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Genotypic Distribution and Mutational Analysis of *Chlamydia trachomatis* in Infertile and Pregnant Women in the Northern Region of Brazil

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Abstract

Introduction: *There is limited data regarding genotyping of Chlamydia trachomatis (CT) among women in the Northern Region of Brazil.*

Objective: *To identify the distribution of genotypes from positive samples of CT using the polymerase chain reaction technique among infertile and pregnant women.*

Materials & Methods: *Clinical information was obtained before medical examination to obtain samples. Positive CT samples underwent sequencing of the ompA gene. Two sets of primers were used: one described in the literature and another designed in this study based on ompA gene sequences deposited in the National Center for Biotechnology Information. Mutation analyses and characterization of variable domains (VDs) of the ompA gene were also performed.*

Results: *In pregnant women, the following genotypes were identified: D [50.0%], E [25.0%], F [12.5%], and I [12.5%]. Among infertile women, genotypes E [16.7%], F [16.7%], and K [66.7%] were detected. Genotype K, associated with chronic infection and infertility, was not found in*

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pregnant women. It also presented the highest number of mutations. Mutational analyses were concentrated in the VD regions, with the greatest number of transition and transversion mutations observed in infertile women with genotype K and in pregnant women with genotype I.

Conclusion: *The high prevalence of genotype K among infertile women and its absence in pregnant women supports its association with chronic chlamydial infection and infertility. This study contributes to understanding CT genotype distribution and mutational patterns, providing insights into the pathogenesis of CT infections in distinct female populations in the Amazon.*

Key words: *Chlamydia trachomatis*, genotypes, *omp*A gene, infertile women, pregnant women, variable domains, Brazil.

INTRODUCTION

Chlamydia trachomatis (CT) is the most common sexually transmitted pathogen worldwide and poses serious public health concerns due to its impact on human reproduction and in pregnant women.[1,2-6]

The identification of the 19 serotypes and variants of CT (A, B/Ba, C, D/Da, E, F, G, Ga, H, I/Ia, J, K) and (L1, L2/L2a, and L3) was based on analysis of immunotyping of epitopes of the Major Outer Membrane Protein (MOMP).[7,8,9,10,11,12] MOMP is the main immunodominant surface antigen of CT, with antigenic determinants located in variable domains (VDI to VDIV). These domains are encoded by the *omp*A gene, and their nucleotide sequences exhibit distinct variations in different genotypes.[9]

Several studies have suggested that CT infections caused by genotypes G, I, and D may be linked to squamous cell cervical carcinoma, while chronic infections with genotype K in women have been recognized as a contributing factor to infertility. Genotypes D through K are associated with urogenital tract infections, with D, E, and F being the most prevalent.[7,8,12,13,14,15,16,17,18,19,20,21]

This study used nucleic acid amplification technology (PCR) to analyze the *omp*A gene encoding MOMP and to identify CT genotypes in infertile and pregnant women in Manaus, the capital state of Amazonas, Brazil.[15,22,23,24,25]

Given the silent nature of CT infection and the lack of genotyping studies among Amazonian women,[25,26,27] understanding the prevalence of CT genotypes is crucial, as this gene plays a key role in the bacterium's antigenicity and has significant implications for women's health in the region.

MATERIALS & METHODS

This study was approved by the Human Research Ethics Committee of the Federal University of Amazonas (UFAM) and conducted in compliance with the Declaration of Helsinki. A total of 149 women were tested for CT using PCR, including 96 infertile women from a local Human Reproduction Clinic (Clininfert) and 53 pregnant women receiving care at the low-risk prenatal clinic of Dona Francisca Mendes University Hospital.

The inclusion criteria for infertile women required patients to: (1) have a physician-confirmed infertility diagnosis based on videolaparoscopy, (2) be residents of Manaus, the capital of Amazonas, and (3) be of reproductive age and unable to conceive after at least one year of attempting pregnancy. For pregnant women, the inclusion criteria were: (1) being in the third trimester of pregnancy (between 29 weeks and 41

weeks + 6 days of gestational age), (2) receiving care at the low-risk prenatal clinic of Dona Francisca Mendes University Hospital, and (3) residing in Manaus.

Endocervical samples were collected using cyto-brushes and suspended in 400 µL of TE buffer (10 mM Tris-HCl, pH 8.0, and 1 mM EDTA), then stored at -20°C until DNA extraction.

The KL1 and KL2 primers were used to amplify a 241 bp fragment of the CT DNA plasmid through PCR out in a PXE 0.2 Thermal Cycler (Thermo Electron Corporation) under the following conditions for 40 cycles.

PCR system for *ompA* amplification

For the PCR amplification of the *ompA* gene of CT, a high-fidelity enzyme was used to ensure accuracy. Each reaction included a negative control, consisting only of ultra-pure water, and a positive control, which was confirmed and sequenced from the Molecular Diagnostic Laboratory of UFAM. Following, a nested-PCR was performed using the NRI/NLI and Sero2A/Pctm3 primers.

The PCR reaction system consisted of: 5.0 µL of DNA for the first reaction and 2.5 µL for the nested-PCR, 10X Buffer, 50 mM $MgSO_4$, 10 mM dNTPs, 10 pmol of each primer (NLO/NRO, Pctm3, Sero2A, and NRI/NLI), Taq DNA Polymerase High Fidelity (1.5 U/µL), ultra-pure water, for a total reaction volume of 25 µL.

PCR was performed using the following thermal cycling program for 40 amplification cycles. pre-denaturation at 95°C for 6 min, followed by denaturation at 95°C for 1 min, annealing at 45°C for 3 min, and extension at 68°C for 2 min.

The nucleotide sequences for the newly designed primers targeting the *ompA* gene were based on CT genotype sequences available in the NCBI GenBank database, identified by their accession numbers. Sequence alignment was performed using BioEdit with the ClustalW tool to generate a consensus sequence for primer design. Conserved regions among the genotypes were identified using MEGA 4.0 and further analyzed with PerlPrimer, ClustalW, and Primer3.

PCR system for *ompA* amplification with new designed primers

The PCR reactions using the new primers (OmpAF/OmpAR and OmpAns1/OmpAns2) followed the same protocol as the previous setup, with one adjustment in the annealing temperatures. For the first PCR with OmpAF/OmpAR primers, the annealing temperature was set to 68°C. For the nested PCR with OmpAns1/OmpAns2 primers, the annealing temperature was set to 62°C. Each PCR reaction included 40 amplification cycles with the following thermal profile: pre-denaturation at 95°C for 6 minutes, denaturation at 95°C for 1 minute, annealing at 68°C for 3 minutes, extension at 68°C for 2 minutes, and a final extension at 68°C for 5 minutes. The nested PCR used the same cycle profile, with the annealing step performed at 62°C for 3 minutes.

The amplified products were analyzed by electrophoresis on a 0.8% agarose gel, stained with ethidium bromide (1 µg/µL), and visualized under ultraviolet light. The expected amplicon sizes were approximately 1,002 bp for OmpAF/OmpAR primers and 677 bp for OmpAns1/OmpAns2 primers. To obtain the desired fragment size, the PCR-amplified products were purified using Exonuclease (Exo) and Alkaline Phosphatase (Sap) enzymes.

The purification system consisted of 10.0 µL of the PCR product, 6 µL of PCR product, 0.34 µL of Exonuclease enzyme (3.3 U), and 0.66 µL of Alkaline Phosphatase

enzyme (0.66 U). The purified samples were sequenced in the variable segments of the *omp*A gene using the primers NLO or NRO, Pctm3 or Sero2A, NLI or NRI, OmpAF or OmpAR, and OmpAns1 or OmpAns2. The Chlamydia nucleotide sequences were analyzed for quality and viability using the PHRED/PHRAP program. To confirm the genotypes of valid Chlamydia nucleotide sequences, the obtained sequences were compared with those available in the World Nucleotide Database GenBank using the BLAST/BLASTN tool. Only results with E-values of e-11 or lower were accepted, while those with E-values of e-10 or higher were disregarded.

Analysis of mutation types and variable domains of the *omp*A gene in *C. trachomatis*

The analysis of the VD of the *omp*A gene in CT was performed using MOMP sequences available in the GenBank Nucleotide Database.

All samples with confirmed genotypes were aligned and compared using the CLUSTAL W tool in BIOEDIT software. The sequences were then analyzed against the VD regions of their respective prototype, based on the GenBank accession number L03754.1.

The types of nucleotide mutations in the genotyped samples, along with their frequency and locations, were analyzed by aligning and comparing the variable site sequences of the VD using MEGA 5.0 software.

Statistical analysis

Statistical analyses were conducted to determine the absolute and relative frequency distributions for categorical data, as well as the median and standard deviation for quantitative data. The Pearson chi-square test was used for categorical data comparisons, and when its application was not feasible, Fisher's exact test was employed.

RESULTS

Among infertile women, 24.0% tested positive for the plasmidial DNA of CT, while in pregnant women, the positivity rate was 39.6%. The average age of infertile patients was 32.3 years (± 4.7 SD), whereas for pregnant women, it was 24.9 years (± 4.9 SD).

Regarding marital status, 84.9% of pregnant women and 92.7% of infertile women reported being in a stable union with their partners. Among the 53 pregnant women studied, two patients did not provide information regarding fertility treatment, leaving a total of 51 patients. All 51 pregnant women (100.0%) reported having no prior fertility treatment.

In the infertility group, 93.8% of women were undergoing infertility treatment for the first time. Additionally, 24 out of 96 infertile women (25.0%) and 16 out of 53 pregnant women (30.2%) reported a history of miscarriage. Regarding the type of infertility, among the 96 infertile women, 64.6% had a history of primary infertility, while 35.4% experienced secondary infertility. Among the 96 infertile women with tubal complications, 42.7% showed tubal factors in the HSG examination. Of these infertile patients, 5.2% experienced an ectopic pregnancy. Additionally, 42.7% of the infertile women had cervicitis observed during the speculum examination, compared to 43.4% of women in the pregnant group, who also showed signs of cervicitis.

Among the 149 women who participated in the study, 22 (22.9%) infertile women and 20 (37.7%) pregnant women tested positive for CT using the KL1/KL2 primers.No statistically significant difference was observed in the PCR results for CT between the two groups of women ($p = 0.045$).

Among the 42 samples positive for CT, only 14 samples (33.3%) showed positive amplification for genotyping. Of these 14 genotyped samples, 8 were from pregnant women, and 6 were from infertile women, using the designed primers. Therefore, 40% (8/20) of the pregnant women's samples and 27.3% (6/22) of the infertile women's samples were successfully genotyped.

Analysis of Different Primers Used in PCR Genotyping

Among the 22 positive samples from infertile women tested with the initially proposed primers, 10 (13.6%) showed amplification of the *omp*A gene, while with the designed primers, 14 (27.6%) samples exhibited *omp*A amplification by nested-PCR.

In the group of 20 positive samples for CT plasmid DNA in pregnant women, 7 (35.0%) samples tested positive for genotyping with the initially proposed primers (Sero2A and Pctm3). With the new primers (OmpAns1 and OmpAns2), 8 samples (40.0%) had the *omp*A gene amplified by nested-PCR. The frequency analysis did not show statistically significant differences, with all p-values above 0.05.

Out of the 14 samples amplified with the new primers, 10 samples were successfully genotyped using the initially proposed primers (NLO/NRO, Pctm3, Sero2A, NLI, and NRI), including 7 samples from pregnant women and 3 from infertile women. Of the 4 non-amplified samples, one belonged to a pregnant woman, while three were from infertile women as shown in Table 11.

Following the sequencing analysis of PCR products obtained with the initially proposed primers, sequencing was also performed on PCR products amplified using the new primers.

All 14 samples amplified by nested-PCR (ompAns1/ompAns2), consisting of 8 samples from pregnant women and 6 from infertile women, showed successful amplification and were sequenced to determine the corresponding genotypes, which are presented in Table 12.

Sequencing analysis using the new primers confirmed the previously obtained results with the initially proposed primers, identifying genotypes D, E, and F. Additionally, the detection of genotype K in infertility samples further validated the prior findings. Additionally, using the new primers, genotype E and genotype K were analyzed and identified in samples from infertile women, while genotype I was found exclusively in a sample from a pregnant woman (Table 11 and Table 12).

According to the analysis performed using GenBank, Table 13 presents the distribution of identified CT genotypes among pregnant and infertile women. A higher frequency of genotype D was observed in pregnant women (50%), while genotype K was predominant in infertile women (66.7%). As shown in Table 13, genotype E was detected in 25% of pregnant women and 16.7% of infertile women. Genotype F was identified in 12.5% of pregnant women and 16.7% of infertile women. Genotype I was found exclusively in pregnant women (12.5%). Genotypes D and I were not observed in the infertile group, while genotype K was absent in pregnant women.

Table 11 – Types of CT identified by BLAST in GenBank. Primers used: NLO/NRO followed by Pctm3/Sero2A and NLI/NRI.

Sample Identification	GenBank Accession Number	Genotype Identified	e-value	Max Identity
Pregnant woman	HM230054.1 FJ943519	D Da	0.0 0.0	98% 98%
Pregnant woman	CP002054.1	D	0.0	99%
Pregnant woman	CP001890.1	E	0.0	99%
Pregnant woman	HM230054.1	D	3e-45	100%
Pregnant woman	CP001890	E	0.0	99%
Pregnant woman	HM230054 FJ943521.1	D Da	0.0 0.0	100% 100%
Pregnant woman	HM230057.1	F	9e-93	98%
Infertile	DQ155300	K	0.0	99%
Infertile	JF437563	F	0.0	99%
Infertile	FJ428246.1	K	0.0	99%

Table 12- Types of CT identified by BLAST in GenBank. Primers used: OmpAF/OmpAR followed by ompAns1/ompAns2.

Sample Identification	GenBank Accession Number	Genotype Identified	e-value	Max Identity
Pregnant woman	AM179412.1	D	0.0	94%
Pregnant woman	AY950628.1	D	0.0	100%
Pregnant woman	DQ890028.1	E	0.0	96%
Pregnant woman	FJ261943.1	D6	0.0	100%
Pregnant woman	JN192145.1	E	2e-170	96%
Pregnant woman	AM179412.1	D	0.0	90%
Pregnant woman	AY950625.1	I	1e91	94%
Pregnant woman	FJ261947.1	F	3e-64	90%
Infertile	DQ890028.1	E	0.0	91%
Infertile	AF414965.1	K	1e-17	95%
Infertile	FJ943529.1	F	2e-133	85%
Infertile	DQ357070	K	0.0	86%
Infertile	FJ428246.1	K	2e-73	82%
Infertile	DQ357070.1	K	3e-143	93%

Table 13 – Distribution according to the frequency of the identified genotype in relation to the group of pregnant women and women undergoing infertility treatment attended at two healthcare units in the city of Manaus – AM.

	Groups				
Identified Genotype	**Pregnant Women**		**Infertility**		
	f_i	%	f_i	%	Total
D	4	50,0	-	-	4
E	2	25,0	1	16,7	3
F	1	12,5	1	16,7	2
I	1	12,5	-	-	1
K	-	-	4	66,7	4
Total	8	57,1	6	42,9	14

Analysis of Mutation Types and Variable Domains in the *omp*A Gene of *C. trachomatis*

The sequences of samples with identified genotypes were analyzed and compared with the VD sequences of their respective prototypes, based on accession numbers deposited in GenBank. These sequences exhibited high variability, with 35 mutation points identified.

Regarding mutation types, both transversions substitutions between purines and pyrimidines (A/G↔C/T) and transitions substitutions within purines (G↔A) or pyrimidines (C↔T) were observed. Among the analyzed genotypes, genotype K displayed the highest number of mutations, particularly of the transition type (C↔T). Additionally, genotype I showed both transversion mutations (GT, TG) and transition mutations (CT, AG). For genotypes D and E, only one transition mutation (AG) and four transversion mutations (AT, TA, TG, CG) were detected, with the CG mutation identified only for genotype D.

The highest number of mutations was observed in samples from both infertile women and a pregnant woman. Based on the analysis, the samples from infertile women were classified as genotype K, while the sample from the pregnant woman was identified as genotype I. For the remaining samples, the VD could not be identified, likely due to insufficient molecular data for analysis. A sample from the infertility group was considered the prototype for this analysis, as it exhibited the most conserved nucleotide sequence among the analyzed samples, with an approximate length of 63 bp. The VD sequence lengths varied between 42 bp and 93 bp. According to the GenBank analysis (accession number L03754.1), the nucleotide sequence length in VD II is 63 bp. Regarding the analysis of the total number of mutations in each study group, a higher number of mutations was observed exclusively in the infertility group.

In the remaining samples, all contained variable domain II. Additionally, it was not possible to identify the variable domain(s) in the other samples.

DISCUSSION

Although the primary focus of this study is the analysis of CT *omp*A gene sequences, we also considered population characteristics to better understand the study groups.

In this study of 96 infertile women, CT was detected in 22 cases. Among these women, 64.6% had primary infertility, while 35.4% had secondary infertility. In comparison, a study by De Lima Freitas et al[25] reported CT positivity rates of 54.4% in women with primary infertility and 44.6% in those with secondary infertility. According to CDC guidelines,[28] CT infections often present with mild or no symptoms; however, the bacteria can cause severe complications, leading to irreversible damage such as tubal lesions and infertility. A study conducted in the Netherlands, suggests that even minimal tubal lesions or asymptomatic endometritis can impair fertility, despite normal laparoscopic findings.[29]

In this study, 24 infertile women (25.0%) and 16 pregnant women (30.2%) reported a history of abortion. Among those who tested positive for CT, 22.9% of infertile women and 37.7% of pregnant women had experienced an abortion. Chlamydia infection has been identified as a potential contributing factor to pregnancy loss. Although some studies have demonstrated a link between CT infection and abortion, it is not possible to attribute pregnancy loss solely to chlamydial infection. This is particularly relevant given that 20 pregnant women (37.7%) in this study tested positive for CT.

In this study, ectopic pregnancy was observed in a small proportion of women diagnosed with infertility, affecting only 5.2% of the 96 participants. Among them, five women reported having experienced at least one episode of ectopic pregnancy. According to Fernandes et al[30], partial or complete damage to the fallopian tubes increases the risk of ectopic pregnancy as a long-term consequence. Additionally, Beslagic, Jasminka, and Mahmutovic reported that pelvic inflammatory disease (PID) is a polymicrobial infection, with CT identified as one of the key pathogens associated with infections leading to ectopic pregnancy.[31]

In this study, clinical signs of cervicitis were observed in 42.7% of infertile women and 43.4% of pregnant women. The positivity rates for CT were 22.9% among infertile women and 37.7% among pregnant women. These findings are significant for women's reproductive health, as cervicitis can lead to complications such as PID or infections during pregnancy, potentially resulting in preterm birth and neonatal infections. Several studies have demonstrated that the etiology of cervicitis may be associated with both *Neisseria gonorrhoeae* and CT.[32]

In this study, 22 patients (22.9%) tested positive for CT. Among the 96 women attending the infertility clinic, 42.7% presented abnormal findings in their HSG examination results. Despite the established study methodology, some samples that tested positive for CT plasmid DNA using PCR did not yield amplification of the *omp*A gene. This raised the possibility of false negatives, as *omp*A was not successfully amplified in all plasmid-positive samples. To address this, we designed new primers and optimized the PCR process.

The accuracy of PCR depends on the choice of the target DNA sequence. According to Brunelle and Sensabaugh,[33] high nucleotide variability exists within the *omp*A gene among CT strains.

In this study, 77.2% (32/42) of samples failed to amplify *ompA* using primers described in the literature. Similarly, as reported by Quint et al,[34] out of 50 positive samples, 14 (28%) were negative for the *omp*A gene through nested-PCR and therefore could not be sequenced. Additionally, commonly used primers in genotyping reactions amplify a large *omp*A fragment (~1.1 kb), and samples with low DNA quality often yield negative results, making genotyping impossible.[35] Consequently, as documented in the literature and observed in this study, not all samples that tested positive for the plasmid showed *omp*A gene amplification, leaving uncertainty regarding the final results.

In our study, we found that out of 149 participants, 28.2% (42/149) tested positive when PCR targeted the plasmid DNA of CT. Based on this, it was expected that all plasmid-positive samples would also test positive for the *omp*A gene. However, among the infertility samples, only 13.6% and 4.5% tested positive using the initially proposed gene amplification primers (Sero2A/Pctm3 and NLI/NRI), while in the pregnant group, 35.0% and 25.0% tested positive with the same respective primers. The failure to amplify the *omp*A gene in all positive samples can be explained by the high genetic variability of this gene among different CT strains, as documented in the literature. The accuracy of PCR is directly influenced by the choice of nucleotide sequences used in the reaction, which can impact amplification efficiency and detection rates.[34] Given the results of this study, new primers were designed for the *ompA* gene region. To enhance PCR performance for genotyping, multiple nucleotide sequences from GenBank were aligned, allowing for the identification of a region with the lowest genetic variability to guide primer design.

Subsequent PCRs using these newly designed primers, which also target the *omp*A gene, resulted in positivity rates of 27.6% for infertility samples and 40.0% for pregnant samples. The development of new primers for nested-PCR was crucial, as they amplify a fragment of 677 bp significantly smaller than the 1.1 kb fragments used in previous approaches. Molano et al[35] noted that shorter sequences are generally more suitable for amplification. Although the results did not reach statistical significance, they demonstrated improved amplification rates compared to previous methods. Therefore, these newly designed primers appear to be more suitable for genotyping PCR in this study.

According to Quint et al[34] the non-amplification of the *ompA* gene in CT-positive samples may be due to the low quantity of DNA in the sample. The detection of CT plasmid DNA is associated with the increased sensitivity of identification methods that target the bacterium's cryptic plasmid, which is present in 7 to 10 copies per cell, compared to single-copy chromosomal targets such as the *omp*A gene.[36] Therefore, literature data suggest that false-negative *omp*A results in plasmid-positive samples may be attributed to the presence of a single copy of this gene. Additionally, all CT plasmids parasitizing humans are highly conserved, with less than 1% nucleotide variation.[37]

Samples from both infertile and pregnant women were genotyped by comparing their sequences with prototype sequences, thereby identifying specific CT *omp*A gene genotypes. We identified genotypes D, E, F, I, and K. As reported by other authors, the prevalence of CT genotypes varies worldwide. Genotypes D (5–48%), variant genotypes of D and E (22–44%), and F (8–20%) are the most commonly associated with urogenital infections. Less frequent genotypes include G (4–7%), Ga, H (<5%), I (6%), variant genotypes of I and J (5–13%), and K (5–10%).[38]

In Brazil, Lima et al reported the distribution of genotypes in endocervical infections: D (33.3%), E (33.3%), F (16.7%), and K (16.7%).[39] Similarly, a recent study conducted by Gallo et al in Argentina identified genotypes E (46.9%), D (21%), and F (16.1%) as the most prevalent in endocervical samples.[40]

Our findings align with those of Lima et al[39] and Bandea et al[41], where genotypes D (50%) and E (25%) were the most prevalent among pregnant women. Additionally, genotypes F and I were detected in equal proportions (12.5%).[39,41] Genotypes D, E, and F are not only the most frequently identified but are also responsible for the majority of genital tract infections caused by CT, as reported by De Haro-Cruz et al[38] Furthermore, according to Anttila et al[42], genotype F is often associated with lower abdominal pain. The predominance of genotype D in this study suggests a biological advantage in our region. The genetic diversity of the *ompA* gene plays a crucial role in ensuring the ecological success of CT within the host.[43] Lampe, Suchland, and Stamm propose that genetic recombination is likely a key mechanism driving the diversification of *omp*A.[44] This hypothesis is supported not only by previous studies, such as those described by Brunham et al[43], but also by the findings of the present study. Additionally, genotype K was not identified among the pregnant women in our study.

In this study, genotype K was the most prevalent (66.7%), followed by genotypes E and F, each at 16.7%. According to findings by Anttila et al, Marrazzo and Stamm, and Morre et al, genotype K has been associated with chronic CT infections and is recognized as a contributing factor to infertility.

Therefore, the exclusive presence of genotype K in the infertile group in our study aligns with the results reported by these researchers.[42,19,20]

When analyzing the sequenced CT samples, most exhibited high genetic variability. Of the 14 sequences obtained, only the VDs of nine samples were successfully analyzed. A possible explanation for the inability to identify the VDs in the remaining samples could be insufficient molecular data or an inadequate number of base pairs for analysis. The fragment sizes of the VDs in the analyzed samples were relatively small (42bp to 93 bp) compared to those deposited in the GenBank database. Notably, one infertility sample, identified as VDII, exhibited the most conserved site and was therefore considered the prototype for analyzing the other samples. In two of the samples with identified VDs, 2 to 3 mutations were observed, while the remaining samples exhibited up to 19 nucleotide variations. The high variability of MOMP has been documented by several authors, with mutations frequently occurring among genotypes.[45] According to Yuan et al and Everett, Bush, and Anderson[46,47], genotypes and variable segments of the domains can be differentiated based on the *omp*A gene sequence. In the present study, the VDII region displayed the highest number of nucleotide variations.

Two infertility samples identified as genotype K and one pregnant sample identified as genotype I exhibited the highest number of both transition and transversion mutations. In the infertility samples, 19 mutation points were observed, with 6 being transversions; in the pregnant sample, 18 mutation points were observed, with 7 being transversions. Transversion mutations, less common than transitions, involve the exchange of a purine for a pyrimidine, or vice versa.

The infertility and pregnant samples described mutations in the VDII variability region and in the VDI and VDIV regions, respectively.

Variations in sequences from VDI to VDIV among genotypes contribute to genetic diversity. This highlights the significance of antigenic peptides in the variable domains VDs for vaccine development.[48,49,50]

Although Bandea et al and De Haro-Cruz et al described genotypes K and I as less common, their frequency in this study was notably high at 66.7% and 12.5%, respectively.[41,38]

In light of this study, the variations observed in the sequences likely result from mutation points or potential recombination of VDs in the MOMP. Among the analyzed samples in this study, the most frequent genotypes were D (50%) and E (25%) in pregnant women, while genotype K (66.7%) was predominant in infertile women. Notably, all these genotypic findings exhibited mutation points in the VDII variability region, except for one infertility sample, which showed mutations in the VDIII region. Although genotype F was identified in this study, the variable domains could not be characterized. This may be due to insufficient information in the sequencing results, which hindered the identification of the VDs.

CONCLUSION

The variability analysis of the CT *omp*A gene in this study provided valuable results that could contribute to epidemiological research, helping to characterize the circulation of CT types in our region. These findings may also serve as a basis for future drug development studies. Therefore, the identification and understanding of mutations in essential genes with pathogenic potential could be utilized for developing effective microbial therapies.

Although some studies have characterized genotypes in Brazil, we believe this is the first study to characterize the genotypes and analyze the variable domains of the CT *omp*A gene in infertile and pregnant women in Manaus, Amazonas.

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