Microbiology and Sampling Techniques

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Aim:
To update and revise Podiatry-relevant microbiology and tissue sampling
Objectives

- To revise the biology of micro-organisms
  - especially those that impact on Podiatric practice
- To consider the nature of infection
  - especially those that impact on Podiatric practice
- To revise the principles of tissue sampling
  - especially those that impact on Podiatric practice
Microbiology

The branch of biology that deals with

- The identification and classification of micro-organisms (MOs) and
- The effects of the presence of MOs on other living organisms
Sampling
Collection of specimens of tissue for

- Microscopy
  - *visual identification* and classification of cellular matter and MOs

- Culture
  - *lab. growth* of tissues and MOs

- Sensitivity
  - *susceptibility* of tissues and MOs to drugs and anti-infective agents
Living Organisms

- Single-celled plants and animals
- Multi-celled plants and animals

The majority of living matter is completely invisible to the naked eye!
Eukaryocytes are organisms that have nucleated cells, which include:

- Single-celled animals, plants, and fungi
- All multi-celled animals (including humans)
- All multi-celled plants and fungi

They form 50% of the world’s biomass. Biomass is defined as the total mass of living matter within a given unit of environmental area.
Single celled eucaryocytic animals, plants and fungi
Multi-celled eucaryocytic animals, plants and fungi
Prokaryocytes

- **Prokaryocytes** = non-nucleated MOs
  - e.g.: bacteria
- Found everywhere - sea water, soil, air, gastrointestinal tracts, hot springs, and in rocks
- Cover all non-sterilized surfaces
- Prokaryocyte organisms are estimated to make up remaining **50% of earth’s biomass**
Micro-organisms (MOs)

MOs = very diverse group of eukaryotic and prokaryotic **unicellular** organisms essential to all ecosystems

- Include **bacteria**, **fungi**, **archaea**, and **protists**; microscopic **plants** (**green algae**); and **animals** such as **plankton** and the **planarian**.

- Some microbiologists also include **viruses**, (others do not, classing viruses to be non-living organic material)
MOs live in all parts of the biosphere where there is liquid water:
- Soil, hot springs, on the ocean floor, high in the atmosphere and deep inside rocks.

MOs are critical to ecosystems:
- Nutrient recycling where they act as decomposers.
- Nitrogen fixation: vital part of the nitrogen cycle.
- Airborne MOs may play a role in precipitation and weather.
Large populations of MOs are normally present on intact skin but do not cause infection due to:

- **Intact stratum corneum**
  - a physical barrier to invasion by MOs
  - slightly acidic pH (fatty acids and other antibacterial polypeptides).

- **Population of diverse commensal, normal skin flora**
  - prevent potentially pathogenic MOs from establishing viable colonies

- **Early defence systems**
  - Inflammation + complement cascade
  - Macrophage (WBC) recruitment
  - Immunoglobulins
Normal commensal flora on skin surface
Infection of subcutaneous and deeper tissues:

occurs when

- the protective epithelial barrier is lost / broached
- the host is immuno-compromised
- inflammatory and healing responses are reduced
- tissue ischaemia prevails
Contamination–Colonization–Infection Spectrum

- **Contamination:**
  - presence of MOs at the wound surface

- **Colonization:**
  - wound surface MOs begin to replicate and increase their metabolic activity.

- **Infection:**
  - invasive growth of MOs within tissues, potentially damaging the tissues and the host
  - Localised
  - Spreading

- Bioburden of $> 10^5$ MOs / g of tissue
  - Triggers infection
  - Suppresses healing
Bioburden assessment
• host resistance
• wound characteristics
• wound cultures.
Infection

- Tissue reaction to MOs
  - Inflammation++
  - Signs of infection (e.g.: cellulitis, pain, pus, malaise)

- Infection
  - Invasion of body tissues by disease-inducing MOs and
  - The reaction of those tissues to the MOs and/or MO-toxins

- Concept of MO load
  - $>10^6$ MOs / cm$^3$ bioburden = tissue infection
  - $>10^3$ MOs / cm$^3$ bioburden = infection in DM wounds
Factors that favour the development of tissue infection

- **Local factors**
  - Large / deep wound
  - Chronic peripheral wound
  - Foreign / necrotic tissue within the wound area
  - On-going contamination
  - Prior or on-going tissue stress / collateral damage
  - Poor local perfusion

- **Systemic factors**
  - Vascular disease / smoking
  - Limb oedema
  - Malnutrition / obesity
  - Diabetes mellitus
  - Renal failure
  - Alcoholism
  - Previous tissue damage
  - Corticosteroid / immunosuppressant therapy
  - WBC defects
Micro-organisms that may cause infection in humans

- Bacteria
- Fungi
- Viruses
- Prions
Bacteria (1)

- Prokaryotic MOs,
- \( \sim 1-5 \times 10^{-6} \text{m} \) long
  - Surrounded by a **cell wall**, provides strength and rigidity
  - *Non-nucleated*
  - Lack membrane-bound intracellular organelles
- Can function and reproduce as individual cells
  - Often *aggregate* into multicellular colonies
- Have a single loop of **DNA**
  - Also harbor small pieces of DNA called **plasmids**
  - Plasmids can transfer via **bacterial conjugation**.
Bacteria (2)

- Reproduce by **binary fission**
  - Do not undergo **sexual reproduction**.
- Under optimal conditions a bacterial colony can double in size / numbers every 10 min
- Some species form extraordinarily resilient **spores**
  - Mechanism for MO survival, not reproduction.
Bacteria: spore formation
Bacteria come in a wide variety of shapes.
Cocci

Baccilli

Spirochetes
Some are pathogenic - (most bacteria are not)

- *Mycobacterium tuberculosis*
- *Streptococcus*
- *Pseudomonas,*
- *Campylobacter*
- *Salmonella*
- *Tetanus*
- *Typhoid fever*
- *Diphtheria,*
- *Syphilis*
- *Hansen's disease,* etc etc.

Bacteria can often be killed by antibiotics
  - ABx destroy bacterial cell wall
Typical bacterial skin, bone and soft tissue infecting agents

- **Staphylococcus aureus**
  - Cause localised infections, and abscess formation

- **Streptococcus pyogenes**
  - Causes radially spreading infections, such as erysipelas

- **Escherichia (E) coli**
  - Can cause deep infections, e.g.: osteomyelitis
Impetigo

Folliculitis

Cellulitis

Acute paronychia

Examples of Staph aureus skin infections
Some Staph. infections are really obvious
Others are more subtle
Examples of Streptococcal soft tissue infection

Examples of Pseudomonal soft tissue infection
Fungi

Fungi are eukaryocytes (have nuclei)

Several unicellular species
- e.g.: baker's yeast (*Saccharomyces cerevisiae*)

Some fungi, e.g.: pathogenic yeast *Candida albicans* can undergo *phenotypic switching*
- grow as single cells in some environments
- or form *filamentous hyphae* (mats) in others

Reproduce
- both asexually, by budding (binary fission)
- and by producing spores (1-40 $10^{-6}$ mm long)
  - *conidia* when produced asexually
  - *basidiospores* when produced sexually.
Fungi are usually **saprophytes**
- *MOs that feeds on dead or decaying matter*

Some can cause diseases in humans, animals and plants
- *most common cause of diseases in crops and other plants*

Life threatening fungal infections in humans most often occur in immunocompromised patients or vulnerable people with a weakened immune system
- Fungal infections affecting skin and nails are more common in the immuno-incompetent population (e.g.: DM, stressed)

Fungi are **non-susceptible to antibiotics**
- both fungi and host has eukaryotic (nucleated) cells
- Antibiotics only affect prokaryotic (non-nucleated) cells.

Most clinical **fungicides areazole- or alaylamine-type drugs**
Fungi affecting skin and nail:

Trichophyton: Pencil Shaped

Microsporum: Spindle shaped

Epidermophyton: club shaped
Common dermatophytes of skin and nail

- **Trichophyton rubrum**
  - ‘Pencils’

- **Epidermophyton**
  - ‘Clubs’

- **Microsprorum**
  - ‘Spindles’
Dermatophyte infections of skin and nails
Some skin conditions can mimic fungal infections
Yeast Infections

Chronic Paronychia

Candida albicans
Viruses

- Sub-microscopic MOs
  - 20-300 nanometers in length \((1\text{nm} = 1 \times 10^{-6}\text{mm} = 1 \times 10^{-9}\text{m})\)
- DNA enclosed within a capsule
  - Virus DNA alters the way host DNA behaves: e.g.: triggers release of inflammatory mediators and cytokines
- *Not susceptible to antibiotics*
  - Reside inside nucleated cells of host
- Pathogenic viruses include
  - Adenoviridae, Picornaviridae, Herpesviridae, Hepadnaviridae, Flaviviridae, Retroviridae, Orthomyxoviridae, Paramyxoviridae, Papovaviridae, Polyomavirus, Rhabdoviridae, Togaviridae.
- Cause diseases including
  - Smallpox, influenza, mumps, measles, chicken pox, ebola, rubella, *warts* (VPs), shingles, polio, hepatitis B, HIV etc etc.
Viral Infections in Skin

- **Molluscum contagiosum**
- **Verruca pedis**
- **Orf (hand, foot and mouth disease)**
- **Herpes zoster (Shingles)**

**Viral Infections in Skin**
Prions

- Abnormal proteins
- Infectious pathogens that *do not contain* nucleic acids.
- Their presence causes e.g.:
  - *scrapie*, bovine spongiform encephalopathy (BSE; ‘mad cow’ disease) and Creutzfeldt–Jakob disease (CJD)
- Prions are *very difficult to destroy by normal sterilization* processes
  - It can require up to 18 autoclave cycles to denature prion protein
Tissue Sampling Techniques
Principles of sampling

- Take samples before any skin prep / wound cleansing
  - Take deep and superficial tissue samples
  - Give the lab as much tissue as is reasonable
- Transport samples in Lab-preferred containers
  - ‘Medium’ tubes – wet samples
    - (close tube tightly to prevent the sample contents drying out)
  - Paper – dry samples
- Tissue biopsy
  - Include normal ‘edge’ tissue as well as diseased area
  - Include deep as well as superficial lesion tissue
- Storage of ‘over-night’ samples
  - Not in the communal fridge!
Labelling samples

- Patient name + DoB
- Patient identification number
- Name of clinician
- Contact details of clinician
- Sample site
- Type of tissue sampled
- Date of sampling
- Any patient co-morbidities
- Medications (e.g.: ABx)
- Type of lab. test required
  - e.g.: Microscopy, culture, sensitivity
Tissue Samples
Harvesting samples of tissue infected and/or affected by bacteria

- Exudate
- Blood
- Pus
- Slough
- Necrotic tissue
- Sequestrae
Bacterial Infection in Wounds

- **2000 wounds tested:**
  - 55.5% of wound showed +ve to bacterial culture

- **Total of 1462 strains of bacteria isolated**
  - ~50% G+ve (e.g.: Staphs)
  - ~47% G-ve (e.g.: E coli)
  - ~3% Anaerobic

Wound swab

Superficial Swab

Deep Swab
Comparison of bacterial cultures from superficial and deep wound swabs

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<tr>
<th>MO</th>
<th>Superficial culture</th>
<th>Deep culture</th>
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<tbody>
<tr>
<td>G+ve</td>
<td>58%</td>
<td>66%</td>
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<tr>
<td>Coag-ve</td>
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<tr>
<td>G-ve</td>
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<td>34%</td>
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<tr>
<td>PsA</td>
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<td>10%</td>
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<tr>
<td>E Coli</td>
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<td>7%</td>
</tr>
<tr>
<td>Klebs</td>
<td>5%</td>
<td>5%</td>
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Path Lab Culture MOs - 1

Transported sample

Sample transferred to nutrient media on plate

Plate incubated at constant temperature in [CO₂] for ~24hrs
Path Lab Culture MOs - 2

Visual inspection of culture
Identification by characteristic features

Culture re-incubated on ABx discs = sensitivity

Written report of results
Path Lab Blood Analysis

Labelled blood sample

Plasma and cells separated in centrifuge

Blood analysis machine

Written report generated
Samples of material affected by fungi

- The ‘leading edge’ is where the active fungi are most likely to be found
- Collected as ‘dry’ samples, dispatched in paper fold
Skin sample collection sites in suspected fungal infection
Nail tissue sampling sites in suspected onychomycosis
Biopsy

- Punch biopsy

- Wide excision biopsy
Summary:

- revised the biology of micro-organisms
  - especially those that impact on Podiatric practice
- considered the nature of infection
  - especially those that impact on Podiatric practice
- revised the principles of tissue sampling
  - especially those that impact on Podiatric practice
Bacteria

- Form *part of normal skin flora*
- **Non-nucleated MOs**
  - Staph aureus, Pseudomonas cause skin infections
  - E Coli can cause osteomyelitis
- **Aerobic / anaerobic**
- **Coagulase +ve / Coagulase –ve**
- Immunocompromise = infection susceptible
- Many Staphs resistant to common ABx
  - MRSA resistant to flucloxacillin and many other ABx
Fungi

- Saprophytes
- Nucleated
  - Non-susceptible to Abx
- Susceptible to broad spectrum drugs
  - *Topical* qzole- and allylamine-based anti-fungal drugs
    - E.g.: miconazole, clotrimazole, terbinafine
  - *Systemic* allylamine-based antifungal drugs
    - E.g.: Terbinafine
- Fungal infections more common in the immunocompromised
Viruses

- Sub-microscopic
  - Within-cell ‘parasite’
  - Incorporated into host cell DNA or RNA
- Infections can be persistant
- Infections more common in immunocompromised patients
- Cure = acquired host immunity
Prions

- Neuroproteins moeity
- Cause of infectious dementia (CJD)
- Not destroyed by cooking
- Not easily destroyed by autoclaving
Tissue sampling

- Collect, transport and label according to protocols
- Collect sufficient sample from deep wound and peripheral skin infections
- Microscopy, culture and sensitivity
- See the advice of the microbiologist for the treatment of less common infections (local protocols)
Thank you for your attention!

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