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1

Molecular Epidemiology of *Chlamydia trachomatis* Urogenital Infection

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1. Introduction

Each year an estimated 340 million new cases of curable sexually transmitted infections occur worldwide, with the largest proportion in the region of South and South East Asia, followed by subSaharan Africa and Latin America and the Caribbean (WHO, 2006). *Chlamydia trachomatis* infections are the most prevalent sexually transmitted bacteria infections recognized throughout the world. World Health Organization (WHO, 2001) estimated that there were 92 million new cases worldwide in 1999 and the incidence of infection has continued to increase each year in both industrialized and developing countries. *C. trachomatis* is now recognized as one the most common sexually transmissible bacterial infections among persons under than 25 years of age living in industrialized nations such as the United States, where the rate of prevalence runs at 4.2% (Miller et al., 2004).

The vast majority of published clearly, show that E, D, F and G, genotypes are isolated from urogenital tract infections with most frequency, however genotypes have yet to be consistently associated with disease severity or even disease phenotype and there is little knowledge of possible *Chlamydia* virulence factors, their expression and how they affect disease severity.

2. Characteristics of bacteria cell

According to the reclassification of the order *Chlamydiales* in 1999, the family *Chlamydiaceae* is now divided in two genera, *Chlamydia* and *Chlamydophila* (Everett., et al 1999).The genus *Chlamydia* comprises the species *C. trachomatis*, *C. suis* and *C. muridarum*.

C. trachomatis are obligate intracellular parasites, possess an inner and outer membrane similar to gram-negative bacteria and a lipopolysaccharide (LPS) but do not have a peptidoglycan layer. Have many characteristics of free-living bacteria, and their metabolism follows the same general pattern; the main difference is their little capacity for generating energy. It has been shown that *Chlamydiaceae* are auxotrophic for ATP, GTP and UTP but not for CTP. (Tipples & McClarty, 1993).

C. tracomatis, is an exclusively human pathogen, with a tropism conjuntival and urogenital, was originally identified by their accumulation of glycogen in inclusions and their sensitivity to sulfadiazine. Based on the type of disease produced, *C. trachomatis* has been divided into biovars, including the lymphogranuloma venereum (LGV) biovar and the trachoma biovar, associated with human conjunctival or urogenital columnar epithelium infections. The original Wang and Grayston classification (Wang & Grayston, 1970) defined 15 *C. trachomatis* serovars, based on antigenic differences, designated A-K and L1-L3, which differ by the antigenicity of their major outer membrane protein (MOMP), codified by gene *omp1*. In addition to these serovars, numerous variants have been characterized. Serovars A, B, Ba and C, infect mainly the conjunctiva and are associated with endemic trachoma; serovars D, Da, E, F, G, Ga, H, I, J and K are predominantly isolated from the urogenital tract and are associated with sexually transmitted diseases (STD), inclusion conjunctivitis or neonatal pneumonitis in infants born to infected mothers. Serovars L1, L2, L2a and L3 can be found in the inguinal lymph nodes and are associated with LGV (Table 1).

Biovar	**Serovar**	**Diseases**
Trachoma	A, B, Ba, C	trachoma
	D, E, F, G, H, I, J, K, Da, Ia	STD, conjuntivitis and pneumonitis
GV	L1-L2, L2a, L3	LGV

Table 1. Biovar and diseased caused by *C. trachomatis*

The genome sequencing projects have shown that *Chlamydia* has a relatively small chromosome at between 1.04 and 1.23 Mbp and contains between 894 and 1130 predicted protein-coding genes. The fully sequence *C. trachomatis* genome consist of a chromosome of approximately 1.0 Mbp plus an extrachromosomal plasmid of approximately 7.5 kbp, with a total of approximately 900 likely protein-coding genes (Read et al., 2000; Carlson et al., 2005) Table 2.

Characteristics	**Dates**
Genome size (pb)	1042519
Genes	894
Plasmid/phage size (bp)	7493
GC%	41.3
No. ORFs	894
No. tRNAs	37
No. rRNA operons	2
% non-coding	9.9

Table 2. Sequences and annotated of *C. trachomatis* D genome

The transcriptional profile of the *C. trachomatis* genome has been analysed by microarrays and RT-PCR (Douglas & Hatch, 2000; Shen et al., 2000). The microarrays and RT-PCR

analysis has showed that 71% or 612 of the 894 genes of *C. trachomatis* continue to be expressed throughout the development cycle, while the others are temporally expressed (Nicholson et al., 2003). Analysis of the profiles of the temporally expressed genes has difficulties in classifying, because of the contrasting results of microarrays analysis on *C. trachomatis* by different groups (Belland et al., 2004; Nicholson et al., 2003).

3. The developmental cycle

C. trachomatis is a small obligate intracellular bacterium, has two developmental stages: -the extracellular elementary body (EB) and -the intracellular reticulate body (RB). EB is the infectious form metabolically inactive (EB), in this stage; the bacteria are in a state similar to that of an endospore, where the outer membrane is resistant to the environment and allows it to exist without a host cell. EB measured from 200 to 400 nm in diameter, is antigenic, non-proliferative, contains few ribosomes, is toxic in cell cultures, and is susceptible to penicillin, resistant to trypsin, osmotic shock and mechanical shock. While RB is intracellular, measured from 500 to 1500 nm in diameter, is not infective or antigenic, is proliferative, contains many ribosomes, is not toxic and is not inhibited by penicillin, is susceptible to trypsin, osmotic shock and mechanical shock.

The eukaryotic cell becomes infected when an EB adheres to the cytoplasmic membrane. The adhesion of EBs to cells is due to multiple weak specific ligand interactions, perhaps involving several molecules. There is evidence that MOMP binds to a heptaran-sulphate receptor on the host cell. The EB penetrates into the cell by endocytosis, remaining within a parasitophorous vacuole also termed inclusion or phagosome. By 2 h after infection within the phagosome EB begin differentiating into RB. Over the next several hours, RB increase in number and in size. RB can be observed dividing by binary fission by 12 h postinfection (hpi). After 18 to 24 h, the numbers of RB are maximized, and increasing numbers of RB begin differentiating back to EB, which accumulate within the lumen of the inclusion as the remainder of the RB continue to multiply. Depending on the species or strain, lysis or release from the infected cell occurs approximately 48 to 72 hpi.

4. The Infection with *C. trachomatis*

The *C. trachomatis* infects columnar epithelial cells of the ocular and urogenital mucosae. These infections have a significant impact on human health worldwide, causing trachoma, the leading cause of preventable blindness, and sexually transmitted diseases (STD) that include pelvic inflammatory disease and tubal factor infertility (Schachter, 1978; Brunham et al., 1988). Chlamydial STDs are also risk factors in cervical squamous cell carcinoma and HIV infection (Chesson & Pinkerton, 2000; Mbizvo et al., 2001).

Trachoma is one of the commonest infectious causes of blindness. The disease starts as an inflammatory infection of the eyelid and evolves to blindness due to corneal opacity. Despite long-standing control efforts, it is estimated that more than 500 million people are at high risk of infection, over 140 million persons are infected and about 6 million are blind in Africa, the Middle East, Central and South East Asia, and countries in Latin America. Trachoma is a communicable disease of families, with repeated reinfection occurring among family members. Transmission is driven by sharing of ocular secretions among young

children in family or community groups, facilitated by the ubiquitous presence of flies. The disease is particularly prevalent and severe in rural populations living in poor and arid areas of the world where people have limited access to water and facial hygiene is poor. Visual loss from trachoma is 2-3-times more common in women than men and is a major cause of disability in affected communities, attacking the economically important middle-aged female population. Global elimination of trachoma as a disease of public health importance has been targeted by WHO for 2020.

The most common site of *C. trachomatis* infection is the urogenital tract. In men, it is the commonest cause of non-gonococcal urethritis and epididymitis however are asymptomatic in approximately 50% of men (Karam et al., 1986; Zimmerman et al., 1990). Urethritis is secondary to *C. trachomatis* infection in approximately 15 to 55 percent of men. Symptoms, if present, include a mild to moderate, clear to white urethral discharge. This is best observed in the morning, before the patient voids. Untreated chlamydial infection can spread to the epididymis. Patients usually have unilateral testicular pain with scrotal erythema, tenderness, or swelling over the epididymis. Men with asymptomatic infection serve as carriers of the disease, spreading the infection while only rarely suffering long-term health problems.

In women, chlamydial infection can lead to a serious reproductive morbidity. Infection of the lower genital tract occurs in the endocervix. It can cause an odorless, mucoid vaginal discharge, typically with no external pruritus**.** Some women develop urethritis; symptoms may consist of dysuria without frequency or urgency. Ascending infection that causes acute salpingitis with or without endometritis, also known as pelvic inflammatory disease (PID), whose long-term consequences are chronic pain, ectopic pregnancy and tubal factor infertility (Stamm, 1999). The 80% of the genital infections are asymptomatic and without clinical evidence of complications and appear to spontaneously resolve, although there only is limited knowledge about the clinical factors that influence the duration of untreated, uncomplicated genital infections (Zimmerman et al., 1990). These infections tend to be chronic and recurring and associated with scarring complications possibly related to hypersensitivity mechanisms.

A *C. tracomatis* infection can infect different mucosal linings, with the majority of cases in the urogenital tract but also the rectum, oropharynx and conjunctiva. Rectal chlamydial infection is often observed in men who have sex with men (Kent et al., 2005; Annan et al., 2009). Contamination of the hands with genital discharge may also lead to conjunctival infection following contact with the eyes. Babies born to mothers with infection of their genital tract frequently present with chlamydial eye infection within a week of birth (chlamydial "*ophthalmia neonatorum*"), and may subsequently develop pneumonia. Furthermore, an existing chlamydial infection increases the risk of contracting HIV (Joyee et al., 2005) and/or Herpes simplex infections (Freeman et al., 2006). This is especially true with the *Lymphogranuloma venereum* (LGV) disease, an invasive and frequently ulcerative chlamydial infection involving lymphatic tissue. LGV occurs only sporadically in North America, but it is endemic in many parts of the developing countries and represent a major risk factor for HIV acquisition (Blank et al., 2005; Schachter & Moncada, 2005; Cai et al., 2010). In addition, it was found that Chlamydial infection can be associated with human

papillomavirus (Oh et al., 2009) and gonorrhea in a 20% of men and 42 % of women (Lyss et al., 2003; Srifeungfung et al., 2009).

5. Detection methods for *C. trachomatis*

Diagnosis of chlamydial infection is even more difficult in asymptomatic and in chronic or persistent infections where the pathogen load would be low. The large pools of asymptomatic infected people are not only at the risk of developing serious long-term sequelae but would also transmit the infection. The development of methods of detection in the laboratory highly sensitive and specific of nucleic acid amplification tests (NAATs) has been an important advance in the ability to conduct population-base screening programmes to prevent complications.

The assays that are used for diagnosis of *C. trachomatis* include conventional diagnostic methods and NAATs. Conventional diagnostic methods involve the isolation by cell culture and application of biochemical and immunological tests to identify. The cell culture is time consuming and laborious, and it has been in many laboratories replaced by antigen detection methods such as enzyme immunoassays (EIA), direct immunofluorescence assays (DFA) and DNA/RNA detection. EIA tests detect chlamydial LPS with a monoclonal or polyclonal antibody while DFA depending on the commercial product used detected LPS or MOMP component. DFA with a *C. trachomatis*-specific anti-MOMP monoclonal antibody is considered highly specific (Cles et al., 1988). DNA/RNA detection is based on the hybridization and its use is suitable for simple and fast diagnosis.

The NAATs includes polymerase chain reaction (PCR), ligase chain reaction (LCR), retrotranscription-PCR (RT-PCR) and real time-PCR. In these probes different DNA or RNA regions are used as target sequences for amplification. The major target sequences are located in cryptic plasmid, *omp1* gene and rRNAs. The cryptic plasmid is present in approximately 10 copies in each *C. trachomatis* organism (Hatt et al., 1988), reason for which some authors suggested that amplification of *C. trachomatis* plasmid DNA is more sensitive (Mahony et al., 1992). However, some studies suggest that plasmid-free variants of *C. trachomatis* may on rare occasions be present in clinical samples (An et al., 1992). Comparative studies of the NAATs suggest that the sensitivity and specificity are quite similar, but of screening tests for *C. trachomatis* NAATs are more sensitive than non-NAATs (Poulakkainen et al., 1998; Ostergaard, 1999; Van Dyck et al., 2001, Black, 1997).

6. Prevalence

The prevalence of urogenital *C. trachomatis* determinate with NAATs from different parts of the world published in the present year and the 2010 is summarized in the table 3. These reports show that the prevalence is high and independent of the country, urban or rural ubication.

Studies amongst clinically healthy population have shown a prevalence rate equal or major to 4%. Two reports show lower prevalence rate of 0.9 % in United States of America (Jordan et al., 2011) and Germany (Desai et al., 2011) for population of military and adolescent students respectively, and the higher prevalence rates are for students in China with 8.8% (Hsieh et al., 2010) and young people in England with 8.3% (Skidmore et al., 2011).

Country	Population studied	% Prevalence	Reference
	Clinically healthy population		
Switzerland	Young male offenders	2%	Haller et al., 2011
England	Young people	8.3%	Skidmore et al., 2011
Croatia	Young adults	6.3%	Božičević et al., 2011
Australia	Young international backpackers	3.5%	Davies et al., 2011
United States of America	Military	0.9 %	Jordan et al., 2011
Germany	Adolescents	0.9%	Desai et al., 2011
United States of America	Adults	5.8%	Jenkins et al., 2011
England	Students	3.41%	Aldeen et al., 2010
Spain	Adolescents and young adult women	4%	Corbeto et al., 2010
Perú	Adults	4.95%	Canchihuaman et al., 2010
United States of America	Athletes	2.7%	Hennrikus et al., 2010
France	General population	2.2%	Goulet et al., 2010
United States of America	General population	1.0%	Chai et al., 2010
Japan.	Students	8.1%	Imai et al., 2010
Switzerland	Undocumented immigrants	5.8%	Jackson et al., 2010
China	Students	8.8%	Hsieh et al., 2010
	Population visiting health services		
United States of America	Pregnant women	4.3%	Roberts et al., 2011
United States of America	Symptomatic adolescent women	19.7%	Goyal et al., 2011
Holland	Pregnant women	3.9%	Rours et al., 2011
Brazil	Women	4.0%	Rodrigues et al., 2011
Brazil	Pregnant women	25.7%	Ramos et al., 2011
Guinea	Women	12.6%	Månsson et al., 2010
United States of America	Women in family planning clinics	10.3%	Gaydos et al., 2011
India	Women	23.0%	Patel et al., 2010
Brazil	Men	13.1%	Barbosa et al., 2010
United States of America	Women with rectal infections	17.5 %	Hunte et al., 2010
Turkey	Pregnant women	7.3%	Aydin et al., 2010
Uganda	Women	7.8%	Darj et al., 2010
Korea	Women with overactive bladder symptoms	7.1%	Lee et al., 2010
Italy	Infertile couples	8.2%	Salmeri et al., 2010
South Africa	Men with urethritis	12.3%	Le Roux et al., 2010

Country	Population studied	% Prevalence	Reference
	high-risk population		
England	Female sex workers	6.8%	Platt et al., 2011
Pakistan	Female sex workers	7.7 %	Khan et al., 2011
China	Men who have sex with men	24%	Li et al., 2011
China	Female sex workers	17.4%	Jin et al., 2011
Indonesia	Female sex workers	37%	Silitonga et al., 2011
Indonesia	Female sex workers	27%	Mawu et al., 2011
Korea	Female rape victims.	28.85%	Jo et al., 2011
Spain	Injecting Drug Users	2.3%	Folch et al., 2011
Switzerland	Adults in a prison	8.3%	Steiner et al., 2010
United States of America	HIV patients	23.93%	Chkhartishvili et al., 2010
France	High-risk population	28%	Fresse et al., 2010
Korea	Female sex workers	12.8%	Lee et al., 2010
Kenia	Fishermen with STI	3.2%	Kwena et al., 2010
Tunisia	Female sex workers	72.9%	Znazen et al., 2010
Indonesia	Female sex workers	43.5%	Tanudyaya et al., 2010
Bangladesh	Female sex workers	2.5%	Huq et al., 2010

Table 3. Prevalence of *C. trachomatis* from different parts of the world published 2010–2011

The reports for the population that visiting health services shown average prevalence rate 11.7%, that ranges from of 4% to 25.7% in Brazil (Rodrigues et al., 2011; Ramos et al., 2011).

The higher prevalence rate reported are for the high-risk population with average of 21.6%; that ranges from of 2.5% in Bangladesh (Huq et al., 2010) up to 72.9% in Tunesia (Znazen et al., 2010) amongst female sex workers.

7. *C. trachomatis* genotypes

C. trachomatis comprises distinct serogroups and serovars. Different genotyping methods are used for determination of circulating *C. trachomatis* serovars within a population can provide information on the epidemiology and pathogenesis of infection, including mapping sexual networks, can allow for monitoring treatment success, and may play a role in developing strategies for improved disease control, such as vaccine design.

Different genotyping methods are available to differentiate between the serovars, and are mainly based on the diversity of the *omp1* gene, which encodes for the MOMP, an antigenically complex that displays serovar, serogroup, and species specificities (Baehr et al., 1988; Stephens et al., 1982). The MOMP is present in all human pathogenic *Chlamydia* species, contains four variable domains designated VS1, VS2, VS3, and VS4 that vary considerably between the species (Stephens et al., 1987; Yuan et al., 1989).

The genotyping methods are basically of two types: Immunological and molecular methods.

The Immunological methods are based in the use of polyvalent and specific monoclonal antibodies that recognized epitopes located on the MOMP of *C. trachomatis*. These methods have been replaced by molecular methods, which are better in specificity and sensitivity.

The molecular methods are based in nucleic acid amplification techniques and are of two types, i) methods that analyzed the *omp1* gene and ii) methods that analyzed several genes.

In methods that analyzed *omp1* gene the amplication products of the *omp1*-PCR are analyzed by restriction fragment length polymorphism (RFLP), nucleotide sequencing, array assay and Real-Time PCR.

In RFLP technical the amplication products of the *omp1*-PCR are cleaved with restriction endonuclease, this test is simple, rapid and its results show a high level of agreement with the results serotyping (Morré et al., 1998)

In array assay the amplication products of the *omp1*-PCR are analyzed by Southern blot hybridization using different DNA probes. These tests are rapid and accurately and also discriminate among multiple genotypes in one clinical specimen (Ruettger et al., 2011; Huang et al., 2008).

The nucleotide sequences of *omp1* show clearly mutations, variants of *omp1* and therefore providing evidence for existence of numerous subspecies. This method has a higher resolution than serotyping and RFLP (Morre et al., 1998), and has been considerate as gold standard for *C. trachomatis* genotyping (Sturm-Ramirez et al., 2000; Watson et al., 2002). However is still very laborious and not suitable for typing the isolates from a large number of clinical samples. A drawback is the difficulties in resolving mixed infections because peaks from different PCR products will be superposed in the chromatograms from sequencing reactions (Pedersen et al., 2009).

In genotyping by real time is evaluated with Taq Man probes in multiplex the *omp-1* gene, the test is specific and convenient for the rapid routine-diagnostic with capacity to detect mixed infections.

The methods that analyzed several genes are system based on hypervariable regions identified as housekeeping genes and polymorphic membrane protein genes. These methods have showed that are capable of identifying high intraserotype variation and greater genetic diversity in comparison to use *omp1* alone. Two types of methods have been described multilocus sequence typing (MLST), which analyzed candidate target regions by PCR and Sequencing (Klint et al., 2007) and the multi-locus variable number tandem repeat (VNTR) analysis and *omp1* or ''MLVA-*omp1*''analized VNTR and *omp1* sequencing together (Pedersen 2008).

8. Genotyping for *C. trachomatis*

The vast majority of published data analyzed mainly with DNA sequencing of *omp1* clearly, show that E, D, F and G, genotypes are isolated from urogenital tract infections with most frequency, but prevalence of individual genotypes has been reported to differ by age, sex, geographic region and racial groups as is summary in the table 4, as studies in China, Holland and Australia from men who have sex with men, which G genotype was more frequent (Li et al., 2011; Quint et al., 2011; Twin et al., 2010). Studies also have shown that nearly of 60% of all typing of clinical isolates in different parts of the world report almost five different genotypes.

Country	Population studied	Genotype found, in descending order of prevalence	Reference
Greece	Men with urethritis	F, E, D, G, B, K, H	Psarrakos et al., 2011
China	Men who have sex with men	G, D, J	Li et al., 2011
Holland	Men who have sex with men	G/Ga, D/Da, J, LGV, L2	Quint et al., 2011
Mexico	Infertile women	F , E, G, K, D, H, LGV L2	De Haro-Cruz et al., 2011
Brasil	Youths and adults	E, F, D, I, J, G, K, H, B	Machado et al., 2011
England	Adults women	D, E, F	Wang et al., 2011
China	Adults	D, F, G, H, J, K	Tang et al., 2011
Australia	Men who have sex with men	D, G, J	Twin et al., 2010
Iran	Women symptomatic	E, F, D/Da, K, I, G, H, J	Taheri et al., 2010
China	Patients attending the STD clinic	E, F, G, D	Yang et al., 2010
Greece	Men with urethritis	E, G, F, Ja, D	Papadogeorgakis et al., 2010
Hungary	Female sex workers	D, E, F, G, H, I	Petrovay et al., 2009
Spain	Adults infected	E, D, G, F, B, H, I, J, K, LGV L2	Piñeiro et al., 2009
Costa Rica	Young women	E, F, D/Da, I/Ia	Porras et al., 2008
Australia	Heterosexual communities	E, F, J/Ja, D/Da, G, K	Bandea et al., 2008
England	Patients attending a genitourinary medicine clinic	E, F, D	Jalal et al., 2007
Brazil	Women attending the STD clinic	D, E, F, K	Lima et al., 2007
China	Women attending the STD clinic and female sex workers	E, F, G, D	Gao et al., 2007
China	Male attending the STD clinic	D, Da, F, K, J, G, H	Yu et al., 2007
Korea	Female sex workers	E, F, G, D, H, J	Lee et al., 2006
China	Clinical specimens	E, D, Da, F, J, K, G, H, Ba	Hsu et al., 2006
Africa	Volunteer students	E, F	Ngandjio et al., 2003
Iceland	Population attending the STD clinic	E, D, J, F, K, G, H, I	Jónsdóttir et al., 2003
India	females with urogenital infections	D, E, F	Singh et al., 2003
Stockholm	Youth health center	E, F, K, D	Sylvan et al., 2002

Country	Population studied	Genotype found, in descending order of prevalence	Reference
Sweden	patients attending the STD clinic	E, F, G, H	Jurstran et al., 2001
Thailand	Pregnant women	F, D, H, K, E, Ia, B, Ja, G	Bandea et al., 2001
Senegal	Female sex workers	E, D/Da, G, F, Ia, K	Sturm-Ramirez et al., 2000
Holland	Adults symptomatic or asyntomatic	D, E, F, Ga, K	Morré et al., 2000

Table 4. Distribution of *C. trachomatis* genotypes from different parts of the world

However MOMP differences and genotypes have yet to be consistently associated with disease severity or even disease phenotype and there is little knowledge of possible *Chlamydia* virulence factors, their expression and how they affect disease severity (Byrne, 2010).

9. Conclusion

Sexually transmitted infections (STI) are responsible for human suffering and carry significant economic costs. Many STI are entirely attributable to unsafe sex. Disease burden linked to unsafe sex amounted in 2004 to 70 millions disability-adjusted life years (DALYs) worldwide, of which 52 million were accounted for by developing countries. Unsafe sex ranked second among the 10 leading risk factor causes of DALYs worldwide, and third among the leading causes of DALYs in developing countries.

Lack of education and communication are contributing factors for the increase in new cases of *Chlamydia*. Also, the stigma surrounding sexually transmitted disease has hindered us in limiting the spread of this disease. Since *Chlamydia* is such a widespread disease, more government funded educational resources should be available to assist individuals in getting information and proper medical attention. Parents also need to be responsible for communicating with their children before a problem exists. If people are properly educated, the spread of *Chlamydia* should decline.

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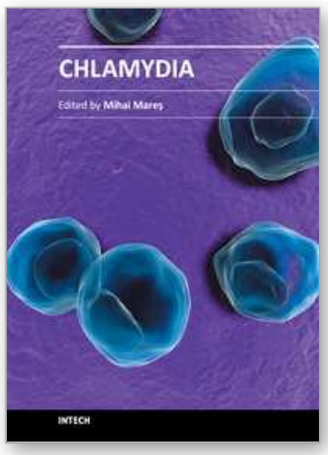

Chlamydia

Edited by Prof. Mihai Mares

ISBN 978-953-51-0470-4
Hard cover, 358 pages
Publisher Intech
Published online 30, March, 2012
Published in print edition March, 2012

Nowadays, Chlamydia still represents a redoubtable pathogen. Among its consequences, the blindness in children and severe impairment of reproductive health in adults are the most mutilating. Worldwide, it is estimated that six million of people suffer from post-trachoma blindness and almost 90 million become sexually infected each year. Due to its silent evolution and sexually transmission, the chlamydial infection can occur in anyone. The book “Chlamydia - A Multifaceted Pathogen” contains an updated review of all-important issues concerning the chlamydial infection. It comprises 18 chapters grouped in four major parts dealing with etiology and pathogenicity, clinical aspects, diagnosis and prevention. The new molecular data about the pathogenicity and the exhaustive presentation of clinical findings bring novelty to the book and improve our knowledge about Chlamydia induced diseases.

How to reference

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Virginia Sanchez Monroy and Jose D'Artagnan Villalba-Magdaleno (2012). Molecular Epidemiology of Chlamydia trachomatis Urogenital Infection, Chlamydia, Prof. Mihai Mares (Ed.), ISBN: 978-953-51-0470-4, InTech, Available from: http://www.intechopen.com/books/chlamydia/molecular-epidemiology-of-chlamydia-trachomatis

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