CHAPTER 6

DISCUSSION

This study focused on the prevalence of the bacterial agents causing respiratory tract infection among children and antibiotic resistance pattern in SRM Medical college hospital. This hospital is located in suburban area of Chennai which caters to people of low socio economic status from surrounding villages. A total of 750 children of age groups 0 to 14 years were included in the study, out of which 57.3%(430) were cases of Upper respiratory tract infection and 42.7%(320) were of Lower respiratory tract infection.

In this present study 30.8% (231/750) cases were reported positive for bacterial pathogens causing acute respiratory tract infection (ARI), other causes may be due to viral or infection due to atypical agents like Chlamydia trachomatis, Mycoplasma pneumoniae etc, identification of which was beyond the scope of this study. Out of the total positives, GAS was 30.7%(71), GCS12.5%(29), GGS 16.9%(39), S.aureus 13%(30), Pneumococci 9.1%(21) Klebsiella pneumoniae 7.3%(17), H.influenzae3%(7), E.coli 2.2%(5), Moraxella catarrhalis 3%(7), Enterococci faecium 0.4%(1), Pseudomonas aeruginosa 0.9%(2), and Acinetobacter baumannii 0.9%(2).

A cross-sectional study from Brazil reported that ARI in children under-five years was 25.6%, among which 76.4% had upper and 23.6% lower respiratory infections[25]. Reports estimated that Bangladesh, India, Indonesia, and Nepal together account for 40% of the global respiratory infection mortality and ARI is responsible for about 30-50% of visits to health facilities and for about 20-40% of admissions to hospitals[2]. It is 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[106].
Among 430 cases of Upper respiratory tract infection 156 (36.3%) showed positive bacterial growth. Among 156 positive samples, most common bacteria isolated was GAS 41%(64), followed by GGS 24.4%(38), GCS 18.6%(29), *Staphylococcus aureus* 11.5%(18), and *Moraxella catarrhalis* 2.6%(4), Pneumococci 0.6%(1), and *Hemophilus influenzae* 1.3%(2). The present study which showed most common isolate among the positives in URI was *Streptococcus pyogenes* 41% which was similar to reports from studies done in Pakistan which showed 44.4% [7]. According to an Indian study done in 2010, prevalence of other beta hemolytic Streptococci was only 22.2%, whereas the present study showed higher percentage of beta haemolytic Streptococci. 43% (GGS 24.4% and GCS 18.6%).[2].

In this present study, among 320 cases of lower respiratory tract infection, only 23.4%(75) cases were positive for bacterial growth. Among the 75 positives the most common isolate was *Streptococcus pneumoniae* 26.6%(20), followed by Klebsiella pneumoniae 22.7%(17), *Staphylococcus aureus* 16%(12), *Hemophilus influenzae* 6.7%(5), *E.coli* 6.7%(5), GAS 9.3%(7), *Moraxella catarrhalis* 3%(4), *Pseudomonas aeruginosa* 2.7%(2), *Acinetobacter baumannii* 2.7%(2) and GGS 1.3%(1) and *Enterococcus faecium* 1.3%(1). A report from Pakistan in the year 2009 showed lesser prevalence of *Streptococcus pneumoniae* 10%, Klebsiella pneumoniae 16%, *Staphylococcus aureus* 7%, and higher percentage of *Hemophilus influenzae* 9%. [7]. Lesser percentage of *Hemophilus influenzae* in this study could be because of stringent Hib vaccination strategy followed in this area. In this study, among the 12 *Staphylococcus aureus* isolated from LRI, 66.7%(8) were Methicillin sensitive *S. aureus* (MSSA) and 33.3%(4) were Methicillin resistant *S. aureus* (MRSA).

A study on bacterial Profile of Lower Respiratory Tract Infections in Egypt in 2013 reported that predominant isolates among 360 patients with Community acquired pneumonia was *S.pneumoniae* (36%), which was higher compared to the present study which had 26.6%, followed by *K. pneumoniae* (10%) which was lower than 22.7% reported in this study. A higher sensitivity was recorded for moxifloxacin, levofloxacin, macrolides, and cefepime; whereas, a
higher rate of resistance was recorded for doxycycline, cephalosporins, ampicillin-sulbactam, and amoxicillin-clavulinate [107].

In a study done in Nepal in 772 children with pneumonia from July 2008 to August 2011, the main bacterial causes of pneumonia were *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (Hib). *Streptococcus pneumoniae* was confirmed in 122 children (15.8%). [108]. This was similar to our studies which showed most common isolates as *Streptococcus pneumoniae*, but followed by *Klebsiella pneumoniae* and *Staph.aureus*. According to WHO bulletin, the leading bacterial cause was found to be *Streptococcus pneumoniae* in 30–50% of pneumonia cases, The second most common organism isolated in most studies was *H. influenzae* type b (Hib; 10–30% of cases), followed by *S. aureus* and *K. pneumoniae*. Prevalence of *H.influenzae* infection is lesser in this study compared to reports from WHO bulletin and Nepal, this could be because of active vaccination followed in this area. [108].

According to a study on epidemiology of LRI in Unites States, Pneumococci stands the leading cause followed by *Haemophilus influenzae* type b (Hib) remains the second important bacterial pathogen of pneumonia. Almost one in 200 children less than 5 years old, developed invasive Hib disease and nearly two-third of Hib infections occurred in children under 18 months. In 2009, a study on children less than 5 years old in the United States showed 32 cases of invasive HIB disease. [106]. In the present study, there was no cases of invasive Pneumococcal or *H.influenzae* infection like Meningitis and septicemia. This again can be explained by the effect of strict vaccination of Hib vaccine and Pneumococcal vaccine.

A Study from United States in 2013 reported *Staphylococcus aureus*, was the third important bacterial organism after *S.pneumoniae* and *H. influenzae* in causing pneumonia. Many of them were Methicillin-resistant *S.aureus* (MRSA). [106]. In this study, among the 12 *Staphylococcus aureus* isolated from LRI, 66.7%(8)were Methicillin sensitive *S. aureus* ( MSSA)and 33.3%(4) were Methicillin resistant *S.aureus* ( MRSA). Among 1545 isolates of *H. influenzae* in the
AWARE Ceftaroline Surveillance Program (2009–2010), 26.3% were non-susceptible to ampicillin. Slight increases in resistance to azithromycin (0.8–1.4%) and trimethoprim/sulfamethoxazole (19.4 vs. 24.4%) were reported during the study period. [109].

In this present study, 5 isolates, *Acinetobacter baumannii* (2) and *Psuedomonas aeruginosa* (2) and one *Enterococcus faecium* were found to be hospital acquired infection. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were isolated in tracheal aspirates from children who were suspected to have ventilator associated pneumonia. Both were multidrug resistant organisms and showed sensitivity to Imepenem and Tazobactum piperacillin only. In a study done in Egypt, the predominant isolates in 318 patients with HAP were, MRSA (23%), *K. pneumoniae* (14%), *E. coli* (11%), *P. aeruginosa* (9%), MSSA (6%), and polymicrobial in 12% . In this study, the number of HAP is too small in number to comment, with *P. aeruginosa* 2.7% and Acinetobacter 2.7% and Enterococci 1.3%. Acinetobacter spp accounts for 3-5% of nosocomial pneumonia [110]. They play a special role in nosocomial pneumonia for the subset of ICU patients requiring mechanical ventilation [111]. In studies from Egypt in HAP cases, high sensitivity were recorded for vancomycin, amikacin, and resistance rates of common pathogens causative of moxifloxacin, levofloxacin, and cefepime. Characteristically, MRSA showed an absolute resistance (100%) for β-lactam-β-lactamase inhibitors, and high resistance rate (92%) for cephalosporins. [107].

Review article on Antimicrobial Surveillance Program (1997-2008) to establish the pathogens of VAP, a consistent 6 organisms (*Staphylococcus aureus* (28.0%), *Pseudomonas aeruginosa* (21.8%), Klebsiella species (9.8%), *Escherichia coli* (6.9%), Acinetobacter species (6.8%), and Enterobacter species (6.3%) caused ~80% of VAP cases in united states. Latin America had an increased incidence of non-fermentative gram-negative bacilli like Acinetobacter. [112]. According to an Indian study of VAP in neonatal intensive care unit, Most common bacterial isolated from endotracheal aspirate of VAP patients was *Klebsiella spp* (32.8%), *E.coli* (23.2%) and *Acinetobacter spp* (17.8%) being the other two
Enterococcus faecium was isolated from a new born patient with respiratory distress. It was confirmed as *Enterococcus faecium* by Automated Vitek system, (Bio murex). It showed resistance to all the antibiotics used: Penicillin, Oxacillin, Erythromycin, Cefotaxime, cefuroxime, Amikacin Co-trimoxazole, Amoxicillin and Vancomycin. Vancomycin resistance was confirmed by minimum inhibitory concentration method and was found to be 64mcg/ml. It was sensitive to Linezolid only. Vancomycin resistant Enterococci (VRE) is a multi-drug resistant Gram positive cocci which is a well-known nosocomial pathogen with significant association with mortality. Among various species of Enterococcus, *E. faecalis* and *E. faecium* are the most common human pathogens. Vancomycin-resistant Enterococcus (VRE) sepsis is emerging as a significant problem in the intensive care setting. The treatment at any age is challenging, but there is a dearth of information on this infection and its treatment in the very premature infant. [114]. Neonatal bacterial sepsis is one of the major causes of morbidity and mortality in neonates Enterococci account for as many as 10% cases of neonatal bacteremia and septicemia. Enterococci may cause early onset (within 7 days of birth) and/or late onset (> 7 days) neonatal sepsis. Most cases of Enterococcal bacteremia in neonates are nosocomial. The drug of choice for VRE is Linezolid. With the ability to transfer some of its plasmids to other Streptococci and Staphylococci and the implications of a possible spread of penicillin and vancomycin resistance to these and other Gram positive species are also of concern [115,116].

In present study, prevalence of GAS was 15% (64/430) in URI and 2.1% (7/320) in LRI cases. According to a cross sectional study in rural community in North India, incidence of infection by other beta haemolytic Streptococcus was 5.5% and GAS 1.3% [66]. In another study done in south India on study of carriage of beta hemolytic Streptococci in school children reported out of the 44 (2.2%) *beta haemolytic Streptococcus* isolated, 38 (86.36%) were GAS, 5 (11.36%) were GCS and one (2.27%) was GGS. [117]. This results are very less compared to the present study. In present study throat infections due to other beta hemolytic
Streptococci was 15.5% (GCS 6.7% and GGS 8.8%) and that of GAS was 14.9%, which is slightly higher when compared to other studies. However this study population consisted of children attending hospital while other studies mentioned above were done as screening of school children. A Study done in South India reported carriage of Beta hemolytic Streptococcus in school going children as 16.3% and GAS as 8.4%, which has lesser prevalence of GAS compared to our studies [18]. The throat carriage of GAS in school children acts as a source of infection.

In this study throat infections due Beta hemolytic streptococci (GGS and GCS) 15.5% and GAS infection 15%, and was slightly higher than finding from South Indian study done by Prabu which showed GGS/ GCS infection to be 10.9% and GAS to be 6.9% [88]. In a Turkish study, 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. This finding shows much lesser prevalence of GCS and GGS compared to the present study, and higher percentage of GAS. This studies shows that other beta haemolytic Streptococci like GGS and GCS are also important in causing throat infections though the prevalence rate differed from region to region. [54].

Accurate diagnosis of Streptococcal pharyngitis followed by appropriate antimicrobial therapy is important for the prevention of acute rheumatic fever; for the prevention of suppurative complications (eg, peritonsillar abscess, cervical lymphadenitis, mastoiditis, and, possibly, other invasive infections); to improve clinical symptoms and signs; for the rapid decrease in contagiousness; for the reduction in transmission of GAS to family members, classmates, and other close contacts of the patient; to allow for the rapid resumption of usual activities; and for the minimization of potential adverse effects of inappropriate antimicrobial therapy[22].

In order to find out the clonal type of GAS present in this locality, emm typing was planned. 30 GAS isolates were chosen, 12 random strains of macrolide sensitive, and macrolide resistant. and 6 strains which showed special features like
cross reactivity to Group C and G types, bacitracin resistance were also included. According to 30 beta hemolytic Streptococci chosen for emm typing. 25 were identified GAS, 4 were other hemolytic Streptococci and 1 isolate could not be amplified. Among the 25 GAS typed, 15 different emm types were identified: emm 12.40, emm 22, emm25.1, emm42, emm 65.1, emm 78.3, emm81.11, emm106, emm113, emm118, emm118.5, emm124, emm152, emm183.2 and emm238.2. GGS typed was found to be stG6792.3, stG6792.2, stG6792 and GCS was found to be stC5345.1.

Among the emm types identified, 5 types were not reported from India from respiratory samples, emm 22, emm 65.1, emm 78.3, emm 124 and emm238.1. StC5345.1 was reported for toxic shock syndrome case in Norway and was not been reported from India.

The present study has only emm 12, and emm 22 which were present in 26 emm type included in the 26 valent Streptococcal vaccine which is under trial[118]. This proves that a detailed knowledge of the emm types prevalent in this region will be helpful to implement a vaccine trial to be effective in this population. There were only limited study about the different emm types of Streptococci reported from India.

The comparison of all the emm types identified from this study and also similar study done worldwide and also in India is given in Table 7[119,120,121,122,123].
Table 6.1 *emm* types identified and comparison with other reports

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th><em>emm</em> type</th>
<th>SRM study</th>
<th>World wide Distribution</th>
<th>Reports from India</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>emm</em>12.40</td>
<td>1</td>
<td>Germany, Taiwan, Spain, Greece, Italy, Europe</td>
<td>Chandigarh, Chennai</td>
</tr>
<tr>
<td>2</td>
<td><em>emm</em>22</td>
<td>1</td>
<td>US, Argentina, Brazil, Chile, Colombia, Denmark, France, Egypt, Korea, and Malaysia,</td>
<td>---</td>
</tr>
<tr>
<td>3</td>
<td><em>emm</em>25.1</td>
<td>2</td>
<td>US, Brazil, India, Nepal</td>
<td>Chandigarh, Haryana</td>
</tr>
<tr>
<td>4</td>
<td><em>emm</em>42</td>
<td>1</td>
<td>US, Chile, Brazil</td>
<td>Chandigarh, Haryana</td>
</tr>
<tr>
<td>5</td>
<td><em>emm</em>65.1</td>
<td>1</td>
<td>South Africa, Bulgaria, Nepal</td>
<td>---</td>
</tr>
<tr>
<td>6</td>
<td><em>emm</em>78.3</td>
<td>1</td>
<td>US, Egypt, France, Argentina, Brazil, Mexico, Nepal</td>
<td>---</td>
</tr>
<tr>
<td>7</td>
<td><em>emm</em>81.11</td>
<td>1</td>
<td>Brazil</td>
<td>Chandigarh, Haryana, Tamil Nadu</td>
</tr>
<tr>
<td>8</td>
<td><em>emm</em>106</td>
<td>2</td>
<td>Malaysia, Egypt, Nepal</td>
<td>Chandigarh, Haryana, Tamil Nadu</td>
</tr>
<tr>
<td>9</td>
<td><em>emm</em>113</td>
<td>3</td>
<td>New Zealand, Thailand</td>
<td>Chandigarh, Tamil Nadu</td>
</tr>
<tr>
<td>10</td>
<td><em>emm</em>118</td>
<td>2</td>
<td>US, Brazil, Italy, Nepal</td>
<td>Chandigarh, Tamil Nadu</td>
</tr>
<tr>
<td>11</td>
<td><em>emm</em>118.5</td>
<td>1</td>
<td>US, Brazil, Italy, Nepal</td>
<td>Haryana</td>
</tr>
<tr>
<td>12</td>
<td><em>emm</em>124</td>
<td>1</td>
<td>Egypt, Malaysia, New Zealand</td>
<td>---</td>
</tr>
<tr>
<td>13</td>
<td><em>emm</em>152</td>
<td>2</td>
<td>---</td>
<td>Vellore, CMC</td>
</tr>
<tr>
<td>14</td>
<td><em>emm</em>183.2</td>
<td>1</td>
<td>---</td>
<td>Vellore, CMC</td>
</tr>
<tr>
<td>15</td>
<td><em>emm</em>238.1</td>
<td>2</td>
<td>Ethiopia, Sweden</td>
<td>---</td>
</tr>
<tr>
<td>16</td>
<td><em>stG</em>6792.3</td>
<td>2</td>
<td>---</td>
<td>Chennai</td>
</tr>
<tr>
<td>17</td>
<td><em>stG</em>6792.2</td>
<td>1</td>
<td>---</td>
<td>Chennai</td>
</tr>
<tr>
<td>18</td>
<td><em>stG</em>6792.0</td>
<td>1</td>
<td>San Francisco</td>
<td>Tamil Nadu</td>
</tr>
<tr>
<td>19</td>
<td><em>StC</em>5345.1</td>
<td>1</td>
<td>Norway-toxic shock syndrome</td>
<td>---</td>
</tr>
</tbody>
</table>

In a study done in Taiwan, *emm* type 12 accounted for 43.4% of all strains. But only 14.3% of the *emm* type 12 strains were associated with invasive
infections. Most were associated with noninvasive infections [124]. Similar to our study as the 1 isolate got in this study was non invasive. A study from Norway reported emm 12,118 and StC 5345 was isolated from skin and soft tissue infection unlike the same emm types were detected in respiratory isolate in present study. [125].

In a report from Western Greece, a statistically significant association was found between macrolide resistance and emm4, emm 77 and emm 22. Whereas emm 1, emm 3, emm 6, emm 12, emm 87 and emm 89 were associated with macrolide susceptibility. In this study also emm 22 was macrolide resistant strain and emm 12 as macrolide sensitive strain. [126].

A study done in Australian aborigin community reports emm 81 as the most common strain. The same study suggest an important implication of their findings was that the associations between emm type and other clinicoepidemiological properties of GAS that may be observed in one geographic region do not necessarily apply to other parts of the world, unless, of course, the emm product is the dominant contributor to the molecular basis for those properties [119].

In a study done in Taiwan, on Streptococci causing skin infection, the emm 106 type was more significantly associated with invasive infection than the other emm types in older patients and those who had malignancy, where as in this study emm 106 was isolated from throat of children who were immune competent [121].

In the Strep-EURO survey(2003 to 2005), emm 1, emm 12, emm 3, and emm 4 accounted for 50% of all cases. Nevertheless, 32 different types were identified in the 11 years, with fluctuations in prevalence over time. Many of the 32 types were responsible for only a few cases but were isolated throughout the study period (e.g., emm 5, emm 11, emm 22, emm 24, emm 28, emm 58, emm 77, emm 78, and emm 118), while others (emm 9, emm 33, emm 53, emm 68, emm 70, emm 76, emm 79, emm 85, emm 87, emm 102, emm 110, and emm 114) were detected only once and have
very seldom been reported in the literature to be circulating in Europe and to be involved in invasive GAS infections. This present study had emm 22 and emm 118 similar to the above study [122]. emm 12 and emm 118 was isolated in a study done in Chandigarh, but emm 118 was seen more in skin isolates unlike this study where we isolated it from throat. [123]. According to cdc data bank. emm118.5 was detected in the year 2006 from GAS pharyngitis isolate it differ from emm118.0 by in-frame deletion of first 7 codons encoding the mature. In this study also 1 strain was emm 118.5.

In a study done in Japan on Streptococcus pyogenes dysgalactea, Three types, stG485, stG6792 and stG2078, predominated among the 42 invasive strains, but the predominance of a specific type was not recognized. [127]. In the present study, 2 stG6792.3 and stG 6792.2 was isolated from throat and stG6792.0 was isolated from Blood.

M protein. emm124.0 was formerly known as st1160. emm238.1 formerly emm1-2.2 and was recovered from acute glomerulonephritis patient in Ethiopia during 1990. Sequence and information concerning this strain supplied by Dr. Wezenet Towodros, Karolinska Institute, Stockholm, Sweden. The emm1-2 sequence was first described in GenBank accession U20096. (ftp://ftp.cdc.gov/pub/infectious_diseases/ biotech/tsemm/) In this study also emm 124 and emm 283.1 was isolated.

Streptococcus pyogenes emm118, emm106, StC 5345, stG 6792 and stG 672.1 isolated was similar to reports from study done in Chennai by T. menon which had emm 118(1 no), emm106 (1 no), StC 5345 (4 number) StG 6792(1no) StG 6792.1(3 no) [128]. In another study done in South India showed emm and emml types which included emm12.0 (28.6%), stG643.0 (28.6%), stC46.0 (17.0%), emm30.11 (8.5%), emm3.0 (2.9%), emm48.0 (5.7%), st3343.0 (2.9%), emm107.0 (2.9%) and stS104.2 (2.9%). Here only emm 12.0 was similar to the present study [129].
In a study from Brazil, thirty-two emm types and subtypes were found, but five \((emm1, emm4, emm12, emm22, emm81)\) were detected in majority(48%) of the isolates. This report had three type: \(emm12\), \(emm22\) And \(emm81\), which were isolated in our studies. Three new emm subtypes were identified \((emm1.74, emm58.14 emm76.7)\) [130].

GAS isolated were completely sensitive to Penicillin and amoxicillin, Cephalothin and cefuroxime but showed only 28.1% resistance to Macrolides, 11.26% to Clindamycin, 12.7% to Ofloxacin and 17% to Chloramphenicol. Pneumococci showed 20% resistance to Penicillin and 15% resistance to Cephalothin and 10% to cefuroxime. Klebsiella pneumoniae showed 47% resistance to Ceftriazone and Ceftazidime 35% to Cefipime. 29% of Hemophilus influenzae was resistance to Ampicillin. Among Staphylococcus aureus isolated, 20% were Methicillin resistant Staphylococcus aureus (MRSA). Our findings were almost similar compared to the surveillance data done in Latin America in 2000 by Mendes C etal [131].

Macrolide resistance in GAS was 28.1% in this study which is in par with 29.4% reported by Capoor MR from New Delhi, where as much lesser than 9.04% reported from Chennai study by SE Jacob [20,87]. This could be because their study in Chennai was screening of healthy school children for GAS, but this present study all the children has pharyngitis. Macrolide resistance in GCS and GGS was 27.6% and 28.2% respectively. In a study done in Portugal, GAS recovered from paediatric pharyngitis (101 isolates) and asymptomatic children (79 isolates), Macrolide resistance was detected in 26.6%, 38.0% and 37.6 % of strains belonging to invasive, asymptomatic and pharyngitis populations, respectively. The Macrolide resistance in pharyngitis (37.6%) is higher than 28.1% reported in this study [122].

In D test done for detecting phenotypes of macrolide resistance in GAS, M type of resistance is the most common type 55%, followed by cMLS 25% and iMLS 10%. Similar results were seen in GGS, M type 56%, cMLS 36%, iMLS 8%. GCS showed only M type 8% and cMLS 10%, and no iMLS.
In a study done in Italy, the phenotypes and genetic determinants for macrolide resistance were determined for 167 erythromycin-resistant \textit{Streptococcus pyogenes} strains. A cMLS phenotype was shown in 18\% of the erythromycin-resistant strains, while inducible resistance was apparent in 31\% and the M phenotype was apparent in 50\% [132]. When compared the present study showed very less Prevalence(10 \%) of i MLS, but higher prevalence of M type and cMLS type of Macrolides resistance. In another study done in Chennai on Group C and Group G Streptococci, \textit{StG 6792.3} and \textit{StG5345.1} was detected. Both were associated with Macrolide resistance. Phenotypic resistance seen was \textit{StG 6792} with 1 each of c MLS and M type and \textit{StG 5345} with i MLS which is similar to this study, \textit{StG 6792} showed c MLS and \textit{StG 6792.1} showed M type of Macrolide resistance. [88].

The 20 Macrolides resistant GAS strains were phenotypically identified as 8 strains c MLS, 1 strain as iMLS and 11 strains M type. Macrolide resistance gene was detected in selected 11 strains of GAS; 5 M type, 5 cMLS and 1 iMLS type for detection of \textit{erm}B gene, \textit{erm} TR and \textit{mef} A gene. All the M type of resistance strains were positive for \textit{mef} A, except 1 which positive for \textit{erm} B. All the cMLS types were positive for \textit{erm}B. The 1 iMLS which was positive by Phenotypic method could not be proved by molecular method, as it gave negative for all 3 genes.In the present study, two distinct genotypes of erythromycin resistance \textit{erm}(B) and \textit{mef} A were documented which suggested a possible polyclonal origin of resistance in this geographical area which is in agreement with previous studies reported from Hongkong, Turkey, [133,134].

In a report for Tamilnadu showed that among the three groups tested, the distribution of \textit{erm}B and \textit{mef}A was high in pharyngitis isolates (30\%) where 10 isolates showed the presence of both genes which was similar to this study which also showed presence of \textit{erm}(B) and \textit{mef} A [135]. In a study done on Erythromycin resistance genes in group A streptococci of different geographical origins from America and Europe, the \textit{erm}TR gene was found in five European countries: Sweden, England, Bulgaria, Italy and Greece, and also outside Europe in Argentina.
and the US. The \textit{ermB} gene was found in four countries: Italy, England, Sweden and the USA. \cite{136,137}.

In a Study done by D Prabu in Chennai reported presence of \textit{erm}(A), \textit{erm}(B) and \textit{mef}(A) were documented in GCS/ GGS isolates which also suggest on Lateral gene transfer from GAS is said to be the contributing factor for the emerging resistance among GCS/GGS . \cite{128}.This study proves that that there are geographical differences in the mechanisms of erythromycin resistance. Macrolides are widely used in India yet regular surveillance of resistant genotypes of beta haemolytic streptococci, is not frequently done. Increase in erythromycin resistance among them is a matter of concern and it may be advisable to use Macrolides only after laboratory tests indicate susceptibility in order to check the multiplication and spread of resistant clones.

In this study, 5 cases of \textit{H.influenzae} were isolated from Pneumonia patients, who were not vaccinated with Hib vaccine. According to Watt et al, Hib disease accounted for 5.6% (3.9–7.7%) of the estimated 6.6 million post-neonatal child deaths and 16% of the estimated 1.8 million pneumonia deaths in HIV-negative children and these Hib-attributable deaths in children are almost entirely vaccine preventable .\cite{52}.At present Hib Vaccine is included in the government since 20 The National Technical Advisory Group on Immunization in India recommended the introduction of \textit{Haemophilus influenzae} type b (Hib) vaccine in their Universal Immunization Program (UIP) in 2008. From December 2011, Hib vaccine in combination with diphtheria, pertussis, tetanus, and hepatitis B has been introduced through the UIP in Kerala and Tamil Nadu states. No \textit{Haemophilus influenzae} was isolated after 2010 in our studies.\cite{138}.

The failure of antibiotic treatment in the eradication of susceptible organisms has recently induced microbiologists to hypothesize the presence of bacteria ordered in communities, attached to surfaces, identified as “biofilms”. \cite{139}. It has been investigated that \textit{S. pyogenes} was able to form biofilm as an alternative method to escape antibiotic treatment and host defenses leading to recurrent infections \cite{64}. A biofilm is a colony of single or multiplebacterial species
embedded in a self-producing polymeric matrix, this matrix guarantees better survival and protection from macrophage action, antibiotics, temperature and pH fluctuations. [140,139]. One of the best known biofilm-specific properties is antibiotic resistance, which can be up to 1000-fold greater than that seen with planktonic cells [141]. So biofilm-associated infections are difficult to eradicate by routine antibiotic doses in compare with planktonic form of bacteria. They need thousands times of doses used for non-biofilm infections[142]. As in biofilm formed by *Streptococcus pyogenes* in pharyngitis patients, which evading high antibiotic concentrations greater than 10-folds minimum inhibitory concentration (MIC) for planktonic *S. Pyogenes* . [141].

In a study done by Shera.J 84 group A streptococcal (GAS) strains representing more than 50 different emm-types was checked for Biofilm formation using Crystal violet. 83 of 84 were able to form biofilms to some degree. Biofilm forming capacity was in general conserved among isolates of the same emm-type. Biofilm formation was not altered by carbon dioxide or sucrose. However, increased glucose concentration resulted in increased biofilm formation [143]. In this study Biofilm formation was checked for all the 71 isolates of GAS by microtitre plate,Biofilm formation was checked with Brain heart infection (BHI) broth and Trypticase soy broth (TSB) with 1% glucose, BHI broth was giving similar results as TSB with 1% broth.This study suggest than Brain heart infusion broth instead of TSB with 1% can be used as an alternative for detection for biofilm formation in GAS.

The serum opacity factor (SOF) of *Streptococcus pyogenes* is a serotyping tool and pathogenesis factor, it is fibronectin-binding surface protein, which has served as a marker for serotyping and distinguishes two *S. pyogenes* lineages. SOF acts as a virulence factor, it is been shown that antisera against SOF can opsonize SOF-positive streptococci in human blood and protect mice against streptococcal infections. The finding that antisera against one type of SOF can opsonize both homologous and heterologous SOF-positive serotypes of group A streptococci suggests that different serotypes of SOF contain a shared epitope(s) that evokes opsonic antibodies.Serum opacity factor (SOF) is a
bifunctional cell surface protein expressed by 40–50% of group A streptococcal (GAS) strains comprised of a C-terminal domain that binds fibronectin and an N-terminal domain that mediates opacification of mammalian sera. [144].

All the strains of GAS screened for presence of SOF, (38/71)53.52% were positive. According to study on High Diversity of Group A Streptococcal emmTypes in an Indian Community: , Thirty-three (55.9%) of the 59 isolates were SOF positive which was similar to 53.2% reported in our studies. In a study done on SOF among GAS isolates from Skin isolates. SOF production was higher (83.3%) in this study than our reports which showed a variation of 53.52% . It could be due to the M type variation. M types may be divided into opacity factor positive and opacity factor negative and hence it is used to subtype the GAS isolates [11].

The relationship between 2 virulence factor biofilm formation and SOF was done. According to 2X2 Fischer’s contingency table test for biofilm formation and SOF, the association between biofilm formation and SOF was considered to be statistically significant by Fischer 2x2 contingency table as P value is less than 0.0001 .

According to 2X2 Fischer’s contingency table test for a biofilm formation and macrolide resistance, P value was found to be 0.0513. This suggests that the association between the two was not statistically significant. Though biofilm formation was known to cause increase in antibiotic resistance in other bacteria, in this study we have more of macrolide sensitive strains which are positive for biofilm formation. Among 25 biofilm formers, 14(56%) isolates were macrolide sensitive. Similar results were reported by various other studies. Studies by L. Baldasseri shows 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 (19). This study suggests that biofilm might have a role in chronic carriage of Streptococcus pyogenes and its difficulty in eradication. The Biofilm hinders the penetration of antibiotics through it, making the otherwise sensitive bacteria, not respond to the antibiotics.