CASE 2: Microbiology Lab

Pseudomonas aeruginosa

A Summary

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BACTERIAL PATHOGENS ASSOCIATED

- *Staphylococcus aureus*
- *Klebsiella pneumoniae*
- *Acinetobacter baumannii*

Staphylococcus aureus

- Gram-positive bacteria
- Opportunistic pathogen: infect burn wounds
- Facultative anaerobic and not motile
- Normal flora in some parts of the human body: skin and upper respiratory
- Illnesses: minor (impetigo and pimples) or severe (pneumonia or sepsis) cases

Staphylococcus aureus

TRANSMISSION

- Direct skin contact or contact through contaminated objects
- Contact with pus from infected wound

VIRULENCE FACTORS

- Toxins, Adhesions proteins, Colonizing proteins, Enzymes, Superantigens

mecA Gene:

- Mutation: encoding a modified penicillin-binding protein
- Offers resistance against methicillin and other Beta-lactam antibiotics
- Difficult to treat

Klebsiella pneumoniae

- Gram-negative bacteria, rod-shaped
- Facultative anaerobe and not motile
- Localization: infect lungs and burn wounds
    - Damage through inflammation and hemorrhage of the lung tissue
- Normal flora of the skin, mouth, and intestines
- K and O antigens: pathogenicity of bacteria
- Possibility of nosocomial infections: infections of the urinary tract, respiratory tract or the skin
- Opportunistic pathogen

Klebsiella pneumoniae

TRANSMISSION

- Person-to-person contact
- Contact from the environment

PATHOGENIC ANTIGENS

- K-antigen: Capsular polysaccharide
- O-antigen: A component of bacterial lipopolysaccharide

RESISTANCE

- Resistant to numerous antibiotics

TREATMENTS:

- 3rd or 4th Generation quinolones and carbapenems
- Monotherapy and combination treatments

Acinetobacter baumannii

- Gram-negative bacteria, rod-shaped
- Facultative anaerobe and not motile
- Those with compromised immunity are more susceptible
- Opportunistic pathogen (residing in water environments)
    - Infections in body systems with high fluid content
    - Respiratory tract, Urinary Tract, and Cerebral Spinal Fluid

Acinetobacter baumannii

VIRULENCE FACTORS

- Biofilms
- Protective capsule surrounding each bacterium cell
- Efflux pumps

MECHANISMS FOR ANTIBIOTIC RESISTANCE

- Small RNA
- Biofilm formation
- Efflux pumps

TREATMENTS

- Resistance to multiple drugs
- Difficult to treat

BACTERIAL SAMPLES FOR THE LAB

SWAB OF BURN WOUND AND PUS

- Method is nominally invasive
- Aseptic methods for obtaining sample (prevent contamination)
- Determining accurate densities of bacteria
    - Collection of multiple surface swabs
- Area of swab: viable tissue
    - Prevent contamination with necrotic tissue or pus

BACTERIAL SAMPLES FOR THE LAB

TISSUE BIOPSY

- Better prediction of septic infection
  - Will provide information on extent and depth of infection
- Limited clinical significance
- Not very practical compared to other methods

BLOOD AND URINE SAMPLES

- To test for systemic infections

HANDLING THE LAB SAMPLES

TRANSPORT OF LAB SAMPLES

- Must be done immediately: inoculation onto culture media within 2 hours of sample collection
- In a sterile container or swab transport system
    - With patient identification, source, and sampling information
- Inoculation of culture media must be done within 24 hours
- Always store at room temperature

IMPORTANCE OF MICROBIOLOGY LAB FOR DIAGNOSIS

- Confirmation of Infection: presence or absence
- Determination of antimicrobial susceptibility of the organisms colonizing the burn wound
- Deciding which treatment approach to use
    - Also consider antibiotic treatment
- More accurate determination of results over clinical presentation using signs and symptoms observed

TEST COMPLETED USING THE SAMPLES

- Gram Staining
- Catalase Test
- Oxidase Test
- Other Tests
- M-PCR

GRAM STAINING

PROCEDURE

- Will provide information of bacterial cell wall composition
- Crystal violet dye stains gram-positive bacteria = VIOLET
- A counterstain (like safranin) stains gram-negative bacteria = PINK

RESULTS

- *P. aeruginosa* is a gram-negative bacteria
    - Observe a counterstained sample = PINK

ENRICHMENT AND DIFFERENTIAL MEDIA

CAN EXPLORE

- Various enzymatic capabilities of different species
- Metabolic pathways of different species

AN EXAMPLE: MacConkey n°3 media

- Selects gram-negative bacteria
- Distinguishes Lac+ bacteria (RED) from Lac- bacteria (YELLOW or COLOURLESS)

CATALASE TEST

PROCEDURE

- Test for the presence of enzyme catalase
  - Resulting from oxygen respiring bacteria
- H_2O_2 applied to sample
- Observe: presence of catalase through BUBBLES
  - Release of oxygen molecules

RESULTS

- Positive result

OXIDASE TEST

PROCEDURE

- Tests for the presence of cytochrome C oxidase
- Positive (with oxidase) = BLUE
- Negative (no oxidase) = NO COLOUR

RESULT

- Positive = BLUE

TRIPLE SUGAR IRON TEST

TYPES OF TESTS IT INCLUDES

- Pyocyanin pigment production
- Analyzing colony morphologies
- Fermentation of carbohydrates like lactose, glucose, and sucrose
- Reduction of sulfur
- Survival at high temperatures (~44°C)

INFORMATION GIVEN

- Potential pathogens residing in infection

VOGES-PROSKAUER (VP) TEST

ABOUT THE TEST

- Identifies bacteria that would ferment glucose to pyruvic acid
  - The metabolize it to acetoin
- Separates Klebsiella-Enterobacter groups from *E. coli* (VP-negative)

METHYL-RED (MR) TEST

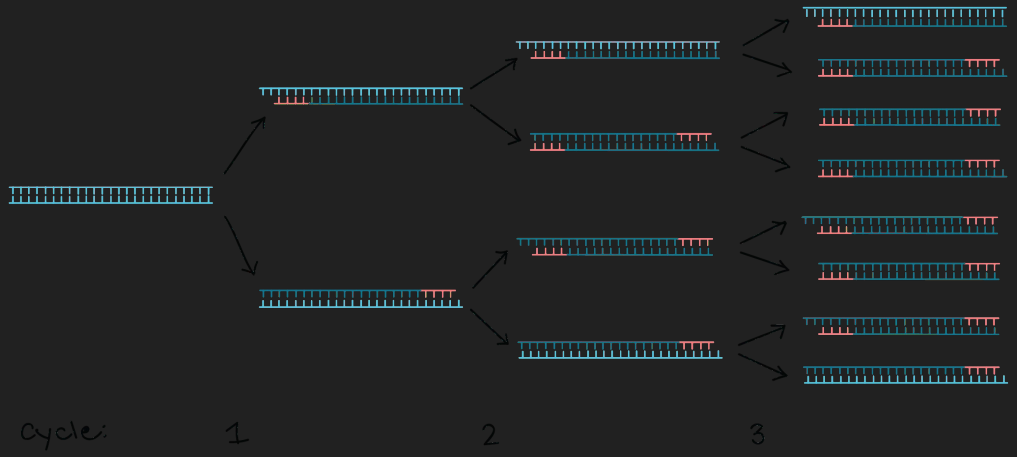
ABOUT THE TEST

- Separates Enterobacteriaceaee and Enterobacter aerogenes
  - Decarboxylate their byproducts
- Create alkaline environment and negative MR test

M-PCR

PROCEDURE

- Create copies of the bacterial genome
  - Sequence will be compared to the genetic database
- Method is used for bacteria that can’t be cultured



Source: KHAN ACADEMY
https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-sequencing-pcr-electrophoresis/a/dna-sequencing

RESULTS EXPECTED

OXIDASE AND CATALASE TEST RESULTS

- *P. aeruginosa* will test positive for both these tests
- Colour change observed with oxidative reagent: DARK PURPLE
- Distinct grape-like odour due to aminoacetophenone production
- Colonies of bacteria detected by fluorescence of MacConkey agar plates when exposed to UV light

M-PCR TEST RESULTS

- Translucent mucoid colonies of MacConkey agar plates: Pale and large
- Presence of water soluble pigments: pyroverdine (yellow-green) pycocyanin (blue-green)
- Amplification of four gene fragements: gyrB, ETA, oprL, and 16S rDNA

RESULTS EXPECTED

COLONY MORPHOLOGY ANALYSIS

- Depending on the site of infection, presence of:
  - Fried Egg Colony
    - Smooth and flat edges
  - Mucoid Colony
    - Smooth