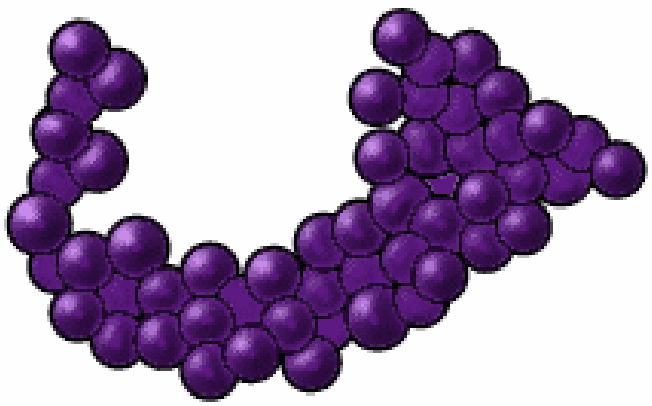
Gram Positive Cocci

Staphylococci

These bacteria, first isolated by Louis Pasteur In 1829 are gram-positive, spherical cells which typically divide in two or more planes to form clusters of cells.

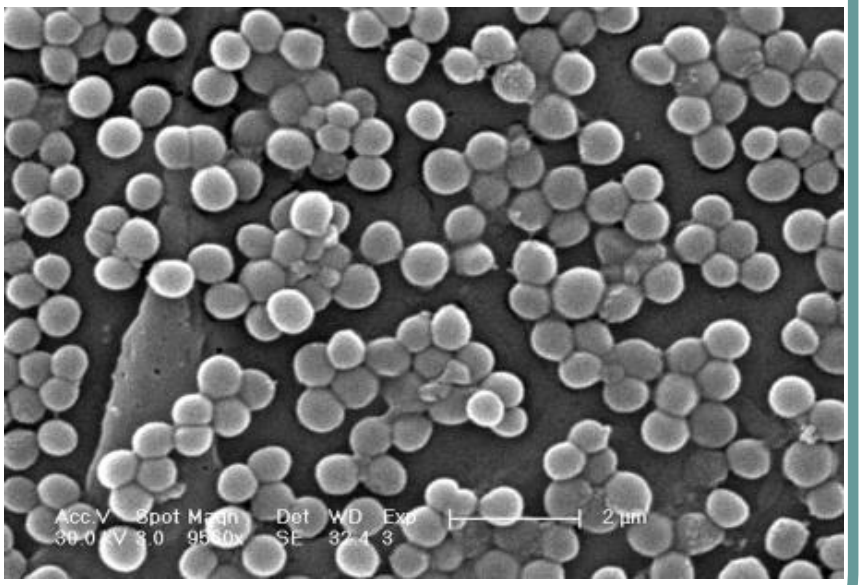


The Family *Micrococcaceae* consists of two the genera, *Staphylococcus* and *Micrococcus*.

- *Staphylococci*: pathogenic or commensal parasites
- *Micrococci*: free-living saprophytes, with little pathogenic potential – but are the occasional cause of opportunistic infections.

Species

- *S. aureus* – the main pathogen – responsible for pyogenic infections: identified by positive coagulase test.
- *S. epidermidis* (*S. albus*) – a universal skin commensal.
- *S. saprophyticus* – similar to S. epidermidis, but resistant to novobiocin.

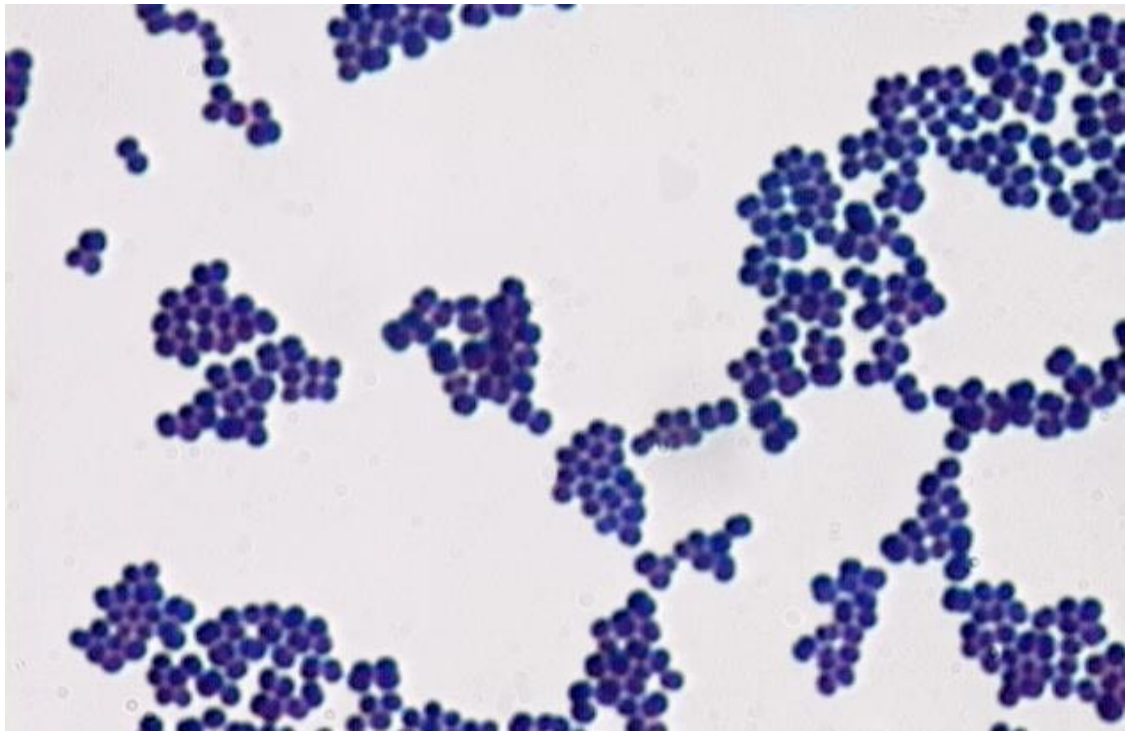


Habitat: the body surfaces and, by dissemination, air and dust.

- S. aureus: the nose – around 50-75% of healthy people carry it: less often, the skin (especially axilla and perineum), throat or gut.
- S. epidermidis: normally present in the resident skin flora. Also the gut or upper respiratory tract.

Morphology and staining:

Gram-positive cocci (diameter about 1 μm), non-motile arranged in grape-like clusters.



Culture:

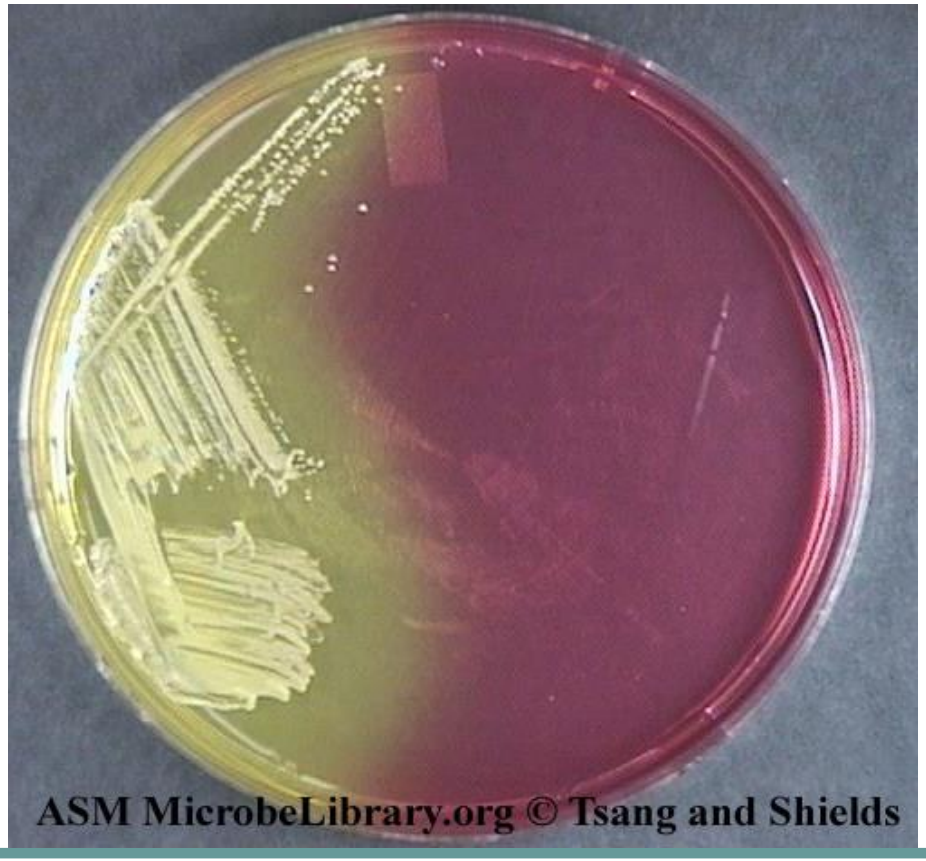
S. aureus is aerobic and grow well on ordinary media aerobically and, although less well, anaerobically; optimal temperature 37ºC.

Colonial appearance: S. aureus – typically golden, but Pigmentation varies from orange to white. After 24 hours' incubation colony is 2 to 4 mm (pinhead size), circular, convex, smooth, shiny, opaque, with entire edge and emulsifies easily. Pigment production occurs at 22oC and only in aerobic culture. ***Blood agar:*** a wide zone of beta haemolysin (clear zone) is produced around colonies. ***Egg yolk medium:*** the organism produces opacity on glucose egg yolk medium through lipolytic enzyme. ***MacConkey medium:*** colonies are small and pink in colour.

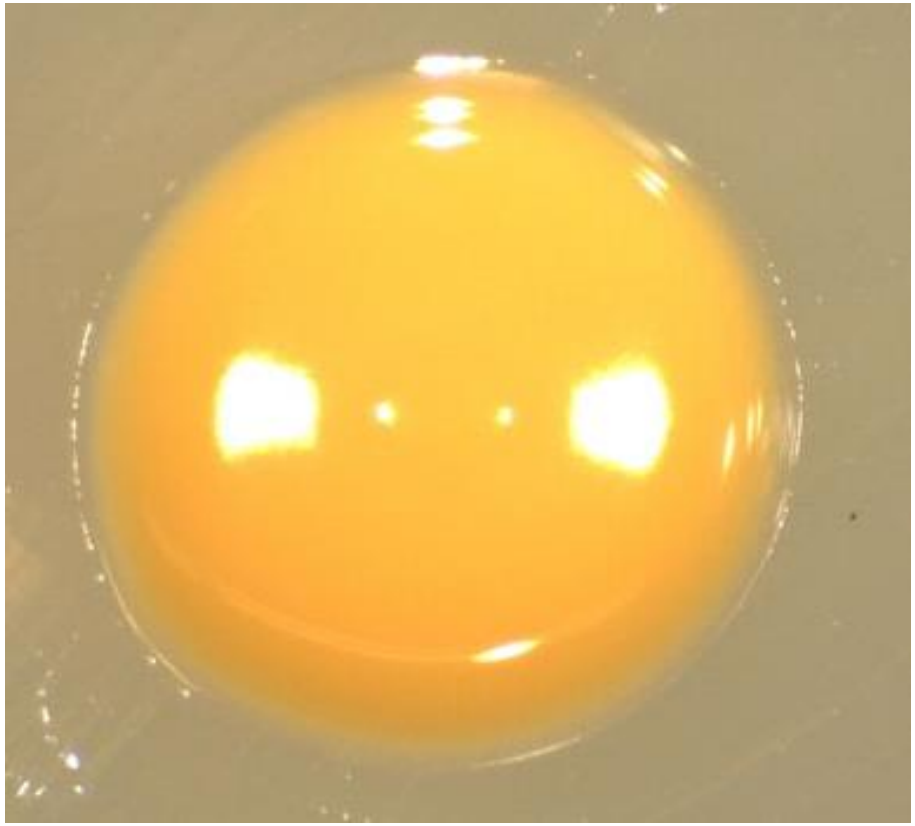
S. epidermidis – white colonies.

Selective media: media containing 8 to 10% NaCl (salt milk agar, salt broth); lithium chloride; tellurite; polymyxin. Staphylococci tolerate to sodium chloride in concentration of 5-10%. Salt-containing media are useful in isolating staphylococci from samples containing large numbers of other bacteria.

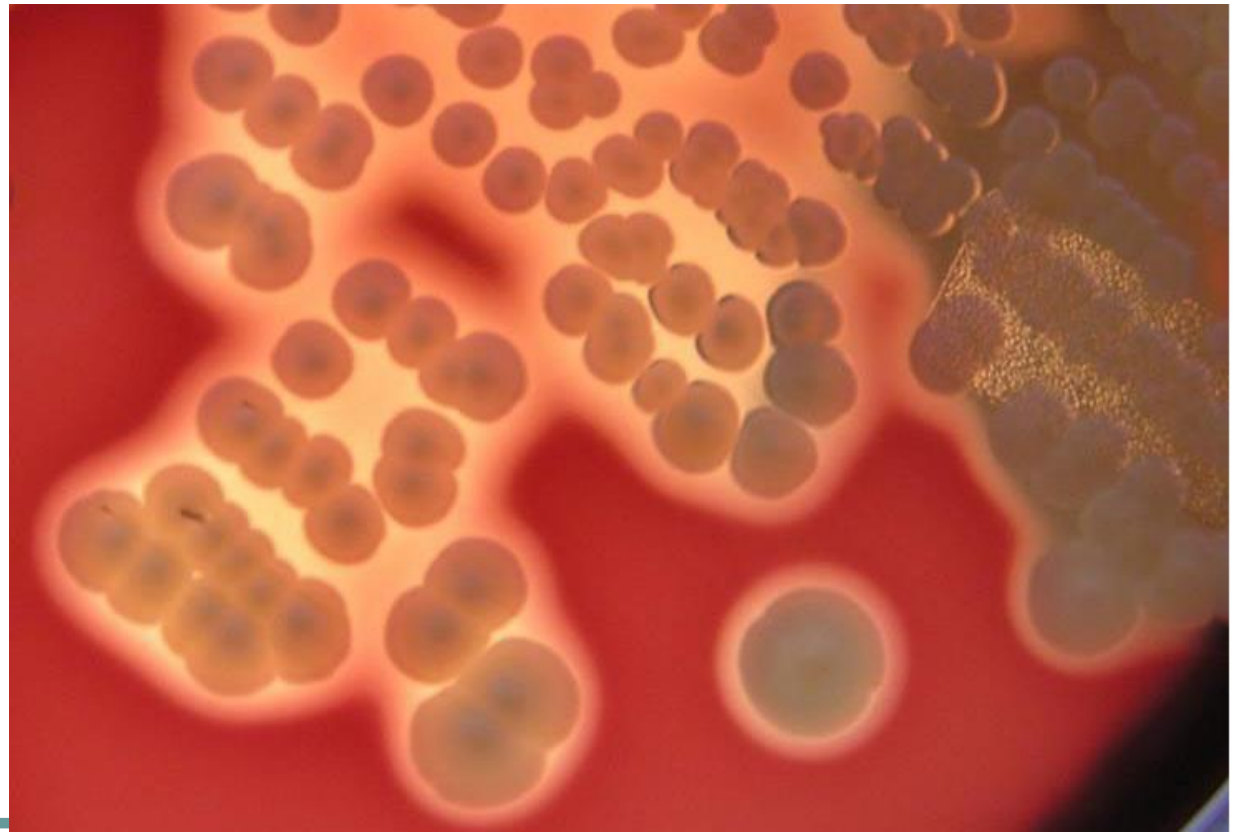
Mannitol salt agar inoculated with *S. aureus* showing fermentation of mannitol (yellow medium)



Staphylococcus aureus cultivated on trypticase soy agar.



Large, creamy white, beta hemolytic colonies typical of *Staphylococcus aureus.*

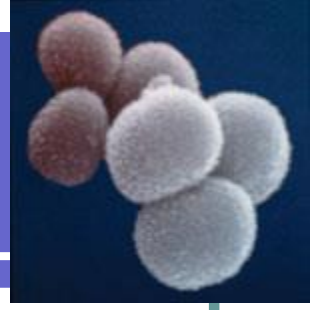


Catalase positive reaction indicating S. aureus infection

- Identification of S. aureus: by the detection of protein A – commercial kits for detecting this surface protein have replaced traditional coagulase test; confirm identity by test for DNAase.
- Typing: strains of S. aureus can be distinguished by the pattern of their susceptibility to an internationally recognized set of over 20 bacteriophages (phages).
- Toxins: S. aureus forms a large number of extracellular toxins and enzymes.

Toxin and toxic components produced by S. aureus

Toxin	Activity
Haemolysins α,β,γ and δ	Cytolytic; lyse erythrocytes of various animal species
Coagulase	Clots plasma
Fibrinolysin	Digests fibrin
Leucocidin	Kills leucocytes
Hyaluronidase	Breaks down hyaluronic acid
DNAase	Hydrolyses DNA
Lipase	Lipolytic (produces opacity in egg-yolk medium)



Toxin	Activity
Protein A	Antiphagocytic
Epidermolytic toxins A and B	Epidermal splitting and exfoliation
Enterotoxin	Causes vomiting and diarrhoea
Toxic shock syndrome toxin-1	Shock, rash, desquamation

Pathogenicity

S. aureus is a an important pyogenic organism, causing:

- superficial infections: pustules, boils, carbuncles, abscesses, impetigo, sycosis barbae, conjunctivitis, wound infections (including postoperative sepsis);
- deep infections: septicaemia, endocarditis, pyaemia, osteomyelitis, pneumonia;
- toxic food poisoning;
- toxic shock syndrome;
- skin exfoliation: toxic epidermal necrolysis (Ritter-Lyell's disease).

S. epidermidis: of lower pathogenicity but an important pathogen of implanted metal and plastic devices and prostheses.

S. saprophyticuc: a cause of urinary tract infection in sexually active women.

Staphylococcal Diseases. The sites of the major staphylococcal infections of humans are indicated by the above numbers.

1 Tissue where S. aureus is often found but does not normally cause disease

Diseases that may be caused by S. aureus are:

2 Pimples and impetigo

3 Boils and carbuncles on any surface area

4 Wound infections and abscesses

5 Spread to lymph nodes and to blood (septicemia), resulting in widespread seeding

6 Osteomyelitis

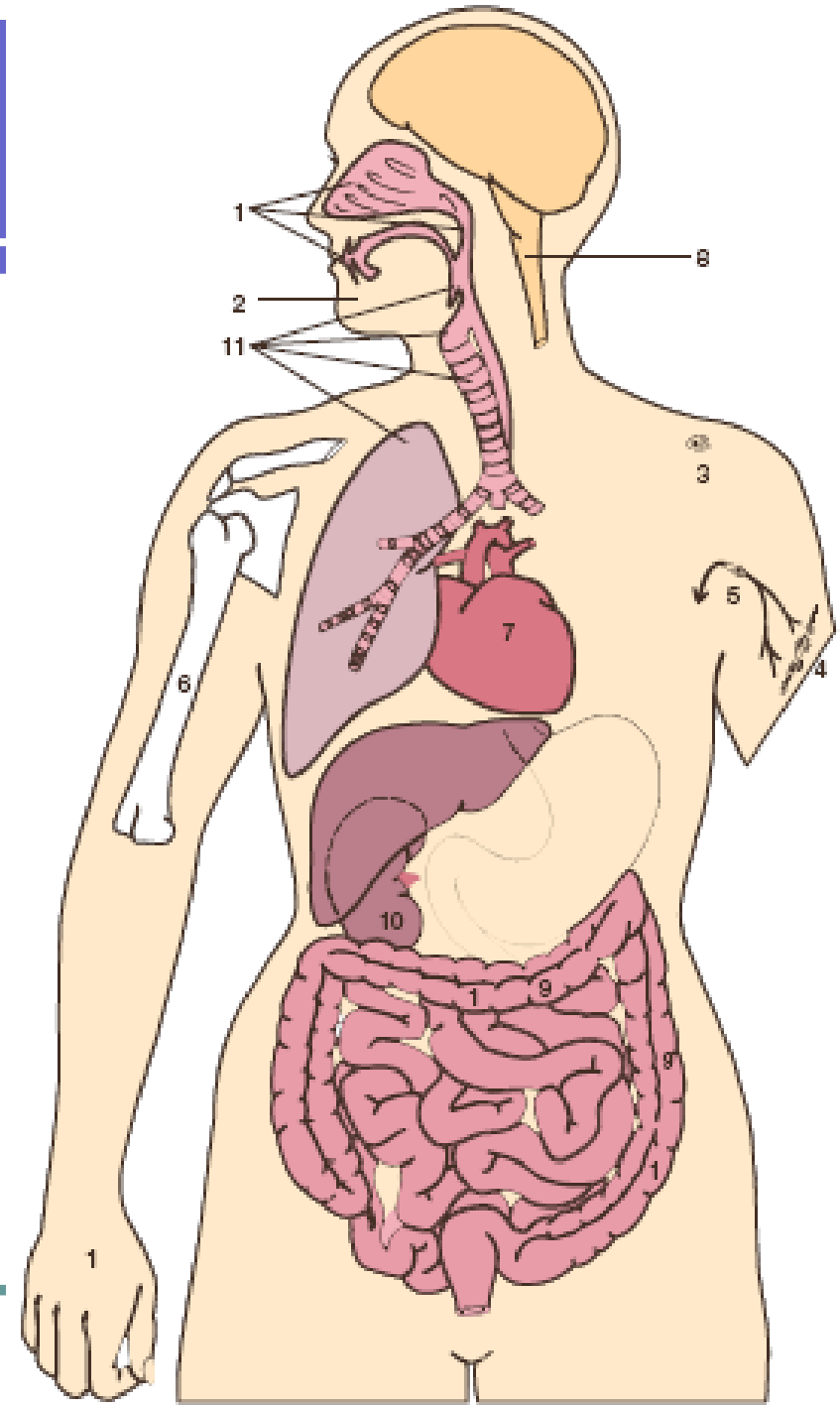
7 Endocarditis

8 Meningitis

9 Enteritis and enterotoxin poisoning (food poisoning)

10 Nephritis

11 Respiratory infections:
Pharyngitis
Laryngitis
Bronchitis
Pneumonia



Staphylococcal folliculitis appears as a red bump, or white pustule.

Staphylococcal folliculitis on the woman’s chest

Staphylococcal scalded skin syndrome, SSSS, also known as **Pemphigus neonatorum** or **Ritter's disease**, is a dermatological condition caused by *Staphylococcus*

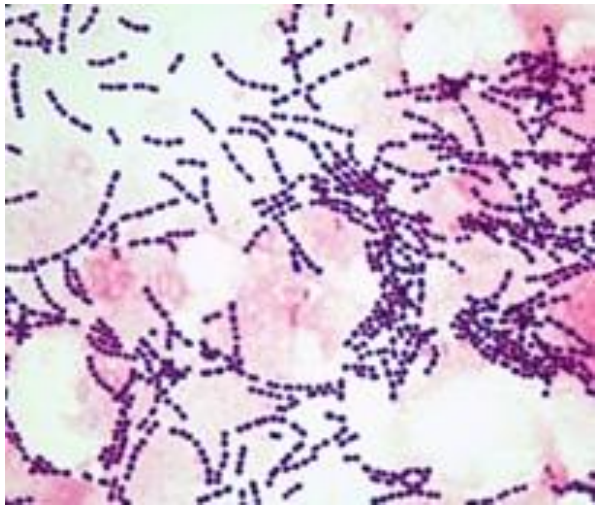
This 1 week old infant presented with erythema toxicum, which can sometimes be confused with “staphylococcal pustulosis”. Erythema toxicum is a benign rash common to newborns, which has an appearance that at times makes its diagnosis quite difficult, for it mimics the characteristic rash produced by a systemic staph infection, i.e. Erythematous maculopapular eruptions.

Antibiotic sensitivity

S. aureus readily appears in multiply resistant form – especially in hospitals. Antibiotics active against S. aureus are: flucloxacillin, macrolides, fusidic acid, vancomicin, cephalosporins.

General characters of Streptococci

Streptococci are Gram-positive spherical or oval cocci arranged in chains or pairs, non-motile and non-sporing, 0.7-0.9 μm diameter.



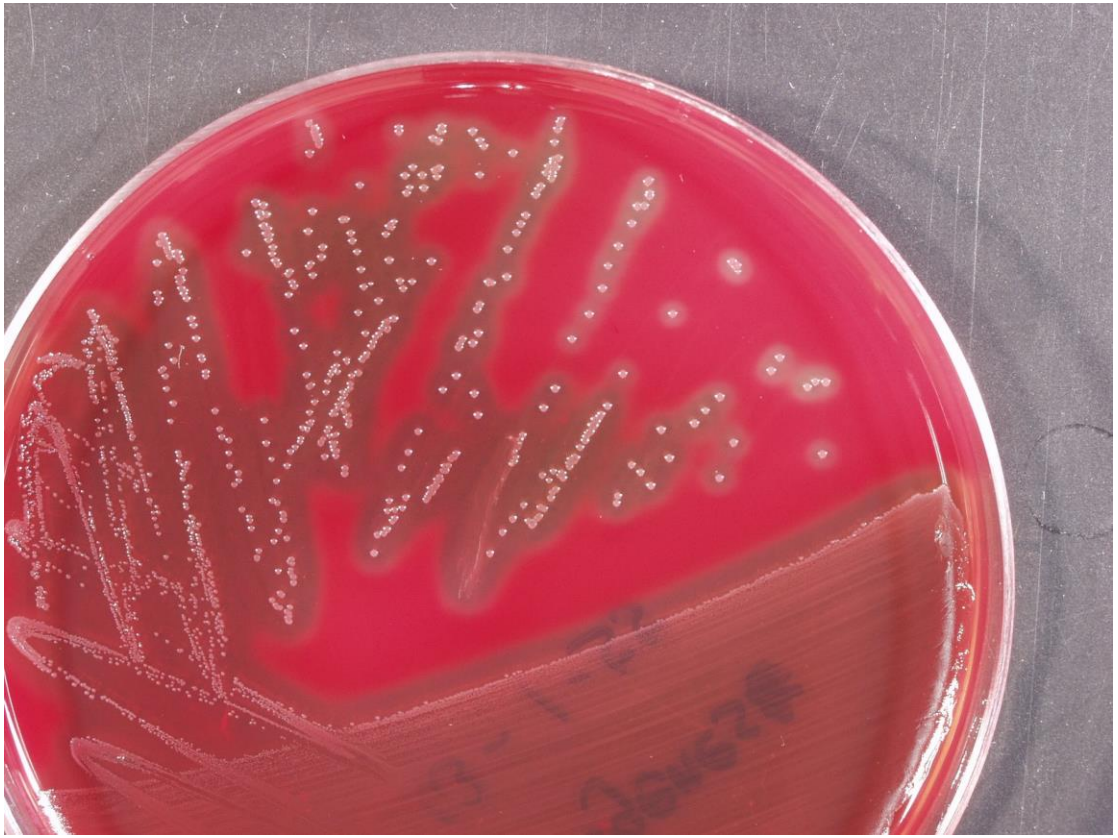
Classification

This is extremely complex, and still incomplete. An important basis for classification is the type of haemolysis produced around colonies growing on blood agar.

Haemolysis	***Appearance***	***Designation***	***Streptococcal class***
Complete	Colourless, clear, sharply-defined zone	β	Pyogenic streptococci
Partial	Greenish discoloration	α	Viridans streptococci
Partial	Greenish discoloration	α	Pneumococci
None	No change	γ or non-haemolytic	Enterococci

- Culture: grow well on blood agar; enrichment of media with blood, serum or glucose may be necessary.
- Colonial morphology: usually small.
- Selective media: media containing an aminoglycoside antibiotic or 1:500 000 crystal violet for inhibit other bacteria in a mixed culture, but permit growth of streptococci.
- Biochemical reactions: all give a negative catalase reaction. Streptococci can be characterized by their biochemical activities.
- Serology: identifies Lancefield groups: 20 are recognized, designated A-H and K-V. The antigens that define the groups are either polysaccharide or teichoic acid.

Streptococcus pyogenes on blood agar



Pyogenic streptococci.

- This class includes the most pathogenic human species: the main pathogen is S. pyogenes. Pyogenic streptococci have polysaccharide Lancefield group antigens in their cell wall: S. pyogenes is in Lancefield group A; other pyogenic streptococci belong to Lancefield groups B, C, G, R and S.
- Habitat: present as a commensal in the nasopharynx of a variable proportion of healthy adults and, more commonly, children. The carriage rate in children is about 10%.

Laboratory characteristics

Culture: blood agar with small, typically matt or dry colonies surrounded by β-haemolysis.

Capsule: some strains produce a hyaluronic acid capsule during the logarithmic phase of growth, and develop mucoid colonies on blood agar.

Toxins: the following extracellular products: streptokinase, hyaloronidase, deoxyribonucleses (DNAases), nicotinamide adenine dinucleotidases (NADase), haemolysins, erythrogenic toxin, leucocidin, protease, amylase.

Serotypes: S. pyogenes (Lancefield group A) can be subdivided into Griffith types, depending on three surface protein antigens:

- M. type-specific, i.e. there is a distinct M antigen for each type or strain: only found in virulent or pathogenic strains. M antigens impede phagocytosis and antibody to them enhances phagocytosis. 65 distinct M serotypes have been identified.
- R: fewer R antigens than there are M antigens, and the same R antigen can be found on several different M types.
- T: each T antigen may be found on several different M types; used in conjunction with M typing for identifying different types of S. pyogenes.

Immunity to infection with S. pyogenes is specific for each individual M type.

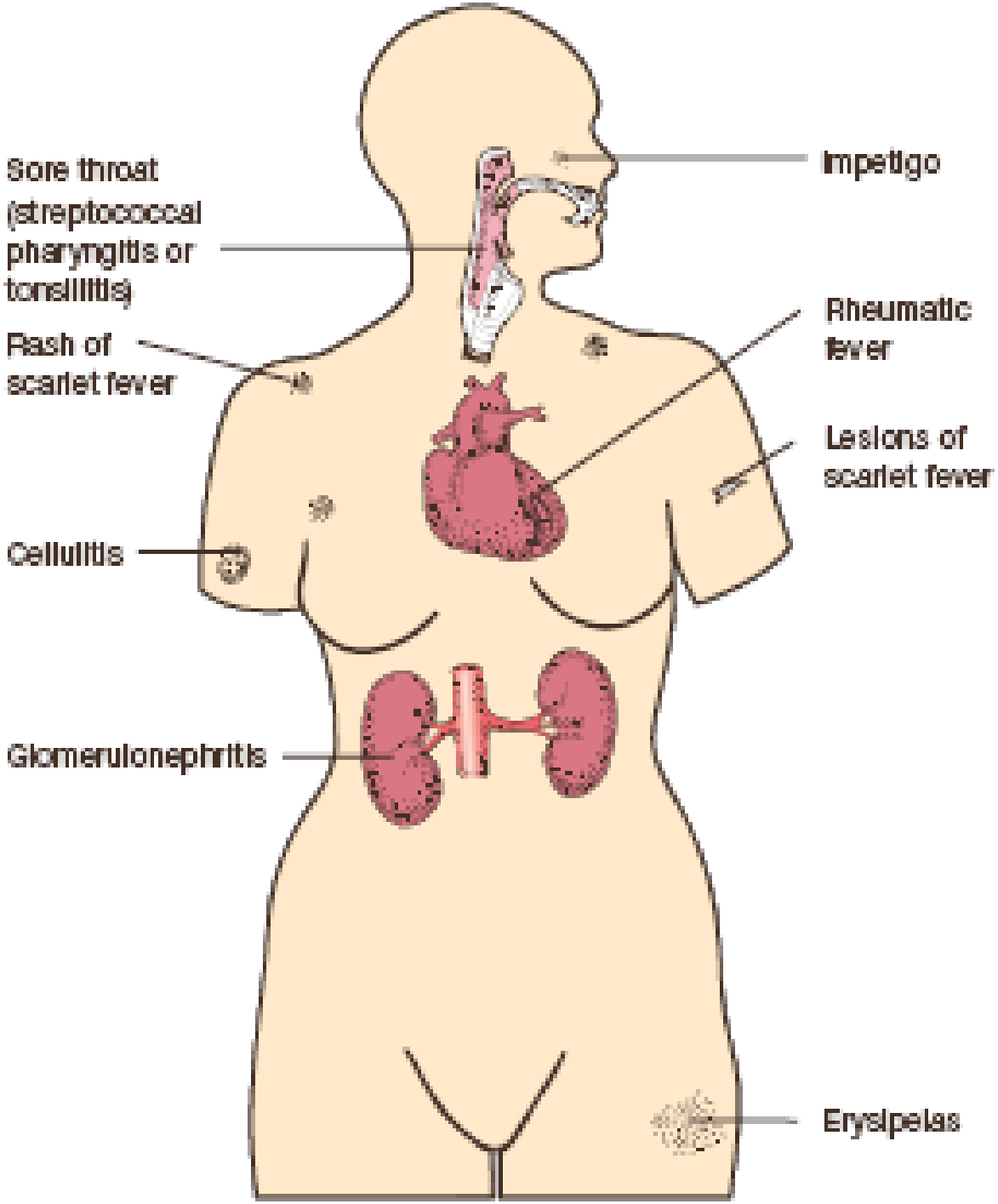
- ***Pathogenicity***

S. pyogenes causes: tonsilitis, pharyngitis, peritonsillar abcess (quinsy), scarlet fever, otitis media, mastoiditis, sinusitis, wound infections, impetigo, erysipelas, puerperal sepsis. Post-streptococcal complications: reumatic fever, glomerulonephritis.

- ***Antibiotic sensitivity***

The drug of choice is penicillin. In patients hypersensitive to penicillin, use erythromycin.

Some of the more prominent diseases associated with group A streptococcal infections, and the body sites affected.



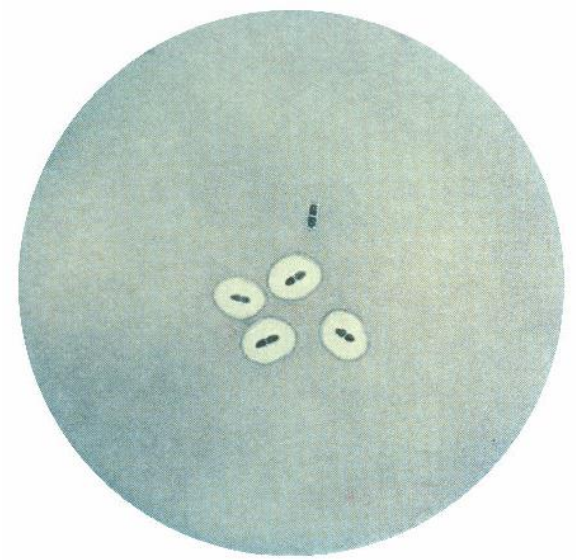
Streptococcus group A infections. Erysipelas is a group A streptococcal infection of skin and subcutaneous tissue.

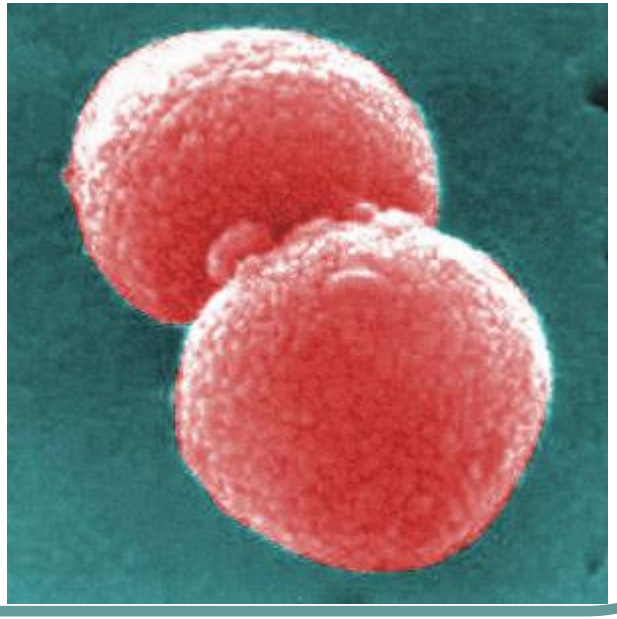
Streptococcal Pharyngitis - Strep Throat

Impetigo is a highly contagious disease caused by streptococcus or staphylococcus bacteria. It causes a superficial skin infection which appears red with yellow or golden crusts. It is seen frequently in children on the face, upper trunk, and arms. Note that the nose is also infected.

Pneumococci

- They are important invasive pathogens. Bacteria are lanceolate diplococci arranged in pairs or short chains and are surrounded by a capsule.
- Habitat: normal commensal of the upper respiratory tract.





- Culture: blood agar.
- Colonies: α-haemolytic, typically “draughtsmen”, i.e. with sunken centre due to spontaneosus autolysis of older organisms. Young colonies may resemble dew-drops, due to large capsules before autolysis.
- Capsule: contains the polysaccharide carbohydrate antigen: type-specific. 84 capsular types are recognized.
- Identification: by a variety of serological tests directed against the antigen. The standard method is capsule swelling – the quelling reaction – observed microscopically when pneumococci are mixed with specific antisera.

- Pathogenicity: Pneumococci are important pathogens and cause a considerable amount of both morbidity and mortality today, despite their sensitivity to penicillin. They may cause: lobar pneumonia; acute exacerbation of chronic bronchitis (often with Haemophilus influenzae); meningitis, otitis media, sinusitis, conjunctivitis, septicaemia (especially in splenectomized patients).
- Antibiotic sensitivity: All strains remain sensitive to penicillin but penicillin resistance is a significant problem in some countries. Resistance to erythromycin, tetracicline and trimethoprim is more commonly encountered.