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RESEARCH ARTICLE

Burden of *Chlamydia trachomatis* in India: a systematic literature review

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One sentence summary: The first systematic literature review on *Chlamydia trachomatis* in India.

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ABSTRACT

Chlamydia trachomatis (hereafter CT) is Gram-negative, obligate intracellular pathogen. It causes the world's most common non-viral sexually transmitted disease. India is home to the world's greatest burden of infectious diseases, yet information on prevalence rates of CT is scarce. This article systematically reviews the literature for the prevalence rates and testing methods in India. A total of 27 studies were included. Four main patients groups (symptomatic women, infertile women, pregnant women and asymptomatic population groups) could be identified with varying rates of CT (0.1%–32% using PCR, 2.4%–75% using ELISA serology). Most of the studies originated from urban settings, 11 of them from New Delhi. In-house PCR was the most common diagnostic technique used generating the following ranges in prevalence for the four group studies: symptomatic women 10%–50%, pregnant women 0.1%–2.5% and asymptomatic populations 0.9%–24.5%. The rates among infertile women were 9%–68% based on serology results. The prevalence rates featured in this paper are in line with other locations across the Indian subcontinent. This review highlights the extreme heterogeneity in the limited studies available in India on CT and the need for standardized guidelines for diagnosis and management of CT in India. The availability of resources should be considered in the formulation of recommendations.

Keywords: Chlamydia trachomatis, India, C. trachomatis, Sexually Transmitted Diseases, Infertility

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INTRODUCTION

Chlamydia trachomatis is a Gram-negative, obligate intracellular pathogen that can lead to a broad spectrum of clinical diseases in human populations. It is known to cause a significant burden of preventable blindness in third world countries. *Chlamydia trachomatis* is also known as the most common bacterial sexually transmitted infection worldwide (Newman *et al.* 2015). With a total of over 130 million new cases per year worldwide in 2015, *C. trachomatis* infections represent a major problem worldwide. Latest trends furthermore seem to indicate that this number is likely to increase (WHO 2008). Urogenital infections with *C. trachomatis* have been associated with a wide range of genitourinary conditions including cervicitis and salpingitis in women as well as epididymitis and urethritis in men. Infection with the pathogen is however often asymptomatic and hence frequently remains undiagnosed, leading to an array of severe long-term consequences (Haggerty *et al.* 2010). Studies indicate that chlamydial infections can lead to severe impairments such as pelvic inflammatory disease, tubal damage and ultimately tubal factor infertility in women if they are not treated in a timely and adequate fashion (den Hartog, Morré and Land 2006; Haggerty *et al.* 2010). Additionally, infections with *C. trachomatis* can severely impact the reproductive health of women, causing severe conditions such as ectopic pregnancies, repeated and spontaneous abortions and stillbirths(Tiller 2002; Baud and Greub 2011). These features render timely identification and reliable diagnosis of *C. trachomatis* an issue of public health importance, as the disease can effectively be treated using antibiotics. This is particularly true in developing countries, where infectious disease already significantly burdens the populations and the healthcare systems (Gangolli, Duggal and Shukla 2005; John *et al.* 2011). The asymptomatic nature of the disease also requires evidence-based guidelines for the implementation of population-wide screening programs. In fact, studies have claimed that, due to the low prevalence of Chlamydia at the population level, screening in the general population may not be cost-effective (Low *et al.* 2007). The identification of high-risk groups or chlamydial infections is however a public health issue (Althaus *et al.* 2010). This aspect is particularly significant for low and middle-income countries, where healthcare resources and budgets are limited (CDC and World Bank 2008).

With a total population of approximately 1.2 billion inhabitants, India is after China the second most populous country in the world (Perianayagam and Goli 2012). It is similarly known to be home to one of the greatest burden of Infectious diseases in the world (John *et al.* 2011). There is however a paucity of data and a lack of overview concerning the burden of *C. trachomatis* infections in India. Insights regarding the strategies for diagnosis and management of *C. trachomatis* in healthcare settings are also limited (Malhotra *et al.* 2013). As a result of the varying levels of specificity and sensitivity of the diagnostic tools utilized in the clinical settings, the choice of method is of primordial importance (Chernesky 2005).

The aim of this paper is to systematically review the available scientific literature to investigate the urogenital *C. trachomatis* burden in India, across different regions and patient groups. The initial scope of this review is to provide an overview of the prevalence of the disease among the different patient groups. Additionally, the techniques used to identify *C .trachomatis* in patients need to be assessed, to lead the way for formulation of future best practices and potential evidence-based guidelines for diagnosis and management of *C. trachomatis* across the Indian subcontinent. Finally, in light of the significant impact of *C. trachomatis* on the female reproductive tract, and the importance of maternal and child health in India, the disease's contributions to the national burden of reproductive health needs to be clarified (Inhorn and Bharadwaj 2007; Ganguly and Unisa 2010; Mahesh 2013) in order to aid policy decisions.

METHODS

Peer-reviewed articles included in this review were obtained from the major databases namely PubMed and Embase using the search headlines '*Chlamydia trachomatis*' and 'India'. Additionally, Google Scholar was screened with the same search criteria to include non-indexed articles and 'gray' literature (Mahood, Van Eerd and Irvin 2014). Lastly, all the references and work cited by the articles included in this review were screened to identify further articles to be included. The above databases were screened for available data from August 2015 until December 2015.

In light of the previously mentioned lack of consistent data originating from the Indian settings, the scope of the literature search was designed to include all the patient groups that are at a higher risk of infection or subsequent sequelae when considering *C. trachomatis*. Taking into account the exploratory nature of the review, the authors screened all articles based on the type of patient population, the diagnostic method utilized to test for a urogenital *C. trachomatis* and the geographical location of the study. All of the studies that suited the scope of the review were included for further analysis based on the abstract and reviewed by at least two of the authors and checked for duplicates. The included articles were then scrutinized individually in their full-text versions. The final inclusion of discordant studies was then discussed with the authors.

If the data screened did not feature population testing, described laboratory techniques in relation with *C. trachomatis* detection or Chlamydia typing of established positive samples, they were excluded from this review. Additionally, all duplicates were excluded.

RESULTS

The combined literature search yielded a total of 27 unique articles; the details of the inclusion are summarized in Fig. 1.

The literature search succeeded in unveiling scientific studies originating from a wide range of geographical locations.

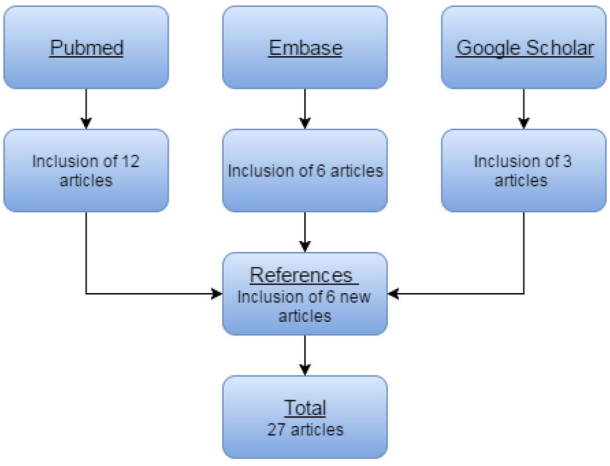

Figure 1. Sources of included articles.

Figure 2. Geographical locations of studies (obtained with Google mymaps).

However, the city of New Delhi was over-represented, as over a third of all the included studies (11/27 = 40%) could be traced back to this location. Similarly, three studies originated from the city of Mumbai and three others from the city of Chennai, respectively. Additionally, it should be noted that most of the studies originated from urban settings, as only two studies were conducted in rural settings (2/27 = 7%). The locations of the different studies included are summarized in Fig. 2.

The differences in the testing methods used to diagnose *Chlamydia trachomatis* and the differences in patient populations enclosed in this review render a meta-analysis of the data difficult. Some distinct patient groups could however be consistently identified and grouped together for analysis. A total of four patient groups could be identified as listed below (see Table 1 for details):

1. Symptomatic patients presenting in healthcare settings: 13 identified studies.
2. Infertile and subfertile women: seven identified studies.
3. Pregnant women: two identified studies and four control groups.
4. Asymptomatic population groups: three studies and one control group included

Symptomatic patients presenting at the gynecology outpatient department (OPD)

The first patient population was the most represented in the review with a total of 13 studies identified. The details of the studies are featured in Table 2. The prevalence of *C. trachomatis* among the studies ranged from 10% in the study by Sood *et al.* (2012) to 50% in the study by Gopalkrishna *et al.* (2000) using polymerase chain reaction (PCR), both in New Delhi (Gopalkrishna *et al.* 2000; Sood *et al.* 2012). However, another study from Chennai by Pushpa Innocent (2010) using ELISA reported a prevalence of 75%. The predominance of studies from New Delhi could also be observed in this group. Eight of the studies conducted on symptomatic patients were in fact conducted in New Delhi.

A similar study testing on men attending the STD clinic in Mumbai was also identified. The authors however did not specify patient details. The study reported 2.2% *C. trachomatis* prevalence using first-void urine (FVU) PCR (Lindan *et al.* 2005).

Infertile and subfertile women

Female patients (repeatedly) infected with *C. trachomatis* are known to be at an elevated risk for late complications,i.e. tubal factor infertility (den Hartog, Morré and Land 2006). Seven

Table 1. The articles are color-coded based on the patient groups they feature: Group 1 (green): symptomatic women; Group 2 (blue): infertile and subfertile women; Group 3 (red): pregnant women; Group 4 (white): asymptomatic population groups.

Main Author	Journal and Year	Location	Study population	Control group	Method of testing*	*C. trachomatis* prevalence
Ghosh *et al.* (2015)	Open microbiology journal	Kolkata (East)	50 infertile women attending the gynecology OPD	50 healthy pregnant women	IgG&IgA ELISA FVU PCR	Study Group:, 15% IgG ELISA, 12.5% IgA ELISA2, 5% PCR Control Group: 2.5% (PCR), 0% IgG, 2.5% IgA
Mohan & Borthakur (2015)	Indian journal of medical microbiology	Guwahati (East)	40 infertile women attending the hospital	40 fertile women	IgG ELISA	Study group: 25% Control group: 7.5%
Bajpaj, Ganesh & Neelesh (2015)	Indian journal of sexually transmitted diseases	Indore (Middle)	111 infertile women attending the IVF center		IgG ELISA	9% after 1 month serology retesting.
Dhawan *et al.* (2014)	Indian Journal of medical research (2014)	New Delhi (North)	200 infertile women		In-house PCR DFA EIA COBAS-Taqman	13.5% (PCR) 9% (DFA) 6.5% (EIA)
Vidwan *et al.* (Vidwan *et al.* 2012)	PLoS one	Vellore (South)	1198 pregnant women		Rapid test (Clearview Chlamydia Test Kits, Iverness) Roche AMPLICOR	10% (Rapidtest) 0.1% (AMPLICOR)
Mania-Pramanik *et al.* (2012)	Journal of reproductive infertility	Mumbai (West)	896 women attending the gynecology OPD (symptomatic)		PCR (In-House)	12.1%
Detels *et al.* (2011)	Sexually transmitted diseases	Chennai (South)	3513 Wine shop patrons and commercial sex workers in Chennai		Roche AMPLICOR PCR	0.9% at baseline
Gita *et al.*(2011)	Infectious diseases in obstetrics and gynecology	New Delhi (North)	2466 women under suspicion of *C. trachomatis* infections (PID, Cervicitis, Salpingitis etc)		DFA Culture In-house PCR (Plasmid) RFLP-based genotyping (MOMP)	15.85% (391/2466DFA) 13.1% (198/1507 Culture) 13.2% (44/333 in-house PCR) 44% (22/50MOMP RFLP)
Sood *et al.*(2012)	Indian journal of dermatology, venerology and leprology	New Delhi (North)	97 symptomatic women presenting at the STD clinic		DFA PCR (plasmid)	11.34% (DFA) 10.3% (PCR)
Patel *et al.* (2010)	Annals of clinical microbiology and Antimicrobials	New Delhi (North)	593 Women attending the Gynecology OPD Symptomatic (discharge)		Group I: Direct fluorescence assay (N=273) Group 2: In house PCR and AMPLICOR	In house PCR/Roche AMPLICOR 23%
Pratibha *et al.* (2010)	Annals of biological research	Chennai (South)	280 patients in 2 groups (discharges and irregular periods	55 women without complaints or symptoms	IgG ELISA	60.9%/75% (patients) 9.1% (control)
Savitha, madhavan, Vinoth Raja (2009)	Journal of pharmaceutical Sciences and Research	Tanjore District, Tamil Nadu (South)	200 women presenting at the Primary Health care center and private clinics		Giemsa stain	10.5%
Malik *et al.* (2009)	Fertility and Sterility	Aligarh (North)	40 infertile women	30 healthy & asymptomatic term pregnant women of similar age	IgG ELISA Culture	55% (IgG ELISA infertile women) 5.5% (IgG ELISA controls)
Dwibedi *et al.* (2009)	Indian Journal of Dermatology, Venerology and Leprology	Cuttack (East)	108 symptomatic married women (71 tested)		MOMP PCR (in-house)	7.04%)
Gupta, Salhan and Mittal (2009)	Journal of Infections in developing countries	New Delhi (North)	355 symptomatic women attending the OPD		IncC and IncB inclusion proteins ELISA MOMP ELISA	30.2% out of which 62 % IncC+ and 59.25% IncB+
Malhotra *et al.* (2008)	Indian Journal of Sexually transmissible disease	New Delhi (North)	276 women with genital discharge or ulcer (symptomatic)		DFA test (Immuno FA) IgG&IgM ELISA	19.9% 10.1% (DFA) 10.9% (ELISA)
Malik *et al.* (2006)	Indian Journal of Medical Research	Aligarh (North)	110 women suffering with primary or secondary infertility	30 Healthy term pregnant women	Culture and ELISA	28.1% (Infertile group) positive by one or more markers, 22% (culture) 3.3% (control Group)
Vinita *et al.*(2006)	Journal of Obstetrics and Gynecology in India	Lucknow (North)	100 Women attending gynecology and Family planning (symptomatic and Asymptomatic)	50 women without complaints	In house urine PCR	14% of symptomatic women 4% of asymptomatic women
Lindan *et al.* (2005)	Journal of clinical Microbiology	Mumbai (West)	690 men attending the STI clinic		First Catch Urine PCR	2.2%
Joyee *et al.* (2004)	International Journal of STD and AIDS	Tajore, Ramnad and dingidul district, Tamil Nadu (South)	Randomly selected adults aged 15-45 1849 participants (1066 females,783 males)		AMPLICOR PCR IgM ELISA	1.1% (PCR) 2.4% (IgM ELISA)

Table 1 *Continued*.

Main Author	Journal and Year	Location	Study population	Control group	Method of testing*	*C. trachomatis* prevalence	
Singh *et al.* (2003)	Journal of clinical microbiology	New Delhi (North)	280 women attending the gynecology OPD for various complaints			Plasmid PCR	28% (18-25) 7.6% (25-35) 3.2% (35-45)
George *et al.* (2003)	Japanese Journal of infectious diseases	Chennai (South)	143 symptomatic patients (80 women and 63 men)			Cell culture, Plasmid-based PCR and Urine based PCR	32.2% (PCR) 25.2% (urine PCR) 19.9% (culture) 38.1% Males 27.5% females
Sharma, Aggarwal & Arora (2002)	Indian Journal of Medical Research	Chandigarh (North)	50 women with infertility 50 women with bad obstetric history		50 health pregnant women	IgG Solid phase EIA (IgG directed against L2 serotype antigen)	68% (infertile women) 50% (bad obstetric history) 10% (controls)
Singh *et al.* (2001)	Acta Cytologica	New Delhi (North)	350 women attending Gynecology OPD for various complaints (symptomatic)		53 Randomly selected slum dwellers	Giemsa stain cytology DFA PCR (In house)	43.1% (OPD Patients) 24.5% (Slum Dwellers)
Gopalkrishna *et al.* (2000)	Clinical microbiology and Infectious diseases	New Delhi (North)	50 symptomatic women attending the STI clinic 50 women with cancerous and precancerous legions of the cervix		30 healthy women of similar age group	IgG ELISA EIA PCR (In house)	STD patients : 50% (PCR), 26% (EIA), 52% (ELISA) 22% of cancerous patients/12% precancerous patients
Paul *et al.* (1999)	Medical Journal of India	New Delhi (North)	Study I: 94 women between 26 and 30 weeks of Gestation Study II: 172 women presenting with spontaneous labor			Chlamydiayzme (Enzyme immunoassay)	Study I 17% Study II 18.6%
Joshi *et al.* (1994)	National medical Journal of India	Mumbai (West)	305 women attending the clinic (mostly asymptomatic)			FITC fluorescence staining (DFA?)	15%

Table 2. *Chlamydia trachomatis* prevalence among symptomatic patients (results given for PCR or most sensitive technique).

Authors	Location	Testing	Cohort size	Prevalence
Mania-Pramanik *et al.* (2012)	Mumbai	PCR	896	12%
Sood *et al.* (2012)	New Delhi	DFA, PCR	97	10%
Gita *et al.* (2011)	New Delhi	DFA, PCR Culture,	2466	15% (DFA)/13.2% (PCR)
Patel *et al.* (2010)	New Delhi	PCR	593	23%
Pushpa Innocent (2010)	Chennai	IgG ELISA	280	60%/75% (two cohorts)
Dwibedi *et al.* (2009)	Cuttack	PCR	108	7.0%
Gupta, Salhan and Mittal (2009)	New Delhi	IgG ELISA	355	30%
Malhotra *et al.* (2008)	New Delhi	DFA, IgG IgM ELISA	276	19%
Vinita *et al.* (2006)	Lucknow	PCR	100	14%
Singh *et al.* (2003)	New Delhi	PCR	280	28%
George *et al.* (2003)	Chennai	Culture and PCR	143	32%
Singh *et al.* (2002)	New Delhi	DFA, PCR	350	43%
Gopalkrishna *et al.* (2000)	New Delhi	IgG ELISA, PCR	50	50%

Table 3. Prevalence among infertile and subfertile women (results given for PCR or most sensitive technique).

Authors	Location	Testing	Cohort size	Prevalence
Ghosh *et al.* (2015)	Kolkata	IgG ELISA, PCR	50	2.5%PCR 15% ELISA
Mohan and Borthakur (2015)	Guwahati	IgG ELISA	40	25%
Bajpai, Ganesh and Neelesh (2015)	Indore	IgG ELISA	111	9%
Dhawan *et al.* (2014)	New Delhi	PCR,DFA, EIA	200	13.5%
Malik *et al.* (2009)	Aligarh	IgG ELISA	20	55%
Malik *et al.* (2006)	Aligarh	Culture, IgG ELISA	110	28.1%
Sharma, Aggarwal and Arora (2002)	Amritsar	IgG EIA	50	68%

studies conducted among infertile and subfertile women were included. The results are summarized in Table 3. The prevalence ranged from 9% in Indore in central region of India to 68% in a study performed in Amritsar, making use of enzyme immunoassay (Sharma, Aggarwal and Arora 2002; Bajpai, Ganesh and Neelesh 2015). A further study from Kolkata in the eastern region of India reported an even lower prevalence using PCR (2.5%) (Ghosh *et al.* 2015). The same study nonetheless reported a seroprevalence of the pathogen of 15% while testing with ELISA (Ghosh *et al.* 2015).

Pregnant women

The third group of interest is pregnant women. Two studies were identified where testing was conducted in pregnant women. Pregnant women were also used as a control group in four previously mentioned studies (Sharma, Aggarwal and Arora 2002; Malik *et al.* 2006, 2009; Ghosh *et al.* 2015). All results are summarized in Table 4. The reported prevalence of *C. trachomatis* in this group for studies reporting the use of PCR ranged from 0.1% in Vellore, South India, to 2.5% in the study by Ghosh *et al.*

Table 4. *Chlamydia trachomatis* prevalence among pregnant women (results given for PCR or most sensitive technique).

Authors	Location	Testing	Cohort size	Prevalence
Ghosh *et al.* (2015)	Kolkata	ELISA, PCR	50	2.5%
Vidwan *et al.* (2012)	Vellore	PCR	1198	0.1%
Malik *et al.* (2009)	Aligarh	IgG ELISA, culture	30	5.5%
Malik *et al.* (2006)	Aligarh	IgG ELISA, culture	30	3.3%
Sharma, Aggarwal and Arora (2002)	Amritsar	IgG EIA	50	10%
Paul *et al.* (1999)	New Delhi	Enzyme immunoassay	94 and 172	17%/18.6%

Table 5. Prevalence of *Chlamydia trachomatis* in population screening (results given for PCR or most sensible technique).

Authors	Location	Testing	Cohort size	Prevalence
Detels *et al.* (2011)	Chennai	PCR	3513 (males and females	0.9%
Savitha, Madhavan and Vinoth Raja (2009)	Tanjore district, Tamil Nadu	Giemsa Stain	200 (females)	10.5%
Joyee *et al.* (2004)	Tanjore, Ramnad and Dingidul districts, Tamil Nadu	PCR	1849 (males and females)	1.1%
Singh *et al.* (2002)	New Delhi	PCR	53 (males and females)	24.5%

in Kolkata, East India (Vidwan *et al.* 2012; Ghosh *et al.* 2015). The prevalence range is even greater in the studies utilizing ELISA tests. These results range from 3.3% in Aligarh reported by Malik *et al.* (2006) to 18.6% in the study by Paul *et al.* (1999). The results featured in this group also display higher prevalence in the studies utilizing serology-based testing.

Asymptomatic population groups

Data from studies performing testing at the population level could only be found in three studies and one control group. The population screening group is hence the least represented group, the results and prevalence of the study are summarized in Table 5. The populations screened in this patient group present with major differences in both sample size, origin and prevalence of *C. trachomatis*. The lowest prevalence (0.9%) was observed by Detels *et al.* among shop owners and commercial sex workers in Chennai, while the highest prevalence (24.5%) was reported for residents of slum areas in New Delhi by Singh *et al.* (2002). Both the aforementioned results were provided using PCR testing.

Testing and diagnostic methods

The methods used to diagnose the disease and identify the pathogen in clinical settings are of pivotal importance. In fact, *C. trachomatis* can be identified through different tests, all of which have different characteristics. Although PCR is currently the gold standard for the identification of the bacteria in human subjects, it however can also be identified through culture (Centers for Disease Control and Prevention 2014; Lanjouw *et al.* 2016). On the other hand, serology-based tests such as ELISA are only suitable for screening in subfertile women. Serology tests help to ascertain the presence of an immune response against the pathogen through the detection of specific antibodies. These antibodies highlight a previous infection which is particularly relevant in the case of *C. trachomatis* infections (Keltz, Gera and Moustakis 2006). Previous infections may in fact be the cause of tubal pathologies which can be traced back as the cause of infertility. Table 6 summarizes the testing methods featured in the studies included in this review. It should be noted that many of the studies made use of more than one diagnostic test.

Table 6. Diagnostic and tests in the studies included in the review.

Testing method	Number of studies
In-house PCR	14
ELISA	13
Commercial PCR tests	5
DFA	4
Culture/Giemsa stain	3

The most commonly reported test in the review was in-house PCR testing, which was performed in 14 studies. ELISA immunological testing was the second most common test, present in 13 studies.

Material for PCR testing

Material for the conduction of (or sample for conducting the) PCR testing can be obtained from different parts of the body as well as different bodily fluids. Each of the products requires a specific sampling procedure which might be more or less invasive. The origin of the sampled material for PCR among the different studies is summarized below. It should be specified that some of the studies featured more than one PCR assays requiring different materials each.

It can be seen that the most commonly used biological material for the conduction of PCR assays were endocervical swabs. It can be seen that the most commonly used biological material for the conduction of PCR assays were endocervical swabs, As featured in Table 7. This method of sampling was used in 11 studies. Other methods included FVU and urine sampling, which were conducted in three studies each. Vaginal samples were used in only two studies.

Table 7. Origin of the material for PCR testing.

Material for PCR testing	Number of studies
Endocervical swabs	11
Urine	3
FVU	3
Vaginal swabs	2

DISCUSSION

This review provides the first overview on the *Chlamydia trachomatis* prevalence in the Indian subcontinent. Articles and studies on the subject could be traced back to various geographical regions of India, and the results could be classified into symptomatic women, infertile and subfertile women and asymptomatic population groups. All of them display varying values *of C. trachomatis* prevalence, which can be attributed to the different settings where testing was conducted, the different population characteristics, but most importantly the tools used to diagnose the pathogen. The findings of this constitute a first attempt at identifying existing discrepancies attributable to differences in population testing techniques and locations regarding the current situation surrounding *C. trachomatis* in India. In fact, in all patient groups investigated in this review, the prevalence results stemming from studies making use of serology-based testing such as ELISA were as expected higher when compared with the results of studies using PCR.

It was observed that, in patient groups 1 and 2, the prevalence reported using ELISA serology is higher than results obtained through PCR testing. This highlights that the seroprevalence is higher among symptomatic and infertile patients when compared to other groups. The presence of an immune response to the pathogen as supported by positive results of serological tests in fact suggests a past or chronic exposure to the pathogen (Horner *et al.* 2013). The high seroprevalences as compared to PCR prevalences suggest that previous *Chlamydia trachomatis* infection is related to long term consequences. In fact, PCR detects bacterial DNA inside of the patient's genital tract, which suggests a current infection rather than a past one. This finding underscores the role played by *C. trachomatis* in chronic infections leading to adverse health outcomes, and particularly, infertility.

Lack of studies featuring male participants could also be observed in group 1. In fact, only one study could be identified, where testing was conducted among men. This study could furthermore not be included in the tables, as the patients tested were not listed as symptomatic, but as attendees of the STD clinic, who may be present only for information. The only testing group where accounts for both men and women could be obtained was the group regarding population screening. Huge variations in terms of sample sizes in this group however restrict the scope for extrapolation. In fact, great differences in sample sizes were reported from this group. The sample size of the featured testing group ranges from 53 participants featured in the control group by Singh *et al.* (2002) in New Delhi to 3513 in the study by Detels *et al.* (2011) performed in the south Indian city of Chennai. This represents a testing group almost 70 times bigger.

The prevalence of *C. trachomatis* across the Indian Territory appears to vary across geographical regions and patient groups. The results suggest that the prevalence rates of *C. trachomatis* may be influenced by a variety of factors. Aspects influencing the prevalence of *C. trachomatis* may be attributable to differences in sensibility and specificity of testing, varied cultural backgrounds as well as healthcare practices and health-related beliefs. Additionally, the performance of local, state-run healthcare delivery systems varies greatly between the different states (Goli *et al.* 2014). Moreover, research in different settings has highlighted the role of host genetic markers in the development of symptoms and consequences related to pathogens such as *C. trachomatis*. Genetic and genomic differences present within the different ethnicities that inhabit the Indian subcontinent could lead to varying prevalence of *C. trachomatis*. Furthermore, it should be stated that, despite the various represented patient groups included in this review, only three individual studies featured male patient groups. It should be furthermore stated that the only patient group where only men were represented was part of the asymptomatic population groups category. This limited data representing the male populations not only questions the completeness of the picture regarding the prevalence of *C. trachomatis* in the population but also raises the question of public health relevance associated with an undetected reservoir of infections, which could be held accountable for propagation of the disease. In addition, there are no mentions of contact tracing and partner testing in the different studies. Nonetheless, the data regarding prevalence featured in this article, with the exception of some very high prevalence numbers, match to some extent the prevalence data from European countries, when considering similar patient groups (ECDC 2014; Redmond *et al.* 2015). Additionally, and in a similar fashion compared with the Indian scenario, data from neighboring countries in similar situations and geographical locations are scarce. Some studies from Sri Lanka and Bangladesh nonetheless seem to suggest similar results across comparable patient groups. A study from the Colombo district in Sri Lanka in fact highlighted a *C. trachomatis* prevalence of 8.3% using PCR amid women attending the STI clinics (Kamani Mangalika *et al.* 2014). In another study from Bangladesh, a prevalence rate of 23% from sexually active women was found using in-house PCR (Hoque *et al.* 2013). The results from these studies support the range of prevalence, which was observed in similar groups in India. These results support the idea that *C. trachomatis* is associated with a significant burden of disease on the Indian subcontinent, particularly among high-risk groups such as sex workers and patients at STI clinics.

An important factor that may render the Indian settings unique regarding testing and identification of *C. trachomatis* and other sexually transmittable diseases in the community is the cultural stigma. Stigma was briefly mentioned in the study by Dwibedi *et al.* (2009) as a significant factor hindering women for seeking assistance and medical care for STI-related symptoms. Although no work could be identified highlighting the stigma against *C. trachomatis*, there exists a body of evidence surrounding the stigma surrounding HIV/AIDS in India. Many articles in fact support the idea that stigma is a major factor, present at multiple levels which hinders proper and timely management of HIV/AIDS, and that impacts the daily life of people affected by the diseases (Bharat 2011; Ekstrand *et al.* 2012, 2013). It is hence plausible that many people in the community shy away from getting diagnosed and treated with a sexually transmitted infection, even in spite of the fact that treatments and cures may exist, like in the case of *C. trachomatis*. This is further supported by experience of co-authors (Indian researchers/medical doctors) in this paper. This highlights the need for additional research into psychosocial factors influencing the health-seeking behavior of Indian patients. Stigma might in fact cause many patients to renounce seeking care and hence expose themselves to long-term health impairments. The absence of acknowledgement of the role of stigma related to STIs may suggest that the issue is widely ignored.

Furthermore, there seems to be discrepancies between the internationally recognized gold standards for *C. trachomatis* detection in industrialized countries and the practices currently implemented in India. As a matter of fact, only slightly short of half of all the studies included in this review featured testing using PCR. Most the PCR tests were performed in-house, while only five studies featured commercially available and internationally acclaimed tests such as the Roche AMPLICOR assay. It should be stressed that, as per the co-authors' experience, due to the elevated prices of some of the commercial PCR assays, in-house PCR tests might represent a valuable alternative for identification of *C. trachomatis* in healthcare settings. More research is nonetheless necessitated to better understand the properties of such tests. The values of specificity and sensitivity of in-house PCR tests ought to be judged against internationally acclaimed tests to ensure appropriate diagnosis. Additionally, there would be a need for evidence-based guidelines regarding the conduction of in-house PCR for the detection of *C. trachomatis*. Research conducted in Indian settings has highlighted that in-house PCR testing displays highly satisfactory results when compared to the AMPLICOR assay (Sachdeva *et al.* 2009). Research from Trinidad and Tobago has further highlighted the use of in-house PCR as a valuable option for settings where the availability of commercial testing kits is limited (Rampersad *et al.* 2007). Further research from South America has highlighted that a set of well-defined in-house PCR primers can be used for the detection of *C. trachomatis*, showcasing high levels of sensitivity and specificity when compared to gold standard kits (Aguilera-Arreola *et al.* 2014).

Furthermore, many of the articles referred to cell culture as the gold standard for the detection and diagnosis of *C. trachomatis* in India. These aspects might be associated with the resource intensity of PCR when compared to more affordable testing methods such as culture, direct fluorescence assay (DFA) or ELISA. PCR testing in fact necessitates a vast array of equipment and expertise and can hence only be performed in some facilities and by trained staff. Identification through culture or serology can conversely be performed in traditional laboratory settings which are present in clinics and primary health centers. This further supports the need for cost-effective diagnostic tools for detection in resource-limited settings such as in India. It should hence be stated that research is needed for the implementation of novel testing and diagnosis methods for *C. trachomatis* and other pathogens in resource-limited settings.

Another important point brought up by the review is the discrepancies in research methodology among the included studies. The control group included in some of the studies did not match with the study populations. This holds in the article by Singh *et al.* (2002) for instance. The study in fact compares female patients in healthcare settings to randomly selected inhabitants of the slums. This also holds for the lack of information regarding the inclusion of these populations. Additionally, in the two studies by Malik *et al.* (2006, 2009), both testing groups composed of infertile women were compared to control groups that were smaller in size. The population tested for *C. trachomatis* ought to be thorough and representative of their populations in order to provide sound information to policy makers. This is especially true in studies aiming at screening populations, as they may guide future screening schemes for sexually transmitted diseases. Overall, there should be a drive towards high-quality research designs, in order to facilitate policy implementations aimed at addressing the burden of *C. trachomatis* among the Indian population. In fact, there is a need for high-quality research to enable the development of evidence-based guidelines for the management of *C. trachomatis* in Indian healthcare settings. The current standard operating procedure regarding the diagnosis and management of sexually transmissible diseases are in fact hard to identify and appear to be incomplete. The current guidelines issued by the government of India indeed miss *C. trachomatis* as a potential cause of vaginal discharge, although it is one of the most common manifestation of *C. trachomatis* infection once it presents with symptoms (Government of India 2014). There is thus a need for clarity at the policy level for the identification, screening, prevention and treatment of pathogens like *C. trachomatis*. In addition of the need for cost-effective and readily implementable testing solutions, there is a need for more research in the policy strata. There is in fact a need for an encompassing policy framework taking all the relevant variables into account in order to foster safe, effective and evidence-based management of *C. trachomatis* infections as well as the long-term consequences they may cause.

This paper touches upon the topic of female infertility in India. This topic remains vastly unexplored, although some accounts suggest that the burden might underreported in India (Jejeebhoy 1998; Malhotra *et al.* 2013). There has been an increase in the number of couples that make use of *in vitro* fertilization and other methods in order to conceive (Widge and Cleland 2009). Experts have also argued that the issue is not properly addressed and is not sufficiently investigated in Indian settings (Pande 2013). Results from national surveys suggest a rise in the number of infertile couples (Mahesh 2013). These factors ought to draw more attention on the burden of infertility, of which *Chlamydia trachomatis* is a major cause alongside other diseases such as *N. Gonorrhea*. In fact, not only is the detection of the pathogen straightforward, but a timely management and treatment of the infection may lead to a full recovery and replenished reproductive health.

CONCLUSION

This review is the first endeavor to systematically review the prevalence of *Chlamydia trachomatis* across India. The studies included for analysis differed greatly based on research methodology, patient populations and testing methods used to diagnose *C. trachomatis* highlighting a need for standardization and guidelines for the identification and diagnosis of *C. trachomatis* in India. The prevalence of the disease across states and patient groups has been shown to vary quite significantly, suggesting the influential role of a wide range of factors including diagnostic tools and perhaps the genetic makeup of the different populations on the Indian subcontinent. More research is needed to develop novel diagnosis tests which are cost-effective and which can be implemented in resource-limited settings such as India. This paper highlights in-house PCR as a technique yielding high levels of sensitivity and specificity for pathogen identification. Tools and techniques however ought to be streamlined. More research is also necessary to ascertain the role of *C. trachomatis* in primary and secondary infertility in India as well as in adverse pregnancy outcomes. Further research will also need to be conducted in order to identify population subgroups that are at a high risk for sexually transmitted diseases and, particularly, for *C. trachomatis*. Policies and guidelines defining the identification and management of *C. trachomatis* in India also ought to be updated to maximize patient outcomes.

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