CHAPTER 8

SUMMARY

This study was conducted among pediatric age group to detect the various bacterial causative agents for respiratory tract infection, hence the results reflects the status in pediatric population. This poses a concern from public health point of view for following reason:

- Streptococcus infection affects mainly the school going age groups. Dissipation of this infection to other children is possible in the school.
- Any resistant strain may have a wide scope for spread to other children, thus causing serious health problem. As per literature review overcrowding in public provide more favorable condition for the spread of Streptococcal infection
- Repeated Streptococcal throat infection is responsible for post infection sequelae like Rheumatic fever, Rheumatic Arthritis etc which is a public health problem. Early detection of Streptococcal infection and treatment will avoid such complication.
- Macrolide resistant Strain detection is important in medical practice. Most of the practitioners use these antibiotics without ant microbiological evidences. Study of resistant strain will be an eye opener for the practitioners. Macrolide like Erythromycin and Azithromycin is still commonly used even though the study indicates 28.1%resistance in GAS. Prevalence of resistance changes periodically and also with geographical areas. Hence periodic screening of Antibiotic resistance pattern is important from public health point of view.
CHAPTER 9

OUTCOME AND FUTURE PERSPECTIVE OF THE STUDY

1. In the present study, strains isolated from throat swab, when subjected to emm typing showed varied emm types. The 26 valent Streptococcal vaccine under trial covered only two types of emm identified in this study( emm 12 and emm 22). This study had 15 different emm types: emm 25.1, emm 42, emm 65.1, emm 78.3, emm 81.11, emm 106, emm 113, emm 118, emm 124, emm 183.2, emm 238.1. This study therefore suggests that in future, if a suitable vaccine has to be formulated for this population, a detailed study of the emm types circulating in this population is necessary.

2. One of the strain isolated form throat swab from this study when typed was shown to belong to StC5345 type, which was reported from a study from Norway to be associated with virulent toxic shock syndrome strain. Further study will be focused to find out the prevalence of such virulent strain among the population under study by emm typing. If prevalence is reasonably high, follow up of such children will be done to prevent the development of severe complication.

3. Macrolide resistance gene was screened for all the phenotypically resistant strain. In future study, screening of all the strains will done, including the sensitive strain to detect the possibility of any virulent gene which is not expressed.


APPENDIX 1

1. MAC CONKEY AGAR  
<table>
<thead>
<tr>
<th>Grams/Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptones (meat and casein)</td>
</tr>
<tr>
<td>Pancreatic digest of gelatin</td>
</tr>
<tr>
<td>Lactose monohydrate</td>
</tr>
<tr>
<td>Bile salts</td>
</tr>
<tr>
<td>Sodium chloride</td>
</tr>
<tr>
<td>Crystal violet</td>
</tr>
<tr>
<td>Neutral red</td>
</tr>
<tr>
<td>Agar</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
<tr>
<td>pH</td>
</tr>
</tbody>
</table>

2. SHEEP BLOOD AGAR  
<table>
<thead>
<tr>
<th>Grams/Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caesin enzymatic hydrolysate</td>
</tr>
<tr>
<td>Peptic digest of animal tissue</td>
</tr>
<tr>
<td>Yeast extract</td>
</tr>
<tr>
<td>NaCl</td>
</tr>
<tr>
<td>Agar</td>
</tr>
<tr>
<td>Distilled water</td>
</tr>
<tr>
<td>pH</td>
</tr>
</tbody>
</table>
### 3. NUTRIENT AGAR

- Peptone: 3.0
- Beef extract: 3.0
- NaCl: 5.0
- Distilled water: 1000 ml
- pH: 7.2
- Agar: 15.0

### 4. NUTRIENT BROTH

- Peptone: 3.0
- Beef extract: 3.0
- NaCl: 5.0
- Distilled water: 1000 ml
- pH: 7.2

### 5. MULLER HINTON AGAR

- Beef infusion: 3.0
- Casein acid hydrolysate: 17.5
- Starch: 1.5
- Agar: 17.0
- pH: 7.3

### 6. CARBOHYDRATE FERMENTATION MEDIA

- Peptone: 10.0
- NaCl: 5.0
- KH2PO4: 0.3
- Phenol red: trace
- pH: 6.5
8. BIOCHEMICAL TESTS

I) INDOLE TEST

PEPTONE WATER

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone</td>
<td>10gms</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5gms</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1000ml</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
</tbody>
</table>

KOVAC’S REAGENT:

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-dimethylamino benzaldehyde</td>
<td>5gms</td>
</tr>
<tr>
<td>Amyl alcohol</td>
<td>75 ml</td>
</tr>
<tr>
<td>Hydrochloric acid (concentrated)</td>
<td>25ml</td>
</tr>
</tbody>
</table>

The p-dimethyl amino benzaldehyde was dissolved in amyl alcohol and to that hydrochloric acid was added.

II) METHYL RED INDICATORS

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methyl red</td>
<td>0.04gm</td>
</tr>
<tr>
<td>Ethanol</td>
<td>40ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100ml</td>
</tr>
</tbody>
</table>

The methyl red was dissolved in ethanol and diluted with water.

III) VOGES PROSKAUER TEST

ALPHA-NAPHTHOL SOLUTION

SOLUTION A

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha napthol</td>
<td>5gm</td>
</tr>
<tr>
<td>Ethanol (100%)</td>
<td>95ml</td>
</tr>
</tbody>
</table>

Alpha napthol was dissolved in ethanol with constant stirring.
SOLUTION B

Potassium hydroxide 40gms
Creatine 0.3gm
Distilled water 100ml

Potassium hydroxide was dissolved in 75ml portion of the Distilledwater, then the creatine was added to the solution and made up to 10ml.

IV) SIMMON'S CITRATE MEDIUM COMPOSITION

Sodium chloride 5 gms
Magnesium sulphate 0.2 gm
Ammonium dihydrogen phosphate 1 gm
Dipotassium hydrogen phosphate 1 gm
Citric acid 2 gms
Bromothymol blue 0.08 gm
Distilled water 1000ml
pH 6.8

The salts are dissolved in water and then citric acid was added.

v) UREASE AGAR

Peptone 0.1g
Glucose 0.1g
Sodium chloride 0.5g
Mono potassium Po4 0.2g
Phenol red 1ml
Agar 2.0g
Distilled water 100 ml
vi) HUGH AND LEIFSON’S OF BASAL MEDIUM

Peptone (tryptone) 2.0
Sodium chloride 5.0
Glucose 10.0
Bromthymol blue 0.03
Agar 3.0
Dipotassium phosphate 0.30
Distilled water 1000ml
pH 7.1

9. REAGENTS

1. GRAM STAINING REAGENTS:

a) Crystal violet solution:

Crystal violet 10
Absolute alcohol 100 ml
Distilled water 1000ml

b) Gram’s iodine:

Iodine 20 mg
NaOH 1ml/L 100ml
Distilled water 900ml

c) Decolourizing agents: Acetone or absolute alcohol (95%)

d) Counter stain: Safranine solution (0.5% in distilled water).

II. Catalase reagents: 3% H₂O₂ - 100ml.

III. Saline: 0.85% NaCl in 100 ml of distilled water.

IV) Oxidase reagent: Tetramethyl-p-phenylenediamine dihydrochloride

V) PYR reagent: L-naphthylamide-β-naphthylamide
## 10. ANTIBIOTIC DISCS

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>30µg</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10 µg</td>
</tr>
<tr>
<td>Bacitracin</td>
<td>0.04 units</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>30µg</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>30µg</td>
</tr>
<tr>
<td>Cefuroxime</td>
<td>30µg</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30µg</td>
</tr>
<tr>
<td>Cephotaxime</td>
<td>30µg</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>30µg</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>30µg</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>30µg</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>10µg</td>
</tr>
<tr>
<td>Imipenem</td>
<td>10µg</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10µg</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>5µg</td>
</tr>
<tr>
<td>Optochin</td>
<td>5µg</td>
</tr>
<tr>
<td>Penicillin</td>
<td>10µg</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>300 U</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>10µg</td>
</tr>
</tbody>
</table>
13) AGAROSE GEL ELECTROPHORESIS

Agarose (0.8%) - 0.8g of agarose was mixed in 100ml of distilled water and the mixture was heated to dissolve the agarose.

1x TAE buffer (pH 8.0)
40mM Tris acetate
1mM EDTA
Gel loading dye
0.25 % (w/v) Bromothimol blue
0.25% (w/v) Xylene cyanal FF 40% (w/v) sucrose in H₂O.
REFERENCES


29. Ramana, K V. “Aetiology and Antimicrobial Susceptibility Patterns of Lower Respiratory Tract Infections (LRTI) in a Rural Tertiary Care Teaching Hospital in Karimnagar, South India. American Journal of Infectious Diseases and Microbiology 1.5 : pp 101-105. 2013.


60. Report of the Adhoc committee to revise the Jones criteria (modified) of the council on rheumatic fever and congenital heart disease of the American Heart Association. Circulation 32: pp 664-668. 1965;


89. Florida Department of Health, 10, 2011.

90. Kenneth Todar, PhD.Todar’s online textbook of Bacteriology, Chapter Streptococcus pneumoinae. Page no1.Kenith


102. CLSI Guidelines 2013, M02-A11 and Mo9-A9, page 112-117


LIST OF PUBLICATIONS


VITAE

SHABANA PRAVEEN
NO 5/3, NEAR RTO, THIRUVALLUVAR NAGAR
THIRUVANMIYUR CHENNAI 600041
Mail id: shabanarazmin@gmail.com; Phone no: 9841249799

EDUCATION

<table>
<thead>
<tr>
<th>SL NO</th>
<th>DEGREE</th>
<th>INSTITUTE/ UNIVERSITY</th>
<th>YEAR PASSED</th>
<th>CLASS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ph.D</td>
<td>SRM UNIVERSITY</td>
<td>WAITING FOR PUBLIC VIVA</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>MPhil</td>
<td>BHARATHIDASAN UNIVERSITY</td>
<td>2007</td>
<td>FIRST CLASS</td>
</tr>
<tr>
<td>3</td>
<td>MSc MEDICAL MICROBIOLOGY</td>
<td>MANIPAL UNIVERSITY</td>
<td>1999</td>
<td>FIRST CLASS</td>
</tr>
<tr>
<td>4</td>
<td>BSc MICROBIOLOGY</td>
<td>MANGALORE UNIVERSITY</td>
<td>1996</td>
<td>DISTINCTION</td>
</tr>
</tbody>
</table>

WORK EXPERIANCE

Teaching

- Presently working as tutor in SRM Medical college, Chennai, India from 25 July 2007.
- Lecturer, Dr. MGR Janaki college, Chennai, (June 2006 to June 2007).

Diagnostic

- Presently working in diagnostic laboratory of SRM Medical college, Chennai, from 25 July 2007.