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Issues with the Diagnosis of *Chlamydia trachomatis* in Cervical Infections in Mexico: From the Causes to the Interventions

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Abstract

In contrast to other countries, Mexico lacks precise estimations on the prevalence and risk factors associated with the *Chlamydia trachomatis* bacterium as well as the impact caused by this infection in different groups of women. There are multiple difficulties, but one of the main reasons is, without a doubt, that cervicovaginal cervicitis caused by this bacterium is not mandatorily reported to epidemiology departments. On the other hand, lymphogranuloma venereal (LGV) is mandatorily reported (including the number of female/male cases, federal entities/districts and age) and the diagnosis is performed using the syndromic management recommended by the World Health Organization (WHO), which is a medical practice with low sensitivity and specificity. A second cause could be the relative difficulty in establishing a diagnosis, considering the laboratory methodology is technically demanding and expensive. A literature review was performed of Mexican research studies on this bacterium, which were presented in congresses or publications, allowing for the identification of a very important number of studies on female patients who visit different governmental and private facilities. These studies report the laboratory methods used to perform diagnostics; furthermore some of these evaluate the sensitivity and specificity. The frequency of this bacterium is varying, which most likely results from the diverse methodologies applied in the cited works.

The latest estimations/projections published by the WHO in 2008 indicate that the number of treatable sexually transmitted infection (STI) cases in adults is approximately 498 million cases, and only 105.7 million cases correspond to Chlamydia. Americas accounted for 25.2 million of these cases. Nevertheless, the WHO acknowledges that "There is, however, a great deal of uncertainty surrounding the global and regional STI estimates. If these uncertainties are to be reduced a determined effort is needed to obtain relevant data, in particular: prevalence data disaggregated by age and sex and estimates of the duration of infection disaggregated by pathogen, the health care seeking behaviour of the population, and access to health care". While *C. trachomatis* is the most common bacterial cause of Sexually transmitted infections (STIs) in the United States, Mexico provides little to the estimations reported by the WHO.

Keywords: Chlamydia; Diagnosis; Mexico

Introduction

The STI aetiology is diverse and those of relevance in Mexico include the following: congenital syphilis, acquired syphilis, genitourinary tract gonococcal infection, LGV by Chlamydia, chancroid, genital trichomoniasis, genital herpes, acquired immune deficiency syndrome, urogenital candidiasis and asymptomatic infection with human immunodeficiency virus (HIV), and there is no surveillance programme for monitoring treatable STIs that are caused by Chlamydiae as well as by mycoplasmas and genital ureaplasmas [1-12].

Chlamydiae are intracellular bacterial pathogens that cause a broad-spectrum of clinically significant diseases in humans. In addition to being the causal agent for genital infections (some of the main bacterial STIs worldwide), *C. trachomatis* also causes trachoma (the main cause of preventable blindness in the world; also, endemic in several developing countries). Based on the main characteristics of the Major Outer Membrane Protein (MOMP), coded by the *ompA* gene, there are 19 serovars recognized for *C. trachomatis* [12-19]. This microorganism can cause asymptomatic urogenital infection, affecting as many as 50% of men and 90% of women. These asymptomatic infections are of great importance because they can go unnoticed, and, if left untreated, they may have grave complications, such as epididymitis in men and pelvic inflammatory disease (PID) in women. PID may lead to ectopic pregnancies and infertility. In new-borns, *C. trachomatis* infection could lead to pneumonia and conjunctivitis. Therefore, it is important to be aware of the existence of this microorganism. Nonetheless, during the bibliographic review, few reports on conjunctival infections and LGV were found. In general, the lack of such reports may be due to the same situations described for cervicovaginal infections [20-24].

Available Methodologies

The methods currently used for diagnosis could be classified into three groups. 1. Isolation of the bacterium using McCoy, HeLa 229, BHK21, and CHO cell cultures, murine peritoneal macrophages or chick embryo yolk sacs ("this is the old gold standard"). 2. Detection using phenotypic methods to demonstrate the presence of the bacteria in direct smears from the clinical sample or through cell culture isolation, which could be performed in different ways, such as in the observation for the reticular bodies (RB) with stains including Giemsa, Gimenez, Machiavello, Iodine-Haematoxylin-Eosin and Papanicolaou,

wherein a vacuole containing the RB can be readily observed. Observation of the elementary bodies (EB) is achieved through direct immunofluorescence (DIF), the ELISA test and immunoperoxidase technique. 3. The genotypic methods to demonstrate the presence of bacterium could include hybridization or amplification. The more commonly used amplification methods are the polymerase chain reaction (PCR), ligase chain reaction (LCR), Q-beta replicase amplification, transcription-mediated amplification (TMA) assay and analysis through restriction fragment length polymorphism (RFLP). To investigate *C. trachomatis* serotypes, PCR has been used, which is followed by analysis through restriction fragment length polymorphism (RFLP-PCR). As for the strain typification, random amplification of polymorphic DNA (RAPD-PCR) and the amplification through enterobacterial repetitive intergenic consensus polymerase (ERIC-PCR) are the most widely used methods [25-29].

Brief Description of The Studies Performed in Mexico

As previously mentioned, the gold standard is to culture the bacterium in McCoy cells, which is followed by the detection and identification of the bacteria through DIF. Nonetheless, using cell culture in diagnostic clinical laboratories is costly because special materials and equipment are required, including a work area with a laminar flow hood and qualified personnel to perform these types of techniques. For this reason, such methodology is only used for research purposes or in highly specialized laboratories. The first studies were performed by isolating the bacterium in cell lines and identifying the bacterium using different stains, the most important of which was the Papanicolaou stain. Later, DIF became the most commonly used technique; currently, PCR is the widespread method used when performing research on this bacterium in women. The antigen detection assays, such as direct immunofluorescence (DIF) and Enzyme-Linked Immunosorbent Assay (ELISA), have facilitated the diagnosis of Chlamidiae infections, but they have low sensitivity and specificity compared with the cell culture. The hybridization of nucleic acids displays a similar sensitivity, but not specificity, to the cell culture. Nucleic acid amplification tests surpass these methodologies in sensitivity and specificity; therefore, they are recommended without hesitation for the diagnosis and screening of Chlamydiae infections. However, the use of these molecular biology tests is limited in Mexico due to their cost and the need for trained personnel to properly perform them. In contrast, most clinical laboratories perform routine diagnostics and rely on the nucleic acid amplification tests (NAATs) recommended methods [30-36].

The reported frequency for this bacterium is highly variable, which is probably as a consequence of the applied methodologies in the studies, population participating in the studies, pathology exhibited by the women and types of samples chosen by the researchers to determine the frequency of the bacterium. The studies that better represent the state and evolutions in the diagnosis of this bacterium in Mexico are those henceforth described (Table 1).

Diagnostic	Cases	Method	Frequency (%)	Year	Ref.
Infectious Vaginitis	258	Bacteriological culture PCR	8.6 young adolescents 23.7 adolescents	1986	[13]
Vaginitis	111	Immunofluorescence	16.7	1987	[29]
Leucorrhoea	200	Giemsa staining Papanicolaou Immunofluorescence	11.5 7.5 15	1992	[16]
Leucorrhoea	200	Papanicolaou	11.5	1994	[10]
Leucorrhoea	200	Isolation in cell culture and immunofluorescence	9.3	1992	[16]
Leucorrhoea	245	Spectrophotometric assay Papanicolaou Immunofluorescence	2.4 2.9 1.6	1996	[35]
Pregnant woman	125	Papanicolaou EIA PCR	3.2 9.6 3	2000	
Vaginitis	200	Giemsa Papanicolaou & Immunofluorescence	12.14	2000	[20]
Urogenital infection	1220	CSF	4.8	2005	[38]
Infertile women	152	PCR-RFLP	15.8	2011	[9]
No disease (women)	105	PCR	10.47	2013	[19]
Infertile women	38	PCR	47.36	2013	[40]

Dead new-born (<1 week)	14	PCR	35.7	2014	[22]
Premature new-born (<2.5 kg)	44	PCR Antibody title	45.5 1.32	2015	[26]

Table 1: Frequency and method for diagnosing *C. trachomatis* selected studies in Mexican literature.

Risk Factors

Written works describe the following as risk factors associated with the natural history of *C. trachomatis* infection in women: young age (<20 yrs.), which could be explained by the anatomic differences found in the cervix of younger women, where the squamo-columnar junction, a primary host target for *C. trachomatis*, is everted and increasingly exposed. Other factors associated with chlamydial infection include unmarried status, nulliparity, black race and a poor socio-economic condition. A history of many sexual partners, a new sexual partner, lack of barrier-contraceptive device use and concurrent gonococcal infection are associated with chlamydial infection. Cervical chlamydial infections are also found to be linked to the use of oral contraceptives. Nevertheless, this information has been described for female populations with different habits and costumes from those of Mexican woman. Perhaps the most complete study on the subject was performed by Guerra-Infante et al., 2003 who not only studied the risk factors on the Mexican female population suffering from infertility, they also evaluated the relationship between infection and cervix abnormalities in a population of Mexican women who visit the infertility clinic. Their results suggest that infection by *C. trachomatis* is associated with a single-partner relationship, marital status and the use of an intrauterine device (IUD) as a pregnancy control method. The presence of ectropion and a friable cervix in obstetrical exploration should be considered an indicator for *C. trachomatis*. However, several independent infection-indicators reported by other authors were not determined in the present work [37-39].

Author´s Opinion About Intervention and Preventive Measures

The first intervention that could be implemented is to broadcast the current knowledge on the clinical relevance of this bacterium in the health field; patients and health care providers should be informed about the facilities where testing could be performed as well as treatment methods and, more importantly, infection prevention from this bacterium should be thoroughly explained. The health institutions should perform campaigns and give talks addressed to the general population. They should provide updated courses to all health personnel. The instructors for such courses should be physicians, researchers and experts in the field.

Along with the informative campaign, efforts should be made to establish a government surveillance program, which should offer the population diagnostic testing. This last intervention should resolve the problem of detecting the bacterium. Therefore, it is important to invest in the health sector to identify equipment and acquire materials as well as to implement newer diagnostic methods with a high sensitivity and specificity, more manageable and less costly NAATs and proper health personnel training for using and implementing the best diagnostic technique to estimate the true prevalence of *C. trachomatis*. The aforementioned interventions will avoid complications associated with an undiagnosed and untreated infection [40,41].

Although the NAATs are presently the best choice, further research on infections caused by *C. trachomatis* should be encouraged, especially in pregnant women and new-borns from developing countries, wherein epidemiological studies are included, which allows for evaluation of the magnitude of the problem and implementation of adequate interventions for each population type. Few studies describe risk factors and the development of better techniques for diagnosing asymptomatic infections; research should be performed to find strains that may be used to create vaccines that control and eradicate infections caused by this bacterium [37,38].

Prevention of infections caused by *C. trachomatis* consists of avoiding high-risk sexual intercourse, i.e., sex without the use of a condom. Once the infection has been diagnosed, it is necessary to implement precise strategies that control the infection, including the registry of these infections as well as provision of adequate treatment for the infected patients and their sexual partners alike [42]. Complication prevention cannot be achieved if the previous conditions are not met. It should be noted that infection with chlamydia can increase the probability of HIV transmission. For the previously mentioned reasons, the implementation of routine monitoring for diagnosis is recommended. Its determination should also be included in the annual gynaecological control and urological evaluations in young men. It is important to educate physicians on the routine need for this test rather than its use as a last resort in patients exhibiting symptoms or in infertile couples. In light of the current antimicrobial resistance problem, standardization of treatment guidelines to avoid the appearance of resistant strains is of utmost importance. The general population should be made aware that initiating sexual intercourse early, lack of barrier-contraceptive methods, frequent changing of sexual partners and lack of consulting specialized professionals increase the risk of acquiring an STI, making a necessity to increase the surveillance and development of awareness and prevention actions.

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Competing Interests

The authors declare that they have no competing interests.

Ethical Aspects

Ethical approval is not required.

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