Sudan University of Science and Technology
College of Graduate studies and Scientific Research

Frequency of Moraxella catarrhalis in Patients with Lower Respiratory Tract Infection in Khartoum State

A Dissertation submitted in partial fulfillment for requirements of M.Sc in Medical Laboratory Sciences (Microbiology)

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July 2011
CHAPTER ONE
CHAPTER TWO
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CHAPTER FIVE
APPENDIX 1
Sudan University of Science and Technology
Faculty of Graduate Studies and Scientific Research

Questionnaire

Hospital name.................................................................

Specimen number............................................................

Age................................. Gender M □ F □ Date........

Residence.................................................................

Clinical remark..............................................................

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Antibiotics administration.............................................

Type of antibiotics........................................................

Time of administration..................................................
APPENDIX 2
Preparation of culture media and biochemical test

2.1 Culture media

2.1.1 Blood Agar, Base (Chocolate Agar, Base)

A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms like *Neisseria*, *Streptococci* etc. The medium is often used to observe the forms of haemolysis from pathogenic microorganisms.

This culture medium can be used without blood for setting up blood cultures and as a base for preparing special culture media.

**Composition:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Grams/Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meat extract</td>
<td>10.0</td>
</tr>
<tr>
<td>Peptone</td>
<td>10.0</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.0</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Final pH 7.3+/−0.2 at 37°C

**Supplements**

These supplements make the media semi-selective for *Moraxella catarrhalis*

<table>
<thead>
<tr>
<th>Supplement</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vancomycin</td>
<td>10µg</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>2µg</td>
</tr>
<tr>
<td>Sodium acetozolamide</td>
<td>10µg</td>
</tr>
</tbody>
</table>

**Directions:**

Suspend 40 g in 1 litre of distilled water. Bring to a boil to dissolve completely. Sterilize by autoclaving at 121°C for 15 minutes. For blood agar, cool to 45-50°C and add aseptically 6% (5-10% is typically) of sterile defibrinated blood.
2.1.2 GC Agar Base

GC Agar Base, with added blood or haemoglobin and other supplements is recommended for selective isolation and cultivation of Gonococci.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone, special</td>
<td>15.000</td>
</tr>
<tr>
<td>Corn starch</td>
<td>1.000</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>4.000</td>
</tr>
<tr>
<td>Monopotassium phosphate</td>
<td>1.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Agar</td>
<td>10.000</td>
</tr>
</tbody>
</table>

Final pH (at 25°C)……………………………. 7.2±0.2

Formula adjusted, standardized to suit performance parameters

**Directions**

Suspend 7.2 grams in 100 ml distilled water, to make a double strength base. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 50°C and aseptically add separately prepared Haemoglobin (FD022) (100 ml sterile 2% solution) and GC Supplement w/ Antibiotics (FD021). Mix well and pour into sterile Petri plates. To increase the selectivity of medium antibiotic supplements such as V.C.N. Supplement (FD023), V.C.N.T. Supplement (FD024), Linco T Supplement (FD026) or Vanclo T Supplement (FD028) may be added. To enhance the nutritional properties of medium, Vitamino Growth Supplement (FD025) or Yeast Autolysate Supplement (FD027) may be added. For Chocolate Blood Agar, prepare single-strength medium using 3.6 grams in 100 ml of distilled water. Sterilize by autoclaving
at 15 lbs pressure (121°C) for 15 minutes and add 5% v/v defibrinated blood. Mix well and heat at 80°C for 10 minutes.

2.1.3 **Mueller Hinton Agar No. 2 M1084**

Mueller Hinton Agar No. 2 is used for testing susceptibility of common and rapidly growing bacteria using antimicrobial discs by the Bauer - Kirby method. Manufactured to contain low levels of thymine, thymidine, calcium and magnesium.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein acid hydrolysate</td>
<td>17.500</td>
</tr>
<tr>
<td>Beef heart infusion</td>
<td>2.000</td>
</tr>
<tr>
<td>Starch, soluble</td>
<td>1.500</td>
</tr>
<tr>
<td>Agar</td>
<td>17.000</td>
</tr>
</tbody>
</table>

Final pH (at 25°C) 7.3±0.2

Formula adjusted, standardized to suit performance parameters

**Directions:**

Suspend 38 grams in 1000 ml distilled water. Heat boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well and dispense as desired.

2.1.4 **Nutrient Agar**

Nutrient Agar is used for the cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

**Composition**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptic digest of animal tissue</td>
<td>5.000</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.000</td>
</tr>
<tr>
<td>Beef extract</td>
<td>1.500</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>1.500</td>
</tr>
</tbody>
</table>
Agar…………………………………………... 15.000
Final pH (at 25°C)…………………………… 7.4±0.2
Formula adjusted, standardized to suit performance parameters

**Directions**

Suspend 28 grams in 1000 ml distilled water. Heat to boiling to
dissolve the medium completely. Dispense as desired and sterilize by
autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well
before pouring.

2.2 **Biochemical tests**

2.2.1 **Nitrate reduction:**

**Composition:**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Gms / Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein Peptone</td>
<td>5,00</td>
</tr>
<tr>
<td>Beef Extract</td>
<td>3,00</td>
</tr>
<tr>
<td>Potassium Nitrate</td>
<td>1,00</td>
</tr>
<tr>
<td>Disodium Phosphate</td>
<td>2,50</td>
</tr>
</tbody>
</table>

Final pH 7,3 ± 0,2 at 25ºC

**Directions:**

Suspend 20 grams of the medium in one liter of distilled water. Mix
well . Dispense into tubes to obtain and Sterilize at 121 (15 lbs .sp) for
15 minutes.

**After incubation**

Nitrate reagents A (sulfanilic acid) and B (naphthylamine) wooden
sticks for zinc zinc powder

: **Nitrocefin disks 2.2.2**
For the rapid detection of β-lactamase enzymes in isolated colonies of Neisseria gonorrhoeae, Moraxella catarrhalis, Staphylococcus spp., Haemophilus influenzae and anaerobic bacteria.

**Composition**

1 package contains 50 disks in a light resistant plastic vial.

.6mm diameter filter paper disks impregnated with Nitrocefin

**Directions**

Place the required number of Nitrocefin disks into a clean empty Petri dish or onto a microscope slide. Disks may be moistened with one drop of deionised water. Do not over-moisten. Using a sterile loop or applicator stick remove several well-isolated and similar colonies and smear onto the surface of a disk. Alternatively: moisten the disk with one drop of deionised water, then holding the disk in forceps, wipe across a colony on an agar plate. Observe the inoculated disk for the development of a red colour.

### 2.2.3 Tributyrin-Strips

It is a diagnostic test for the differentiation between Branhamella and Neisseria. The test principle is an enzyme hydrolysis of tributyrin. This reaction causes colour change of acidobasic indicator. The result of the reaction is read after 18-20 h.

**Composition:**

(1 package contains 300 test strips) contains strips saturated with tributyrin and acidobasic indicator

**Directions:**

Using a sterile forceps, throw one tributyrin strip into the suspension of tested strain in 1 ml of buffered saline (pH 7.2). Incubate the test sample at 37°C (without CO2). Preliminary results can be read after several hours when the red colour changes to yellow in the case of positive result. Perform the final evaluation of result after 18-20 hours incubation.