CHAPTER 2

REVIEW OF LITERATURE

2.1 PREVALENCE OF RESPIRATORY TRACT INFECTION

Respiratory tract infection is the most common acute illness affecting paediatric population and it ranges from self limited infection like common cold to life threatening infections like pneumonia and epiglottitis. Respiratory tract infection can be classified as Upper respiratory tract infection and lower respiratory tract infection. Upper respiratory tract infection (URI) involves infection of nasal passage, pharynx, tonsil and epiglottis. Clinical presentation involves Pharyngitis, rhinosinusitis, nasopharyngitis, tonsillitis and epiglottitis. Lower respiratory tract infection (LRI) involves the infection of bronchi and lungs which include Pneumonia, Bronchitis, Bronchiolitis, Pleuritis etc.

It is estimated that Bangladesh, India, Indonesia, and Nepal together account for 40% of the global Acute respiratory infection mortality (ARI). ARI is responsible for about 30-50% of visits to health facilities and for about 20-40% of admissions to hospitals [21]. According to a study done in Delhi slums on 1307 children, 14.6% had at least one attack of ARI every year [22]. Another community-based study carried out in a rural area of Delhi also reported the prevalence of ARI to be 12.1% among under-five year old children. A cross-sectional study from Ahmadabad reported that prevalence of ARI was 22% and age group of 4-5 years were mostly affected (47.3%) [23]. A community-based study in a coastal village of Karnataka reported 6.42 episodes of ARI per child per year; the incidence of pneumonia was significantly higher among infants [24]. A cross-sectional study from Brazil reported that ARI in children under-five years was 25.6%, among which 76.4% constituted upper and 23.6% lower respiratory infections [25].
2.2  PREDISPOSING FACTORS FOR BACTERIAL RTI

Viral infection is thought to predispose the respiratory niche to bacterial colonization by different mechanisms. Viruses make the epithelium more susceptible to bacterial colonization by altering mucosal surfaces. Mucociliary function of the respiratory epithelium is decreased as cilia is damaged. Integrity of the epithelial layer is damaged by viruses, enhancing colonization and translocation of bacteria [26].

2.3  BACTERIOLOGICAL PROFILE OF ARI

Etiological agents of RTI vary from region to region, and also during various climates. The bacteriological agents causing ARI are listed below.

Table 2.1  Bacteriological profile of ARI. [27]

<table>
<thead>
<tr>
<th>Presenting symptoms</th>
<th>Specimen to be taken</th>
<th>Bacteria potentially associated with infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>URI:Throat and pharynx</td>
<td>Swab of posterior pharynx, Swab of tonsil, Nasopharyngeal Swab.</td>
<td>Beta hemolytic Streptococci, Corynebacterium Diphtheriae Neisseria gonorrhoeae Bordetella pertussis</td>
</tr>
<tr>
<td>URI- Nose, sinus</td>
<td>Nasopharyngeal swab, Sinus washings.</td>
<td>Streptococcus pyogenes. Streptococcus pneumoniae, Staphylococcus aureus Hemophilus influenzae Klebsiella spp Other Enterobacteriaceae Anaerobes</td>
</tr>
</tbody>
</table>
In a study done in Tamil nadu on RTI among the positives, various bacteria isolated were *S.aureus* (45.61%), β hemolytic *Streptococci*(22.81%), *Klebsiella pneumoniae*(14.91%), *Pseudomonas aeruginosa* (8.33%), and *α hemolytic Streptococci*(14.83%), *E.coli* (2.91 %) and *H.influenzae* (1.32%).[28] Another study done in Karimnagar, Andhra Pradesh, reported that among the 106 respiratory samples tested 56 (52.83%) were positive for various bacteria, 6 (5.6%) grew fungi and 44 (41.5%) revealed no pathogens. *Klebsiella pneumoniae* (45.1%) was the predominant pathogen followed by *Citrobacter freundii* (12.9%), *Pseudomonas aeruginosa* (9.6%), and *Staphylococcus aureus* (9.6%) [29]. Overall carriage rate of respiratory pathogens was 30% with *Streptococcus pneumoniae*, *H. influenzae* and GAS accounting for 22%, 5% and 4.5% respectively. Antibiotic resistance was highest in *Strep. pneumoniae* with 66.7% of strains resistant to penicillin. MDR strains were also encountered. Erythromycin resistance was observed in both *H.influenzae* (28.4%) and GAS (22%)[30].

In a Nationwide surveillance of bacterial respiratory pathogens conducted in Japan in 2007, 1108 strains were isolated, which included 226 *Staphylococcus aureus*, 257 *Streptococcus pneumoniae*, 6 *Streptococcus pyogenes*, 206 *Haemophilus influenzae*, 120 *Moraxella catarrhalis*, 122 *Klebsiella pneumoniae*, and 171 *Pseudomonas aeruginosa.*[31]. In a study done in Kenya on etiology for ARI in children under five, 24% were positive for bacterial cultures, *S.pyogenes* (15.4%) and *Streptococcus viridans*(6.4%) were the most predominant bacterial agents. *Streptococcus pneumoniae* and *Staphylococcus aureus* were isolated in only a few specimens.[32] In a report from Bangladesh, 31.4% culture positivity was seen in ARI cases. Predominant bacterial isolates from URI were *Staph aureus* (12.4%) and *S. pyogenes* (9.8%) whereas predominant agent from LRI were *Strep pneumoniae* (14.7%) and *Haemophilus influenzae* (8.6%)[33]. In another study from Netherlands, Rhinovirus was most common in case patients (24%), followed by influenza virus type A (11%) and Coronavirus (7%).
2.3.1 Upper Respiratory Tract Infection

Risk factors for contracting a URI include the following:

- Contact: Close contact with small children in places such as school or daycare.

- Inflammation: Inflammation and obstruction due to allergic rhinitis or asthma can predispose to infections

- Travel to crowded places: The incidence of contracting a URI is increased because of exposure to large numbers of individuals in closed settings

- Immunocompromised conditions that affect cellular or humoral immunity: Weakened immune function may result from splenectomy, HIV infection, use of corticosteroids, immunosuppressive treatment after stem cell or organ transplantation, multiple medical problems, or common stress; cilia dyskinesia syndrome and cystic fibrosis also predispose individuals to URIs

- Anatomic changes due to facial dysmorphisms, previous upper airway trauma, and nasal polyposis

- Carrier state: Some people are chronic carriers of group A streptococci.[34].

Identification of causative agent of URI is very challenging due to the presence of bacterial flora of upper respiratory tract. Oropharyngeal flora of normal individual consist of viridian Streptococci, *Streptococcus pneumoniae, Staphylococcus aureus, Hemophilus influenzae, Moraxella catarrhalis*, many anaerobic bacteria and yeast such as Candida apart from viruses like Adenovirus and Herpes virus, without causing any symptoms. [35].
Some of the common symptoms of URI are listed below

**Acute bacterial rhinosinusitis:** In children, acute bacterial sinusitis is defined as a URI with any of the following: Persistent nasal discharge or cough lasting 10 days or more without improvement, worsening course after initial improvement. Severe onset of fever of 102°C or greater with nasal discharge for at least 3 consecutive days. In older children and adults, symptoms (e.g., pain, pressure) tend to localize to the affected sinus.[36].

**Group A Streptococcal pharyngitis:** The following physical findings suggest infection with group A Streptococcal disease: Erythema, swelling, or exudates of the tonsils or pharynx, temperature of 38.3°C (100.9°F) or higher, tender anterior cervical nodes (≥ 1 cm).

**Viral nasopharyngitis:** Nasal mucosal erythema and edema are common. Profuse discharge is more characteristic of viral infections than bacterial infections; initial clear secretions typically become cloudy white, yellow, or green over several days, even in viral infections. Fever is common in children with Rhinoviral infections[36].

**Epiglottitis:** This condition is more often found in children aged 1-5 years, who present with a sudden onset of the following symptoms: Sore throat, drooling, difficulty or pain during swallowing, globus sensation of a lump in the throat. Dry cough or no cough, dyspnea, fever, fatigue or malaise (may be seen with any URI) [36].

**Laryngotracheitis and laryngotracheobronchitis:** Nasopharyngitis often precedes laryngitis and tracheitis by several days. Swallowing may be difficult or painful. Patients may experience a globus sensation of a lump in the throat. Hoarseness or loss of voice is a key manifestation of laryngeal involvement [36].
2.3.2  Lower Respiratory Infections

Pneumonia

Pneumonia is among the top ten most common causes of death among all age group in United states. Successful treatment of Pneumonia poses a great challenge because of the involvement of a variety of pathogens. No single antimicrobial regimen can cover all the possible etiological agents[37].

**Table 2.2 Bacterial agents of Acute Pneumonia** [38].

<table>
<thead>
<tr>
<th>Common</th>
<th>Uncommon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus pneumoniae,</td>
<td>Acinetobacter spp</td>
</tr>
<tr>
<td>Hemophilus influenzae</td>
<td>Bacillus spp</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>Moraxella catarrhalis</td>
</tr>
<tr>
<td><strong>Mixed Anaerobes</strong></td>
<td>Campylobacter fetus</td>
</tr>
<tr>
<td>Bacteroides spp</td>
<td>Neisseria meningitides</td>
</tr>
<tr>
<td>Fusobacterium spp</td>
<td>Nocardia spp</td>
</tr>
<tr>
<td>Peptostreptococcus spp</td>
<td>Pasteurella maltocida</td>
</tr>
<tr>
<td>Peptococcus spp</td>
<td>Proteus spp</td>
</tr>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>Enterococcus spp</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>Streptococcus pyogenes</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td></td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td></td>
</tr>
<tr>
<td>Serratia spp</td>
<td></td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
</tr>
<tr>
<td>Legionella spp</td>
<td></td>
</tr>
<tr>
<td>Bordetella</td>
<td></td>
</tr>
</tbody>
</table>

**Types of Pneumonia**

Pneumonia can be broadly classified into Community acquired Pneumonia and Hospital acquired Pneumonia.
Community Acquired Pneumonia (CAP)

CAP is defined as pneumonia acquired outside the hospital or long-term care facility. It occurs within 48 hours of hospital admission or in a patient presenting with pneumonia who does not have any of the characteristics of healthcare-associated pneumonia ie, hospitalized in an acute care hospital for 2 or more days; resided in a nursing home or long-term care facility; received recent intravenous antibiotic therapy, chemotherapy, or wound care within the past 30 days of the current infection; or attend a hospital or haemodialysis clinic [39,40].

CAP presents with sudden onset of chills followed by fever, pleuritic chest pain and cough that produces mucopurulent sputum. Fatigue, Anorexia and sweat may be seen. White blood count is in the range of 15000 to 35000 / mm³. Sputum is thick and purulent and may be rust colored. Gram’s stain reveals numerous neutrophils and bacteria, often a single type of bacteria predominating. Chest film shows area of parenchymal involvement with alveolar filling. But even with rigorous laboratory evaluations, a microbiological diagnosis may be made in only 20-70% cases of CAP. [41,42].

Typical bacterial pathogens that cause CAP include Streptococcus pneumonia, Haemophilus influenzae, and Moraxella catarrhalis and account for approximately 85% of CAP cases. CAP is usually acquired via inhalation or aspiration of a pulmonary pathogen into a lung segment or lobe. Less commonly, CAP results from secondary bacteremia from a distant source, such as Escherichia coli from urinary tract infection and/or bacteremia. Aspiration pneumonia is the only form of CAP caused by multiple pathogens (eg, aerobic/anaerobic oral organisms). Klebsiella pneumoniae CAP occurs primarily in persons with chronic alcoholism and Staphylococcus aureus may cause CAP in patients with influenza. Pseudomonas aeruginosa is a cause of CAP in patients with bronchiectasis or cystic fibrosis.[39]. Atypical pathogen CAP manifests a variety of pulmonary and extrapulmonary findings (eg, CAP plus diarrhoea). Atypical CAP can be divided into those caused by either zoonotic or non zoonotic atypical pathogens. Zoonotic atypical CAP pathogens include Chlamydia psittaci (psittacosis), Coxiella burnetii (Q fever),
and *Francisella tularensis* (tularemia). Nonzoonotic atypical CAP pathogens include *Mycoplasma pneumoniae*, *Legionella* species, and *Chlamydia pneumoniae*. These organisms account for approximately 15% of all CAP cases[43].

*Streptococcus pneumoniae* is the leading cause of acute CAP ranging from 16-60%. *H.influenzae* is the second most common, ranging from 3-38%. The use of Hib vaccine has decreased the incidence of *H.influenzae* type b infection, but there is a striking increase in infections caused by non typeable *H.influenzae*. 

*Staphylococcus aureus* accounts for 2-5% of CAP, but increased incidence in post influenza pneumonia. Community acquired MRSA (CA MRSA) pneumonia are associated with mortality rate of 29-60%. Among Gram negative bacilli, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter* spp are most common[44].

### Hospital Acquired Pneumonia (HAP)

According to American Thoracic Society (ATS) guidelines, nosocomial pneumonia or hospital-acquired pneumonia (HAP) is defined as a lung infection that begins in a non-intubated patient within 48 hours of admission. Ventilator-associated pneumonia (VAP) is a form of nosocomial pneumonia that begins more than 48 hours after the patient is intubated. Healthcare-associated pneumonia (HCAP) occurs within 90 days of a hospitalization that lasts 2 days or more, a stay at a nursing home, or a hospital-based clinic or hemodialysis facility. Healthcare-associated pneumonia also describes pneumonias that occur within 3 days of receiving antibiotics, chemotherapy, or any type of wound care [45].

HAP is the second leading type of Nosocomial infection, it accounts for 13-18% of total infection. It is the leading cause of infection related deaths in hospitalized patients with mortality rate of 33-50%. Higher mortality rates were seen when patients are bacteremic or have Pneumonia caused by *Pseudomonas aeruginosa* and *Acinetobacter* spp. Approximately 60% of HAP are caused by aerobic Gram negative bacilli like *Klebsiella pneumoniae*, *E.coli*, *Serratia marcesens*, *Acinetobacter* spp *Enterobacter* spp and *Pseudomonas aeruginosa*. 
*S. aureus* cause 13-40% cases of HAP. MRSA and multi drug resistant (MDR) organism are the major etiological agent of pneumonia[44].

**Bacterial agents causing LRI**

A study from US involving one hundred fifty-four hospitalized children with LRIs reported 79% of children were positive for microbial growth. Respiratory bacterial pathogens were identified in 60% (of which 73% were *Streptococcus pneumoniae*). Children with typical bacterial or mixed bacterial/viral infections had the greatest inflammation and disease severity. [46]. A systematic review of literature reported aetiologies for 5919 Community acquired pneumonia(CAP) patients diagnosed between May 1995 and December 2012 which included 1421 (24.0%), 3571 (60.3%) and 927 (15.7%) from Cambodia, Thailand and Vietnam, respectively. *Streptococcus pneumoniae* and *Haemophilus influenzae* were the most common pathogens. Gram-negative bacteria such as *Burkholderia pseudomallei* and *Klebsiella pneumoniae* were also frequently isolated, particularly in bacteremic CAP in Thai adults and Cambodian children. Reports from study on LRI in hospitalized children in New Caledonia showed 87.9% cases were positive and bacteria represented 18.4% of the pathogens detected[47]. In a study done on LRI in children’s emergency hospital Timisoara, One hundred twenty bacterial strains were isolated from 69 children. Gram-negative bacteria represented 77.5% of isolates, 20% were Gram-positive and the rest were Non-fermenting Gram-negative strains. Gram-negative isolates *Pseudomonas aeruginosa* (31.11%), *Klebsiella pneumoniae* (23.65%) and Enterobacter spp (12.90%) were the majority. *Staphylococcus aureus* (79.1%) and Coagulase negative Staphylococci (12.5%) dominated among Gram-positive cocci[48].

In a south Indian study on LRI, the bacteria isolated were *Klebsiella* spp (51.1%), *Pseudomonas aeruginosa* (19.1%), *E. coli* (8.6%), *Acinetobacter spp* (7.3%), *Staphylococcus aureus* (6.9%), *Streptococcus pneumoniae* (5.8%) and *Enterococci* (1.3%) [49].
Complications of Pneumonia

Patients with pneumonia can be treated often successfully with medication. But some people, especially those in high-risk groups, may experience complications, including:

**Bacteremia:** Bacteria that enter the bloodstream from the lungs can spread the infection to other organs, potentially causing organ failure.

**Lung abscess:** An abscess occurs if pus forms in a cavity in the lung. An abscess is usually treated with antibiotics. Sometimes, surgery or drainage with a long needle or tube placed into the abscess is needed to remove the pus.

**Pleural effusion:** Pneumonia may cause fluid to build up in the thin space between layers of tissue that line the lungs and chest cavity (pleura). If the fluid becomes infected, you may need to have it drained through a chest tube or removed with surgery.

**Dyspnea:** If pneumonia is severe or the patient have chronic underlying lung diseases.

2.4 ANTIBIOTIC RESISTANCE OF BACTERIAL AGENTS OF RTI

A clinical report from American academy of Paediatrics, widely documented that inappropriate antibiotic prescribing, especially for upper respiratory tract infections (URIs) of viral origin, is common in ambulatory care. As many as 10 million antibiotic prescriptions per year are directed toward respiratory conditions for which they are unlikely to provide benefit. Recent evidence shows that broad-spectrum antibiotic prescribing has increased and frequently occurs when either no therapy is necessary or when narrower-spectrum alternatives are appropriate. This can lead to drug resistance to newer antibiotics and unwanted medical expenses. The threats of antibiotic resistance can be prevented by promoting judicious antibiotic prescribing, which encompasses both reducing overuse and ensuring that appropriate agents are prescribed.
According to reports from a systematic review by Sophie Goyet et al., *S. pneumoniae* displayed low resistance to penicillin A (mean 6.4%, range 1.6–11.0) and amoxicillin-clavulanic acid (mean 3.1%, range 0.0–14.0), varying degrees of resistance to Cephalosporins (to ceftriaxone: mean 9.8%, range 5.7–33.3, to cefuroxime: mean 47.5%, range 47.5–47.4); moderate resistance to chloramphenicol (mean 48.3%, range 12.0–78.5) and high level of resistance to trimethoprim/sulfamethoxazole (SXT) (mean 78.2%, range 51.6–100.0). *H. influenzae* displayed high levels of resistance to SXT (mean 76.8%, range 42.8–83.0), with a rising trend over time. Of all 98 *K. pneumoniae* isolates tested in Thailand, Vietnam or Cambodia, 26.5% showed resistance to amoxicillin-clavulanic acid. Resistance in *K. pneumoniae* to cephalosporins was reported but carbapenems and amino-glycosides remained generally active on most isolates[50].

In a study from Timisoara, Colistin was the most efficient antibiotic active on Gram-negative bacteria, followed by Levofloxacin and Imipenem. All Gram-positive isolates were susceptible to Vancomycin and Linezolid [51].

**Antibiotic Agents**

Vast majority of children diagnosed with pneumonia in the outpatient setting are treated with oral antibiotics. High-dose amoxicillin is used as a first-line agent for children with uncomplicated community-acquired pneumonia, which provides coverage for *S. pneumoniae*. Second or third generation cephalosporins and Macrolide antibiotics such as azithromycin are acceptable alternatives but should not be used as first-line agents because of lower systemic absorption of the cephalosporins and pneumococcal resistance to macrolides. Macrolide antibiotics are useful in school-aged children, because they cover the most common bacteriologic and atypical agents (*Mycoplasma, Chlamydia phila, Legionella*). However, increasing levels of resistance to macrolides among pneumococcal isolates should be considered (depending on local resistance rates). Many suggest that penicillin and Macrolide resistance among *S. pneumoniae* isolates has been increasing.

Hospitalized patients can be safely treated with narrow-spectrum agents such as ampicillin, and this is the mainstay of current guidelines for pediatric
community-acquired pneumonia. Children who are toxic appearing should receive antibiotic therapy that includes vancomycin (particularly in areas where penicillin-resistant pneumococci and Methicillin-resistant \textit{S.aureus} [MRSA] are prevalent) along with a second- or third-generation cephalosporin. If gram-negative pneumonia is suspected beta-lactam antibiotics are administered.

**Antibiotic Treatment guidelines for RTI**

**URI**

1. Initiation of empiric anti-microbial therapy
   - Amoxicillin-clavulanate
   - High dose amoxicillin-clavulanate
   - Doxycycline
   - Third generation oral cephalosporin (cefixime or cefpodoxime) plus clindamycin, as indicated

2. Empiric treatment in adults and children with penicillin allergy
   - Doxycycline (not suitable for children)
   - Respiratory fluoroquinolone (levofoxacin, moxifloxacin)
   - Combination therapy (clindamycin plus third generation oral cephalosporin [cefixime or cefpodoxime])

3. Duration of therapy before considering alternative management strategies
   - If symptoms worsen after 48–72 hours of initial empiric antimicrobial therapy
   - If symptoms fail to improve despite 3–5 days of initial empiric antimicrobial therapy
   - Intranasal saline irrigation (physiologic or hypotonic saline[52]).
1. Outpatient anti-infective treatment
   - Amoxicillin
   - Macrolide antibiotics
   - Influenza antiviral therapy

2. Inpatient anti-infective treatment
   - Ampicillin or penicillin G
   - Empiric therapy with a third-generation parenteral cephalosporin
   - Empiric combination therapy with a macrolide (oral or parenteral), in addition to a beta-lactam antibiotic
   - Vancomycin or clindamycin in addition to a beta-lactam (for methicillin-resistant *Staphylococcus aureus* [MRSA][53].

2.5 *STREPTOCOCCUS PYOGENES*

2.5.1 Historical Perspectives

*Streptococcus pyogenes* was first described by Billroth in 1874 in patients with Erysipelas and wound infections. In 1853, Fehleisen isolated chain forming organism in pure culture from peri-erysipelas lesions. In 1884, Rosenbach named the organism *Streptococcus pyogenes*. Studies by Schottmueller in 1903 and J.H. Brown in 1919 led to knowledge of different patterns of hemolysis described as Alpha, Beta and Gamma hemolysis. A later development in this field was the Lancefield classification of Beta hemolytic *Streptococci* (BHS) by serotyping based on M-protein precipitin reactions. Lancefield established the critical role of M protein in disease causation [54].
In early 1900’s, Doctez, George and Dick identified hemolytic Streptococcal infection as the cause of Scarlet fever. The epidemiological studies of the mid 1900’s helped to establish the link between GAS infection and acute Rheumatic fever (ARF) and acute glomerulonephritis[17].

**Current Taxonomy**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Firmicutes</td>
</tr>
<tr>
<td>Class</td>
<td>Bacilli</td>
</tr>
<tr>
<td>Order</td>
<td>Lactobacillales</td>
</tr>
<tr>
<td>Family</td>
<td>Streptococcaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Streptococcus</td>
</tr>
<tr>
<td>Species</td>
<td>Pyogenes</td>
</tr>
</tbody>
</table>

2.5.2 **Classification of Streptococci**

The genus Streptococcus includes important pathogens and commensal of mucosal membranes of the Upper Respiratory Tract (URT) and for some species, the intestines.

The classification of *Streptococci* is based on

- Colony morphology and hemolytic reactions on Blood agar.
- Serologic specificity of the cell wall, group-specific substance and other cell wall or capsular antigens.
- Biochemical reactions and resistance to physical and chemical factors.
- Ecologic features.
Figure 2.1 Classification of Streptococci
Table 2.3 Characteristics of Medically important Streptococci [55]

<table>
<thead>
<tr>
<th>Name</th>
<th>Group specific substance</th>
<th>Hemo lysis</th>
<th>Habitat</th>
<th>Important laboratory criteria</th>
<th>Common &amp; important diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Streptococcus pyogenes</em></td>
<td>A</td>
<td>Beta</td>
<td>Throat, Skin</td>
<td>Large colonies (&gt;0.5mm), PYR test positive, inhibited by Bacitracin, Ribose not fermented</td>
<td>Pharyngitis, impetigo, Rheumatic fever, Glomerulo nephritis, toxic shock</td>
</tr>
<tr>
<td><em>Streptococcus agalactiae</em></td>
<td>B</td>
<td>Beta</td>
<td>Female genital tract, lower GI</td>
<td>Hippurate hydrolysis, CAMP test Positive.</td>
<td>Neonatal sepsis and meningitis, bacteremia in adults.</td>
</tr>
<tr>
<td><em>Streptococcus dysgalactiae subspecies equisimilis, others</em></td>
<td>C,G</td>
<td>Beta</td>
<td>Throat</td>
<td>Large (&gt;0.5mm) colonies, Ribose and Trehalose fermentation</td>
<td>Pharyngitis, endocarditis, pyogenic infections.</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em> (and other Enterococci)*</td>
<td>D</td>
<td>Gamma, Alpha</td>
<td>Colon</td>
<td>Growth in presence of bile, hydrolyze esculin, growth in 6.5% NaCl, PYR-positive</td>
<td>Abdominal abscess, UTI, endocarditis.</td>
</tr>
<tr>
<td><em>Streptococcus bovis</em> group</td>
<td>D</td>
<td>Gamma</td>
<td>Colon, biliary tree</td>
<td>Growth in presence of bile, hydrolyze esculin, no growth in 6.5% Nacl, degrades starch</td>
<td>Endocarditis, common blood isolate in colon cancer, biliary disease.</td>
</tr>
<tr>
<td><em>Streptococcus anginosus group</em> (S.anginosus, S.intermedius, S.constellatus,S.milleri group)*</td>
<td>F(A,C,G) and untypeable</td>
<td>Alpha, Beta, Gamma</td>
<td>Throat, colon, female genital tract.</td>
<td>Small (&lt;0.5mm) colony variants of β-hemolytic species. Group A are bacitracin-resistant and PYR-negative. Carbohydrate fermentation patterns</td>
<td>Pyogenic infections, including brain abscesses.</td>
</tr>
<tr>
<td><em>Viridans streptococci</em> (many species)*</td>
<td>Usually not typed or untypeable</td>
<td>Alpha, Gamma</td>
<td>Mouth, throat, colon, female genital tract</td>
<td>Optochin-resistant. Colonies not soluble in bile. Carbohydrate fermentation patterns</td>
<td>Dental caries(S.mutans), endocarditis, abscesses(with many other bacterial species), some species, such as S.mitis, have high-level resistance to Penicillin</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em></td>
<td>None</td>
<td>Alpha</td>
<td>Nasopharynx</td>
<td>Susceptible to Optochin. Colonies soluble in bile, quelling reaction-positive</td>
<td>Pneumonia, meningitis, endocarditis, otitis media, sinusitis</td>
</tr>
<tr>
<td><em>Peptostreptococcus</em></td>
<td>None</td>
<td>Gamma, Alpha</td>
<td>Mouth, colon, female genital tract</td>
<td>Obligate anaerobes</td>
<td>Abscesses (with multiple other bacterial species)</td>
</tr>
</tbody>
</table>
2.5.3 Prevalence of GAS

Group A Streptococci cause approximately 5-15% of all pharyngitis infections, accounting for several million cases of Streptococcal pharyngitis each year. This infection is rarely diagnosed in children younger than 2 years[34]. *Streptococcus pyogenes* remains as carrier in healthy children, serving as a source of infection. In a study done in Turkish healthy children, a total of 61 isolates of beta-hemolytic *Streptococci* were obtained from healthy children. Of the isolates, 75.4% (46 of 61 isolates) were group A, 3.3% (2 isolates) were group B, 4.9% (3 isolates) were group C, 9.8% (6 isolates) were group G, and 6.6% (4 isolates) were non-A,B,C,G.(56). A report from Washington showed *Streptococcus pyogenes* prevalence during pharyngitis was 20.2% [57].

Predisposing factors for Streptococcal infections

Increased exposure to respiratory pathogens e.g.: - crowded settings as those found in

- Over crowded schools and daycare favor the colonization.
- Environmental factors, e.g: passive smoking, exposure to pollutants
- Defects in the immune system: eg, familial disposition, Immunological factors like deficiency in IgG (seen in young children), deficiency in IgA.

Group A Streptococci

Group A Streptococci (GAS) or *Streptococcus pyogenes* is a beta hemolytic Streptococci responsible for most cases of Streptococcal illness. Several virulence factors contribute to the pathogenesis of GAS, such as M protein, hemolysin and extracellular enzymes. They cause a wide variety of human infections ranging from mild skin infections or a sore throat to severe, life-threatening condition.
2.5.4 Virulence Factors of *S.pyogenes*

**Figure 2.2 Cell surface structure of *S.pyogenes***

**Intrinsic constituents**

*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.

**M-protein**

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 highly depends on M protein. Strains lacking M protein are
essentially nonpathogenic. Streptococci isolated from chronic pharyngeal carriers contain little or no M protein and are also relatively avirulent[56].

Pathogenesis

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, 80 M protein 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[57].

emm Typing

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[58]. This is based on sequence at the 5’ end of a locus (emm) that is present in all isolates. More than 150 emm types have been described. Based on identification of more than 160 nucleotide base at 5’ terminals of hyper variable region, it is assigned into various emm type. Accurate identification of GAS is an essential part of epidemiological and pathogenic studies of Streptococcal diseases.

Hyaluronic acid capsules

Most Streptococcal strains are enveloped by hyaluronic acid capsule that serves as an accessory virulence factor by inhibiting phagocytosis.
Lipoteichoic acid and protein F

These are cell wall constituents. They propagate the adherence of *Streptococcal pyogenes* to fibrinoecitin on the surface of human epithelial cells. This is an important event in the initiation of the infectious process.

Serum Opacity Factor (SOF)

SOF was first discovered in 1938 by Australians; Ward and Rudd. 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. SOF production was reported to be 83.3% from cases of pyoderma. Various other reports give 34-50% of prevalence. The difference in SOF by various strains positivity can be due to variation in M proteins of each strains [58].

T and R-protein

T and R protein are other Streptococcal protein, which does not appear to be a virulence factor but shows significant antigenic variation among clinical isolates. Therefore, T typing is a useful adjunct to M typing studies of GAS infections. They are present in many serotypes of *Streptococcus pyogenes*. R-protein is present in some types of *Streptococcus pyogenes* (types 2,3,28 and 48)[59].

Extracellular enzymes and toxins

In addition to somatic constituents, GAS produces a wide variety of extracellular enzymes and toxins important in pathogenesis.

1. *Streptococcal pyogenic exotins* (SPE)

They are also called ‘erythrogenic’ toxin. This is because according to Dick test, erythematous reaction was produced on injection into susceptible individuals. The family of SPE’s include A, B, C, and F.
Functions

They are responsible for the rash of scarlet fever. They are also responsible for other pathogenic effects on the host including pyrogenicity, cytotoxicity and enhancement of susceptibility to endotoxin. SPE B is a precursor for a cysteine protease that is another determinant of virulence.

GAS isolates associated with streptococcal toxic shock syndrome encode certain SPEs (ie. A, C,E) capable of functioning as super antigens. These antigens induce a marked febrile response, induce proliferation of T lymphocytes and induce synthesis and release of multiple cytokines, including Tumor necrosis factor, Interleukin-1 beta and Interleukin-6. This activity is attributed to the ability of the super antigen to simultaneously bind to the V-beta region of the T cell receptors and to class II MHC antigens of antigen-presenting mononuclear cells, resulting in widespread nonspecific t-cell proliferation and increased production of Interleukin-2.

Hemolysins

*Streptococcus pyogenes* also elaborates 2 distinct Hemolysin; Streptolysin-O and Streptolysin S.

**Streptolysin-O**

Streptolysin-O activity is seen only in pour plates and not in surface cultures and it is highly immunogenic. Streptolysin O is toxic to a wide variety of cell types. It may be important in contributing to virulence having two important activities: Cardiotoxic and Leucotoxic.

Streptolysin O is highly immunogenic, and determination of the antibody responses engendered to this protein Anti Streptolysin O (ASO) titre is often useful in the serodiagnosis of recent infection. An ASO titre in excess of 200 IU is considered significant in adults and excess of 333 IU in pediatrics, and suggests recent or recurrent infection with Streptococci [59].
**Streptolysin S**

It is responsible for haemolysis seen around streptococcal colonies on the surface of blood agar plates. It may not be immunogenic. It is capable of damaging polymorphonuclear leukocytes and sub cellular organelles. Streptolysin ‘S’ and ‘O’ are produced not only by GAS, but also by some strains of GCS and GGS [20].

**Streptokinase (fibrinolysin)**

It is an antigenic protein. Neutralizing antibodies against it appears in convalescent sera. It promotes the lysis of human fibrin clots by activating a plasma precursor (plasminogen).

**Deoxyribonucleases**

It is also called Streptodornase. It helps in depolymerisation of DNA. It helps to liquefy the thick pus and may be responsible for thin serous character of streptococcal exudates. Four distinct DNAase have been recognized. DNAase A, B, C, D. DNAase B is the most pathogenic to human beings.

**Nicotinamide adenine dinucleotidase (NADase)**

It is antigenic in nature and neutralized by the antibody in convalescent sera. It may be leucotoxic.

### 2.5.5 Pathogenicity

Group A Streptococci are extracellular bacterial pathogens which produce a variety of pyogenic infections involving the mucous membranes, tonsils, skin, and deeper tissues, including pharyngitis, impetigo, pyoderma, erysipelas, cellulitis, necrotizing fasciitis, toxic streptococcal syndrome, scarlet fever, septicemia, pneumonia, and meningitis. Infections may be mild to extremely severe. Complications such as sepsis, pneumonia, and meningitis can occur, which may lead to a fatal outcome.
Pharyngitis

Group A streptococci are the most common bacterial cause of pharyngitis and primarily affect school-age children 5 to 15 years of age. All ages are susceptible to spread of the organism under crowded conditions, such as those at schools and military facilities. Pharyngitis and its association with rheumatic fever are seasonal, occurring in the fall and winter. This is in contrast to pyoderma or skin infection, which occurs in the summer and can be associated with the production of acute glomerulonephritis. Groups C and G can also cause pharyngitis and must be distinguished from group A organisms after throat culture. Although they are not considered normal flora, pharyngeal carriage of group A Streptococci can occur without clinical symptoms of disease. Certain M protein serotypes, such as M types 1, 3, 5, 6, 14, 18, 19, and 24 of *S. pyogenes*, are found associated with throat infection and rheumatic fever. [61].

Pyoderma and Streptococcal Skin Infections

Group A Streptococci which invade the skin and cause impetigo are different M protein serotypes from those that cause pharyngitis. In addition, some of the skin strains are associated with production of acute post Streptococcal glomerulonephritis. The skin infections and nephritis are seasonal, usually occurring during the summer months and in temperate climates. The infection is limited to the epidermis, usually on the face or extremities, and is highly contagious.

Group A streptococcal strains may enter the skin through abrasions and other types of lesions to penetrate the epidermis and produce erysipelas or cellulitis. Erysipelas is a distinctive form of cellulitis with characteristically raised and erythematous superficial layers of the skin, while cellulitis affects subcutaneous tissues. Cellulitis may occur from infected burns or wounds. Both erysipelas and cellulitis can be caused by Streptococcal groups A, B, C, and G.
Invasive infection of *Streptococcus pyogenes*

Bacteraemia, Toxic shock syndrome, Necrotizing fasciitis (commonly called flesh-eating disease) are the invasive infections caused by GAS. Untreated group A Streptococcal pharyngitis can result in the following: Acute rheumatic fever, Acute glomerulonephritis, Peritonsillar abscess, Toxic shock syndrome, Impetigo, Cellulitis or abscess, Otitis, Sinusitis, Conjunctivitis, Bronchitis.

Mortality from GAS pharyngitis is rare, but serious morbidity or death may result from one of its complications. Streptococcal pharyngitis without complications rarely poses significant risk for morbidity. However, retropharyngeal, intraorbital, or intracranial abscesses may cause serious sequelae. The risk of mortality is significant in patients who progress to streptococcal toxic shock syndrome, which is characterized by multiorgan failure and hypotension[62].

**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. The nature of biofilm structure and the physiological attributes of biofilm formers confer an inherent resistance to antimicrobial agents, like antibiotics, disinfectants, or germicides. Mechanisms responsible for resistance may be one or more of the following: (i) delayed penetration of the antimicrobial agent through the biofilm matrix, (ii) altered growth rate of biofilm organisms, and (iii) other physiological changes due to the biofilm mode of growth[63].

Biofilm formation by *Streptococcus pyogenes* supports the failure of its antibiotic therapy, in spite of less antibiotic resistance shown by the bacteria. Various recent studies suggest that some subtypes of *Streptococcus pyogenes* produce biofilm and may have a role in chronic carriage of Streptococcus in pharynx.

Studies by L. Baldasseri showed erythromycin sensitive strains showed more thicker biofilm than resistant strains [64]. Studies done by Yukthi Sharma
reported only 7/22 biofilm formers showed Macrolide resistance. This shows biofilm might have a role in chronic carriage of *Streptococcus pyogenes* and its difficulty in eradication [19].

**Post-Streptococcal sequelae**

**Rheumatic fever:** Until 1960, it was a leading cause of death in children and a common cause of structural heart disease. Rheumatic fever causes chronic progressive damage to the heart and its valves. It is the most serious sequelae of hemolytic Streptococcal infections.

This is an autoimmune response secondary to molecular mimicry following GAS pharyngitis. Certain strains of GAS contain cell membrane antigens which is similar to human heart tissue, therefore antibodies formed against such antigen cross react with human heart tissue antigens. Beta Streptococcal serotype (e.g. M types 3,5,18,19,24) is linked directly to acute rheumatic fever. It is characterized by an exudative and proliferative inflammatory lesion of the connective tissue especially that of the heart, joints, blood vessels and subcutaneous tissue. Morbidity from acute Rheumatic fever (ARF) is directly proportional to the rate of Streptococcal infections. The mortality rate has declined steadily over the last 3 decades [65].

The first clinical evidence of RF in India came from Punjab by Wig in 1935 and in studies on rheumatism in childhood and adolescence by Kutumbiah in 1940. Further surveys established the presence of RF/RHD and considered it to be the commonest heart disease in the country by mid 1950s[66].

Primary prevention of Rheumatic fever/ Rheumatic heart disease requires identification of GAS sore throat and use of penicillin to eradicate the Streptococci. The requirement for primary prevention consist of (i) public awareness regarding danger of RF from sore throat (ii) identification of sore throat as being due to GAS infection, and (iii) use of injectable penicillin to cure the infection[66].
Acute Glomerulonephritis

Acute glomerulonephritis is a disease characterized by the sudden appearance of edema, hematuria, proteinuria, and hypertension with or without oliguria. It can follow Streptococcal infections. Post streptococcal glomerulonephritis follows infection with only certain strains of Streptococci, designated as nephritogenic. Acute post Streptococcal glomerulonephritis (APSGN) follows pyodermatitis with streptococci M types 47, 49, 55, 2, 60, and 57 and throat infection with streptococci M types 1, 2, 4, 3, 25, 49, and 12.

APSGN is believed to be an immune-mediated disease, in which an immune complex containing a Streptococcal antigen is deposited in the affected glomeruli. Urea-nitrogen retention, high blood pressure, low levels of serum complement is the symptoms. Majority recover completely whereas some develop chronic glomerulonephritis and ultimate kidney failure[67].

Prevalence of GAS

Group A Streptococcal bacteria cause approximately 5-15% of all pharyngitis infections, accounting for several million cases of Streptococcal pharyngitis each year. This infection is rarely diagnosed in children younger than 2 years [34]. Healthy children harbouring Streptococcus pyogenes as carrier serve as source of infection. In a study conducted on Turkish healthy children, a total of 61 isolates of beta-hemolytic Streptococci were obtained from healthy children. Of the isolates, 75.4% (46 of 61 isolates) were group A, 3.3% (2 isolates) were group B, 4.9% (3 isolates) were group C, 9.8% (6 isolates) were group G, and 6.6% (4 isolates) were non-A,B,C,G[54]. A report from Washington showed Streptococcus pyogenes prevalence during pharyngitis was 20.2% [68].

Other Beta hemolytic Streptococci causing RTI

Group C streptococci (GCS) and group G streptococci (GGS) are normal commensal flora of the human upper airway and frequently are asymptomatic.
colonizers of the skin, gastrointestinal tract and female genital tract. They can occasionally cause infections of the skin and soft tissues, pharyngitis, bacteremia and endocarditis, septic arthritis and osteomyelitis, puerperal infections, and meningitis.

GCS and GGS of human origin are now considered to constitute a single subspecies, *Streptococcus dysgalactiae* subsp. *equisimilis*. A comparison of the complete genome sequence of a clinical isolate of GGS *S.dysgalactiae* subsp. *equisimilis* with that of other Streptococcal species demonstrated that it is most closely related to *S. pyogenes*, with 72 percent sequence similarity (69) 

* S. *dysgalactiae* subsp. *equisimilis* shares many virulence determinants with *S. pyogenes*, including the antiphagocytic M protein, streptolysin O, streptolysin S, streptokinase, and one or more pyrogenic exotoxins similar to those implicated in streptococcal toxic shock [69].

2.6 GROUP C STREPTOCOCCI

Group C Streptococci (GCS) accounts for about 5% of all cases of streptococcal infection in adult humans. These may be divided into 4 groups biochemically. They are *S.dysgalactiae*, *S.equi*, *S.equisimilis*, *S.zooepidemicus*. *S.equisimilis* is isolated from human sources.

**Pathogenicity**

The symptoms of sore throat caused by beta GCS in humans can range in severity from very mild to incredibly severe presentation. Aside from the sore throat, other symptoms include low to high fever, neck swelling, enlarged tonsils, and hoarseness and even acute to moderate nausea. More severe symptoms included arthritis, pneumonia and even bacteraemia which could lead to toxic shock[35].
2.7 GROUP G STREPTOCOCCI (GGS)

Identified in 1935 by Lancefield and Hare, GGS are part of normal flora of the pharynx, Gastro intestinal tract, Genital tract and skin. It is also present as commensal in throats of monkeys or dogs. These are typical chain forming gram positive cocci, facultative anaerobes which produce small or large colonies on sheep blood agar. It is usually not inhibited by Bacitracin disc.

Pathogenicity

In recent years, GGS have been reported to cause a variety of human infections, such as sore throat, pharyngitis, cellulitis, meningitis, infection of the heart valves (endocarditis) and sepsis. The spectrum of GGS infections ranged from mild skin and soft tissue infection. Studies show that it can lead to invasive diseases accounting to 46%, including urogenital infection (10%); lower respiratory tract infection 10%; pharyngitis 8%; endocarditis and catheter infection (7%); and others(19%) such as peritonitis, pelvic abscess, rectal abscess, and septic arthritis. Four of the 6 persons with pharyngitis were assumed to be colonized with the organism. Eight (24%) of 34 skin and soft tissue infections were associated with bacteremia, 5 (15%) with osteomyelitis, and 20 (59%) with polymicrobial infections[70].It is reported that mortality rates for patients with GGS bacteremia also vary ranging from 5% to 30% [71].It is an important cause of endocarditis, septicemia and septic arthritis.

They cause isolated exudative or epidemic pharyngitis and cellulitis, indistinguishable clinically from GAS disease [72]. Beta hemolytic GCS and GGS and hemolysin deficient variants cause epidemics of exudative pharyngitis pharyngio- tonsillitis. Rapid diagnosis of pharyngeal carriage of GCS and GGS strains that lead to glomerulonephritis, toxic-shock syndrome and Rheumatic fever, may prevent unnecessary death and disability [73].
In a study done in Chennai, a total of 131 GCS/GGS isolates included in the study, 94 were throat isolates from asymptomatic school children, the remaining 37 were clinical isolates from blood (2), throat swabs (25), skin swabs (5), pus (4) and pleural fluid (1). Of the 131 isolates, 15 (11.4%) were GCS and 116 (88.5%) were Group G Streptococcus[74].

2.8 STREPTOCOCCAL VACCINES

A multivalent vaccine containing amino-terminal M protein fragments from 26 different serotypes of GAS was constructed by recombinant techniques. (M type 24, 5, 6, 19, 29, 14, 1.0, 12, 28, 3, 1.2, 18, 2, 43, 13, 22, 11, 59, 33, 89, 101, 11, 114, 75, 76, 92). The vaccine was free of tissue-cross-reactive epitopes and was believed to have the potential to induce broadly protective antibody responses in humans that could have a significant impact on the overall disease burden caused by group A streptococci [75].

More than 80 different M serotypes cause infections, only a limited number of M protein serotypes are practical for a type-specific vaccine. In addition, it has been observed that M serotypes which cause infection are cyclic in populations and also that different M serotypes are responsible for rheumatic fever in different parts of the world. Vaccines which targeted epitopes common to all M proteins also appeared to be effective against colonization. Common group A Streptococcal antigens other than M protein have also been under investigation as vaccines for protection against colonization and infection.

Currently there is no commercial GAS vaccine available as the development of safe GAS vaccines is challenging, with greater than 150 M types, antigenic variation within the same serotype, large variations in the geographical distribution of serotypes, and the production of antibodies cross-reactive with human tissue which can lead to host auto-immune disease. So researches are focused on Cell wall anchored, cell membrane associated, secreted and anchorless proteins have all been targeted as a potential GAS vaccine candidates[76]
2.9 ANTIBIOTIC RESISTANCE IN GAS

Penicillin is the drug of choice for the treatment of GAS infection. For patients sensitive to beta-lactam antibiotics, Macrolides are often the recommended substitute. Penicillin resistance has not yet been described in *S.pyogenes*, but resistance Macrolides has been widely reported [77]. Azithromycin which can be given orally, once a day makes it an attractive option for Pharyngitis. Clindamycin is given for multiple recurrent infection of Streptococcus [78].

The relationship between *S.pyogenes* resistance to erythromycin and macrolide consumption in Spain was studied. Erythromycin resistance was highly correlated with the consumption of total Macrolides. A progressive increase in the erythromycin resistance curve was seen after the consecutive introduction of both twice a day and once daily Macrolides, which contributed to the increase in the total Macrolide consumption, replacing tds Macrolide prescription[79].

**Macrolides**

These drugs (erythromycin, azithromycin, clarithromycin, and roxythromycin and ketolide, telithromycin) bind to the 50s subunit of the ribosome, and the binding site is a 23s rRNA. They may interfere with formation of initiation complexes for peptide chain synthesis or may interfere with amino acyl translocation reactions.

Some Macrolide-resistant bacteria lack the proper receptor on the ribosome (through methylation of the rRNA). This may be under plasmid or chromosomal control. Over the last several decades there has been increased resistance to Macrolides reported from several countries. European surveillance in Italy identified that 32% of GAS isolates exhibits resistance to Macrolides. France has reported a steady escalation of Erythromycin resistance reaching 23%. Portugal identified 11% of GAS isolates as resistant to Macrolides. Resistance in other European countries during 1990’s fell between 1% and 7% [17].
CLASSIFICATION

Types of Macrolide resistance

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

2.9.1 Genes encoding for Macrolide Resistance

The genes classes that encode for the phenotypes are also of two main types, “erm” and “mef” gene.

erm- The bacterial gene class coding for erythromycin ribosomal methylase, which methylates a single adenine in 23s rRNA, itself a component of 50s rRNA, multiple erm gene types are recognized. There can be both constitutive (always on) and inducible variants of erm, the phenotypes of which are designated as either cMLSb
or iMLS\textsubscript{B} respectively. \textit{erm} gene regulation is complex and may involve more than one mechanism. cMLS\textsubscript{B} is coded by \textit{erm} B gene and iMLS\textsubscript{B} by \textit{erm} TR gene.

\textit{mef} – macrolide efflux pump. \textit{mef} gene actively pumps out macrolides (14 and 15 ring), but not lincosamides, streptogramins or ketolides[77].

### 2.9.2 Phenotypic Characterizations of Macrolide Resistance

Double Disk susceptibility Testing (D Test) is performed for the detection of phenotypic characterization of Macrolide and clindamycin resistance by placing Erythromycin and clindamycin disks is placed 12 mm distance on a blood agar. Three types of results are found: iMLS\textsubscript{B} – The clindamycin zone is blunted towards the erythromycin because the erythromycin induces clindamycin resistance. cMLS\textsubscript{B} – No zone is forms around either erythromycin or clindamycin because \textit{erm} gene is fully expressed at all times. M type – No change in the clindamycin zone induced by erythromycin because \textit{mef} A gene does not pump out clindamycin regardless of erythromycin presence. [80].

### 2.9.3 Prevalence of Macrolide Resistance in \textit{Streptococcus Pyogenes}

Many factors are probably involved in the emergence and spread of antibiotic resistance, but antibiotic consumption seems to be the main driving force. In Argentina, although prevalence of erythromycin resistance in Streptococci is still low, it has increased significantly in recent years in most regions [80]. Studies in Japan, Finland and elsewhere show a strong correlation between national macrolide consumption and resistance in GAS. Between 1998 and 2001 a statistically significant increase in GAS resistant to Erythromycin and Azithromycin was observed in Spain[81].

Various rates of Macrolide resistance of \textit{Streptococcus pyogenes} are reported worldwide, like 37% in Pennsylvania, France 6.2%, Poland 12%, etc [83]. According to Indian studies; from Chennai 16.2% of Macrolide resistance was reported, 38.23% from Mangalore and 29.4% from North India by MR.Capoor[19,20].
2.9.4 Prevalence of Macrolide Resistance in Group C and Group G Streptococcus

Various studies have reported different rates of macrolide resistance in GCS and GGS. Report by Jun Yin, Sangjie Yu et.al. from China reported 43.8% resistance in 2010[84]. Studies from Netherland by Pavlovi ljiljana et. al reported very less Macrolide resistance in GCS(6.9%), followed by, GGS(4.6%) in 2006[85]. In studies done in Spain by Merino Díaz L et.al. (2007) Erythromycin resistance was 5.3% in GCS and 33.3% in GGS [86].

In Indian studies; Report from Chennai in 2006 showed Among macrolide resistant strains, M phenotype accounted for the majority (73.6%) and only 26.31% showed cMLS phenotype[87]. In another report in 2013, twelve out of 16 (75%) erythromycin resistant GCS/GGS isolates showed MLSB resistance and four isolates (25%) were M phenotype. Of the 12 isolates which exhibited MLSB resistance, seven were of cMLSB phenotype and five showed iMLSB phenotype. Among the 16 erythromycin resistant GCS/ GGS isolates, nine (56.25%) were positive for \textit{erm} (B) gene, three (18.75%) for \textit{erm} (A) gene and four (25%) were positive for \textit{mef} (A) gene. The seven cMLSB phenotype isolates were positive for \textit{erm} (B) gene[88].

2.10 OTHER BACTERIA CAUSING RTI

2.10.1 \textit{Streptococcus Pneumoniae}

\textit{S. pneumoniae} is a leading cause of morbidity and mortality in the United States, resulting each year in an estimated 3,000 cases of meningitis, 50,000 cases of pneumonia, and 7,000,000 cases of otitis media [89].

\textit{S. pneumoniae} are Gram-positive, lanceolate shaped diplococci (elongated cocci with a slightly pointed outer curvature), but they may also occur singly and in short chains. When cultured on BA, they produce alpha haemolytic colonies. Individual cells are between 0.5 and 1.25\,\mu m in diameter. They are non-sporing, non-motile, catalase negative and ferment glucose to lactic acid. Unlike
other Streptococci, they do not display an M protein, they hydrolyze inulin, and their cell wall composition is characteristic both in terms of their peptidoglycan and their teichoic acid.

*Streptococcus pneumoniae* often colonizes the nasopharynx. They are also common causes of pneumonia (lobar type), paranasal sinusitis and otitis media, sepsis and meningitis which are usually secondary to one of the former infections. It also causes osteomyelitis, septic arthritis, endocarditis, peritonitis, cellulites and brain abscesses. *S.pneumoniae* is currently the leading cause of invasive bacterial disease in children and elderly.*S.pneumoniae* is known in medical microbiology as the *Pneumococcus*, referring to its morphology and its consistent involvement in Pneumococcal pneumonia[89].

**Pathogenesis**

They produce no toxins of significance. The virulence of the organism is a function of its capsule, which prevents phagocytosis or delays infection.

**Hyaluronate lyase**

Hyaluronate lyase is another major surface protein of *S.pneumoniae* with potential antigenetically variable properties that might be essential for full pneumococcal virulence.

**Pneumolysin**

It is yet another virulence factor of Pneumococci that penetrates the physical defenses of the host. Pneumolysin is a 53-kDa protein produced by all clinical isolates of the pathogen [90].

**Autolysins**

These are members of a widely distributed group of enzymes that degrade the peptidoglycan backbone of bacterial organisms. The action of these cell
wall-degrading enzymes ultimately leads to cell lysis. These enzymes are located in the cell envelope and are also postulated to play roles in a variety of physiological cell functions associated with cell wall growth[90].

**Predisposing factors**

Predisposing factors such as patients at extremes of age younger age weaning from breast milk in less than 6 months of age,RTI that damage surface cells, abnormal accumulation of mucus which protect Pneumococci from phagocytosis, abnormal circulatory dynamics, Other mechanisms like malnutrition, general debility, and anaemia.

**Pathogenicity**

Fever, chills and sharp pleural pain, tachypnoea, crackles and wheezing are the usual clinical presentation. Sputum is rusty coloured and similar to alveolar exudate. Pneumococcal pneumonia must be differentiated from pulmonary infarction, atelactasis, neoplasm, congestive heart failure. From the Respiratory Tract they disseminate to other parts of the body including ears e.g,otitis media. Infection sometimes extends from the mastoid to the meninges. Bacteremia from pneumonia has a triad of severe complications, meningitis, endocarditis and septic arthritis.

2.10.1.1 **Antibiotic resistance of S.pneumoniae**

Clinical resistance to penicillin in *S.pneumoniae* was first reported by researchers in Boston in 1965, subsequently this phenomenon was reported from Australia (1967) and South Africa (1977). Since these early reports, penicillin resistance has been encountered with increasing frequency in strains of *S.pneumoniae* from around the world [91].

In South Africa, strains resistant to penicillin and Chloramphenicol as well as multi-resistant strains have been isolated. Similar patterns have been reported from Spain. (Peter C. Appelbaum, Vol.15. pg 77-83). *S.pneumoniae* was once among the most highly penicillin susceptible bacteria however, reports of multi
drug resistant strains have been published since the late 1970s. The rapid spread of resistant clones and the emergence of new variants of resistance mechanisms call for effective surveillance systems and collaboration among clinicians, scientists, the pharmaceutical industry, and regulatory and public health agencies[92].

Unfortunately, *S.pneumoniae* is reported to be becoming increasingly resistant to variety of antibiotics. Penicillin non-susceptible *S.pneumoniae* ranges from 25% to 50% and rates of macrolide resistance among *S.pneumoniae* are reported as high as 31%. A high prevalence of resistance to other antimicrobial classes are prevalent among Penicillin-resistant strains. Newer quinolones (eg.Gatifloxacin, Gemifloxacin and Moxifloxacin) have better anti-pneumococcal activity invitro[92].

In Atlanta, the annual incidence of invasive Pneumococcal infection was 30 cases per 100,000 populations. Isolates from 25% of the patients were resistant to penicillin and 26% were resistant to Trimethoprim-sulfamethoxazole, 15% resistant to Erythromycin, 9% to Cefotaxime, 25% to multiple drugs[93]. Asian Network for Surveillance of Resistant Pathogens (ANSORP) during 1996-1997 documented high prevalence rates (>60%) of erythromycin resistance among clinical isolates of *S.pneumoniae* in Taiwan, Korea, Japan and Vietnam. Surveillance reported that Hong Kong (79.3%) and Japan (71%) showed an alarming high prevalence of erythromycin resistance in Pneumococci [94].

### 2.10.1.2 Pneumococcal vaccines

Pneumococcal conjugate vaccine (PCV) is vaccine used to protect infants and young children against disease caused by the bacterium *S.pneumoniae*. There are currently three PCV vaccines available on the global market: Prevnar, Synflorix, Prevnar 13. Protein-polysaccharide conjugate vaccines against *S.pneumoniae* promise to be an effective public health intervention for children, especially in an era of increasing antimicrobial resistance.
Several most common serotypes are included in the 7-valent polysaccharide conjugate vaccine (PCV7). Serotype coverage of PCV7 was 76.5% in those aged less than 5 years but 88.9% in those aged one year. The introduction of PCV7 into the childhood immunization schedule would reduce the burden of pneumococcal disease in children[95].

2.10.2 Haemophilus Influenzae

*Haemophilus influenzae* is a pleomorphic gram-negative coccobacillus. *H. influenzae* may be either encapsulated (typeable) or unencapsulated (nontypeable). There are six encapsulated serotypes (designated a–f) that have distinct capsular polysaccharides[96,97].

Clinical Features

The most common types of disease caused by *Haemophilus influenzae* include pneumonia, bacteremia, meningitis, epiglottitis, septic arthritis, cellulitis, otitis media, purulent pericarditis, and other less common infections such as endocarditis and osteomyelitis [96,97].

Risk Groups

Unimmunized children younger than 4 years of age and household contacts and day-care classmates of a person with Hib disease are at increased risk of Hib disease.

Incidence

Due to routine use of the Hib conjugate vaccine since 1990, the incidence of Hib disease in infants and young children has decreased by 99% to fewer than 1 case per 100,000 children younger than 5 years of age. In the United States, Hib disease occurs primarily in underimmunized children and among infants too young to have completed the primary immunization series. In developing countries, where
routine vaccination with Hib vaccine is not widely available, Hib remains a major cause of lower respiratory tract infections in infants and children.

The epidemiology of invasive *H. influenzae* disease in the United States has decreased after the introduction of the Hib vaccine. The largest burden of invasive *H. influenzae* disease now occurs in children <5 years of age and older adults ≥ 65 years of age. Nontypeable *H. influenzae* now causes the majority of invasive *H. influenzae* disease in all age groups [97].

### 2.10.2.1 Hib vaccine

Hib vaccine is one among the recommended routine childhood immunizations in the United States. Currently, three monovalent conjugate Hib vaccines and three combination vaccines that contain Hib conjugate are available. Vaccination schedule followed is 6,10,14 weeks and a booster dose at 12 to 18 months. Since licensure of conjugate vaccines for infants (1990) and children (1987), rates of Hib disease among children younger than five years old have declined by 99% in the United States, while rates for adults have remained stable.

The use of Hib vaccines in the private sector is widespread in India for almost a decade. The Ministry of Health and Family Welfare (MoHFW), Government of India (GoI), decided to introduce the vaccine initially in two states tamil nadu and kerela in april 2008. Government of India has introduced Hib as liquid pentavalent vaccine (LPV) combined with DPT and HepB in 10-dose presentation. It is estimated that Hib disease prevention through vaccine use has the potential to reduce India’s under-5 mortality rate by 4 percentage points. The introduction of LPV in India is a major milestone and a step forward to accelerate child survival in India, and progress towards achieving national health goals and Millennium Development Goal 4 [98].
2.11 ANTIBIOTIC RESISTANCE AMONG BACTERIA CAUSING RESPIRATORY TRACT INFECTION

The antimicrobial susceptibility pattern of the isolated bacteria from RTI indicated the varied level of multidrug resistance. The production of Extended spectrum beta lactamases (ESBL) (75%) and metallo betalactamases/carbapenemases (MBL) (25%). Occurrence of Methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant *Staphylococcus aureus* (VRSA) was noted. Imipenem and Amikacin were found to show greater activity against gram negative bacterial isolates where as linazolid, amikacin, ciprofloxacin, ofloxacin and co-trimoxazole were effective against gram positive bacterial isolates. Among the gram positive bacterial isolates *Streptococcus pneumoniae* was found to be susceptible to most of the antimicrobials test [99].