Definition

Medical Microbiology – a branch of microbiology that focuses on organisms that cause human disease, and cover aspects of transmission, diagnosis, treatment and prevention.

Bacteria

Prokaryotic Cells

- 0.2 – 2 um in diameter
- 1 - 10 um in length
- **Single copy of genetic material**, usually circular double stranded DNA located in the nucleoid
- No nuclear membrane, nucleoli or membrane bound organelles
- Cell wall composed of **peptidoglycan**, no cholesterol
- 70s ribosomes
- Contain **inclusion bodies** – granules of organic or inorganic material that are stockpiled by the cell for use as energy stores
- **Endospores**

Taxonomy

- **Classification** – based on classical characteristics
- **Nomenclature**
- **Identification** – determine which group it belongs to

Endospores

Formed within normal vegetative cell in response to environmental stress and can survive for long periods of time and germinate when given appropriate environmental stimuli.

Occur due to unequal division resulting in formation of spores with own ribosomes and DNA.

Spores often do not take us dye.

Cell Wall

Peptidoglycan layers composed of two sugar derivatives, normally composed off sheets and linked by a tetrapeptide:

- NAG
- NAM

Gram-Positive Cell Wall

Consists of thick layer of **peptidoglycan** – highly cross-linked.

Also contains:

- **Teichoic acid** – covalently linked to peptidoglycan layer. Can elicit an immune response.
- **Lipoteichoic acid** – linked to plasma membrane

Gram-Negative Cell Wall

More complex than G(+) cell.

Thinner peptidoglycan layer. No teichoic acids.

Surrounded by **outer membrane** composed of **lipopolysaccharide**:

- The **polysaccharide** component of the outer membrane, O-polysaccharide, is an antigen used in identification (**O-antigen**)
- The **lipid** component can act as a potent **endotoxin** – can be involved in sepsis

Bacterial Capsules

Usually consist of polysaccharides and extend from the surface of the cell.

- Prevent bacteria from dehydration
- Involved in attachment to various body systems
- Survive harsh conditions
- Escape host defences – poorly antigenic and anti-phagocytic

Fimbriae

Short, thin, hairlike appendages

- Involved in attachment to surfaces in infection – can attach to epithelial in UTI
- Some required for motility

Sex Pili

Similar to fimbriae but much longer and less numerous. Usually required for mating (conjugation).

Flagella

H-antigen.

- Thin protein tubes 20nm in diameter
- Different arrangements can be seen in different bacteria, therefore can be diagnostic
- Flagellum rotates like a propeller, counter-clockwise rotation causes forward motion

Laboratory Diagnosis

Potential pathogens can be identified asd on:

- **Phenotype and physiology -** gram staining
- **Biochemistry**
- **Molecular techniques** – DNA/RNA sequencing
- **Immunologic techniques** – testing serum for bacterial antigens or antibody production

Microscopic Examination and Gram Stain

First step in identifying the organism is to prepare a smear slide, stain it and view under light microscope.

Growth of Potential Pathogen

To positively identify a pathogen, it is necessary to isolate it in pure culture – containing only one type of bacterium.

Bacteria often have different growth requirements:

- Temperature
- Oxygen requirements

Oxygen Requirements

- **Obligate anaerobes** – killed by oxygen
- **Facultative anaerobes** – grow in presence and absence of oxygen
- **Anaerobes** – can grow in absence of oxygen
- **Obligate aerobes** – require oxygen for growth
- **Aerobes** – grow in presence of oxygen

Growth Media

Enriched Media

Enriched media – infused with blood.

- **Horseblood agar** – whole blood
- **Chocolate agar** – lysed red blood cells

Also allows the identification of organisms that can damage red blood cells (e.g. haemolytic streptococci):

- **Beta haemolytic** – complete lyses of red blood cells (colonies surrounded by zones of clearing)
- **Alpha haemolytic** – partial lyses of red blood cells (colonies surrounded by green colour)
- **Gamma haemolytic** – no lysis

Selective and Differential

Contains substances that inhibit unwanted bacteria while allowing desired bacteria to grow. Also contains a pH sensitive dye that allows bacteria on the plate to be distinguished.

Contains selective agent and carbohydrate (carbon source).

- **Macconkey agar** – selects for gram negative organisms
  - **Selective agent –** bile salts (to select for agents that can grow in **gut**)
  - **Carbohydrate (differential) agent** – lactose

- **Mannitol salt agar**
  - **Selective agent** – salt (to select for agents that can grow on **skin**)
  - **Carbohydrate (differential) agent** – mannitol

Semi-Automated Identification (API) System

Different tubes contain various carbohydrates – bacteria is added.

Based on whether bacteria is positive for any of these, each one is assigned a number which generates a code that can be compared to a database.

Rapid Single Enzyme Tests

Catalase Test

Some pathogens possess the catalase enzyme which can inactivate oxygen radicals produced by immune cells.

Catalase converts hydrogen peroxide to water and oxygen.

Differentiates gram-positive cocci, **staphylococcus (+ve)** from **streptococcus (-ve)**

Coagulase Test

Enzyme causes a clot to form when bacteria are mixed with plasma.

Differentiates ***staphylococcus aureus*** **(+ve)** from **coagulase-negative staphylococci (CONS**).

Oxidase Test

The enzyme cytochrome c is part of the electron transport chain found in some bacteria – can accept electrons from artificial substances to produce a dark oxidised product.

Differentiates gram-negative bacteria:

- Enterobacteriaceae are -ve
- Pseudomonas aeruginosa are +ve

Immunologic Detection of Microorganisms

Used to detect patient's response to infection (antibody response) or detect the presence of antigen in patient's body fluid.

Latex Agglutination Test

Latex beads coat antigen or antibody and added to plate with patient serum.

Nucleic-Acid Based Tests (PCR)

Amplification of DNA.

Can be used to rapidly identify the presence of a pathogen's DNA in various clinical specimens. The **bacterial 16S RNA gene** has emerged as the most useful marker for microbial detection and identification.

Particularly useful in identifying bacteria that are not easily cultured – i.e. intracellular/atypical bacteria.

Antibiotic Susceptibility Testing

Once a pathogen is cultured, its sensitivity to specific antibiotics serves as a guide to choosing the appropriate antimicrobial therapy.

Disk-Diffusion Method

Qualitive method – put disk containing different antibiotics onto agar plate with pure culture.

Zone of inhibition of growth means that the organism is sensitive to the particular antimicrobial agent.

No zone of inhibition means that the organism is resistant to the particular antimicrobial agent.

Quantitative method – use a strip with increasing concentration of antibiotics and analyse zone of no growth to determine minimum inhibitory concentration.