• **Classification** is arrangement of bacteria into groups (the same organisms can be classified differently according to the view: serotype classification, antimicrobial resistance classification…)

• **Nomenclature** (name) is the mean of communicating - it is binominal

• **Taxonomy** - science of classification, identification, nomenclature and making a system

• **Identification** is practical use of classification criteria to distinguish certaine organism from others
Graduating

- **subspecies:** serotyping *Streptococcus pneumoniae* type 8
- **species** - distinct organism with certain characteristic features, similar organism within genus *Streptococcus pneumoniae, S.sp*
- **genera:** *Streptococcus*,
- **families:** *Micrococaceae*,
- **orders**
Approaches to taxonomy

• Numerical,
• phylogenetic,
• genome size,
• Guanin+cytosine content,
• DNA relatedness, thermal stability of DNA sequences, DNA relatedness under supraoptimal conditions…….
• In practice - polyphasic approche - depending on importnace and purpose of nomenclature
Bacterial identification in practice

• Pure culture
• Colony morphology
• gram staining - preliminary identification $G+G$-
• growth characteristics aerobic, anaerobic
• Biochemical properties genus + species
• antigenic properties type
• binominal nomenclature - clinical purposes – in italics
• Molecular and genetic characteristic
• G+C, DNA homology, thermal stability -epidemiological and forensic puroposes
Principles of diagnosis

• Symptomatic patient - manifestation of infection
• Suspection of microbial ethiology - exogenous or endogenous
• Specimen selection, collection -
• Specimen processing and microbiological examination - specific techniques
• Result communication and consultation
• Asymptomatic patient
Practical taxonomy

**Bacteria**

**Gram positive**
- **G+cocci**
  - anaerobic: Peptostreptococcus, Peptococcus, Sarcina, Ruminococcus
  - aerobic: Staphylococcus, Micrococcus, Streptococcus, Enterococcus
- **Gram+rods**
  - anaerobic: Clostridium, Corynebacterium, Arcanobacterium, Rhodococcus
  - aerobic: Bacillus, Propionibacterium, Mobiluncus, Bifidobacterium
- **G-cocci**
  - anaerobic: Veillonella, Megasphoera
  - aerobic: Neisseria, Moraxella, Branhamella

**Gram negative**
- **G-rods**
  - aerobic: Vibonaceae, Campylobacter, Helicobacter, Bacteroides, Porphyromonas, Prevotella, Fusobacterium
  - anaerobic: Leptotricha, Wolinella

**Miscellaneous bacteria**
- Mycoplasma, Ureaplasma, Chlamydia
- Rickettsiaceae
- Spirochetales - Spirochetes, Leptospira, Borrelia
- Mycobacterium
- Nocardia, Actinomycetes,
Micrococcaceae – family (G+ cocci)  
Staphylococcus – genus  

- Pathogens of man  
- Divided in 2 groups acc to plasmacoagulase production - artificial  

PC negative - common comensals of human skin and animals, some of them cause infections, but only in certain circumstances (Staphylococ. epidermidis, warneri, haemolyticus, saprophyticus, hyicus) - facultative pathogens  
PC positive - Staphylococcus aureus, (St. Intermedius)
Staphylococcus aureus G+ coccus

- colonizes nasal passage and axillae
- able to grow on salt medium (10%), catalase +,
grows in clusters, PC positive - distinguishing factor
G+ cocci in clusters
gram staining
Cocci in clusters in fluorescein preparation
Growth characteristic – growing on salt media – staphylococci
Fermenting manitol – yellow – *St. aureus vs. St. epidermidis*
Staphylococcus aureus G+cooccus

- **Structure:** capsule, peptidoglycan, protein A, teichooic acid, clumping factor, cytoplasmic membrane
- **Toxins:** alfa, beta, delta, gama, leukocidin, exfoliating, toxic shock syndrome TSST 1, TSST 2 - enterotoxin, SSST
- **Enzymes:** coagulase, catalase, hyaluronidase, staphylokinase, lipases, fibrinolysin, nuclease, penicilinase
Toxins

- **alfa** - cytotoxic, disrupting cell membrane and smooth muscle of vessels, necrotising
- **beta** - sfingomyelininase C, termolabil, hydrolysis of phospholipids, destruction of tissue, production of abscess
- **delta** – termostabil, cytolysis, detergent properties
- **gamma** - erytrocytolysis
- **leukocidin** – enhancement of permeability and formation of poresin the cell wall, resistance to phagocytosis
- **exfoliative toxin** – responsible for SSS – skin scaled sydrome, disruption of intercelular junction – desmosomes in the stratum granulosum of the skin, protective antibodies – disease of children
- **TSS-1** – in some strains of *St.aureus* TSS – toxic shock syndrome –
- **TSS-2 enterotoxins** – resistent to hydrolysis with gastric acid, termostabil, 5 types A-E, B – pseudomembranous colitis, neurotoxin, diarrhoea and vomiting
Enzymes

• **Coagulase** - bound (fibrinogen - fibrin) and free, formation of fibrin layer and **abscess** – production from phagocytosis

• **Catalase** – transformation of toxic \( \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2 \)

• **Fibrinolysin** - staphylokinase – dissolves fibrin clot

• **Lipase** – different types, surviving of staphylococci in seboroic area - transmission to the skin and subcutaneous space, formation of skin infections

• **Hyaluronidase** – disruption of mucopolysaccharides in the connective tissue, spreading

• **Nuclease** - termostabil E

• **Penicilinase** - beta lactamase, enzyme disrupting betalactam ring of PNC ATB
Virulence factor and patogenesis

- Multifactorial
- adherence
  - surface proteins (att. to fibronectin),
  - fibrinogen/fibrin binding protein (att. to clots and traumatised cell, the most important part of slime on biomaterial of indwelling devices),
  - fibronectin
- avoidance of host defence
  - capsule polysaccharide,
  - protein A - bind IgG - disruption of opsonisation,
  - leukocidin - toxic for PMNL
- damage of host tissue
  - toxins and enzymes
Clinical manifestations caused by 

**St. aureus**

- **Skin** – pus forming disease of skin: ulcers, abscesses, styes, carbuncul, impetigo
- **Systemic** - endocarditis, pneumonia, bacteraemia, osteomyelitis, septic arthritis, flebitis, mastitis, meningitis, infection of urinary tract - blade
- Hospital infections, infections of skin, catheters, burnings
- **GIT** infection – enterotoxin – vomiting, diarrhoea
- **TSS** - toxic shock syndrom
- **SSS** - scalded skin syndrom

Pyogenic - pus

Toxin
Superantigen: enterotoxins and TSST

• **TSST** - exotoxin secreted during the growth of some strains, connected with superabsorbent tampons - fever, hypotension, shock, rash, desquamation, multi organ involvement
TSST-1: 75% menstrual TSS., TSST-2 enterotoxin B and C: 50% non menstrual TSS

• **Enterotoxins** A-E- resistant to hydrolysing gastric enzymes and to heat 100°C (neurotoxin)

• **Superantigen**: superstimulation of T cells nonspecifically 1 of 5 cells instead of 1 of 10 000 with APC - cytokines released in large amounts - (any T cell with Vb element is stimulated)
Superantigens

- Definition

**Conventional Antigen**

- Monoclonal/Oligoclonal response
  - $1:10^4 - 1:10^5$

**Superantigen**

- Polyclonal response
  - $1:4 - 1:10$
Clinical manifestation of *St. aureus*

- **Skin** - stye, soil, carbuncle, impetigo, endocarditis, pneumonia,
- **General pyogenic** Nosocomial and wound infection, infection of indwelling devices, burns, osteomyelitis, immunosuppression, phlebitis, mastitis, meningitis
- **Toxic** GIT infections - vomitus, diarrhea
  TSS - toxic shock syndrome
  SSS scalded skin syndrome
impetigo
folikulitis
carbuncul
Staphylococcal scaled skin syndrome
SSSS– Riter’s disease
1. **Diseases caused:**
   a) Skin infections with pus formation: boils (furuncles) and carbuncles; impetigo.
   c) Toxin-mediated diseases: SSSS, TSS, food poisoning.

2. **Pathogenesis:**
   a) Skin infections result from a break in primary defenses (skin barrier); invading bacteria produce numerous virulence factors (e.g., toxins) which inhibit normal phagocytosis and cause pus formation (dead PMNs).
   b) Capsular polysaccharides may enhance virulence in deep infections.
   c) Different toxins exhibit specific pathologic effects; e.g., enterotoxins type A through E can affect nerve and intestinal cells directly in food poisoning or act as superantigens in TSS (enterotoxin F). Exfoliative toxin causes killing of cells at the junction of dermis and epidermis, resulting in sloughing of the skin.

3. **Immunity:** Integrity of skin. No permanent immunity due to virulence factors.

4. **Epidemiology:** Found in anterior nares of ca. 30% of normal individuals. Hospital-acquired strains often have more antibiotic resistance (e.g., methicillin-resistant *S. aureus* [MRSA]).

5. **Diagnosis:**
   a) Coagulase (+);
   b) catalase (+);
   c) growth on 7.5% NaCl-mannitol agar;
   d) ± gold-colored, ± hemolytic colonies on BAP;
   e) gram-positive cocci in clusters among RBCs in blood culture.

6. **Control:** Proper hand washing; hospital disinfection to prevent spread and promote eradication of MRSA. Vancomycin treatment is the last resort. © ASM Press, 2003
Bacteria

Staphylococcus aureus
(staf-i-h-low-cock-us aw-ree-us)
© 2007 nanobugs, inc
St. epidermidis and other PC negative

- Infection of **indwelling devices** - catheter, shunt, artificial joint
- Infection of valve - artificial or damaged - **endocarditis** - indolent course - 1 year after surgery
- *Staphylococcus saprophyticus* - urinary infection of young sexually active women
- Contamination or ethiological agens
Bacteria

Staphylococcus epidermidis

(staf-ih-low-cock-us ep-ih-derm-ih-dis)

© 2007 nanobugs, inc
Staphylococcus epidermidis

1. **Diseases caused:** Colonization of artificial devices including indwelling catheter lines, joints, and valves.

2. **Pathogenesis:** Bacteria have polysaccharide outer layer (glycocalyx) that binds strongly to plastics. This also helps prevent penetration of antibiotics, making treatment more difficult.

3. **Immunity:** Normal, healthy, unbroken skin is the main protection. Infection occurs when extreme measures compromise skin and artificial devices are implanted.

4. **Epidemiology:** These bacteria are part of the NF of skin. Infection is usually hospital-acquired.

5. **Diagnosis:**
   - *a*) Gram-positive (purple) cocci in clumps;
   - *b*) usually white, non-hemolytic colonies on blood agar;
   - *c*) coagulase negative;
   - *d*) culture of blood or urine;
   - *e*) peripheral signs of endocarditis (Osler’s nodes, etc.).

6. **Control:** Proper sterile technique for insertion of artificial devices and maintenance of IV catheters.
ATB susceptibility and resistance

- **Penicillin**, staphylococal penicillin - oxacillin, **methicillin**, TTC, CMP, ERY, KANA, GEN, STM, Fluoroquinolones

- Quick development of resistance: **penicilinase**

- Now some strains are resistant to conventional ATB

- **Hospital strains** resistant to many ATB - including glycopeptides, vancomycin, teicoplanin

- **MRSA** - meticilin resistant St.aureus

- **VRSA** - vancomycin resistant St.aureus - resistance transferred from enterococci

- **St.epidermidis** - often meticilin resistant.
Methicillin-resistant Staphylococcus aureus
(meth-ih-sil-in ree-sist-ant staf-ih-low-cock-us aw-ree-us)
© 2007 nanobugs, inc

Staphylococcus aureus
(staf-ih-low-cock-us aw-ree-us)
© 2007 nanobugs, inc
Nosocomial problem

- MRSA
- multiple resistance
- VRSA
- resistance to antiseptics and disinfectants
- Mechanism of resistance acquiring:
  1) extrachromosomal plasmids, transposons or DNA insertion
  2) mutation in chromosomal genes
G+cocci, catalase negat.

- genus
- **Streptococcus:**
- **Enterococcus**
- Aerococcus, Gemella, Lactococcus, Leuconostoc, Pediococcus
  - seldom pathogenic for human
shape

G+ cocci in chains or diplococci
Streptococcus - taxonomy and properties

- **Taxonomy**
  - according to **haemolysis** on blood agar:
    - alfa - incomplete, alfa hemolytical, viridant streptococci
    - beta - complete, beta hemolytical streptococci
    - gama - without haemolysis
  - according to **serological groupes sc. Lancefield** A-H, K-U, not all streptococci have this groupe specific antigen of cell wall
- **Group A (GAS)**  S. pyogenes  *bacitracin* beta hemolysis
- **Grpup B**  S. agalactiae  *CAMP* beta hemolysis
- **Group C**  S. anginosus  beta, alfa
- **Grouzp D**  S. bovis  alfa, gama
-  -  S. pneumoniaiae  *optochin* + alfa
-  -  S. salivarius  *optochin* - alfa
Streptococci with alfa hemolysis

- *S. pneumoniae* optochin + alfa
- *S. salivarius* optochin - alfa
- Streptococcus beta hemolyticus

Streptococcus alfa hemolyticus
Streptococci with beta hemolysis

A  \( S.\ pyogenes \)  beta hemolysis (bacitracín+)

B  \( S.\ agalactiae \)  beta hemolysis (CAMP+)
group A streptococcus – GAS
Streptococcus pyogenes

- Forme long chains in microscopy if cultivated in the liquid broth media
- **Antigenic structure** - capsule - hyaluronic acid - identical with connective tissue - nonimmunogenic
- peptidoglycan
- **groupe and type specific antigens** -
  - most important **M** antigen - on the surface of fimbriae, responsible for virulence
  - T protein, R protein, F protein – structural proteins of the wall
- lipoteichoic acid
Factors of pathogenesis and immunity

- Capsule nonimmunogennic - inhibits phagocytosis
- **M protein** - protection against phagocytosis and cooperation with C’. Type 1,3,18 - invasive diseases., M3 a M18 - rheumatic fever
- F protein - receptor for fibronectin (matrix of eukaryotic cells - adhesin
- Lipoteichoic acid - ?
Toxins

- **Exotoxins** - *erytrogenic toxin* - termolabil, type A,B,C - hypersensitivity, endotoxic, cytotoxic, non specific mitogen for T and immunosuppressive for B lymphocytes activity, rash in scarlatina
- Dick´s, Schultz´s test
- **Streptolysin S** - oxygen stable, lysis of ery, nonimmunogenic
- **Streptolysin O** - reversibly inactivated by oxygen, immunogenic, antibodies against streptolysine O - **ASLO**, killing leukocytes
- **Streptokinase** - lysis of blood thrombus, spread of bacteria
- **DN-ase** - noncytolytic, depolymerisation of free DNA - in pus, declines viscosity of pus, spread
Streptococcal infections

- **Streptococcus pyogenes HSA**
  - pharyngitis - dif dg. from viral
  - scarlat fever - pharyngitis with exanthem - infectious strain gained by lysogenic conversion ability to produce erytrogenic toxin (rash with desquamation of skin, circumoral whiteness, strawberry thongue)
  - toxic shock syndrome - cellulitis, necrotising fasciitis, hypotension, erytrodermia, multiorgan failure, bakteraemia, M1, M3, M18 - types
  - erysipelas, pyodermia - purulent skin diseases
Pharyngitis - streptococcal
Erysipelas
Flesh eating streptococcus

Toxic shock syndrome – celulitis and necrotising faciitis - superantigen
Bacteria

Streptococcus pyogenes
(strep-toe-cock-us pie-ah-jen-eez)

© 2007 nanobugs, inc
**Streptococcus pyogenes**

1. **Diseases caused:** Group A Streptococcus (GAS) can cause:  
   a) pharyngitis ("strep throat");  
   b) a spectrum of skin infections including:  
      i) impetigo,  
      ii) erysipelas, and  
      iii) necrotizing fasciitis (flesh-eating disease);  
   c) toxin-mediated diseases like scarlet fever and toxic shock-like syndrome (TSS);  
   d) diseases associated with sequelae of infection, e.g., rheumatic fever and acute glomerulonephritis.

2. **Pathogenesis:** Bacteria bind to cells by filaments of M protein and lipoteichoic acid.  
   A number of exoenzymes (e.g., hyaluronidase) can facilitate spread of the organisms in tissue. Erythrogenic toxins can act as superantigens in TSS.

3. **Immunity:** Antibody to M protein facilitates phagocytosis, but protection is limited to the specific type of M protein recognized by the antibody. Neutralizing antibody can also prevent some toxin-mediated diseases.

4. **Epidemiology:** Bacteria are spread by aerosol and fomites. Asymptomatic carriers provide a reservoir for further spread. Both respiratory and skin infections are most common in children.

5. **Diagnosis:**  
   a) Gram stain shows purple cocci in chains.  
   b) Usually white, beta-hemolytic bacitracin-sensitive colonies on blood agar.  
   c) Group A by Lancefield; M protein subtyping; presence of antibody to streptolysin O.  
   d) Rash in scarlet fever, TSS.  
   e) Typical lesions in impetigo (see 1bi); kidney failure in acute glomerulonephritis; heart murmur in rheumatic fever.

6. **Control:** Penicillin is often given for pharyngitis and skin infections to prevent more serious sequelae.

© ASM Press, 2003
Streptococcal infections- late sequelae

- **Febris rheumatica** - rheumatic fever - nonpurulent streptococcal disease - inflammatory disease of heart, joints, vessels and submucosis - **autoimmune disease** related to some serotypes of M protein (types specific antigen, factor of patogenity, numbered M18, M3….) RF sequelae present only after respiratory infections. Not after skin infection

- **Poststreptococcal acute glomerulonefritis** - specific nefritogen strains, after skin and respiratory infections., immunocomplexes deposition
Diagnosis - laboratory

- **Microscopy** - gram stain, **G+ cocci in chains**, not colonising skin, in skin swab only if together with leu is significant for disease. Of any value from URT sample
- **Detection of antigens** - directly from clinical material (also from URT), detection of groupe specific antigens of cell wall, specific, not sensitive - negative tests should be confirmed by cultivation
- **Cultivation, Identification** - **hemolysis**, bacitracin test
- **Antibodies** - **ASLO** - confirmation of preliminary streptococcal infection in patients with RF a GNF (+ anti **Dnase** antibodies
Streptococcus agalactiae, HSB, GBS

• **newborn infections**
  - meningitis, pneumonia, puerperal - postpartum sepsis
  /colonisation of URT, GIT and vagina - danger of
  contamination of newborn during prolonged and preterm
  labour, importance of maternal immunity/

• G+ cocci in chains,

• beta hemolysis: dif dg from HSA - CAMP test - strengthened
  hemolysis of *Staf. aureus* - butterfly
Streptococcus agalactiae, HSB, GBS

- Structure - polysaccharid capsule,
  - cell wall - peptidoglycan with type and groupe specific antigens and lipoteichoic acid,
  - cell membran

- Antigenic structure - antibody against capsule antigens are protective - diseases in newborns

- Enzymes - Dnase, hyase, protease, hemolysin – responsible for the spread of infection
1. **Diseases caused**: Group B *Streptococcus* (GBS) causes
   a) neonatal meningitis and b) infections of mothers during childbirth.

2. **Pathogenesis**: GBS has a polysaccharide capsule of sialic acid that contributes to invasiveness into the CSF. Mothers carrying the organisms in their vaginas may pass them on to their babies during childbirth, or become more seriously infected themselves.

3. **Immunity**: Maternal antibody to GBS organisms can be protective to the neonate by enhancing opsonization of organisms.

4. **Epidemiology**: GBS can be found as NF of vagina. Infection of the neonate can occur during vaginal birth.

5. **Diagnosis**: a) Gram-positive cocci in i) chains grown from blood or ii) clusters in CSF; b) beta hemolytic on blood agar; c) Lancefield group B; d) hippurate hydrolysis.

6. **Control**: Certain circumstances (e.g., premature or prolonged rupture of membranes) warrant antibiotic treatment of pregnant women to prevent infection in the newborn.
Other streptococci

- Beta hemolytical group C,F,G
  - URT and skin infections never late complications
- **Viridant - alfa and nonhemolytical streptococci**
  - *Streptococcus salivarius*, **viridans** - bacteremia, subacute endokarditis, caries of teeth, intraabdominal purulent infections
- Important condition for development of disease is the preliminary damage of tissue (tooth, valve)
- **Carries** - formation of dextran from glucose
  - *Streptococcus pneumoniae* – viridant (lecture 2)
Bacteria

Streptococcus mutans

(strep-toe-cock-us mew-tanz)

© 2007 nanobugs, inc
Streptococcus mutans

1. **Diseases caused:** Common in the mouth where they can
   a) lead to dental caries or b) cause endocarditis if they get
   into the bloodstream of individuals with
   damaged heart valves (e.g., during dental
   extraction). Conjunctival petechiae, a sign
   of bacterial endocarditis, is shown.

2. **Pathogenesis:** Bacteria form
   polysaccharide coats (glycocalyx) that
   allow them to stick to teeth. They
   produce acid from sugars in the saliva
   and can lead to erosion of tooth
   enamel and dentin. Glycocalyx also
   promotes adhesion to damaged heart
   valves if the bacteria gain access to
   the bloodstream.

3. **Immunity:** Normal body defenses are
   usually adequate to prevent disease.

4. **Epidemiology:** NF of the oral cavity.

5. **Diagnosis:** a) Gram-positive cocci in
   chains; b) alpha-hemolytic on blood agar; c) Lancefield
   negative; d) optochin resistant and bile resistant.

6. **Control:** Good oral hygiene including regular dental
   checkups. Prophylactic antibiotics can be given for major
   dental work on people with damaged heart valves.
**ATB susceptibility**

- **Str. pyogenes** - 100% susceptible for PNC, (in allergies macrolides ERY)
- **Str. agalactiae** - good susceptibility for PNC, some strains tolerate - inhibition, not resistance, resistance to ERY, TTC
- Str. salivarius - susceptible for PNC,
- **Str. pneumoniae** - PNC, TTC, CMP, CEF., resistance for PNC - multiresistence - decline of affinity of ATB to PBP
Enterococcus

- G+ cocci, colonising in great quantities intestine and colon, able to grow in the presence of bile - dif. dg. - esculin media
- E. faecalis
- E. faecium
- Urine tract infections, intraabdominal abscesses, bakteraeaemia
- ATB nonbactericidal for enterococcie, resitence - synergic therapia: aminoglykosides + cell wall acting
- Resistance plasmid transferable - (on staphylococci too - vancomycine)
Bacteria

Vancomycin-resistant Enterococcus

(van-koh-my-sin ree-sist-ant en-ter-oh-cock-us)

© 2007 nanobugs, inc
**Enterococci**

1. **Diseases caused:** a) Urinary tract infections, b) abdominal or pelvic wound infections; c) bacteremia.

2. **Pathogenesis:** Organisms are found throughout the environment and are common residents of the GI flora.

3. **Immunity:** The barrier function of mucus in the GI tract and the flushing mechanism of urine in the urinary tract provide adequate protection in normal individuals. Organisms are not particularly invasive but will thrive in areas outside the gut if given the opportunity.

4. **Epidemiology:** Part of NF of GI tract. Infections and disease are usually hospital-acquired. *Enterococcus faecalis* is the most common clinical isolate.

5. **Diagnosis:** a) Gram-positive, facultative anaerobic cocci singly, in pairs, or in short chains. b) Growth on i) special media with bile and esculin or ii) BAP. c) Catalase negative, salt tolerant (6.5% NaCl), and growth at 45°C. d) Most enterococci possess the Lancefield group D cell wall antigen (these organisms were formerly categorized with the streptococci).

6. **Control:** Antibiotic therapy (especially in combination) is warranted for immunocompromised individuals. Isolation of vancomycin-resistant enterococci (VRE) is increasing, a worrisome trend.

© ASM Press, 2003