Chlamydia trachomatis infection prevalence and serovar distribution in a high-density urban area in the north of Italy

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The aim of this study was to assess Chlamydia trachomatis (CT) infection prevalence and serovar distribution in a high-density urban area in the north of Italy, by comparing different groups of subjects divided on the basis of the type of care provider they referred to (STI Clinic, gynaecologists or general practitioners). From January 2011 to May 2014, all the specimens submitted to the Microbiology Laboratory of St Orsola Hospital in Bologna for CT detection were tested by PCR assay. For positive specimens, molecular genotyping based on RFLP analysis was performed. Total prevalence of CT infection was 8.1%, with significant differences between subgroups (P<0.01) but stable during the study period. The STI Clinic was mainly responsible for CT diagnosis, whereas the lowest infection prevalence was detected in gynaecological clinics, despite the high number of tests performed. Extra-genital samples were almost exclusively collected from males at the STI Clinic. Interestingly, 13.3% of patients providing extra-genital specimens were positive for CT on rectal and/or pharyngeal swabs, and 4.4% of cases would have been missed if extra-genital sites had not been tested. The most common serovar was E, and serovar distribution was influenced by gender (P<0.01), age (P<0.01), care provider (P=0.01) and anatomical site (P<0.01). The L2 serovar was detected only in extra-genital samples from males at the STI Clinic. Knowledge about care providers’ contributions in CT testing and diagnosis is essential for infection control. CT typing is crucial for appropriate management of specific infections, such as lymphogranuloma venereum in extra-genital samples of high-risk populations.

INTRODUCTION

Chlamydia trachomatis (CT) is the causative agent of the most common bacterial sexually transmitted infection (STI) worldwide (Stevens et al., 2010). In Europe, an overall rate of 182 notifications per 100,000 population in countries with comprehensive surveillance systems was reported in 2013 (ECDC, 2015).

Urogenital CT infections, such as urethritis in men and cervicitis in women, are often asymptomatic, and for that reason they can remain unnoticed and untreated, leading to several sequelae and complications including pelvic inflammatory disease, tubal infertility, ectopic pregnancy and epididymo-orchitis (Price et al., 2013; Menon et al., 2015).

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

Besides urogenital localizations, it is well known that this microorganism can be found at extra-genital sites, such as pharyngeal and rectal mucosa, according to the sexual repertoires of the couples (van Liere et al., 2013, 2014; den Heijer et al., 2015; Allan-Blitz et al., 2016). Extra-genital CT infections are often asymptomatic or characterized by non-specific symptoms, acting as an important reservoir for further transmission and potentially leading to sequelae both in men having sex with men (MSM) and in women (van Liere et al., 2013, 2014; Peters et al., 2014; Patton et al., 2014).

CT infections are also responsible for trachoma-associated blindness (Derrick et al., 2015) and for lymphogranuloma venereum (LGV), a systemic infection characterized in
Western countries by outbreaks of symptomatic ulcerative procto-colitis mainly in MSM (de Vrieze & de Vries, 2014; Foschi et al., 2014). The different clinical manifestations of CT infections are associated with specific distinct CT serovars, divided on the basis of their antigenic reactivity with specific monoclonal antibodies. In particular, serovars A to C are associated with trachoma, serovars D to K with typical urogenital infections and serovars L1 to L3 with LGV (Stevens et al., 2010). Since CT serotyping is time- and labour-consuming, nowadays genotyping methods are currently used and a variety of molecular techniques, such as PCR-RFLP, real-time PCR or nested-PCR, followed by sequencing, have been reported (Pedersen et al., 2009; de Vries et al., 2015).

The gene encoding the major outer membrane protein (omp1), is widely used as a molecular target, since it contains four spaced variable domains (VDI to VDIV) flanked and interspaced by five conserved domains (Bom et al., 2011).

The availability of simple and reliable methods for CT genotyping has led to a great variety of scientific investigations on CT molecular epidemiology, showing variable results depending on the geographical area, the selection of population, the type of samples (genital vs extra-genital specimens) and the molecular assay (Papadogeorgakis et al., 2010; Marangoni et al., 2012; Mejuto et al., 2013; Yang et al., 2014; Versteeg et al., 2015).

Genotyping of CT strains within a population may provide useful information on the pathogenesis of the infection and enables associations with clinical and epidemiological findings (Lagergard et al., 2010; Bom et al., 2011). Furthermore, genotyping can allow monitoring of contact tracing or evaluation of treatment success (Kapil et al., 2015), and may play a role in developing strategies for vaccine design (Geisler et al., 2003).

Finally, CT genotyping has a critical role in the choice of appropriate antibiotic treatment in cases where LGV is suspected, because when L1–L3 serovars are detected, patients should receive a 3 week therapy regimen (doxycycline 100 mg orally twice a day for 21 days), instead of a 1 week approach (Korhonen et al., 2012; Workowski & Bolan, 2015).

The aim of this study was to assess CT infection prevalence in a high-density urban area in the north of Italy, by comparing different groups of subjects divided on the basis of the type of care provider they referred to. In particular, the relative contributions in CT testing and in CT infection positivity were assessed for an STI Clinic, for gynaecologists and for general practitioners. Moreover, an investigation about CT serovar distribution was also performed.

### Table 1. Primary demographic characteristics and specimens provided by the subjects enrolled

The contribution of each subgroup (STI Outpatients Clinic, gynaecological clinics and general practitioners) to the whole population is reported as a percentage in parentheses. Differences between the three subgroups are expressed by P value.

<table>
<thead>
<tr>
<th></th>
<th>Whole population</th>
<th>STI Clinic</th>
<th>Gynaecology</th>
<th>GPs</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. subjects</td>
<td>8906</td>
<td>4081 (45.8)</td>
<td>3173 (35.6)</td>
<td>1652 (18.6)</td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>6090</td>
<td>1886 (31)</td>
<td>3072 (50.4)</td>
<td>1132 (18.6)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Males</td>
<td>2816</td>
<td>2195 (78)</td>
<td>101 (3.6)</td>
<td>520 (18.4)</td>
<td></td>
</tr>
<tr>
<td>Mean age ± sd (years)</td>
<td>34.8 ± 10.6</td>
<td>32.6 ± 10.3</td>
<td>36.1 ± 9.4</td>
<td>37.7 ± 12.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>14–18</td>
<td>113</td>
<td>44 (38.9)</td>
<td>39 (34.5)</td>
<td>30 (26.6)</td>
<td></td>
</tr>
<tr>
<td>19–23</td>
<td>993</td>
<td>689 (69.4)</td>
<td>189 (19.0)</td>
<td>115 (11.6)</td>
<td></td>
</tr>
<tr>
<td>24–28</td>
<td>1698</td>
<td>1078 (63.5)</td>
<td>407 (24.0)</td>
<td>213 (12.5)</td>
<td></td>
</tr>
<tr>
<td>29–33</td>
<td>1655</td>
<td>753 (45.5)</td>
<td>610 (36.9)</td>
<td>292 (17.6)</td>
<td></td>
</tr>
<tr>
<td>34–38</td>
<td>1609</td>
<td>498 (31.0)</td>
<td>790 (49.1)</td>
<td>321 (19.9)</td>
<td></td>
</tr>
<tr>
<td>39–43</td>
<td>1310</td>
<td>395 (30.1)</td>
<td>639 (48.8)</td>
<td>276 (21.1)</td>
<td></td>
</tr>
<tr>
<td>44–48</td>
<td>656</td>
<td>267 (40.7)</td>
<td>234 (35.7)</td>
<td>155 (23.6)</td>
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</tr>
<tr>
<td>&gt;48</td>
<td>872</td>
<td>357 (41.0)</td>
<td>265 (30.4)</td>
<td>250 (28.6)</td>
<td></td>
</tr>
<tr>
<td>No. samples submitted</td>
<td>9314</td>
<td>4477 (48.1)</td>
<td>3177 (34.1)</td>
<td>1660 (17.8)</td>
<td></td>
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<tr>
<td>Urogenital</td>
<td>8918</td>
<td>4085 (45.8)</td>
<td>3175 (35.6)</td>
<td>1658 (18.6)</td>
<td></td>
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<tr>
<td>Extra-genital</td>
<td>396</td>
<td>392 (99)</td>
<td>2 (0.5)</td>
<td>2 (0.5)</td>
<td>&lt;0.01</td>
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<td>Pharyngeal swabs</td>
<td>227</td>
<td>225</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Rectal swabs</td>
<td>169</td>
<td>167</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No. patients providing extra-genital samples</td>
<td>293</td>
<td>291 (99.4)</td>
<td>1 (0.3)</td>
<td>1 (0.3)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Females</td>
<td>60</td>
<td>58</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>233</td>
<td>233</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>
METHODS

Study group. In the period from January 2011 to May 2014, data about all the consecutive clinical samples submitted to the Microbiology Laboratory of St Orsola Hospital in Bologna for CT detection were retrospectively collected.

The specimens were obtained from three different groups of patients: subjects attending the STI Outpatients Clinic of the hospital, patients attending gynaecological clinics and people referring to general practitioners’ offices.

Patients seeking care from the STI Outpatients Clinic usually met one or more of the following criteria: having STI-related symptoms, having sexual contacts with an infected partner, or having unsafe intercourse with new or multiple sexual partners. The population consulting gynaecological clinics was mainly composed of women seeking generic gynaecological consultation, pregnant women looking for screening for mother-to-child transmissible infections and couples seeking infertility counselling and support. Finally, subjects referring to general practitioners were generally patients with non-specific symptoms related to urogenital disorders.

Subjects under 14 years or not sexually active were excluded from the study. For all the patients, data about gender and age were recorded and at least one sample of urogenital origin (first-catch urines or urethral, vaginal and endocervical swabs) was obtained. In addition, in cases reporting unsafe anal and oral intercourse or complaining of rectal and vaginal and endocervical swabs) was obtained. Both the urogenital and the extra-genital swabs were taken by the clinicians during the visit, avoiding self-collection of specimens.

Extra-genital samples were processed for CT DNA detection from January 2012, after a preliminary internal validation study, to assure the suitability of the molecular commercial assay for this kind of sample (Marangoni et al., 2015).

The urine specimens were collected in Siemens Urine Transport medium (UTK), whereas E-Swabs were used for the collection of genital and extra-genital secretions (Copan). E-Swabs were preferred to eNAT medium (UTK), whereas E-Swabs were used for the collection of genital (Marangoni et al., 2015). After the PCR step, the amplified product was digested with Alul, Ddel and/or HinfI restriction enzymes (Promega) and visualized after electrophoresis run in ethidium bromide-stained 12% polyacrylamide gel (Mini-PROTEAN 3 Cell; Bio-Rad). CT serovar identification was made by analysis of the specific restriction pattern.

Only the specimens with a cycling threshold <36 in the real-time PCR were regarded as suitable for further typing. In fact, samples with a low starting amount of DNA (cycling threshold >36) by commercial NAATs (nucleic acid amplification tests) are usually negative by the in-house typing systems, because of the lower sensitivity of these methods, as already described (Marangoni et al., 2015; Veeresteg et al., 2015).

Data analysis and statistics. First, a descriptive analysis was performed to assess the main characteristics of all the subjects enrolled in the study (number of subjects, proportions of males and females, mean age, number and type of specimens submitted for CT detection). The relative contribution of the three subgroups (STI Outpatients Clinic, gynaecological clinics and general practitioners’ offices) to the whole population was set up as well.

Second, an evaluation of CT infection prevalence stratified by sex, age and anatomical site was conducted, considering both the entire population and the single subgroups. CT infection prevalence was calculated based on the number of patients and not on the number of specimens submitted. In particular, a patient was considered infected when at least one of the samples submitted was reactive for CT DNA detection. Finally, an assessment about CT serovar distribution stratified by sex, age, anatomical localization and subgroup was performed.

CT genotyping. In the case of a positive result, the corresponding remaining eluate was recovered from the Versant CT/GC DNA 1.0 assay (Siemens Healthcare Diagnostics), a duplex real-time PCR, simultaneously detecting the presence of CT and GC DNA.

The Versant molecular system consists of a sample preparation module designed for automated sample preparation and an amplification/detection module designed for real-time PCR and detection. CT-specific primer and probes were designed to target the nucleic acid sequence of the 7.5 kb cryptic plasmid outside the 377 bp deletion (nvCT mutant) (Kerndt et al., 2011).

CT genotyping. In the case of a positive result, the corresponding remaining eluate was recovered from the Versant PCR plate and used for CT molecular genotyping. Molecular genotyping was performed by an ompI gene semi-nested PCR followed by RFLP analysis as previously described (Gallo Vaulet et al., 2010; Foschi et al., 2014). Briefly, the first product of 1033 bp was amplified using the following paired primers: SER01A (5’-TGATAAAAACCTGCAGATCGG-3’) and SER02A (5’-TTCCTAGATCTCATTCTTGTT-3’). The reaction was performed in a final volume of 50 µl containing 1.5 mM MgCl2, 0.05 mM each deoxy-nucleotide triphosphate, 0.32 µM each primer, 2 U Taq DNA polymerase (Promega) and 10 µl template. Cycling conditions began with a 7 min denaturation step at 94°C, followed by 40 cycles of denaturation at 95°C for 1 min, annealing at 45°C for 3 min and extension at 72°C for 3 min. An additional 7 min extension at 72°C was performed at the end of the 40 cycles.

Then, 1 µl of the first-round PCR product was used for the semi-nested PCR, amplifying a 978 bp fragment. The second PCR round was performed with the same reagents and conditions as the first round and with the following primers: SER02A and PCTM3 (5’-TCC TTGCAAAGCTGCTGTGGGAATCTCT-3’). After the PCR step, the amplified product was digested with Alul, Ddel and/or HinfI restriction enzymes (Promega) and visualized after electrophoresis run in ethidium bromide-stained 12% polyacrylamide gel (Mini-PROTEAN 3 Cell; Bio-Rad). CT serovar identification was made by analysis of the specific restriction pattern.

Fig. 1. Contribution of each care provider to CT positivity. Setting 100% as the total, the relative contribution of each type of care provider (STI Outpatients Clinic, gynaecological clinics and general practitioners) to CT prevalence, to male and female positivity, to extra-genital site positivity and to GC co-infection is expressed as a percentage.
In order to evaluate statistically significant differences between the three subgroups of subjects, ANOVA was used to compare quantitative data, and categorical data were analysed with the $\chi^2$-test. Data were analysed with GraphPad Prism version 5.02 for Windows (GraphPad Software). A value of $P<0.05$ was considered statistically significant.

### RESULTS

#### Study group and specimens provided

Primary demographic characteristics and specimens provided by the subjects enrolled, with the relative contribution of the different subgroups to the whole population, are shown in detail in Table 1.

During the study period, a total of 9314 samples, including 8918 specimens of urogenital origin and 396 from extra-genital sites, were collected from a total of 8906 patients. A total of 293 patients, almost exclusively from the STI Outpatient Clinic (99.4%; $P<0.01$) and mainly males (79.5 vs 20.5%), submitted at least one extra-genital sample in addition to a specimen of urogenital origin. In particular, 103 patients underwent both rectal and pharyngeal swab collection; 124 provided only a pharyngeal swab and 66 only a rectal one. Moreover, 12 patients submitted two different samples of urogenital origin.

Females represented 68.4% of the total number of subjects recruited. Overall, the STI Outpatients Clinic and the gynaecological clinics accounted for more than 80% of CT tests performed. The former tested the largest proportion of men (78%), whereas the latter tested the largest proportion of women (50.4%).

Subjects attending the STI Outpatients Clinic were significantly younger compared with the other groups of patients ($P<0.01$) and globally 87.6% of subjects enrolled were older than 23 years.

#### CT infection prevalence

Table 2 shows CT infection prevalence stratified by age, gender and anatomical site in the whole population and in the three subgroups, whereas the contribution of each care provider type to CT positivity is presented in Fig. 1.

Total prevalence of CT infection was 8.1% (718/8906) with a significant difference between the three populations analysed ($P<0.01$). In particular, the highest CT infection prevalence was observed in the group of females attending the STI Outpatients Clinic.

In order to evaluate statistically significant differences between the three subgroups of subjects, ANOVA was used to compare quantitative data, and categorical data were analysed with the $\chi^2$-test. Data were analysed with GraphPad Prism version 5.02 for Windows (GraphPad Software). A value of $P<0.05$ was considered statistically significant.

### Table 2. Overall CT infection prevalence and CT infection rates (%) stratified by age, gender and anatomical site

Data are reported first for the whole population and then for the three subgroups (STI Outpatients Clinic, gynaecological clinics and general practitioners). Differences between the three subgroups are expressed by $P$ value.

<table>
<thead>
<tr>
<th></th>
<th>Whole population</th>
<th>STI Clinic</th>
<th>Gynaecology</th>
<th>GPs</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$N$</td>
<td>8906</td>
<td>4081</td>
<td>3173</td>
<td>1652</td>
<td></td>
</tr>
<tr>
<td>Overall CT prevalence*</td>
<td>8.1 (718/8906)</td>
<td>13.0 (531/4081)</td>
<td>3.4 (109/3173)</td>
<td>4.7 (78/1652)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td><strong>CT positivity by gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>46.6 (335/718)</td>
<td>37.5 (199/531)</td>
<td>95.4 (104/109)</td>
<td>41.0 (32/78)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Males</td>
<td>53.4 (383/718)</td>
<td>62.5 (332/531)</td>
<td>4.6 (5/109)</td>
<td>59.0 (46/78)</td>
<td></td>
</tr>
<tr>
<td>M : F ratio</td>
<td>1.08</td>
<td>1.66</td>
<td>0.04</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td><strong>CT positivity by age</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age (years ± sd)</td>
<td>30.8 ± 9.1</td>
<td>30.2 ± 9.1</td>
<td>31.7 ± 8.7</td>
<td>33.5 ± 9.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>14–18</td>
<td>1.2 (9/718)</td>
<td>1.5 (8/531)</td>
<td>1.0 (1/109)</td>
<td>0.0 (0/78)</td>
<td></td>
</tr>
<tr>
<td>19–23</td>
<td>21.2 (152/718)</td>
<td>23.7 (126/531)</td>
<td>13.7 (15/109)</td>
<td>14.1 (11/78)</td>
<td></td>
</tr>
<tr>
<td>24–28</td>
<td>29.5 (212/718)</td>
<td>31.1 (165/531)</td>
<td>27.5 (30/109)</td>
<td>21.8 (17/78)</td>
<td></td>
</tr>
<tr>
<td>29–33</td>
<td>17.1 (123/718)</td>
<td>16.0 (85/531)</td>
<td>21.1 (23/109)</td>
<td>19.2 (15/78)</td>
<td></td>
</tr>
<tr>
<td>34–38</td>
<td>11.8 (85/718)</td>
<td>10.5 (56/531)</td>
<td>16.5 (18/109)</td>
<td>14.1 (11/78)</td>
<td></td>
</tr>
<tr>
<td>39–43</td>
<td>8.6 (62/718)</td>
<td>7.1 (38/531)</td>
<td>10.1 (11/109)</td>
<td>16.6 (13/78)</td>
<td></td>
</tr>
<tr>
<td>44–48</td>
<td>4.9 (35/718)</td>
<td>5.1 (27/531)</td>
<td>2.7 (3/109)</td>
<td>6.4 (5/78)</td>
<td></td>
</tr>
<tr>
<td>&gt;48</td>
<td>5.6 (40/718)</td>
<td>4.9 (26/531)</td>
<td>7.3 (8/109)</td>
<td>7.7 (6/78)</td>
<td></td>
</tr>
<tr>
<td><strong>CT positivity by anatomical site</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urogenital site only</td>
<td>94.6 (679/718)</td>
<td>92.7 (492/531)</td>
<td>100 (109/109)</td>
<td>100 (78/78)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Urogenital + extra-genital site</td>
<td>1.0 (7/718)</td>
<td>1.3 (7/531)</td>
<td>0.0 (0/109)</td>
<td>0.0 (0/78)</td>
<td></td>
</tr>
<tr>
<td>Extra-genital site only</td>
<td>4.4 (32/718)</td>
<td>6.0 (32/531)</td>
<td>0.0 (0/109)</td>
<td>0.0 (0/78)</td>
<td></td>
</tr>
<tr>
<td>GC co-infection</td>
<td>6.4 (46/718)</td>
<td>7.7 (41/531)</td>
<td>1.8 (2/109)</td>
<td>3.8 (3/78)</td>
<td>0.04</td>
</tr>
<tr>
<td>Females</td>
<td>15.2 (7/46)</td>
<td>14.6 (6/41)</td>
<td>50 (1/2)</td>
<td>0 (0/3)</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>84.8 (39/46)</td>
<td>85.4 (35/41)</td>
<td>50 (1/2)</td>
<td>100 (3/3)</td>
<td></td>
</tr>
</tbody>
</table>

*Patients were considered positive when at least one of the specimens submitted was found reactive for CT DNA.

http://jmm.microbiologyresearch.org
was found in the STI Outpatients Clinic (13%), followed by general practitioners (4.7%) and gynaecological clinics (3.4%). Overall, 74% (531/718) of the CT-positive cases were observed in the STI Outpatients Clinic, 15.2% (109/718) in gynaecological clinics and 10.8% (78/718) in general practitioners’ offices. In each group, CT infection prevalence was stable during the study period (data not shown) (P=0.056).

Overall, the male-to-female ratio (M:F) of the CT-positive patients was 1.06, but, in contrast to subjects attending the STI Outpatients Clinic (M:F=1.66) and general practitioners (M:F=1.43), in the gynaecological clinics CT positivity was mainly found in females (M:F=0.04).

The mean age of the positive patients was lower than that of the negative subjects (30.8 vs 35.1 years; P<0.01) and CT positivity peaked in the 24–28 year group in the three groups. CT-positive patients in the STI Outpatients Clinic were younger than in other groups (P<0.01).

Considering CT positivity by anatomical site, we found that 7.8% (699/8918) of urogenital specimens, 20.7% (35/169) of rectal swabs and 2.2% (5/227) of pharyngeal swabs tested resulted positive for CT DNA detection. Urogenital samples contributed 94.6% of the total of CT-positive specimens, whereas extra-genital samples represented 5.4% of all the reactive specimens found.

Thirty-nine out of the 293 patients (13.3%) who underwent collection of at least one extra-genital sample were found positive for CT on rectal and/or on pharyngeal swab and in seven cases we found contemporaneous positivity at both the urogenital and extra-genital sites. None of the extra-genital tests performed outside the STI Clinic was found positive.

Patients positive at extra-genital sites were mainly males (34/39; 87.2%).

Considering only the patients providing rectal and/or pharyngeal swabs in addition to samples of urogenital origin, we noticed that CT infections were mainly detected at extra-genital rather than urogenital sites. In particular, in this group, in 82% (32/39 cases) of positive patients no urethral involvement was detected and only the extra-genital site was found CT-reactive.

It is worth underlining that 4.4% (32/718) of CT cases would have been missed if rectal and pharyngeal sites had not been tested.

GC co-infection was detected in 6.4% of CT-positive patients, mainly in males (84.8%), and it was more common in subjects attending the STI centre (89.1%) than in other groups (P=0.04).

**CT serovar distribution**

Out of the 718 CT-positive cases, 563 (78.4%) were available for typing. The most common serovar in our population was E (42.6%) followed by F (14.7%), G (14%) and D (11.7%). Altogether, these serovars accounted for approximately 83% of CT infections.

CT serovar distribution was statistically different among men and women (P<0.01) and it was influenced by age (P<0.01), by the group of patients analysed (P=0.01) and by the anatomical site tested (P<0.01). Detailed results are presented in Table 3. The main aspects arising from this analysis concern the L2 serovar. In particular, this serovar was identified as the most common CT genotype in rectal specimens (62.8%) and was detected only in extra-genital samples (22 rectal swabs and 1 pharyngeal swab from 22 patients), and only in males attending the STI Outpatients Clinic. The mean age of LGV-positive patients was much higher than that of patients infected by other CT serovars (P<0.01).

Data about serovar typing for extra-genital samples have been available only since 2012, when the use of the Versant CT/GC DNA 1.0 assay was extended to pharyngeal and rectal swabs.

Evaluating the serovar distribution per year (Fig. 2), we noticed that prevalence of F and K serovars varied significantly throughout the study period (P=0.01 and 0.02, respectively).

**DISCUSSION**

The aim of this study was to evaluate CT infection prevalence and serovar distribution in a high-density urban area in the north of Italy, by comparing different groups of subjects divided on the basis of the care providers they referred to. To our knowledge this is the first broad survey conducted in our country about CT infection epidemiology and can contribute in developing strategies for CT prevention and control.

We found that total prevalence of CT infection was 8.1%, ranging from 3.4% in the gynaecological clinics to 13% when assessing subjects from the STI Outpatients Clinic. This figure is in agreement with European reports about the epidemiology of CT infections, stating that the proportion of positive CT tests is generally 5–10% in sexually active young people (ECDC, 2015) and it is similar to other surveys conducted in different countries worldwide (Yang et al., 2014; Corsenac et al., 2015; Samarawickrema et al., 2015)

Overall, in our population, three-quarters of CT infections were diagnosed in the STI Outpatients Clinic (74%) and the remainder were almost equally distributed among gynaecological clinics (15.2%) and general practitioners (10.8%). When comparing CT infection prevalence in the three subgroups, CT positivity in gynaecological clinics was the lowest, although this setting contributed more than one-third of the total subjects tested.

In a recent Dutch paper, den Hejer and colleagues (den Hejer et al., 2015) showed that gynaecologists perform a substantial proportion of CT testing although general practitioners and STI clinics are mainly responsible for CT diagnosis. In contrast to this work, which found an almost
equal distribution of CT-positive cases between STI clinics and general practitioners, we found that the STI Outpatients Clinic represents the predominant and major source of CT-positive cases.

As already described, if considering subjects at high risk for STI transmission, such as sex workers or MSM with multiple sexual partners, CT infection prevalence can easily exceed 10–15% (Petrovay et al., 2009; Mejuto et al., 2013; Yang et al., 2014; Verhaegh-Haasnoot et al., 2015).

In our context, the STI Outpatients Clinic basically tests high-risk groups and young patients with STI-related symptoms, who represent a reservoir for CT infection and transmission. On the contrary, gynaecologists mainly screen low-risk older asymptomatic subjects in order to rule out a CT infection in fertility counselling or during general clinical evaluations. In this setting, CT testing is often part of a panel of infectivity testing, performed in the absence of particular risk factors.

General practitioners probably represent a sort of middle course between the other two groups, targeting a more varied population. These differences have an influence on the proportion of CT-positive tests, as clearly highlighted by our results.

We found no significant differences when comparing CT prevalence per year in all the populations analysed. The constant prevalence of CT infections found in our study underlines the need for innovating strategies for CT control in our country, such as primary prevention, case-management guidelines, partner notification and opportunistic testing (ECDC, 2014).

Similarly to other studies conducted in different countries, the majority of CT tests (68.4%) were performed in women (Kufeji et al., 2003; Hughes et al., 2007; Dimech et al., 2014; den Heijer et al., 2015).

In agreement with European reports (ECDC, 2015), the male-to-female ratio of all positive CT cases found in our study was close to 1, but significant differences were noticed among the three subgroups. The STI Outpatients Clinic tested almost the same proportion of men and women, but CT positivity was much higher in men (M:F=1.66), whereas general practitioners tested twice as many women as men, finding a male-to-female ratio of 1.43. The latter figure resembles that reported by Hughes et al. (2007), who stated that in UK general practice, low rates of testing in men, together with high positivity, prove to be a missed opportunity for CT diagnosis and contact tracing in general practice.

Evaluating the age distribution of CT-positive patients, we noticed that, in contrast to the majority of surveys, which state that CT infections are more often detected in young people aged under 24, the highest rates in our study were found in 24–28-year-old subjects (ECDC, 2015; Veersteg et al., 2015). These findings probably reflect the high contribution in our population of patients aged over 23. As

### Table 3. Overall CT serovar prevalence and serovar distribution by age, sex, anatomical site and subgroups analysed

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Males</th>
<th>Females</th>
<th>M:F</th>
<th>Mean age ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>11.7 (68)</td>
<td>4.2 (23)</td>
<td>2.8</td>
<td>32.24 ± 11.0</td>
</tr>
<tr>
<td>E</td>
<td>31.4 (169)</td>
<td>38.7 (185)</td>
<td>1.2</td>
<td>34.7 ± 8.7</td>
</tr>
<tr>
<td>F</td>
<td>14.7 (83)</td>
<td>15.2 (77)</td>
<td>1.0</td>
<td>31.5 ± 8.5</td>
</tr>
<tr>
<td>G</td>
<td>2.6 (15)</td>
<td>1.6 (9)</td>
<td>1.6</td>
<td>36.8 ± 5.5</td>
</tr>
<tr>
<td>H</td>
<td>2.5 (15)</td>
<td>1.2 (4)</td>
<td>2.1</td>
<td>26.0 ± 9.0</td>
</tr>
<tr>
<td>I</td>
<td>6.0 (34)</td>
<td>2.3 (13)</td>
<td>2.6</td>
<td>22.6 ± 5.5</td>
</tr>
<tr>
<td>J</td>
<td>3.9 (22)</td>
<td>3.2 (8)</td>
<td>1.2</td>
<td>29.6 ± 10.9</td>
</tr>
<tr>
<td>K</td>
<td>2.6 (15)</td>
<td>2.1 (7)</td>
<td>2.4</td>
<td>40.0 ± 9.7</td>
</tr>
</tbody>
</table>

P-value: <0.01

Prevalence is referred to the number of CT cases available for typing analysis (563/718) for contemporary positivity at urogenital and extra-genital sites (7 cases), the serotyping result was attributed to the extra-genital site group.
argued in other papers (den Heijer et al., 2015) and similarly to our context, it appears difficult to reach at-risk younger subjects for different reasons (i.e. not feeling at risk, being unaware of the possible implications of CT infections, refusing testing etc.).

Regarding extra-genital tests, we noticed that anorectal and pharyngeal swabs were almost exclusively collected by the STI Outpatients Clinic, mainly from males. Although the current guidelines suggest screening rectal and pharyngeal sites in addition to genital specimens on the basis of a patient’s sexual history (Workowski & Bolan, 2015), almost no extra-genital tests were performed by general practitioners and gynaecologists in our sample. Probably, this aspect is not linked to the absence of unsafe oral and anal intercourse in subjects attending general practitioners and gynaecological clinics but to a low awareness of these care providers about extra-genital CT testing and to patients’ feelings of STI-related shame and STI-related stigma (Hocking et al., 2008; Cunningham et al., 2009).

Interestingly, we found that 13.3 % of patients who provided at least one extra-genital sample were positive for CT on rectal and/or on pharyngeal swab. Moreover, we detected many cases of rectal and pharyngeal CT infections without urethral involvement and we noted that 4.4 % of CT-positive patients would have been missed if extra-genital sites had not been tested.

Since patients with single positive extra-genital samples were almost exclusively males, our data support the recommendation that MSM need to be tested at multiple anatomical locations (van Liere et al., 2014; den Heijer et al., 2015).

The availability of high-quality tests for CT detection in extra-genital specimens as well as strategies to encourage extra-genital CT testing in MSM and high-risk women outside the STI clinic are therefore critical components of healthcare (den Heijer et al., 2015).

Nowadays, NAATs have become the reference method for CT detection in both urogenital and extra-genital samples, representing a reliable, fully automated, easy-to-use, high-throughput method (Moncada et al., 2009; Bachmann et al., 2010).

Besides its good sensitivity and specificity, a major benefit of the Versant CT/GC DNA 1.0 assay is that the remaining eluate can be used for additional molecular tests (Bongaerst et al., 2011; Marangoni et al., 2015). Taking advantage of this opportunity, we investigated CT serovar distribution in our settings, performing a molecular genotyping assay based on omp1 gene semi-nested PCR followed by RFLP analysis.

On the whole, the most common serovars found in our epidemiological context were E (42.6 %), F (14.7 %), G (14 %) and D (11.7 %). The present finding is similar to many other surveys conducted in both high-risk and low-risk subjects (Petrovay et al., 2009; Weill et al., 2010; Yang et al., 2014; Veersteg et al., 2015; Giffard et al., 2016). Effectively, in many countries worldwide, serovars E, F, D, G and K represent the most common serovars among patients with urogenital infections, accounting for 60–80 % of positive patients (Millman et al., 2006; Pedersen et al., 2009; Lagergard et al., 2010; Veersteg et al., 2015).

Depending on different studies and populations, CT serovar distribution has been demonstrated to be influenced by epidemiological or clinical characteristics, such as gender, sexual orientation or presence of symptoms (Marangoni et al., 2012; Gharsallah et al., 2012; Yang et al., 2014; de Vries et al., 2015), reflecting the creation of specific CT infection reservoirs. Anyway, other studies found no correlations between serovar distribution and specific patient characteristics (Weill et al., 2010; Lagergard et al., 2010; Veersteg et al., 2015; Giffard et al., 2016).

In our population, CT serovar distribution was significantly influenced by age, gender and type of population assessed. This aspect, together with the variations in serovar distribution per year during the study period, can help elucidate various clinical, evolutionary and epidemiological questions and can be useful to develop strategies for CT control and prevention (de Vries et al., 2015).

CT serovar distribution for extra-genital samples deserves a separate discussion. As already described, since 2003 (Nieuwenhuis et al., 2004; Foschi et al., 2014) LGV serovars with rectal localization have been found in high-risk populations, mainly in MSM, leading to moderate or severe ulcerative proctitis with various ano-rectal disorders (Martin-Iguacel et al., 2010). In the present study, the L2 serovar was identified as the most prevalent CT genotype on rectal specimens, and, as previously reported (White, 2009; Foschi et al., 2014), LGV-positive patients were significantly older than LGV-negative ones.

Fig. 2. CT serovar distribution per year. Statistically significant differences were noted for serovars F (P=0.01) and K (P=0.02). Since data about serovar typing in extra-genital samples were only available starting from 2012, L2 serovar distribution per year was evaluated excluding 2011.
In accordance with international data, the L2 serovar was detected only in extra-genital samples from only males belonging to the STI Outpatients Clinic, with no cases of urethral involvement (Ward et al., 2009; Foschi et al., 2014; Heiligenberg et al., 2014; Stoner & Cohen, 2015). Even though recently some cases of urethral LGV in a group of MSM were recorded (de Vrieze et al., 2013) and several cases of LGV in women were reported (Rodriguez-Dominguez et al., 2014), we had no opportunity to detect similar cases.

As underlined by our findings, CT serovars other than L2 can be found in rectal and pharyngeal mucosa with different rates on the basis of the population enrolled (Korhonen et al., 2012; Mejuto et al., 2013; Labiran et al., 2015). In this context, the correct identification of CT serovar has a crucial role in establishing adequate antibiotic treatment, since, in a case of L1–L3 serovar detection, an LGV regimen should be started (Mohrmann et al., 2014; Workowski & Bolan, 2015).

The particular tropism of L2 serovar for rectal mucosa and the sporadic cases of urethral LGV highlighted the importance of performing CT NAAT testing on extra-genital sample and of carrying out CT genotyping on this kind of specimens (Korhonen et al., 2012; Mejuto et al., 2013; Mohrmann et al., 2014).

Since commercial NAATs for CT detection do not allow differentiation between serovars or the identification of LGV, many laboratories have set up in-house methods, even in the absence of US Food and Drug Administration (FDA) clearance or Conformité Européene (CE) marking (Klint et al., 2006; Jalal et al., 2007; Stevens et al., 2010; Korhonen et al., 2012). As reviewed by Pedersen et al. (2009) and Xia & Xiong (2014), many molecular methods, mainly based on omp1 or pmplH gene amplification and characterization, are nowadays available, showing different performances in terms of sensitivity, specificity and ability in the detection of mixed infections.

Although high-resolution genotyping methods, such as multilocus variable number of tandem repeat analysis (MLVA), multilocus sequence typing (MLST) or microarray assay (Christerson et al., 2011; Ruettger et al., 2011; Bom et al., 2011; Wang et al. 2011; Satoh et al., 2014; Hermann et al., 2015), have been recently described, their use in routine diagnostic practice is largely restricted by their complexity and high costs. So far these assays have basically been used in epidemiological and phylogenetic analysis or in the evolutionary surveillance of specific clones, but what is really needed in the near future is the advent of rapid and cost-saving methods for faster and reliable management of patients (Pedersen et al., 2009; Xia & Xiong, 2014).

Aware of its low resolution and its insufficient ability to find genovariants and mixed infections, we found RFLP a simple and cost-saving method, useful and appropriate for our setting. Although 22 % failure of molecular typing seems a large proportion, this percentage is consistent with other reports showing that only 60 to 80 % of samples CT-positive by commercial NAATs are subsequently successfully genotyped by in-house methods, because of the better sensitivity of the former assays (Yang et al., 2014; Veersteg et al., 2015).

In conclusion, this work highlights major issues about CT infection prevalence and serovar distribution. First, in our context, the characteristics of the tested population differ between care providers: the STI Outpatients Clinic is mainly responsible for CT infection diagnosis, whereas the gynecological setting has the lowest CT prevalence, despite the high number of tests performed.

Secondly, extra-genital CT testing needs to be encouraged, especially among care providers outside the STI Clinic, because almost no extra-genital tests are performed by general practitioners and gynaecological clinics.

Finally, besides the role that CT molecular typing plays in finding association with epidemiological features and in increasing knowledge about serovar distribution worldwide, the identification of a specific serovar in a particular setting (i.e. LGV in extra-genital samples of high-risk population) is crucial for the appropriate management of patients in terms of antibiotic treatment and prevention of complications (Korhonen et al., 2012; Mejuto et al., 2013).

Nevertheless, the lack of information about symptoms and sexual orientation of patients could have affected our results and the use of a typing system with inadequate performance could have potentially led us to miss particular associations between serovar and epidemiological findings.

Further studies are needed to better understand the dynamics in CT testing among care providers with the perspective of optimizing and improving the cooperation between them. The role of specific CT serovars in the pathogenesis of infection needs to be elucidated with the goal of developing better strategies for CT infection control and prevention.

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