

RISSERIA

Introduction

> Pathogens are:-

N.meningitidis

N.gonorrhoeae



Selective media

1.Thayer-Martin medium contains vancomycin, colistin, nystatin. Vancomycin kills all Gram Positive organisms **Colistin** (polymyxin) kills all Gram Negative organisms (except Neiserria) Nystatin kills all Fungi 2. Modified New York City medium (MNYC)

N. meningitidis

Morphology :

Arranged in pair with adjacent surfaces flattened





Cultural characteristics

□ Strict aerobe, required 5-10% CO2 35-36°C.

chocolate agar/ Muller
 Hinton agar/ Modified
 Thayer Martin medium



Biochemical reactions

- Oxidase test: Positive
- Catalase test: Positive
- Glucose fermentation: Acid only
- Maltose fermentation: Acid only

Pathogenicity

Cerebrospinal meningitis

Meningococcal septicemia

Laboratory diagnosis

Sample collection

 \Box C.S.F.

□ Blood for culture

□ Nasopharyngeal swab (West's post nasal swab)

Sample processing

≻ <u>CSF</u>:

➤ Gram stain

- Culture deposit into chocolate agar, Modified Thayer Martin agar & Muller Hinton agar.
- ≻ Incubate in 5-10% CO₂ at 35-36°C.
- Id : cultural characteristic and biochemical reactions.
- Capsular antigen detection: From the CSF by passive agglutination test.

In case of meningitis CSF of the patient is divided in three parts and processed as follow

N. gonorrhoeae

Morphology

- Gram negative, kidney shaped cocci
- Arranged in pair with adjacent surfaces
 concave

Cultural characteristics

More difficult to grow than meningococci

Colony characteristics

Biochemical reactions

□ Oxidase Positive

□ Catalase test

Positive

Glucose Acid only

□ Maltose Not fermented

Gonorrhea

Vulvovaginitis in prepubertal girls

Proctitis

Ophthalmia neonatorum

Laboratory diagnosis

Sample collection

In male

Purulent urethral discharge (morning drop of secretion or obtained by prostatic massage), Urine

Transportation

 Transport media: Transgrow or JEMBEC

Immediately / if delay
 Swab transported in
 amies transport
 medium

Sample processing

- Specimen: urethral, cervical smears & swabs (transport medium).
- Gram film: intracellular Gram -ve diplococci
- Culture: selective media
- > Oxidase +ve
- > acid production from glucose
- Latex agglutination

Neisseria gonorrhoeae and Neisseria meningitidis Flow Chart

Questions

• Enumerate bacteria causing purulent meningitis. Three primary pathogens:

N. meningitidis, H.Influenza, S.pneumoniae

N.menningitidis - all ages

H.Influenza - 2m-5y

S.pneumoniae - all ages but more common in adult with underlying illnesses.

E.coli & other coliforms **Strept.group B** Flavobacteria

After surgery or trauma

S.aureus

S.pneumoniae

Listeria

Salmonella spp.

All common in **neonates**

How meningococci reach to the meninges?
 Meningococcal meningitis, as a spread from nasopharynx blood stream meninges <u>in</u> susceptible hosts.

Direct spread to meninges

- Enumerate the media used for isolation of meningococci and gonococci.
- Which antimicrobial agents are added in modified Thayer-Martin medium? Why?
- Write full form of MNYC medium.

PRACTICAL 17A

GRAM POSITIVE BACILLI

Classification of Gram Positive Bacilli (GPB)

CORYNEBACTERIUM

• Genus: Corynebacterium

Species: C. diphtheriae ,C. ulcerans, C. pseudotuberculosis, C.xenopi

Morphology

Cuneiform (Chinese letter pattern) arrangement

> volutin granules or metachromatic granules.

On <u>Albert</u> staining: Bacilli - green colour & Granules - blue colour Corynebacterium diphtheriae

Cultural characteristics

• Loffler's serum medium

• Tellurite blood agar

• Modified Tinsdale's medium

Modified Tinsdale's agar

Tellurite agar

Loeffler's Serum slope

Biochemical reactions

- C. diphtheria ferments glucose & maltose with production of acid.
- Urease test Negative.
- Virulence test:
- (i) In vivo test:
- (a) Subcutaneous test Animal = Guinea pig
 (b) Intracutaneous test Animal = Guinea pig
 (ii) In vitro test:
 - (a) Elek's gel precipitation test(b) Tissue culture test

Elek's gel precipitation

- 1 = *C. diphtheriae* (non-toxigenic) negative control
- 2 = *C. diphtherise* test organism
- 3 = *C. diphtherise* (toxigenic) positive control
- 4 = *C. diphthenise* test organism
- 5 = ANTITOXIN STRIP
- 5 = lines of precipitate

Laboratory diagnosis

• Throat or nasopharyngeal swabs

• Direct staining

• Culture on selective media

 confirmation done on the basis of colony morphology, biochemical reaction and by in vitro virulence test.

Questions

1. Why diphtheria bacillus is known as KLB?

2. Difference between C. diphtheriae and diphtheroid.

3. Enumerate types of diphtheria.

4. What is responsible for toxigenicity of C. diphtheriae?

Praetical 170

bae# lus

GENUS: Bacillus

MORPHOLOGY

B. anthracis - cause Anthrax disease.

Species:

Specimens must be marked HIGH RISK.

Smears are stained by Gram's stain and polychrome methylene blue stain. (McFadyean's reactions). B. subtilis - cause contamination in the laboratory.

✤ B. cereus — cause food poisoning.

Spores of Bacillus sterothermophilus are used to validate autoclave process

M'Fadyean's reaction

> Medusa Head appearance

Inverted fir tree appearance

String of pearls reaction

> PLET(polymixin, lysozyme, EDTA, thallous acetate) added to heart infusion agar.

Questions

Enumerate modes of transmission of Anthrax.

