جامعة السودان للعلوم والتكنولوجيا

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

تكرار حدوث موراكسيلا كاتارهاليس في مرضى التهابات الجهاز التنفسي السفلي في ولاية الخرطوم

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

By

Eltaib Mahmoud Abd Elrhman Abd Ellah

B. Sc (Honor) Medical Laboratory Science, Omdurman Islamic University (2009).

Supervisor

Dr. Yousif Fadlalla Hamed Elnil

July 2011

CHAPTER ONE

CHAPTER TWO

CHAPTER THREE

Chapter four

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…….……

…….

…….

…….

…….

……..…

…….

Antibiotics administration……...……

Type of antibiotics……..…….

Time of administration…….

APPENDIX 2

Preparation of culture media and biochemical test

2.1Culture 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 observate 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:

Ingredients	Grams/Litre
Meat extract…….	10.0
Peptone ……	10.0
Sodium chloride ……	5.0
Agar……	15.0

Final pH 7.3+/-0.2 at 37°C

Supplements

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

Vancomycin ……..……10µg

Amphotricin B……..……2µg

Sodium acetozolamide……10µg

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

Ingredients	Gms / Litre
Peptone, special	15.000
Corn starch	1.000
Dipotassium phosphate	4.000
Monopotassium phosphate	1.000
Sodium chloride	5.000
Agar	10.000
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

Ingredients Gms / Litre

Casein acid hydrolysate…….. 17.500

Beef heart infusion…….……. 2.000

Starch, soluble ……1.500

Agar…….…….… 17.000

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

Ingredients Gms / Litre

Peptic digest of animal tissue ……..…..5.000

Sodium chloride……..… 5.000

Beef extract…… 1.500

Yeast extract ……..….1.500

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.

2.2Biochemical tests

2.2.1 Nitrate reduction:

Composition:

Ingredients	Gms / Litre
Casein Peptone	5,00
Beef Extract	3,00
Potassium Nitrate	1,00
Disodium Phosphate	2,50

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

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

.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