0001).

The infectious diseases centres showed the largest increase in NG absolute cases, rising from 9 in 2018 to 175 in 2023, primarily driven by expanded testing (75 subjects tested in 2018 vs 2085 in 2023). An increasing prevalence was observed among individuals aged >45 years, along with a rise in the male-to-female (M:F) ratio, from 5.9 in 2018 to 10.2 in 2023. The highest NG prevalence was detected in multi-site sampling accesses (≥3 specimens: 1070/6701, 16.0%) and in extragenital single-site specimens, particularly rectal swabs (60/447, 13.4%) and pharyngeal swabs (42/501, 8.4%). In contrast, female genital single-site swabs exhibited very low prevalences (endocervical:

**Table 3** Absolute and percentage distribution of positive samples of *Chlamydia trachomatis* (CT)-positive samples per single healthcare access stratified by biological samples for diagnostic testing, age group, sex and testing location in Bologna, Italy, 2018–2023

| | | CT n | CT n (%) | Positive samples by age n (%) | | | | Positive samples by sex n (%) | | Positive samples by STI testing location n (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total accesses | Pos. | 18–25 | 26–34 | 35–44 | ≥45 | Male | Female | STI | ID | GYN | FAM | COMM |
| Biological samples for diagnostic testing | ≥3 biological samples | 6701 | 803 (12.0) | 169 (14.2) | 285 (12.1) | 203 (11.4) | 146 (10.6) | 756 (12.0) | 47 (12.4) | 472 (14.6) | 331 (9.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | 2 biological samples | 3583 | 269 (7.5) | 86 (9.5) | 112 (8.3) | 42 (5.5) | 29 (5.1) | 186 (7.0) | 83 (8.9) | 198 (8.9) | 67 (5.6) | 2 (6.1) | 1 (1.0) | 1 (2.7) |
| | Urine only | 25 213 | 1829 (7.3) | 740 (11.2) | 784 (8.1) | 195 (3.4) | 110 (3.5) | 977 (8.2) | 852 (6.4) | 1515 (9.7) | 18 (3.2) | 44 (3.3) | 78 (2.3) | 174 (4.1) |
| | Pharyngeal swab only | 501 | 13 (2.6) | 8 (6.1) | 3 (1.6) | 1 (1.0) | 1 (1.1) | 11 (2.6) | 2 (2.4) | 12 (2.8) | 1 (1.3) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Rectal swab only | 447 | 78 (17.4) | 15 (20.5) | 22 (19.5) | 19 (15.2) | 22 (16.2) | 77 (18.0) | 1 (5.3) | 64 (17.7) | 13 (15.7) | 0 (0.0) | 0 (0.0) | 1 (33.3) |
| | Endocervical swab only | 5973 | 189 (3.2) | 89 (8.5) | 63 (3.1) | 29 (1.5) | 8 (0.9) | 0 (0.0) | 189 (3.2) | 22 (7.6) | 0 (0.0) | 43 (2.5) | 76 (3.4) | 48 (2.8) |
| | Vaginal swab only | 3697 | 122 (3.3) | 67 (6.5) | 37 (2.7) | 12 (1.1) | 6 (2.9) | 0 (0.0) | 122 (3.3) | 9 (5.7) | 0 (0.0) | 25 (3.7) | 77 (3.0) | 11 (3.5) |
| | Urethral swab only | 348 | 22 (6.3) | 2 (4.0) | 14 (10.9) | 3 (2.9) | 3 (4.5) | 17 (10.5) | 5 (2.7) | 1 (50.0) | 1 (9.1) | 5 (3.6) | 0 (0.0) | 15 (8.4) |
| | Conjunctival swab only | 21 | 3 (14.3) | 0 (0.0) | 2 (40.0) | 1 (16.7) | 0 (0.0) | 3 (23.1) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (15.0) |
| | Other* | 17 | 3 (14.3) | 1 (33.3) | 0 (0.0) | 0 (0.0) | 2 (28.6) | 3 (27.3) | 0 (0.0) | 0 (0.0) | 3 (42.9) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Total accesses | | 46 501 | 3331 (7.2) | 1177 (10.7) | 1322 (7.7) | 505 (4.3) | 327 (5.0) | 2030 (9.2) | 1301 (5.3) | 2293 (10.3) | 434 (8.0) | 119 (3.0) | 232 (2.8) | 253 (3.9) |

Unit of analysis=access (unique combination of patient and date). An access is considered CT-positive if ≥1 CT result within that access=positive.
Values are given as absolute counts (n) and percentages in parentheses (%) indicating the proportion of CT-positive accesses within the same subgroup. Example: 'Urine only=1829 (7.3%)' means 1829 CT-positive samples, representing 7.3% of all 'Urine only' accesses.
*Other specimens mainly include synovial fluids, intestinal and gynaecological biopsies.
COMM, community services/other access points; FAM, family counselling centres; GYN, gynaecology/obstetrics centres; ID, infectious diseases centres; STI, dermatology/STI clinic.

40/5973, 0.7%; vaginal: 19/3697, 0.5%). Urine-only accesses showed a low prevalence (668/25 213, 2.6%) but nonetheless accounted for a substantial absolute number of cases (n=668) with a M:F ratio of 5.9. Detailed results are reported in table 4.

## DISCUSSION

In this study, we assessed the laboratory-confirmed prevalence of CT and NG infections in a high-density urban area in northern Italy over a 6-year period (2018–2023), comparing different groups of subjects based on age, sex and the healthcare facilities they visited. This broad survey on CT/NG epidemiology contributes valuable insights that can aid in developing strategies for STI prevention and control.

Overall, we found a CT infection prevalence of 7.2%, consistent with other surveys conducted globally.[14 15] In our metropolitan area, nearly three-quarters of CT infections were detected at the STI outpatients clinic (69%), followed by the infectious disease centres (13%). The remaining cases were distributed across community services/general practitioners (7.6%), family counselling centres (6.9%) and gynaecological clinics (3.6%). Although the latter two healthcare facilities accounted for approximately one-third of all subjects tested (26%), their CT infection prevalence was the lowest (around 3%).

A similar study by den Heijer *et al* showed that gynaecologists conduct a substantial proportion of CT testing, although general practitioners and STI clinics are primarily responsible for CT diagnosis.[16] In contrast, our findings show that the STI clinic and infectious disease centres—mainly serving male patients and high-risk groups—were the primary sources of CT-positive cases. These high-risk groups include younger individuals, patients with STI-related symptoms, MSM engaging in unprotected sex with multiple partners and PrEP users. In these core groups, CT infection prevalence often exceeds 10–15%.[17 18] Conversely, attendees of gynaecology and family centres—mainly low-risk, asymptomatic women seeking fertility counselling or general screening—represent a lower prevalence population. General practitioners serve a more heterogeneous group, including both males and females, and thus represent an intermediate category. These differences in testing populations and practices may help explain the varying prevalence rates.

Regarding age distribution, the highest CT prevalence was observed among individuals aged 18–25 years (10.7%), followed by those aged 26–34 years (7.7%). These results align with recent European data showing the highest proportion of CT cases in the 20–24 (40%) and 25–34 (27%) age groups.[15] These data highlight young adults as a priority target for interventions aimed at reducing CT burden.[19] Therefore, efforts must focus on improving access to sexual health services and strengthening sexual education programmes to address common barriers in this group, such as perceived low risk, unawareness of CT implications and reluctance to undergo testing.[20]

The male-to-female (M:F) ratio of CT-positive cases in our study was 1.5, aligning with the overall European ratio of 0.9.[21] However, significant variability across countries (eg, Portugal 2.3, Greece 0.3) likely reflects differences in testing strategies, public health policies and surveillance systems.

It is worth underlining that a large proportion of samples from women were represented by urines. Considering that urine samples are not optimal for CT detection due to their lower sensitivity compared with genital swabs,[22] it is possible that the prevalence of CT infection in women has been underestimated.

**Table 4** Absolute and percentage distribution of positive samples of *Neisseria gonorrhoeae* (NG)-positive samples per single healthcare access stratified by biological samples for diagnostic testing, age group, sex and testing location in Bologna, Italy, 2018–2023

| | | NG n | NG n (%) | Positive samples by age n (%) | | | | Positive samples by sex n (%) | | Positive samples by STI testing location n (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Total accesses | Pos. | 18–25 | 26–34 | 35–44 | ≥45 | Male | Female | STI | ID | GYN | FAM | COMM |
| Biological Samples for Diagnostic Testing | ≥3 biological samples | 6701 | 1070 (16.0) | 245 (20.6) | 388 (16.4) | 276 (15.5) | 161 (11.7) | 1039 (16.4) | 31 (8.2) | 636 (19.7) | 434 (12.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | 2 biological samples | 3583 | 320 (8.9) | 88 (9.7) | 134 (10.0) | 58 (7.6) | 40 (7.0) | 281 (10.6) | 39 (4.2) | 255 (11.5) | 65 (5.4) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Urine only | 25213 | 668 (2.6) | 166 (2.5) | 239 (2.5) | 137 (2.4) | 126 (4.0) | 571 (4.8) | 97 (0.7) | 617 (3.9) | 15 (2.3) | 5 (0.4) | 5 (0.1) | 26 (0.6) |
| | Pharyngeal swab only | 501 | 42 (8.4) | 13 (9.9) | 12 (6.5) | 9 (9.4) | 8 (9.1) | 37 (8.8) | 5 (6.1) | 37 (8.7) | 5 (6.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Rectal swab only | 447 | 60 (13.4) | 19 (26.0) | 14 (12.4) | 13 (10.4) | 14 (10.3) | 60 (14.0) | 0 (0.0) | 52 (14.4) | 8 (9.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Endocervical swab only | 5973 | 40 (0.7) | 12 (1.1) | 12 (0.6) | 4 (0.2) | 12 (1.3) | 0 (0.0) | 40 (0.7) | 7 (2.4) | 0 (0.0) | 10 (0.6) | 11 (0.5) | 12 (0.7) |
| | Vaginal swab only | 3697 | 19 (0.5) | 7 (0.7) | 4 (0.3) | 5 (0.5) | 3 (1.4) | 0 (0.0) | 19 (0.5) | 2 (1.3) | 0 (0.0) | 9 (1.3) | 7 (0.3) | 1 (0.3) |
| | Urethral swab only | 348 | 12 (3.4) | 5 (10.0) | 3 (2.3) | 3 (2.9) | 1 (1.5) | 11 (6.8) | 1 (0.5) | 1 (50.0) | 1 (9.1) | 1 (0.7) | 0 (0.0) | 9 (5.0) |
| | Conjunctival swab only | 21 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| | Other* | 17 | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Total accesses | | 46501 | 2231 (4.8) | 555 (5.0) | 806 (4.7) | 505 (4.3) | 365 (5.6) | 1999 (9.1) | 232 (1.0) | 1607 (7.2) | 528 (9.7) | 25 (0.6) | 23 (0.3) | 48 (0.7) |

Unit of analysis=access (unique combination of patient and date). An access is considered NG-positive if ≥1 NG result within that access=positive.
Values are given as absolute counts (n) and percentages in parentheses (%) indicating the proportion of NG-positive accesses within the same subgroup. Example: 'Urine only=668 (2.6%)' means 668 NG-positive samples, representing 2.6% of all 'Urine only' accesses.
*Other specimens mainly include synovial fluids, intestinal and gynaecological biopsies.
COMM, community services/other access points; FAM, family counselling centres; GYN, gynaecology/obstetrics centres; ID, infectious diseases centres; STI, dermatology/STI clinic.

In contrast to CT, the epidemiology of NG infection in our setting was markedly different. NG infections were almost exclusively detected in high-risk settings (ie, STI clinic and infectious disease centres), with minimal detection in other healthcare services. With an overall prevalence of 4.8%, NG infection peaked in individuals older than 45 years, although the highest absolute number of cases was among those aged 26–34 years. The M:F ratio was notably high at 8.6.

These findings reflect the recent European epidemiological report about gonorrhoea, confirming a high M:F ratio (overall ratio in 2023 was 3.9), with the largest proportion of cases reported among the age group 25–34 years.[23] As in many other settings,[24] MSM likely represent a significant proportion of NG-positive individuals in our cohort.

Despite the distinct epidemiological profiles of CT and NG, several common trends were observed.

*Rising case numbers over time:* Although overall prevalence remained stable, the absolute number of CT and NG cases increased significantly, primarily among males. This trend correlates with broader testing availability and awareness campaigns, particularly in high-risk groups. The rise in positive cases at Infectious Disease centres, often associated with PrEP users, is notable. Since 2016, European guidelines have recommended regular STI screening among PrEP users, a policy that was further incentivised in Italy following the reimbursement of PrEP by the National Health Service in April 2023.[10] Additional factors contributing to this rise may include high-risk sexual behaviours (eg, condomless sex, multiple or anonymous partners, chemsex), HIV care protocols and the implementation of guidelines recommending extragenital testing.[25] Moreover, our data showed a shift in CT and NG infections towards older age groups, possibly reflecting evolving sexual behaviours.[26]

*Impact of the COVID-19 pandemic:* We observed a significant decline in reported cases in 2020, followed by a rebound in 2021 and 2022. The initial drop likely reflects reduced social/sexual activities, reduced access to STI services, lower testing rates and under-reporting as resources were redirected to pandemic response.[27] The rebound may be explained by increased testing availability (including home testing), higher consultation rates in sexual health clinics and changes in sexual behaviour post-lockdown.[21 22]

*Significant extragenital involvement:* A notable proportion of CT and NG cases were diagnosed at extragenital sites, particularly rectal chlamydia and pharyngeal gonorrhoea. These findings align with the biology of CT and NG, which can persist and replicate in the mucosa of the anorectal and oropharyngeal tracts.[28 29] In our study, extragenital tests were predominantly performed by the STI clinic and infectious disease centres, primarily on male patients. Although current guidelines recommend site-specific testing based on sexual history,[16] extragenital testing remains underutilised in other healthcare settings. This may stem from low provider awareness or patient-related factors such as stigma and shame.[16] Our findings support expanding extragenital testing beyond specialised STI and infectious disease centres.[16] The importance of this approach is further highlighted by the fact that many CT and NG infections were located exclusively at extragenital sites without genital involvement.

It should be noted that heterogeneity among subjects with respect to sex, age, testing location and type of specimens submitted may have influenced our findings. For instance, most samples (and nearly all extragenital swabs) were collected at the STI clinic and the infectious diseases centres, which likely led to an overestimation of the overall CT/NG prevalence. Moreover, the low CT positivity rate observed in vaginal swabs (3.3%) may

be explained by the fact that the majority of these specimens originated from low-prevalence settings, such as family counselling (70%) or gynaecological centres (19.6%). Finally, the marked increase in testing volume at infectious diseases centres (75 subjects in 2018 vs 2085 in 2023), primarily involving older male patients, may account for the observed shifts in the demographic profile of CT-infected and NG-infected individuals, namely, a higher positivity in older age groups together with an increased M:F ratio. According to the Italian System for Reporting Infectious Diseases (PREMAL), both CT and NG infections must be notified to the national surveillance system, a requirement that is vital for monitoring public health and detecting outbreaks (available at: https://www.gazzettaufficiale.it/eli/id/2022/04/07/22A02179/sg; last accessed on 25 May 2025). However, these systems are inherently subject to underestimation, resulting in uncertainty about the true incidence of infection.[30] Our findings may therefore enable more accurate estimation of under-reporting within the study area and inform public health strategies to address this gap, optimising the awareness of the importance of CT/NG reporting among all healthcare workers involved in STI management.

In conclusion, this study highlights important aspects of CT and NG epidemiology, emphasising differences based on population characteristics, healthcare settings and external factors such as the COVID-19 pandemic and PrEP availability. Our findings underscore the need to enhance regional and national STI screening policies as a core strategy for controlling CT and NG transmission.

Furthermore, innovative approaches to CT/NG prevention and control are essential, including primary prevention, standardised case management, partner notification and opportunistic testing. In this context, our data highlight the potential value of the laboratory system as a tool for integrated epidemiological surveillance.

A major limitation of this study is the lack of information on symptom status and sexual orientation of tested individuals, as well as detailed data on PrEP usage, which may have influenced our findings. Future studies are needed to better understand CT/NG testing dynamics and to improve coordination among various healthcare providers.

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