2

Review of Literature
2.1 Historical review of Chlamydia

In 1964, Schechter et al., revealed the truth about Chlamydia when he found the presence of RNA, DNA as well as cell wall structures demonstrated by electron microscopy. Earlier, these bacteria were believed to be viruses, due to their obligate intracellular mechanism. However, Chlamydia was grouped under Rickettsia until the genus Chlamydia was established and two different strains, Chlamydia trachomatis and Chlamydia psittaci were isolated. Later on, several new different Chlamydial strains have been isolated. Chlamydophila pneumoniae-like bacterium was first reported and described in 1965 (Kuo et al., 1995) was believed to be an atypical strain of C.psittaci.

The bacterium was first isolated from the eye of a child during a trachoma vaccine trial in Taiwan, and thus given the name TW-183. The first report of Chlamydophila pneumoniae causing clinical manifestations came in 1983, when the bacterium was isolated in the United States from a throat swab of a university student suffering from pharyngitis. This isolate was termed AR39 due to its isolation as an acute respiratory pathogen. This group of organisms was eventually called TWAR (Grayston et al., 1990), which was an acronym for the first two isolates, i.e. TW183 and AR39. During that time, TWAR was considered a human C. psittaci strain spreading among humans without an avian or mammalian host. Interestingly, TWAR was found to be a serologically unique group among the known C.psittaci isolates. These displayed differential and milder pathogenic properties when grown in cell culture or inoculated into mice or chicken embryos. In the year 1989, Chlamydia pneumoniae strain was first reported (Grayston, 2000).
2.2 Chlamydia Terminology and Classification

The genus Chlamydia is associated with trivial names such as noun "Chlamydia" (singular) "chlamydiae" (plural) and with the adjective "Chlamydial". The Chlamydiaceae, which currently has only the genus Chlamydia, is divided into two genera Chlamydia and Chlamydophila gen. nov. Two new species, Chlamydia muridarum sp. and Chlamydia suis sp. join Chlamydia trachomatis in the emended genus Chlamydia, Chlamydophila gen. assimilates the current species, Chlamydia pecorum, Chlamydia pneumoniae and Chlamydia psittaci, to form Chlamydophila pecorum comb., Chlamydophila pneumoniae comb and Chlamydophila psittaci comb. Three novel Chlamydophila species are derived from Chlamydia psittaci: Chlamydophila abortus gen. nov., sp. Chlamydophila caviae gen. nov., sp. and Chlamydophila felis gen. nov., sp. Emended descriptions for the order Chlamydiales and for the family Chlamydiaceae are provided (Everett, 1999). Chlamydiae are disseminated by aerosol or by contact, requiring no alternate vector. Of the fifteen or more major groupings in the domain Bacteria (Stackebrandt, 1997; Van de et al., 1994), Chlamydiales is the only lineage whose known members are exclusively intracellular parasites of members of the domain Eucarya.

The members of the order Chlamydiales are obligate intracellular gram negative bacteria. They demonstrate a two-stage major developmental form of replication: the infectious bacterial form is endocytosed by eukaryotic cells and resides within a cytoplasmic inclusion known as Elementary Body (EB), where it transforms later into a vegetative form and replicates by binary fission, which is known as Reticulate Body (RB) (Moulder, 1991). The taxonomical classification of the Chlamydiales is a debatable issue. There are two recognized types of classification in Chlamydial species i.e. 'Old classification' and 'New...
Chapter 2

Classification based on early physiological taxonomy. The recent phylogenetic results show four related groups within the genus Chlamydia, as discussed earlier though Chlamydiaceae exhibits similar developmental forms, they differ morphologically and in some biochemical traits this has an impact on biochemical characteristics which correlate with different phylogenetic traits of the species. This data contributed to the creation of two additional species, *Chlamydophila pneumoniae* (Grayston et al., 1989) and *Chlamydophila pecorum* (Fukushi and Hirai, 1992). In acceptance with the arguments against the genus division, the old classification has been considered in the present work.

**Classification**

![Chlamydial classification based on Old and New classification](http://www.idexx.com/)

2.2.1 *Chlamydophila psittaci*

*Chlamydophila psittaci* primarily infects birds. The former 'mammalian' *Chlamydia psittaci* abortion, feline and guinea-pig strains have been moved to three new species. *C. psittaci* in birds is often
systemic and infections can be in apparent, severe, acute or chronic with intermittent shedding. Most organs become infected, as well as the conjunctiva, respiratory system and gastrointestinal tract. Stress will commonly trigger onset of severe symptoms, resulting in rapid deterioration and death. *C. psittaci* serovar A is endemic among psittacine birds and has caused sporadic zoonotic disease in humans, other mammals and tortoises (Seth-Smith *et al.*, 2011).

**2.2.2 Chlamydophila pecorum**

*Chlamydophila pecorum* strains are generally non-invasive in a mouse model of virulence, and are serologically and pathogenically diverse, having been isolated only from mammals: cattle, sheep, goats (ruminants), koalas (marsupials), and swine. *C. pecorum* causes reproductive disease, infertility and urinary tract disease and has associated with abortion, conjunctivitis, encephalomyelitis, enteritis, pneumonia and polyarthritis (Garner *et al.*, 2004).

**2.2.3 Chlamydophila felis**

*Chlamydophila felis* is endemic among house-cats worldwide, primarily causing conjunctivitis, rhinitis and respiratory problems. It can be recovered from the stomach and reproductive tract. Zoonotic infection of humans with *C. felis* has been reported. FP Cello strain (feline pneumonitis strain) has an extrachromosomal plasmid and produces lethal disease in mice, whereas FP Baker strain does not. An attenuated FP Baker strain is used as a live vaccine for cats (Azuma *et al.*, 2006).
Figure 2.1: Pictures depicting Chlamydial members (1) *Chlamydophila pneumoniae* (2) *Chlamydophila psittaci* (3) *Chlamydophila pecorum* (4) *Chlamydophila felis* (5) *Chlamydophila caviae* (6) *Chlamydophila abortus* (7) *Chlamydophila trachomatis*
2.2.4 *Chlamydophila caviae*

*C. caviae* can be recovered from the conjunctiva of guinea-pigs suffering from ocular inflammation and eye discharge. There are five known *C. caviae* isolates, and the *ompA* (omp1) sequences of these isolates are virtually identical. The natural site of *C. caviae* infection is the conjunctiva, but are capable to infect the genital tract of guinea-pigs with *C. caviae* and elicit a disease that is very similar to human *C. trachomatis* infection. *C. caviae* is remarkably specific for guinea-pig and attempts to infect mice, hamsters, rabbits and gerbils have been generally unsuccessful, except for one gerbil (Gordon *et al.*, 1966).

2.2.5 *Chlamydophila abortus*

*C. abortus* strains are endemic among ruminants and efficiently colonize the placenta. They have a distinctive serotype and nearly 100% conservation of ribosomal and *ompA* sequences. *C. abortus* is the reference strain for determining whether a new strain belongs to the *Chlamydiaceae* (16S- or 23S-rRNA should be > 90% identical to the *C. abortus* genes). An extrachromosomal plasmid has not been identified in any strain of *C. abortus*. It is primarily associated with cases of abortion and weak neonates. Sporadic zoonotic abortion due to *C. abortus* has been confirmed by genetic analysis of isolates from women who work with sheep. Typical isolates have been obtained from cases of abortion in sheep, cattle and goats worldwide (e.g. strains B577, EBA, OSP, S26/3 and A22) and its genome has been sequenced and published (Entrican *et al.*, 2001).
2.3 *Chlamydia pneumoniae* and its pathogenicity

Chlamydia and Chlamyphila species cause serious health problems in both humans and mammals where *C.pneumonia* (Cpn) is a tiny obligate intracellular gram negative bacterium which is most often illustrious for causing a type of pneumonia. Until 1970's, the bacterium was not even isolated and was mistaken for a virus. In the year 1989 J.Thomas Grayston and his associates classified and named it as a separate species of the Chlamydiae (*Chlamydia*). The organism was originally called as TWAR strain from the names of the two original isolates -Taiwan (TW-183) and an acute respiratory isolate designated AR39. *C.pneumonia* is a human respiratory pathogen with a unique biphasic life cycle characterized by an obligate intracellular (replicative) and an extracellular (infectious) form of the organism which is difficult to culture in invitro condition. It can survive only by growing in the invivo condition (Leinonen, 1993).

*C.pneumonia* is widely distributed via the respiratory route and infects the majority of the world's population. The majority (70%) of acute human *C.pneumonia* respiratory tract infections are asymptomatic or only mildly symptomatic. But a minority (30%) of them causes more severe respiratory illnesses, including community-acquired pneumonia, bronchitis, sinusitis, emphysema and a variety of upper respiratory tract infections.

Recent studies suggest that it is also involved in chronic diseases like atherosclerosis (Sriram et al., 2005), multisclerosis, Alzheimer's disease (Balin et al., 2008) and lung cancer (Littman, 2005). After acute infection the *C.pneumonia* intracellular life cycle is characterized by the development of metabolically inert (and thus antibiotic resistant) atypical
"persistent" inclusions. It changes its form from a vegetative reticulate body into an infectious elementary body during the late stage of its infection cycle.

This biologic behavior correlates with a clinical course following an acute symptomatic illness that is characterized by persistence of symptoms and the comprehension of the molecular events in the morphological change is important to understand the switching mechanism between acute and chronic infection. It is deemed to relate to the pathogenesis of COPD. An emerging body of evidence, links C.pneumonia infection with a spectrum of chronic inflammatory lung diseases of currently unknown etiology namely asthma, chronic bronchitis and chronic obstructive pulmonary disease (COPD).

2.4 Chronic Obstructive Pulmonary Disease (COPD)

Chronic obstructive pulmonary disease (COPD) is an umbrella term used to describe progressive chronic lung diseases that cause obstruction in lung airflow. It is also known by various other names, such as chronic obstructive lung disease (COLD), chronic obstructive airway disease (COAD), chronic airflow obstruction (CAO), chronic airway (or airflow) limitation (CAL), or simply as chronic bronchitis and emphysema. Chronic obstructive pulmonary disease, which includes chronic bronchitis and emphysema, is a progressive disease characterized by airflow limitation/obstruction, which is either not reversible at all or only partially reversible.

It is generally difficult to separate out the two conditions; hence these are grouped together as COPD. The airflow obstruction in COPD is associated with an abnormal inflammatory response of the lungs to chronic inhalation exposure from smokes, dusts and other air pollutants.
COPD can cause coughing that produces large amounts of mucus (a slimy substance), wheezing, shortness of breath, chest tightness, and other symptoms (Beaty et al., 1991).

2.4.1 Main risk factors for COPD

COPD is preventable. The primary cause of COPD is tobacco smoke (including secondary or passive exposure). Other risk factors include:

- Indoor air pollution (such as solid fuel used for cooking and heating);
- Outdoor air pollution;
- Occupational dusts and chemicals (vapors, irritants, and fumes);
- Frequent lower respiratory infections during childhood.

Total deaths from COPD are projected to increase by more than 30% in the next 10 years unless urgent action is taken to reduce the underlying risk factors, especially tobacco use.
Figure 2.3: Picture depicting the condition of patient suffering from COPD, the inner image shows the alveoli sacs filled with fluid

Courtesy: http://iahealth.net/

2.4.2 Symptoms

Diagnosis of COPD should be considered in any patient who has symptoms of a chronic cough, sputum production, dyspnea (difficult or labored breathing) and a history of exposure to risk factors for the disease. Wherever spirometry is unavailable, clinical symptoms and signs, such as abnormal shortness of breath and increased forced expiratory time, can be used to help with the diagnosis. A low peak flow is consistent with COPD, but may not be specific to COPD because it can be caused by other lung diseases and by poor performance during testing (Seemungal et al., 2001). Chronic cough and sputum production often precede the development of airflow limitation by many years; although not all
individuals with cough and sputum production go on to develop COPD. Because COPD develops slowly, it is most frequently diagnosed in people aged 40 years or over.

2.4.3 Diagnosis and treatment

A chronic obstructive pulmonary disease (COPD) diagnosis is confirmed by a simple test called “spirometry”. It measures how deeply a person can inhale and exhale and how fast air can move inside and outside of the lungs. As COPD develops slowly, it is frequently observed in people aged 40 or older. Presently serological testing is also the most common method used for the diagnosis of *C. pneumonia* infection. Available assays include microimmunofluorescence (MIF) tests, enzymed immunoassays (EIAs) and enzymed-linked immunosorbent assays (ELISAs), performing a variety of commercial and in-house versions. Grayston *et al.*, have proposed a set of criteria for the definition of both an acute and past infection. An acute infection was defined as single IgM≥1:16, a single IgG titer of ≥1:512, or a fourfold rise in the IgG titer. Past or preexisting infection was defined as an IgG titer of ≥1:16 and b1:512. With the recent advent of molecular technology, PCR is unquestionably a useful diagnostic tool, which has the ability to amplify rapidly small amounts of specific DNA or RNA. Unlike culture, PCR can detect organisms that are non-cultivable in persistent infection and are rendered non-viable during transport, although the latter limits its clinical usefulness since it cannot distinguish between viable and nonviable organisms after antibacterial therapy (Pauwels *et al.*, 2012).

The uses of genus or species specific antibodies with a peroxidase for antigen detection (immunohistochemistry and immunocytochemistry), or a fluorescent (immunofluorescence) have been used to detect
C. pneumonia in vascular tissue. However, most of these methods experience a subjective reading. Immunofluorescence technique also has the difficulty of a non-specific staining combined with the morphological heterogeneity of C. pneumonia elementary bodies (Verkooyen et al., 1997). Various forms of treatment can help control its symptoms and increase quality of life for people with the illness. For example, medicines that help dilate major air passages of the lungs can improve shortness of breath. The availability of treatment options for COPD differs across varying resource settings.

2.4.4 Who is at risk?

Earlier, COPD was more common in men, but because of increased tobacco use among women in high-income countries, and the higher risk of exposure to indoor air pollution (such as solid fuel used for cooking and heating) in low-income countries, the disease now affects men and women almost equally. Almost 90% of COPD deaths occur in low- and middle-income countries, where effective strategies for prevention and control are not always implemented or accessible (Mannino et al., 2007).

2.5 Chlamyphila pneumoniae infection and COPD exacerbation

C. pneumoniae infection is associated with higher rates of exacerbation and airway microbial colonization in patients with COPD. It has been recognized as a common cause of respiratory tract infections affecting all age groups. The organism has been implicated as an infectious trigger for acute exacerbations of COPD. Moreover, the intracellular existence of this pathogen and the ability to cause chronic respiratory infections has led to a number of studies that investigated its possible association with disease development.
Chapter 2

Review of Literature

COPD is unquestionably one of the major leading causes of death and disability worldwide; its prevalence is expected to increase over the next 20 years. It is well documented that as COPD progresses, the inflammatory process increases, resulting in parenchymal destruction and fibrosis of the small airways. Since only a small proportion of smokers develop chronic bronchitis, and even a smaller proportion of them finally suffer from COPD. The possible role of recurrent or persistent infections in the development of this disease gains a growing interest (Papaetis et al., 2009). The possible correlation of acute *C.pneumonia* infection with episodes of acute exacerbation of COPD (AECOPD) has been examined in a variety of studies with conflicting results reported. Moreover, the intracellular existence nature of this pathogen and its ability to cause chronic respiratory infections, have made it difficult to eradicate completely. This has created a diagnostic challenge to examine whether it has a role both in the pathogenesis and progression of COPD (White et al., 2003).

After its entrance into the host cell, the EB differentiates into the larger, metabolically active RB, which forms inclusion bodies and multiplies in the cytoplasm with binary fission from 8 to 12 rounds. RBs are later reorganized asynchronously to EBs, which are released from the cell to begin another infection cycle as a result of exocytosis or host cell lysis; two to three days post infection (Fig. 2.4). Under suboptimal growth conditions and in certain cell types, such as monocytes, biphasic developmental cycle completion is prevented or at least slowed below the detection limit of EBs. In this persistent form, aberrant non-infectious but viable forms of this pathogen are exhibited, showing a reduced metabolic activity and resistance to antibiotic therapy. Persistence represents a state of infection in which the microbe cannot be inflicted by the host and
results in continuing damage during the time (Fig. 2.4) (Kalman et al., 1999).

As it is generally the case with intracellular bacteria, T-helper-1 (Th1) type response plays a pivotal role in the resolution of C. pneumonia infection. Cytokines such as gamma interferon (IFN-\(\gamma\)), tumor necrosis factor alpha (TNF-\(\alpha\)), lymphotoxin and interleukin 1 (IL-1) have been shown to inhibit Chlamydial infection (Papaetis et al., 2009). The results of most in vitro studies have shown that IFN-\(\gamma\) treatment, tryptophan deficiency and antibiotics such as penicillin and ampicillin are factors that lead to persistence (Hogan et al., 2004). Persistent C. pneumoniae infection was correlated with continued expression of genes related to DNA replication, but not with those associated with bacterial cell division. Chronic infection may also modulate cellular apoptosis so that the organism survives and multiplies and is, thus, protected from fragmentation (Hammerschlag, 2002).

Additionally, many factors which promote persistence are associated with the host include older age, smoking, male gender, glucocorticoid use and concomitant diseases (Hertzen, 1998). Glucocorticoids strongly downregulate many aspects of cell-mediated immunity and enhance the shift from Th1 to Th2 response. Thus, severely deteriorating the host's ability to eradicate the C. pneumoniae infection and trigger the re-activation of persistent Chlamydia to actively growing forms. Many earlier studies have shown that children who were on steroid therapy were infected with remarkably high titers of C. pneumoniae (Papaetis et al., 2009). In vitro studies also suggest that the addition of hydrocortisone in HEp-2 infected cells with C. pneumoniae significantly
increased the number of inclusions observed. Cortisol has a stabilizing effect on autophagosomes and suppresses the premature release of infectious particles, modifying host susceptibility to this pathogen (Tsumura et al., 1996). Smoking, the principal risk factor for the development of COPD, may enhance deeper invasion of \textit{C.pneumoniae} and increase cortisol levels in the circulation, making its eradication difficult. The role of humoral response to an established \textit{C.pneumoniae} infection seems to be rather ineffective. It probably has a contributory role in reinfection, although antibodies have an important diagnostic role (Waites et al., 2008).
2.6 Prevalance of COPD

As per WHO released GOLD update report the existing COPD prevalence data show remarkable variation due to differences in survey methods, diagnostic criteria, and analytical approaches. Despite the complexities, some conclusions are drawn based on the emerging data regarding COPD prevalence is not least due to increased data quality control. A systemic review and meta-analysis suggests that the prevalence of COPD is appreciably higher in smokers and ex-smokers than in non-smokers, in those over 40 years of age than those under 40 years and in men than in women. According to WHO estimates, 65 million people have moderate to severe chronic obstructive pulmonary disease (COPD). More than 3 million people died of COPD in 2005, which corresponds to 5% of all deaths globally.

Most of the information available on COPD prevalence, morbidity and mortality come from high-income countries. Even in those countries, accurate epidemiologic data on COPD are difficult and expensive to collect. It is known that almost 90% of COPD deaths occur in low-income and middle-income countries. At one time, COPD were more common in men, but because of increased tobacco use among women in high-income countries and the higher risk of exposure to indoor air pollution (such as biomass fuel used for cooking and heating) in low-income countries, the disease now affects men and women almost equally.

In 2002 COPD was the fifth leading cause of death, total deaths from COPD were projected to increase by more than 30% in the next 10 years unless urgent action is taken to reduce the underlying risk factors, especially tobacco use. The newer projected estimate shows that COPD will be the third leading cause of death worldwide in 2030. This increased
mortality is mainly driven by the expansion of epidemic of smoking. The earlier studies reveals the primary risk factors like smoking, occupational exposure to environmental pollutants (i.e., tobacco smoke, occupational dust and fumes, respiratory infection, outdoor air pollution, and indoor air pollution caused by biomass or traditional fuels and coal) and socioeconomic status can cause COPD.

It is likely that both genetic and acquired host factors as well as environmental exposures, either separately or in combination may contribute to the development of the disease. Many studies indicate association of C.pneumoniae infection and COPD which is more noxious and increases the mortality rate of the patients due to interaction between host factors (i.e., genes, airway hyper responsiveness, and lung growth). COPD is associated with the significant economic burden. In the European Union, the total direct costs of respiratory disease are estimated to be about 6% of the total health care budget, with COPD accounting for 56% of the direct costs of COPD. Its exacerbations account for the greatest proportion of the total COPD burden on the health care system. In developing countries, direct medical costs may be less important than the impact of COPD on workplace and home productivity.
Figure 2.5: Map showing the prevalence of the infected COPD population worldwide; the intensity of the color indicates the severity of COPD infected patients.

Courtesy: http://www.who.int/en

COPD may force two individuals to leave the workplace—the affected individual and a family member who is meant to care for the disabled one. It may cause a serious damage on the economy of the developing countries since human capital is the most important national asset. In 1990, COPD was the twelfth leading cause of DALY’s (Disability adjusted life years) lost in the world, but by 2030 COPD will be the seventh leading cause of DALYs lost worldwide (Mannino et al., 2007) (GOLD report, 2013).
2.7 Human respiratory diseases caused by

*Chlamydia pneumoniae*

2.7.1 Community acquired pneumonia and respiratory infections

*C. pneumonia* is a cause of community acquired pneumonia throughout the world; it has been confirmed by many serological studies. In many instances, the diagnosis has been based on a significant increase in specific IgG titers. It is unlikely that all seroconversion episodes were associated with community acquired pneumonia. It was never the most common aetiological agent and co-infection with a second organism was often present (Fang et al., 1990; Kauppinen et al., 1995). If it is assumed in adults, that the annual incidence of community acquired pneumonia is 0.1%, that *C. pneumoniae* is responsible for 10% of these and that the annual seroconversion rate is 1.5%, then only 1 in 150 *C. pneumoniae* infections will result in pneumonia. Apart from pneumonia, there is limited evidence that *C. pneumoniae* can also cause upper respiratory tract infections (Hammerschlag, 2000). But it has been suggested that 90% or more of *C. pneumoniae* infections are asymptomatic (Kleemola et al., 1988). In one study, only 3 of 8 culture positive patients had serologic evidence of acute infection (Chirgwin et al., 1991).

Apart from acute respiratory infections, *C. pneumoniae* has also been implicated in chronic respiratory carriage and culture studies have shown that asymptomatic infection may persist for at least a year. The seroprevalence of *C. pneumoniae* IgG antibodies in adults is 50% or more (Wong et al., 1999) but as there is no evidence to suggest that any particular IgG titer can distinguish between current infection and past exposure. It cannot be assumed that half the adult population is chronically infected. Studies have shown that *C. pneumoniae* can be
isolated from the nasopharynx of up to 4.7% of subjectively healthy subjects (Gnarpe et al., 1991; Hyman et al., 1995; Miyashita et al., 2001). Hypothetically, if chronic C. pneumoniae infection were highly endemic, then large epidemics would not be expected because of herd immunity. Reports of generalized outbreaks of C. pneumoniae infection have been retrospective, based on serology. Nevertheless, a population based seroprevalence study has implied that C. pneumoniae epidemics are frequent and affect significant numbers of the population (Karvonen et al., 1993). It was found that periods of low and high prevalence (IgG titre ≥ 16), ranging from 44 to 67%, alternated in an epidemic cycle of approximately 10 years. It is difficult to reconcile these figures, which suggest that 20% of the population are involved, with the much smaller figures found in the other studies. As this study did not attempt to measure seroconversion, it does not provide definitive evidence that large scale epidemics occur.

2.7.2 Asthma

Asthma is a chronic inflammatory condition characterized by reversible narrowing of the bronchial airways. It is diagnosed by the response to bronchodilator drugs, the measurement of the forced expiratory volume in one second and the peak expiratory flow rate, both of which show reductions and a marked diurnal variation.

The cause is unknown, but asthma attacks can be precipitated by a number of factors including allergens, exertion, excitement, cold air and respiratory infections. Wheezing is a symptom of asthma and as it is also a feature of C. pneumoniae infection, it was wondered whether the two were linked. The first report of an association was published in 1991 (Hahn et al., 1991). Unfortunately, it cannot be certain that some of the
studies conducted were accurate because pulmonary function tests were not obtained. Moreover, rather than asthma, it is likely that some of the patients had post infective bronchial hyper-reactivity, a well known clinical syndrome which usually improves by 6 months (Cook et al., 1998; Hahn, 2005).

2.8 Chronic diseases & Chlamydophila pneumoniae

2.8.1 Coronary artery disease

Coronary heart disease, carotid artery stenosis, aortic aneurysm, claudication (occlusion of the arteries of the lower extremities), are among the commonest causes of death worldwide. Seroepidemiologic studies have associated C.pneumoniae antibody with coronary artery disease, myocardial infarction, carotid artery disease, and cerebrovascular disease. Considerable knowledge of the epidemiology of C.pneumoniae infection has been derived from serologic studies using the C.pneumoniae specific microimmunofluorescence test. Virtually everyone is infected at some point in life, and reinfection occurs commonly (Kuo et al., 1995).

C.pneumoniae infection has been suspected as a probable cause because of raised total leucocyte count (TLC), C-reactive protein (CRP), ESR and fibrinogen levels, in the acute coronary syndrome (Gupta et al., 2012). There have been several studies in industrialized countries linking infection with coronary artery disease (CAD). In one of the study, it shows the results for and against the assumption of infection (leven and Hoymans, 2005). Worldwide, according to the 2005 WHO estimate, out of 17 million deaths, 2/3 occurs in low income and medium income countries including India, deaths in India due to heart disease is 29 percent where there has been a 7-fold increase in 4 decades (1960-2000) of the prevalence of IHD in epidemiological studies (WHO, 2005; Yusuf et al.,
2004; Bahl et al., 2001). Meanwhile, a key question is whether *C. pneumoniae* plays a causal role in these conditions, or is simply associated with them because it has a predilection for damaged tissue (the hitch-hiker hypothesis).

### 2.8.2 Atherosclerosis

Atherosclerosis is an important component of cardiovascular disease. Cardiovascular diseases are the leading cause of death worldwide (30.4%) (WHO, 2011), atherosclerosis is associated with the formation of atherosclerotic lesions which are asymmetric focal thickenings of the innermost layer of the artery, the intima. They consist of cells, connective-tissue elements, lipids, and debris. (Stary, 1995) Blood-borne inflammatory and immune cells constitute an important part of an atheroma, the remainder being vascular endothelial and smooth-muscle cells. The atheroma is preceded by a fatty streak, an accumulation of lipid-laden cells beneath the endothelium (Stary, 1994). Most of these cells in the fatty streak are macrophages, together with some T cells. Fatty streaks are prevalent in young people, never cause symptoms, and may progress to atheromata or eventually disappear. In the center of an atheroma, foam cells and extracellular lipid droplets form a core region, which is surrounded by a cap of smooth-muscle cells and a collagen-rich matrix. T cells, macrophages, and mast cells infiltrate the lesion and are particularly abundant in the shoulder region where the atheroma grows (Jonasson et al., 1986).

Many of the immune cells exhibit signs of activation and produce inflammatory cytokines. Although there are well-defined risk factors for atherosclerosis, these factors do not account for all incidences of the disease. Because atherosclerotic processes are typified by chronic
inflammatory responses, which are similar to those that are elicited by chronic infection, the role of *C. pneumoniae* infection in promoting or accelerating atherosclerosis has received renewed attention with recent studies evidence that chronic infection with *C. pneumoniae*, might contribute to atherosclerotic lesion progression (Campbell, 2004).

### 2.8.3 Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory condition. It is often a debilitating disease in which human body's immune system eats away at the protective sheath (myelin). It covers human body nerves which facilitate to attack the central nervous system (CNS) which is made up of the brain, spinal cord, and optic nerves. Damage to the myelin causes interference in the communication between your brain, spinal cord and other areas of your body. This condition may result in deterioration of the nerves themselves, and the process is irreversible. This damage slows down or blocks messages between your brain and your body, leading to the symptoms of MS. They can include Visual disturbances, muscle weakness, trouble with coordination and balance, sensations such as numbness, pricking, or "pins and needles", thinking and memory problems; there is no single diagnostic test for MS. Doctors use a medical history, physical exam, neurological exam, MRI, and other tests to diagnose it.

It may be an autoimmune disease, which happens when your immune system attacks healthy cells in your body by mistake. Multiple sclerosis affects women more than men. It often begins between the ages of 20 and 40. Usually, the disease is mild, but some people lose the ability to write, speak, or walk. Classically, demyelinated plaques are distributed in both time and space. The cause is unknown, but one theory is that
disease is produced by the host immune response to an infectious agent or
to autoantigen Viruses have most often been implicated as potential
agents of MS, but as long ago as 1983 it has been hypothesized
that \textit{Chlamydia} might play a role (Perlmutter and Darvish, 1983).

In \textit{C.pneumoniae} DNA, the corresponding figures were an
astonishing 97\% versus 18\%, respectively (Sriram \textit{et al.}, 1999). The
studies have cautiously concluded that, although the organism could
represent the trigger for MS, it might simply represent secondary infection
of damaged central nervous system tissue (Swanborg \textit{et al.}, 2003). The
studies point out that, there is good evidence for a possible infectious
component in the development of multiple sclerosis and that more than 20
agents have been implicated.

\subsection*{2.8.4 Alzheimer's disease}

Alzheimer's disease (AD) is a progressive brain disease that leads
to dementia in the elderly population. It slowly destroys memory and
thinking skills and, eventually evens the ability to carry out the simplest
tasks of daily living and is totally irreversible. It is also customary to
distinguish between early familial FAD and late-onset AD (LOAD). The
development of LOAD, the most prevalent form of AD, is believed to be a
multifactorial process that may also involve infections with bacterial or
viral pathogens. One in five people over the age of 80 are affected by
Alzheimer's disease (Dobson \textit{et al.}, 2003). As long ago as 1987, a possible
association of \textit{Chlamydiae} and other infectious agents with Alzheimer's
disease was investigated using serology (Renvoize and Hambling, 1987),
although no relationship was found.

However, Balin \textit{et al} (1998) reported that they were able to
find \textit{C.pneumoniae} DNA in the brains of 17 out of 19 persons with
sporadic Alzheimer's disease, but in only 1 out of 19 controls. Chlamydial RNA gene transcripts were detected, indicating that the Chlamydia were metabolically active. C.pneumoniae was also isolated by culture from tissue homogenates of the two specimens that afforded enough tissue on which to make the attempt. Immunohistology indicated that the Chlamydia were primarily located in diseased areas of the Alzheimer brain in glial cells, perivascular macrophages and intravascular monocyes. It has been suggested that C.pneumoniae may be neurotropic and involved in a number of neuro-inflammatory conditions including multiple sclerosis, Guillain-Barre syndrome (Haidl et al., 1992; Balin & Hammond, 2012), stroke and meningoencephalomyeltis (Guglielminotti et al., 2000). Since the first report on the presence of C.pneumoniae in brains of patients with AD appeared in 1998, this bacterium has most often been implicated in AD pathogenesis. However, while some studies demonstrate a clear association between Cpn infection and AD, others have failed to confirm these findings.

2.8.5 Lung Cancer

Lung cancer is one of the most common and serious types of cancer. It is the number one cause of cancer deaths in both men and women worldwide. Although smoking is known to cause lung cancer in almost one billion men and 250 million women in the world (Mackey and Eriksen, 2002). Strong evidence of a link between smoking and lung cancer has existed since 1950 (Doll et al., 2004). The lung cancer mortality is now sometimes used as a measure of a population's past exposure to smoking. Cancer that begins in the lungs is called a primary lung cancer, cancer that begins in another part of the body and spreads to the lungs is
known as secondary lung cancer. There are two types of lung cancer, which grow and spread differently.

The small cell lung cancers (SCLC)—a less common type that usually spreads faster than non-small-cell lung cancer and non-small cell lung cancers (NSCLC)—the most common type, accounting for more than 80% of cases; can be either squamous cell carcinoma, adenocarcinoma or large-cell carcinoma. Lung cancer mainly affects older people. It is rare in people younger than 40, but the rates of lung cancer rise sharply with age. Lung cancer is most commonly diagnosed in people aged 70-74 years, although people who have never smoked can develop lung cancer. Tobacco use is the most important risk factor for cancer causing 22% of global cancer deaths and 71% of global lung cancer deaths.

According to WHO (2008) report, deaths due to lung cancer is estimated about 1.37 million deaths worldwide and it is projected to continue rising, with an estimated 13.1 million deaths by 2030. Many serologic studies reported suggest epidemiologic evidence of elevated relative risk on the association between C.pneumoniae infection and risk of lung cancer. In the earlier studies identified, serologic evidence of past infection with C.pneumoniae had higher lung cancer risks than those without such evidence (Littman, 2005).

2.9 Chlamydophila pneumoniae pathogenicity in animals

A recent discovery states that in the last couple of years, humans are not the only natural hosts with which C.pneumonia is the primary cause for the disease. Successively, the C.pneumonia was isolated from horses, koala bears affected by ocular and genital infection, Australian and African frogs, from a Tanzanian chameleon, a green sea turtle living in the Cayman Islands, an iguana, puff adders and a Burmese python. All of the
animals in which the *C. pneumonia* was confirmed, were suffering from some form of illness that is also typical in humans when affected by this Chlamydia species. All strains also showed a high similarity with the human *C. pneumoniae* pathogen (Pospisil, 2004). With this evidence we can strongly conclude that *C. pneumoniae* has extended its pathogenicity in a wide range of hosts.

### 2.10 *Chlamydophila pneumoniae* genome architecture

At present there are 4 fully sequenced *C. pneumoniae* genomes (strains: AR39, CWL029, TW-183 and J138) (Kalman *et al.*, 1999; Read *et al.*, 2000; Shirai *et al.*, 2000) the first sequenced and published genome was 1.23 Mb. The information about the gene organization is based on comparative genome analysis, which is predicted to carry 1,122 genes, including 1,052 protein coding genes and 296 SNPs (Kalman *et al.*, 1999). Except AR39 (2 chromosomes) strain all remaining strains enclose single chromosome, sequence revealed that Chlamydiae have a small circular genome of 1,230,230bps.

No extrachromosomosal elements were identified for this species, yet the complete genome had 40. 6% GC content (coding capacity 88%). From the 1074 predicted protein coding genes in *C. pneumonia* AR39, 636 (60%) was assigned function through similarity searches, 251 (23%) were found to be similar to hypothetical genes in other bacteria, and the remaining 186 genes (17%) had no homologues to any other proteins sequenced. *Cpn* CWL029 sp consist 1096 coding genes and 1033 proteins is the smallest genome compared to where *Cpn* J138 consists 1,110 coding genes and 1069 proteins, *Cpn* TW183 consists 1,155 coding genes and 1113 proteins and the zoonotic koala strain *Cpn* LPCoLN sp consists of 1,154 coding genes and 1,105 proteins.
Figure 2.6: Graph depicting *C. pneumonia* genome wide distribution of genes based on functional class (generated by Bluejay).
Figure 2.7: An illustration of *Chlamydia pneumoniae* genome map with features
Ring 1: Genes + strand and Genes - strand    Ring 5: Subcellular Locations + strand
Ring 2: COGs + strand
Ring 3: 80 tRNA
Ring 4: GC%

(*Chlamydia pneumoniae* AR39 Genome map generated using Bluejay)
2.11 Comparative genomics

Comparative genomics is a modern field of biology, the fully sequenced wide range of genomes belonging to species like human, mouse, bacteria and a wide variety of other organisms from virus to whale are compared. By simply comparing the sequences of genomes of different organisms, researchers can derive conclusions, at the molecular level to distinguish different life-forms between each other. Comparative genomics also provides a powerful tool for studying evolutionary transformation among organisms, by helping to identify genes that are conserved or common among species, as well as genes that give each organism its unique characteristics. A simple comparison of the general features of genomes such as genome size, number of genes, and chromosome number presents an entry point into comparative genomic analysis.

The higher resolution comparisons are possible by direct DNA sequence comparisons between species. Comparison of discrete segments of genomes is also possible by aligning homologous DNA from different species. By using computer-aided analysis it is possible to zero down to the genomic features which have been preserved by multiple organisms over millions of years. Researchers are able to locate the signals that represent the location of genes, as well as sequences that may regulate gene expression. Indeed, much of the functional parts of the human genome have been discovered or verified by this type of sequence comparison (Lander et al., 2001). This is now a standard component of the analysis of every bacterial genome sequence, where the information that is gained by genome comparison. It can be used to identify the pathogenicity of various diseases by discovering the specific virulence genes and can be considered as potential drug targets. Later on the
identified drug targets can be successfully included into the drug discovery pipeline (Thomas et al., 2003).

2.12 Synteny in bacterial genomes

Synteny, the conservation of the genes and their relative positions in the genomes of different species, reflects fundamental constraints on natural evolution, as genes are not located randomly along genomes. The position of genes along genomes affects their function and evolution. In bacterial genomes, functional constraints are thus responsible for the concentration of highly expressed genes near the origin of replication, and the clustering of co-functional genes into operons of co-regulated genes. Similarly, evolutionary constraints on gene order are evidenced in bacteria by the highly variable rates of recombination over different chromosomal regions, and by the propensity of co-localized genes to be co-displaced through horizontal transfer (Lawrence et al., 2003). Thus, while genes may be lost, gained, duplicated and rearranged during evolution. The comparison of evolutionary related species shows a remarkable stability of genomic organization. This conservation of genomic organization often referred to as "synteny". The synteny based genome comparison answers the queries like (i) How to infer, from a comparison of multiple genomes, the pairs of genes with significant conservation of proximity? (ii) How to describe, beyond pairwise relationships, the organization of conserved properties of co-localization? (iii) How to explain, from an evolutionary perspective, the origin of this organization?

By performing synteny based genome analysis, Identification of these syntons corresponds to functional features of bacterial genomes encompassing operons. Based on the distribution of their size partition they can be classified into two classes: large syntons, associated with
fundamental functions of bacterial cells, and smaller ones, which in many cases do not correspond to previously defined genomic units. Inferring synteny from multiple genomes presents several difficulties: (a) the classification of genes into orthology classes, which is a remarkably difficult problem; (b) an highly non-uniform sampling of genomes, both because natural genomes are phylogenetically related and because sequencing efforts have not been distributed evenly across strains and species; (c) the definition of a non-ambiguous criterion for assessing significant conservation of proximity between genes.

In the present work the novel approaches to infer pairs of co-localized genes which are crucial from multiple genomes to identify and assign them as potential drug targets have been adopted, which describe the organization of bacterial genomes (chlamydial), it has paved a way to study their evolutionary history. In bacterial genomes, we thus identify synteny units, or "syntons", which are clusters of proximal genes that encompass and extend operons. The size distribution of these syntons divides them into large syntons, which correspond to fundamental macromolecular complexes of bacteria, and smaller ones, which display a remarkable exponential distribution of sizes. This distribution is "universal" in two respects: it holds for vastly different genomes, and for functionally distinct genes.

2.13 Interactomics

Interactomics is a discipline of Bioinformatics which comprises the study of interactions and their consequences between various proteins as well as other cellular components. The network of all such interactions known as the 'interactome', aims to provide a better understanding of genome and proteome functions. Interaction studies of proteins with
various biomolecules help in deciphering and understanding the functions of various proteins in the complex network of cellular pathways. Proteins interact with other biomolecules such as nucleic acids, lipids, hormones, etc. to execute a multitude of functions in living organisms such as signal transduction, growth and regulation and metabolism, to mention a few.

In bacterial pathogens the behavior, morphology and response to stimuli biological systems are dictated by the interactions between their components. These interactions, as observed in this study, are therefore shaped by genetic variations and selective pressure. Similar to what has been achieved by comparing genome structures and protein sequences. It is hopefully possible to obtain valuable information about bacterial systems' evolution by comparing the organization of interaction networks and by analyzing their variation and conservation. Interactomics is an example of "top-down" systems biology, which takes an overhead, as well an overall view of a bio-system or organism (Cesareni et al., 2005). Large sets of genome-wide and proteomic data are collected, and correlated between different molecules are inferred. In order to ensure their function(s) in the cell, proteins are organized in machineries, underlaid by a complex network of interactions.

Identifying protein interactions are thus crucial to our understanding of cell functioning (Rual et al., 2005) of most organisms, except for some model organisms, are largely unknown. Experimental methods, including high-throughput techniques are highly resource intensive. Therefore, computational discovery of protein-protein interactions can accelerate biological discovery by presenting "most-promising" pairs of proteins that are likely to interact. For many bacteria, genome sequence, and thereby genomic context of proteomes, is readily
available; additionally, for some of these proteomes, localization and functional annotations are also available, but interactomes are not available.