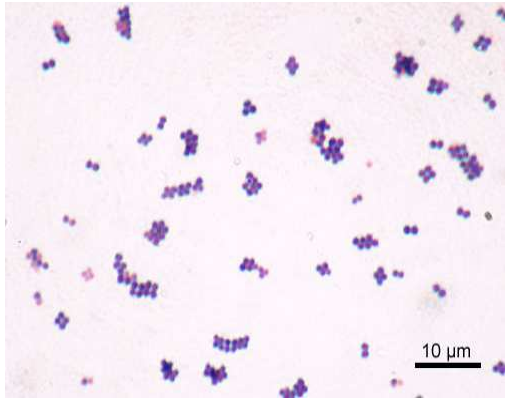
Staphylococcus

Staphylococcus species



- Gram positive facultative anaerobes (anaerobic – *S. saccharolyticus*, *S. aureus* subsp. *anaerobius*), non-motile, non-spore forming, unencapsulated, arranged in grape-like clusters
- Staphylé – grapes
- Small-colony variants – *S. aureus*
  - Non-pigmented, non-hemolytic, fail to express number of virulence factors
- Tolerate high concentration of salt (10%)

Staphylococcus species (~70 species)

- **1. Coagulase positive**: ***S. aureus***

+ 8 other species (*S. intermedius*, *S. lugdunensis*, …..)

- **2. Coagulase negative:**

S. epidermidis
S. saprophyticus
S. haemolyticus
S. hominis
S. warneri
and many others.....

Staphylococus aureus - epidemiology

- Widespread in nature

- present on the skin and mucous membranes (colonization) in of mammals and birds

- Symbiotic relationship with host BUT after disruption of natural barriers become pathogenic - penetrates into tissues and causes:
  purulent skin infections, abscesses in organs, osteomyelitis

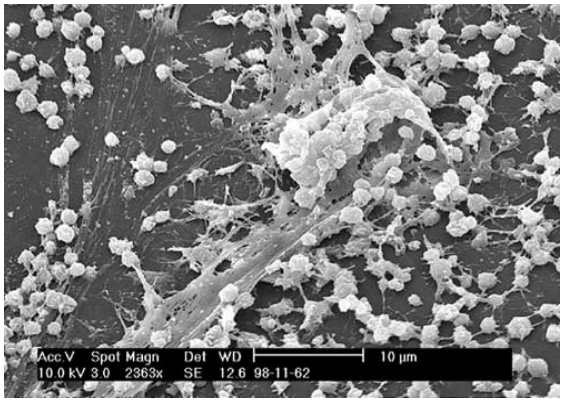
- *S. aureus* – major species in human - about 30% of healthy individuals is colonised
  - *S. aureus* – anterior nares
  - long-term, transient colonisation

Staphylococci – can produce biofilm

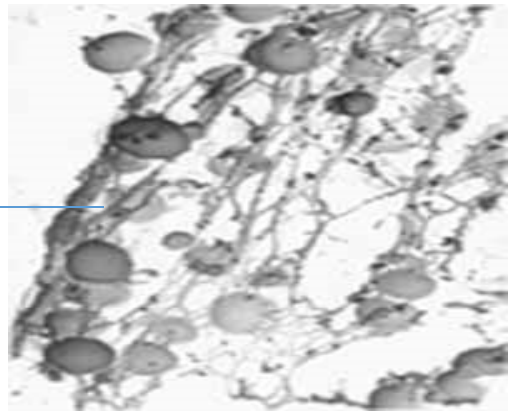
- **A biofilm is a community of microbes living in the extracellular matrix.**

- **Bacteria adhere to the living and inert surfaces**
- **biofilm has a complex structure**

- ***Staphylococcus epidermidis*** **anchored in extracellular slime (glycocalyx) adhering to the surface of the catheter**



Micrograph of staphylococcal biofilm on surface of surgical suture



Staphylococci – can produce biofilm

- Staphylococci (*S. epidermidis* with its adhesins adhere to mucosal surfaces or objects (plastic, catheters, catheters, etc.)
- Formation of extracellular polymers by chemical signals – information carried on genes
- Microcolonies are formed with the slime that differentiates into the biofilm. Gradually, the separation of the bacteria and the spread of colonization, also suitable conditions for the transfer of information, plasmids - Catheter sepsis.
- Staphylococci are protected from adverse environmental conditions, antibiotics and immune mechanisms.

Staphylococcus aureus

- Slime layer - adhesion

- *Staphylococcus aureus* cell wall contains **peptidoglycan**- (murein) **teichoic acid** + **surface adhesion proteins**
- Staphylococcal protein A reacts non-specifically with the Fc fragment of immunoglobulins and thus protects *Staphylococcus* from phagocytosis (not opsonization)
  - Anticomplementary effect

Staphylococcus aureus - pathogenesis

Evade immunity, colonise skin/mucous membranes, produce enzymes/toxins

- **Cytotoxins** – toxic for erythrocytes, leukocytes, fibroblasts
  - alpha toxin (betahemolysis)
  - beta toxin (sfingomyelinase C – hot-cold hemolysis – effect on erythrocytes is more profound at low temperature)
  - PVL toxin (pore-forming leukocidin)
- **Exfoliative toxins** – serine proteases
- **Enterotoxins (A - X)**
- **Toxic shock syndrome toxin-1**

- **Enzymes**
  - Coagulase (free coagulase – conversion of finrinogen to fibrin)
  - Hyaluronidase (facilitate bacterial spread)
  - Fibrinolysin (dissolve fibrin clots)
  - Lipases
  - nucleases

S. aureus – clinical significance

- Human colonizer

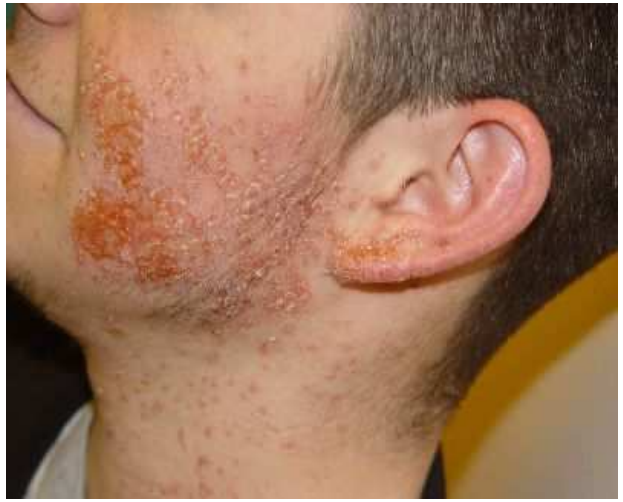
- **Community-acquired infections**
- **Hospital-acquired infections**

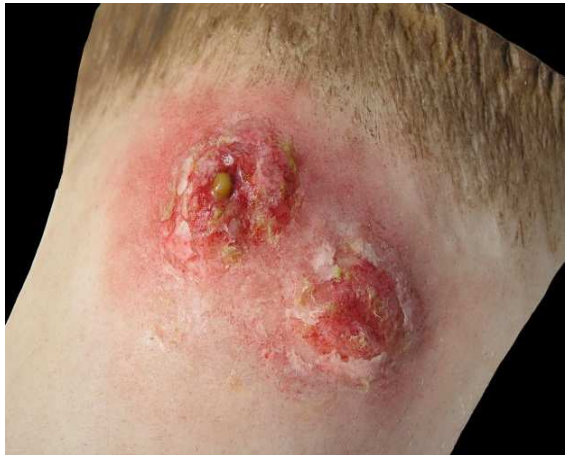
1. Toxin-mediated diseases (food poisoning, scalded skin syndrome, toxic shock syndrome)
2. Infections of the skin and tissue/organs (furuncles, cellulitis, impetigo, joint/bone infections, heart valve..)
3. Infections of lung and urinary tract

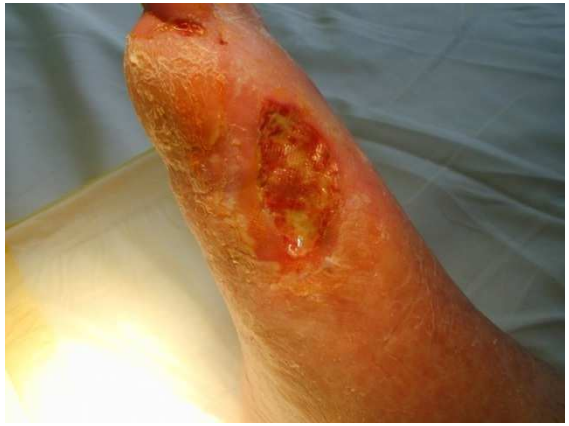
Hospital-acquired infections

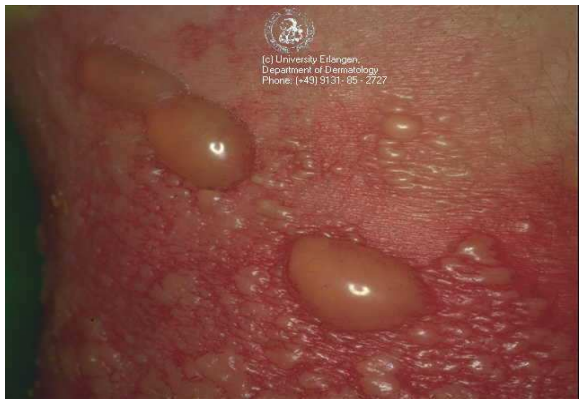
surgical wound infections, ventilator-associated pneumonia, catheter-related bacteremia, infections of prosthetic materiál (shunts, vascular grafts, prosthetic joints…)

Staphylococcal impetigo, carbuncle, infection, chronic wounds, septic blisters on the skin.









Toxin-mediated diseases (food poisoning, scalded skin syndrome, toxic shock syndrome)

- Symptoms are results of toxin activity
- From self limiting to life threatening
- Some toxins (toxic shock syndrome toxin – TSST-1, enterotoxins) act as superantigens

Staphylococci - superantigens

- Superantigens are antigens that do not require for its reaction with the immune system processing in antigen presenting cells. Bind directly to the T-cell receptor (TCR) and MHC II macrophage.

- Superantigens cause rapid polyclonal activation of lymphocytes, induce cytotoxic activity, overproduction of cytokines and react with the antigen receptor on the cell membrane.

- The number of T cells that can directly react with superantigens, is considerably higher than that with conventional antigens

Toxic shock syndrome toxin (TSST)

- a superantigen, rapid activation of the immune system, increases endothelial permeability, multiorgan failure
- uniform antigen protein
- synthesis is controlled by chromosomal genes
- acts as a superantigen, joins the MHC-II binding molecules (SS2 domains) macrophages and TCR (VSS domains) lymphocytes
- overproduction of cytokines, symptoms of shock syndrome
- mitogenic effects on T lymphocytes
- pyrogenic and also with direct action on the thermoregulatory center in the hypothalamus, inhibiting the synthesis of macromolecules endothelial function and damaging a number of internal organs
- Ireversibly affects renal tubular epithelium
- there is increased sensitivity of the host organism to the effects of LPS G-bacteria (Gram negative)

Toxic shock syndrome

- Diasease caused by strains producing superantigens - TSST-1 toxin or enterotoxin B or C
- High-absorbancy menstrual tampons
- Non-menstrual cases – postoperative colonisation with *S. aureus* producing superantigen
- Clinical symptoms
  - High fever, hypotension, erythematious rash (desquamation after 1 – 2 weeks), multiple organs involved – disseminated intravascular coagulation
- 5 % mortality rate

S. aureus- enterotoxins A-X

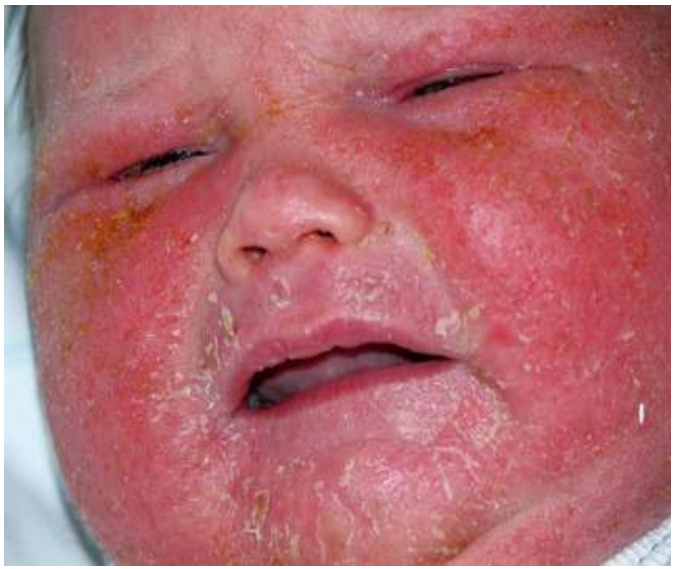
- thermostable, vomiting and diarrhea
- basic proteins resistant to proteolytic enzymes GIT
- resist to boiling for 30 minutes
- synthesis is controlled chromosomally, eventually carried on plasmids
- more than 20 types of these toxins – most common A (C, and D)
- food poisoning, enterotoxicosis
- may cause symptoms of toxic shock syndrome like TSST-1 (enteroxin B or C)
- toxins stimulate visceral receptors in the intestinal mucosa and thus cause accelerating peristalsis and diarrhea, irritation of vegetative nervous system leads to a secondary stimulation of the emetic center in CNS

Food poisoning

- incubation period 2 – 6 hours
- Clinical symptoms
  - Nausea
  - Vomiting
  - watery diarrhea
  - headache
- Source – contaminated milk products, meat
  - Human carriers
- Self-limiting disease – resolved within 24 h

Staphylococcal scalded skin syndrome - SSSS

- Primarily affects neonates and young children
- ***S. aureus* exfoliative toxins** (dissolve proteins in skin)
- toxic epidermolysis (Ritter syndrome)
- Localised form – Bullous impetigo
- Large cutaneous blisters, gradually cracking, peeling skin
- Only top layer of epidermis is involved
- fluid in the blisters is sterile (toxic syndrome)
- Resolved within 7 – 10 days (antibodies against toxin)
- Secondary bacterial superinfection



Staphylococcal infections

Cutaneous

- Impetigo, folliculitis, furuncles, carbuncles
- Young adults
- Impetigo – secondary infection of *S. pyogenes* infection

Wound infection (foreign body)

- Community-acquired meticilin resistant *S. aureus* (MRSA)

Systemic infections

- Bacteremia – focus in lung, bones…
- Endocarditis
- Pneumonia
  - Aspiration pneumonia
  - Necrotizing pneumonia
    - Associated with production of PVL toxin
    - Often associated with CA-MRSA

Panton Valentine leukocidin PVL toxin

- Staphyloccoccal cytotoxin
- Encoded by LukS-PV/LukF-P genes
- Produced by strains that cause skin infections, and pneumonia
- Community-acquired MRSA strains
- PVL creates pores in neutrophils and macrophages that leads to lysis and the patient's inability to resist the infection

Coagulase negative staphylococci

- ***Staphylococcus epidermidis, S. hominis, S. haemolyticus*** and about forty other species - major part of the normal bacterial flora of the skin, also present in the animal population

- Evaluation and interpretation of the presence of these staphylococci on skin x blood culture, catheters, ets.

- Hospital-acquired infections – use of prosthetic and indwelling devices, increasing number of imunocompromised patients

- Catheter-related bacteremia, endokarditis (native and prosthetic valve), wound infections, bone and joint infections, peritonitis, eye infections, etc.

- ***S. saprophyticus*** – cause of urinary tract infections (young, sexually active females), non-gonococcal urethritis, prostatis

Coagulase negative staphylococci

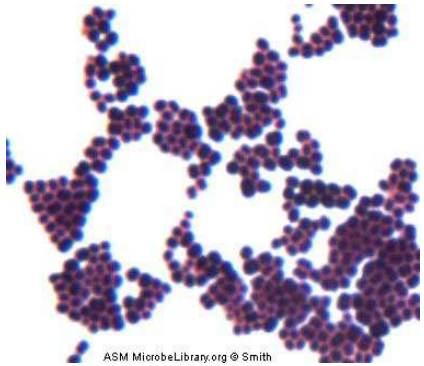
- heterogeneous group
- some (*S. haemolyticus*) even produce hemolysins BUT in general – they do not produce number of virulence factors as *S. aureus*
- They have the ability to grow in the biofilm, adhere well to the plastics - catheter sepsis!

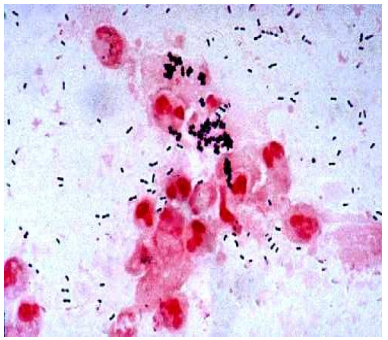
Staphylococci - laboratory diagnostics

- Microscopy: sputum, TAS, BAL, pus ....
- G + cocci (about 1µm) in clusters, grapes - unencapsulated
- Cultivation on blood agar and other cult. media
- Hemolysins – the species with ß hemolysis:

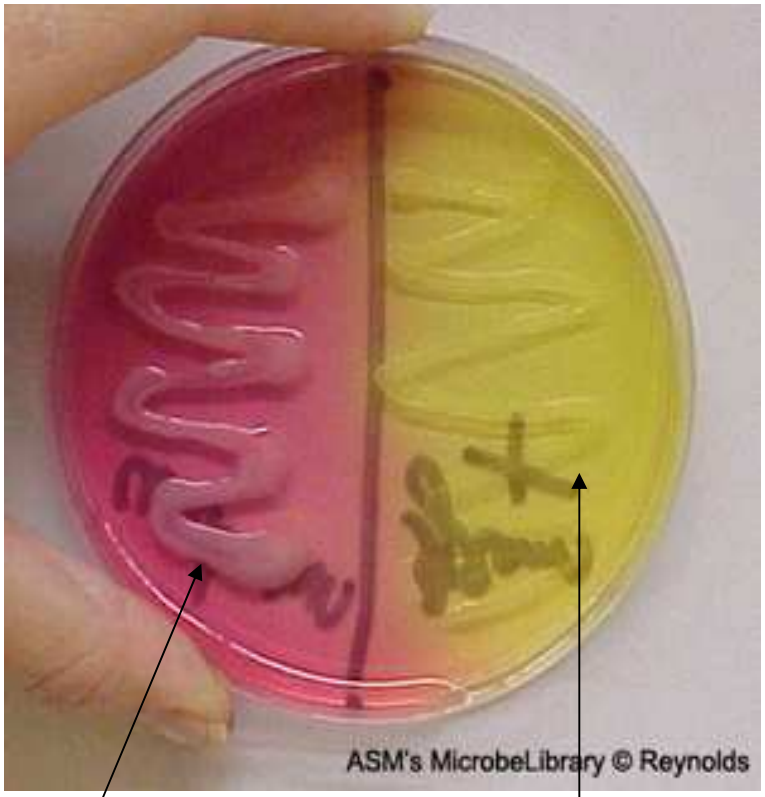
  S. aureus, S. haemolyticus

- *Staphylococcus aureus* produce yellow ("golden = aureus") carotenoid pigment
- Tolerate NaCl - grow on agar media with higher content of salt = "salty agar"





Staphylococci - laboratory diagnostics



S. epidermidis

S. aureus, manitol +

Staphylococci tolerate high salt content

Salty mannitol agar to differentiate ***S. aureus***

(ferments mannitol, therefore changing color from red to yellow)

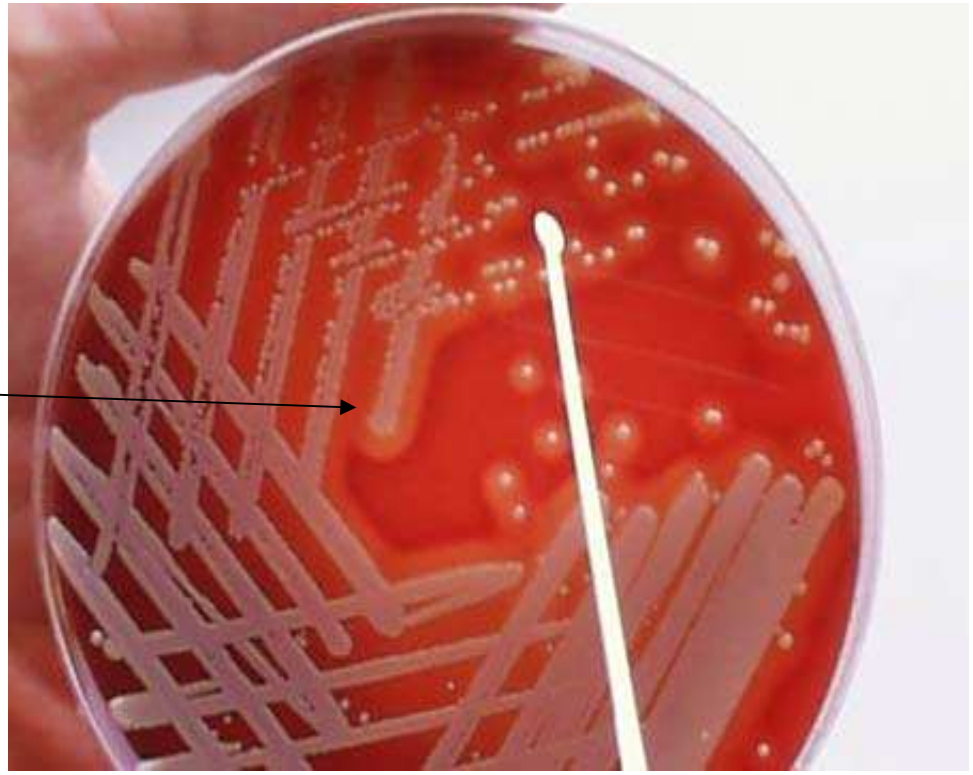
from ***S. epidermidis***

(does not ferment manitol, left – red)

Staphylococcus aureus

Cultivation of *S. aureus* on the blood agar

Around yellowish colonies ("golden pigment"), is a zone of beta hemolysis – complete brightening of blood agar (dissolution of erythrocytes)



Staphylococcus epidermidis

Cultivation

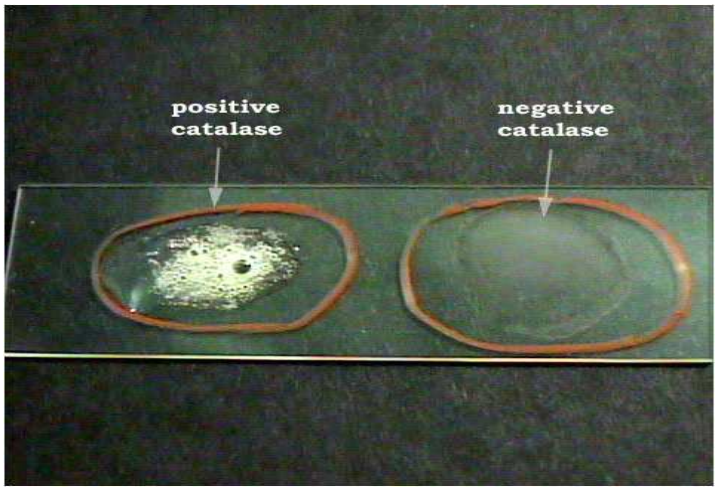
S. epidermidis on blood agar

Colonies are white

The hemolysis around colonies is not present

Staphylococci – laboratory diagnostics

- **catalase test**
  - distinguish staphylococci from streptococci (both gram positive coccus)
  - reaction with hydrogen peroxide – bubbles formation



positive : Genus *Staphylococcus*

Negative: Streptococci

Identification of staphylococci

- Coagulase test
  - Free coagulase of *S. aureus* (not bound to the cell wall) precipitates the plasma fibrinogen into fibrin and thus delimits inflammatory process (abscess formation)

- Slide agglutination test
  - On the surface of *Staphylococcus aureus* there is „bound“ coagulase (clumping factor) which act as an adhesin for fibrinogen
  - Commercial kits detect more surface protein adhesins (clumping factor, protein A, …)

Laboratory demonstration of free coagulase

- Tube method for differentiation of coagulase +, positive staphylococci (*Staphylococcus aureus*) from other staphylococci (coagulase -)
  - insert bacteriological loop with few colonies into the tube with rabbit plasma

- coagulation of plasma = positivity

***Staphylococcus aureus* coagulase positive**

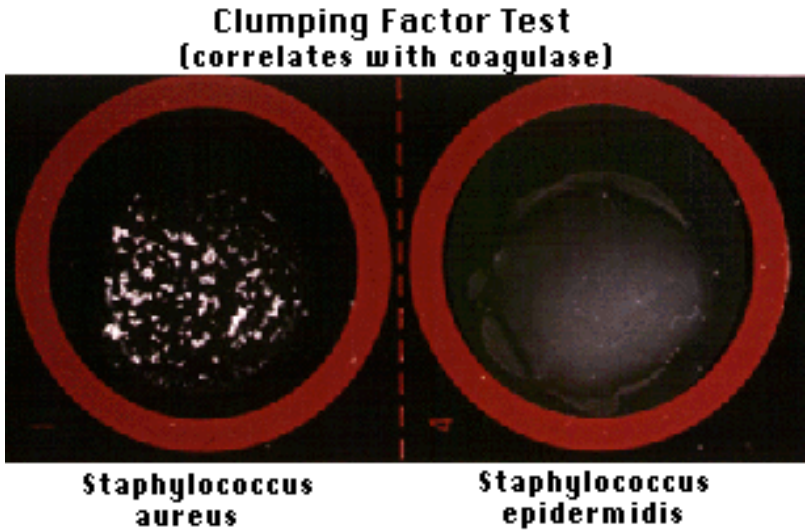
Laboratory demonstration of coagulase bound to the cell wall

(slide rapid method)

Test determines the formation of bound coagulase and presence of a protein A in the cell wall of staphylococci

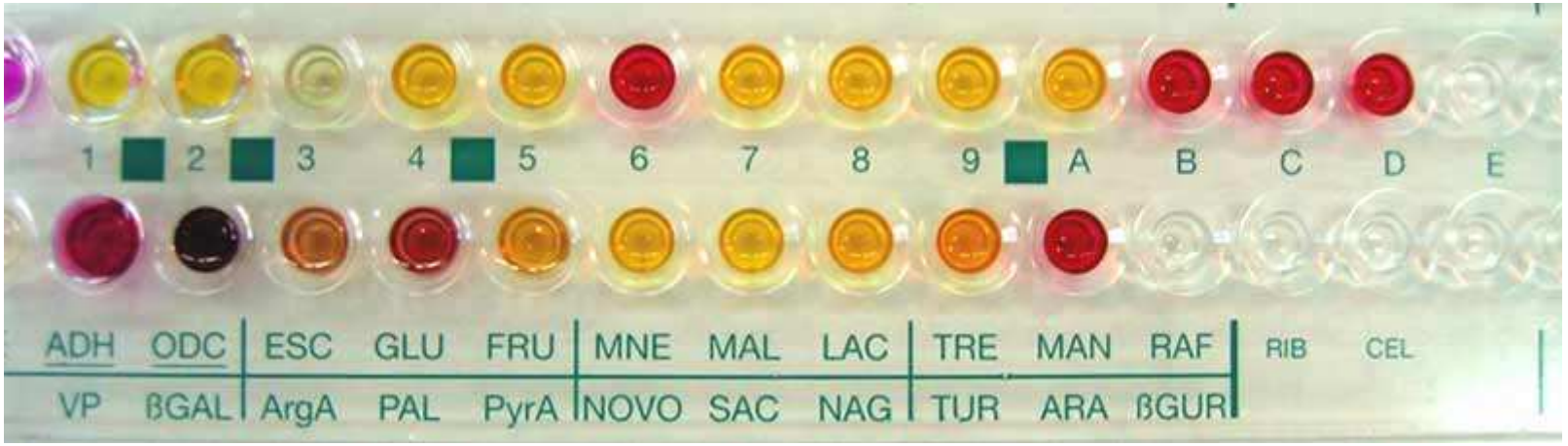
***S. aureus* – positive**

***S. epidermidis* - negative**



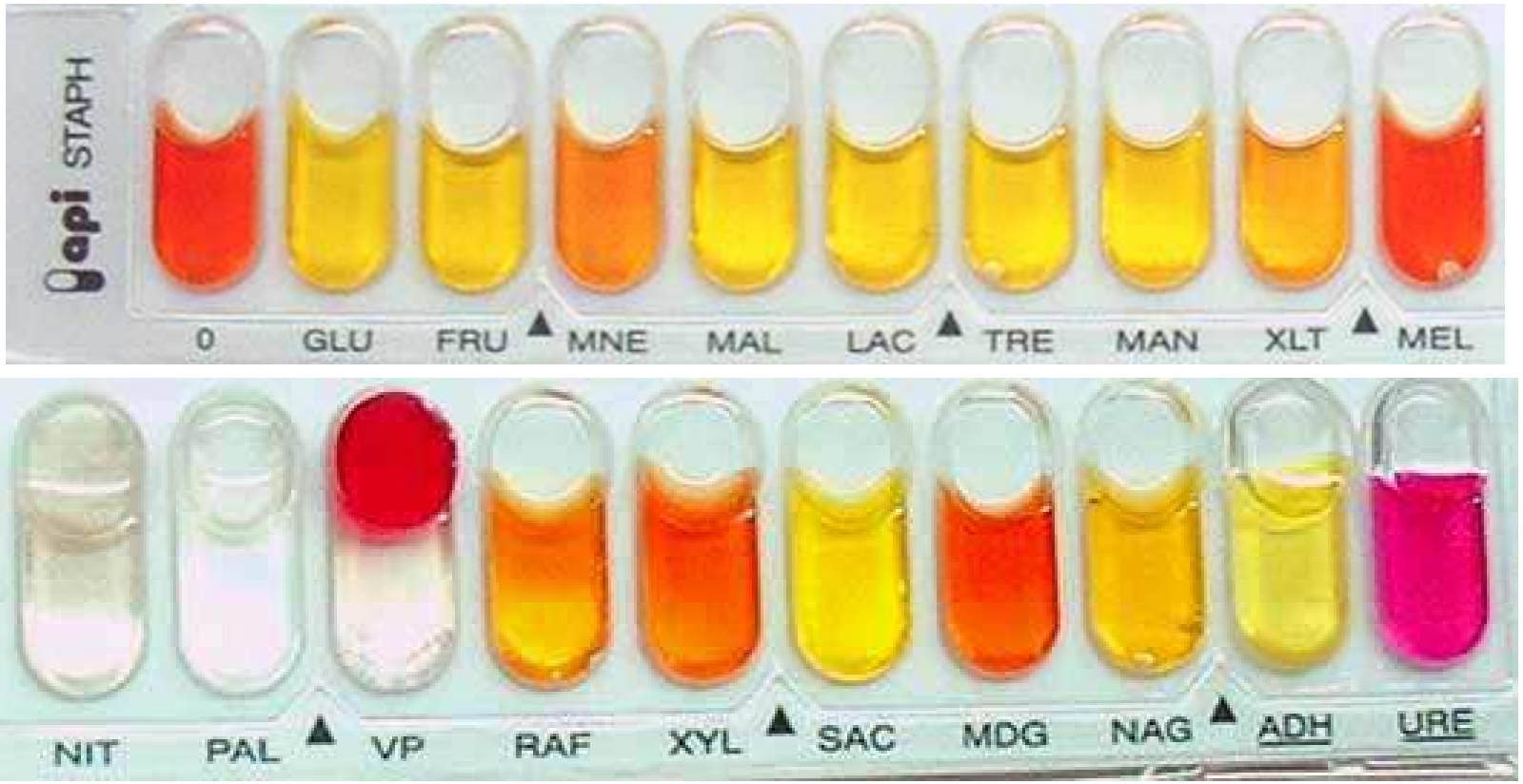
Api Staph - Detection of biochemical reactions, staphylococci are biochemically active, produce enzymes and toxins

- the combination of different biochemical tests - positive reaction is shown by changing the color of the contents of the reaction well (microtube) - photometric evaluation



Test API-Staph (determination of staphylococci by biochemical properties - fermentation of saccharides, reactions with different chemicals, etc.).

- test is evaluated visually by the change of color



Treatment of staphylococcal infections

- Localized infection – surgery (incision, drainage)

- 90% of staphylococci resistant to penicillin G due to production of betalactamase (penicillinase) cleaving betalactam structure of penG

- Isoxazolylpenicillins – semisynthetic, resistant to betalactamase
  - Methicillin, **oxacillin**, **cloxacillin**, **flucloxacillin**

- Resistance to isoxasolylpenicillins (*mecA*/*mecC* genes – MRSA)
  - Glycopeptides – **vancomycin**

  - Alternatively
    - **trimethoprim/sulfamethoxazole** (if susceptible for po therapy)
    - **ceftarolin** – antiMRSA cephalosporin
    - **linezolid** – Panton-Valentine Leukocidin (PVL) positive strains (staphylococcal pneumonia)
    - **Mupirocin** – topical ATB for nasal carriers (MSSA and MRSA)

Staphylococcus aureus – antibiotic resistance

- Gene transfer of antibiotic resistance among strains of *Staphylococcus aureus* is performed frequently with transduction, i.e. transferring the resistance gene tempered (attenuated) bacteriophage (Siphoviridae)

- Resistance to antibiotics is further transmitted and spread through R plasmids during conjugation (formation of the enzyme - staphylococcal penicillinase)

Staphylococcus aureus - MRSA

- Staphylococci resistance to methicillin / oxacillin (MRSA) is formed by incorporating *mec*A or *mec*C gene into the chromosome (induces change of the penicillin-binding proteins – it changes the locus on bacteria where antibiotic binds to)
- PBP change to PBP2b - inability links PBP2 at PNC

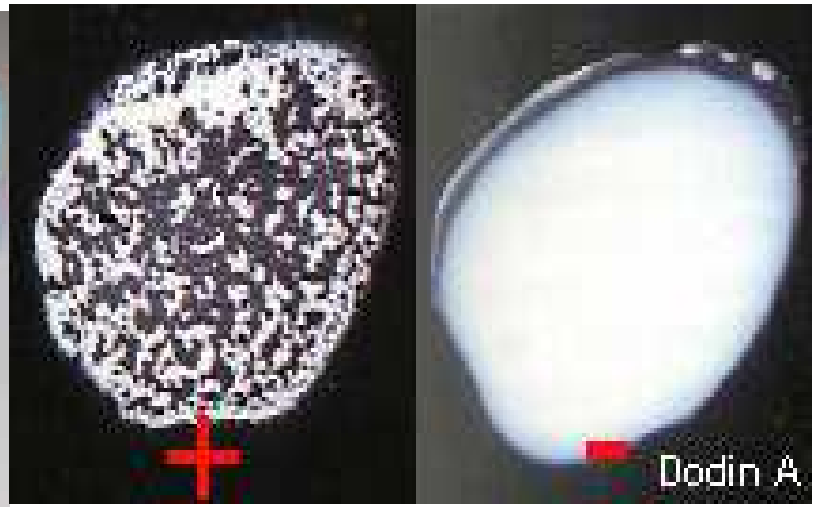
- MRSA is at the same time resistant to all beta-lactam antibiotics (except antiMRSA cephalosporin – ceftarolin)

MRSA – methicilin resistant

S. aureus

- Cultivation:
- selective agar medium MRSA Select - which contains higher concentration of NaCl + cefoxitin

MRSA - latex agglutination



Usage: to distinguish the methicillin-resistant *Staphylococcus aureus* - MRSA (altered of Penicillin Binding Protein - PBP 2b due to the presence of *mecA* gene) from other staphylococci (i.e. susceptible to methicillin/oxacillin)