Ample distribution of the *Chlamydia trachomatis* F genotype in the genital infections of women from of the city of Belém, in the amazon region of Brazil

Ampla distribuição do genótipo *Chlamydia trachomatis* F nas infecções genitais de mulheres da cidade de Belém, na região amazônica do Brasil

Amplia distribución del genotipo F de *Chlamydia trachomatis* en las infecciones genitales de mujeres de la ciudad de Belém, en la región amazónica de Brasil

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**Abstract**

*Chlamydia trachomatis* uroinfection has become, in recent years, the most prevalent sexually transmitted infection worldwide, affecting quality of life and causing severe morbidity and mortality. This infection is silent and can lead to long-term reproductive complications. This study evaluates the prevalence and distribution of *C. trachomatis* genotypes in sexual infections of women from Belém, Amazon, Brazil. A cross-sectional, descriptive and prospective study was carried out from March 2019 to February 2020. Endocervical secretions were collected and DNA was extracted from these samples for *ompA* gene amplification by nested PCR and agarose gel electrophoresis. Positive samples were genotyped by nucleotide sequencing. The strains were genotyped by comparing the sequences of this study with those deposited in a public database. The Odds Ratio test was used to assess the relationship between infection and the variables investigated. A total of 230 samples were included in the analyses, of which 11 were positive for infection with a mean age of 44.18 years. We found no significant association between infection rates and the different variables investigated. One of the sequenced samples was diagnosed as genotype D, while all other samples were genotype F. The low prevalence of *C. trachomatis* in the sexual infections recorded in the present study, and the prevalence of genotype F,
may be related to the relatively advanced age of the participants. Our results reinforce the importance of preventing late sequelae in the female population of the Amazon.

**Keywords:** Chlamydia trachomatis; Genotyping; Women's health; Sexually Transmitted Diseases.

### 1. Introduction

*Chlamydia trachomatis* is responsible for the world’s most prevalent bacterial Sexually Transmitted Infection (STI). An estimated 100 million new cases of this infection occur each year USA (CDC, 2021), Europe (ECDC, 2020), Africa and Latin America (Huai et al., 2020). The vast majority (80%) of infected women are asymptomatic. The lack of an early diagnosis of infection in female populations can hamper the understanding of the epidemiology of this STI, resulting in the evolution of cases with the late appearance of complications in the female reproductive tract (Hoenderboom et al., 2019). The *Chlamydia trachomatis* STI cases showed remarkable evidence even within the period of social isolation of the 2019 Coronavirus Disease (COVID-19) pandemic (García et al., 2021).

The definition of the different *C. trachomatis* genotypes is normally based on the analysis of the four variable domains of the *ompA* gene, which codifies the Major Outer Membrane Protein (MOMP). This analysis can differentiate 19 genotypes, which are distributed in three subgroups The A, B, Ba, and C genotypes are related to trachoma, especially in children who are in fragile socioeconomic conditions (Ndisabiye et al., 2020; Odonkor et al., 2021), while the L1, L2, L2a, and L3 genotypes are related to a systemic and invasive STI, venereal lymphogranuloma venereum, being frequently reported in Men who have Sex with Men (MSM) (Puchant et al., 2020). The D, Da, E, F, G, Ga, H, Ia, J, and K genotypes are all associated with asymptomatic...
STI. In women, untreated *C. trachomatis* sexual infection may undergo clearance or may progress to a fibrosing infectious-inflammatory syndrome that ascends the genitourinary tract, called Pelvic Inflammatory Disease (PID) (Nuradilova et al., 2021), bringing loss women's quality of life (Gonullu et al., 2021).

The few data that are available for Brazilian populations indicate a prevalence of infection by *C. trachomatis* ranging from 4% to 20.5% in young, asymptomatic women and those seeking gynecological care. The D, E, and F genotypes are the most prevalent in the Brazilian population (Costa-Lira et al., 2017; Azevedo et al., 2019; Rodrigues et al., 2019; Brasilienese et al., 2016; Santos et al., 2016; Santos et al., 2017; Santos et al., 2018; Tavares et al., 2014; Travassos et al., 2016; Ribeiro et al., 2020; Silveira et al., 2020; Suehiro et al., 2021). Even with few studies on *C. trachomatis* genotyping in Brazil, it is possible to verify a genotypic distribution similar to that of different parts of the world (Feodorova et al., 2018; Rawre et al., 2019; Chung et al., 2020; Hurtado et al., 2021).

In Brazil, public health programs that target STIs do not include *C. trachomatis* in their screening schedule, which means that this has become a neglected infection (Brazil 2012, Brazil, 2017). The populations that reside in peripheral and low-income neighborhoods of the country’s major cities are characterized by social indices that may contribute to the spread of infection by *C. trachomatis* (Somayaji et al., 2017).

In the present study, we evaluate the prevalence of infection by *C. trachomatis* and the distribution of the different genotypes of this bacterium in women resident in the peripheral neighborhoods of the city of Belém, in the Amazon region of Brazil.

2. Material and Methods

2.1 Study population and data collection

This is a cross-sectional, descriptive and prospective study was conducted March 2019 to February 2020, aiming at women from the less privileged neighborhoods of the city of Belém, capital of the state of Pará, in northern Brazil, who voluntarily sought gynecological care at the University Extension Program of the Institute of Sciences Biology of the Belém of the Federal University of Pará. This study was conducted by a multidisciplinary team of researchers from the Center for Tropical Medicine and the Institute of Biological Sciences of the Federal University of Pará, Brazil.

The present study included women aged between 18 and 76 years, who live in peripheral and low-income neighborhoods of the Amazonian city of Belém, and who have either never had a Pap smear or had their more recent exam more than one year previously. The exclusion criteria were pregnancy, menstruation or not providing free and informed consent. The participants were invited to respond to a socioepidemiological questionnaire.

The variables investigated were age, marital status, family income, schooling, age at first sexual intercourse, the number of lifetime sexual partners, miscarriages, condom use, gynecological disorders, and the frequency of the Pap test. All the data provided by the participants were anonymized.

2.2 Collection of the biological samples

Samples were collected at the Cytopathology Laboratory of the Health Sciences Institute of the Federal University of Pará in Belém, northern Brazil. Endocervical secretions were collected during routine pelvic exams using an endocervical brush, and the samples were stored in cryogenic tubes containing 1 ml of Tris-EDTA (TE) at a temperature of -20°C for later testing. The clinical data were collected together with each biological sample. The test Papanicolau follow the recommendations of the Ministry of Health of Brazil (Brasil, 2016).
2.3 Extraction of the DNA

The DNA was extracted using a Mini Spin Plus kit (BIOPUR/Curitiba/Paraná/Brazil), according to the manufacturer's instructions, and stored at -20°C until analysis. The protocol of Greer et al. (1991) was used to perform a Polymerase Chain Reaction (PCR) of the human β-globin gene was conducted prior to the detection of C. trachomatis to confirm the suitability of the samples.

2.4 Detection of the ompA gene of C. trachomatis

Chlamydia trachomatis was detected using the nested PCR protocol modified of Jalal et al. (2007), which amplified 394 base pairs (bps) of the ompA gene of C. trachomatis. The first reaction used 6.0 μL of GoTaq Green Master Mix (Promega, Madison, WI, USA), 0.5 μL (20 pmol/μL of each) of the primers P1 (A) and P2, 2 μL of the genomic DNA, and 3 μL of sterile water for a final volume of 12 μL. The second reaction used 0.5 μL of the solution of the first reaction, 6.0 μL of Go Taq Green Master mix (Promega, Madison, WI, USA), 4.5 μL of sterile water, and 0.5 μL (20 pmol/μL) of the primers P3 and P4.

In both stages, the initial activation consisted of warming the samples to 95°C, for 5 min (first stage) or 1 min (second stage), followed by 35 cycles of denaturation at 94°C for 40 s, annealing at 54°C for 30 s, and extension at 72°C for 90 s, with a final extension at 72°C for 7 min. The amplified products were visualized through electrophoresis in 1% agarose gel.

2.5 DNA sequencing

For the nucleotide sequencing of positive samples of DNA from C. trachomatis, the nested PCR protocol adapted from Lysén (2004) was used. A fragment of approximately 990 bps of the ompA gene was amplified by nested PCR, using the primers P1(B) (5′-ATGAAAAAACCTTTGAAATCGG-3′) and OMP2 (5′-ACTGTAACCTGGATTTTGTCG-3′) and, whenever re-amplification was necessary, using the inner primers MOMP87 (5′-TGA ACCAAGCCTATGATCGA CGGA-3′) and RVS1059, 5′-GCAATACCGAAAGATTCTAGATTTCATC-3′ (Casillas-Vega et al., 2017). The first step of the nested PCR was run in a 0.5 μL volume containing 20 pmol/μL of each primer (P1[B] and OMP2) and 5.0 μL of the DNA extracted from the endocervical secretion, 14 μL of sterile water, 1.0 μL of MgCl₂, 1.0 μL of deoxynucleoside triphosphate (10mM), 2.5 μL of 10x buffer, and 0.5 μL of Hotstar Taq DNA Polymerase 1.5U (Qiagen). The amplification was run in a final volume of 25 μL.

The temperature cycle steps used for nested PCR were the same used by Lysén et al (2004). In the first step of the nested PCR, amplification conditions began with an initial denaturation at 95°C for 5 min, followed by 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, and a final extension at 72°C for 7 min. In the second step, the MOMP87-RVS 1059 primers were used with 1.5 μL of the product of the first step, which was added to a final volume of 25 μL. The conditions of the second step were the same as those described above, except for the annealing temperature, which was 60°C, and the addition of 17.5 μL of sterile water (Lysén et al., 2004).

The products of the nested PCR were purified using a BigDye Xterminator Purification kit (Applied Biosystems, Foster City, CA, USA) for the sequencing of both strands. A BigDye Terminator Cycle kit (Foster City, CA, USA) was used for the sequencing reaction, according to the manufacturer's instructions. The reaction mixtures were sequenced in an ABI 3130 automatic sequencer (Applied Biosystems, Foster City, CA, USA).

2.6 Phylogenetic analysis and genotyping

The sequences were assembled using the CAP3 software, aligned in MAFFT v 7.221, and edited in Bioinformatic Geneious v 8.1.7. The consensus sequences were compared with known C. trachomatis lineages (24) using the BLAST search tool at the National Center for Biotechnological Information.
The phylogenetic analysis was run in three stages. The first stage involved the use of IQ-TREE v 1.3.2 for the selection of the most adequate evolutionary model for the maximum likelihood analysis. The phylogenetic reconstruction was also run in IQ-TREE. The standard error was obtained using a bootstrap value of 0.03 for 2000 repetitions. In the third stage, FigTree v 1.4.2 was used to edit the phylogenetic tree produced by the analyses.

2.7 Statistical analysis

The prevalence was calculated by dividing the number of individuals infected by the total sample. An Odds Ratio test was used to evaluate the relationship between infection and the socioepidemiological variables, with the 95% Confidence Interval (CI) being estimated in each case. All analyses were run in BioEstat 5.0, and a p-value = 0.05 significance level was considered in all cases.

2.8 Ethics Statement

This study was submitted to and approved by the research ethics committee of the Health Sciences Institute of the Federal University of Pará, in accordance with the CAAE certificate 55516416.4.0000.0018, based on decision no. 1,566,268. All the women that participated in the study were aware of its objectives and agreed formally to take part by signing a standard informed consent term.

3. Results

A total of 230 samples were included in the analyses, of which, 11 were positive for infection by C. trachomatis, with an overall prevalence of 4.8% (95% CI: 2.0–7.5%). The mean±SD age of the participants was 44±13.4 years, with a median age of 56 years old (range 20–76 years), and 92.6% of the individuals being over 24 years old. Just over half (52.2%) of the participants were single, and 82.2% had an income of the Brazilian minimum wage (approximately US$250 per month) or less. Most (74%) had initiated sexual activity after 15 years of age, and the majority (73%) had had 1–3 sexual partners during their lifetimes. Approximately two-thirds (64.8%) had never had a miscarriage, and a similar proportion (67.4%) reported using condoms. Slightly more individuals (68.3% of the total) complained of gynecological problems, and 62.6% confirmed having an annual Papanicolaou exam.

The infected individuals had a mean age of 44.18 years, with a median of 44 years (interquartile interval: 35.0–54.0 years). Some variation was found in the prevalence of infection of different groups, although no significant differences were found in the bivariate analyses of the prevalence of infection in the different subgroups. In the case of the number of sexual partners, for example, the exact same prevalence (4.8%) was recorded in the least active (three partners or less) and more active (more than three partners) groups. At the opposite extreme, none of the women in the higher income group were infected, as against 5.8% in the low-income group, although the small number of individuals in the former group limited the statistical power of the comparison.

The highest prevalence (8.0%) was recorded in the group that did not use condoms, although, once again, there was no significant difference in relation to the group that did use protection. The second highest prevalence (7.7%) was recorded for the group of women that were sexually inactive, which appears to be counter-intuitive, although once again, the result may have been influenced primarily by the small sample size, plus the fact that infection may reflect past sexual activity. A relatively high prevalence (7.5%) was also recorded in single women, as against a relatively low prevalence of 1.8% in married women, the second lowest prevalence recorded in any subgroup. The next highest prevalence (7.4%) was recorded in women who had reported having had a miscarriage. A much lower prevalence (6.1%) was recorded in the group that do not have a regular Pap
smear test, and all other values recorded here were less than 6%, that is, most were similar to the overall mean of 4.8% (Table 1).

Table 1. Socioepidemiological variables evaluated in the present study, and the results of the bivariate analysis of their influence on \textit{C. trachomatis} infection in low-income women from the city of Belém, Pará, in the Amazon region of northern Brazil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total (n=230)</th>
<th>\textit{C. trachomatis} positive (n=11/4.8%)</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)\textsuperscript{a}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 24</td>
<td>17</td>
<td>1</td>
<td>0.788</td>
<td>0.0948–6.551</td>
<td>0.692</td>
</tr>
<tr>
<td>&gt; 24</td>
<td>213</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conjugal status\textsuperscript{a}</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single</td>
<td>120</td>
<td>9</td>
<td>0.228</td>
<td>0.0482–1.0813</td>
<td>0.078</td>
</tr>
<tr>
<td>Married</td>
<td>110</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household income\textsuperscript{a} (Number of Brazilian minimum wages)</td>
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<td></td>
<td>0.187</td>
<td>0.0108–3.2378</td>
<td>0.174</td>
</tr>
<tr>
<td>≤ 1</td>
<td>189</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 1</td>
<td>41</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years) at first sexual intercourse\textsuperscript{b}</td>
<td></td>
<td></td>
<td>1.621</td>
<td>0.3402–7.7246</td>
<td>0.792</td>
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<tr>
<td>≤ 15</td>
<td>60</td>
<td>2</td>
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<tr>
<td>&gt; 15</td>
<td>170</td>
<td>9</td>
<td></td>
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<tr>
<td>Sexual partners in lifetime\textsuperscript{a}</td>
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<td></td>
<td>1.016</td>
<td>0.2610–3.9625</td>
<td>0.741</td>
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<tr>
<td>≤ 3</td>
<td>168</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3</td>
<td>62</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sexually active</td>
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<td></td>
<td>0.491</td>
<td>0.1380–1.7483</td>
<td>0.471</td>
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<tr>
<td>Yes</td>
<td>178</td>
<td>77.4</td>
<td></td>
<td></td>
<td></td>
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<tr>
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<td>52</td>
<td>22.6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Miscarriage</td>
<td></td>
<td></td>
<td>2.304</td>
<td>0.6808–7.7978</td>
<td>0.303</td>
</tr>
<tr>
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<td>81</td>
<td>35.2</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>No</td>
<td>149</td>
<td>64.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Condom use\textsuperscript{a,b}</td>
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<td></td>
<td>0.383</td>
<td>0.1131–1.2992</td>
<td>0.221</td>
</tr>
<tr>
<td>Yes</td>
<td>155</td>
<td>67.4</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>No</td>
<td>75</td>
<td>32.6</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>
The *C. trachomatis* *ompA* nucleotide sequences identified in the present study were deposited in GenBank (NCBI) under GenBank accession numbers MN106259–MN106269 (Table 2) shows the accession number of the *Chlamydia trachomatis* sequences used in the phylogenetic analysis of this study.

**Table 2.** The accession number of sequences of the *Chlamydia trachomatis* used in the phylogenetic analysis of this study.

<table>
<thead>
<tr>
<th>Accession Number</th>
<th>Strain</th>
<th>Genotype</th>
<th>Neighborhoods*</th>
</tr>
</thead>
<tbody>
<tr>
<td>MN106259</td>
<td>4143</td>
<td>F</td>
<td>Belém do Pará</td>
</tr>
<tr>
<td>MN106260</td>
<td>4151</td>
<td>F</td>
<td>Belém do Pará</td>
</tr>
<tr>
<td>MN106261</td>
<td>4153</td>
<td>F</td>
<td>Belém do Pará</td>
</tr>
<tr>
<td>MN106262</td>
<td>4157</td>
<td>F</td>
<td>Belém do Pará</td>
</tr>
<tr>
<td>MN106263</td>
<td>4161</td>
<td>F</td>
<td>Belém do Pará</td>
</tr>
<tr>
<td>MN106264</td>
<td>4162</td>
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<td>MN106265</td>
<td>4164</td>
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<td>4170</td>
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<td>MN106267</td>
<td>4236</td>
<td>F</td>
<td>Belém do Pará</td>
</tr>
<tr>
<td>MN106268</td>
<td>4362</td>
<td>D</td>
<td>Belém do Pará</td>
</tr>
<tr>
<td>MN106269</td>
<td>5938</td>
<td>F</td>
<td>Belém do Pará</td>
</tr>
</tbody>
</table>

Belém of Pará, Amazon, Northern Brazil. Source: Authors.

One of 11 samples sequenced (9% of the total) was diagnosed as genotype D, whereas all the other samples (91%) were genotype F. The phylogenetic analysis revealed 99–100% between the sequences collected during the present study, and a mean similarity of 87.9% in comparison with the reference lineages of *C. trachomatis* (Figure 1).
Figure 1. Results of the phylogenetic analysis of the *ompA* gene sequences of *C. trachomatis* detected in the endocervical samples of women from low-income neighborhoods in the city of Belém, in Pará, northern Brazil. The samples collected in the present study are shown in green, and all others were obtained from GenBank (https://www.ncbi.nlm.nih.gov/genbank).

Source: Authors.

4. Discussion

We recorded a low prevalence of *C. trachomatis* in the women resident in peripheral neighborhoods of one of the largest cities in the Brazilian Amazon region. This prevalence is similar to that recorded in number of previous studies in Brazil, such as patients in gynecological clinics in Amazonas state (Rocha et al., 2014), with a prevalence of 6.8% in Rio Grande do Sul (Silveira et al., 2020), with a prevalence of 6.2% among young adult women from Goiás (Ribeiro et al., 2020), with a prevalence of 1.8%, as well as women from riverside communities on the Marajó Archipelago in the state of Pará (Santos et al., 2018), where a prevalence of 4% was recorded. Prevalences similar to that recorded in the present study were found in populations from
a number of other countries, such as Tunisia (5%), Italy (4.4%), China (4%), Argentina (4%), Gambia and Senegal (4%), and Chile, with 1% (Herrmann et al., 2015; Bianchi et al., 2016).

The low prevalence of *C. trachomatis* recorded in the present study may have been related, at least in part, to the relatively mature ages of the participants, given that previous studies (Chen et al., 2017) have shown that *C. trachomatis* is less common in older women. Higher rates of infection have been recorded in younger women in the Brazilian Amazon region, including pregnant individuals in Amazonas state (11%) (Azvedo et al., 2019), and parturients (18%) (Santos et al., 2016) and university students (11.9%) (Santos et al., 2017).

Sexual infection by *C. trachomatis* is a major preoccupation for public health services throughout the world. In some developed countries, official public screening programs have improved the potential for the reliable quantification of cases, which has enabled public authorities to better identify epidemiological patterns and invest in more systematic and effective strategies of tracking, prevention, and treatment (Jordá et al., 2018). However, it is believed that in the Amazon region the situation is more worrying, as there is no screening system that informs us about the silent epidemiology of this sexual infection in order to mobilize control measures and prevention of possible late sequelae.

Most disadvantaged Brazilian populations have limited access to good quality public reproductive health services, and in particular the molecular diagnosis of *C. trachomatis* infection. The lack of an effective public screening system in Brazil for this STI is one of the primary determinants of the lack of reliable epidemiological data on this sexual infection. In Brazil, few data are available on the prevalence, risk factors or the *C. trachomatis* genotypes. As this infection is not included in the official STI screening programs for women of reproductive age, *C. trachomatis* persists as a potentially grave public health problem that goes virtually unnoticed by the public health authorities (Galvão et al., 2019). Inadequate socioeconomic conditions are widely-known to be an important risk factor for STI. These conditions encompass low income, unstable employment, reduced education levels, a lack of knowledge on the prevention of STIs, inadequate public sanitation, and reduced access to public reproductive health services. A disadvantaged environment is known to have a major influence on the sexual life and the formation of the personality of young women. Populations in the Amazon are in a situation of socioeconomic vulnerability that may be linked to the high rates of this STI, since it can be favored by the absence of a national screening and early diagnosis system, and by logistical difficulties in adhering to public programs of primary care for health of our unified health system (Garnelo et al., 2020; Machado et al., 2021).

The present study recorded the almost complete predominance of *C. trachomatis* genotype F in the study population. Although no clear geographic pattern has yet been identified in the distribution of *C. trachomatis* genotypes, relatively high frequencies of genotype F have been recorded in other Brazilian populations (Lima et al., 2007; Brasiliense et al., 2016; Machado et al., 2011; Santos et al., 2018). The sexual DK genotypes of *C. trachomatis* do not seem to present a differentiated pattern of distribution in exclusive populations, as well as there are no reports of greater or lesser pathogenicity in their infections, which is perhaps why our genotypes showed a distribution similar to that of other populations of several regions in the world (Feodorova et al., 2018; Rawre et al., 2019; Chung et al., 2020; Hurtado et al., 2021).

The principal limitation of the present study is its reduced sample size which, while achieved by spontaneous demand, is probably not representative of the study population. Given this, the present study can be considered to be no more than a pilot study that provides important insights and guidelines for further research, which will be fundamental to the identification of the possible determinants of the relatively ample distribution of *C. trachomatis* genotype F in mature women from the Amazon region.
5. Conclusions

The low prevalence of the *C. trachomatis* sexual infection recorded in the present study and the predominance of the F genotype in the infections may be related to the relatively mature age profile of the sample group. Similar findings have been produced by studies in other regions of Brazil, and in other regions of the world. The findings of the present study reinforce the importance of the adequate screening of this infection for the prevention of late sequelae in the female population of the urban and peripheral areas of Brazil. Future studies will be important to elucidate the epidemiological situation of *C. trachomatis* genotypes in sexual infections in female and male populations from Amazon.

References


