Enterobacteriaceae

The Enterobacteriaceae include the following genera: *Escherichia* spp. *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., *Serratia* spp., *Shigella* spp., *Salmonella* spp. and *Yersinia* spp.

Table 8.1 Enterobacteriaceae infections

Genus/species	Common infections
Escherichia coli	Urinary tract infection
	Intra-abdomal infection
	Wound infection
Klebsiella spp	Urinary tract infection
	Pneumonia
	Intravascular catheter-related infection
Enterobacter spp	Hospital-acquired pneumonia
Serratia spp	Wound infection
Proteus spp	Urinary tract infection
Salmonella serotypes Typhi and Paratyphi	Enteric fever and bloodstream infection
Other salmonellae	Enteritis
Shigella spp	Enteritis
Yersinia enterocolitica	Enteritis
Yersinia pestis	Plague
Yersinia pseudotuberculosis	Mesenteric adenitis

They are Gram-negative bacilli, which are found as commensals in the intestinal tract of mammals. They are also referred to as coliforms or enteric bacteria.

Definition

Aerobic and facultatively anaerobic growth; optimal growth normally at 37 °C; grow readily on simple media; ferment wide range of carbohydrates; oxidase-negative; some are motile; bile tolerant and grow readily on bile-salt-containing media, e.g. MacConkey's agar.

Morphology and identification

. Fermentation of lactose to produce pink colonies on MacConkey's agar is characteristic of *Escherichia*, *Enterobacter* and *Klebsiella*.

Salmonella, *Shigella*, *Serratia*, *Proteus* and *Yersinia* do not ferment lactose and form pale colonies on MacConkey's agar.

. Various tests are used for identification:

(a) oxidase (negative);

(b) carbohydrate fermentation reactions;

(c) production of urease (which splits urea with release of ammonia);

(d) hydrogen sulphide production;

(e) amino acid decarboxylation;

(f) indole production.

Commercial kits based on these biochemical tests are available for identification of the Enterobacteriaceae.

. Enterobacteriaceae possess a variety of antigens: these may include lipopolysaccharide ('O'), flagellar ('H') and capsular polysaccharide ('K') antigens. These are used to subdivide (serotype) some genera and species, e.g., *Escherichia*, *Salmonella*.

Escherichia

The genus Escherichia currently contains several species. However, *E. coli* (Figure 8.1) is the species most frequently isolated from humans.

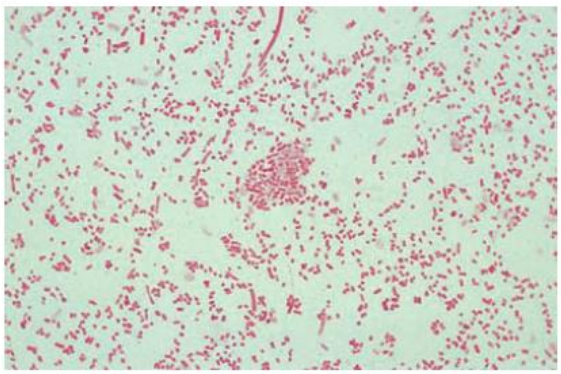


Figure 8.1 Gram stain of *Escherichia coli* showing Gram-negative bacilli (2 µm × 0.5 µm).

Epidemiology and associated infections

Although E. *coli* is a harmless commensal of the human intestine, some strains (identified as particular O, H, and K serotypes) can cause infections of the gastrointestinal tract, urinary tract, biliary tract, lower respiratory tract, bloodstream, haemolytic-uraemic syndrome (HUS), hemorrhagic colitis and neonatal meningitis

Pathogenicity

. Specific fimbriae facilitate adherence to mucosal surfaces and colonization of the intestinal and urinary tracts.

. The lipopolysaccharide (endotoxin) in the cell wall is liberated when Gram-negative bacteria lyse, resulting in production of inflammatory mediators (cytokines and nitric oxide) and complement activation. This results in endotoxic shock and intravascular coagulopathy.

. The K1 capsular polysaccharide antigen is associated with neonatal meningitis.

. A number of distinct infections are mediated by the different protein toxins produced by *E. coli*.

. VTEC (verocytotoxin-producing *E. coli*, particularly the O157:H7 serotype, are an important cause of diarrhea and HUS. These are also referred to as enterohaemorrhagic *E. coli* (EHEC).

. Most are sporadic cases and have the following key features:

zoonotic infections mainly from cattle, but also from vegetables washed in contaminated water;

low infecting dose; acquired by eating undercooked contaminated meat and vegetables; damage gut endothelium, resulting in hemorrhagic colitis HUS occurs in about 5% of patients, which results in renal failure, oliguria, thrombocytopenia.

Diarrhea caused by other *E. coli*:

. Enteropathogenic (EPEC): cause of infantile diarrhea;

. Enterotoxigenic (ETEC): travelers' diarrhea, non-invasive;

. Enteroinvasive (EIEC): causes dysentery-like illness;

. Enteroaggregative (EAEC): watery diarrhea without fever.

Laboratory diagnosis

. Diagnosis is by direct isolation of the microorganism from clinical samples, e.g. feces, urine and blood.

. Identification of some pathogenic strains, e.g. VTEC, EPEC, may be achieved by serotyping.

Antibacterial therapy

E. coli is commonly resistant to penicillin and ampicillin by production of b-lactamase enzymes. Production of extended spectrum b-lactamases (ESβLs), which inactivate many penicillins and cephalosporins, is an increasing problem. Antibiotics often used to treat *E. coli* infections include the cephalosporins, trimethoprim, ciprofloxacin and aminoglycosides; strains isolated from hospitalized patients are often more resistant to antibiotics and therefore local antibiotic sensitivity patterns need to be considered.

Klebsiella

The genus *Klebsiella* contains a number of species, including *K. pneumoniae* and *K. oxytoca*; these can be distinguished on the basis of biochemical tests. Pathogenicity is associated with capsular polysaccharide and lipopolysaccharide (endotoxin) production. There are many capsular serotypes. Klebsiellae are widespread in the environment and in the intestinal flora of humans and other mammals. Infections are often opportunistic and associated with hospitalization, particularly in high-dependency units. They include pneumonia, urinary tract and wound infection, and neonatal meningitis. Outbreaks of healthcare-associated infection occur. *Klebsiella* spp. often produce β-lactamases and are resistant to ampicillin. Cephalosporins (e.g. cefotaxime), β-lactamase

inhibitor/penicillin combinations (e.g. co-amoxiclav) and aminoglycosides (e.g. gentamicin) can be used to treat Klebsiella infections, but multiply resistant strains may limit antibiotic choice. Extensive use of broad-spectrum antibiotics in hospitalized patients has led to development of multi-drug-resistant strains that produce ESβLs.

Enterobacter and Serratia

Enterobacter spp. and *Serratia* spp. are closely related to *Klebsiella* spp. Infections occur principally in hospitalized patients and include the lower respiratory and urinary tracts. Hospital cross-infection with antibiotic-resistant strains is a particular problem. ESβL production in strains of Enterobacter is an increasing problem.

Salmonella

The genus Salmonella contains a large number of species (more correctly, serotypes). Salmonella serotype Typhi and Salmonella serotype Para typhi cause enteric fever (typhoid or paratyphoid); other salmonellae cause enteritis.

Classification

. Over 2,000 serotypes are distinguished, most of which belong to the species *S. enterica*. However, many of these have been given binomial names (e.g. *Salmonella typhimurium* and *Salmonella enteritidis*), although they are not separate species.

In clinical practice, laboratories identify microorganisms according to their binomial name.

. *Salmonella* spp. have both H and O antigens. There are over 60 different O antigens, and individual strains may possess several O and H antigens; the latter can exist in variant forms, termed ‘phases. Salmonella serotype Typhi also has a capsular polysaccharide antigen referred to as ‘Vi’ (for virulence), which is related to invasiveness.

. Agglutination tests with antisera for different O and H antigens form the basis for the serological classification of *Salmonella* spp. Further strain differentiation of *Salmonella* spp. for epidemiological purposes can be achieved by phage typing.

Epidemiology

Salmonella spp. are commensals of many animals, including poultry, domestic pets, birds and humans. Transmission is via the fecal-oral route.

The infective dose is relatively high (~10^6 microorganisms) and multiplication in food is important for effective transmission. A chronic carrier state can occur.

Morphology and identification

. *Salmonella* spp. are motile and produce acid, and occasionally gas, from glucose and mannose.

. They are resistant to sodium deoxycholate, which inhibits many other Enterobacteriaceae.

Deoxycholate agar is used as a selective media to isolate *Salmonella* spp. from stool specimens.

. *Salmonella* spp. do not ferment lactose and form pale colonies on MacConkey's medium; on xylose lysine deoxycholate (XLD) agar, many *Salmonella* spp. form pale colonies with black centres as a result of H_2S production. This aids recognition of Salmonella colonies in mixed cultures.

. Further biochemical tests are required for definitive identification. Serotyping of O and H antigens by slide agglutination is used for speciation.

Pathogenicity

Salmonella spp. can survive the acidic pH of the stomach and invade the gut, resulting in an inflammatory response and subsequent diarrhea.

Associated infections

. Salmonella infections: (caused by non-typhoid salmonellae;

. Enterocolitis/gastroenteritis: rarely associated with bloodstream infection, osteomyelitis, septic arthritis or abscesses;

. Enteric fever: caused by Salmonella serotype Typhi and Salmonella serotype Para typhi.

Enteric fever is prevalent in Asia, South America and Africa; approximately 300 cases per year in the UK. Salmonella spp. may persist in biliary and urinary tracts after recovery.

Laboratory diagnosis

. Enterocolitis: culture of stool samples on selective media, e.g. XLD, DCA (deoxycholate citrate agar), and enrichment media, e.g. selenite broth; identification of Salmonella spp. by biochemical and agglutination tests. Phage typing can be used for typing individual strains.

. Enteric fever: isolation of Salmonella serotypes Typhi or Para typhi from blood cultures (first week of infection), urine (second week) or feces (first week onwards). Serology (Widal's test) is now rarely performed, because of unreliable results.

Treatment and prevention

. Enteric fever: ciprofloxacin (though resistance is increasing);

.Enterocolitis: self-limiting; antibiotics (e.g. ciprofloxacin and cefotaxime) reserved for severe or invasive infection, particularly in the elderly, very young or 'immunocompromised' individuals;

. Typhoid immunization; avoidance of contaminated water/food.

Shigella

Classification

The main pathogenic species are *S. sonnei*, *S. boydii*, *S. dysenteriae* and *S. flexneri*. They are distinguished by biochemical reactions and antigenic characteristics ('O' antigens).

Epidemiology

Obligate human pathogens with no animal reservoirs; transmission via fecal-oral route with low infective dose (10–200 microorganisms). Direct person-to-person spread is common; chronic carrier state is rare.

Morphology and identification

Shigella spp. are non-motile (they have no flagella). They are resistant to sodium deoxycholate and grow on deoxycholate agar (see Salmonella). They are non-lactose or late lactose (*S. sonnei*) fermenters. Further biochemical tests are carried out for definitive identification and serotyping by slide agglutination is used for speciation.

Pathogenicity

Shigella spp. express an intestinal adherence factor, which aids colonization within the gut. They cause disease by invasion and destruction of the colonic mucosa, and also produce an enterotoxin (cytotoxin) known as Shiga toxin, which can cause microangiopathy, HUS and thrombocytopenic purpura.

Associated infections

Self-limiting diarrheal illness, dysentery (diarrhea with blood and pus, fever, abdominal pain) (HUS and bloodstream infection are rare).

Laboratory diagnosis

Stool culture on selective media, e.g. XLD.

Treatment

Antibiotics (e.g. ciprofloxacin) reserved for severe cases (often caused by *S. dysenteriae*).

Proteus

The genus Proteus contains a number of species, e.g. *P. mirabilis* and *P. vulgaris*. Characteristics include:

. non-lactose fermenting, produce pale colonies on MacConkey's agar;

. motile, tendency to 'swarm' on blood agar;

. important cause of urinary tract and occasionally abdominal wound infection.

Yersinia

The genus Yersinia contains three human pathogens: *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*; these species are identified by biochemical tests.

Y. pestis

Y. pestis is the cause of plague (black death). Although mainly of historical interest in Europe, plague remains endemic in some areas of the world. It is primarily a pathogen of rodents and is transmitted to humans via infected fleas; lymph nodes associated with the flea bite enlarge to form a bubo (bubonic plague). Bloodstream invasion and pneumonia may follow (pneumonic plague).

Person-to-person spread via droplets occurs in pneumonic plague. *Y. pestis* can be isolated from blood, bubo aspiration, sputum, throat swabs and skin scrapings. Treatment for *Y. pestis* infection is with an aminoglycoside or tetracycline. Laboratory diagnosis is by microscopy and culture of clinical material.

Y. pseudotuberculosis

This is primarily an animal pathogen, but in humans it is an occasional cause of mesenteric adenitis, rarely septicemia. It is probably transmitted to humans via contaminated food. Laboratory diagnosis is through culture of feces on selective agar, e.g. CIN agar and cold enrichment of feces.

Y. enterocolitica

Y. enterocolitica causes diarrheal disease, terminal ileitis and mesenteric adenitis. Infection may be complicated by septicemia or reactive polyarthritis.

A zoonotic infection of domestic and wild animals, transmission is via the fecal-oral route.

Laboratory diagnosis is by culture of feces on selective agar, e.g. CIN agar, cold enrichment of feces, culture of blood specimens or serology.

Proteus spp.

Classification

The tribe Proteeae is classified into three genera *Proteus*, *Morganella* and *Providencia*. Most of them except for some Providencia strains, produce a powerful urease which rapidly hydrolyses urea to ammonia and carbon dioxide. A characteristic feature which distinguishes tribe Proteeae from other enterobacteria is the presence, in all members of the tribe, of the enzyme phenyl alanine deaminase which converts phenyl alanine to phenyl pyruvic acid (PPA reaction). All members of this tribe fail to ferment lactose.

Proteus

Proteus Bacilli

Proteus bacilli are normal intestinal commensals and opportunistic pathogens like coliforms. name 'Proteus' refers to their pleomorphism, after the Greek god Proteus who could assume any shape.

Genus Proteus has four species: *P. mirabilis*, *P. vulgaris*, *P. myxofaciens and P. penneri. P. mirabilis*, *P. vulgaris* are widely recognized as human pathogens. These are motile, gram-negative bacilli, characterized by swarming growth on agar.

Morphology

They are gram-negative coccobacilli, 1–3 mm long and 0.6 mm wide. Pleomorphism is frequent—short coccobacilli to long filaments. In young swarming cultures, many of the bacteria are long, curved and filamentous. They may be arranged singly, in pairs or in short chains. They are actively motile with peritrichous flagella. They also have more type of fimbriae and are non-capsulated.

Cultural Characteristics

They are aerobe and facultative anaerobes. All grow well on laboratory nutrient media. Proteus organisms are usually first recognized by their characteristic putrefactive odor described as **'fishy'** or **'seminal'** and swarming appearance on non-inhibitory solid media, such as nutrient agar and blood agar.

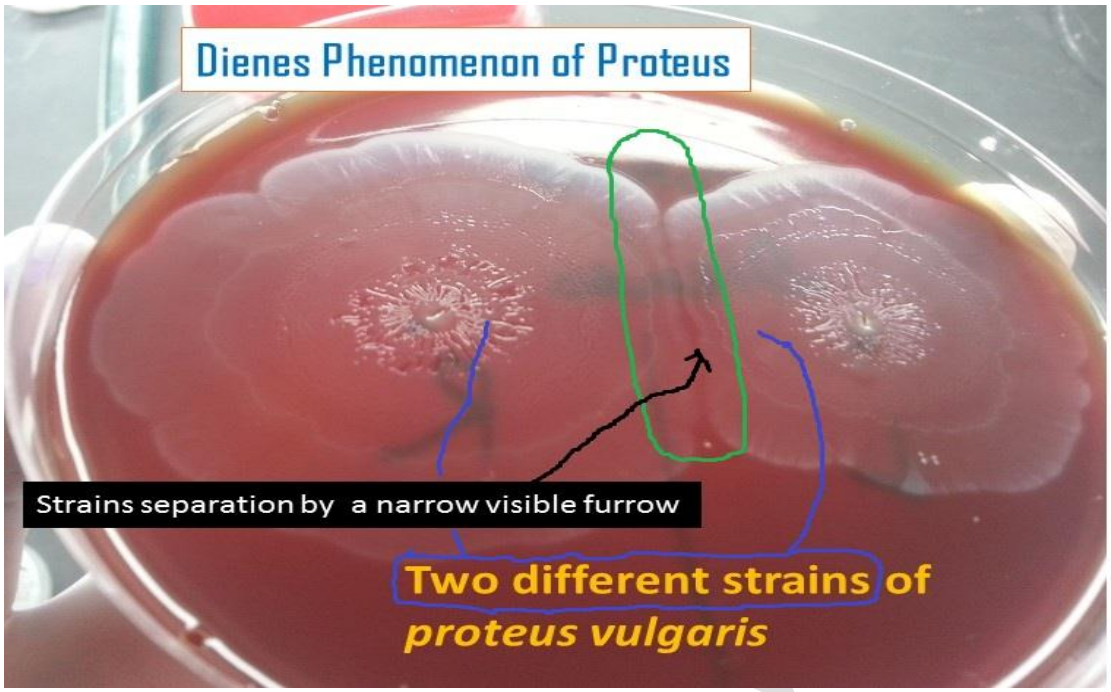
Swarming is a striking feature of *Pr. mirabilis* and *Pr. vulgaris*. Swarming of *Proteus* appears to be due to vigorous motility of the organism although the exact cause is not yet established.

Swarming growth is a problem in the laboratory when mixed growth is obtained in which *Proteus* bacilli are present with other bacteria. A number of methods have been devised to inhibit swarming. Swarming of *Proteus* can be inhibited by (i) increasing concentration of agar (6%) and by (ii) incorporation of chloral hydrale (1: 500), sodium azide (1:500), alcohol (5–6%), sulfonamide, surface. active agents or boric acid (1:1000).

Swarming does not occur on MacConkey's medium, on which smooth colorless (NLF) formed. *Proteus* produces uniform turbidity with a slight powdery deposit and an ammoniacal odor in liquid medium (peptone water).

Diene's phenomenon: When two identical strains of *Proteus* are inoculated at different places of the same culture plate, the resulting swarms of growth coalesce and no line is formed between swarming culture of the same strain. When, however, two different strains of *Proteus* species are inoculated, the spreading films of growth fail to coalesce and remain separated by a narrow but easily visib1e furrow. This

is known as Diene's phenomenon. It has been used to determine the identity or non-identity of various strains of *Proteus.*



Biochemical Reactions

The distinctive characters of this genus are:

i. **PPA test**—Deamination of phenyl alanine to phenyl pyruvic acid (PPA test) is always positive.

ii. **Urea hydrolysis**—Urea hydrolysis by enzyme urease is another characteristic of *Proteus*, but is negative in some *Providencia* strains.

iii. All species of *Proteus* produce acid from glucose.

iv. Lactose is not fermented.

v. They are malonate utilization negative.

vi. Indole is formed by *Pr. vulgaris* but is negative in *Pr. mirabilis*.

vii. They are MR positive and VP negative.

viii. H_2S is produced by *Pr. vulgaris* and *Pr. mirabilis*.

ix. Nitrate reduction positive.

Antigenic Structure

Proteus bacilli possess somatic O and flagellar H antigens, which are of considerable historical interest. Weil and Felix (1916) studying *Proteus* bacilli observed that flagellated strains growing on agar formed a thin surface film resembling the mist produced by breathing on glass and named this variety the 'Hauch' form (from *Hauch*, meaning film of breath). Non-flagellated variants grew as isolated colonies without the surface film and were called 'Ohne Hauch' (meaning without film of breath). These names came to be abbreviated as the Hand 0 forms. Subsequently, the H and O were extended to refer to the flagellar and somatic antigens of other bacilli as well.

Weil and Felix also observed that certain nonmotile strains of *Pr. vulgaris*, called the 'X strains', were agglutinated by sera from typhus fever patients. This heterophilic agglutination due to the sharing of an alkali stable carbohydrate antigen by certain strains of *Proteus* (OX2, OX19 and OXK) and rickettsiae forms the basis of the Weil-Felix reaction for the diagnosis of some rickettsial infections. Three nonmotile *Proteus* strains OX2, OX19 and OXK are used in the agglutination test.

OX19, OX2 are the strains of *P. vulgaris* serotype 01 and serotype 02 and OXK is the strain of *P. mirabilis* serotype 03.

Typing methods: Phage typing, bacteriocin (proticin) typing and serotyping schemes have been developed for *Proteus* and *Providencia* species. Swarming *Proteus* strains exhibit the Dienes phenomenon and this forms the basis for a precise method of differentiation among such strains.

Pathogenesis

Proteus bacilli are widely distributed in nature as saprophytes, being found in decomposing animal matter, in sewage, in manured soil and in human and animal feces. They are frequently present on the moist areas of the skin. They are opportunistic pathogens, commonly responsible for urinary and septic infections, often nosocomial.

P. mirabilis accounts for the majority of human infections seen with this group of organisms. All members of the tribe can cause urinary tract infections (UTI), wound infections, pneumonia, infection of the ear, respiratory tract infection, septicemia and nosocomial infections. Strains of *Pr. mirabilis* are a prominent cause of urinary tract infection in children and in domiciliary practice. UTI caused by *Proteus* tends to be more serious than that caused by *E. coli* and other coliforms.

It produces urease which splits urea into carbon dioxide and ammonia. Ammonia inactivates complement, damages renal epithelium and makes the urine alkaline. This increase in pH causes precipitation of calcium and magnesium salts from the urine and results in the formation of urinary calculi.

Laboratory Diagnosis

Culture: Laboratory diagnosis of the infections caused by species *Proteus* can be carried out by culture of the specimen on MacConkey agar or Deoxycholate Citrate Agar (DCA).

Identification: The isolate is identified by its morphological, biochemical and agglutination reactions.

Treatment

Proteus bacilli are resistant to many of the common antibiotics. An exception is *P. mirabilis* which is sensitive to ampicillin and cephalosporins.

Staphylococcus spp.

Definition

Gram-positive cocci; usually arranged in clusters; non-motile; catalase positive; non-sporing; grow over a wide temperature range (10–42 °C), with an optimum of 37 °C; aerobic and facultatively anaerobic; grow on simple media.

Classification

1 Colonial morphology: *S. aureus* colonies are grey to golden yellow; *S. epidermidis* and *S. saprophyticus* colonies are white. Staphylococci may produce haemolysins, resulting in haemolysis on blood agar.

2 Coagulase test: *S. aureus* possesses the enzyme coagulase, which acts on plasma to form a clot. Other staphylococci (e.g., *S. epidermidis* and *S. saprophyticus*) do not possess this enzyme and are often termed, collectively, 'coagulase negative staphylococci' (CoNS). There are three methods to demonstrate the presence of coagulase:

(a) tube coagulase test: diluted plasma is mixed with a suspension of the bacteria; after incubation, clot formation indicates *S. aureus*

(b) slide coagulase test: a more rapid and simple method in which a drop of plasma is added to a suspension of staphylococci on a glass slide; visible clumping indicates the presence of coagulase.

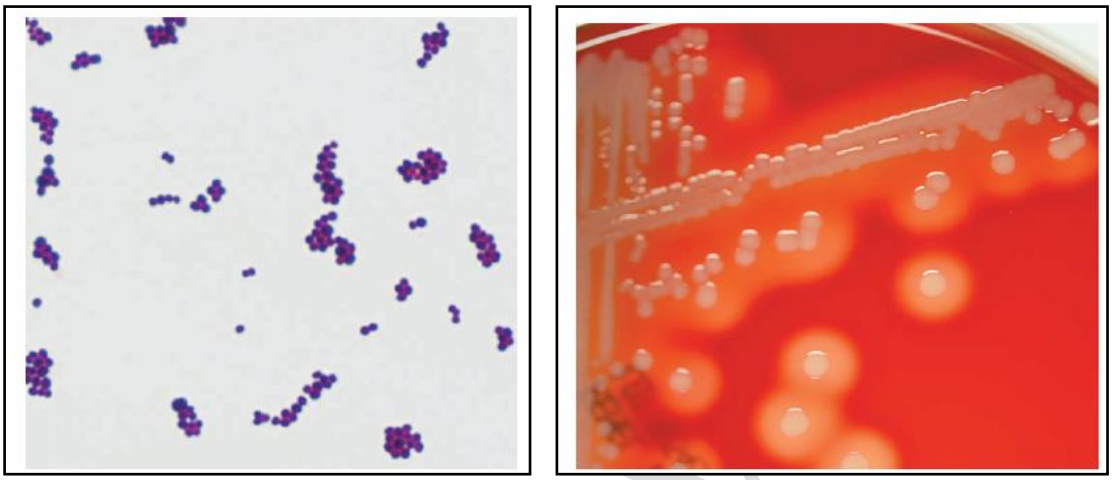
(c) latex agglutination test: cells are mixed with coated latex particles; visible agglutination provides simultaneous detection of staphylococci containing coagulase and/or protein A.

3 Deoxyribonuclease (DNAase) production: *S. aureus* possesses an enzyme, DNAase, which de-polymerizes and hydrolyses DNA; other staphylococci rarely possess this enzyme.

4 Protein A detection: *S. aureus* possesses a cell-wall antigen, protein A; antibodies to protein A agglutinate *S. aureus* but no other staphylococci.

5 Novobiocin sensitivity: useful for differentiating between species of coagulase-negative staphylococci; *S. saprophyticus* is novobiocin resistant and *S. epidermidis* is sensitive.

S. aureus



Gram stain of *Staphylococcus aureus* showing gram-positive cocci in pairs, tetrads, and clusters. Original magnification ×1000. (Courtesy of L. Ching).

Colonies of *Staphylococcus aureus* on a blood agar plate after 24 hours incubation. The yellow-gray colonies are 3 to 4 mm in diameter on the 10-cm plate. The colonies are surrounded by clear zones of hemolysis about 1 cm in diameter. (Courtesy of H. Reyes.)

Epidemiology

S. aureus is a relatively common human commensal: nasal carriage occurs in 30–50% of healthy adults, fecal carriage in about 20% and skin carriage in 5–10%, particularly the axilla and perineum. *S. aureus* is spread via droplets and skin scales, which contaminate clothing, bed linen and other environmental sources.

Morphology and identification

On microscopy, *S. aureus* is seen as typical Gram-positive cocci in ‘grape-like’ clusters. It is both coagulase and DNAase positive; Other biochemical tests can be performed for full identification.

Pathogenicity

S. aureus causes disease because of its ability to adhere to cells, spread in tissues and form abscesses, produce extracellular enzymes and exotoxins, combat host defenses and resist treatment with many antibiotics.

Adhesins

S. aureus has a wide repertoire of adhesins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), which mediate adherence to host cells; these include protein A, fibrinogen and fibronectin-binding and collagen-binding protein.

Exotoxins and enzymes

Coagulase and Clumping Factor

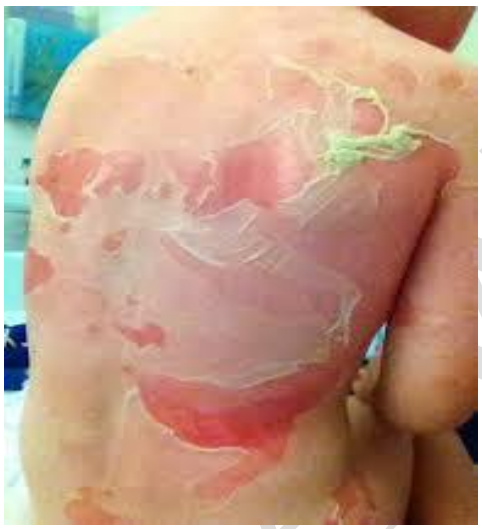
Coagulase: *Staph. aureus* produces coagulase, an enzyme-like protein that clots oxalate or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells.

Clumping factor: it is another example of an MSCRAMM that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *Staph. aureus* forms clumps. Clumping factor is distinct from coagulase. However, no human vaccines against this factor are available to date.

Haemolysin, leukotoxin and leukocidin: several exotoxins are produced by *S. aureus*; α-toxin (haemolysin) lyses erythrocytes and damages platelets; β-toxin degrades sphingomyelin and is toxic for many types of cells, including erythrocytes; leukocidin (Panton Valentine leukocidin, PVL) lyses white blood cells and damages membranes and susceptible cells.

Enterotoxins: there are six soluble enterotoxins that are produced by almost half of all *S. aureus* strains. They are heat stable (resistant at 100 °C for 30 min), unaffected by gastrointestinal enzymes and are a cause of food poisoning, principally associated with vomiting.

Exfoliative/epidermolytic toxin: some strains produce a toxin that can result in generalized desquamation of the skin (staphylococcal scalded skin syndrome) SSSS.



SSSS

Toxic shock syndrome toxin (TSST): this is associated with shock and desquamation of skin, and is usually related to an underlying *S. aureus* infection.

Staphylococcal enterotoxins, TSSTs, and exfoliative toxins are 'superantigens', all of which bind non-specifically to specific white cells, resulting in overproduction of cytokines, giving rise to a toxic shock-like presentation.

Other enzymes: *S. aureus* may also produce staphylokinase (results in fibrinolysis), hyaluronidase (dissolves hyaluronic acid), proteases (degrades proteins) and lipases (solubilizes lipids).

Cell envelope

Over 90% of all clinical isolates of *S. aureus* strains possess a polysaccharide capsule that interferes with opsonization and phagocytosis. *S. aureus* also possesses a cell-wall protein (protein A) that binds the Fc component of the antibody, preventing complement activation.

Antibiotic resistance

Many strains of *S. aureus* are resistant to the antibiotic methicillin and are termed 'methicillin-resistant *S. aureus*' (MRSA). Most resistance depends on the production of an additional penicillin-binding protein, which is encoded by an acquired *mecA* gene. Many strains of MRSA are now resistant to multiple antibiotics.

Laboratory diagnosis

Laboratory diagnosis is by microscopic detection of the microorganism in clinical samples, direct isolation from the infected site or blood cultures, and detection of serum antibodies to staphylococcal haemolysin and DNAase. *S. aureus* strains can be typed ('fingerprinted') by conventional methods, including biotype and antibiogram. *S. aureus* can also be genotyped by molecular methods, including pulsed field gel electrophoresis (PFGE). Typing of *S. aureus* is useful in epidemiological studies.

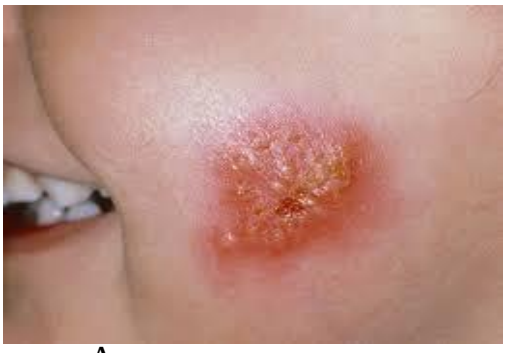
Treatment and prevention

Antimicrobial agents, such as flucloxacillin, remain the first-line treatment for sensitive strains of *S. aureus*; however, the increase in infections caused by MRSA has required the use of glycopeptide antibiotics such as vancomycin. Resistance to vancomycin has been reported but is still rare. MRSA can cause sepsis, ranging from wound infections to urinary tract infections and severe sepsis and septic shock. Epidemic strains of MRSA(EMRSA) have also been recognized.

Prevention of spread through effective infection control procedures, including MRSA decolonization, is therefore important.

Associated infections

. Skin: impetigo, boils, furuncles, folliculitis, carbuncles, paronychia, blepharitis, wound infections, staphylococcal scalded skin syndrome (SSSS) causes by stimulating the release of large amounts of interleukins IL-1 and IL-2 in the body.



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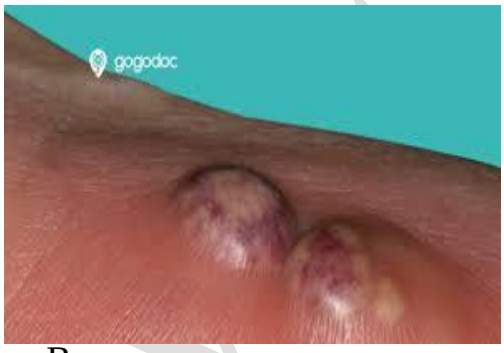

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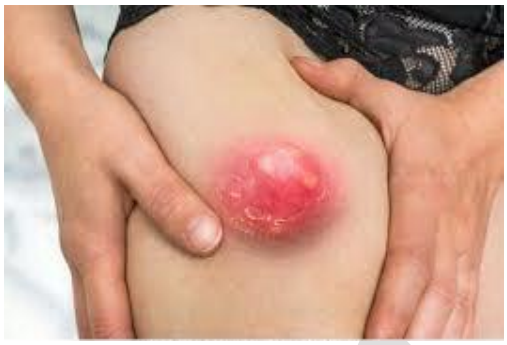

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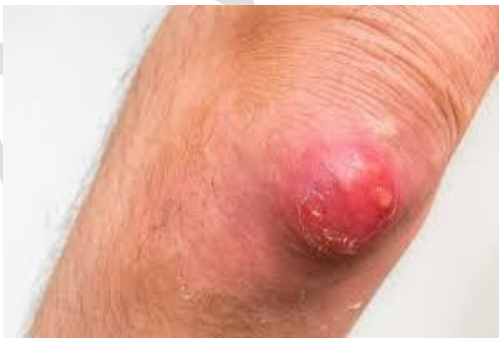

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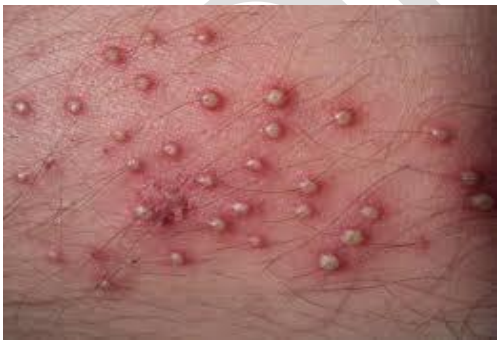

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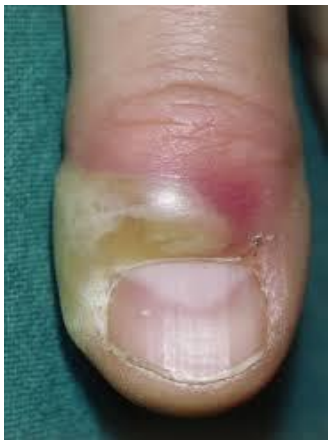

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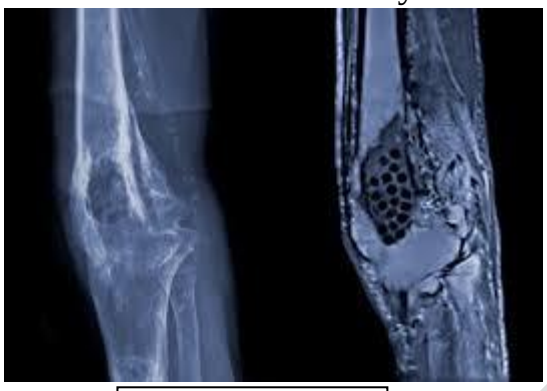
A: Impetigo, B: Boiles, C: Furuncles,
D: Carbuncles, E: Folliculitis,
F: Paronychia, G: Blepharitis

. Respiratory: pneumonia, lung abscesses, exacerbations of chronic lung disease;

. Skeletal: most common cause of osteomyelitis and septic arthritis



osteomyelitis

. Invasive: bloodstream infection, infective endocarditis, deep abscesses (brain, liver, spleen),

toxic shock syndrome;

. Gastrointestinal: toxin-mediated food poisoning;

. Device related: indwelling catheters, prosthetic joints and heart valves.

S. epidermidis

. *S. epidermidis* is both coagulase and DNAase negative and is present in large numbers on the human skin and mucous membranes.

. *S. epidermidis* is a cause of bacterial endocarditis, particularly in patients with prosthetic heart valves and in drug addicts. It is also a major cause of infections of implanted devices such as cerebrospinal shunts, hip prostheses, central venous and peritoneal dialysis catheters.

. The microorganism colonizes implanted devices by attaching firmly onto artificial surfaces. Some strains also produce a slime layer (glycocalyx), which appears to facilitate adhesion and protect the microorganism from antibiotics and host defenses. The increased use of implanted devices, particularly central venous catheters, has

resulted in S. epidermidis becoming one of the most frequently isolated microorganisms from blood cultures. *S. epidermidis* occasionally causes

urinary tract infections, particularly in catheterized patients. When isolated from hospitalized patients, *S. epidermidis* is often resistant to antibiotics such as flucloxacillin and erythromycin, necessitating the use of glycopeptide antibiotics (e.g., vancomycin).

S. saprophyticus

S. saprophyticus is both coagulase and DNAase negative and is frequently associated with urinary tract infections in sexually active young women, occasionally resulting in severe cystitis with haematuria

Streptococcus spp.

Definition

Gram-positive cocci arranged in pairs or chains; facultatively anaerobic; non-sporing; non-motile; catalase-negative; most are capsulate; optimum growth at 37 °C; sometimes require enriched media; many species exhibit characteristic haemolysis on blood agar. Many streptococci are human commensals (most notably of the upper respiratory tract).

Classification

Streptococci are classified by:

1 The type of haemolysis observed on blood agar:

(a) α-haemolysis: a greenish zone forms around colonies due to partial haemolysis of erythrocytes. An example of an a-haemolytic species is *Streptococcus pneumoniae*.

(b) β-haemolysis: a clear zone form around colonies due to complete haemolysis of erythrocytes.

(c) γ-haemolysis: no zone is formed, as erythrocytes are not lysed. These streptococci are more commonly referred to as non-haemolytic streptococci.

2 Serological detection of cell wall antigens: streptococci can be classified alphabetically according to the possession of specific cell wall antigens (Lancefield groups A–H and K–V). Antibodies that react with these antigens are used to group streptococci and are particularly useful in the identification of β-haemolytic species. These groups are important to distinguish, as they can cause specific infections.

3 Biochemical reactions: some streptococci are difficult to classify by the above characteristics, therefore biochemical tests can be useful in their identification.

α-Haemolytic streptococci

Streptococcus pneumoniae (pneumococcus)

Epidemiology

S. pneumoniae is a commensal of the upper respiratory tract.

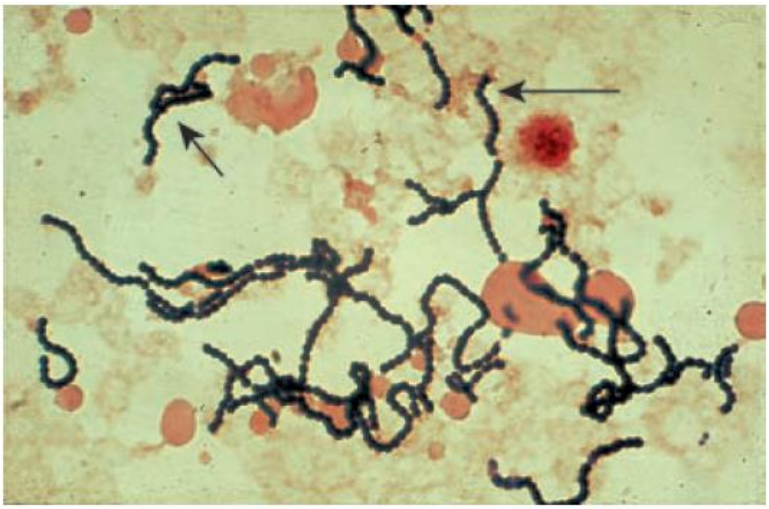


Figure 4.1 Gram stain of streptococci showing long chains.

Figure 4.2 Colonies (each 0.5 mm diameter) of α- (left) and β-haemolytic (right) streptococci on a blood agar plate.

Morphology and identification

Cocci is most commonly observed in pairs or chains and often have a polysaccharide capsule. Their growth is enhanced in the presence of additional carbon dioxide (CO_2) and colonies are typically disc shaped with central depressions (giving a 'draughtsmen' appearance)

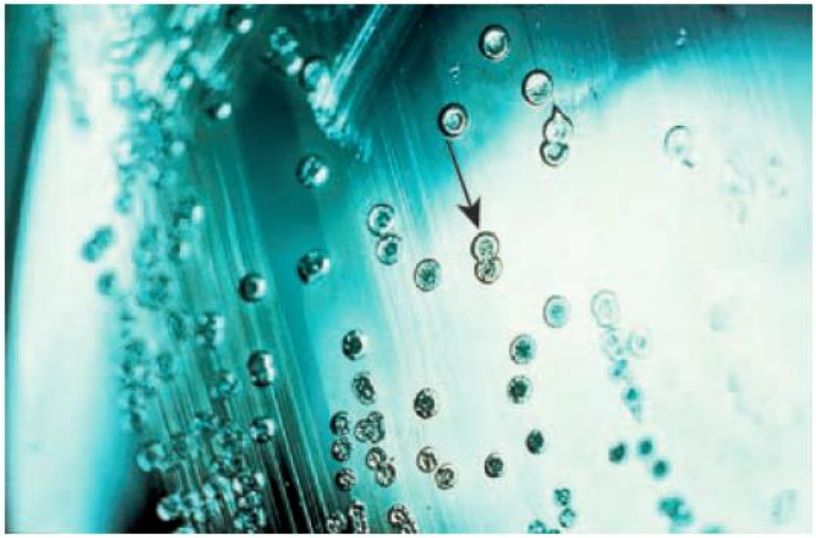


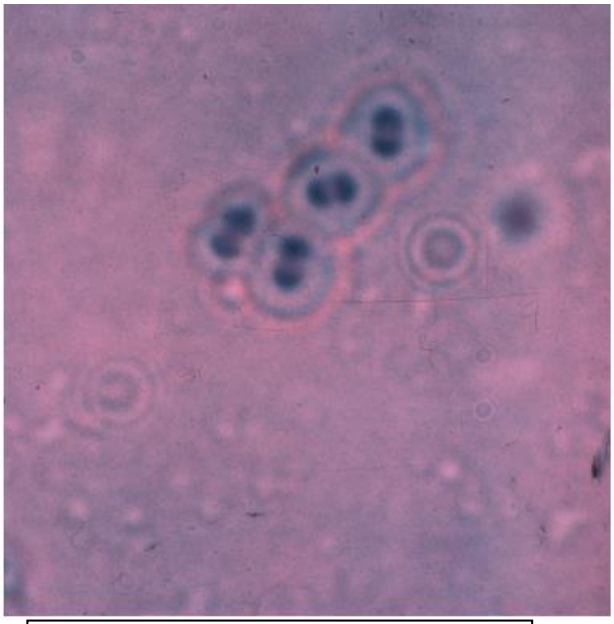
Figure 4.3 *Streptococcus pneumoniae* colonies (arrowed) with a characteristic 'draughtsman'-like appearance (1 mm diameter).

S. pneumoniae may be differentiated from the 'viridans' streptococci by its sensitivity to optochin and its solubility in bile salts.

optochin

Quellung Reaction

When pneumococci of a certain type are mixed with specific anti-polysaccharide serum of the same type or with polyvalent antiserum on a microscope slide, the capsule swells markedly, and the organisms agglutinate by crosslinking of the antibodies.



S. pneumoniae quelling reaction

This reaction is useful for rapid identification and for typing of the organisms, either in sputum or in cultures. This test is rarely used because of the high reagent costs and the expertise required in assay performance and interpretation.

Pathogenicity

There are in excess of 80 antigenic types of pneumococcal polysaccharide capsules. A limited number of serotypes account for the majority of cases of infection. The capsule inhibits phagocytosis, unless antigen-specific opsonic antibody is present.

Pneumococci also produce pneumolysin, a membrane- damaging exotoxin.

Associated infections

. Respiratory tract: otitis media, sinusitis, lower respiratory tract infection (particularly community-acquired pneumonia);

. Musculoskeletal: septic arthritis;

. Gastrointestinal: spontaneous bacterial peritonitis;

. Central nervous system: meningitis.

Laboratory diagnosis

This is by microscopy and microbiological culture of specimens from the infected site, sputum, blood, peritoneal fluid or CSF. Direct detection of pneumococcal antigen in specimens can be undertaken by various techniques. Pneumococcal DNA can be detected in blood, CSF and other samples by PCR.

Treatment and prophylaxis

S. pneumoniae is sensitive to a wide range of antibiotics. However, penicillin-resistant strains have now emerged worldwide.

A pneumococcal conjugate vaccination (PCV) schedule is currently recommended in infants. In addition, a pneumococcal polysaccharide vaccine (PPV) is recommended for adults 65 years of age or older, and for patients at particular risk of infection.

'Viridans' or 'oral' streptococci

The ‘viridans’ streptococci are a group α- or non-haemolytic streptococci, which are predominantly found in the oral cavity and so are commonly referred to as the ‘oral’ streptococci. Examples include the *Streptococcus mitis*, *Streptococcus mutans*, *Streptococcus salivarius* and *Streptococcus sanguinis* groups.

Epidemiology

The ‘viridans’ streptococci are commensals of the upper respiratory tract.

Morphology and identification

They are mostly resistant to optochin and insoluble in bile salts. Biochemical tests are often used for their identification.

Pathogenicity

Various carbohydrates facilitate the attachment of these streptococci to teeth adjacent to the gingivae.

Some species, particularly *S. mutans*, produce acid involved in the development of dental caries.

Associated infections

. Cardiovascular: they are a common cause of infective endocarditis;

. Dental: these streptococci, particularly *S. mutans*. are the most common cause of dental caries and periodontal disease.

Laboratory diagnosis

Diagnosis is by isolation of the microorganism from infected sites.

Treatment and prophylaxis

The 'viridans' streptococci are usually sensitive to a wide range of antibiotics.

β-Haemolytic streptococci

Group A (*Streptococcus pyogenes*)

Epidemiology

S. pyogenes is an upper respiratory tract commensal.

Morphology and identification

Group A streptococci will not grow on media containing bile. The identity of *S. pyogenes* is normally confirmed by Lancefield grouping and biochemical testing. Strains of *S. pyogenes* can be further differentiated according to the presence of surface proteins M, R and T (Griffith types). Epidemiological typing can be carried out, based on the possession of different M-proteins.

Pathogenicity

S. pyogenes produces a wide range of virulence factors including:

. A capsule composed of hyaluronic acid: provides protection against phagocytosis.

. Fimbriae/pili: facilitate adherence to host cells. They consist of lipoteichoic acid (an adherence factor).

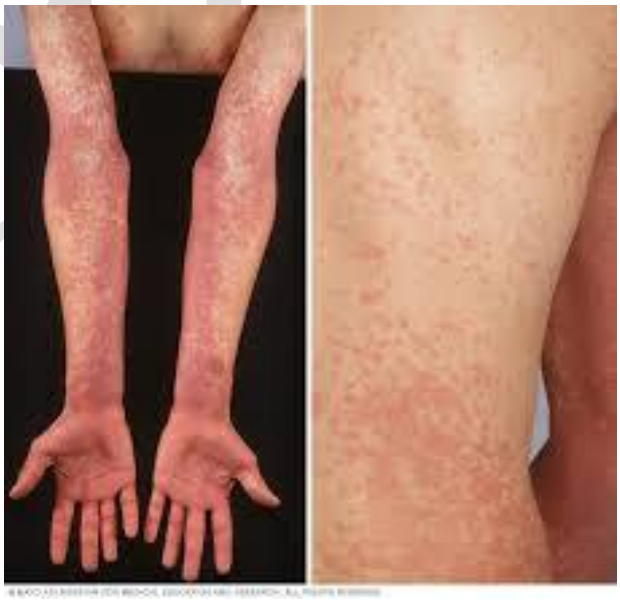
. M-proteins: surface proteins which are antiphagocytic and also bind host proteases.

. F-proteins: surface proteins that bind to fibronectin.

. Streptolysins (haemolysins): streptolysins O and S lyse erythrocytes and are cytotoxic to leukocytes and other cell types.

. Other enzymes: include streptokinase (prevents the formation of a fibrin mesh), hyaluronidase (breaks down hyaluronic acid in connective tissue), deoxyribonucleases (DNAases), nicotinamide adenine dinucleotidase (NADase) and C5a peptidase (inactivates the C5a component of the complement system).

. Streptococcal pyrogenic exotoxins (SPEs) (erythrogenic toxins): responsible for the rash of scarlet fever.



Scarlet Fever (Skin Rash)

These are 'superantigens', which facilitate release of cytokines, potentially leading to shock.

Associated infections

. Respiratory tract: retropharyngeal, sinusitis, tonsillitis, otitis media, pneumonia;

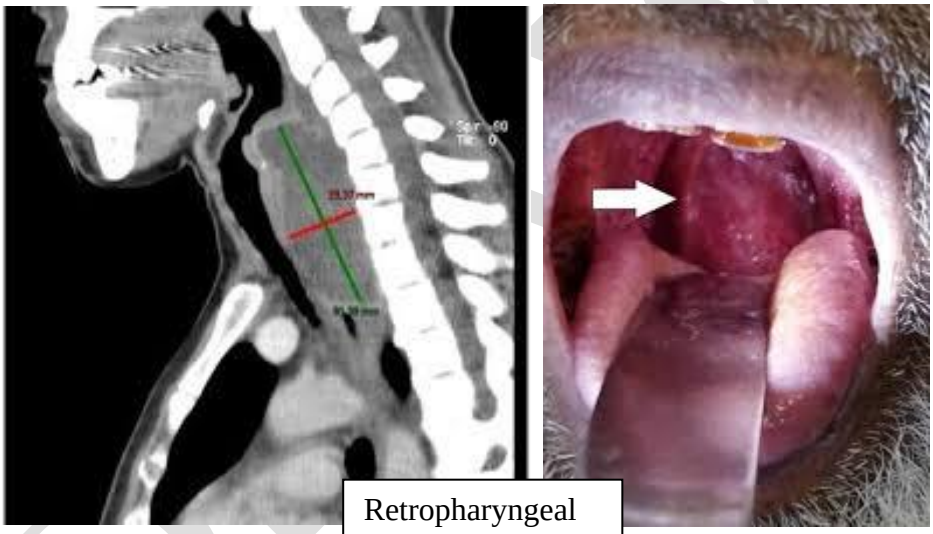
. Musculoskeletal: septic arthritis;

. Gastrointestinal: spontaneous bacterial peritonitis;

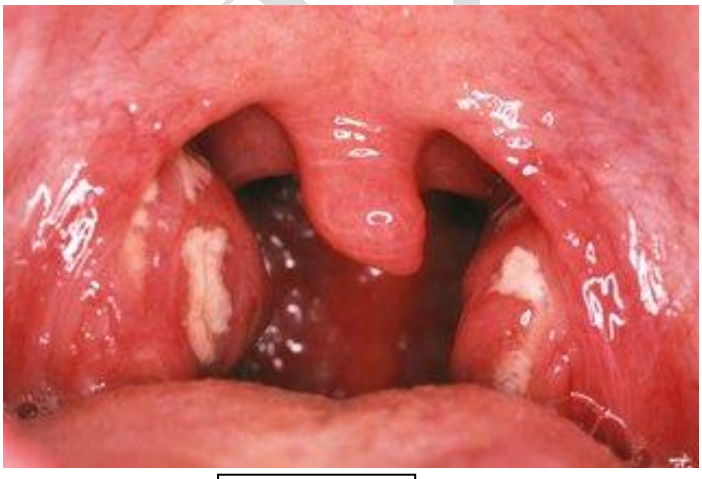
. Skin and soft tissue: cellulitis, impetigo, erysipelas, scarlet fever, wound infection, necrotizing fasciitis;

. Genitourinary: puerperal sepsis;

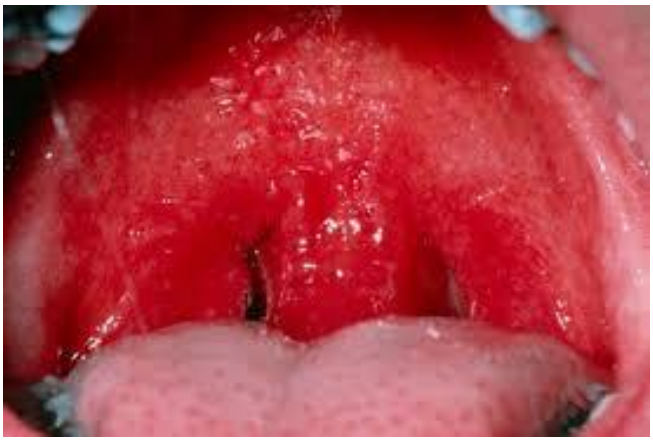
. Cardiovascular: infective endocarditis.



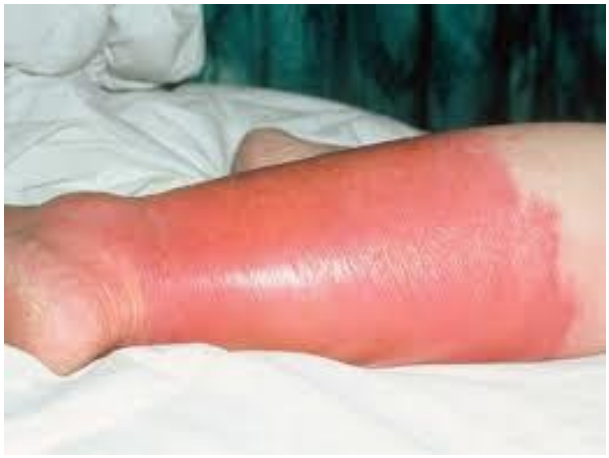
Retropharyngeal



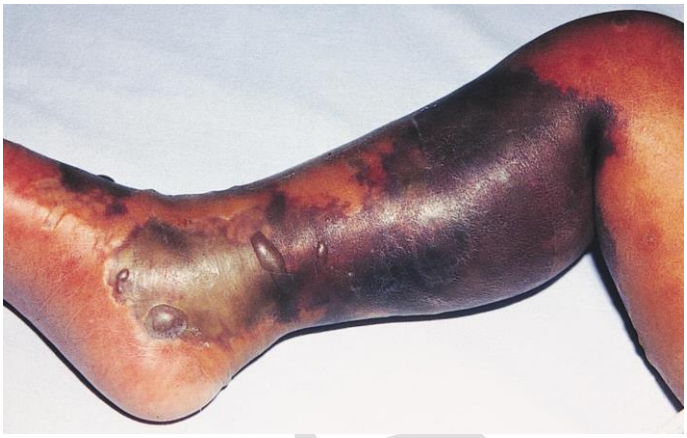
Tonsillitis



Pharyngitis



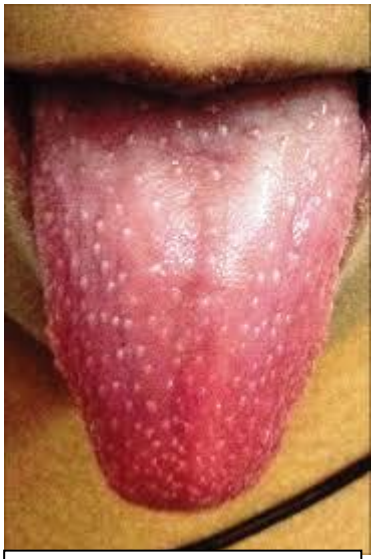
erysipelas



necrotizing fasciitis

Scarlet Fever

Infection with strains that elaborate any of the Streptococcal Pyrogenic Exotoxins (SPEs) may superimpose the signs of scarlet fever on a patient with streptococcal pharyngitis. In scarlet fever, the buccal mucosa, temples, and cheeks are deep red, except for a pale area around the mouth and nose. Punctate hemorrhages appear on the hard and soft palates, and the tongue becomes covered with a yellow-white exudate through which the red papillae are prominent (strawberry tongue). A diffuse red "sandpaper" rash appears on the second day of illness, spreading from the upper chest to the trunk and extremities.



strawberry tongue

Streptococcal Toxic Shock Syndrome (STSS)

STSS may begin at the site of any group A streptococcal infection even at the site of seemingly minor trauma. The systemic illness starts with vague myalgia, chills, and severe pain at the infected site. Most commonly, this is in the skin and soft tissues and leads to necrotizing fasciitis and myonecrosis. STSS continues with nausea, vomiting, and diarrhea followed by hypotension, shock, and organ failure.

Post-infection complications

Antibodies produced as a result of infection with *S. pyogenes* may cause non-pyogenic complications at other anatomical sites post-infection. Indeed, rheumatic fever and acute glomerulonephritis may develop up to 3 weeks after the streptococcal infection. Inflammation of the cardiac muscle occurs in rheumatic fever, whilst acute glomerulonephritis is characterized by inflammation of the renal glomerulus.

Laboratory diagnosis

Diagnosis is by isolation of the microorganism from infected sites (throat, skin, blood). The detection of serum antibodies to streptolysin O (ASOT: anti-streptolysin O titer) is particularly useful for the diagnosis of post-infection complications, such as rheumatic fever or acute glomerulonephritis.

This is because the microorganism is often no longer present at the time of clinical presentation.

Treatment

S. pyogenes is sensitive to many antibiotics. Penicillin remains the drug of choice for treatment of infection with this microorganism.

Group B (*Streptococcus agalactiae*)

Epidemiology

S. agalactiae forms part of the normal fecal, perineal and vaginal flora in females.

Morphology and identification

These microorganisms grow readily on blood agar and are identified by Lancefield grouping.

Group B streptococci will grow on media containing bile.

Pathogenicity

The virulence factors for group B streptococci are less well defined than for group A. However, more type-specific antigens and lipoteichoic acid are present in strains isolated from serious infection, thus these factors appear to be important in its virulence.

Associated infections

. Respiratory tract: pneumonia in neonates and the elderly;

. Musculoskeletal: septic arthritis, osteomyelitis;

. Skin and soft tissue: cellulitis;

. Genitourinary: (in the post-partum period) septic abortion, endometritis, urinary tract infections;

. Cardiovascular: infective endocarditis;

. Central nervous system: neonatal meningitis (neonatal acquisition of *S. agalactiae* is most frequently via transmission from the colonized mother in utero or at the time of birth.

Laboratory diagnosis

This is by isolation from the infected site. Direct detection of antigen in body fluids can be undertaken by various techniques.

Treatment

Penicillin is the drug of choice for treatment of infection with *S. agalactiae.*

Group C and G β-haemolytic streptococci

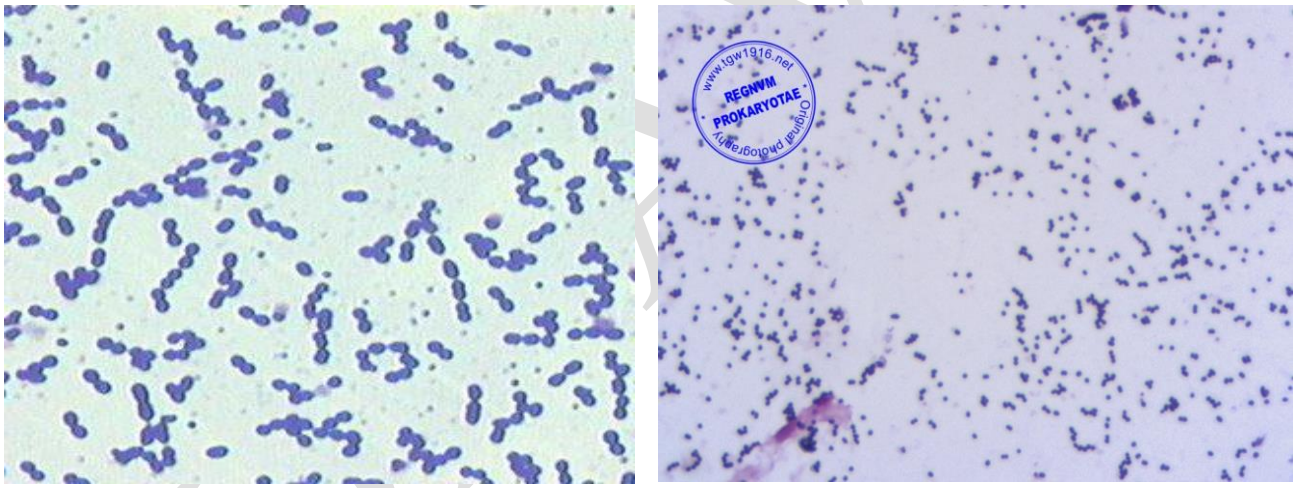
. Group C and G streptococci contain multiple species.

. They will not grow on media containing bile and are identified by Lancefield grouping and other commercial identification kits.

. Whilst infection with these microorganisms is less common, it can be severe. Examples of infections caused by these microorganisms include respiratory tract, puerperal and skin infection, endocarditis, meningitis, bacteremia and the post-streptococcal infection complication, acute glomerulonephritis.

. Infections are often treated with penicillin.

Enterococci (previously classified as streptococci)



Enterococcus spp. under microscope

Enterococcus faecalis and *Enterococcus faecium* are most commonly associated with human infection; however, infection with other enterococcal species also occurs.

Definition

Gram-positive cocci arranged in pairs or chains; facultatively anaerobic; non-sporing; non-motile (except *Enterococcus casseliflavus* and *Enterococcus gallinarum*); mostly catalase-negative (some strains

produce a pseudo catalase); some strains are encapsulated; can grow over wide temperature range.

Epidemiology

Enterococci are commensals, most notably of the gastrointestinal and vaginal tract.

Morphology and identification

Enterococci are usually nonhemolytic, but are occasionally α-hemolytic, or rarely β-hemolytic, it is belonged to Lancefield group D. Speciation can be performed using biochemical tests. They will grow on media containing bile and hydrolyze aesculin in the presence of 40% bile. Since group D streptococci (the *Streptococcus bovis* group) also share these properties, differentiation is achieved by the fact that group D streptococci, unlike enterococci, are unable to hydrolyze Pyrrolidonyl-β-naphthylamide PYR) and arginine and are not heat resistant.

Pathogenicity

Enterococcal strains can produce cytolysin, which causes lysis of a variety of cells, including erythrocytes and other mammalian cells. Other virulence factors include gelatinase, hyaluronidase, Enterococcal Surface Protein (a surface adhesin) and extracellular superoxide production.

Associated infections

They cause a number of (mainly health care associated) infections. These include:

. Gastrointestinal: peritonitis;

. Genitourinary: urinary tract infection;

. Cardiovascular: infections associated with indwelling catheters, infective endocarditis.

Laboratory diagnosis

Diagnosis is by isolation of the microorganism from infected sites.

Treatment

Enterococcal infections are often treated with ampicillin. Enterococci, (particularly *E. faecium*), have developed resistance to penicillin. In addition, the emergence of resistance to glycopeptide, antibiotics such as vancomycin is of concern.

These bacteria are referred to as ‘vancomycin resistant enterococci’ (VRE).