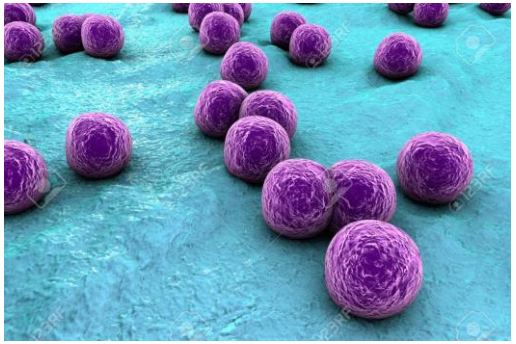
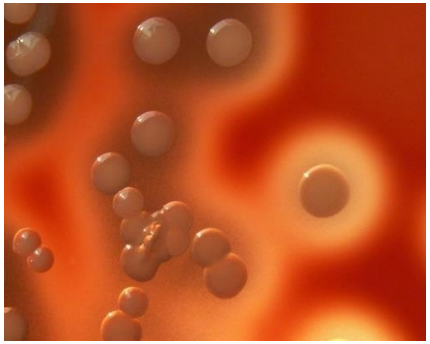
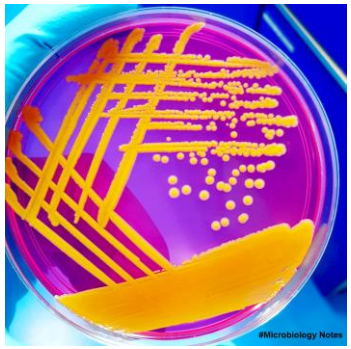
Staphylococci







„The Grapes of Wrath“

Jan Tkadlec

jan.tkadlec@lfmotol.cuni.cz

2. LF UK

FN MOTOL

Alas, Poor Caravaggio! Did The Famous Painter Die From Staphylococcus?

Billy Idol, 66, fighting MRSA super bug

What is a staph infection? Ozzy Osbourne's health scare explained

UFC

Dustin Poirier shows gnarly staph infection on his foot, spent 3 nights at a hospital

Revealed: Kardashian clan 'freaking out' over Khloe's staph infection...

'It was exacerbated by stress': Khloe Kardashian gives update on staph infection battle, is healing thanks to 'tons of love'

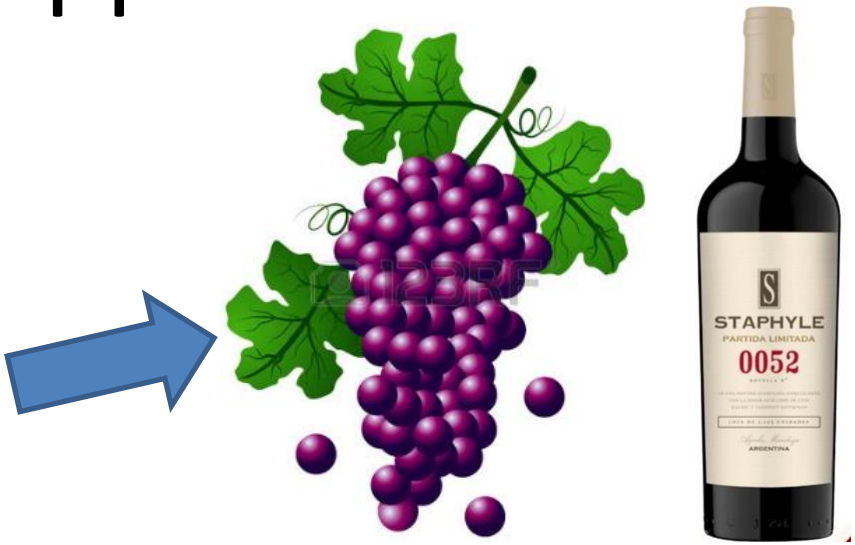
By MIKE LARKIN FOR DAILYMAIL.COM

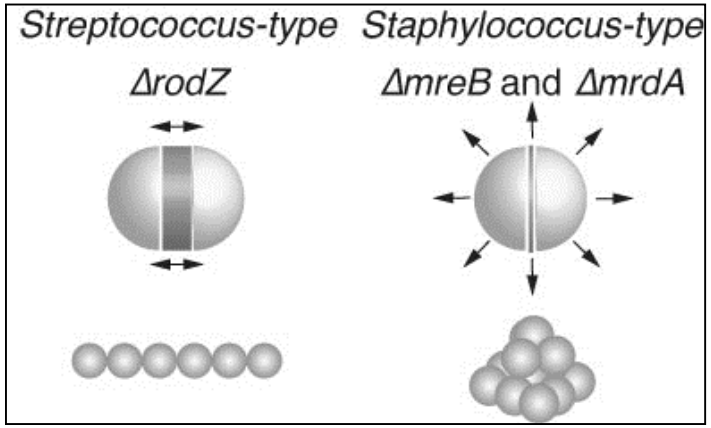
PUBLISHED: 01:38 GMT, 22 November 2015 | **UPDATED:** 21:49 GMT, 22 November 2015

Share 12 8

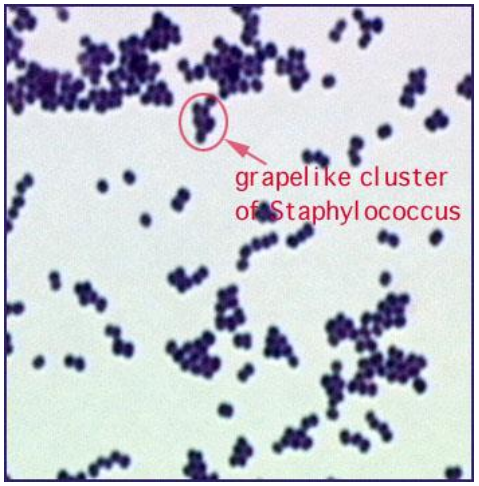
Microscopic appearance

- Cell morphology - coccus
- 0,5-1,5 µm in diameter
- **Gram-positive (purple)**
- Arranged in grapes-like clusters (*staphylé* = bunch of grapes)
- May have a capsule

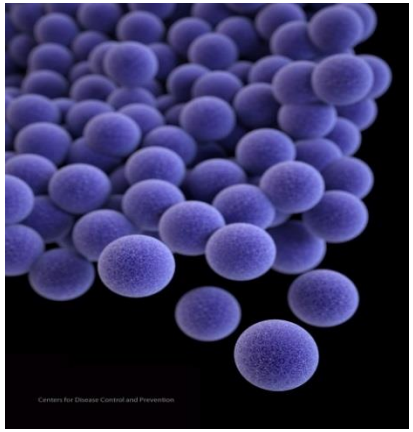




Clusters are formed as a result of cell division mechanism



Light microscopy - Gram staining



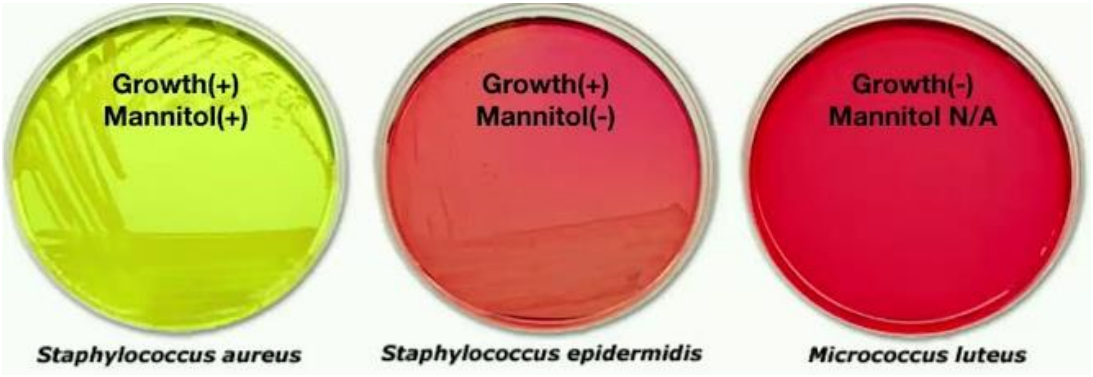
Electron microscopy

Cultivation of Staphylococci

- Easy to grow on common media
  - Columbia blood agar (hemolysis – *S. aureus*)
- Tolerance to high salt concentration (up to 10%)
  - Selective culture – manitol salt agar

Staph on blood agar

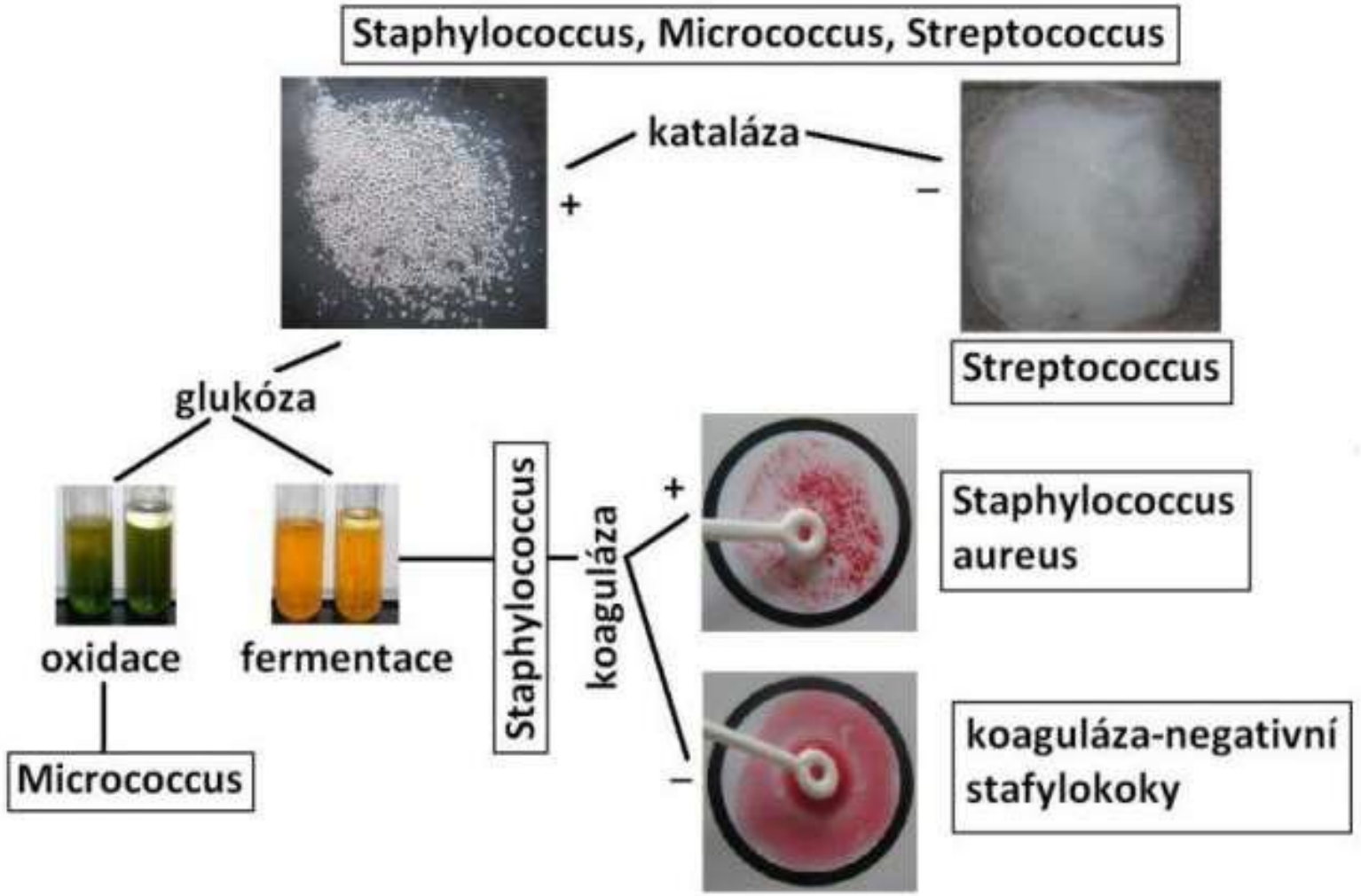
  - NaCl prevents growth of non-staphylococcal species, while fermentation of manitol (color change) differentiate *S. aureus* (yellow) from CoNS



S. aureus *S. epidermidis*

Easy to grow is the problém

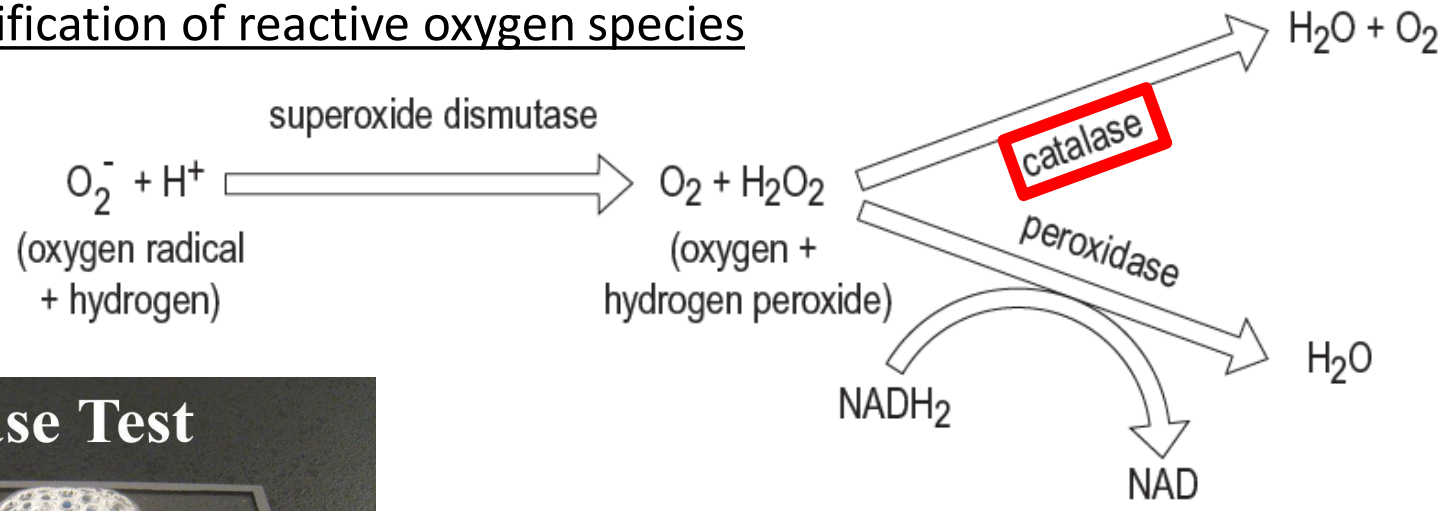
G+ cocci:

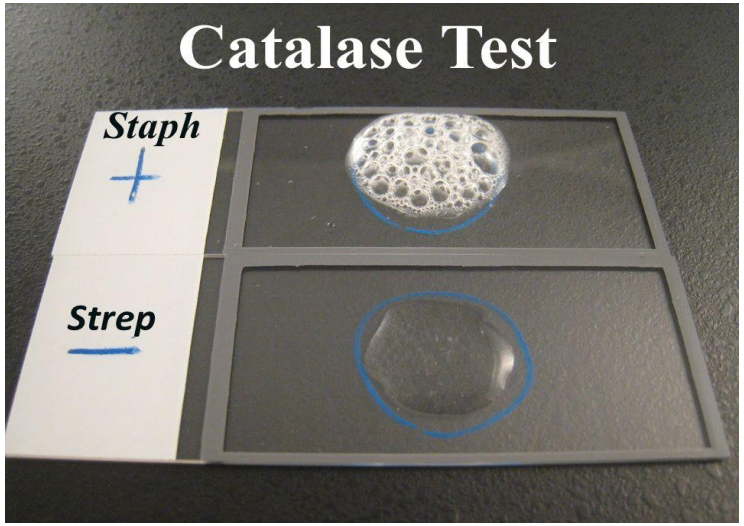


Obr. 6.6 Screeningové testy pro rodovou identifikaci grampozitivních aerobních koků

Biochemical properties

- facultative anaerobes
  - fermentation is primary source of energy generation
- grows in presence of oxygen
  - <u>detoxification of reactive oxygen species</u>



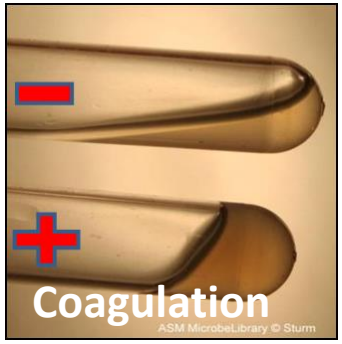


Staphylococci are catalase positive

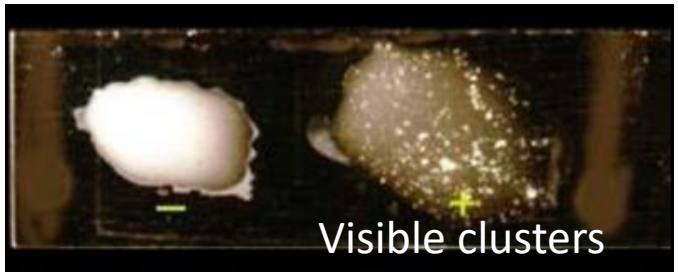
- Differentiation from streptococci (catalase negative)

Detection of coagulase

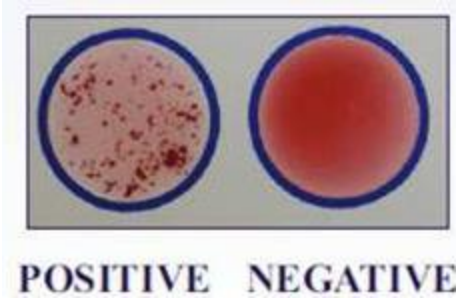
- Tube coagulase test:
- Coagulase converts fibrinogen to fibrin (insoluble)
- **free coagulase**
  - Rabbit plasma inoculated with staphylococcal colony, incubation 37°C for 1.5 hours



- Slide coagulase test (Clumping factor) – detection of **bound coagulase**



- Coagulase detection by latex agglutination



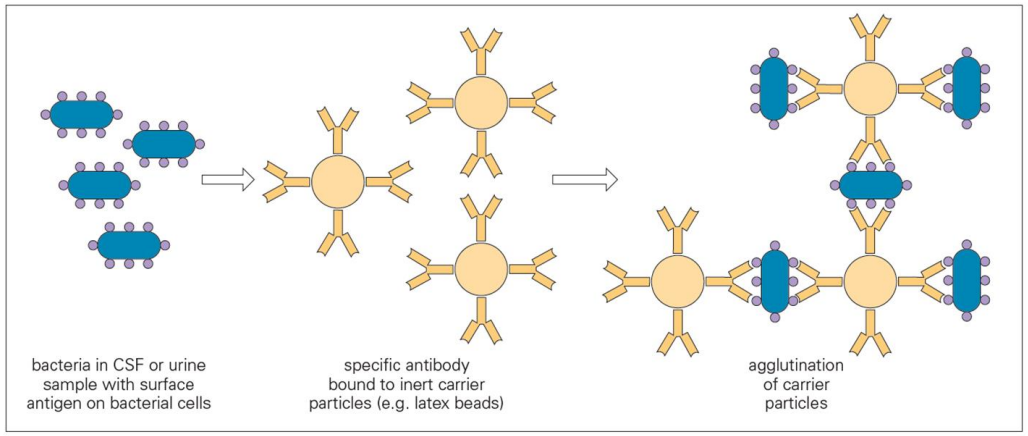
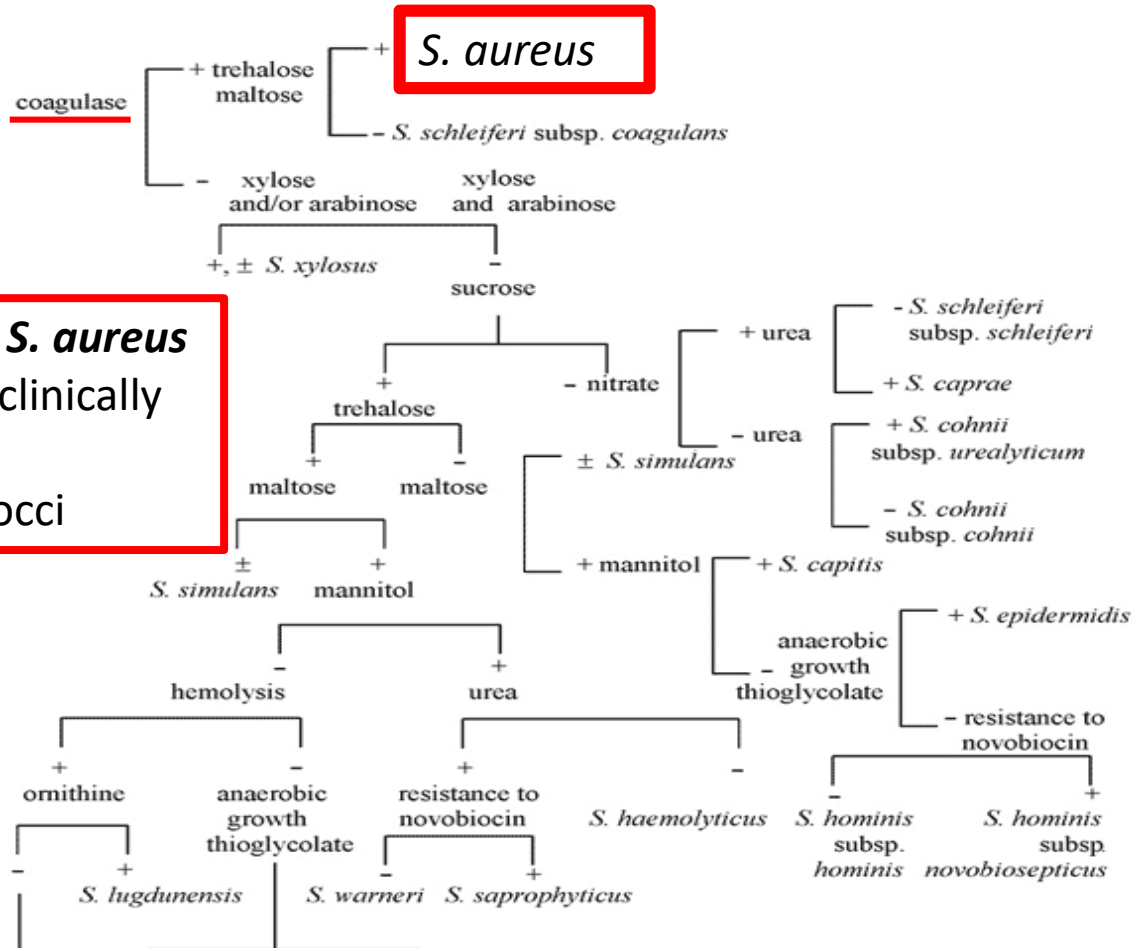


Figure 32.9 When a specimen of cerebrospinal fluid (CSF) containing bacteria (e.g. *Haemophilus influenzae*) is mixed with a suspension of latex particles coated with specific antibody (e.g. *H. influenzae* anticapsular antibodies), the interaction between antigen and antibody causes an immediate agglutination of particles, which is visible to the naked eye.

Biochemical differentiation of *Staphylococci*

Production of coagulase differentiate ***S. aureus*** (+) from other staphylococci that are clinically less important (mostly)
CoNS – coagulase negative staphylococci



Note: there are rare strains of *S. aureus* that did not produce coagulase
Other coagulase positive species includes: *S. delphini*, *S. hyicus*, *S. intermedius*, *S. lutrae*, *S. pseudintermedius*, and *S. schleiferi* subsp. *Coagulans,* however these are rather rare in human clinical material

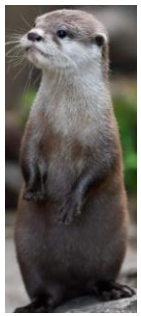
Ecology

- **<u>Body surface and mucous membranes</u>** of human and animals
- Host
  - Specialist

S. caprae

S. equorum

S. devriesei

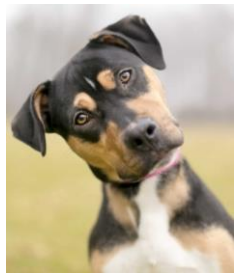


S. lutrae

S. felis

  - Generalist

S. aureus:



And more….

Animals could be source of infection!

Pathogenic potential

Oportunistic pathogens
Low virulence
Limited repertoir of virulence factors

S. epidermidis
S. haemolyticus
S. hominis
S. capitis

S. saprophyticus

S. epidermidis group

S. aureus
S. argenteus
S. lugdunensis

Oportunistic pathogens
High virulence
Broad repertoir of virulence factors

S. carnosus
S. xylosus
S. equorum

Rarely found in human infection
Lacks virulence factors

S. epidermidis group

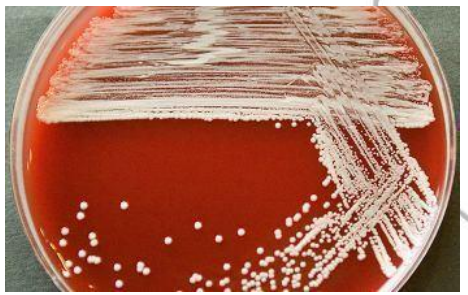
Microflora of skin and epitelia - *S. epidermidis* up to 97% of human

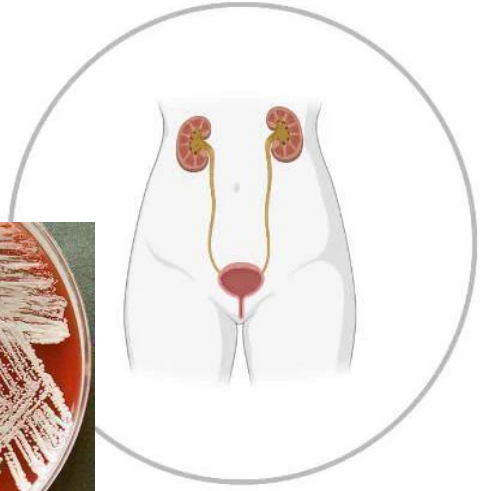
+ S. haemolyticus, *S. hominis*, *S. capitis*

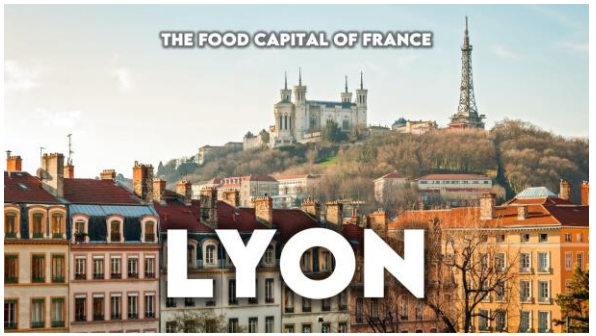
- Non pigmented and non hemolytic colonies
- Low virulence
- Common inhabitants of skin and mucous membranes- *S. epidermidis* up to 97% of people
- High antibiotic resistance
- Increasing number of infection
- Hospital aquired
- Prostetic devices and catheter related infections
- Not severe but hard to treat infections

S. saprophyticus

- Coloniser of urogenital tract (women)
- 2nd most common cause of UTI (10-20%)
- Young women
- Infection related to sexual activity

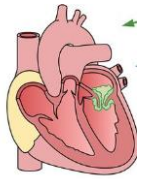






S. lugdunensis

- Hemolytic, pigmented
- Produce bound but not free coagulase
- Skin comensal
- Skin and soft tissue infections
- endokarditis, osteomyelitis, sepsis, …

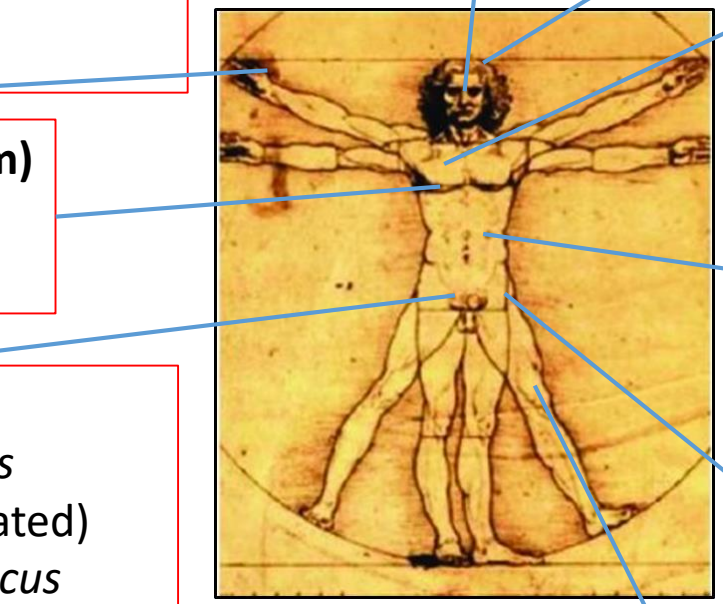


Staphylococci in clinical samples

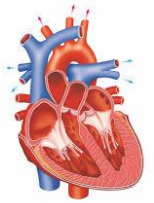
Nose and throat (swabs)
CoNS – colonisation
S. aureus colonisation or upper-airway infection

CSF
Meningitidis
S. aureus and CoNS

Skin (swab, pus, tissue)
S. aureus infection or colonisation
CoNs – colonisation (infection is rare)



Heart (valves)
Infective endokarditis:
S. aureus and CoNS



Lungs (sputum)
Pneumonia
S. aureus

Blood (Bloodculture)
BSI: *S. aureus*
CoNS (catheter related BSI)

Urine
UTI: *S. aureus* (catheter related)
S. saprophyticus

Stool
S. aureus rarely cause of colitis

***S. aureus* toxicoses (toxin detection)**
Enterotoxicosis – food poisoning
Staphylococcal toxic shock syndrome

Bone and joint infection (pus, tissue)
Osteomyelitis, septic arthritis, prosthetic joint infection
S. aureus and CoNS

Staphylococcus aureus

Golden staph

S. aureus carriage

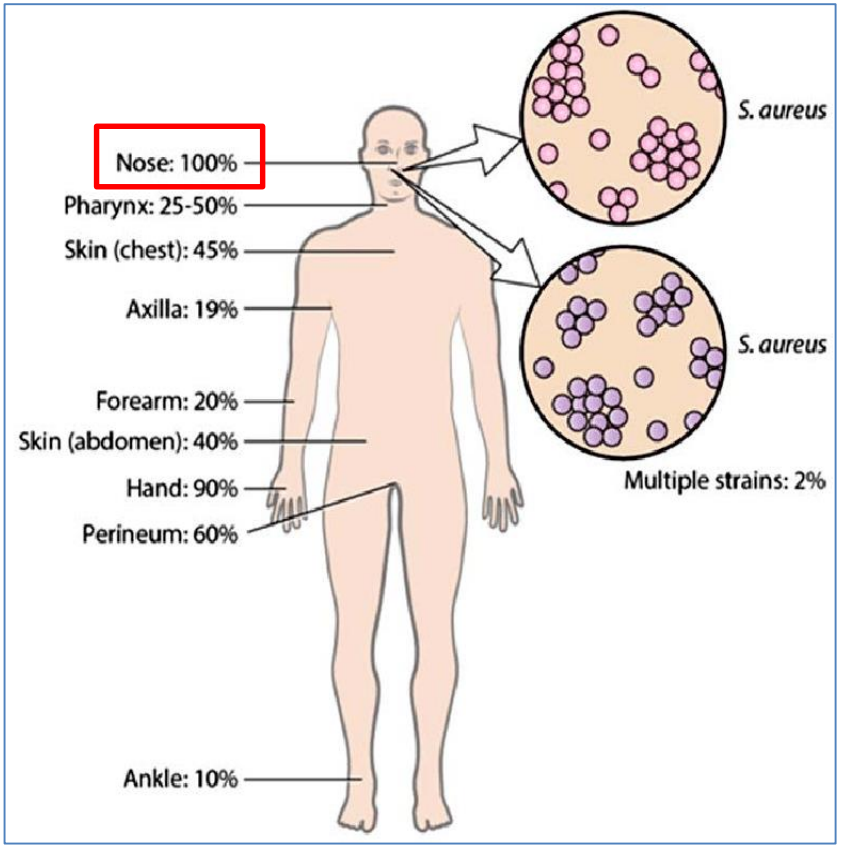
Carriage rate varies 10 - 60% of healthy adults

1. Persistent carriers – higher amount of bacteria for longer time
2. Intermitent carriers – lower amount of bacteria for shorter time
3. (Non-carriers)

- Varies with geographic location, ethnicity, age, sex and body niches

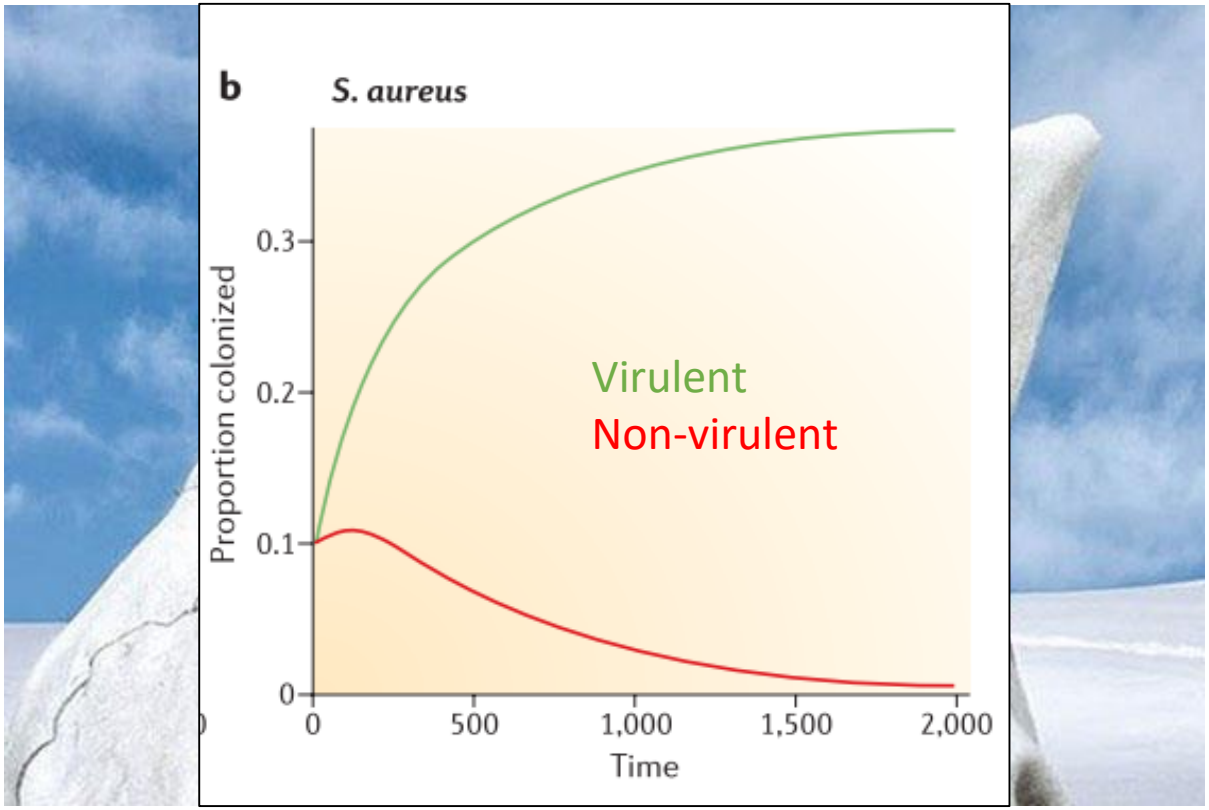
- Risk factors for Staph carriage:
  - Hormonal contraceptives, crowding and healthcare exposure, low vitamin D status, atopic dermatitis, male gender, diabetes

Body niches colonised in *S. aureus* carriers



- Colonisation is risk factor for the *S. aureus* infection
  - Surgery, hospitalization, wounds

Nose to nose
How *S. aureus* spread



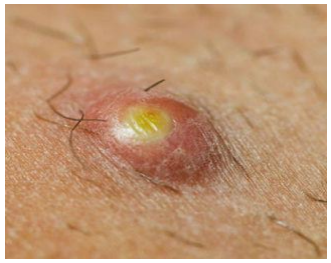
Massey et al. *Nat Rev Microbiol. 2006*

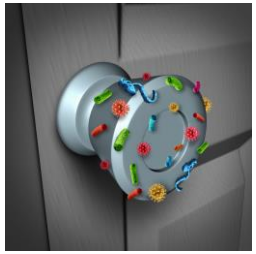
Problem: in frequent direct nose to nose contact complicated by presence of competing microflora

Virulence in *S. aureus* life cycle

Carrier

Carrier with skin infection





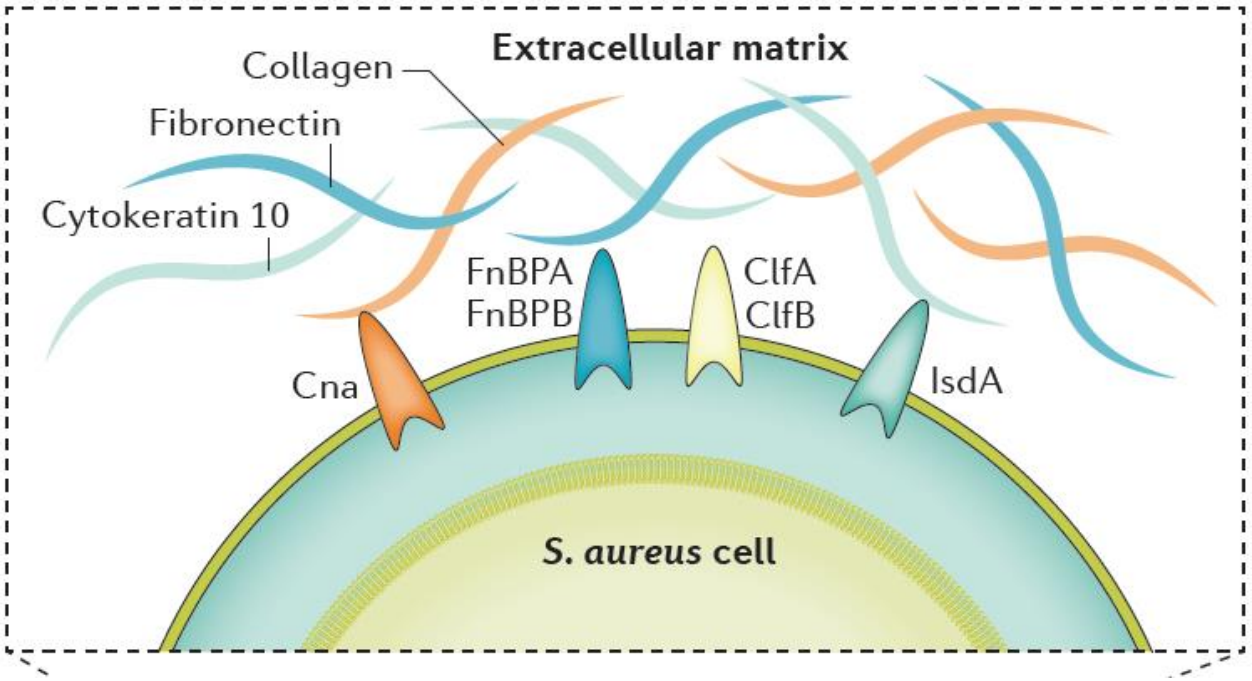
- Skin infection most common form of S. aureus infection
- Mild, in most case does not require treatment

Adhesion

Adhesins binding:

Extracellular matrix

- collagen
- Fibronectin
- Cytokeratin
- Etc.



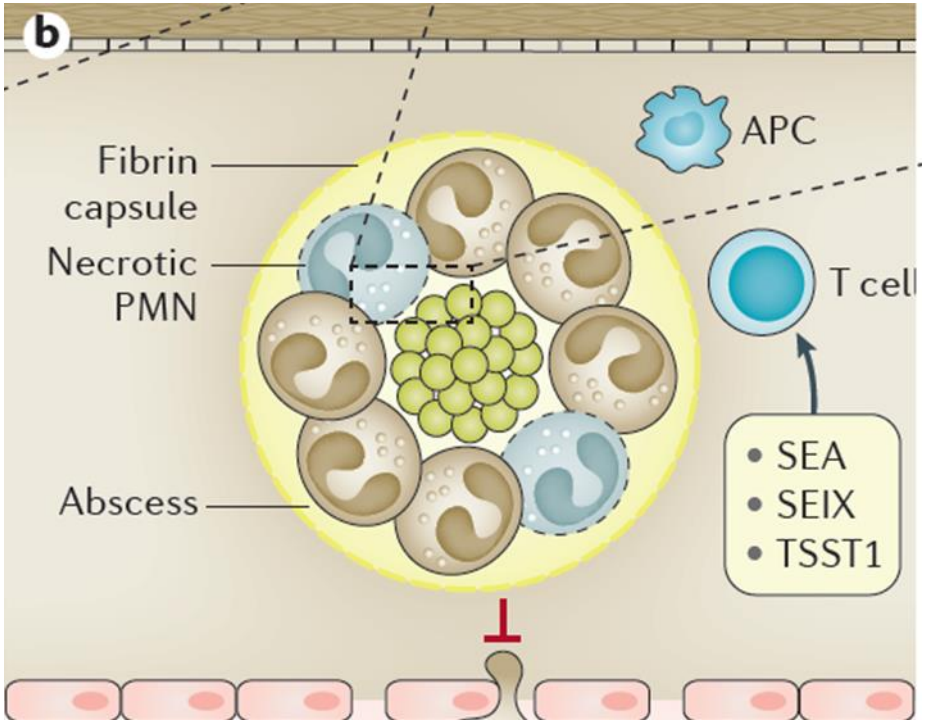
Production of factors inhibiting chemotaxis of leucocytes to place of infection

- chemotaxis inhibitory protein of *S. aureus* (CHIPS)
- FPRL1 inhibitory protein (FLIPr))

Localised infection
- hold your ground

Coagulase forms fibrin coating around the infection foci (absces)

- Hiding of the antigens
- Limited acces for leucocytes
- Accumulation of cytotoxins



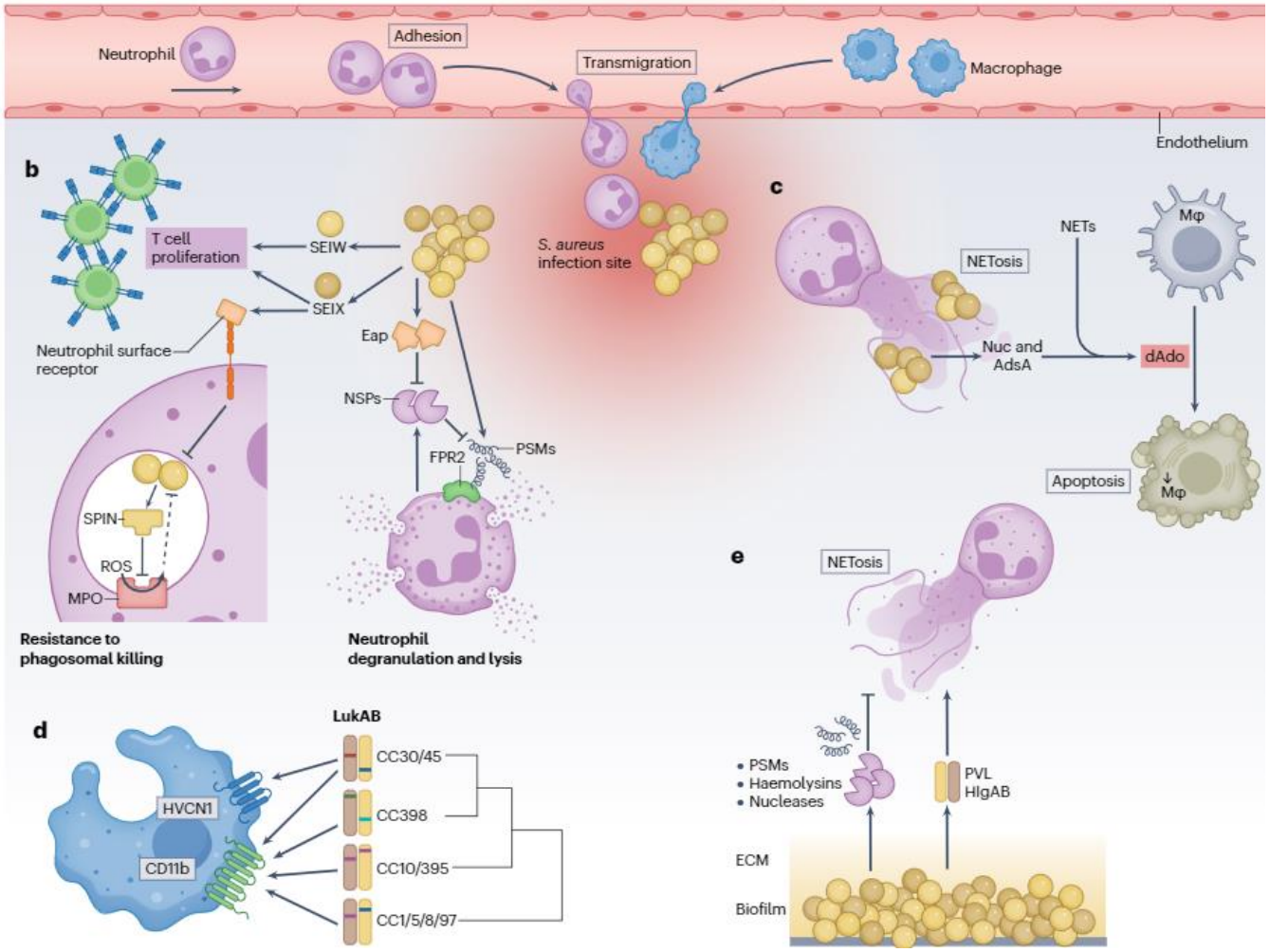
Opsonisation and complement inhibition

- Protein A
- capsule

Cytotoxins – lysis of immune cells

- Leukocidins – PMN, neutrophiles, macrophages
- α – haemolysin – T-cells, monocytes, platelets etc.
- Phenol soluble modulines - neutrophiles

S. aureus interacting with immmunity



Example of *S. aureus*

Inhibition of neutrophil chemotaxis – CHIPS

Dysregulation of immune response– superantigens (SEA, SEIW, SEIX)

Phagocytosis inhibition –SEIX

Release from NETosis – *nuc* nuclease

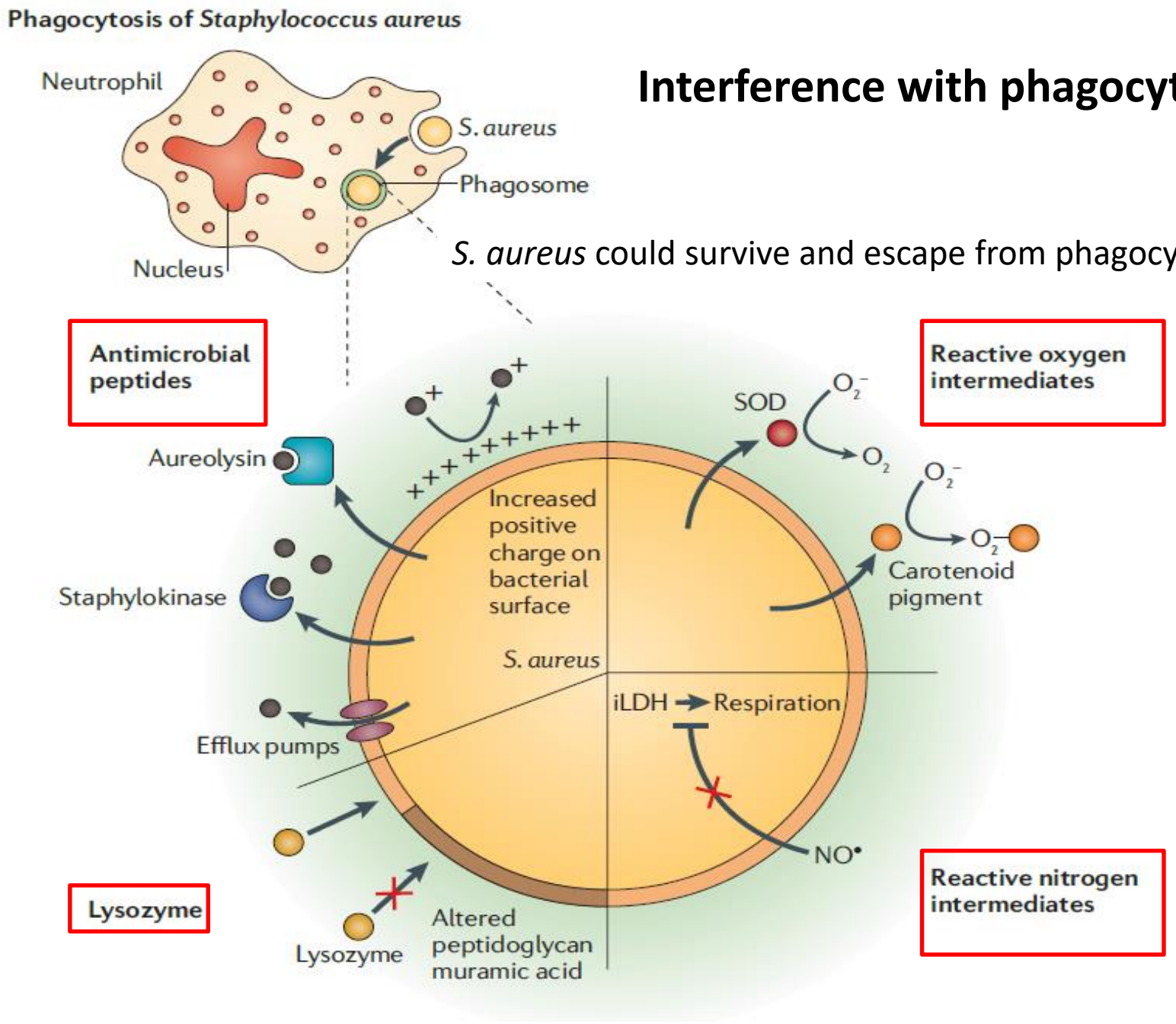
Macrophage apoptosis- AdsA

White blood cells lysis– PSM, hemolysins, leukocidins (LukAB, PVL)

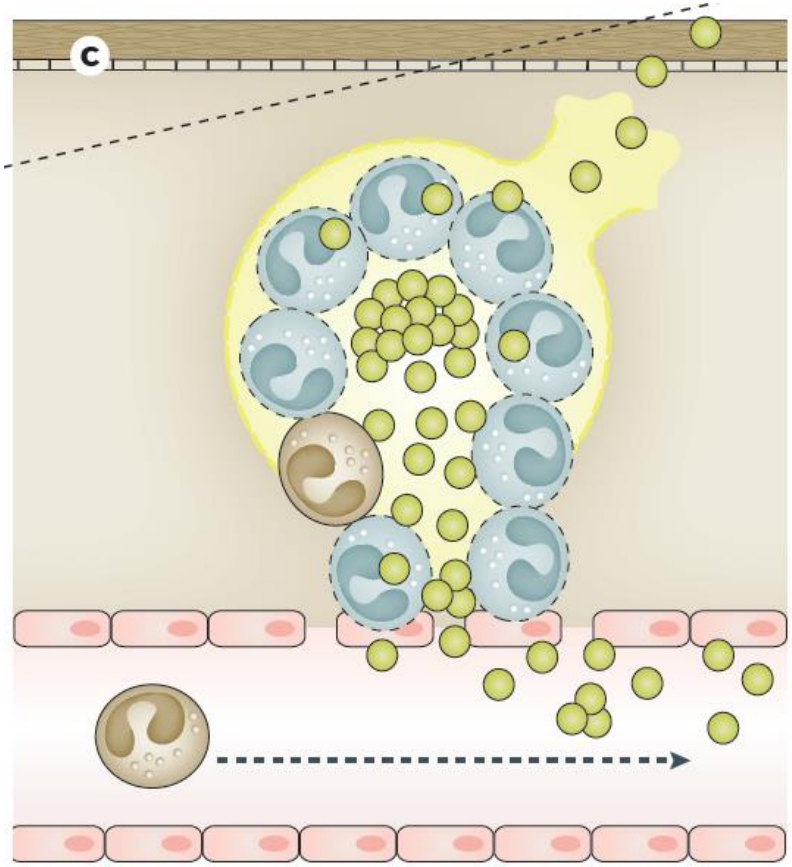
Howden et al, Nature Reviews Microbiology 2023

Interference with phagocytosis

S. aureus could survive and escape from phagocytic cells



Systemic spread



Dissemination

Disintegration of fibrin envelope – stafphylokinase
Lysis of extracellular DNA (NET) – nuclease
Destruction of extracellular matrix - hyaluronidase
Release of bacteria from the place of infection

- invasion of surrounding tissue
- Systemic spread
- Invasion of bloodstream
- Metastatic foci

(endokarditis, osteomyelitis, pneumonia, organ abscesses…)

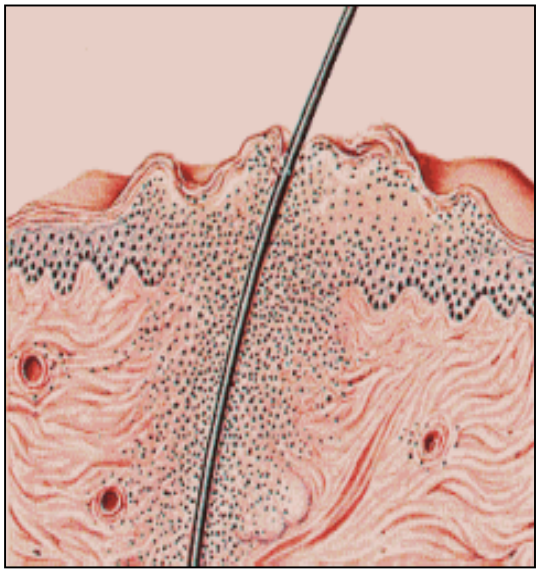
<u>Outcome of the imune system vs Staph aureus strugle:</u>

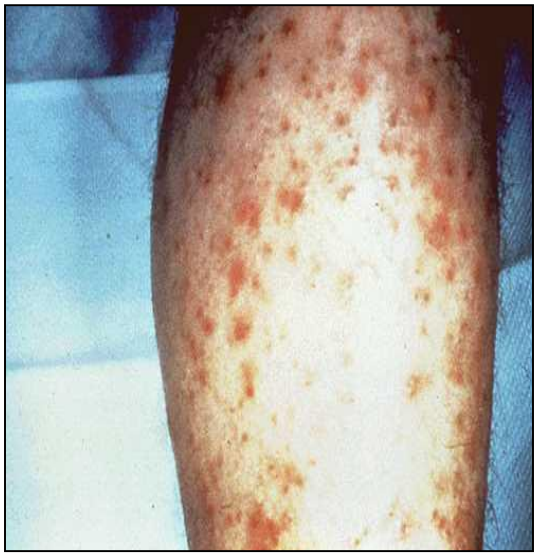
1. Elimination of infection, drainage of abscess
2. invasion of surronding tissue and bloodstream

Infections caused by *Staph. aureus*

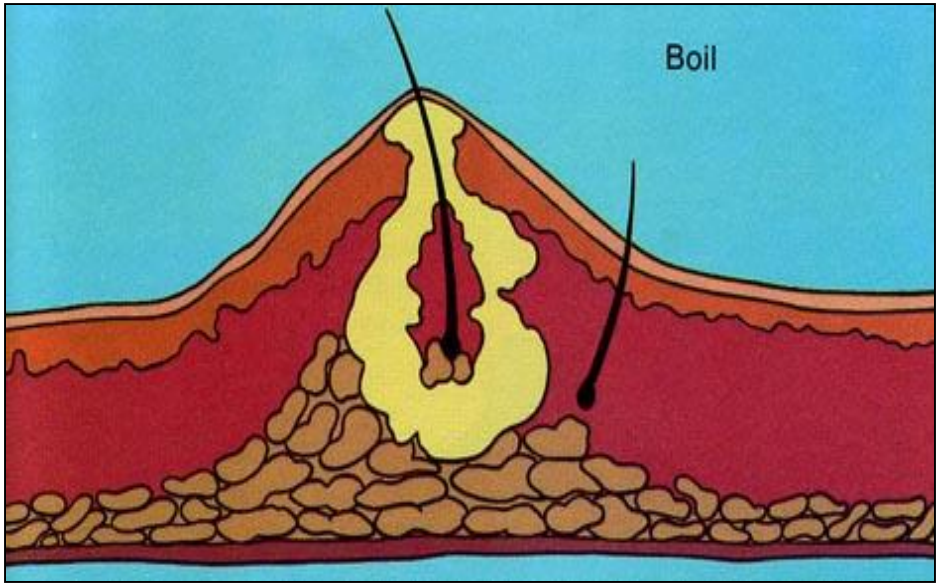
Skin and soft tissue infections

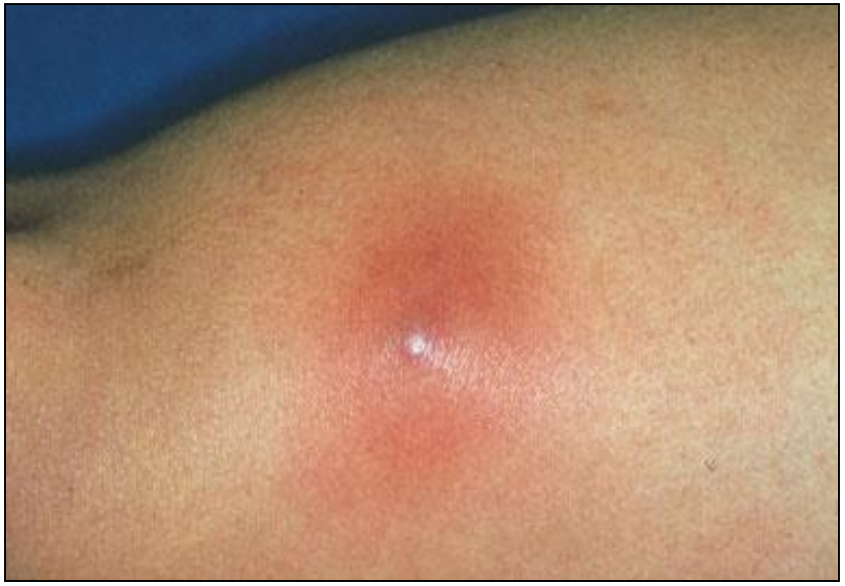
- *S. aureus* is most common cause of SSTI
  - Cutaneous abscess (folliculitis, carbuncles, furuncles) is hallmark of *S. aureus* SSTI



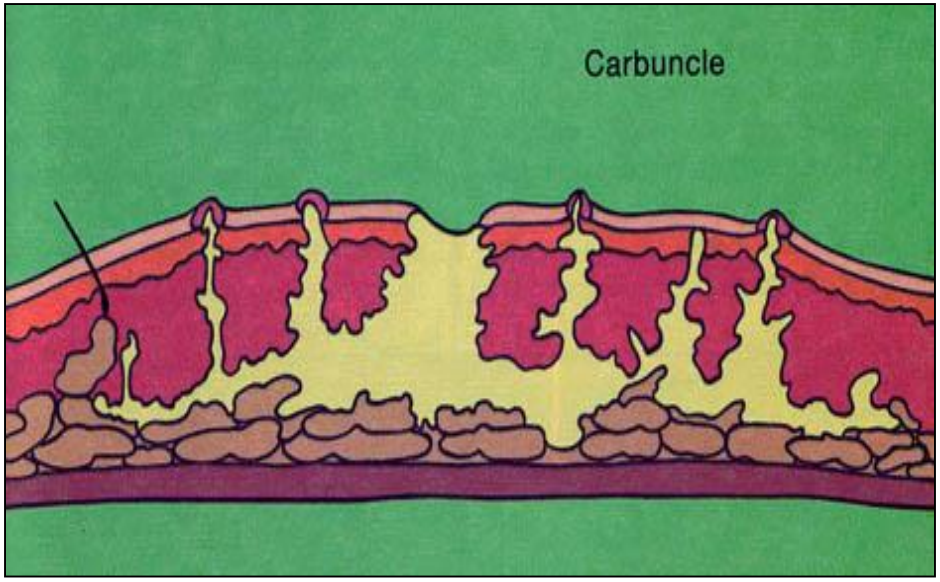


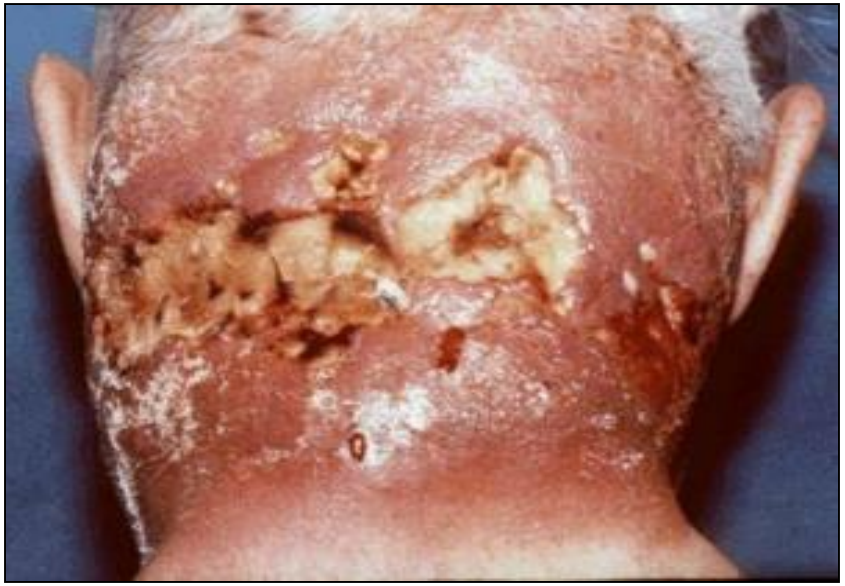
Folliculitis – infection of hair foliculle



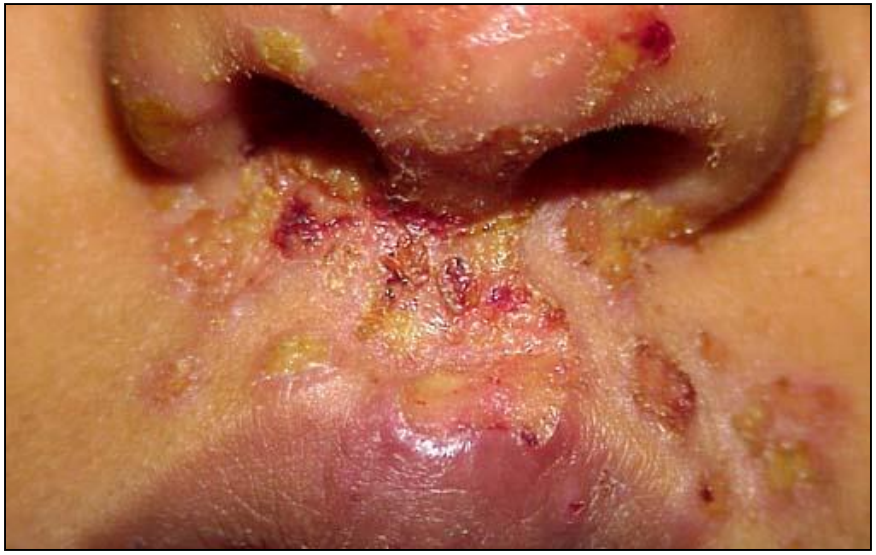


Furuncle (abscess formed as a result of hair follicule or sweat gland infection)



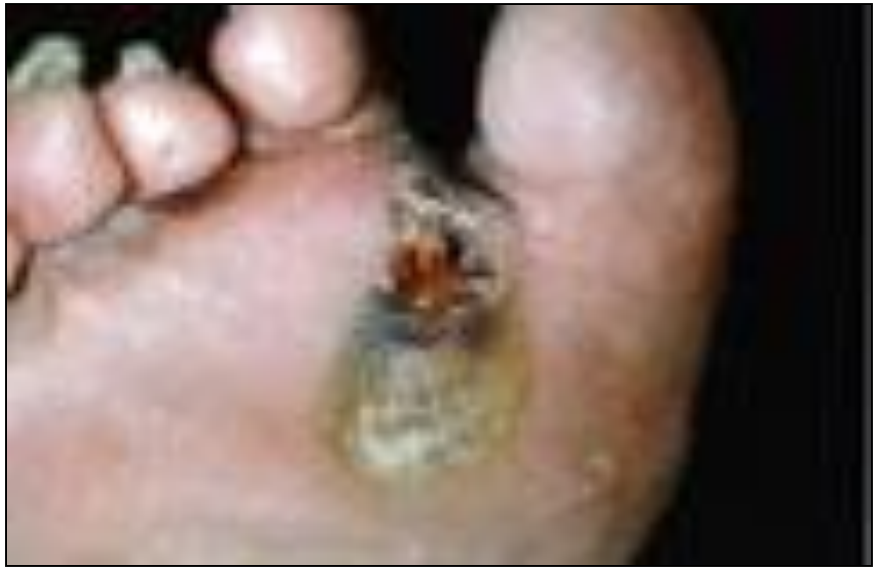


Carbuncle (large abscess or group of abscesses –furuncles)

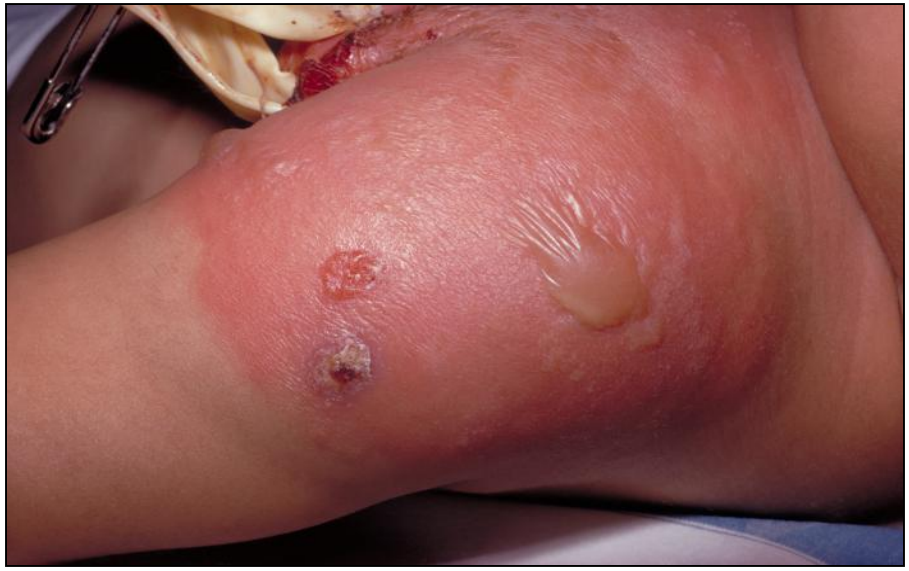


Impetigo

Paronychia



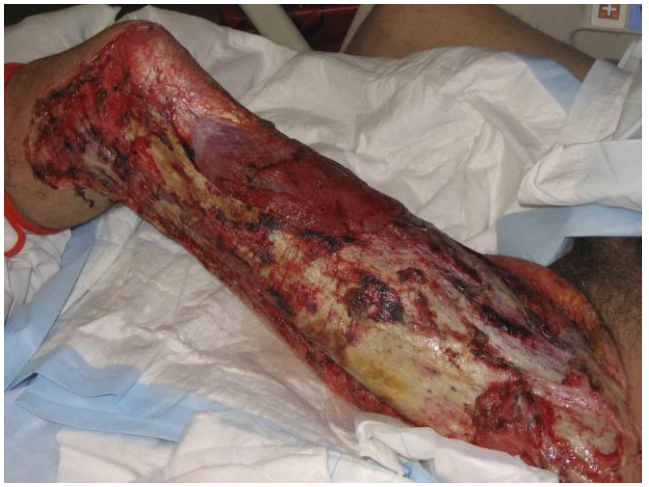
Wound infection

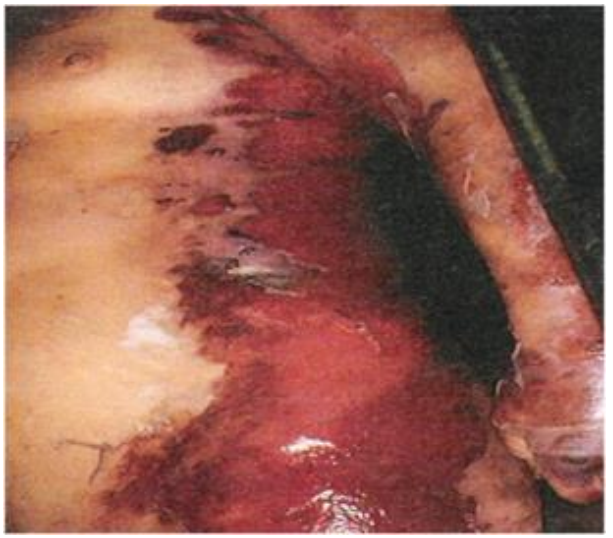


Cellulitis

Other SSTI

- **Necrotizing fasciitis** – severe rapidly progresing infection of the fascial plane deep to the subcutaneous tissue
- **Pyomyositis** – purulent infection of skeletal muscle that arises from hematogenous spread, usually with abscess formation, tropical countries
- **Wound infection** and surgical site infections
  - postoperative mediastinitis – complication of cardiac surgery
  - Nosocomial infections
- Mastitis - infection of one or both of the mammary glands
- Scalded skin syndrome – exfoliatins (toxins)





Treatment and diagnosis of SSTI

- drainage if there is drainable focus
- In case of uncomplicated SSTI antibiotics are often not necessary
- complicated cases: oral or parentheral antibiotic therapy – co-trimoxazole, antistaphylococcal penicilins, clindamycin. In case of MRSA: vancomycin, daptomycin, linezolid.
- Superficial infection (impetigo) could be treated by topical treatment (fusidic acid or mupirocin)
- Dx: swabs or collected pus

Staph BSI managment

Uncomplicated *S. aureus* bloodstream infection	(1) Exclusion of endocarditis (2) No implanted joint prostheses or intravascular devices/foreign bodies (3) Negative follow-up blood cultures performed obtained 2–4 days after initiation of effective antimicrobial therapy (4) Defervescence within 72 h of initiating effective antimicrobial therapy (5) No evidence of metastatic sites of infection (or deep-seated focus such as, e.g., osteoarticular infection or visceral abscess)
Complicated *S. aureus* bloodstream infection	Cases with positive blood cultures that do not meet criteria (1)–(5)

BSI by *S. aureus*

- Always clinically significant
- High risk of secondary metastatic complications
- Continuous blood culture positivity and fever are indication of complications – endocarditis, infection foci…
- It is necessary to remove source of infection

S. aureus bacteremia (SAB)

- Incidence 10-30 per 100,000 person-years
- Risk factors:
  - Age –highest risk up to first year of life and above 70 years
  - Gender – males higher chance of SAB
  - Ethnicity – afro-american population in US and indigenous Australians has increase risk of SAB
  - HIV – as much as 24 times increase risk of SAB compare to non-HIV population
  - Intravenous drug users – non-sterile needles
  - Hemodyalisis patients (catheters)
  - In general, *S. aureus* colonisation – prevention via decolonization before surgery, etc.

Primary foci of SAB

  - catheter, SSTI, pulmonary inf., osteoarticular inf., endocarditis

- Mortality depending on foci ranges from 10 (SSTI) to 70 % (pulmonary)
- Treatment : i.v. betalactams and glycopeptides (vancomycin), daptomycin, etc.

Infective endocarditis (IE)

- *Staphylococci* form a biofilm on the hearth valve (native or prosthetic), bacteria are release into the blood and could cause infection of other organs
- *S. aureus* is most common cause of IE
- Risk factor is healthcare contact, injection drug use, SAB, stroke, diabetes
- Treatment: prolonged i.v. therapy

Osteoarticular infections

Infection via haematogenous spead or a direct trauma

S. aureus is most common cause

Osteomyelitis

Septic arthritis

Prosthetic joint infection

Often chronic and recurent, requires prolonged antibiotic treatment and often surgery

Pneumonia

- Community acquired pneumonia (CAP)
  - Often superinfection after influenza, severe when PVL toxin production – necrotizing pneumonia
- Hospital acquired pneumonia (HAP)
- Ventilator associated pneumonia (VAP)

S. aureus is common cause in all three types

- VAP and HAP – secondary pneumonia =hematogenic spread from other foci of infection
  - High mortality

Case report – PVL associated necrotising pneumonia

Clinical description

- 17.3. 2016 a 21 years old man admitted to emergency
- Signs of respiratory failure and severe pneumoia
- Progressing to septic shock
- Extracorporal oxygenation therapy
- Empirical therapy: ciprofloxacin, **clarithromycin**, piperacilin /tazobactam, **oseltamivir** (flu)

Laboratory 18.3.

- **Leukopenic**
- **CRP** 196 mg/l
- **Temperature** 37 °C
- **Renal insuficiency**

Microbiology 18.3:

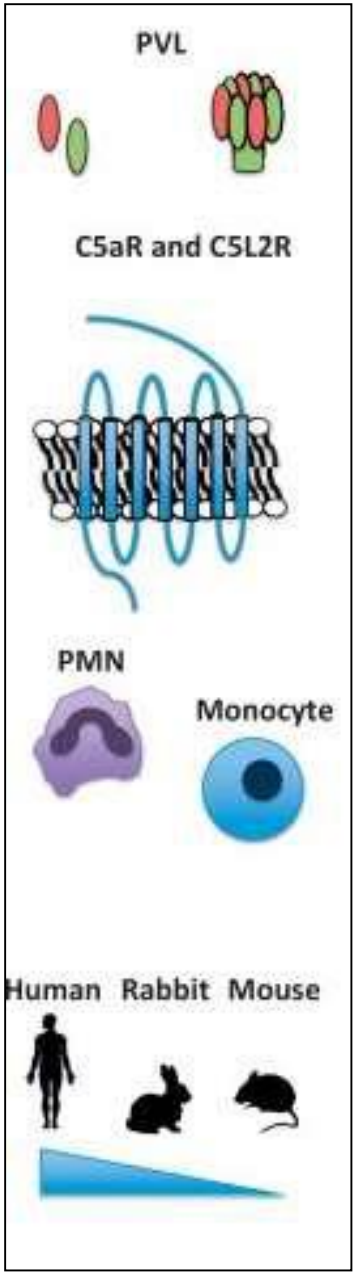
- Antigen *Streptococcus pneumoniae*:: negative
- Antigen *Legionella pneumophila* typ 1:: negative

- **PCR Influenza A**
- Microscopy of sputum: G+ cocci in clusters
- Culture *S. aureus* MSSA – **PCR PVL positive**
- **linezolid**

18.3. 2016 14:15 *Exitus letalis*

PVL toxin

- Panton-Valentin Leukocidin (1932)
- Bicomponent pore-forming toxin
  - the LukS-PV and the LukF-PV subunits
  - Targets: neutrophils, monocytes and macrophages
- High host specificity
  - Induce rapid activation and cell death in human (and rabbit) neutrophils
  - But didnt affect murine or simian cells
  - 2-3% of *S. aureus* clinical isolates



PVL in pathogenesis

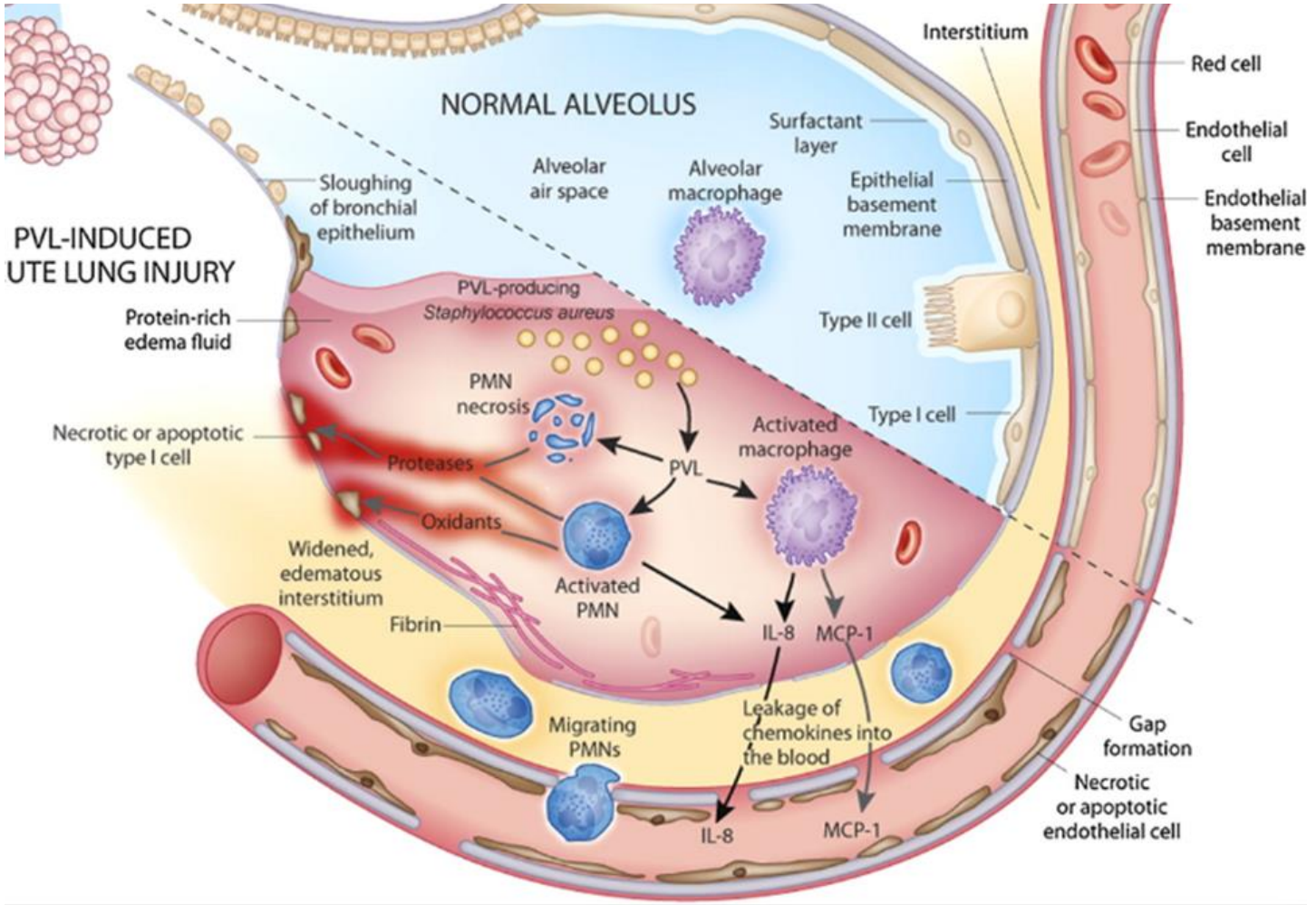
Acute, severely necrotising skin infections and **necrotising pneumonia**

- Pneumonia often preceded by influenza
- High mortality, rapid progress

PVL gene mostly in community acquired *S. aureus* and MRSA (CA-MRSA) infections

PVL action in necrotising pneumonia

Inflammation (influenza) cause migration and accumulation of PMNs in lungs.
Production of PVL by *S. aureus* cause rapid lysis of PMNS and release of toxic antimicrobial compounds.
Released serine proteases starts to digest lung tissue causing massive damage



Food poisoning

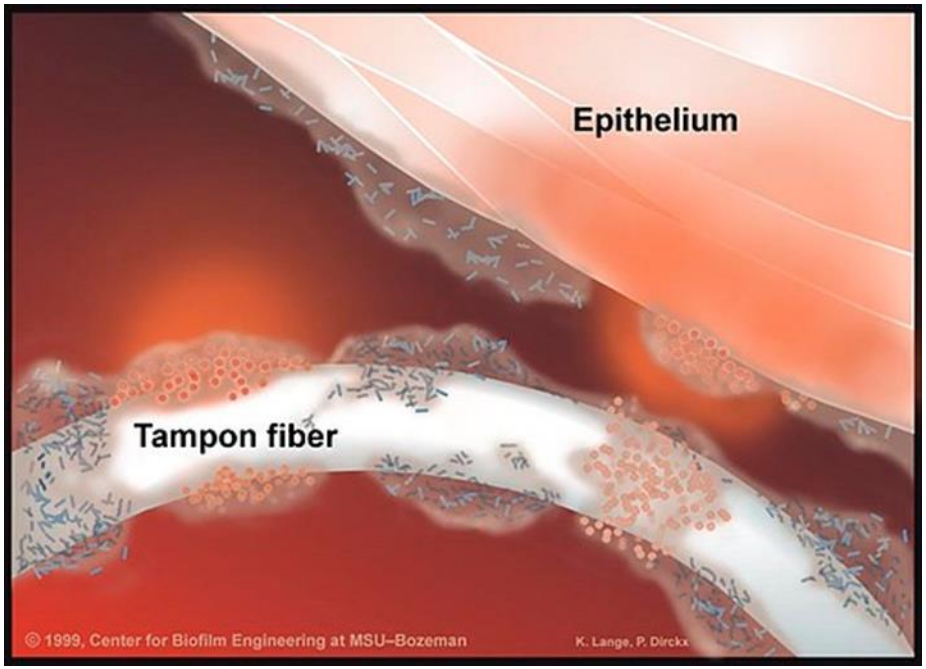
- Enterotoxins are chemoresistant and heat-stable secreted polypeptids
  - More than 20 genes – *sea*, *seb*, *sec*…
    - Clinical isolate has on average 6 enterotoxin genes
  - It can withstand boiling at 100°C for a few minutes
  - Infected food (milk products, meat, ice cream, …)
- Incubation period 2-6 hours
- Interfere with intestine function
- Clinical symptoms - nausea, vomiting and diarrhea
- Self limited, recovery in day or so

Toxic Shock Syndrom

- TSST 1 (Toxic Shock Syndrome Toxin)
- Toxin producing *S. aureus* is usually localized at mucosal sites (nasopharynx, vagina – tampons) or in abscesses
- released toxin circulate in blood and stimulate release of IL-1, IL-2, TNF-α and other cytokines
- high fever, rash, desquamation, diarrhea, vomiting, hypotension even multiple organ failure
- Fatal outcome isn´t rare

Staphylococcal toxic shock syndrome

- Caused by superantigen
- High similarity to septic shock
  - Excesive production of cytokines by activatet T-cells
  - fever
  - **Colaps of immune and regulátory homeostasis**
  - **Systemic patological changes**



biofilm formed on tampon fibres- source of toxin producing staphylococci

Treatment of *S. aureus* infection

	MSSA	MRSA
uncomplicated/non-invasive SSTI	antistaph. **β-lactams** (oral)	clindamycin, TMP-SXT, doxycyklin, linezolid (oral)
Bacteremia (I.V.)	semisyntetické antistaf. β - laktamy (oxacillin, nafticilin) cefalosporiny	**vancomycin**, daptomycin, (ceftarolin, ceftobiprol)
Endocarditis (I.V.)	- II -	- II -
Pneumonia (I.V. switch to oral)	- II - , dlindamycin, linezolid	vancomycin, communty:tigecycline, ceftarolin, PVL: linezolid
Osteomyelitis (I.V. switch to oral)	Semisyntethic antistaf. β – lactams (oxacillin, nafticilin) cephalosporines	vancomycin, daptomycin, TMP-SXT+rifampicin/linezolid/clindamycin

TMP-SXT=trimetoprim+suplhametaxazol=co-trimoxazol; I.V. intravenous administration

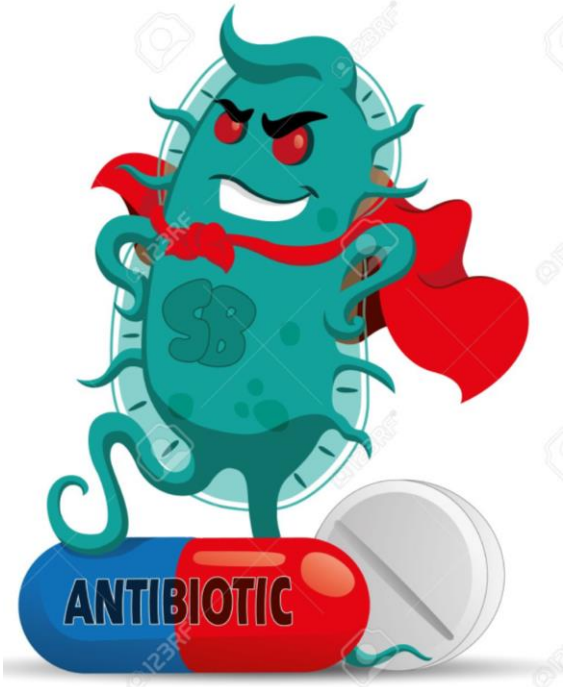
Samples for microbiology must be taken before start of therapy!!!
Empiric treatment must reflect local trends in antimicrobial susceptibility
Source of the infection must be found and removed – otherwise there is risk of recurence
For uncomplicated SSTI antimicrobials are not needed
Vancomycin only for MRSA, β-lactams a cephalosporines works muc better in sensitive strains
When the infection is caused by toxin producing bacteria antibiotics targetting proteosyntesis are prefered (e.g. Linezolid)

Unfortunate story of *S. aureus* vaccine development

- Vaccination is clever solution for antibiotic resistance, and prophylaxis
- Why there is no vaccine against *S. aureus*?
- So far, any candidate vaccine did not demonstrate efficacy.

- StaphVax – testing in humans does not show protection from *S. aureus* infection

- V710 – failed to elicit the immunity
  - Even worse, vaccinated patients that get infected have worse outcome
  - all 12 V710 recipients (but only 1 of 13 placebo recipients) with low IL2 levels prior to vaccination and surgery died after postoperative *S. aureus* infection



Hard to kill

Antibiotic resistance in *S. aureus*

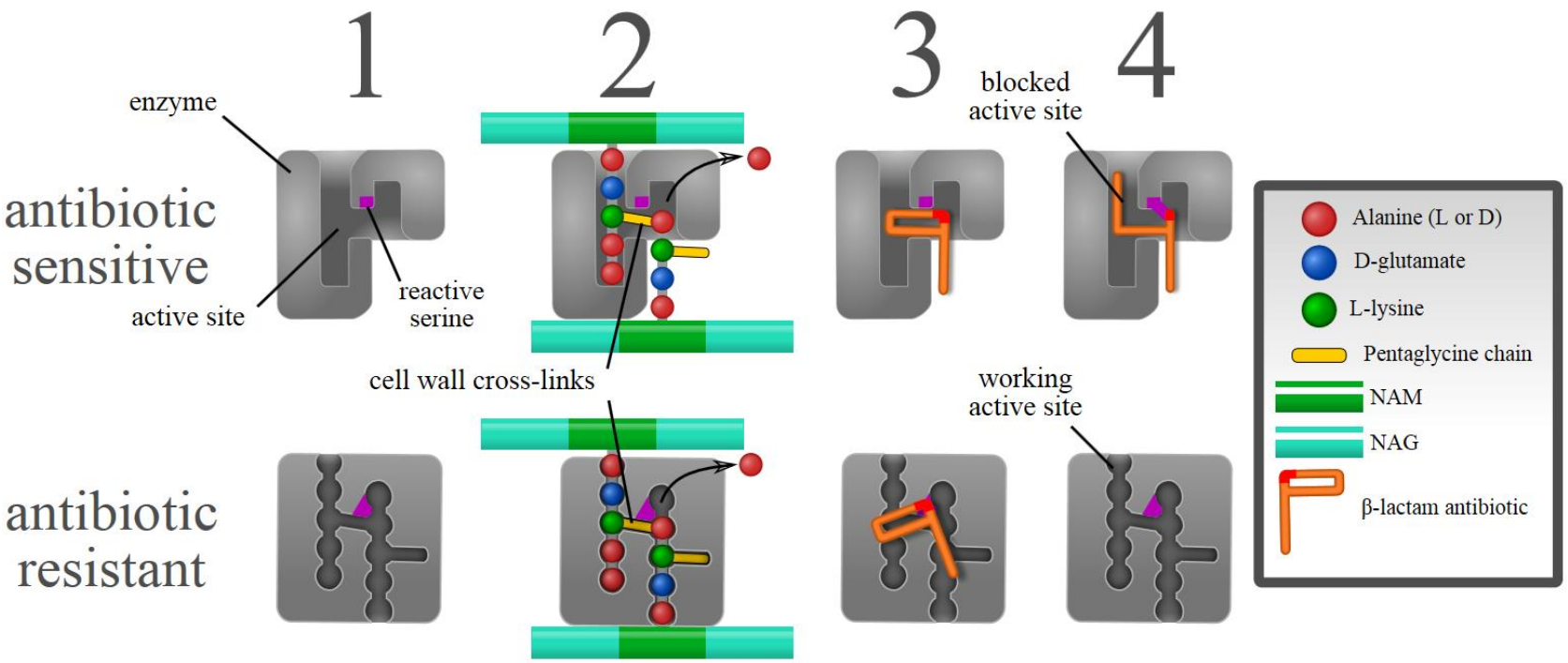
First step: resistance to penicillin

- Penicillin (Fleming 1928, introduction 1940s)
- *S. aureus* infection mortality was about 80% before introduction of penicillin
- Penicillin resistence in mid 1940s
- Enzyme penicillinase – plasmid encoded
  - Hydrolysis of the beta - lactam ring
  - Resistance to penicillin G, ampicillin and similar drugs
- Nowdays almost all *S. aureus* produce penicillinase

Second step: MRSA

- Introduction of methicillin (1956)
  - Semisynthetic derivate of penicillin
  - Resistant to penicillinase
- MRSA (methicillin resistant *S. aureus*) 1961
  - Resistance to penicillin, methicillin (oxacillin) and cefalosporins
  - often resistant to tetracyclines, macrolides and aminoglycosides
  - Susceptible to vancomycin, linezolid, daptomycin
  - Higher mortality and morbidity compare to MSSA

Mechanism of MRSA resistance



In susceptible bacteria beta-lactams binds PBP2 (penicillin binding protein) and prevents formation of peptidoglycan crosslinks, leading to disruption of cell wall

In MRSA beta-lactams are unable to bind PBP2**a** (coded by *mecA* gene)

Price of MRSA infection

REVIEW ARTICLES

Methicillin-resistant *Staphylococcus aureus* (MRSA): burden of disease and control challenges in Europe

R Köck[1,2], K Becker[2], B Cookson[3], J E van Gemert-Pijnen[4], S Harbarth[5,6], J Kluytmans[7,8], M Mielke[9], G Peters[2], R L Skov[10], M J Struelens[11,12], E Tacconelli[13], A Navarro Torné[12], W Witte[14], A W Friedrich (alexander.friedrich@ukmuenster.de)[1]

Köck et al, EuroSurveil (2010)

TABLE 3

Estimates from recently published (2001–2009) studies of hospital fin **Length of stay** nfections c **Additional cost**

Type of infection, setting of study	Number of patients	Effect on hospital length of stay	Effects on costs	Reference
Bacteraemia, one teaching hospital, USA, 1997–2000	96 MRSA vs 252 MSSA	Median LOS: 9 days (MRSA) vs 7 days (MSSA), p=0.045; MRSA independent risk factor for increased LOS (1.3–fold, p=0.016)	Hospital charges after *S. aureus* bacteraemia: USD 26,424 (MRSA) vs USD 19,212 (MSSA), p=0.008	Cosgrove SE *et al.* [18]
Haemodialysis-related infections, one teaching hospital, USA, 1996–2001	54 MRSA vs 89 MSSA	Median LOS: 11d (MRSA) vs 7days (MSSA), p<0.001	Adjusted median costs for initial hospitalisation: USD 21,251 (MRSA) vs USD 13,978 (MSSA), p=0.012 and	Reed SD *et al.*
Surgical site infections, one tertiary care and one community hospital, USA, 1994–2000	121 MRSA vs 165 MSSA vs 193 uninfected controls	Medi… vs 23… (MRS…	…MSS… …5 day…	
BSIs, one tertiary care hospital, USA, 2000–2003	95 MRSA vs 87 MSSA	LOS a… p=0.… not significant)		
Ventilator-associated pneumonia, 16 teaching and 43 nonteaching hospitals, USA, 2002–2003	95 MSSA vs 59 MRSA	Total inpatient LOS: 20 days (MRSA) vs 15d (MSSA), p=0.04. M… patients consumed excess resources of 3.8 inpatient days, p=0.08	of USD 7731 (p=0.035) in total costs	[28]

MRSA vs MSSA
+ 2 - 10 days

MRSA vs MSSA
+ 7000 - 60 000 USD/patient
(lowest estimations - 2000 USD)

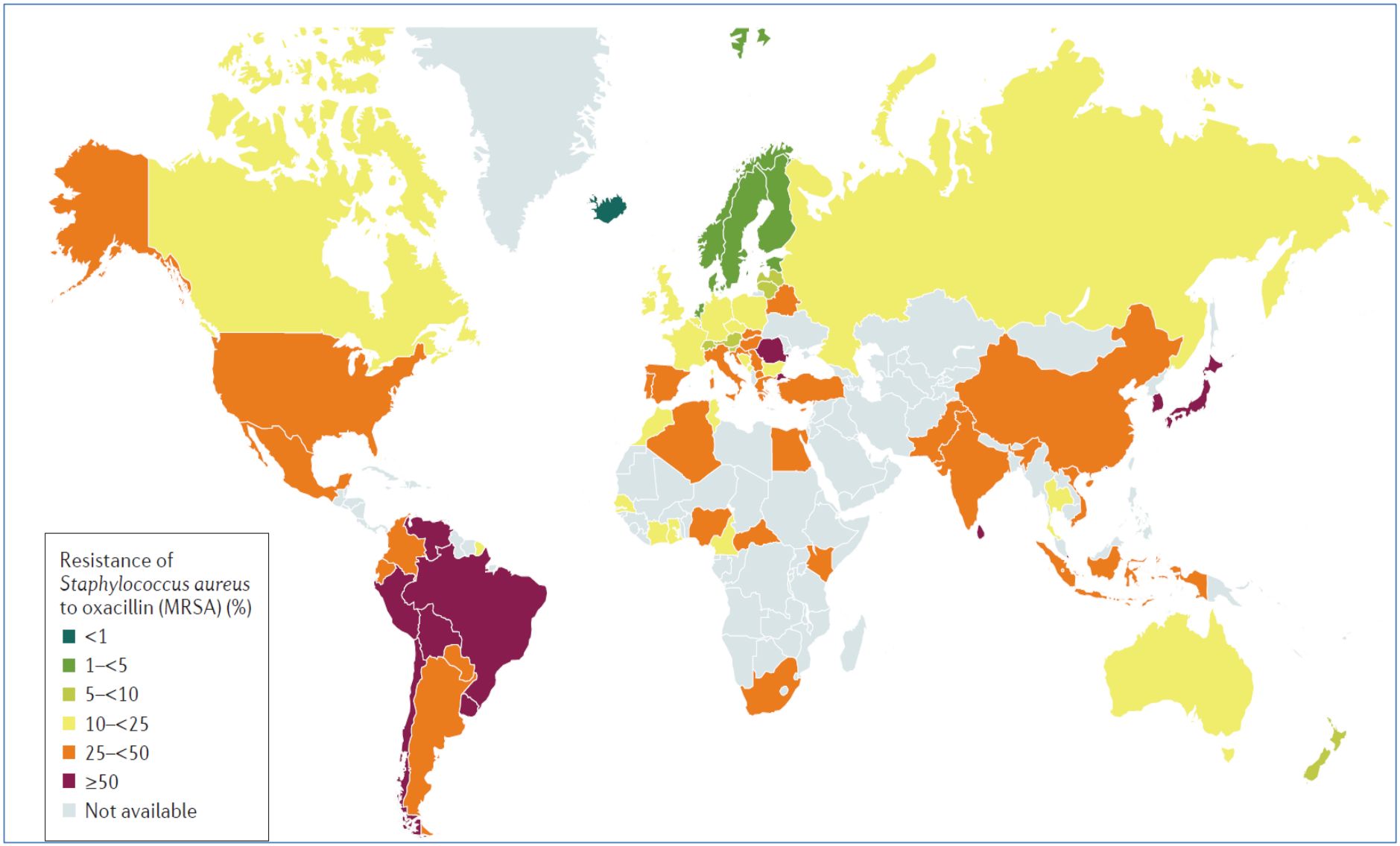
BSI: bloodstream infection; ICU: intensive care unit; LOS: length of stay; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-susceptible *Staphylococcus aureus*; SSI: surgical site infection; USA: United States of America; USD: United States dollars; VAP: ventilator-associated pneumonia.

FNM: cca 200 new MRSA cases/year

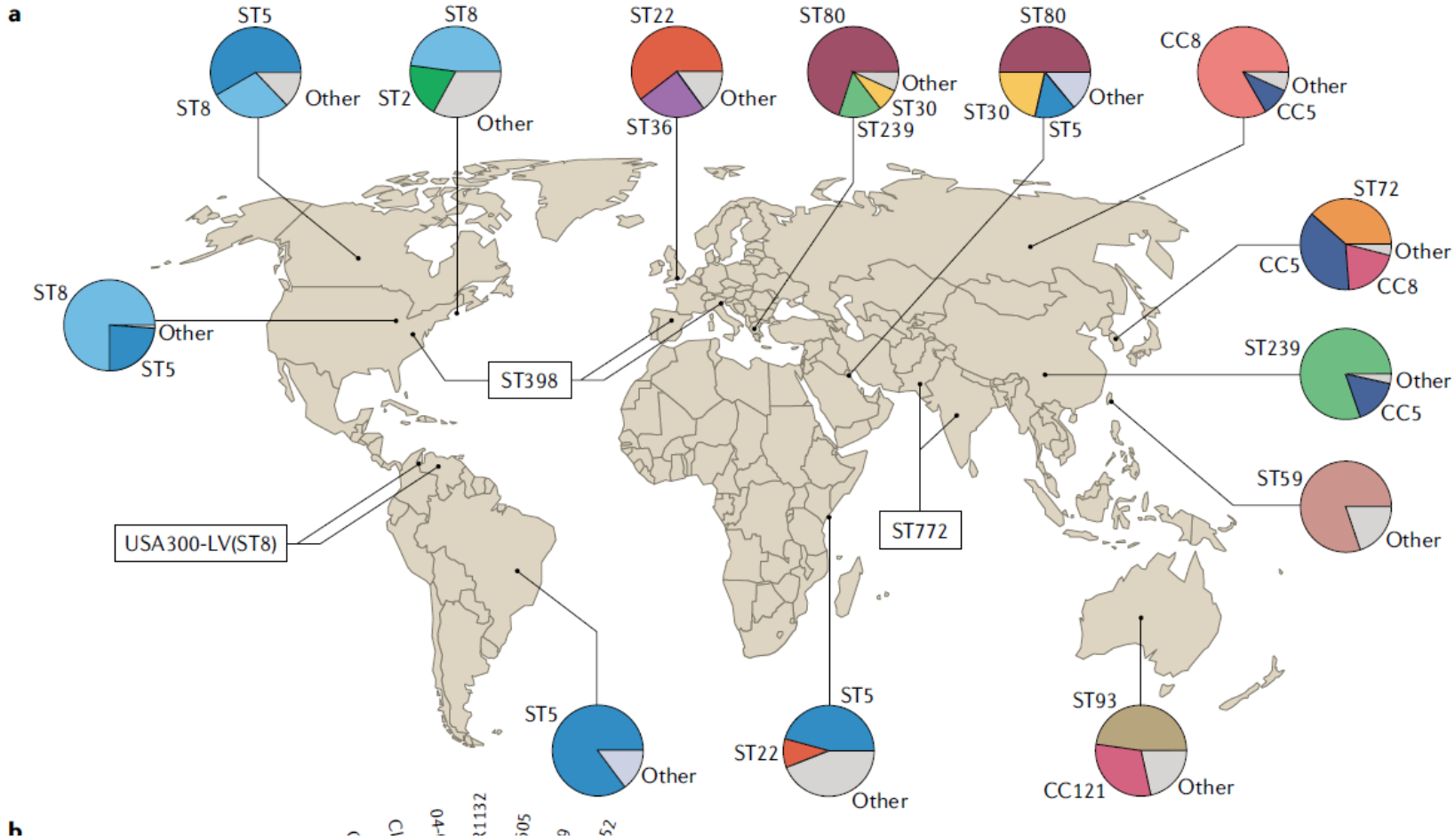
Nosocomial infection cost CZ: 8-9 billions CZK/year

Mortality: MRSA 2x higher

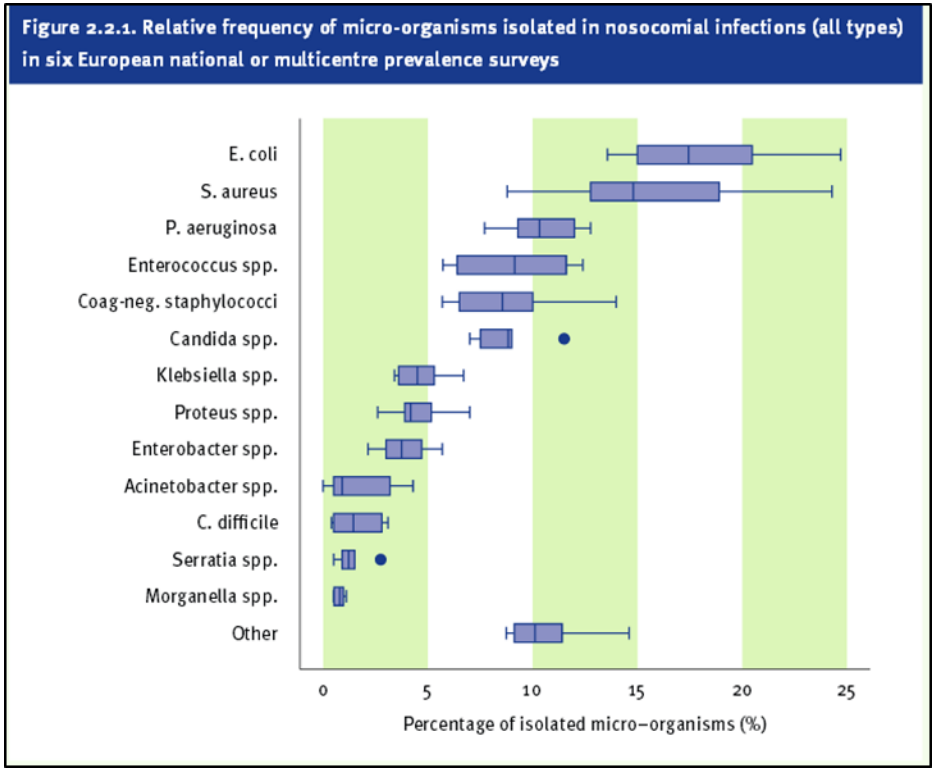
Global MRSA prevalence



There is not one MRSA – local clones



- MRSA according to their origin:
  - Healthcare-associated (HA-MRSA)
    - Multiple resistance
    - nosocomial or hospital infection
    - Major form of MRSA until 1996
    - Nosocomial pneumonia
    - Catheter related UTI
    - BSI
    - E.g. CC5, CC30
  - Community-associated (CA-MRSA)
    - Often only methicilin resistance
    - Severe SSTI and rare necrotizing pneumonia
    - Often PVL toxin
    - Growing prevalence
    - Children, adults and otherwise healthy people
    - Prevalent in USA and Asia
    - CC8 **(USA300)**, CC80, CC59



Cellular targets of drugs effective agains MRSA

Couple of new anti-MRSA drugs introduced recently

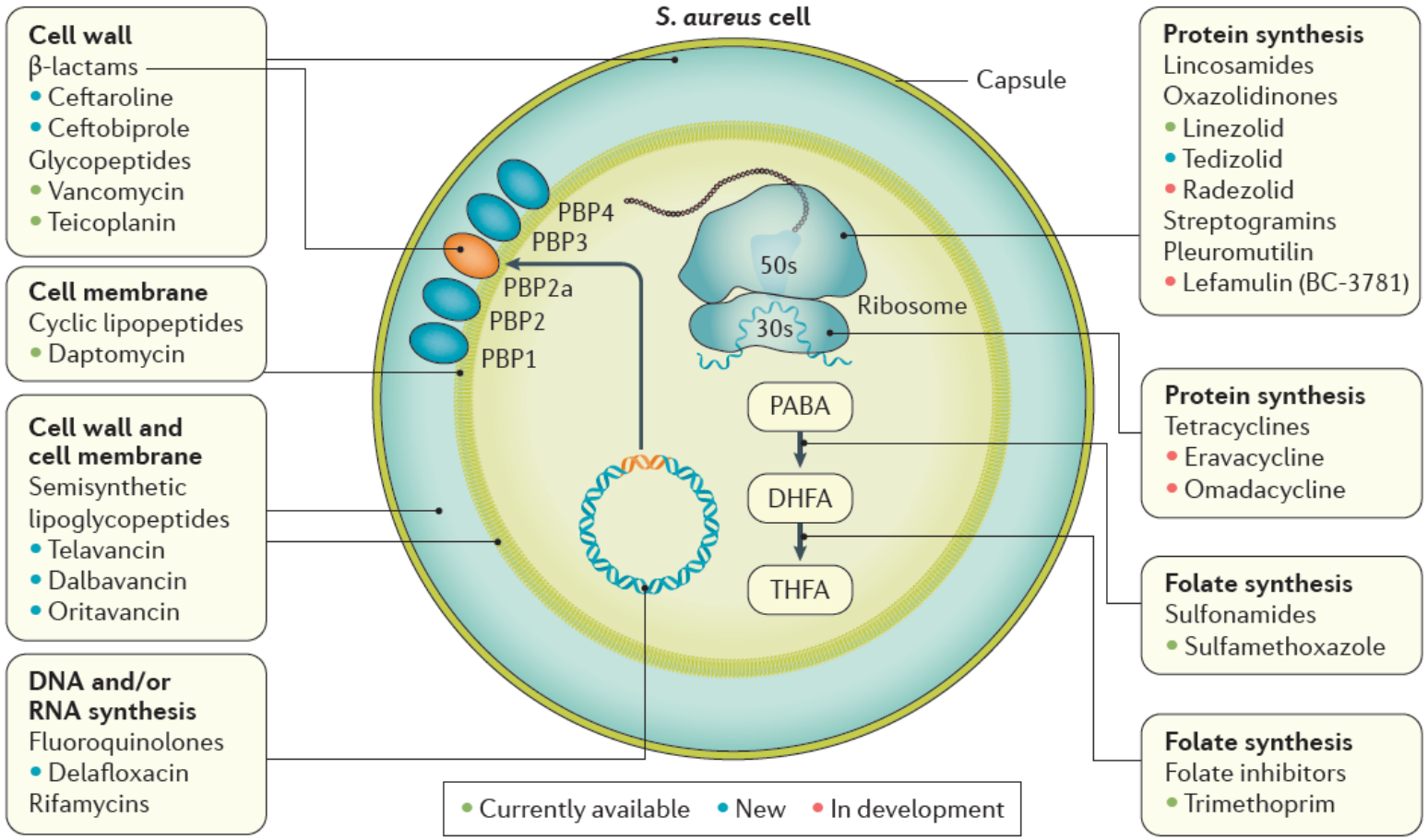
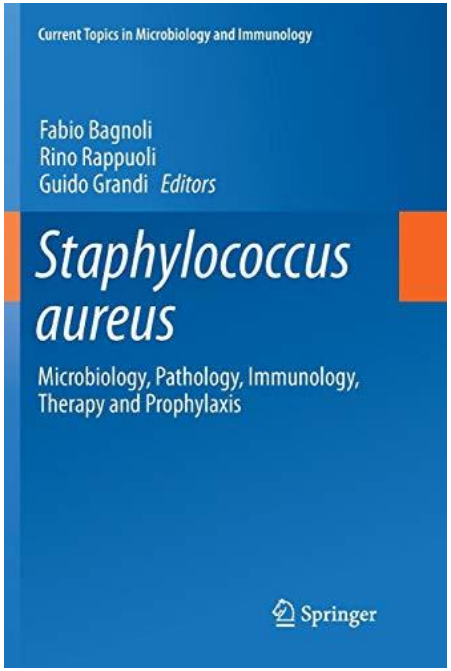
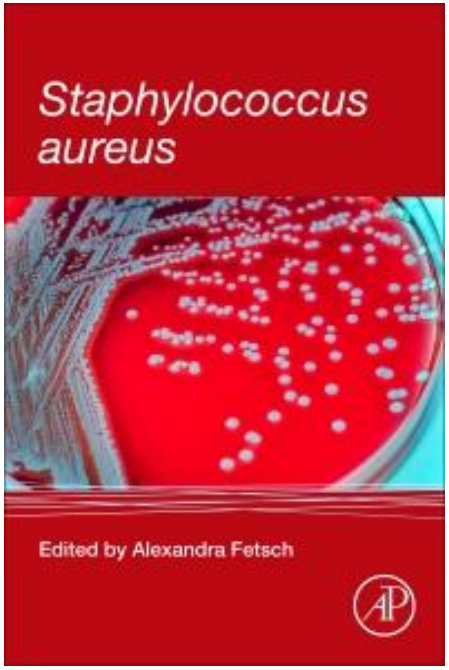
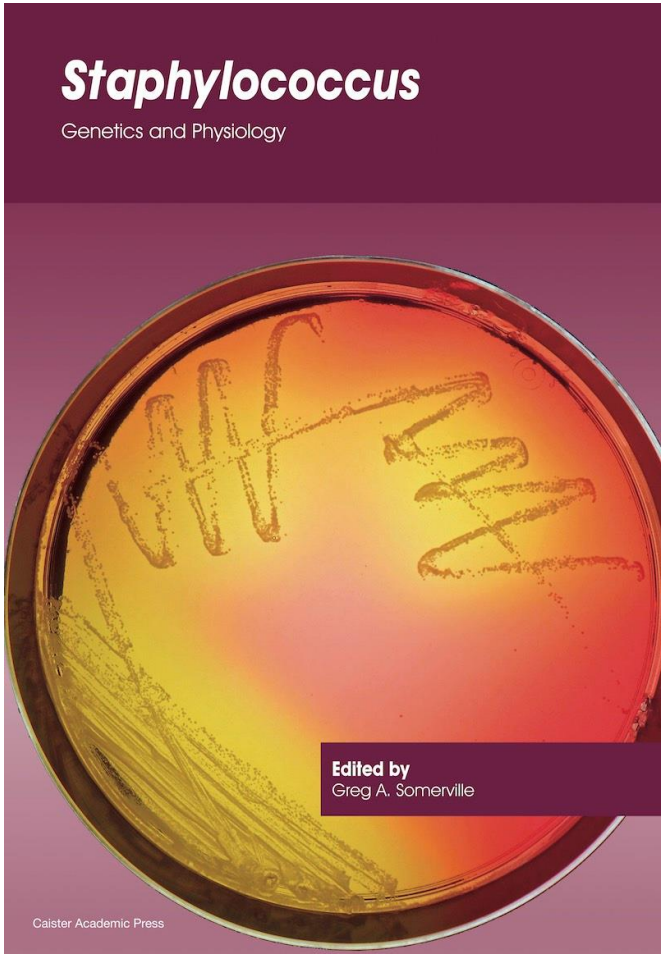


Figure 5 | **Bacterial targets of antibiotics active against MRSA.** Antibiotics have diverse mechanisms of action and target different bacterial structures or metabolic pathways. Existing antibiotic options are in green, new antibiotics approved and on the market are in blue and antibiotics in the pipeline are in orange. DHFA, dihydrofolic acid; PABA, para-aminobenzoic acid; PBP, penicillin-binding protein; *S. aureus*, *Staphylococcus aureus*; THFA, tetrahydrofolic acid. Figure adapted from REF.[229], Macmillan Publishers Limited.

Sources







Review *S. aureus* infections
https://cmr.asm.org/content/28/3/603.long
Review MRSA
https://www.nature.com/articles/nrdp201833
Guidelines for MRSA treatment
https://academic.oup.com/cid/article/52/3/e18/306145

Thank you for your attention!