BASIC MICROBIOLOGY

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CONTENTS

This chapter includes a review of:

- Micro-organisms
- Bacteria
- Viruses
- Chlamydiae
- Fungi
- Ocular pathogens
- Anti-microbial agents
- Protozoal parasites – amoeba

INTRODUCTION

It is essential for optometrists to understand:

- Patients with ‘red eye’.
- Prophylaxis following foreign body removal and contact tonometry.
- Understanding microbiology important for differential diagnosis of Red eye
- How disinfectants remove the potential for infection from contact lens solutions
- Contact lens solutions.
- Constituents of any eye drops.

MICRO-ORGANISMS

- Microbiology covers:
  - Protozoa
  - Fungi
  - Bacteria
  - Chlamydia,and
  - Viruses.
- Eucaryotic cells: similar to higher organisms protozoa and fungi
- Procaryotic cells: simple cells
- Protozoa and fungi have eucaryotic cells and can be either parasitic or free living.
- Bacteria are procaryotic cells
- Viruses are the simplest organism
  - can only multiply by invading host cells
  - neither procaryotic or eucaryotic

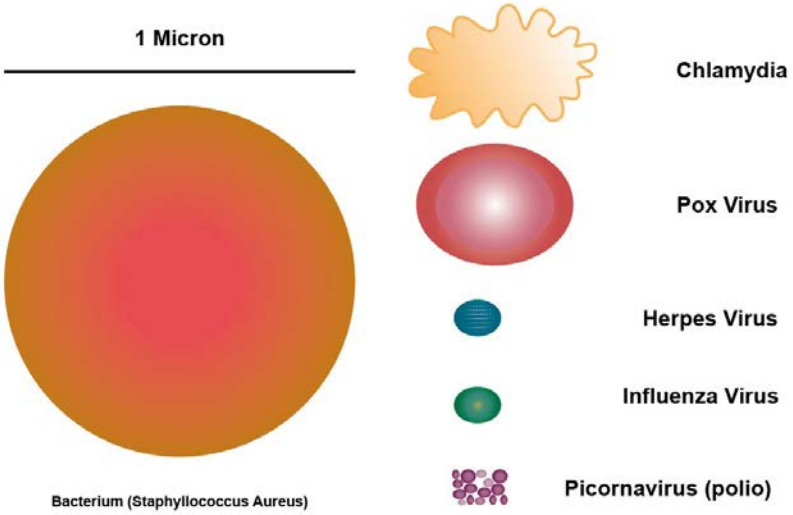

Figure 10.1 *Relative size of different micro-organisms. Relative sizes: viruses 10-300 nm (0.3 micrometers). Bacteria 0.5 to 50 micrometers. Smallest bacteria same as largest virus*

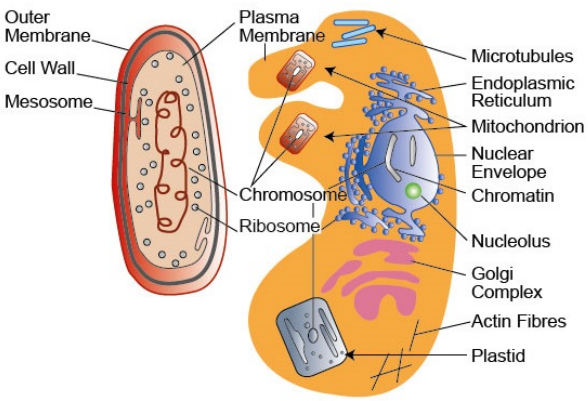

Figure 10.2 Structures of a micro-organism: procaryotic cell (left), eucaryotic cell (right)

- Procaryotic (P): bacteria
- Eucaryotic (E): fungi, plants and animals
- Size
    - E: larger
    - P: smaller
- Eucaryotic cells: nucleus and Endoplasmic reticulum
- Procaryotic cells: lack of nuclear membrane
- DNA
    - P: circular
    - E: linear
- Mitochondrion
    - P: absent
    - E: 1-several dozen
- Cell division
    - P: binary fission
    - E: mitosis or meiosis

BACTERIA

- Ability to infect and multiply in varied environments.
- Ability of many types to cause disease (pathogenicity)

Bacteria are important because of their pathogenicity, which is the ability of an organism to cause disease in another organism, thus called pathogen.

It is often used interchangeably with the term "virulence", although some authors prefer to reserve the latter term for descriptions of the relative degree of damage done by a pathogen. Pathogens are infectious agents that cause disease and include viruses, bacteria, fungi, protozoa and higher parasites.

Bacterial Cell

- Procaryotic cell.
- Absence of discrete structures found in eucaryotic cells.
- No mitochondria, no endoplasmic reticulum, no golgi apparatus. Ribosomes free in cytoplasm. No nucleus, no nuclear membrane and no mitosis.
- Single strand of DNA
    - Unlike eucaryotic cell where it is organised into chromosomes

Micro-organisms Requirements

- Nutritional
    - Nitrogen, Carbon, Oxygen, Hydrogen
- Oxygen
    - Water or atmospheric

- Conditions
    - Temperature and pH
- Metabolic

For a micro-organism to exist and multiply there are certain basic requirements:

- Nitrogen and Carbon – nutritional
- Oxygen and Hydrogen – water
- O_2: some require atmospheric, some cannot exist in O2 environments
- Can exist at high and low temperatures and pH
- Nitrogen and Carbon: most critical requirements
- Some have bacterial chlorophyll and can synthesise simple organic compounds using sunlight

Bacterial Reproduction

- Binary fission
- Four phases
    - Lag phase (A) - organisms remain dormant. There is no increase in numbers
    - Log phase (B) - exponential growth
    - Stationary phase (C) - number new organisms = number of dying organisms
    - Decline phase (D) - number of viable organism decline

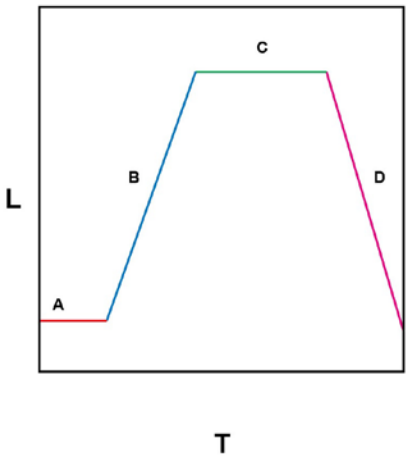

Figure 10.3 *The four phases of bacterial reproduction*

Bacterial Classification

- Microscopy and staining e.g. gram staining
    - Often necessary to determine exactly which bacteria present, especially for treatment
- Differential media and biochemical tests
    - Supports the growth of some bacteria and not others
- Serological testing
    - Specific antibodies
- Bacteriophage typing
    - Viruses that attack bacteria

VIRUSES

- Smaller than bacteria (18-300 nanometres)
- Animal viruses, plant viruses, bacteriophages.
- RNA or DNA surrounded by protein capsule or membrane.
- RNA or DNA - never both

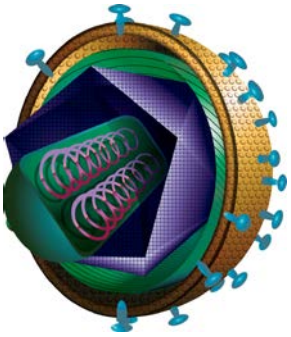

Figure 10.4 *Representation of a virus*

- Outer cover: vital role in infection and spread
    - Responsible for recognising host and binding to receptor molecule
- Capsid which is the outer shell has a rigid structure
- Protective against physical, mechanical, chemical and enzymatic damage

Virus Reproduction

- Virus particle, a viron, becomes attached to the surface of the cell via receptors.
    - There are specific receptors for specific conditions
- Virus particle passes into the cell.
- Virus particle loses its coat and releases genetic material into host.
- Into cytoplasm if RNA and into nucleus if DNA
- Viral genetic material induces cell to produce different macromolecules essential for production of new viral particles.
- Assembly of new virus particles takes place within the host cell and are released.

Virus Classification

- Single strand DNA virus e.g. Parvovirus
- Double strand DNA virus e.g. Herpes viruses
- Single strand RNA viruses e.g. Rubella
- Double strand RNA viruses e.g. Californian tick fever.

Main groups of animal viruses:

- Parvovirus: carried by dogs and can infect humans
- Colorado tick fever (CTF): muscle ache, headache, fever etc.

CHLAMYDIAE

- More complex than viruses but less complex than bacteria.
- Contain both DNA and RNA.
- Multiply by binary fission.
- Susceptible to certain antibiotics

Forms of Chlamydiae

- Elementary body (300nm)
    - Exists on outside of body – infections
- Reticulate body (100nm). Inside cell
    - Not infectious
    - Attack mucous membranes
    - Inhibit host cell protein synthesis
    - Obtain ATP from host
    - Cannot generate its own ATPthis
    - Synthesises own nucleic acid and proteins
    - E.g. chlamydia trachomatis

FUNGI

- Only 100 species are pathogenic out of tens of thousands.
- Infections ranging from trivial such as athlete's foot to severe such as filamentous keratitis.
- Can colonize non-living structures, e.g. SCL's.
- Eucaryotic cells: have nucleus and mitochondria
    - Includes moulds and yeasts

OCULAR PATHOGENS

Eye is at risk from a large range of opportunistic pathogens. For example, if cornea is compromised by a CL induced abrasion. The lids and conjunctiva at greatest risk of infection. In order to combat possible infection, tears contain antimicrobial agents- immunoglobin A, G and M (antibodies or proteins that identify and fight infections).

Possible sources of ocular infection

- Opportunistic pathogens
- Invasive organisms
- Congenital infections
- Direct contact
- Airborne infections
- Insect-borne infections
- Bacteria from nasopharynx
- Trauma
- Infected contact lenses
- Infected eye drops
- Infected instruments

COMMON OCULAR PATHOGENS

BACTERIA

- **Gram positive cocci**
  - Staphlycoccus aureus
    - some are normal inhabitants of skin, Some very serious: fatal septicaemia. Resistance is common, including MRSA (methicillin resistant).
    - infection of lids, lacrimal apparatus, conj, cornea, acute chronic blepharitis.
  - Streptococci
    - Cause sore throat, conjunctivitis, dacrocystits, blepharitis.
- **Gram negative cocci**
  - Neisseriae meningitis
    - Cause neisseriae gohonorrhae.
- **Gram positive rods**
  - Corynebacterium diptheriae
    - Can cause conjunctival infections.
- **Gram negative rods**
  - Pseudomonas aeruginosa
    - Most notorious of bacteria causing ocular problems.
    - Found in small numbers in gut and skin.
    - Normally controlled by bodys natural defences – but not in immunocompromised patients.
    - Cause meibomitis, conj, corneal ulcers, ophthalmia neonatorum.

VIRUSES

- Herpes virus
  - Herpes Zoster (HZ): chicken pox virus in children
    - Shingles in adults
    - Virus stored in trigeminal nerve – can affect ophthalmic division
    - Re- activated
    - Cytomegalovirus
  - Herpes Simplex (HS) – dendritic ulcers
- Adenovirus
  - causes 5–10% of upper respiratory infections in children
  - Can cause conjunctivitis, ear infection, throat infection
  - Highly contagious
- Varicella zoster virus
  - chicken pox and shingles

FUNGI

- Candida albicans
- Aspergillus niger

- Opportunistic fungus, can strike after corneal abrasion or surgery
- Normally found in mucous membranes of eye
- Cause corneal ulcers, conjunctivitis, uveitis
- Also Aspergillus Niger: severe local infection in eye

CHLAMYDIA

- Causes trachoma
    - Affects around 40 million people around the world (Burton, Mabey, 2009).
    - Areas where over 90% affected.
    - Thrives in poor living conditions
    - Passed on by insects and contaminated objects e.g. bed linen.
    - Causes conjunctivitis, corneal pannus, severe scarring, trichiasis (lashes grow towards eye).

ANTI-MICROBIAL AGENTS

- Physical agents
- Antimicrobial preservatives
- Chemotherapeutic agents

Many agents have an ability to kill micro-organisms, e.g. white blood cells circulating antibodies in blood.

- Sterilization
    - killing or removal of all viable organisms from object or pharmaceutical product
- Disinfection
    - capacity of an object to cause infection is removed
- Chemotherapeutic agents
    - treatment of disease by chemicals.

PHYSICAL AGENTS

- Heat
- Freezing
- Ionizing
- UV radiation
- Filtration
- Ultrasonics

Heat:

- One of the best known disinfecting or sterilizing agents
- Used for solutions, instruments, dressings

- Heat at 60 degrees will kill most viruses as well as pathogenic bacteria and fungi
- Boiling – spores of pathogenic bacteria

Autoclaves:

- Heating product in steam at 121 degrees for 15 mins – sterilization
- Dry heat is less effective

Freezing:

- Reduce number of bacteria
- Damaged by formation of ice crystals
- Some will survive in a dormant phase

Ionizing radiation:

- Fluorescein impregnated strips
- Alpha, beta and gamma rays used

UV radiation:

- Light is bactericidal at low wavelengths (240-280nm) UV region

Filtration:

- 0.22 µmmicro m filter
- Retains all bacteria – not viruses

Ultrasonics:

- Sound will kill bacteria
- Ultrasonic CL cleaners

ANTI-MICROBIAL PRESERVATIVES

- Benzalkonium chloride
    - Detergent action
    - Disruption of cell membrane
    - Most commonly used preservative
    - Often combined with a chelating agent to enhance effect of preservative
- Mercury components
    - Thiomersal – hypersensitivity and toxic to some patients
- Chlorhexidine
    - Alternative. Toxic to corneal endothelium in high concentration
- Oxidizing agents
    - H_2O_2 3-6%. >10% for spores

CHEMOTHERAPEUTIC AGENTS

Different chemotherapeutic agents have specific action against certain groups of micro-organisms:

- Inhibition of cell wall formation e.g. penicillin
- Inhibition or damage to cell membrane e.g. polymixin B
    - Important in prokaryotic cell. Affects cell respiration.
- Inhibition of protein synthesis e.g. tetracyclines
- Inhibition of nucleic acid synthesis e.g. quinolines
- Antimetabolites e.g. sulphonamides
    - interfere with production of new genetic material.

PROTOZOAL PARASITES - AMOEBA

Acanthamoeba

Common protozoa which is found in water and soil.

- Can cause keratitis.
- Associated with soft contact lens wear and cleaning regimes involving water (85%).
- 1 in 30,000 contact lens wearers
- Difficult to treat
- Rare
- Painful

In developed world, keratitis caused by acanthamoeba is almost always associated with contact lens wear. In developing world, this is due to poor hygiene.

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