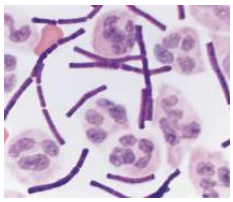
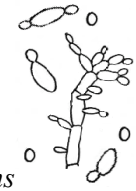
Experiment	**Microbiota of the nose**
Advisors	Prof. Dr. Brigitte Berger-Bächi, bberger@immv.unizh.ch , Department of Medical Microbiology, University of Zürich, Gloriastrasse 32, 8006 Zürich, Tel 044 634'26'50 Fax 044 634'49'06
Reading	Chapters in BBOM 10th: BBOM : Madigan M.T., J.M. Martinko and J. Parker: "Brock - Biology of Microorganisms", 10th Edition (2003), Prentice Hall. **21.1-21.8**, 21.9-21.14, **26.9**
Objectives	To analyze the normal flora of the nose on selective media and identify staphylococci by biochemical tests.
Background	**Normal bacterial flora.** Body surfaces such as skin and mucous membranes are colonized by large numbers of microorganisms that are highly adapted to their particular environment. These organisms, also called commensals, constitute the body's normal flora. Positive effects of commensals are the continuous stimulation of the immune system of the host and protection from pathogens. When members of the normal flora from one anatomical site gain access to an otherwise sterile location, then infection may result. Sometimes these are relatively trivial, but others may be life-threatening. Such infections are known as endogenous. Predisposition for endogenous infections is destruction of the natural barriers against infection, such as lesions of the skin, or reduced immunity, or carriage of indwelling devices. Figure 1. Some bacteria of the skin and upper respiratory tract. Characteristic shape and staining properties. Blue, Gram-positive; red, Gram-negative. **Skin** • Coagulase-negative staphylococci  • Proionibacteria  • Corynebacteria  • Yeast, fungi, especially *Candida albicans* 

Nasopharynx

- α-hemolysing streptococci (i.e.: *Strept. pneumoniae*)

- γ-hemolysing streptococci (i.e..: *Strept. mutans*)

- Staphylococci

- Neisseria

Identification of bacteria. The bacterial species can be determined according to their morphology and shape, Gram-staining properties, phenotypic appearance of the colonies on different media, biochemical characteristics, and by DNA sequence analysis of the 16s RNA DNA sequence. Most but not all medically important bacteria can be grown on growth-media.

Growth media can be classified according to their function into different classes:

General growth media: Non-selective media that allow growth of most bacteria. Example: blood plates

Selective media: Media that allow growth of specific bacterial groups, and inhibit growth of other bacteria. Example: MacConkey plates allow growth of Gram-negative bacteria, but inhibit growth of Gram-positive bacteria.

Differential media: Some bacteria need specific factors for growth such as addition of serum, vitamins, and other substances (i.e. *Haemophilus influenzae* needs NAD and hemin).

One plate may have both: differential- and selective properties.

Plates	**Selection**	**Selective properties**
Sheep blood agar	No	Hemolysis
MacConkey-agar	Bile-salt and cristall-violett inhibit Gram-positive bacteria	Lactose und indicator (neutral red): degradation of lactose produces acid , the pH indicator turns red
CNA-agar	Nalidixic acid and colistin inhibit Gram-negative bacteria	Hemolysis

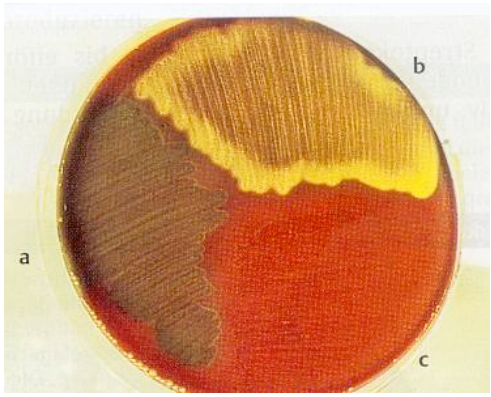
Hemolysins are virulence factors. The result of the hemolysin action can be seen on blood plates.

α-hemolysis: Bacteria producing α-hemolysin are surrounded by a green hemolysis zone. There is partial hemolysis of the erythrocyte membrane and conversion of hemoglobin into met- and sulf-hemoglobin.

β-hemolysis: Bacteria producing β-hemolysin are surrounded by a clear transparent lysis zone. It is due to the complete hemolysis of all blood cells and complete degradation of hemoglobin to colourless products.

γ-hemolysis: This term describes the absence of any macroscopic visible hemolysis.

Fig. 2. Example of different hemolysis



The nose is an important reservoir of *Staphylococcus aureus*. 30 % of the healthy population can be transient- or permanent carrier of staphylococci. Nasal carriage of *S. aureus* is a well-defined risk factor for subsequent infection in nearly all categories of hospitalized patients. Persons at risk for staphylococcal infection are immunocompromised patients, carriers of indwelling devices such a catheters and foreign bodies, intravenous drug abusers, and persons with skin lesions or undergoing surgery.

We will analyze the micro flora of the nose by fractionating the bacteria on different media and search for colonies that look like staphylococci.

Characteristics of *S. aureus*:
- white to yellow colonies
- α-hemolytic
- catalase-positive
- coagulase-positive

Precautions when working with *S. aureus*

Staphylococci can cause a variety of diseases, including superficial infections such as wound infects, furuncles, carbuncles, to more severe- and life-threatening diseases such as endocarditis, pneumonia, and osteomyelitis. However, many healthy people are carriers of *Staphylococcus aureus.* Staphylococci can colonize the nose, throat, and humid areas such as axilla and groin. In most cases resident staphylococci do not cause disease. Nevertheless we have to be careful with cultures of S. *aureus*.

Literature

www. Links

Normal flora: http://gsbs.utmb.edu/microbook/ch006.htm

Practical Work

First week:
Each student will obtain:
a sheep-blood plate
a CNA plate
a McConkey plate
Write your name on the back of each plate.

Take a sterile cotton tip, dip it into sterile NaCl solution, and insert it about 1-2 cm into your nose and rub the interior nare with a light turning movement. Roll the cotton tip gently over about 1/3 of the plate (1st fraction). Fractionate it into a 2nd and 3rd fraction. Incubate the plates at 37 °C.

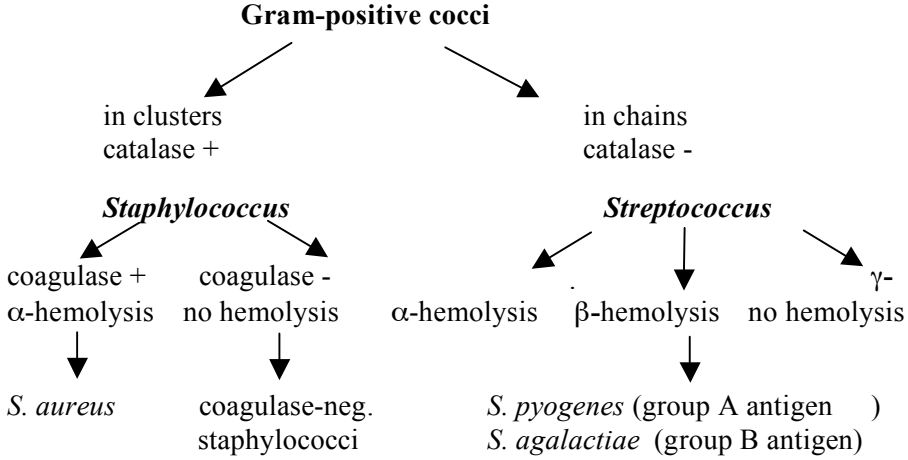
Next week:
describe the colony morphology
color
amount of bacteria
hemolysis type

	Morphology of the colonies	**Hemolysis**
sheep blood		
CNA		
McConkey		

Biochemical characterisation of the isolates.

Each group will receive one *S. aureus* and one *Staphylococcus epidermidis* strain. If you have own colonies from the nose that look like staphylococci and show α-hemolysis on the sheep blood plate, analyse them as well.

Diagram for the identification of Gram-positive cocci



Catalase
The catalase degrades the toxic hydrogen peroxide into water and oxygen.

$$2\ H_2O_2 \rightarrow 2\ H_2O + O_2$$

The catalase is a defence mechanism of bacteria against the peroxide that is produced by certain immune cells of the host to kill bacterial invaders (see BBOM Fig. 6.29)

Mix a loop full of bacteria with a drop of 3 % H_2O_2 on a glass slide. The immediate appearance of bubbles is indicative of the presence of catalase.

Tube coagulase test
The coagulase is an enzyme that causes clotting of blood plasma.

Mix a loop full of bacteria with plasma, incubate for several hours at 37 °C and look for fibrin clotting. The pathogenic *S. aureus* produces coagulase that is excreted into the medium, while coagulase-negative staphylococci produce no coagulase.

3c. Agglutination test for *S. aureus* (Staphaurex Plus):

The surface-bound clumping factor, which is specific for *S. aureus*, causes an agglutination of the plasma, similarly as the coagulase. The Staphaurex-Plus slide agglutination kit recognizes a somatic and a capsular antigen, in addition to clumping factor and staphylococcal protein A, and serves to distinguish between the clumping factor-positive *S. aureus* and clumping factor-negative coagulase-negative staphylococci. The yellow latex particles are coated with fibrinogen (a ligand of the clumping factor) and IgG antibodies that react with the type 5 capsule, which is often produced by clinical *S. aureus* isolates. By mixing *S. aureus* with a drop of the latex solution, a rapid agglutination of the particles occurs by the reaction of

- fibrinogen with the clumping-factor
- IgG with the staphylococcal capsule.

The reagents used should be at room temperature. Mix them well before use.
For each test add one drop of test-latex in a circle-, and one drop of control latex (not coated latex) in the second circle of the reaction cardboard.

Mix a loopful of the same bacteria in the drop of the control- and test-latex. *S. aureus* should be suspended homogeneously in the control-latex and should produce a precipitate in the test-latex.

Results

Reaction	**Strain 1**	**Strain 2**	**Strain 3**
Catalase			
Coagulase			
Clumping factor			

Material and Experimental Protocols	Material and equipment needed: Differential and selective plates, sterile cotton tips and loops, glass slides, H_2O_2 solution, tube plasma, Staphaurex-Plus kit Procedures employed: sampling of bacteria of the nose, fractionation of bacteria on plates, biochemical tests for differentiation
Laboratory Rules & Precautions	**Laboratory rules:** - Do not drink or eat in the room. - Wear a lab coat (obligatory). - Persons with skin lesions wear gloves (gloves are provided). - Disinfect the table with 70 % ethanol if you spill organisms. - Clean and disinfect the table after the experiments with 70 % ethanol. - Disinfect your hands before leaving the course.
Goals & Experiences gained	Identification of bacteria on selective growth media biochemical characterization of staphylococci
Timing	1st day, 30 min; 2nd day 45 min
Reporting	Results, Discussion, Explanations
Questions to be answered	How can you distinguish between *S. aureus* and *S. epidermidis*? Which strain is more pathogenic: *S. aureus* or *S. epidermidis*? How can you determine experimentally if a strain produces hemolysins? What are risk factors for staphylococcal infections? How do staphylococci spread from person to person? What precautions would you suggest to prevent spreading of staphylococci between patients? How many out of all students participating in the practicum are carriers of S. *aureus*?