

Genital and extra-genital *Chlamydia trachomatis* and *Neisseria gonorrhoeae* infections in young women attending a Sexually Transmitted Infections (STI) clinic

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SUMMARY

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the most common agents of bacterial sexually transmitted infections (STIs) worldwide.

Here, we evaluated genital and extra-genital *C. trachomatis* and *N. gonorrhoeae* infection prevalence in a cohort of young women attending an STI Outpatients Clinic in Italy.

From May 2019 to December 2019, 134 women aged 18-26 years were enrolled. A vaginal, a pharyngeal and a rectal swab for the molecular detection of *C. trachomatis* and *N. gonorrhoeae* were collected from each patient. Chlamydia-positive samples underwent a molecular genotyping based on *pmpH* gene.

Total prevalence of *C. trachomatis* and *N. gonorrhoeae* infections was 17.9% and 11.2%, respectively. Chlamydial infections were prevalent in the urogenital (16.4%) and rectal (13.4%) sites, whereas *N. gonorrhoeae* predominated in the genital (7.4%) and pharyngeal (6%) mucosa. Overall, 5.2% of cases would have been missed if extra-genital sites had not been tested. Notably, 60% of women with a rectal infection did not report anal sex. A history of sexual contacts with a positive partner ($P=0.03$) and a history of ≥ 3 partners in the last 6 months ($P=0.0075$) were highly predictive of a bacterial STI. No cases of lymphogranuloma venereum were found.

These data could help set up effective strategies to prevent the spread of STIs.

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INTRODUCTION

Chlamydia trachomatis and *Neisseria gonorrhoeae* are the most common agents of bacterial sexually transmitted infections (STIs) worldwide (WHO, 2015).

Urogenital *C. trachomatis* and *N. gonorrhoeae* infections in women (i.e., urethritis and cervicitis) are often asymptomatic and, if left untreated, can lead to several sequelae and complications, including pelvic inflammatory disease (PID), tubal infertility and ectopic pregnancy (Price *et al.*, 2013; Stevens and Criss, 2018).

Moreover, urogenital infections are associated with increased likelihood of HIV infection transmission and acquisition (Haggerty *et al.*, 2010).

Besides the common urogenital localizations, these microorganisms can be found at extra-genital sites, as pharyngeal and ano-rectal mucosa, on the basis of the sexual repertoires of the couples (van Liere *et al.*, 2014; den Heijer *et al.*, 2015; Foschi *et al.*, 2016; Danby *et al.* 2016; Gaspari *et al.*, 2019). In women, the prevalence of extra-genital infections can be up to 5-15% in high-risk populations (Chan *et al.*, 2016).

Extra-genital *C. trachomatis* and *N. gonorrhoeae* infections are often asymptomatic or characterized by non-specific symptoms, acting as an important reservoir for further transmission (Peters *et al.*, 2014; van Liere *et al.*, 2014). Moreover, untreated pharyngeal gonococcal infections can be crucial for the emergence of multi-drug resistance in *N. gonorrhoeae* (Unemo and Shafer, 2014). Indeed, it has been reported that the mosaic *penA* alleles, responsible for the cephalosporin resistance, has emerged by DNA recombination with partial *penA* genes from commensal *Neisseria* species found in the oro-pharynx (Osaka *et al.*, 2008).

In the last few years, nucleic acid amplification techniques (NAATs) have become the reference methods for the diagnosis of genital and extra-genital *C. trachomatis* and *N. gonorrhoeae* infections, due to their excellent specificity and sensitivity and rapid turn-around time (Workowski and Bolan, 2015; Bristow *et al.*, 2017).

At present, international guidelines recommend an annual screening for genital *C. trachomatis* and *N. gonorrhoeae* infections in all sexually active women aged <25 years, or in older women at increased risk for infection (e.g., those who have a new sex partner, more than one sex partner, a sex partner with concurrent partners, or a sex partner who has a sexually transmitted infection) (LeFevre, 2014). Moreover, there is increasing evidence that heterosexual women should be tested for extra-genital chlamydia and gonorrhoea, when oral and anal sexual activity is reported (Garner *et al.*, 2015).

In Italy, no national official guidelines for genital *C. tra-*

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chomatis and *N. gonorrhoeae* screening are currently available; nevertheless, the Ministry of Health suggests a chlamydial screening in women aged <25 years and or in older women at increased risk for infection (available at: http://www.salute.gov.it/portale/salute/p1_5.jsp?lingua=italiano&id=116&area=Malattie_sessualmente_trasmissibili). For extragenital infections no specific recommendations have been issued so far in Italy.

In this context, extensive knowledge about the prevalence and characteristics of genital and extra-genital *C. trachomatis* and *N. gonorrhoeae* infections in women could help set up effective strategies to prevent the acquisition and spread of STIs.

To the best of our knowledge, no detailed information about extra-genital infections due to *C. trachomatis* and *N. gonorrhoeae* in young women in Italy is currently available. Therefore, the aim of this study was to assess genital and extra-genital *C. trachomatis* and *N. gonorrhoeae* infection prevalence in a cohort of young women (<26 years) attending an STI Outpatient Clinic in the North of Italy.

MATERIALS AND METHODS

Study population and sample collection.

From May to December 2019, all the consecutive women attending the STI Outpatients Clinic of St. Orsola-Malpighi Hospital in Bologna (Italy) and meeting one of the following criteria were enrolled for the study:

- 1) presence of STI-related symptoms (e.g., vaginal discharge, abnormal bleeding, dyspareunia);
- 2) presence of risk factors for *C. trachomatis* and *N. gonorrhoeae* infections (i.e., new or multiple sexual partners, unprotected intercourse, history of a partner positive for STIs). This free-access clinic basically tests high-risk groups and young subjects by following patients for counselling, prevention, diagnostic testing, and therapeutic management of STIs.

Exclusion criteria included: being younger than 18 or older than 26 years, being pregnant, having used any antibiotics in the last month, refusing to perform extragenital sampling (i.e., rectal and pharyngeal swabs) for *C. trachomatis* and *N. gonorrhoeae* detection, and denying consent to the study.

After a preliminary interview, a clinical visit was carried out for each patient. Personal data (e.g., age, sexual orientation) and information about symptoms, number of sexual partners in the last 6 months and type of sexual practices were recorded. Afterwards, a vaginal, a pharyngeal and a rectal swab (E-Swab, Copan, Brescia, Italy) for the molecular detection of *C. trachomatis* and *N. gonorrhoeae* were collected. Both the urogenital and the extra-genital swabs were taken by the clinicians during the visit, avoiding self-collection of specimens.

To avoid biases related to prior antimicrobial treatment, only samples collected at the first visit were considered, excluding specimens obtained during the follow-up period. Moreover, patients were managed for human immunodeficiency virus (HIV), hepatitis B and C virus (HBV and HCV) and syphilis infections, following the regular STIs evaluation of the Clinic, as previously reported (Foschi *et al.*, 2018). The protocol was approved by the Ethics Committee of Sant'Orsola-Malpighi Hospital in Bologna (7/2016/U/Tess). The study was carried out in accordance with the Declaration of Helsinki, following the recommendations of the Ethics Committee of the Hospital.

C. trachomatis and N. gonorrhoeae detection

Urogenital specimens and extra-genital samples were processed by Versant CT/GC DNA 1.0 Assay (Siemens Healthcare Diagnostics, Terrytown, USA), a commercial real-time PCR, simultaneously detecting the presence of *C. trachomatis* and/or *N. gonorrhoeae* DNA (Marangoni *et al.*, 2015). This molecular assay has proved to be extremely sensitive in the detection of *C. trachomatis* and *N. gonorrhoeae* infections (limit of detection: 1.0 copies/ml for *N. gonorrhoeae* and 10 copies/mL for *C. trachomatis*), with excellent specificity (e.g., no false positive results due to the presence of non-gonococcal *Neisseria* species) (Kerndt *et al.*, 2011; Marangoni *et al.*, 2015).

An estimate of the bacterial load of *C. trachomatis* and *N. gonorrhoeae* NAAT-positive specimens was obtained using the cycle threshold (Ct) values (Bissessor *et al.*, 2015).

C. trachomatis typing

Positive samples underwent *C. trachomatis* molecular genotyping, using the correspondent remaining eluate from Versant PCR plate. Molecular genotyping was performed by a real-time PCR targeting the polymorphic membrane protein H gene (*pmph*), as previously described (Chen *et al.*, 2008). This assay allows the distinction between L and non-L chlamydial serovars on the basis of a unique 36-base-pair deletion region of *pmph* gene that occurs only among LGV strains (Chen *et al.*, 2008).

Statistical analysis

All statistical analyses were performed by using GraphPad Prism software (GraphPad Prism version 5.02 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com).

To evaluate statistically significant differences between the subgroups of subjects, t-test, Mann-Whitney, and Kruskal-Wallis test were used to compare quantitative data, whereas categorical data were analysed with chi-square or Fisher's exact test. A *P* value <0.05 was considered statistically significant.

RESULTS

Study population

From May to December 2019, 320 women were eligible for the study but 206 of them (64.3%) refused to perform extra-genital sampling or denied consent to the study, thus being excluded. Therefore, a total of 134 women, with a mean age of 23.1±1.9 years were enrolled. Of them, 30.6% (41/134) complained of various uro-genital symptoms, including mainly vaginal discharge (59.5%), abnormal bleeding (11.9%), dyspareunia (9.5%) and genital itching (9.5%). Contrariwise, the patients reported no pharyngeal (e.g., pharyngeal pain, hoarseness) or rectal symptoms (e.g., rectal discharge, anal pain, tenesmus).

A total of 11 subjects (8.2%) reported a history of sexual contacts with a partner positive for *C. trachomatis* and/or *N. gonorrhoeae*. All the women reported vaginal and oral intercourse, whereas only 32.8% (44/134) claimed to have had anal intercourse. Out of the 134 patients, 64.9% had <3 sexual partners in the last 6 months, while 35.1% reported a history of ≥3 sexual partners in the last 6 months.

As for sexual orientation, most of the women were heterosexual (82.8%), whereas bisexuals and lesbians accounted for 14.1% and 3.0% of the total, respectively.

Overall, no cases of HIV, syphilis, HCV, or HBV were found in the cohort of subjects enrolled.

C. trachomatis and *N. gonorrhoeae* infection prevalence

Total prevalence of bacterial STIs (i.e., at least one microorganism in at least one anatomical site) was 26.1% (35/134). Prevalence of single *C. trachomatis* and *N. gonorrhoeae* infections was 14.9% (20/134) and 8.2% (11/134), respectively, whereas 3.0% (4/134) of the women showed a contemporary chlamydial/gonococcal infection in at least one site (Figure 1 A).

When stratified for the anatomical sites (Figure 1 B), the highest prevalence of chlamydial infections was found in the genital tract (16.4%; 22/134), followed by the rectum (13.4%; 18/134) and the oro-pharynx (4.4%; 6/134). For gonorrhoea, most of the cases were found in the genital (7.4%; 10/134) and pharyngeal (6.0%; 8/134) mucosa, with only 3.0% (4/134) of rectal *N. gonorrhoeae* infections.

Out of the 35 women with a bacterial STI, 37.1% showed

contemporary genital and rectal positivity, whereas 17.1% were positive in all the three anatomical sites tested. Only 8 women (22.8% of the positives) showed a single genital infection.

It is worth emphasising that 7 women were characterized only by extra-genital infections without genital involvement. Therefore, 20% of *C. trachomatis* and *N. gonorrhoeae* cases (5.2% of all women enrolled) would have been missed if rectal and pharyngeal sites had not been tested.

STI predictors, clinical/epidemiological associations

As shown in Table 1, the mean age of STI-positive patients was similar to the negative subjects (23.0±1.7 years vs 23.1±1.9 years; $P=0.7$).

Interestingly, more than 70% of women with a genital infection were totally asymptomatic.

Overall, 19.5% (8/41) of symptomatic women were positive for genital *C. trachomatis* and/or *N. gonorrhoeae* infections, whereas in the group of asymptomatic patients, 21.7% (20/92) of participants were found positive for a genital STI. Gonococcal infections were characterized by the presence of genital symptoms more often than chlamydial infections (50.0% vs 16.6%; $P=0.09$). Overall, the presence of genital symptoms was not a significant predictor of a genital bacterial STI (28% of STI-positive women vs 32% of STI-negative patients; $P=0.8$; Table 1).

A history of sexual contacts with a partner positive for *C. trachomatis* and/or *N. gonorrhoeae* (17.1% of STI-positive women vs 5% of STI-negative patients), as well as a history of ≥3 sexual partners in the last 6 months (54.2% of STI-positive women vs 28.2% of STI-negative patients) were highly predictive of a bacterial STI ($P=0.03$ and $P=0.0075$; Table 1). Contrariwise, no significant association was found between sexual orientation and presence of chlamydial/gonococcal infections (97% of heterosexuals/bisexuals both in STI-positive group and in STI-negative women; $P=1$).

It is worth noting that 60% of women with a rectal infection did not report a history of anal intercourse. Therefore, no association between rectal STIs and anal intercourse was found (40% of STI-positive women reporting unsafe anal sex vs 31.5% of STI-negative women reporting anal intercourse; $P=0.45$).

The presence of symptoms was not related to higher bacterial loads at the genital site (*C. trachomatis* median Ct values: 22.8 in the case of symptoms vs 25.0 in asymptomatic women; $P=0.48$; *N. gonorrhoeae* median Ct values: 22.7 in the case of symptoms vs 31.6 in asymptomatic women; $P=0.28$). Similarly, no significant associations between bacterial loads and the different anatomical sites (genital, pharyngeal and rectal mucosa) were found (*C. trachomatis* median Ct values: 23.2 for genital infections vs 22.3 for pharyngeal infections vs 26.9 for rectal infections; $P=0.45$;

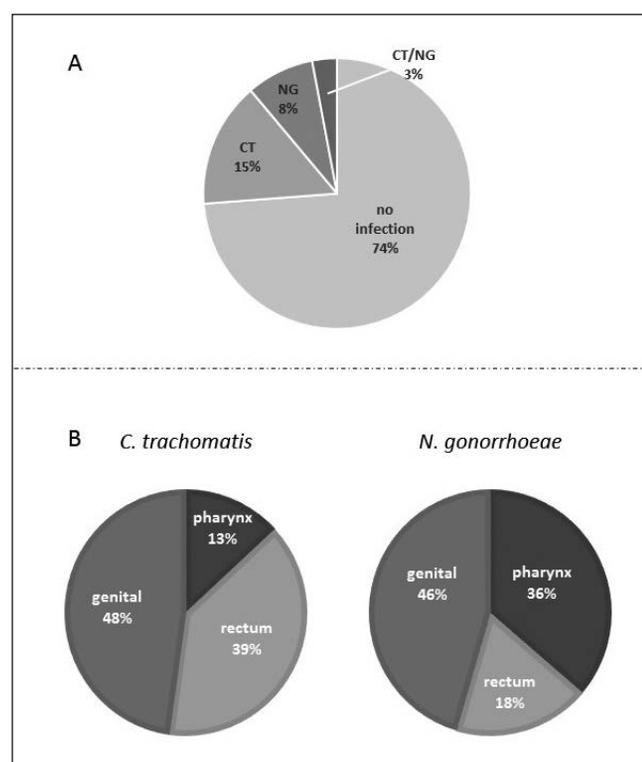


Figure 1 - Prevalence of *C. trachomatis* and *N. gonorrhoeae* infections.

A) Prevalence of single *C. trachomatis* and *N. gonorrhoeae* infections and mixed cases. B) Distribution of *C. trachomatis* and *N. gonorrhoeae* infections is stratified by the different anatomical sites (genital tract, oro-pharyngeal mucosa, rectum). CT: *C. trachomatis*; NG: *N. gonorrhoeae*.

Table 1 - Characteristics of women enrolled, stratified by the STI-positivity.

	STI-positive women* (n=35)	STI-negative women (n=99)	P value
Mean age (years ± SD)	23.0 ± 1.7	23.1 ± 1.9	0.7
Genital symptoms ^o	8/28 (28%)	34/106 (32%)	0.8
Chlamydia and/or gonorrhoea positive partner	6/35 (17.1%)	5/99 (5%)	0.03
≥3 sexual partners in the last 6 months	19/35 (54.2%)	28/99 (28.2%)	0.0075
Heterosexuals/Bisexuals	34/35 (97%)	96/99 (97%)	1

*At least one microorganism (*C. trachomatis*, *N. gonorrhoeae*) in at least one anatomical site (genital, oro-pharyngeal and rectal mucosa).

^oThe percentages are calculated on the genital positivity/negativity.

N. gonorrhoeae median Ct values: 25.1 for genital infections vs 31.4 for pharyngeal infections vs 24.9 for rectal infections; $P=0.48$).

Finally, similar chlamydial loads were detected at the rectal site between women reporting or not reporting anal intercourse (median Ct values: 26.9 vs 26.6; $P=0.9$).

C. trachomatis typing

Molecular genotyping revealed that all cases of chlamydial infections were due to non-L *C. trachomatis* serovars.

DISCUSSION

In this study we evaluated genital and extra-genital *C. trachomatis* and *N. gonorrhoeae* infection prevalence in a group of young women (18-26 years) attending an STI Out-patients Clinic in the North of Italy.

Overall, we found that total prevalence of *C. trachomatis* and *N. gonorrhoeae* infections was 17.9% and 11.2%, respectively. The high rate of chlamydial/gonococcal infections found in our cohort reflects the type of population enrolled. Indeed, if considering women at higher risk for STI transmission (e.g., sexually active young people, history of unsafe intercourse, multiple sexual partners), *C. trachomatis* and *N. gonorrhoeae* infection prevalence can easily exceed 10-15% (Petrovay *et al.*, 2009; Peters *et al.*, 2014; Foschi *et al.*, 2016).

Our findings agree with European reports stating that, in women, *C. trachomatis* and *N. gonorrhoeae* infection cases peak among 20-24-year-old subjects and that chlamydial infections are more frequent than gonococcal ones (ECDC, 2015).

Similarly, previous Italian reports highlighted a significant burden of bacterial STI infections in young women, *C. trachomatis* being the most reported one (Bianchi *et al.*, 2016; Salfa *et al.*, 2016; Martinelli *et al.*, 2019; Sabbatucci *et al.*, 2019).

The surprisingly high rate of gonorrhoea found in our cohort underlines the need for close surveillance of *N. gonorrhoeae* infections in the female population as well. Although the growing trend in the number of reported gonorrhoea cases continues to be driven mostly by increased cases of 'men having sex with men' (MSM), a marked increase among heterosexual women has been reported in the last years in many countries (ECDC, 2019). The high rate of reported *N. gonorrhoeae* infections across Europe indicates continuing high levels of risky behaviour. This is especially of concern considering the increasing levels of antimicrobial resistance in NG reported by the European Gonococcal Antimicrobial Surveillance Programme (Euro-GASP) (Cole *et al.*, 2019). Thus, further strengthening of prevention activities aimed at increased testing uptake and testing frequency in women most at risk are certainly expected.

When looking exclusively at positive cases stratified by anatomical site, we found that *C. trachomatis* infections were prevalent in the urogenital (48%) and rectal (39%) sites, whereas *N. gonorrhoeae* infections predominated in the genital (46%) and pharyngeal (36%) mucosa. Besides the creation of specific sexual networks in the population enrolled, this aspect could reflect the different bio-pathological behaviour of the two microorganisms. Indeed, *Neisseria* species are able to find in the oro-pharynx a suitable ecological niche to replicate and persist over time (Quilin and Seifert, 2018; Marangoni *et al.*, 2020a; Marangoni *et al.*, 2020b), whereas *C. trachomatis* benefits from the high lev-

els of indole found in the intestinal tract (Jordan *et al.*, 2017; Ziklo *et al.*, 2018).

Considering the presence of symptoms, we confirmed that, in the female population, most of *C. trachomatis* and *N. gonorrhoeae* infections are completely asymptomatic (Chan *et al.*, 2016; Grant and Crane, 2017; Unemo *et al.*, 2019). Actually, we found that more than 70% of genital infections and all extra-genital cases were characterized by the absence of symptoms. When present, genital symptoms were not associated with higher bacterial loads; other factors probably contribute to the onset of symptoms, such as local immunity, individual susceptibility, bacterial strain virulence and type of endogenous microbiota.

As previously reported (Chan *et al.*, 2016; Llata *et al.*, 2018), we detected several cases of rectal and pharyngeal infections without genital involvement. Therefore, about 5% of positive patients would have been missed if extra-genital sites had not been tested.

Besides the well-established role of a genital screening (Grant and Crane, 2017), our data support the recommendation that high-risk women (e.g., those who are sexually active with concurrent or non-mutually monogamous partners, regardless of reported exposure sites) need to be tested at multiple anatomical locations, corroborating the idea of a universal extra-genital screening program (Garner *et al.*, 2015; Andersson *et al.*, 2017).

Due to the frequency of asymptomatic pharyngeal/rectal infections and the inaccuracy of testing based on self-reported behaviour, extra-genital *C. trachomatis* and *N. gonorrhoeae* screening should not be based on clinical symptoms and should be considered in high-risk settings such as STI clinics (Chan *et al.*, 2016; den Heijer *et al.*, 2016). However, extra-genital screening protocols among sexually active women are not currently widespread, and further study is needed to evaluate the impact on sexual health outcomes. In Italy, no current recommendations for extra-genital screening are available; the data from this study suggest that new policies and guidelines for extra-genital *C. trachomatis* and *N. gonorrhoeae* screening in women are urgently needed as a critical component of the healthcare. In the absence of a routine screening program, our results underline that several predictors could help identify subjects at higher risk for *C. trachomatis* and *N. gonorrhoeae* infections. In particular, a history of sexual contacts with a partner positive for chlamydia and/or gonorrhoea, as well as the history of ≥ 3 sexual partners in the last 6 months were significantly associated with positivity for a bacterial STI.

In this context, extensive knowledge of the characteristics and the risk factors of *C. trachomatis* and *N. gonorrhoeae* infections in women is crucial to set up effective prevention programs and to significantly reduce the transmission and spread of STIs (Francis *et al.*, 2020).

Other interesting data emerged when focusing on rectal infections. We noticed that:

- 1) rectal chlamydia and/or gonorrhoea were frequently associated with a concurrent genital infection;
- 2) most of the women testing positive for rectal infections did not report anal sex;
- 3) no differences in rectal bacterial loads were found between women reporting and not reporting anal intercourse.

In line with previous reports (Barry *et al.*, 2010; Dukers-Muijers *et al.*, 2015), these results underline the significant contribution of non-sexual inoculation of the rectum with infected cervico-vaginal secretions.

The high rate of concurrent genital and rectal chlamydial infections raises another important issue. It has been shown that doxycycline can be more effective than azithromycin for rectal chlamydia treatment (Kong *et al.*, 2015; Workowski and Bolan, 2015). Therefore, European guidelines recommend doxycycline as the treatment of choice for rectal infections (Lanjouw *et al.*, 2010). Based on our results, we can speculate that, even in absence of a rectal testing, women with a chlamydial genital infection should probably be treated with doxycycline, in order to 'cure' an undetected rectal localization.

Finally, when looking at *C. trachomatis* serovar distribution, we found that all cases of chlamydial infections were associated with D-K serovars, with no cases of lymphogranuloma venereum (LGV).

As previously reported, in our setting, LGV is found mainly in the rectal site of high-risk HIV-positive 'men having sex with other men' (MSM) (Foschi *et al.*, 2014; Foschi *et al.*, 2018).

Although sporadic cases of LGV in women were recently reported in Europe (Rodriguez-Dominguez *et al.*, 2014; Juzlova *et al.*, 2018), we had no chance to detect similar cases in the present study.

Therefore, in an epidemiological context where LGV prevalence is extremely low (e.g., female population), the need for routine *C. trachomatis* genotyping should be evaluated with caution, assessing the cost-benefit ratio, and limiting the test to selected cases.

In conclusion, the main findings of this study are as follows:

- 1) young women (18-26 years) reporting risky sexual behaviour are a significant reservoir for *C. trachomatis* and *N. gonorrhoeae* infections;
- 2) in order not to miss infections in women, extra-genital sites should be part of the routine testing of multiple anatomical localizations for *C. trachomatis* and *N. gonorrhoeae* detection;
- 3) rectal infections are very common, asymptomatic and often associated with non-sexual activities;
- 4) probably, in our setting, LGV has very limited diffusion in the female population.

This survey can contribute to developing strategies for the prevention and control of STIs. Further studies with larger numbers of women are needed for an in-depth evaluation of the dynamics occurring in the female population in order to set up 'tailored' preventive and surveillance approaches.

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