CHAPTER 1

INTRODUCTION

1.1 PREVALENCE OF RTI

Respiratory tract infection (RTI) is one of the major Public health problems reported from developing countries. Acute respiratory tract infection (ARI) is reported as the major cause of death in infants and children, and attributes to 15-30% of death in under 5 age group in India.[1]. It is estimated that Bangladesh, India, Indonesia, and Nepal together account for 40% of the global Acute respiratory infection mortality. ARI is the cause for about 30-50% of visits to healthcare facilities and for about 20-40% of admissions to hospitals [2].

According to WHO bulletin 2008, more than half of the world’s annual new pneumonia cases are concentrated in just five countries where 44% of the world’s children aged less than 5 years live: India (43 million), China (21 million) and Pakistan (10 million) and in Bangladesh, Indonesia and Nigeria (6 million each).[3]

Acute pharyngitis is an inflammatory condition of the pharynx due to infection by several microorganisms. Most cases are of viral aetiology and include common cold and influenza. It’s most important bacterial cause is Streptococcus pyogenes (S.pyogenes) It is important to differentiate bacterial causes from viral causes, since bacterial causes respond well to antimicrobial therapy.

Laryngitis is characterized by hoarseness or huskiness of voice along with dry cough. The most common etiological agents are beta haemolytic Streptococci, Moraxella catarrhalis and Haemophilus influenzae
Acute epiglottitis is cellulites of the epiglotis and adjacent structures that has potential for causing abrupt or complete airway obstruction. *H. influenzae* is the major cause of epiglottitis in children, followed by Pneumococci, *Staphylococcus aureus* and beta hemolytic Streptococcus.

Etiological agents of RTI vary from region to region, and also during various climates. Although virus accounts for most of the respiratory tract infections, bacterial infections are also known to cause primary infection or superadded secondary infection and in most cases require antibiotic therapy[4]. In upper Respiratory tract infections (URI) most common isolates were *Streptococcus pyogenes* and other beta hemolytic streptococci, *Staphylococcus aureus* (*S.aureus*), *Moraxella catarrhalis*, and *Hemophilus influenzae* (*H.influenzae*). Others were *Streptococcus pneumoniae* (*S.pneumoniae*) and *Corynebacterium diphtheriae* (*C.diphtheriae*)[5]. Bacterial causative agents for lower Respiratory tract infection in children vary from *Streptococcus pneumoniae* (8-61%), *Hemophilus influenzae* (1-15%), Gram negative bacilli (3-5%), atypical agents like Chlamydia (1-14%), Mycoplasma (7-24%)[6,7]. Viral pathogens causing respiratory tract infections account for 44-49% of the cases.[8]. It has been known that viral infections are the main causes of mild to moderate pneumonia (especially in the first years of life) while bacterial infections are the leading cause of severe pneumonia. Next to *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib) remains the second important bacterial pathogen of pneumonia. Almost one in 200 children less than 5 years of age developed invasive Hib disease and nearly two-third of Hib infections occurred in children under 18 months. *Staphylococcus aureus* is the third important bacterial causative in pneumonia. In the United States, *S. aureus* has escalated in the past decade and many of them are methicillin-resistant *S.aureus* (MRSA). WHO bulletin 2008, also indicated the leading bacterial cause of pneumonia as *Str.pneumoniae* which was identified in 30–50% of cases, followed by *H. influenzae* type b 10–30% followed by S. aureus and K.pneumoniae.[4]
1.2 **STREPTOCOCCUS PYOGENES**

*S.pyogenes* causes both local as well as systemic invasive infections associated with immunologically mediated Post Streptococcal sequelae. Therefore this study focuses on infection by *Streptococcus pyogenes* and other beta haemolytic Streptococci.

*S.pyogenes* cell wall contain proteins (M,T,R antigens), Carbohydrate (group specific) and peptidoglycans. Hair-like pili project through the capsule of Group A Streptococci. The major virulence factor of the organism is the M protein. M-protein is a stable dimer, anchored to the cell membrane; it traverses and penetrates the cell wall. The proximal portion of the molecule is highly conserved among Group A isolates, whereas the distal portion contains type-specific epitopes localized on the tips of fibrils (fimbriae) that protrudes from the cell surface. The ability of GAS to initiate disease is highly depends on M protein. Strains lacking M protein are essentially non-pathogenic. Streptococci isolated from chronic pharyngeal carriers contain little or no M protein and are also relatively avirulent.[9]

Molecular mechanisms by which M protein mediates pathogenesis are complex. In the non-immune host, M protein mediates an antiphagocytic effect by inhibition of activation of the alternate complement pathway. Acquired immunity to Streptococcal infection is based on the development of opsonic antibodies directed against the antiphagocytic epitopes of M protein. Although such antibodies protect from infection against a homologous M protein type, more than 80 M proteins types have been recognized. Community based outbreaks of particular streptococcal diseases tend to be associated with certain M types; therefore, M serotyping has been very valuable for epidemiological studies [9].

Another typing scheme that characterizes and measures the genetic diversity among isolates of *Streptococcus pyogenes* is *emm* typing. The *emm* gene encodes the M protein, which forms the basis of a serological typing scheme [9].
1.2.1 Serum–Opacity Factor (SOF)

Serum opacity factor is a virulence determinant expressed by GAS has the ability to opacify serum by disrupting the structure of high density lipoproteins resulting in the formation of large lipid vesicle to cause serum to appear cloudy. SOF is a multifunctional protein by which it binds to various host proteins like fibronectin, fibrinogen and fibulin, which are involved in bacterial adhesion. It is an Alpha lipoprotein that is able to opacify media containing mammalian serum. It is known to be produced by 29 different M serotypes. [10].

SOF production was reported to be 83.3% from cases of pyoderma. Various other reports has given 34-50% of SOF positivity. The difference in SOF positivity can be due to variation in M proteins of each strains.[11].

1.2.2 Biofilm Formation in Streptococcus Pyogenes

Biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances produced by them, and exhibit an altered phenotype with respect to growth rate and gene transcription.

1.2.3 Streptococcal Vaccines

Most recent vaccine strategies have targeted either the type-specific N-terminal region of the M protein or the highly conserved carboxy-terminal region of the M protein molecule. Vaccination against the N-terminal type-specific region induced protective bactericidal and opsonic antibody against the specific M protein serotype, while vaccination against the conserved carboxyl-terminal region of the M protein protected against multiple serotypes and prevented colonization at mucosal surfaces. Investigators have worked over the past two decades to develop a safe and efficacious M protein vaccine to be used for immunization, especially against rheumatogenic serotypes. In addition, with the resurgence of serious Streptococcal infections, it is prudent to pursue a GAS vaccine against serotypes that produce invasive disease[12].
Streptococcal infection often fails to antibiotic therapy, leading to chronic throat carriage and recurrent infection. This could not be because of antibiotic resistance, as rate of antibiotic resistance is very less in *Streptococcus pyogenes* compared to other bacteria. Various recent studies suggests that some subtypes of *Streptococcus pyogenes* produce Biofilm and may have a role in chronic carriage of Streptococcus.

### 1.2.4 Antibiotic Resistance of *Streptococcus pyogenes*

Streptococcus species is uniformly susceptible to Penicillin, but Macrolides remains drug of choice for patient allergic to penicillin. Over the last several decades there has been increased resistance to Macrolides being reported from several countries.

There are two major recognized Macrolides resistance phenotypes – MLS\(_\beta\) and ‘M’ type. MLS\(_\beta\) is the resistance to Macrolides,Lincosamides and Streptogramins conferred by methylation of a single adenine in the bacterial 50s ribosome that binds to erythromycin encoded by erm gene. It can be either inducible (iMLS\(_\beta\)) or constitutive (cMLS\(_\beta\)).M-type – resistant to Macrolides, but not to lincosamides or Streptogramin B, conferred by Macrolide efflux pump encoded by mef gene[13].

Various rates of Macrolide resistance of *Streptococcus pyogenes* are reported worldwide, like 37% in Pennsylvania, France 6.2%, Poland 12%, etc[14,15,16]. European surveillance in Italy identified that 32% of GAS isolates exhibits resistance to Macrolides. France has reported a steady escalation of Erythromycin reaching 23% to date. Portugal identified 11% of GAS isolates were resistant to Macrolides [17]. According to Indian studies; Reports from Chennai showed 16.2 % of Macrolide resistance, 38.23% of resistance was reported from Mangalore and 29.4% from North India[18,19,20].