MSS Module Practical 2021-2022

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Diagnosis

- Sample collection and Transportation
- Direct smear Microscopy
- Culture
- Biochemicals
- Typing of Staphylococcus aureus
- Antibiotic Sensitivity Testing (AST)

Sample Collection

Type of sample depends on the site of

Infection	Specimen
Suppurative lesion	Pus, wound swab
Respiratory infection	Sputum
UTI	Mid stream urine
PUO, Bacteremia	Blood
Food poisoning	Feces, Vomitus, food
Carriers	Nasal and perianal swab



Oxidative fermentative (OF)

Direct Smear Microscopy

- Staphylococcus measuring 0.5-1.5 microns
- Present within and outside PMNs



Culture

- Blood agar
 - Colonies are betahemolytic
- MSA (Selective media)
 - 1% Mannitol + 7.5%
 NaCl + phenol red







S. aureus on mannitol salt agar (live in high salt media, yellow)

Biochemical Reaction

- Catalase : positive
- Coagulase test : positi
- Oxidase : negative



- Ferment glucose, lactose, maltose, sucrose and mannitol, with production of acid but no gas
- Indole : negative
- MR (Methyl Red) test : positive
- VP (Voges-Proskauer) test : positive
- Gelatin liquefaction : positive
- Phosphatase : positive

DNA Hydrolysis

- Used to determine the ability of an organism to hydrolyze DNA (produce deoxyribonuclease or Dnase).
- Agar medium: this medium is <u>pale green in</u> <u>color because of DNA-methyl green (indicator)</u> <u>complex</u> (Note: Methyl green is binds to the negatively-charged DNA). organism that <u>hydrolyze DNA fade to colorless zone</u> on the blue/green agar.
- -Positive: Staphylococcus aureus
 -Negative: Staphylococcus epidermidis

DNA Hydrolysis test

Positive: Staphylococcus aureus



Negative: Staphylococcus epidermidis

Antibiotic Sensitivity Testing

- This is important as staphylococci develop
- resistance to drugs readily.

MRSA

- Methicillin-resistant S. aureus.
- First reported in 1960s.
- May colonize mucosal or epithelial surfaces, (common : anterior nares)
- Nosocomial pathogen.
- Shows Resistant to penicillins, cephalosporins, carbapenems, onmobactams.
- Hospital-acquired (HA MRSA)
- Community-acquired cases now (CA MRSA

Lab Diagnosis

1- Gram staining (Microscopy): Gram-positive cocci

2-Blood Agar

- Small (0.5-1mm), circular, semi-transparent colonies.
- Produce wide zone of β hemolysis.
- Catalase negative.

3- Biochemical reactions

- Bile insoluble
- Ferments sugars producing acid but no gas.





Lab Diagnosis

4- Antigen detection tests (ELISA) or agglutination tests

5- Antibody detection

- ASO titer for respiratory disease
- antiDNAse & antihyaluronidase for skin infections (Titres higher than 300 are taken)

Propionibacterium acnes

Acne

• A type of folliculitis (inflammation of hair follicle and sebaceous gland)

o Most common skin disease in humans

• Resolves in early adult life

OTreated by skin care, keratolytics, and antibiotics

oPathophysiology

The production of sebum is under the control of androgens. During puberty the androgens stimulate the production of sebum plus increased keratinization and desquamation in sebaceous duct. This causing blockage of ducts and this turns the gland as a sac for the multiplication of *P. acnes* and other flora (yeast, staph, micrococci). In addition to the fatty acids produced by *P. acnes* and enzymes released from bacteria and neutrophils stimulate inflammation.

Laboratory Diagnosis of *Propionibacterium acnes* Infection

Laboratory Examination

- No laboratory examinations required.
- If there is suspicion of an endocrine disorder, free testosterone, follicle-stimulating hormone, luteinizing hormone should be determined to exclude hormonal imbalance.
- Transaminases (ALT, AST), triglycerides, and cholesterol levels may be required if systemic isotretinoin treatment is planned



Propionibacterium acnes Pleomorphic, gram-positive rods

Onchocercus volvolus (River Blindness worm)

Morphological characters

- I. Adults:
 - Male: shorter than female.
 - Female: Layes micrifilaria in subcutaneous nodules.
- II. Microfilaria:
 - Smooth curves.
 - Non sheathed.
 - Anterior end & tail free of nuclei.





Pseudomonas aeruginosa on Triple Sugar Iron agar, Use: to determine bacterial ability to ferment glucose & lactose or sucrose and form hydrogen sulfide (H2S). Growth; red slant, red buttom (because alkaline condition, no fermentation), no gas, no H $_2$ S produced