86 Chlamydial infection

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Chlamydia trachomatis is an obligate intracellular pathogen, which is now recognized as the most prevalent sexually transmitted bacteria in the world (1). In 1999, the World Health Organization (WHO) estimated that of the 340 million cases of sexually transmitted infections, 92 million were due to *C. trachomatis* infection. This high prevalence rate makes it an important public health concern. In women, *C. trachomatis* infection has detrimental effects on reproductive health and carries considerable health-care costs. Genital infection in women is primarily localized to the endocervix, causing a clinical syndrome of mucopurulent cervicitis. The majority (70–90%) of women infected with *C. trachomatis* are asymptomatic (2). In untreated women, this infection can ascend into the endometrium and fallopian tubes to establish a chronic infection that can result in pelvic inflammatory disease (PID) and salpingitis. Of women with a single episode of PID, 9% develop ectopic pregnancy and 11% develop tubal factor infertility (3). In pregnant women, *C. trachomatis* infection is associated with premature rupture of membranes and an increased risk of preterm delivery (4). This infection can also be vertically transmitted, causing pneumonia and conjunctivitis in newborns.

In addition to its role in genital diseases, *C. trachomatis* infection of the eye (trachoma) is one of the world's leading causes of preventable blindness. In 2002, the WHO reported that this infection accounts for about 3.6% (1.3 million) of total blindness worldwide. This disease is endemic in 56 countries including many in Africa, the Middle East and some parts of Asia, Latin America, and the Western Pacific. Trachoma is transmitted through contact with eye droplets from contaminated hands, clothing, and eye-seeking flies. The most affected populations are those living in poverty where resources are limited, hygiene is poor, and conditions permit facile transmission of the infection (5).

C. trachomatis can also cause a systemic sexually transmitted disease, Lymphogranuloma venereum (LGV), which is characterized by genital ulceration and inguinal lymphadenopathy (buboes). This disease can progress and result in hemorrhagic proctocolitis, rectogenital scarring, and lymphedema. LGV is endemic in some areas of Africa, Asia, South America, and the Caribbean but is uncommon in industrialized countries (6).

TAXONOMY

C. trachomatis is a member of the family chlamydiaceae. Three species under this family are commonly associated with human diseases: *C. trachomatis*, *C. pneumoniae*, and *C. psittaci*. Under a classification revised in 1999, chlamydiaceae is divided into two genera, chlamydia and Chlamydophila. *C. trachomatis* is under the genus chlamydia, while *C. pneumoniae* and *C. psittaci* are classified under the genus Chlamydophila (7). Some of the

properties that differentiate the three chlamydiaceae species that represent important human pathogens are summarized in Table 1.

The two *C. trachomatis* biovars (trachoma and LGV) are further differentiated into 19 serovars based on antigenic differences in the major outer membrane proteins (MOMPs). The trachoma biovar includes serovars A, B, Ba, and C, which primarily infect the conjunctiva, and serovars D, Da, E, F, G, Ga, H, I, Ia, J, and K, which primarily infect the urogenital tract. The LGV biovar consists of serovars L1, L2a, L2b, and L3 and primarily infects the inguinal lymph nodes.

In addition to these serovars, several other variants have been previously identified (8,9). One new variant of *C. trachomatis* (nvCT) was discovered in Sweden in 2006 (10). It carries a deletion in the target sequences used for standard nucleic acid amplification-based diagnostic methods and escaped detection until recently. In 2007, nvCT spread throughout Sweden, accounting for 20% to 65% of chlamydia cases in that country (11). The majority of those cases that were detected in other countries had epidemiologic links to Swedish contacts (12).

MICROBIOLOGY AND PATHOGENESIS

The major disease symptoms associated with *C. trachomatis* infection are caused not by direct pathogen activity, but rather by the host immune response to infection. The conventional view holds that manifestations of chlamydial diseases result from classical delayed-type hypersensitivity (DTH) reactions. Clinical and animal studies suggest that DTH is stimulated by chlamydial heat shock protein 60 (13–16). However, other studies also suggest that the inflammatory response that is elicited by the infected host cells themselves is sufficient to cause chronic inflammation, tissue remodeling, and subsequent scarring. Following in vitro infection with *C. trachomatis*, genital epithelial cells produce and secrete large quantities of pro-inflammatory chemokines, including IL-8, GRO α , granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1 α , IL-11, and IL-6 (17,18). IL-1 has been shown to initiate human fallopian tubal damage during *C. trachomatis* infection in an ex vivo model (19). Overall, the increase in epithelial secretion of pro-inflammatory chemokines results in the recruitment of immune cells to the site of infection. Persistence of the inflammatory response within the genital tract results in tissue scarring (20).

C. trachomatis infection can also directly destroy infected genital epithelial cells during replication. *C. trachomatis* is an obligate intracellular bacterium that has a unique developmental life cycle. It enters target host cells as a small (300–400nm) elementary body (EB), metabolically inactive but infectious. Chlamydial organisms never contact the host cell cytoplasm. Rather, the entry of chlamydial EB induces the formation of a

Table 1 Human Pathogenic Chlamydiaceae

Property	<i>Chlamydia trachomatis</i>	<i>Chlamydia pneumoniae</i>	<i>Chlamydia psittaci</i>
Host	Primarily human	Primarily human	Primarily birds and lower mammals; occasionally humans
Biovars	Trachoma and LGV	TWAR	Many
Diseases	Ocular trachoma, oculogenital diseases, infant pneumonia and lymphogranuloma venereum	Upper and lower respiratory tract infection	Psittacosis
Inclusions stain with iodine	Yes	No	No
Susceptibility to sulfonamides	Yes	No	No

Abbreviations: LGV, lymphogranuloma venereum; TWAR, Taiwan acute respiratory agent.

new intracellular compartment partially derived from the host cell's Golgi apparatus. This compartment, called an inclusion body, inhibits phagolysosomal fusion. chlamydial EBs within the inclusion body utilize host adenosine triphosphate for energy. Within 6 to 8 hours after entry, chlamydial EBs undergo primary differentiation and transform into larger (800–1000nm) actively dividing reticulate bodies (RBs). RBs are metabolically active and noninfectious. RBs continuously divide over the next 18 hours by binary fission to produce 300 to 500 progeny. At 48 to 72 hours after infection, *C. trachomatis* RBs transform back into EBs through secondary differentiation. Upon completion of their developmental cycle within the host cell, EBs exit the epithelial cell to infect neighboring uninfected cells.

An alternate growth mode of chlamydia, commonly referred to as persistent growth, has also been observed in vitro. Persistent growth is induced by factors such as amino acid deprivation (21), penicillin treatment (22), and exposure to suboptimal levels of interferon gamma (23). This growth mode is characterized by enlarged and pleomorphic RBs that do not undergo binary fission and differentiation into EB. During persistent growth in the host cell, chlamydial organisms are viable but not cultivable. This condition, however, can be reversed upon removal of the persistence-inducing factor (24). Although direct evidence of persistent growth in vivo has not been clearly established, this phenomenon may explain chronic infection resulting from reactivation of these alternate persistent forms. Cycles of dormancy and reactivation may contribute to the pathologic changes observed in chronic disease (Fig. 1).

EPIDEMIOLOGY

In the United States, 1,108,374 chlamydial infections were reported to the Centers for Disease Control and Prevention (CDC) in 2007, corresponding to a rate of 370.2 cases per 100,000 people. This rate translates to a more than fourfold increase from 1988 when 87.1 cases were reported per 100,000 people (25). Independent studies in different cities in the United States and in different countries around the globe, however, have demonstrated variation in prevalence rates depending on several factors, including high-risk sexual behavior and age (26,27). A comparative study conducted in the Philippines between high-risk female sex workers attending a social hygiene clinic and low-risk women attending an

obstetrics and gynecology clinic showed that high-risk women have a fivefold higher chlamydial prevalence rate compared with low-risk women (28). In addition, multiple studies have shown that young age is the factor most closely associated with *C. trachomatis* infection (29,30). Infection rates in sexually active adolescents are consistently at least two- to threefold higher than that in adult women (31).

In the same 2007 CDC report, the overall rate of chlamydial infection in women in the United States was 543.6 cases per 100,000. The rate of chlamydial infection in men is lower than that in women with 190.0 reported cases per 100,000 people. This lower rate in men may reflect the higher number of women screened for *C. trachomatis* and may also indicate that sex partners of infected women may not be diagnosed or reported. This is a major deterrent to efforts at controlling the rate of infection in women because men provide a continuous, untreated reservoir for transmission of new and recurrent infections.

Approximately 5% to 15% of pregnant women in the United States are infected with *C. trachomatis* (32). Independent studies in other countries show that prevalence rates in pregnant women are widely divergent (Table 2). Assessment of *C. trachomatis* infection prevalence rates, however, is complicated by the use of several different diagnostic methods among different studies. These methods may vary markedly in their sensitivities (see section on Diagnosis and Screening) and specificities. Comparison among studies is also compounded by biases in the selection process and heterogeneity in inclusion criteria among investigations. An evaluation limited to those persons seeking medical care in clinics may not be representative of the entire population. Thus, there exist significant limitations in generalizations made based on these studies.

Risk Factors of chlamydia Infection in Pregnancy

The risk factors for chlamydial infection in pregnant women were summarized in a review article by Tiller et al. and include young age (<20 years of age), coinfection with other sexually transmitted diseases, abacteriuric pyuria, unprotected sex within the past 3 months, a partner with nongonococcal urethritis, and more than one sexual partner in the last 3 months (41). Like studies in nonpregnant women, young age is consistently associated with chlamydial infection among pregnant women.

Figure 1 Chlamydial growth cycle. Chlamydia travels between susceptible cells as small, metabolically inert forms, called the elementary bodies (EBs). EBs attach to cells and enter into a phagosomal compartment partially derived from the host cell Golgi apparatus. Within the phagosomal compartment, now called an inclusion, EBs transform into larger, metabolically active forms called reticulate bodies (RBs). RBs reproduce by binary fission, filling the enlarging inclusion. Late in the chlamydial life cycle (36–48 hours), RBs reorganize into EBs and EBs are released into the extracellular space to infect surrounding susceptible cells. Under certain in vitro conditions, chlamydial RBs undergo differentiation into large atypical forms that may persist within the infected cell for prolonged periods of time. Clinical and epidemiologic characteristics of chlamydial infections also support persistence. [Reproduced with permission from The Curators of the University of Missouri (Copyright 2011, The Curators of the University of Missouri)].

Table 2 Prevalence of <i>Chlamydia trachomatis</i> Infection in Pregnant Women		
Reference	Site	Prevalence rate (%)
Chen et al. 2009 (33)	Melbourne, Australia	3.2
Lujan et al. 2008 (34)	Tete, Mozambique	4.1
Lubbad et al. 2007 (35)	Gaza, Palestine	8.3
Chen et al. 2006 (36)	Fuzhou, China	10.1
Kadzhaia et al. 2005 (37)	Tbilisi, Georgia	33.0
Apea-Kubi et al. 2004 (38)	Accra, Ghana	3.0
Shimano et al. 2004 (39)	Hokkaido, Japan	4.2
Chotnopparatpattara et al. 2003 (40)	Bangkok, Thailand	10

C. trachomatis infection in pregnant women increases the risk of pre-term delivery, premature rupture of membranes, and perinatal mortality. Thus, prenatal screening of high-risk pregnant women and treatment of newly diagnosed infections can help to prevent adverse pregnancy outcomes and transmission to neonates. The CDC recommends screening at the onset of prenatal care, then again in the third trimester.

Perinatal Risks of chlamydial Infection

Based on a recent study of vertical transmission of *C. trachomatis* in China, the transmission rate of *C. trachomatis* from infected mother to infants born through vaginal passage is approximately 66.7%. In comparison, the risk to those born by cesarean section is 8.3% (42)

Transmission of *C. trachomatis* infection from mother to infant may also occur via exposure to intrauterine infection during late pregnancy (43). Intrauterine infection may be responsible for chorioamnionitis, a frequent cause for prematurity and respiratory insufficiency at risk neonates (44). chlamydial infection may also result in neonatal conjunctivitis and in infant pneumonia that may progress to obstructive lung disease.

Early spontaneous pregnancy loss has been attributed to *C. trachomatis* infection-based seroepidemiologic evidence and on the detection of chlamydial antigens, in endometrial curettage samples (45,46). This correlation, however, is not without controversy (47). An association between *C. trachomatis* infection and premature rupture of the membranes has also been suggested and intrauterine lung infection by

C. trachomatis has been demonstrated has been demonstrated in premature infants (48).

DIAGNOSIS AND SCREENING

Traditional diagnostic testing for chlamydial infection involves cell culture techniques using urethral-, cervical-, or conjunctival-derived patient specimens. Specimens are inoculated on cycloheximide-treated McCoy cells, a susceptible tissue culture cell line, and examined after 24 and 72 hours of incubation. Chlamydia inclusions are then stained using immunofluorescent chlamydia-specific antibodies and examination of chlamydial growth is performed using standard microscopy. In competent hands, this method can have a specificity as high as 100%; therefore, culture-based detection of chlamydial infections has long been used as a reference standard for assessing the validity of new diagnostic tests and is the method of choice for medicolegal purposes. The cell culture method, however, is technically difficult, time consuming, and expensive. Furthermore, its sensitivity has been estimated to be only 60% to 85% (49,50). The performance of these techniques can vary between handlers and even within a laboratory. Thus, although some laboratories have maintained culture techniques for their high specificity, most others now use nonculture-based alternatives. These simpler techniques are more practical for routine work and offer higher sensitivity. They include detection of chlamydial antigens using directly labeled fluorescent antibodies (DFAs) and enzyme immunoassays (EIAs) and detection of chlamydial genetic material (RNA/DNA) using nucleic acid amplification tests (NAATs).

DFA involves the use of fluorescein-conjugated antibodies to directly detect chlamydial EBs within patient specimens. This test is rapid and its sensitivities and specificities range from 70% to 100% and 87% to 99%, respectively, for men and 68% to 100% and 82% to 100%, respectively, for women (51,52). The use of this technique is much faster than cell culture techniques but also requires highly skilled personnel. The EIA assay, on the other hand, does not require highly skilled personnel and has sensitivities and specificities that range from 62% to 97% and 92% to 100% for men and 64% to 100% and 89% to 100% for women (53). The sensitivity of the EIA method, however, has recently been questioned (54,55).

Because chlamydial genetic material can be shed into the urine, NAAT can be performed on specimens collected noninvasively. The major target sequence used in nucleic acid amplification techniques for the diagnosis of chlamydial infection is the *omp1* gene, which codes for the MOMP present in all three human pathogenic chlamydial species (56). Because this method includes an amplification step that improves sensitivity, NAAT has a better sensitivity than EIA for detecting chlamydia in urine specimens. NAAT is now also considered an effective method for detecting asymptomatic chlamydial infections. Currently, there are several NAAT platforms available for chlamydial diagnostic and screening purposes. A recent evaluation using first void urine specimens and three automated NAAT systems [ProbeTec ET reagents on Viper platform (BD Diagnostics), Aptima Combo 2 reagents on Tigris platform (Gen-Probe) and Abbott RealTime CT/NG reagents on an m200 platform (Abbott Molecular Diagnostics)] revealed that these approaches are all suitable for

C. trachomatis detection and each offers high sensitivity and specificity (57).

The use of urine specimens for NAAT testing is also the method of choice for screening men for chlamydial infections. It is noninvasive and differences in sensitivity and specificity between urethral swabs and urine samples are not significant. Screening targeted to men is beneficial from a public health perspective, as men provide a reservoir for continuous transmission to women. The rates of *C. trachomatis* infection in men vary from 4% to 6% of which approximately 90% are asymptomatic (58–60). The availability of an effective noninvasive method for screening men may provide more widespread detection of asymptomatic *C. trachomatis*-infected individuals in the community. Furthermore, this methodology can be readily used in school-based health centers in high-prevalence communities. Such noninvasive diagnostics could greatly increase the identification of infected individuals for treatment and help to prevent the spread of infection.

The use of vaginal swabs for NAAT testing offers an effective alternative for screening women for chlamydial infection. An evaluation of the use of vaginal swab specimens in the Aptima NAAT system revealed that NAAT provides excellent sensitivity and specificity. Using NAAT for detecting chlamydia on patient-collected vaginal swabs gives a sensitivity and specificity of 98.3% and 96.5% respectively, and for clinician-collected swabs, 97.2% and 95.2% respectively (61). Thus, the use of vaginal swab specimens for NAAT provides an excellent alternative to pelvic examination and endocervical specimen collection. To prevent the adverse effects of chlamydial infection on pregnancy outcomes, the screening of all pregnant women is recommended by the CDC. For these purposes, the noninvasive NAAT is the method of choice. In short, widespread, noninvasive screening for chlamydial infection in men and women should lead to early detection and treatment, thereby reducing reproductive tract morbidities and preventing vertical and horizontal transmission.

TREATMENT AND MANAGEMENT

Treatment of *C. trachomatis*-infected patients is essential not only for clearing infection and eliminating symptoms, but also for the prevention of transmission to sex partners. In pregnant women, treatment is also given to prevent vertical transmission to infants. Based on the latest CDC guidelines (62), the recommended first-line treatment for *C. trachomatis* is either a single 1-g oral dose of azithromycin or a twice daily, 100-mg oral dose of doxycycline for 7 total days. In populations in which adherence to the 7-day doxycycline regimen is typical, azithromycin and doxycycline are equally efficacious, with microbial cure rates of 97% and 98% respectively. However, in populations with poor compliance or unpredictable follow-up, cure rates for the azithromycin regimen exceeded those using doxycycline. Since the majority of gonococci in the United States are susceptible to azithromycin and doxycycline, either of these treatment regimens may also provide coverage for chlamydia patients coinfecting with *Neisseria gonorrhoeae*. Recommended alternative regimens for *C. trachomatis* treatment include erythromycin, ofloxacin, and levofloxacin. Ofloxacin and levofloxacin, however, offer no dosage advantages and are more expensive than erythromycin-based treatments.

For pregnant women, doxycycline, ofloxacin, and levofloxacin are contraindicated. Thus, the recommended first-line treatment for a chlamydia-infected pregnant woman is the single 1-g oral dose of azithromycin or a 500-mg oral dose of amoxicillin, three times a day for 7 days. Alternative regimens include erythromycin base or erythromycin ethylsuccinate. Frequent occurrence of gastrointestinal side effects with use of erythromycins discourages compliance and consequently reduces treatment efficacy. Lower erythromycin doses for longer duration are alternative regimens that may improve gastrointestinal tolerance. Notably, erythromycin estolate is contraindicated during pregnancy because of drug-related hepatotoxicity. To ensure therapeutic cure, testing for chlamydial infection must be repeated 3 weeks after the completion of therapy in pregnant subjects. This reduces the risk of maternal and neonatal sequelae with persistent disease. It is also recommended that sexual partners of pregnant women be tested and treated if they have sexual contact within 60 days after treatment. If either partner is positive for chlamydial infection, he/she should be instructed to avoid sexual contact until both have been cured and are without symptoms.

For infants with ophthalmia neonatorum or pneumonia due to chlamydial infection, the recommended regimen is erythromycin base or erythromycin ethylsuccinate. Efficacy using

erythromycin treatment is only 80%, so follow-up testing on infants is recommended. If infection was not cleared, a second course of similar therapy is recommended (Table 3).

PREVENTION AND FUTURE RESEARCH

Implementation of a comprehensive *C. trachomatis* screening program can lead to a 60% reduction in the incidence of PID (63). With the availability of noninvasive, highly specific, and sensitive methods for the detection of *C. trachomatis* infection, opportunistic chlamydia screening has great potential for controlling the spread of infection through early detection and early treatment. Importantly, this includes the detection and treatment of subjects with asymptomatic infection. Combined, these screening efforts should help to prevent the development of adverse sequelae and further transmission of the infection.

Despite the efficacy of antibiotics in treating *C. trachomatis* infection, treatment is primarily given to those with symptomatic infections. Continued transmission by untreated individuals with asymptomatic infection hampers control efforts difficult. To achieve more effective control measures, the development of a safe and efficacious human vaccine is necessary, but has eluded researchers to date. Ongoing animal studies, however, do have promise. Novel antigens that induce protective immunity in *C. trachomatis* mouse models have been identified (64,65) and provide vaccine candidates that may be suitable for human study. Also in murine models, the intranasal administration of a secreted chlamydial protein was found to enhance resolution of infection and provide protection against oviduct pathology (66). These advances in chlamydia vaccine development should ultimately lead to better chlamydial control measures and a reduction in the global prevalence rate of *C. trachomatis* infection.

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Table 3 Treatment Recommendations for Chlamydial Infections

Recommended regimens
Azithromycin 1g orally, single dose, or
Doxycycline 100mg orally twice a day for 7 days
Alternative regimens
Erythromycin base 500mg orally four times a day for 7 days, or
Erythromycin ethylsuccinate 800mg orally four times a day for 7 days, or
Ofloxacin 300mg orally twice a day for 7 days, or
Levofloxacin 500mg orally once daily for 7 days
For pregnant women
Recommended regimens
Azithromycin 1g orally, single dose, or
Amoxicillin 500mg orally three times a day for 7 days
Alternative regimens
Erythromycin base 500mg orally four times a day for 7 days, or
Erythromycin base 250mg orally four times a day for 14 days, or
Erythromycin ethylsuccinate 800mg orally four times a day for 7 days, or
Erythromycin ethylsuccinate 400mg orally four times a day for 14 days
For newborns
Erythromycin base or ethylsuccinate 50mg/kg/day orally divided into four doses daily for 14 days
For children who weigh <45kg
Erythromycin base or ethylsuccinate 50mg/kg/day orally divided into four doses daily for 14 days
For children who weigh >45kg but who are aged <8 years
Azithromycin 1g orally, single dose
For children aged >8 years
Azithromycin 1g orally, single dose, or
Doxycycline 100mg orally twice a day for 7 days

Source: From Centers for Disease Control and Prevention 2010 treatment guidelines (62).

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