General Introduction
To Pneumonia
1.0. General introduction

1.1. Infectious diseases

An infectious disease is a clinically evident illness resulting from the presence of pathogenic microbial agents, including pathogenic viruses, bacteria, fungi, protozoa, multicellular parasites and aberrant proteins known as prions. These infectious pathologies are also called communicable diseases or transmissible diseases due to their potential of transmission from one person or species to another by a replicating agent (as opposed to a toxin) cause major threats to human health. Infections are prevalent in developing countries, where co-infection is common. The adverse impact of infectious diseases is most severe among the poorest people, who have the fewest material, physical and financial resources to draw from and limited or no access to integrated health care, prevention tools and medications. An estimated 13.6 million children and adults died in 2008 from an infectious disease (Mathers et al., 2005; WHO, 2008) (Figure 1.1) and more than half are the results of bacterial infections including tuberculosis, pneumococcal infections, pertussis, bacterial diarrhoea, tetanus, diphtheria, leprosy and meningitis (Moxon et al., 2002). In particular, diseases commonly result from exposure to gram positive bacteria, such as Staphylococcus aureus, Streptococcus pneumoniae and group A Streptococcus, and gram negative bacteria, such as Escherichia coli and Helicobacter pylori.

Figure 1.1. Annual Deaths Due to Selected Infectious Diseases

Overall, the lack of adequate control of bacterial and other microbial infections continues to be a major global public health challenge and priority. Currently,
there are few cellular functions targeted in bacterial pathogens (Moir et al., 1999). To combat the growing problem of antibiotic resistant bacteria, targeting new proteins will provide an important role in drug discovery. In fact, number of crucial pathways remains untargeted today.

The increased resistance to commonly used antibiotics, a growing prevalence of infections and the emergence of new pathogenic bacteria challenge current use of antibiotic therapy. Since last 10 years, the large number of antibiotic resistant strains has been evolved and threatening whole world community. The acquisition of drug resistance property is usually of three different approaches.

1. Inactivation or chemical modification of the antibiotic
2. Modification and replacement of the target site
3. Reduced uptake of antibiotics into the bacterial cell, or active exclusion from the cells.

By increasing morbidity and mortality with emerging these diseases, it is necessary effort to achieve the sixth Millennium Development Goal (MDG), which focuses on stopping and reversing the spread of infectious diseases by 2015 (UNMDG, 2007).

1.2. Pneumonia

Pneumonia is one of the leading causes of morbidity and mortality in children under five years of age, it has been identified as the major "forgotten killer of children" by the United Nations Children's Fund (UNICEF) and WHO (World Health Organization). The incidence in this age group is estimated to be 0.29 episodes per child-year in developing and 0.05 episodes per child-year in developed countries. This translates into about 156 million new episodes each year worldwide, of which 151 million episodes are in the developing world. Most cases occur in India (43 million), China (21 million) and Pakistan (10 million), with additional high numbers in Bangladesh, Indonesia and Nigeria (6 million each) (Rudan et al., 2008). Recent estimates from the WHO suggest that pneumonia
is responsible for 19% of deaths in the above age group, leading to 4 million deaths per year (WHO, 2008) (Figure 1.2).

Of these deaths, two thirds occur during infancy and more than 98% occur in the developing countries (WHO, 2006). In India, recent estimates in under-fives suggest that 13% of deaths and 24% of National Burden of Disease is due to pneumonia. Hospital based studies have reported that 20–30% of admissions in under-fives are due to pneumonia. People with AIDS stand a much greater chance of dying from pneumonia. Substantial evidence revealed that the leading risk factors contributing to pneumonia incidence are lack of exclusive breastfeeding, under nutrition, indoor air pollution, low birth weight, crowding and lack of measles immunization. Based on limited available evidence, recent studies have identified *S. pneumoniae*, *Haemophilus influenza* and respiratory syncytial virus as the main pathogens associated with childhood pneumonia. (Rudan et al., 2008).

Figure 1.2. Major infectious diseases causes of child mortality

Although various pathogens may cause pneumonia, either singly or in combination, the available evidence, including the effectiveness of case management, suggests that two bacteria are the leading causes: *H. influenzae* type b (Hib) and *S. pneumoniae* (pneumococcus) (Sazawal et al., 2003). WHO estimates that in 2000, Hib and pneumococcus together accounted for more than 50% of pneumonia deaths among children aged 1 month to 5 years (CDC-MMWR, 2006). It concludes that for preventing and reducing death from pneu-
Pneumonia requires efficient vaccine and antibiotics. Antibiotics can effectively treat pneumonia at the community level and are very inexpensive, costing less than a dollar per dose. Tragically, only an estimated 1 of every 5 children with signs of pneumonia receives life saving antibiotics. Due to the inevitable evolution of antibiotic resistance in pneumonia causing pathogens, the development of novel antibiotics is essential to overcome widespread and growing antibiotic resistance. Of all pneumonia deaths, 47.7% occur in the least developed countries, (UNICEF/WHO, 2006) most of which are eligible to get support for the purchase of vaccines and development of their immunization programmes through the GAVI Alliance (Black et al., 2003). For more than 30 years, vaccines have played an important part in pneumonia prevention. Vaccines against Hib and S. pneumoniae, efficacious case management, breast feeding promotion and zinc supplementation are cost-effective in reducing pneumonia mortality. Environmental and nutritional interventions reduce pneumonia and provide other benefits. These strategies combined may reduce total child mortality by 17% (Niessen et al., 2009). Urgent action is needed to deliver life saving Hib and pneumococcal vaccines to all children, but childhood vaccination is widely regarded as one of the most cost-effective disease prevention interventions. (Laxminarayan et al., 2006) compare with antibiotics.

1.3. Origin of S. pneumoniae

S. pneumoniae (Figure 1.3) was first identified as a major respiratory pathogen shortly after its isolation by Pasteur in 1881. It was originally named Diplococcus pneumoniae in 1926 but later renamed as S. pneumoniae (in 1974) because of its growth in chains in liquid media. Due to its role as the etiologic agent of pneumonia, it has long been known informally as the pneumococcus. The bacterium is usually seen as pairs (diplococci) but may also occur as short chains or single cells. It is an important bacterial pathogen worldwide, can cause invasive infections such as pneumonia, meningitis, and septicemia, as well as localized infection in children known as Otitis Media (DeVelasco et al., 1995; Jedrzejas et al., 2001; Simell et al., 2001; Michelow et al., 2002).
The pneumococcal outer surface consists of a cell wall covered by a polysaccharide capsule. The encapsulated strains have been shown to be more virulent than those without a capsule. The study of pneumococcal capsule was the object of many investigations that led to important scientific discoveries. In 1928, Griffith showed that when heat-killed encapsulated pneumococci and live strains lacking a capsule were together injected into mice, the none capsulated strain could be converted into encapsulated pneumococci and kills the mice. Years later, the carrier of this genetic information was shown to be DNA (Avery et al., 1944). It completely envelopes the pneumococcal cells and protects from phagocytosis. The pneumococcal capsules are polysaccharides excreted outside the cell and are composed, generally, of repeating units of simple sugars that remain attached to the outer surface of the bacterium, possibly in a covalent form (Lopez and Garcia, 2004). Capsule polysaccharides are highly heterogeneous. By 1940, 80 serotypes, defined by different capsular polysaccharides, had been described (CDC, 2005), and the number has now risen to over 100 different capsule types that now form the basis of antigenic serotyping of the organism (Bogaert et al., 2004a). These serotypes are grouped in 46 serogroups, based on immunological similarities (Hausdorff et al., 2005).

The next layer below the capsule, the cell wall, consists of polysaccharides, teichoic and lipoteichoic acid and several cell wall-associated surface proteins (Bogaert et al., 2004b). The cell wall of *S. pneumoniae* consists of covalently linked polysaccharide and polypeptide chains. This framework is known as peptidoglycan (or murein) is a macromolecule constitutes up to 50% of the cell wall mass. The Cell wall synthesis can be divided into three separate stages that
occur in distinct subcellular compartments, the cytoplasm, the membrane, and, finally, the cell wall itself (Figure 1.4). The polysaccharide component consists of linear chains of alternating β-(1-4)-linked N-acetylglicosamine (NAG) and N-acetylmuramic acid (NAMA, which consists of N-acetyl-D-glucosamine in an ether linkage with D-lactic acid. The NAMA lactic acid residue forms an amide bond with a D-amino acid-containing tetrapeptide to form the peptidoglycan-repeating unit. Neighbouring parallel peptidoglycan chains are covalently cross-linked through their tetrapeptide side chains. In S. pneumoniae, whose tetrapeptide has the sequence L-Ala- Glu- Lys- D-Ala-, this cross link consists of branched stem peptides that contain short Ala-Ala or Ala-Ser substituent that from the terminal carboxyl group of one tetrapeptide to the ε-amino group of the Lys in a neighboring tetrapeptide (Figure 1.5).

The surfaces of Gram-positive bacteria are covered by teichoic acids (Figure 1.6). They are polymers of glycerol or ribitol linked by phosphodiester bridges. The hydroxyl groups of this sugar-phosphate chain are substituted by D-Ala residues and saccharides such as glucose or N-acetylglicosamine. Teichoic acids are anchored to the peptidoglycans via phosphodiester bonds to C6-OH groups of their NAG residues. Lipoteichoic acid is chemically identical to the teichoic acid but is attached to the cell membrane by a lipid moiety (Voet, 1995). Both the teichoic acid and the lipoteichoic acid contain phosphorylcholine (Tomasz, 1967), a very unusual component in bacteria (Catterall, 1999). Phosphorylcholine is not only targeted by the choline-binding domain (CBD) of choline-binding proteins (CBPs) but functions itself as an adhesin by recognizing the platelet-activating factor receptor (PAFr) of host cells (Cundell et al., 1995a).

On the basis of genomic analysis (S. pneumoniae R6 GenBank accession number AE007317; S. pneumoniae TIGR4 GenBank accession number AE005672), it is estimated that the pneumococcus contains more than 500 surface proteins (Todar, 2003). Some are membrane-associated lipoproteins and others are physically associated with the cell wall. As mentioned above, a unique group of proteins on the pneumococcal surface is the family of choline-binding proteins.
CBPs are noncovalently bound to the choline moiety of the cell wall and are used to anchor various different functional elements onto the bacterial surface (Rosenow et al., 1997).

Figure 1.4. Biosynthetic pathway of Cell wall assembly, in which Peptidoglycan synthesis mainly occur in cytoplasm, membrane and extracellular face of the cytoplasmic membrane.

Figure 1.5. Schematic representation of the pneumococcal peptidoglycan. The variable presence of branched stem peptides is indicated on the left. (Adapted from Normark and Normark, 2002).
1.3.1. Impact of \textit{S. pneumoniae} on human health

\textit{S. pneumoniae} is a bacterial pathogen that affects children and adults worldwide. Those most commonly at risk of pneumococcal disease are children between 6 months and 4 years of age and adults over 60 years of age (ACIP, 1997). The risk of pneumococcal infection is much increased in persons with impaired IgG synthesis or decreased responsiveness to polysaccharide antigens due to immunosuppressive conditions such as human immunodeficiency virus infection or chronic lymphocytic leukaemia (ACIP, 1997). Also susceptible are persons with impaired phagocytosis, or defective clearance of pneumococci. In particular, the absence of a functional spleen, through congenital asplenia, splenectomy, or sickle-cell disease predisposes one to a more severe course of pneumococcal infection (WOIN, 2004). The bacterium is currently the leading cause of invasive bacterial disease in children and the elderly (Todar, 2003). \textit{S. pneumoniae} is the most commonly identified bacterial pathogen causing community-acquired pneumonia (CAP) (Catterall, 2004) and mortality rates in CAP can be alarming. However, despite the name, \textit{S. pneumoniae} causes many types of disease in addition to pneumonia. These include upper respiratory diseases such as acute sinusitis, otitis media and tracheobronchitis (WOIN, 2004). The pneumococcus also causes a broad-spectrum of invasive diseases such as meningitis, osteomyelitis, septic arthritis, endocarditis, peritonitis, pericarditis, cellulitis and brain abscess, all of which account for substantial hospitalisation and death worldwide (Sinave, 2004). \textit{S. pneumoniae} is the most common cause of bacte-
rial meningitis in adults, and is one of the top two isolates found in otitis media (Ryan and Ray, 2004). Neurological sequela and/or learning disabilities can occur in meningitis patients, and hearing impairment can result from recurrent otitis media. Virtually every child will experience pneumococcal otitis media before the age of 5 years (Bogaert et al., 2004). The WHO report on child deaths (Bryce et al., 2005) shows that, worldwide, 73% of deaths in children younger than age 5 years are attributable to six causes, with pneumonia accounting for 19% of the burden (Figure 1.7). Furthermore, the report indicates that sepsis or pneumonia in neonates and pneumonia in older children constitutes 26% of all deaths (Figure 1.7).

![Figure 1.7. Worldwide major causes of death in children younger than age 5 years and in neonates (yearly average for the period between 2000-2003) (from Bryce et al., 2005).](image)

In the United Kingdom, the pneumococcus is responsible for 30-50% of community and 8% of nosocomial Pneumonia, and it may be the cause of most cases of pneumonia with no identified causative organism (Sinave, 2004). In Ireland, there are approximately 8,000 hospital admissions from pneumonia annually and almost 2,000 people die from the disease – 90% of these are over 65 years old (WOIN, 2004). In the USA, *S. pneumoniae* infections caused 1,00,000 to 1,35,000 hospitalizations for pneumonia, 6 million cases of otitis media, and 60,000 cases of invasive disease, including 3300 cases of meningitis until the year 2000 (CDC, 2005). Death occurred in 14% of hospitalized adults with invasive disease. In children living in the developing world, the incidence of invasive pneumococcal disease is several times higher than the incidence in
industrialized countries. It is estimated that 60-90% of lower respiratory tract infections in Gambian children younger than 5 years are caused by pneumococcal disease (Adegbola, 2006). Although exact numbers are difficult to obtain, it is estimated that pneumococcus infection is responsible for more than one million of the 2.6 million annual deaths resulted due to acute respiratory infection in children younger than 5 years, 10.6 million children aged less than 5 years have been found affected with pneumococcal disease every year. The information gleaned from these sources suggests that the incidence of mortality from pneumococcal disease is high; however, further studies are greatly needed (Fedson et al., 1999). WHO estimates that, worldwide 1.6 million deaths have occurred by pneumococcal disease in 2005, with 7.00,000 to 1 million of these occurring in children younger than 5 years (WHO, 2007). Even patients in developed countries, invasive pneumococcal disease carries a high mortality rate an average of 10–20% in adults with pneumococcal pneumonia, with much higher rates in those with risk factors for disease.

1.3.2. Antimicrobial therapy and vaccination

1.3.2.1. Antimicrobial therapy

At one time, disease caused by *S. pneumoniae* could be reliably treated with antibiotics. The inhibition of bacterial growth through antibiotics targeting cell wall biosynthesis has been a proven mode of action since the beginning of the antibiotic era (Walsh et al., 2003). Treatment of pneumococcal infections is usually with β-lactam antibiotics (Normark and Normark, 2002; Catterall, 2004), which include benzyl penicillin (penicillin G), ampicillin, cephalosporin C, ceftriaxone and aztreonam. These compounds are bactericidal and inhibit penicillin-binding proteins (PBPs) (Charpentier and Tuomanen, 2000). PBPs catalyse the transpeptidation of the stem peptides required for cross-linking the peptidoglycan cell wall, as well as the DD-carboxy-peptidation step required to remove terminal D-alanine from the stem peptides (Normark and Normark, 2002). The β-lactam antibiotics also stimulate the activity of pneumococcal autolysins. The mechanism of penicillin resistance in clinical isolates of *S.*
*pneumoniae* involves the alteration of PBPs, so as to reduce their affinity for the antibiotic molecule (Charpentier and Tuomanen, 2000). In the 1940s, penicillin antibiotics became available and were used effectively to treat pneumococcal infections. In the 1960s, nearly all strains of *S. pneumoniae* were susceptible to penicillin, but since that time, there has been an increasing prevalence of resistance, especially in areas of high antibiotic use. The first reports on resistance to penicillin were reported in 1967 in Australia and New Guinea (Hansman *et al.*, 1971). From 1970 to 1990, resistance to *S. pneumoniae* has increased significantly, presumably due to the increased use of antibiotics. The first reports of penicillin-resistant *S. pneumoniae* strains appeared in the US in the early-1980s (Krause *et al.*, 1982), then beginning in the 1990s their share increased from less than 5% of all isolates to approximately 35% in 2002 (File, 2006). Similarly, resistance to macrolides is increasing, with 24% of all isolates being resistant in 2000 (Jacobs *et al.*, 2003). To further complicate the issue, multidrug resistance is increasingly present among *S. pneumoniae* isolates, documented by a recent study showing 22% of all isolates to be resistant to at least three antibiotics (Doern *et al.*, 2005) and now has been reported all over the world (Hennessy *et al.*, 2002). Currently, penicillin resistance in *S. pneumoniae* varies from 0% resistance in the Netherlands to 71.5% in South Korea (Walsh and Amyes, 2004). The rate of decreased penicillin susceptibility is expected to be as high as 30% in some communities in the USA (Sinave, 2004). Macrolides are a group of drugs (typically antibiotics) which belong to the polyketide class of natural products. The mechanism of action of the macrolides is inhibition of bacterial protein synthesis by binding reversibly to the bacterial ribosome, thereby inhibiting translocation of peptidyl-tRNA. Pneumococcal resistance to macrolides occurs by alteration of the structure of the bacterial ribosome (Charpentier and Tuomanen, 2000). In many countries macrolides resistance is higher than penicillin resistance and does not appear to be arresting. For example, in Hungary and Italy, where penicillin resistance is estimated to range 2 to 10%, erythromycin resistance can be as high as 42% (Walsh and Amyes, 2004). Isolates that are susceptible to penicillin also are susceptible to nearly all
other antibiotics (excluding the macrolide group). However, if penicillin minimum inhibitory concentration (MIC) raises, the MIC of the most active β-lactam drugs cefotaxime, ceftriaxone (both broad-spectrum third generation cephalosporins) also rises. Multiple antibiotic resistant strains of *S. pneumoniae* first emerged in the early 1970s in Papua New Guinea and South Africa but now cover the globe and have rapidly increased since 1995 (Todar, 2003). The cause of this international spread was mostly due to a few multidrug-resistant clones of serotypes 6B, 9V, 14, 19A, 19F, and 23F (Bogaert *et al.*, 2004b). Genotyping studies on *S. pneumoniae* have identified a group of 16 multi-drug resistant clones circulating around the world (Walsh and Amyes, 2004).

The fluoroquinolones group of broad-spectrum antibiotics (a quinolone subset) remains active. Among the respiratory quinolones, moxifloxacin is currently the most active against pneumococci, followed by gatifloxacin (Sinave, 2004). Quinolones and fluoroquinolones are related to nalidixic acid and target DNA gyrase, that catalyses DNA supercoiling during replication, and topoisomerases IV complex C2E2, which is essential for chromosome segregation (Charpentier and Tuomanen, 2000). However, the excessive use of this class of antimicrobial agents is resulting in developing resistance that is of major concern. So what are the antibiotics of last resort for the treatment of macrolide resistant, penicillin and multi-drug resistant *S. pneumoniae*. Currently they are linezolid and vancomycin (oxazolinidone and glycopeptide class of antibiotics, respectively) (Walsh and Amyes, 2004). Most pneumococcal strains remain susceptible to vancomycin (Sinave, 2004), but this is a less desirable antibiotic because of dosing and tissue penetration issues. Vancomycin is another lytic antibiotic inhibiting peptidoglycan synthesis, but it does so at an earlier step than penicillin. It exerts its antibacterial activity by binding to D-Alanine termini of peptidoglycan precursors, preventing these from being incorporated into the growing peptidoglycan wall (Normark and Normark, 2002). Recently, however, vancomycin tolerance emerged in pneumococci (Novak *et al.*, 1999), both as mutants of laboratory strains and as naturally occurring clinical isolates (Normark and Normark, 2002). Even when antimicrobial therapy is effective, pneumococcal diseases remain
associated with significant mortality. Austrian and Gold (1964) compared data from the pre-antibiotic and post-antibiotic eras and demonstrated similar mortality rates during the first few days of the disease in patients with bacteremic pneumonia.

Antibiotic-resistant \textit{S. pneumoniae} infections may require higher doses of antibiotic, longer duration of treatment and hospitalization, the use of more expensive medications, or use of medications with greater side-effect potential. The spread of these resistant bacteria increased dramatically during the 1990s. Understanding the evolution of resistant pneumococci and their spread among people is critical to developing effective prevention strategies and is an extremely important public health priority.

However, today antibiotic resistance in \textit{S. pneumoniae} is common and increasing. In the United States, drug-resistant pneumococci cause at least 15,000 cases of meningitis, 7,000 cases of sepsis/bacteremia, 1,50,000 cases of pneumonia, and more than 1,000,000 cases of otitis media annually. Between 3-to-35\% of pneumococcal illness is due to drug-resistant strains. This proportion varies widely with location and over time, but resistance has been reported throughout the world. This reinforces the need for an effective vaccine and/or effective new drugs against pneumococcal infection and disease. In light of this growing resistance problem, the identification and characterization of more potential targets for antibiotic therapy is of paramount importance.

\textbf{1.3.2.2. Vaccination}

More than 90 serotypes exist; of strains causing invasive disease, 88\% are serotypes included in the 23-valent polysaccharide vaccine. This vaccine has been available for the prevention of pneumococcal disease since 1977. Since 1983 to date, a 23-valent vaccine has been licensed. This polysaccharide vaccine (effective in adults and children above 2 years old) is comprised of capsular polysaccharide antigens purified from the 23 serotypes (1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F)
accounting for 85-90% of pneumococcal diseases in the UK (Catterall, 2004). The vaccine confers serotype-specific protection in adults and induces exclusively humoral immune response (Brown et al., 2001). However, the polysaccharide vaccine remains one of the most controversial of the currently used vaccines because of its variable efficacy against all manifestations of pneumococcal infections. The vaccine is about only 60% effective in preventing invasive diseases and is less effective at preventing pneumonia and other localised respiratory tract infections (McDaniel and Swiatlo, 2004). Furthermore, there are major disadvantages in the use of the polysaccharide vaccines:

1) The lack of efficacy in children under 2 years of age (that fails to respond immunologically to purified polysaccharides) and in immunocompromised patients (WHO, 1999)

2) They are not effective against acute otitis media (Wadwa and Feigin, 1999)

3) They do not induce a T-cell dependent immune response (Bogaert et al., 2004b).

Polysaccharides are T-independent antigens, and their effect is mediated by B cells without the involvement of T cells, and as such has very low immunogenicity in children under two. This class of antigens also induces poor memory immune responses, so boosting does not result in significantly higher antibody titres than a single immunisation (Swiatlo and Ware, 2003). However, these weaknesses can be overcome by covalently linking polysaccharides with a carrier protein (De Roux and Lode, 2005), a technique that has been successful in the \textit{H.influenzae} Type b and group C meningococcal vaccines (Booy et al., 1994; Girard et al., 2006).

In 2000, a heptavalent protein-polysaccharide conjugate vaccine (Prevnar-Wyeth, USA) was licensed for use in infants (less than 5 years of age) in the USA. This vaccine, that contains polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F and 23F conjugated to diphtheria CRM197 protein (Shinefield et al., 1999) has been approved by the Food and Drug Administration (USA) and the
Committee on Proprietary Medicinal Products (Europe) for the prevention of invasive disease in children (Bogaert et al., 2004b). Since the polysaccharide is conjugated to a protein carrier, infants' immune systems respond to the polysaccharide in a T-dependent manner and produce memory B cells (McDaniel and Swiatlo, 2004). In fact, it has been shown capable of inducing antibody production and immunological memory in very young children with an immature immune system (De Roux and Lode, 2005). The vaccine can be administered with other childhood vaccines and is generally well tolerated (McDaniel and Swiatlo, 2004). In a study conducted by Black et al., (2000) the vaccine was shown to be highly effective (>90%) in preventing invasive disease in infants caused by the 7 serotypes included in the vaccine. This vaccine also showed to significantly reduce pneumococcal carriage and to decrease episodes of otitis media (Lopez and Garcia, 2004). In addition, several studies have shown a significant reduction in nasopharyngeal carriage of vaccine type pneumococci in infants (Ghaffar et al., 2004). Unfortunately, this vaccine is expensive to produce and will be difficult to implement on a large scale in developing countries (Swiatlo and Ware, 2003; Bogaert et al., 2004b). Another added complication with the conjugated 7-valent vaccine is the replacement of vaccine types with non-vaccine types, and the potential of these non-vaccine types to also cause invasive disease (Jomaa et al., 2005). Also the limited number of various capsular polysaccharides that can be included in the vaccine is considered a drawback (Ling et al., 2004). Furthermore, the need for varied formulations including different serotypes for different regions of the world is another issue that will affect the global impact of this vaccine (McDaniel and Swiatlo, 2004). The current pneumococcal vaccines elicit immune responses to the polysaccharide capsules. However, there are two major disadvantages associated with these capsular-based vaccines. First, the requirements to conjugate a number of polysaccharide types to a protein carrier to give efficacy in young children who are unable to mount protective immune responses to polysaccharides (as mentioned above); and secondly, conjugation restricts the number of polysaccharide types that can be included in any given formulation.
Finally, the ability to prevent infections could improve through expanded use of 23-valent polysaccharide vaccine among adults and through use of the conjugate vaccine for infants and young children. Campaigns for judicious use of antibiotics along with the new vaccine may slow or reverse emerging drug resistance.

An alternative approach that may offer broader coverage, be less costly and can have the potential to be used in all age groups is a vaccine based on one or more proteins common to all serotypes (McDaniel and Swiatlo, 2004). Protein-based vaccines have the advantages of being antigenically conserved across capsular types, comparatively inexpensive to produce by recombinant DNA techniques and able to induce memory responses which are long lasting (Swiatlo and Ware, 2003). Several studies performed with animals have demonstrated the ability of protein-based vaccines to protect against experimental pneumococcal disease (Jomaa et al., 2005). However, there is a need for a better understanding in the role in pathogenicity of several pneumococcal proteins that are being studied for their potential to induce protective immune responses. Hence, there is urgent need a renewed interest in the research and development of new diagnostics, vaccines and drug treatments (WHO, 2006; Brown, 2007).

1.4. *S. pneumoniae* R6

*S. pneumoniae* R6 (SpR6) remains a suitable laboratory strain for basic experiments on fundamental genetic and physiological mechanisms in pneumococcus. However, because laboratory strain R6 contains so many mutations compared to the D39 strain, it cannot be assumed that physiological and metabolic properties studied in strain R6 will extrapolate to pathogenic strain D39. To date, there are three *S. pneumoniae* genomes that have been sequenced completely and are publicly available. The genomes are from different strains namely; a 19F strain sequenced by Glaxo-SmithKline, a type 4 strain sequenced by TIGR and strain R6 by Eli Lilly (Chan et al., 2003). Strain R6 is a descendant of the type 2 capsule clinical isolate used by Avery and coworkers to demonstrate the genetic function of DNA (Avery et al., 1944), and it is used worldwide as a standard
laboratory strain. The R6 strain was reported to be 2,038,615 bp and 40% G + C content. It contains 2,043 predicted protein coding regions and 73 noncoding RNA genes (Hoskins et al., 2001). As a consequence of the capacity of *S. pneumoniae* to take up DNA, its genome is littered with genes that are apparently derived from other bacteria. There are 40 ORFs that are similar to genes in gram-negative bacteria and that have not been found in other gram-positive genome sequences (Hoskins et al., 2001). This is not surprising, because *S. pneumoniae* occupies the same niche in the human respiratory system as several gram-negative species such as *H. influenzae*. Additionally, at least 2% of the genes were found to be significantly truncated relative to orthologous genes characterized in other bacteria. Transporters are the most frequently truncated genes and it was shown that there are five ORFs that are similar to genes encoding drug efflux pumps (Hoskins et al., 2001). The virulent isolate sequenced by TIGR was shown to have 2,160,837 base pairs with G + C content of 39.7%. It contains 2236 predicated coding regions; of these, 1140 (64%) were assigned a biological role (Tettelin et al., 2001). By comparing the nucleotide and protein sequences of the two strains i.e R6 and TIGR exhibited differences and similarities (Jothi et al., 2007). Glaxo-SmithKline presented sequences and functional annotations for the 2.1 Mbp of a 19F strain which was shown to have 2,046 open reading frames (Dopazo et al., 2001).

From this study, it concludes that the capacity to identify all potential genes within this pathogen should greatly facilitate the identification of novel targets for antibiotic discovery as well as new candidates for vaccine development. This process will be significantly enhanced by the comparison of the *S. pneumoniae* R6 sequence to that of pathogenic strains of *S. pneumoniae* (www.tigr.org and http://genome.microbio.uab.edu/strep/) (Figure 1.8). These comparisons, in concert with genetic and gene expression studies, should catalyze expansion of *S. pneumoniae* biology.
The outer circle shows predicted coding regions on the plus strand, color-coded by role categories: salmon, amino acid biosynthesis; light blue, biosynthesis of cofactors and prosthetic groups and carriers; light green, cell envelope; red, cellular processes; brown, central intermediary metabolism; yellow, DNA metabolism; green, energy metabolism; purple, fatty acid and phospholipid metabolism; pink, protein fate/synthesis; orange, purines, pyrimidines, nucleosides, and nucleotides; blue, regulatory functions; gray, transcription; teal, transport and binding proteins; black, hypothetical and conserved hypothetical proteins. The second circle shows predicted coding regions on the minus strand, color-coded by role categories. The third circle shows predicted transcription termination signals. The fourth circle shows an atypical nucleotide composition curve. The fifth circle shows the GC-skew curve. The sixth circle shows IS elements in red. The seventh circle shows RO.UP elements in green. The eighth circle shows BOX elements in blue. The ninth circle shows tRNAs in green. The tenth circle shows the rRNAs in blue and structural RNAs in red.

Figure 1.8. Circular representation of the S. pneumoniae R6 genome map. (This figure kindly provided by Dr. Hervé Tettelin from The Institute for Genomic Research (TIGR).

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