Clinically-important Micro-organisms.

Staphylococci

Staphylococci are very common and important human pathogens. The microscopic grape-like clusters were described by Robert Koch in 1878, and grown by Louis Pasteur in 1880, who stated '*osteomyelitis is a boil in bone marrow'.*

Staphylococcus is by far the most important genus in the family of Gram-positive cocci called Micrococcaceae. Two other genera in the family, *Stomatococcus* and *Micrococcus,* very rarely cause human infections.

Staphylococcus aureus, which produces the enzyme coagulase and usually has golden-yellow colonies, is the major human pathogen. It is pyogenic (pus-producing), causing abscesses in skin and most other organs, leading to bacteraemia and endocarditis, and also produces many toxins. It has four special characteristics:

- Virulence - causing severe disease in normal hosts
- Difference - causing different disease in different sites, by different mechanisms, and involving different strains
- Persistence - both in the environment, and on humans, who are frequently asymptomatic carriers
- Resistance - to many antibiotics that were previously effective.

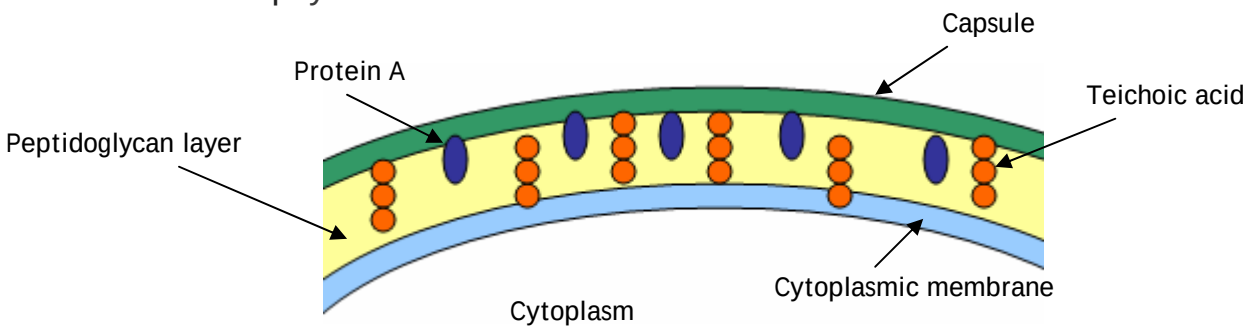
The rest of the genus are coagulase-negative staphylococci (CNS), and the most important are S. *epidermidis* (formerly S. *albus* because colonies are usually white), and S. *saprophyticus.*

Structure

Staphylococci have the typical bacterial procaryotic internal structure and Gram-positive cell walls. In addition to the usual peptidoglycan (murein), S. *aureus* has two special components in the cell wall:

Protein A - unique to S. *aureus.* It is linked to the peptidoglycan with an outer end that binds to the Fc receptor of IgG, protecting the microbe from opsonisation. This property is used in some serological tests for other organisms, to carry an antibody against them.

Teichoic acids - polyribitol glycerophosphates are found in all staphylococci and are involved in complement activation and attachment to mucosal surfaces as they bind to fibronectin. Anti-teichoic acid antibodies are a research test for systemic staphylococcal infections.



Other components include:

Capsules - rare in culture but more common in infected tissue. The capsule protects from complement, antibodies and phagocytes

Slime layers - found in some coagulase-negative staphylococci, which assist adherence to synthetic catheters, grafts, and prostheses while hindering chemotaxis and phagocytosis.

Pathogenesis and virulence

S. *epidermidis* and related species are members of the normal skin flora, and opportunist pathogens in hosts with impaired defences. They particularly infect by attaching to foreign, synthetic materials like intravascular catheters or joint prostheses, often assisted by slime production. They lack most of the toxins and enzymes of S. *Aureus.*

S. *saprophyticus* has special adherence factors for urinary tract

epithelium. S. *aureus* by contrast, has many virulence factors. These are toxins, enzymes and the actual structure of the organism itself. Its success as a pathogen also results from its lack of antigenicity and the consequent lack of protective antibodies.

Clinical syndromes

Staphylococcal infection presents with a wide range of syndromes affecting many tissues and caused by three mechanisms:

- local destruction (abcess)
- blood spread
- toxin production.

Chemotherapy

Penicillinase-resistant penicillins such as oxacillin and flucloxacillin are used for serious infections. First or second generation cephalosporins such as cephalothin, cephalexin and cefuroxime are usually safe in patients who are hypersensitive to penicillins. Vancomycin is usually effective for methicillin-resistant staphylococci. Erythromycin and its newer relatives are used in milder infections.

Control is both important and difficult in hospitals as staphylococci can persist for months in dust, curtains and linen, and human carriage is often permanent. Reservoirs, routes of spread and ruptures of skin and mucous membranes differ, so different measures are appropriate in different circumstances. These include cleaning, hand disinfection, air-control, decrease in direct and indirect contact, and portal protection by aseptic or no-touch technique for all invasive procedures.

KEY LEARNING POINTS.

1. **Staphylococci are Gram-positive cocci are important pathogens, particularly the coagulase-positive** *S. aureus.*
2. **Coagulase-negative staphylococci include** *S. epidermidis*, **important in infecting prostheses and catheters, and** *S. saprophyticus*, **a cause of urinary infections.**
3. *S. aureus* **is a virulent primary pathogen causing many different infections in many different tissues by three major mechanisms: by abscess formation, by blood spread (bacteraemia and endocarditis) and by toxins.**
4. **Cell structure includes the usual Gram-positive cell wall plus protein A and teichoic acid.**
5. **Virulence factors include many enzymes, toxins and the bacterial structure.**
6. **Management includes antibiotics (testing for susceptibility) and minimising infection by cleansing techniques, control of air and contact, and aseptic procedures.**

Streptococci and Enterococci

Classification and description

Streptococci and enterococci have certain characteristics that contribute to their ability to cause disease:

- the ability to live as normal flora on our skin and mucosal surfaces, mainly in the nasopharynx, gut and vagina
- *Strep. pyogenes* and *Strep. pneumoniae* are aggressive pathogens with numerous virulence factors, which give the ability to adhere, invade and damage tissues.
- other strains are 'opportunistic pathogens': normal flora that can become pathogenic in abnormal sites or in abnormal hosts
- infection is followed by spread locally, to distant organs and to other people.

The description of these organisms is:

- Gram-positive cocci (GPC), usually in chains, sometimes in pairs
- non-motile, non-sparing and may be capsulated
- facultatively anaerobic
- nutritionally fastidious, needing blood or other rich media
- catalase negative.

The classification of streptococci is confusing, as three separate criteria are used:

- biochemical into species
- serological based on specific polysaccharide antigens in the cell wall
- haemolytic by the lysis seen when cultured on sheep blood agar: beta means a clear zone; alpha, a green zone (viridans means 'making green' in Latin) and gamma means no haemolysis. E.g. beta-haemolytic streptococci.

Enterococci are now placed in a separate genus because of different

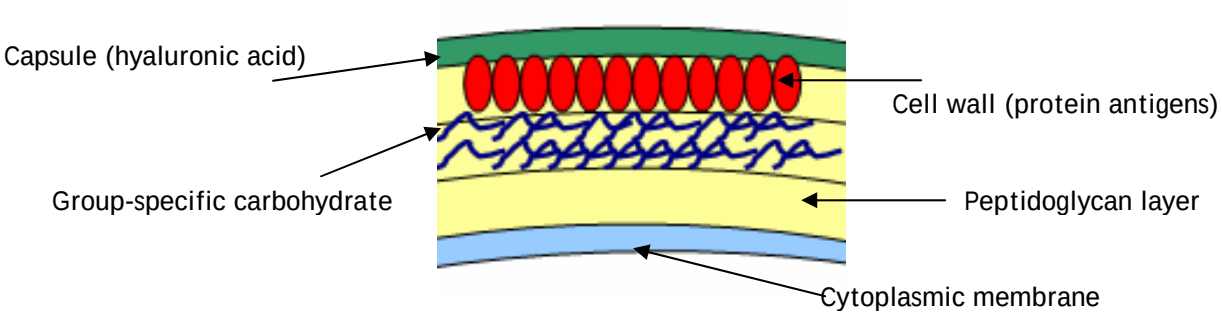
characteristics, including resistance to bile, 6.5% NaCl and antibiotics.

Streptococci that are obligate anaerobes are also placed in a separate genus, called *Peptostreptococcus*.

Structure and Function

Streptococci have a complex cell wall. The biological principle that structure relates to function is illustrated by the components:

- pneumococcal capsule gives resistance to phagocytosis
- lipoteichoic acid on pili helps adhesion to host cells
- type-specific M-Protein in group A gives virulence
- Lancefield group-specific carbohydrate protects peptidoglycan
- linear peptidoglycan with cross-linking gives rigidity.



Pathogenesis and virulence

Virulence factors are found in the aggressive pathogens *Strep. pyogenes* and *Strep. pneumoniae* and are related to surface antigens and extracellular products. Some appear to help the spread of disease, but it is not yet possible to link every individual toxin with particular clinical infections.

Clinical Syndromes

Disease is caused by:

- direct invasion and inflammation
- local spread
- distant spread

- distant toxin effects, e.g. scarlet fever
- immune mechanisms, e.g. rheumatic fever.

There is therefore a wide range of clinical syndromes associated with streptococcal infections:

Strep. Pyogenes - throat infections, wound and burn infections, puerperal sepsis, scarlet fever, rheumatic fever, glomerulonephritis, septicaemia etc.

Strep. Agalactiae - neonatal pneumonia and meningitis, puerperal sepsis

Enterococci - urinary tract and wound infections, endocarditis, septicaemia

Strep. Pneumoniae - bronchitis, pneumonia, bacteraemia, meningitis

Strep. viridans **group** – caries, endocarditis, bacteraemia.

Chemotherapy

In general streptococci are very sensitive to penicillin, while enterococci are quite resistant to most antibiotics, except ampicillin or vancomycin. However, pneumococci with partial or complete resistance to penicillin and vancomycin-resistant enterococci are increasingly common.

A multivalent pneumococcal vaccine is used to protect those particularly at risk, including splenectomised and immunocompromised patients. Locating carriers (nose, throat, skin or perineal carriage) is important in controlling outbreaks, especially in hospitals or closed communities.

KEY LEARNING POINTS.

1. **Streptococci are Gram-positive cocci, usually growing in chains, facultative anaerobes, nutritionally fastidious and catalase negative.**
2. **Enterococci are more resistant than streptococci to bile, salt and antibiotics.**
3. **Identification depends on Gram stain, haemolysis, biochemical tests and Lancefield grouping.**
4. **The complex cell wall and enzymes and toxins have important functions, including adhesion, virulence and spread.**
5. ***Strep. pyogenes*** **and** ***Strep. pneumoniae*** **are aggressive pathogens, invasive and virulent even in normal hosts.**
6. **Other streptococci are opportunistic pathogens, i.e. normal flora that cause disease in abnormal sites or abnormal hosts.**
7. **Disease is caused by invasion and spread, toxin effects and immune mechanisms.**

Clostridium

Clostridia are Gram-positive, strictly anaerobic, spore-forming rods, mainly free-living in soil. There are four major human pathogens, but numerous other species occasionally cause infections. The four important pathogens are:

- *C. perfringens*, causing skin, soft tissue, and muscle infections, ranging from simple cellulitis to gas gangrene and also causing food poisoning and enteritis necroticans
- *C.tetani*, the cause of tetanus
- *C. difficile*, implicated in antibiotic-associated colitis
- *C. botulinum*, the cause of botulism.

Features used to distinguish species include colony appearance, the shape and position of spores, motility, biochemical tests and toxin production.

Clostridium perfringens

C. perfingens (formerly *C. welchii*) is a large, spore-forming Gram-positive rod which is an obligate anaerobe, although it can tolerate exposure to air for up to 72 hours. Unlike most other clostridia it is non-motile, although colonies spread rapidly on agar plates. It is haemolytic, and metabolically active. It is found in soil, the gut, the female genital tract and nearby skin.

C. perfingens produces many toxins and extracellular enzymes, with alpha toxin as the most important, lysing red and white blood cells, platelets and endothelium, causing severe haemolysis, bleeding and tissue destruction. Beta toxin causes vascular leakage and is also important in necrotising enteritis. Delta toxin haemolyses red cells. Theta toxin causes haemolysis, pulmonary oedema and cardiac arrhythmias.

Production of extracellular enzymes helps the organism spread rapidly through tissues. The enterotoxin works differently, acting like cholera toxin on the adenylate cyclase system of ion transport to cause fluid loss and diarrhoea.

Clinical syndromes

In skin, soft tissues and muscle there is a spectrum from cellulitis to gas gangrene and rapid death. In the gut there are two different conditions, infective 'food poisoning', and necrotising enteritis.

Management

Penicillin is given for the tissue infections, but urgent surgery and consultation concerning antitoxin and hyperbaric oxygen are essential. Antibiotics are not needed for food poisoning and are of little help in necrotising enteritis.

Clostridium tetani

C. tetani is a large anaerobic Gram positive rod usually found in soil. Tetanus, which is caused by the toxin produced by growing *C. tetani*, has been known since antiquity, particularly related to war injuries. When a wound is contaminated with tetanus spores, tetanus occurs only if the tissue conditions are suitable for spore germination, i.e. necrosis and anaerobiosis.

Pathogenesis and virulence

Germinating growing organisms produce tetanospasmin, one of the two most potent poisons known. It binds to peripheral nerve membranes, then moves by retrograde neuronal transport to anterior horn cells where it blocks the release of inhibitory neurotransmitters, thus causing spasms and spastic paralysis.

Confirmatory tests

Diagnosis is clinical, as *C. tetani* may be isolated from contaminated wounds in which it has not released toxin and, conversely, often cannot be found in the wound (which may be trivial but must be anaerobic) causing tetanus.

Clinical syndrome and management

Tetanus is the clinical syndrome which develops. Treatment includes antitoxin, penicillin ± metronidazole, surgical wound care, sedation, and often paralysis and ventilation. Since patients with severe injuries usually receive antitoxin, most cases of tetanus arise from relatively trivial injuries involving contamination with soil or foreign bodies. Active immunisation with tetanus toxoid before injury or after recovery gives excellent immunity.

Clostridium botulinum

C. botulinum is an anaerobic, motile, spore-forming Gram-positive rod with oval, subterminal or central spores. The spores from soil or vegetables are relatively heat resistant

Pathogenesis and virulence

Food-borne disease is produced (like tetanus) not by infection but by intoxication with an extremely potent heat-labile toxin, usually types A, B or E, in uncooked or improperly cooked foods (the toxin is destroyed by boiling at 100'C for 10 minutes). The heavy chain of the toxin binds to cholinergic nerves, blocking acetylcholine release and hence blocking transmission. Infant botulism is an actual infection caused as the organisms (often from honey) multiply in the gut and liberate toxin there. Wound botulism is very rare and is produced by toxin from multiplying organisms in an infected wound.

Clinical syndromes and management

The three forms are food-borne, infant, and wound botulism. Penicillin treatment is used, but early antitoxin before all the toxin binds to nervous tissue is necessary and ventilatory support may be needed. Control depends on education in correct cooking and home bottling methods.

Clostridium difficile

C. difficile was only recognised as a pathogen in the early 1970s. It is an obligate anaerobe with typical Gram-positive structure, and resistant spores. It is more antibiotic resistant than other clostridia. It is part of normal bowel flora in most children and some adults. Its spores persist in hospital and other environments, and some infections are exogenous.

Pathogenesis and virulence

C. difficile produces two toxins, an enterotoxin (toxin A) causing secretory, haemorrhagic diarrhoea, and a cytotoxin (toxin B) causing a destructive cytopathic effect in tissue culture cells. Both cause changes in experimental animals, and each appears important in pathogenesis when the balance of normal flora in the colon is upset by antibiotic therapy.

Clinical syndrome and management

C. difficile produces antibiotic-associated diarrhoea, varying in severity from several loose stools daily to severe pseudomembranous colitis. Withdrawal of the causative antibiotic is sufficient in mild cases, but oral metronidazole, vancomycin or bacitracin is effective in more severe cases, though relapse is common. Control is by careful use of antibiotics, and care in hospital practice to avoid cross-infection.

Other species including *C. septicum, C. novyi* and *C. tertium* can cause cellulitis and gangrene similarly to *C. perfingens*. Apparently spontaneous infection with *C. septicum* is often a sign of undiagnosed colonic cancer.

KEY LEARNING POINTS.

1. **Clostridia are anaerobic spore-forming Gram~positive rods.**
2. **They produce severe disease by numerous very potent toxins.**
3. *C. perfringens* **causes a range of skin, soft tissue and muscle disease, from simple cellulitis to fatal gas gangrene; it also causes food poisoning.**
4. *C tetani* **causes tetanus.**
5. *C. botulinum* **causes food~borne, infant and wound botulism.**
6. *C. difficile* **causes antibiotic-associated diarrhoea and pseudomembranous enterocolitis**

Pseudomonads

Pseudomonad is an umbrella term for Pseudomonas species plus new genera (previously Pseudomonas) including Burkholderia and Stenotrophomonas. These are Gram-negative rods and are:

- strict aerobes (a few can grow anaerobically using nitrate)
- non-lactose fermenting and motile
- oxidase positive, with oxidative metabolism, never fermentative
- able to survive with few nutrients, e.g. acetate, glucose
- widely distributed, therefore in nature, in fluids, in hospitals
- normal bowel flora in few healthy people
- opportunist pathogens infecting those with impaired defences

Their structure is typical of Gram negative bacteria. From a fluid reservoir by various routes they need a portal of entry such as burnt skin, intravenous drug abuse or medical use, instrumentation or disease. Then their numerous virulence factors overcome weakened host defences, including neutropenia or immune defects.

Clinical syndromes

P. aeruginosa causes lung infections, especially in cystic fibrosis, septicaemia , wound, burn, ear and other organ infections. Other Pseudomonas spp. mainly cause opportunistic infections, with wound infections, bacteraemia or organ infections, e.g. pneumonia.

Management

Usually two antibiotics are used, as pseudomonads are intrinsically quite resistant, and host defences are often impaired. Gentamicin (or tobramycin) with either an anti-pseudomonal penicillin like ticarcillin or a third-generation cephalosporin, especially ceftazidime, are usual.

KEY LEARNING POINTS.

1. **Pseudomonads are strict aerobes widely distributed in nature.**
2. **They are mainly opportunist pathogens infecting those with impaired defences.**
3. **Virulence factors enable spread of infection from the site of entry, including septicaemia.**

Candida

Candida are small round yeasts which multiply by budding forming pseudohyphae. They are classified into species by carbohydrate assimilation and fermentations. *C. albicans* is the commonest and with *C. tropicalis*, *C.glabrata* and *C. parapsilosis* is part of the normal oropharyngeal, gut and vaginal flora, but Candida are uncommon on the skin unless it is macerated or damaged.

Virulence and pathogenesis

Candida spp. have typical fungal cell structure. They have minimal virulence for normal hosts, and pathogenesis depends on one or more defects in host defence, including:

- impaired skin-mucous membrane defences caused by mechanical breaches including catheters, by changes in normal flora owing to disease or drugs, or bs, maceration, trauma or disease
- impaired neutrophil or eosinophil function
- impaired monocyte or macrophage function
- impaired lymphocyte function
- impaired alternative complement pathway.

Antibody is usually produced but is probably not protective. The known adherence of *Candida spp*. to many cells may be important

Clinical syndromes

These include:

- local skin or nail disease including nappy rash, intertrigo or paronychia , occurring in hosts with only locally impaired defences
- local mucous membrane disease such as oral thrush or vaginitis with locally impaired defences

- local but severe chronic mucocutaneous candidiasis (CMC) in children with specific T-cell defects
- organ system infection, including CNS, pulmonary, cardiovascular, urinary, eye, bone, joint or disseminated infection, in the immunocompromised patient .

Chemotherapy

Local imidazoles or nystatin are used for local infections, ketoconazole for CMC. Deep, systemic or disseminated infections need specialised treatment with amphotericin B, fluconazole or caspofungin.

Control

This depends on treating or removing the predisposing defects in host defences. Chemoprophylaxis is used in vulnerable patients.

KEY LEARNING POINTS.

1. **Round yeasts which multiply by budding.**
2. ***C albicans* is the commonest yeast in the normal flora of the oropharynx, gut and vagina but is not found on normal skin.**
3. **Local infection of skin, nails or mucous membrane occurs when local, first-line defences are impaired.**
4. **Systemic opportunistic infection occurs in the immunocompromised patient.**
5. **Imidazoles or nystatin are used for local infections and for prophylaxis. Systemic infections need amphotericin B, fluconazole or caspofungin**

Fungi infecting skin and adjacent tissues

The superficial mycoses are fungal infections of the outermost layers of skin, and of hair.

Superficial mycoses –

Tinea versicolor (pityriasis versicolor)
This results from skin infection by Pityrosporum orbiculare, a lipophilic yeast. Budding yeasts and short mycelial fragments are seen in potassium hydroxide-treated skin scrapings from the scaly hypo-pigmented infection. Tinea versicolor should be differentiated from erythrasma, caused by Corynebacterium minutissimum, which fluoresces pink under LW light.

Cutaneous mycoses -

Fungi known (wrongly) as dermatophytes ('skin plants') infect the keratinised surface of the body producing 'tinea' or 'ringworm'. The infections are named after the body area affected:

- tinea capitis: scalp
- tinea barbae: beard
- tinea corporis: body
- tinea cruris: groin
- tinea pedis: feet (*T. Mentagrophytes* var *interdigitale*, *T. rubrum*)
- tinea unguium (nails).

There exist around 40 species, from three genera :Trichophyton, Microsporum and Epidermophyton. Many contain keratinases and infect only keratin-containing tissue.

Certain generalisations may be made:

- Each species of *Trichophyton* and *Microsporum* can cause infection of skin, hair or nails.
- *T. rubrum*, *T. mentagrophytes* and *M. canis* are most common, but frequency varies widely between tropical and temperate areas.
- *Epidermophyton* does not cause tinea capitis or barbae and very rarely causes tinea unguium.
- Some zoophilic species may be recognised by their names; these particularly infect the skin and hair of children, and the beard areas of rural men owing to greater contact. They usually provoke greater host inflammation response in humans.
- Geophilic species particularly infect the heads of children, again related to their greater contact with soil.

Confirmatory tests

Scrapings from the lesions are treated with potassium hydroxide to dissolve the keratin and expose the fungal bodies on direct microscopy.

Clinical syndromes

Tinea of the skin, hair and nails is common. Chemotherapy is with topical antifungals including imidazoles such as clotrimazole. Oral terbinafine, itraconazole or griseofulvin is used for hair, nail and severe skin disease.

KEY LEARNING POINTS.

Superficial fungal infections -

- **Superficial mycoses are caused by four specific fungi and result in hypopigmentation or black skin, or black or white hairs.**
- **Diagnosis is clinical and by microscopy.**
- **Infected skin is treated topically, and infected hair clipped or shaved.**

Cutaneous fungal infections ('Tinea') -

- **Cutaneous mycoses result from infection by Microsporum, Trichophyton or Epidermophyton spp.**
- **Sources are humans, animals or the soil.**
- **Diagnosis is by microscopy and culture.**
- **Treatment is topical for mild skin disease, or oral terbinafine, itraconazole or griseofulvin for hair, nail or severe skin disease.**