13. Streptococci and enterococci

13.1. GENERAL FEATURES

Streptococci and enterococci are gram-positive cocci usually arranged in pairs (diplococci) or chains. Some are members of the normal flora that are potential pathogens (*E. faecalis* – urinary tract infection). However some of them are true pathogens always causing disease in the body (e.g. *S. pyogenes* – sore throat, necrotizing fasciitis, cellulitis, toxic shock syndrome*).* The most common type of streptococcal infections are pyogenic (suppurative).

13.2. CLASSIFICATION

Haemolytic properties are detected around colonies growing on blood agar. α haemolytic organisms produce *partial haemolysis (*viridation), caused by bacterial H_2O_2 that oxidizes haemoglobin of the red cells to green methemoglobin. Alpha haemolytic organisms include a large group of commensal species called Viridans streptococci, some enterococci and also the serious pathogen *S. pneumoniae*. β haemolytic streptococci cause complete haemolysis. This is caused by streptolysin. Beta haemolytic organisms include the pathogenic streptococci (e.g. *S. pyogenes*). γ haemolytic organisms are non-haemolytic. This group includes some species of oral streptococcal flora and some enterococci.

Serologic (Lancefield) groupings are a subdivision of β hemolytic streptococci based on detection of particular C-carbohydrates in the cell wall (groups A-U). The most medically relevant are streptococci of groups A (*S. pyogenes*) and B (*S. agalactiae*).

13.3. VIRULENCE FACTORS AND PATHOGENESIS

Virulence factors are genetic, biochemical, or structural features that enable an organism to produce disease. **Capsule:** the hyaluronic acid mimics human connective tissue and is therefore non-immunogenic, which prevents the bacteria from being phagocytosed. **Cell wall:** Fimbriae are anchored in the cell membrane and extend through the cell wall and capsule (composed from antigenic M protein). Their structure resembles cardiac tissue and can therefore create an autoimmune-like reaction (rheumatic fever). **Enzymes and toxins:** Hyaluronidase digests connective tissue. Dnases A to D digest DNA. Streptokinase dissolves fibrin clots by activating plasmin. Pyrogenic exotoxin is a superantigen that enhances the release of proinflammatory cytokines by the immune system. It is the cause of the red rash of scarlet fever and is strongly involved in streptococcal toxic shock syndrome and necrotizing fasciitis. Streptolysins O and S (Oxygen labile and oxygen stabile, respectively) and pneumolysin (produced by *S. pneumonia*) cause cell lysis. C5a peptidase inactivates C5a of the complement.

13.4. INFECTIONS AND POSTSTREPTOCOCCAL DISEASES

Agent - beta haemolytic streptococci (group A, *S. pyogenes*)

1. Suppurative local infections: acute tonsilopharyngitis (purulent inflammation of oropharynx and tonsillae), pyoderma (pustules that form on the skin, burst and then form a yellow crust)

2. Suppurative invasive infections: cellulitis, necrotizing fasciitis, puerperal fever,

bacteraemia/sepsis, streptococcal toxic shock syndrome (distinguished from staphylococcal toxic shock syndrome by it being accompanied by bacteremia or necrotizing fasciitis)

3. Poststreptococcal diseases (rheumatic fever, acute glomerulonephritis) cause autoimmune inflammatory changes of the heart or renal glomerulus because of antigenic similarity of streptococcal antigens and human tissue. This could progress to damage of the heart valves or glomerules.

Agent - beta haemolytic streptococci (group B, *S. agalactiae*)

Usually cause early-onset (bacteraemia, pneumonia, meningitis) or late-onset neonatal disease (bacteraemia with meningitis). Other diseases are puerperal (childbed) fever of mothers shortly after childbirth (could also be by *S. pyogenes* or others).

S. pneumoniae causes serious infections with sudden onset of symptoms (pneumonia, empyema, meningitis and otitis), especially in children.

Enterococci are mainly responsible for urinary tract infections in catheterized patients. They can cause endocarditis in bacteraemic patients.

13.5. TREATMENT, PREVENTION & CONTROL

Streptococcus pyogenes: penicillin G alone or in combination with clindamicin (fasciitis), in allergic patients use macrolides

S. agalactiae: penicillin G, ampicillin plus aminoglycosides in serious infections

S. pneumoniae: almost penicillin susceptible if not a cephalosporin of 3rd generation (cefotaxim) or glycopeptide (vancomycin);

Enterococci: useful combination of β lactams and aminoglycosides (e.g. ampicillin & gentamicin) or glycopeptides (rarely resistant)

Viridans streptococci: susceptibility varies, treatment is always based on susceptibility testing

13.6. LABORATORY DIAGNOSIS

a) Specimens: Swabs, pus, blood aspirates, cerebrospinal fluid and other clinical material depending on the localization of the process.

b) Microscopy: Streptococci are gram-positive cocci arranged in diplococci or chains (fig. 1).

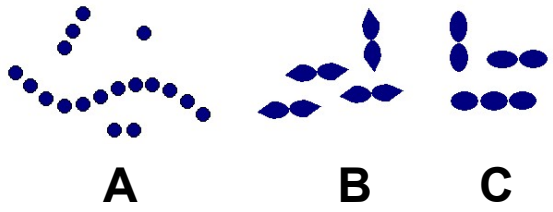


Fig.1. The characteristic arrangement of these bacterias (A). Exceptions that could be seen are lancet shaped diplococci in S. pneumoniae (B) or coccobacili in enterococci (C).

c) Culture: Streptococci grow on general enriched culture media as matt or smooth (if encapsulated) colonies. Hemolytic properties differentiates α, β and γ hemolytic species (fig. 2). In case of suspected competetive flora some selective or diagnostic media are suggested to use (eg. chromogenic agar where streptococcal colonies have a specific colour).

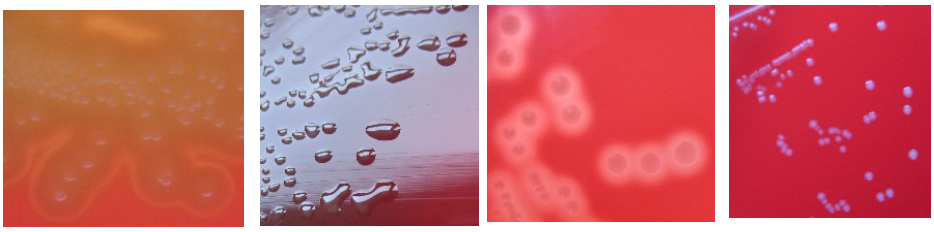


Fig. 2. Colonies surrounded by zone of α (S. salivarius) (A) and mucoid colonies of S. pneumoniae (B), β (S. pyogenes) (C) and γ hemolysis (Enterococcus faecalis) (D)

d) Phenotypical identification is focused on detection of specific biological properties of streptococci (eg. enzyme and structural protein detection) (see chapter – Identification) to be able to define the subtype.
Screening tests (preliminary identification): detection of some phenotypic properties are showed in fig.3.

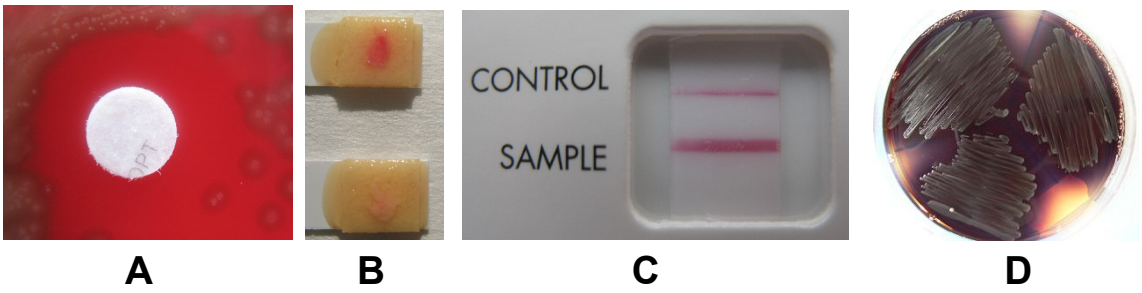


Fig. 3. Detection of susceptibility to optochin in S. pneumoniae (A), production of pyrolidonyl naphtylamidase in S. pyogenes and enterococci – PYR test (B), detection of C-carbohydrates specific for S. pyogenes detected by enzymatic immunoassay EIA (C) and production of esculin by enterococci (D).

Biochemická identifikace. Produkce metabolických enzymů je vyhodnocena pomocí dichotomického klíče nebo numerickou identifikací (obr. 4) (viz – Identifikace).

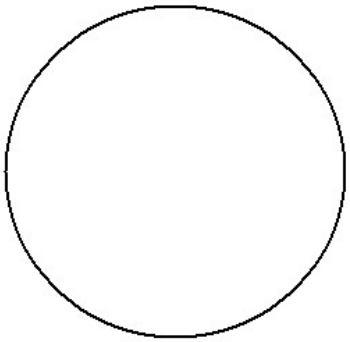
Fig. 4. Biochemical identification using diagnostic kit. Various substrates are metabolized in the microtubes. Positive and negative reactions are noted and using data of known metabolic profiles the strain being analysed data is compared differentially and a species is identified.

Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry - (MALDI – TOF MS): analyses molecular structure (mainly proteins) of an unknown microbial isolate, as the mass spectral pattern consisting of a number of structurally related mass spectral peaks, and comparing with a known patterns analyzing the isolate. The mass spectrometer first ionizes, then mass separates and finally detects time of the ions flight, thus producing a mass spektrum and comparing it with known mass spektra analyse the studied microorganism (see also in chapter 6 - Identification).

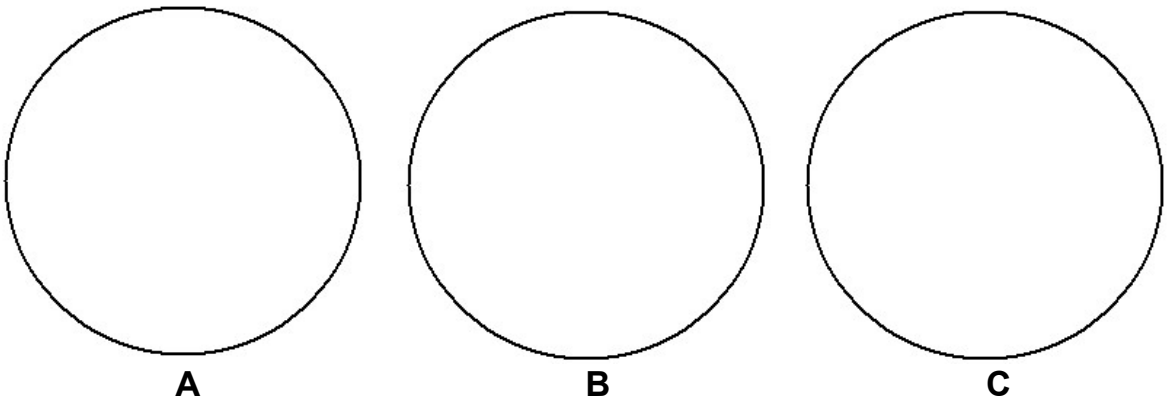
e) Genotypical identification. There are several ways to identify a strain or species using genotype. For instance, comparing electrophoresed fragments allows visualization of the restriction profile. Alternatively, homology of highly conserved regions, such as the 16S RNA gene, can assist identification of the species. (see also Chapter 6 – Identification).
f) Susceptibility testing. Qualitative methods (disk diffusion method), quantitative methods or their combination (E-test) are used to test the susceptibility (see also chapter – ATB testing).
g) Serologic diagnosis of poststreptococcal diseases is based on detection of various antibodies to structural parts of S. pyogenes antigens (e.g. Anti-DNase, antihyaluronidase, antistreptokinase). The antistreptolysin A antibodies (ASO) in patient sera is the most widely used.

13.7. PRACTICAL PART – STREPTOCOCCI & ENTEROCOCCI

Exercise 1: MICROSCOPY: Prepare a gram-stained smear of purulent material evacuated from a subcutaneous abscess caused by pyogenic streptococci. After drying, fixing and gram-staining draw the morphology of the specimen that you can see in the microscope.



Exercise 2: MICROSCOPY: Stain of alpha, beta and gamma hemolytic streptococcal colonies. After drying, fixing and gram-staining draw the specimen's morphology that you can see in microscope view. Do they vary in their cell morphology or not?



Exercise 3: *PRELIMINARY IDENTIFICATION OF STREPTOCOCCUS PYOGENES. Perform a Gram stain on a suspected bacterial culture and then perform a catalase test. Mix the culture with 3% H_2O_2 – if bubble appear the test is positive and then the pyrrolidonyl amidase test PYR (using commercial strip and ingredients). Draw and note the procedures in the space below.*

Exercise 4: *INTERPRET THE RESULT OF BIOCHEMICAL IDENTIFICATION AND SUSCEPTIBILITY TESTING. Using producer recommendation interpret the result of biochemical identification of suspected staphylococcal strain. Using standardized criteria interpret also the result of disk diffusion method or determine minimal inhibitory concentration of the antibiotics tested.*

13.7. LAB QUIZ

1. Specify some streptococcal virulence factors.
2. Specify the infections caused by streptococci. How can these infections be categorised generally?
3. Describe the principles of the treatment of streptococcal infections.
4. Specify the direct detection methods used in diagnostics of streptococcal diseases.
5. Specify the prevention and control of serious streptococcal infections.