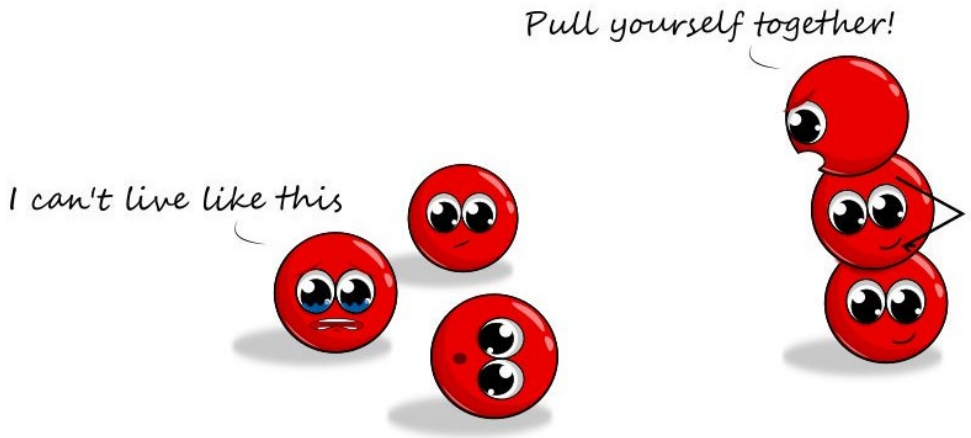
Streptococci and enterococci

Marcela Krutova



marcela.krutova@lfmotol.cuni.cz

FN MOTOL

Streptococcus spp. – general features

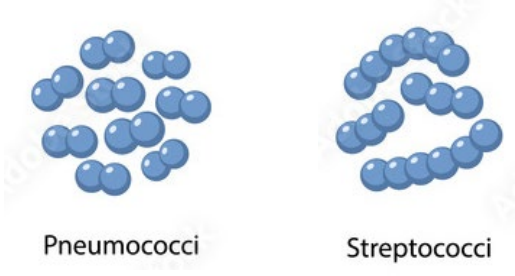
The genus Streptococcus is a diverse collection of **gram-positive cocci** typically arranged in **pairs** (diplococci) **or chains.**

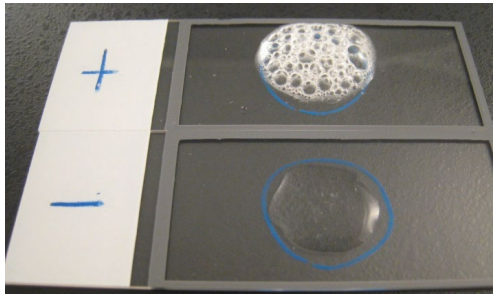
Most species are **facultative anaerobes** and some grow only in an atmosphere enhanced with carbon dioxide **(capnophilic growth).**

Complex nutrition requirements – serum or **blood-enriched media.**

Carbohydrates are fermented resulting in the production of lactic acid.

Unlike *Staphylococcus* species, Streptococci are **catalase-negative*.**





*Catalase test

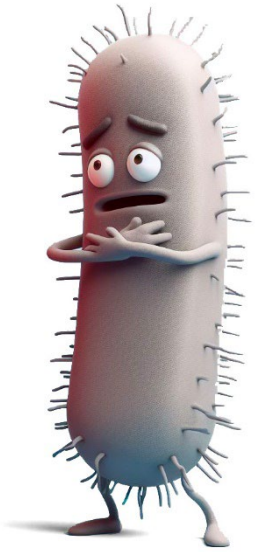
Catalase is an enzyme produced by microorganisms that live in oxygenated environments to **neutralize the bactericidal effects of toxic forms of oxygen metabolites such as hydrogen peroxide** (H_2O_2). The catalase enzyme protects aerobes and facultative anaerobes from oxidative damage.

- superoxide dismutase (SodA)
- thiolperoxidase (TpxD)
- alkyl hydroperoxidase (AhpD)

Streptococcus spp. – clinical relevance

Primary pathogens (colonisation increases risk of infection, high virulence, able to cause infection of the healthy host).

Opportunistic pathogens (requires lowered immunity/defence, lower virulence).



Commensals - part of the natural microflora /microbiota (oral cavity, intestine, skin, vagina...)

Interpretation of microbiological findings:
Identification of species, diagnosis of the patients, site of sampling, and clinical conditions of the patient.

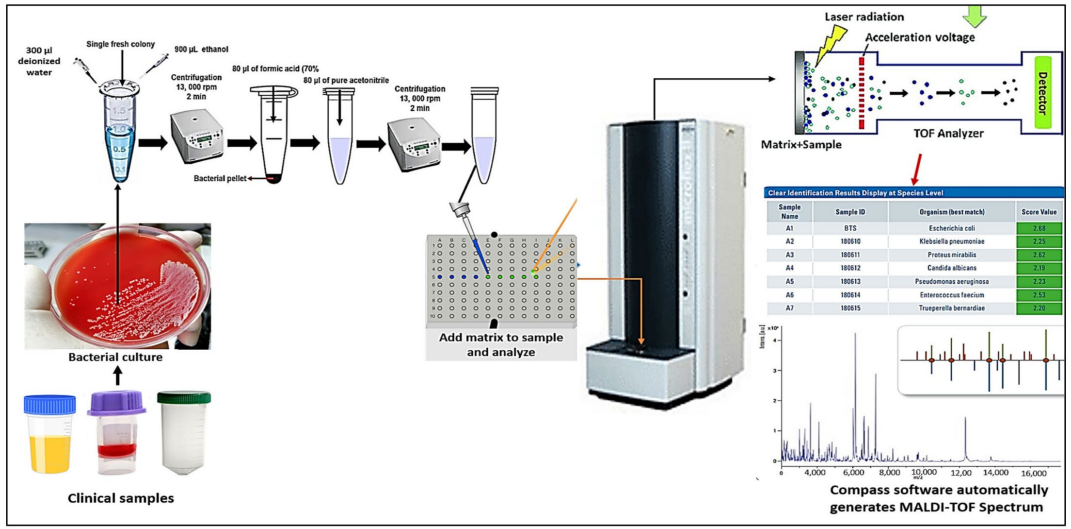
Differentiation between *Streptococcus pneumoniae* and other viridans group streptococci by MALDI-ToF

➢ R.Y. Yahiaoui et al. **2020**

496 *S. pneumoniae* and 83 *non-S. pneumoniae*
495 of 496 *S. pneumoniae* isolates were identified as *S. pneumoniae* and one isolate was identified as *non-S. pneumoniae*
Of the **83 *non-S. pneumoniae* isolates**, 37 were correctly identified as *non-S. pneumoniae*, and **46 isolates as *S. pneumoniae***.
MALDI-TOF MS sensitivity was 99.8% (95% confidence interval (CI) 98.9-100) and **the specificity** was **44.6%** (95% CI 33.7-55.9).

➢ T. Wan et al., **2023**

A total of 103 nonpneumococcal VGS and 29 *S. pneumoniae* blood isolates at a medical center in northern Taiwan
-3 different machines, **none of the nonpneumococcal VGS isolates were misidentified as pneumococci by the latest Biotyper system 4.1, and vice versa**. a specific gene sequencing test is still needed to precisely differentiate the species of strains **in the *S. mitis* and *S. bovis* group**.



Workflow of MALDI-ToF, Elbehiry, A et al., 2022.

References: Wan T, Lee T, Chen X, Hunag Y, Teng L, Hsueh P, Chiu H. 2023. Performance assessment of the Bruker Biotyper MALDI-TOF MS for the identification of difficult-to-identify viridans group streptococci. J Clin Microbiol 61:e01143-23. https://doi.org/10.1128/jcm.01143-23
Yahiaoui RY, Goessens WH, Stobberingh EE, Verbon A. Differentiation between *Streptococcus pneumoniae* and other viridans group streptococci by matrix-assisted laser desorption/ionization time of flight mass spectrometry. Clin Microbiol Infect. 2020 Aug;26(8):1088.e1-1088.e5. doi: 10.1016/j.cmi.2019.11.024.
Elbehiry, A.; Aldubaib, M.; Abalkhail, A.; Marzouk, E.; ALbeloushi, A.; Moussa, I.; Ibrahem, M.; Albazie, H.; Alqarni, A.; Anagreyyah, S.; et al. How MALDI-TOF Mass Spectrometry Technology Contributes to Microbial Infection Control in Healthcare Settings. *Vaccines* **2022**, *10*, 1881. https://doi.org/10.3390/vaccines10111881.

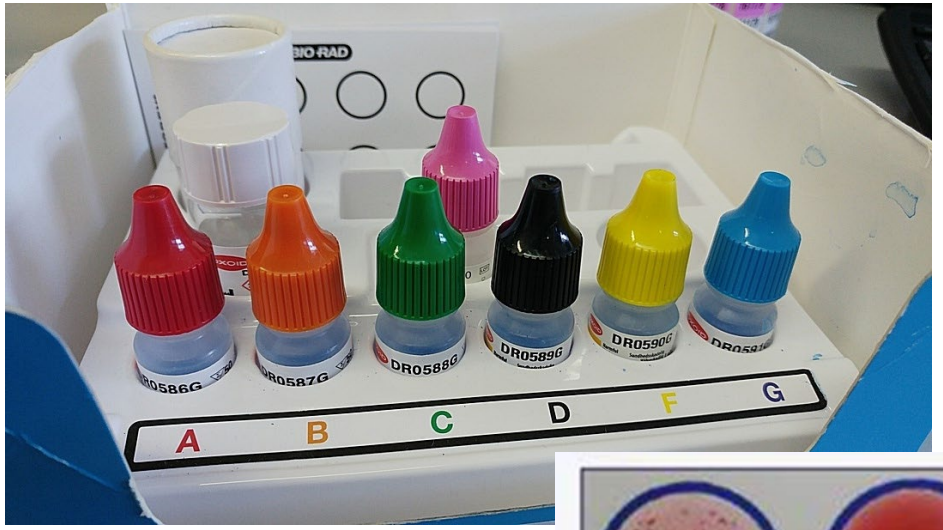
Classification of common Streptococci

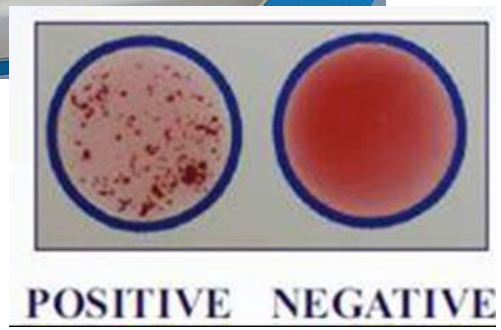
Biochemical Classification	Serological classification*	Hemolysis patterns
S. pyogenes (bacitracin **S,** PYR test)	A	β
S. agalactiae (hippurate hydrolysis, CAMP test)	B	β; occasionally nonhemolytic (ϒ)
S. dysgalactiae	C, G	β
S. anginosus group	Nongroupable (reports C, F, G)	β; occasionally α or nonhemolytic (ϒ)
S. bovis	D	nonhemolytic (ϒ) ; occasionally α; β
Viridans group	Nongroupable	α or nonhemolytic (ϒ)
S. pneumoniae (optochin **S**, bile solubility)	Nongroupable	α

- Rebecca Lancefield, serological classification scheme based on group-specific antigens (cell wall carbohydrates), 1933.
- Other groups H….Z are mostly not clinically important

Adapted from Murray et al., Medical Microbiology, 5th edition

Serological classification and haemolysis patterns





Lancefield grouping is based on the carbohydrate composition of bacterial antigens found on their cell walls. The test utilizes latex particles sensitized with group-specific antibodies which agglutinate in the presence of homologous antigens.

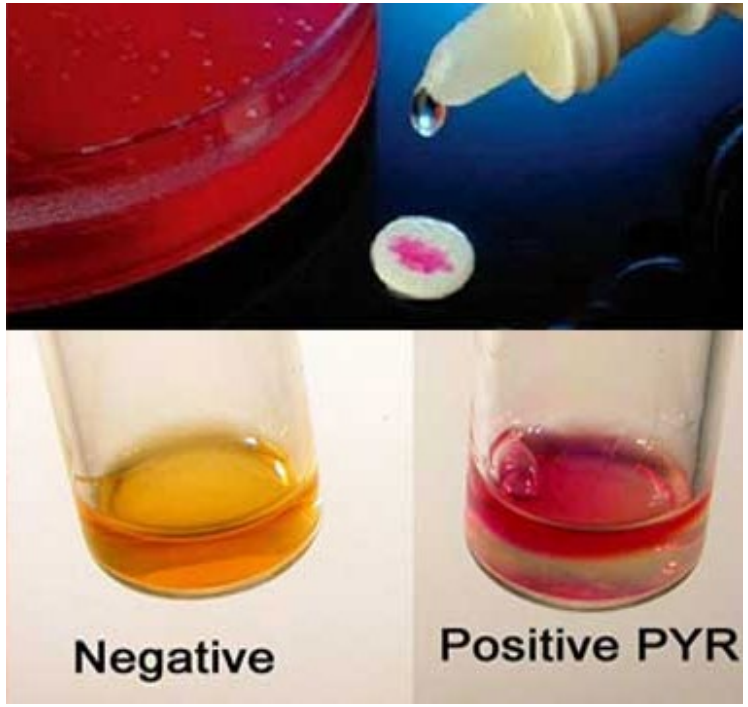
The ability of bacterial colonies to induce haemolysis when grown on blood agar. Haemolysis is caused by **haemolysin**.

β – full (complete) haemolysis

α - *incomplete haemolysis* and *partial haemolysis (green)*

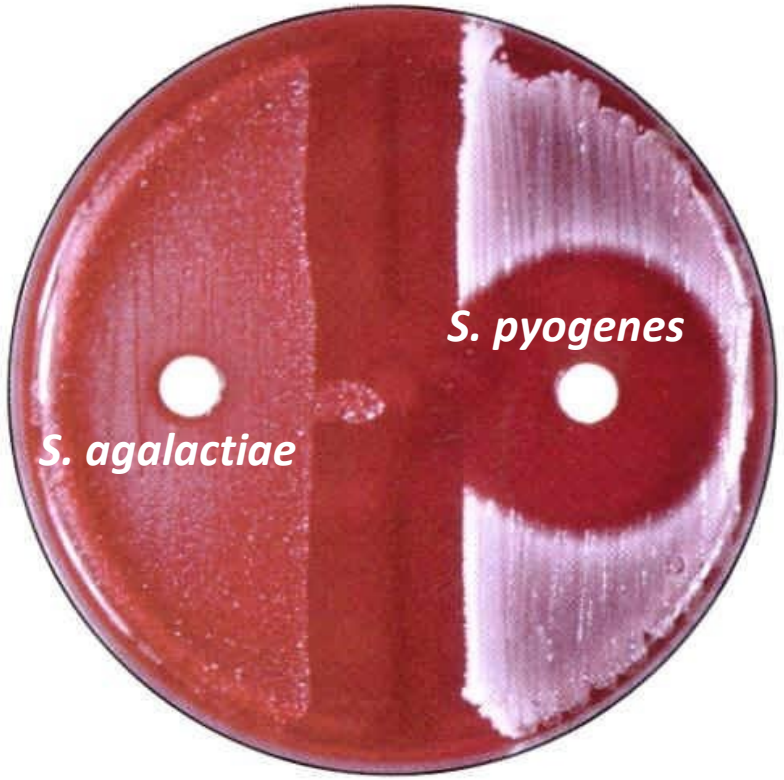
ϒ – no haemolysis, *non-haemolytic*

Other tests for differentiation of streptococci



PYR Test

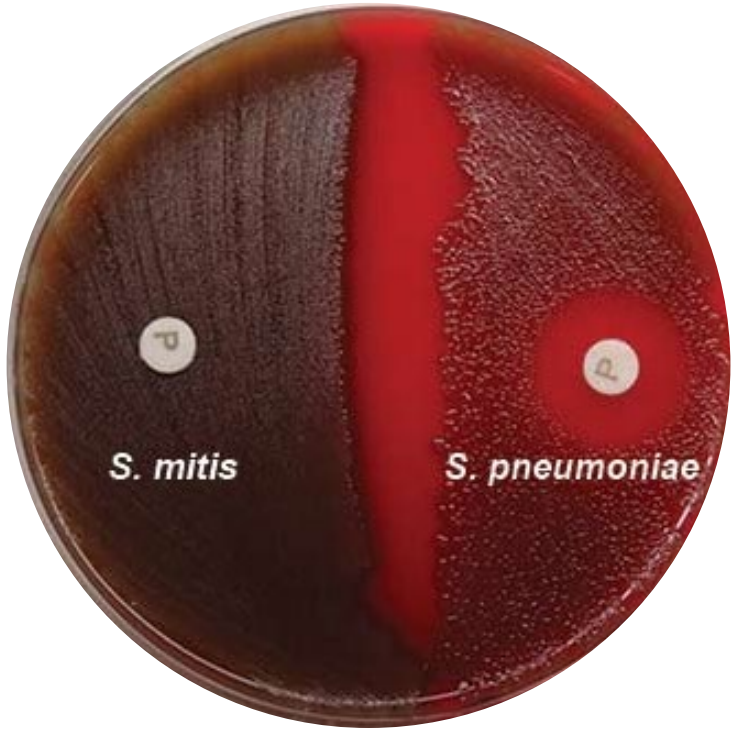
detection of **pyrolidonyl arylamidase** activity in ***Streptococcus pyogenes*, *Enterococcus* spp.** Free b-napthylamide is then detected by the addition of the diazo dye complex, N , Ndimethylaminocinnamaldehyde. The development of a red colour is indicative of PYR hydrolysis.



Bacitracin Susceptibility test

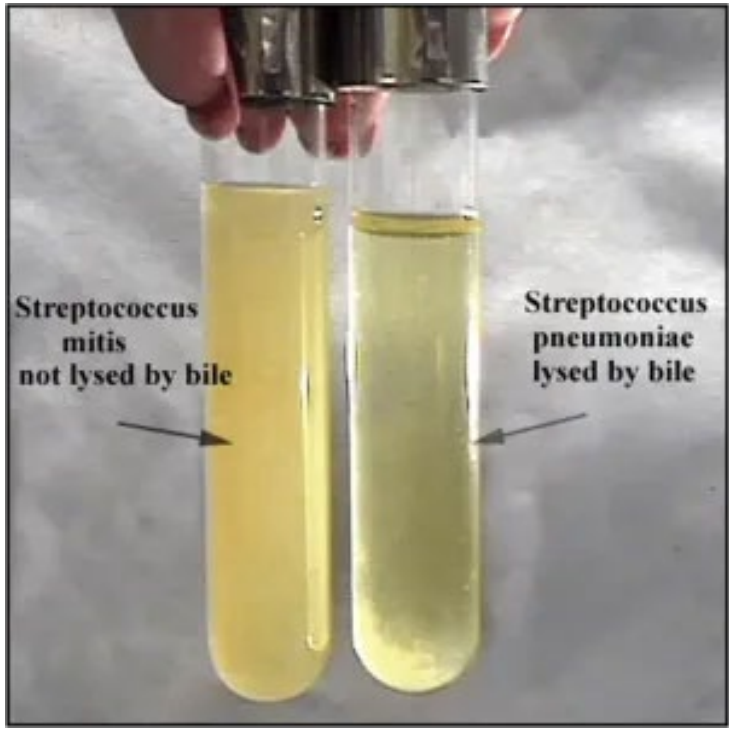
Bacitracin is a polypeptide antibiotic. Differentiation of beta-haemolytic group A streptococci (*Streptococcus pyogenes*- susceptible) from other beta-haemolytic streptococci).

Other tests for differentiation of streptococci



Optochin susceptibility test

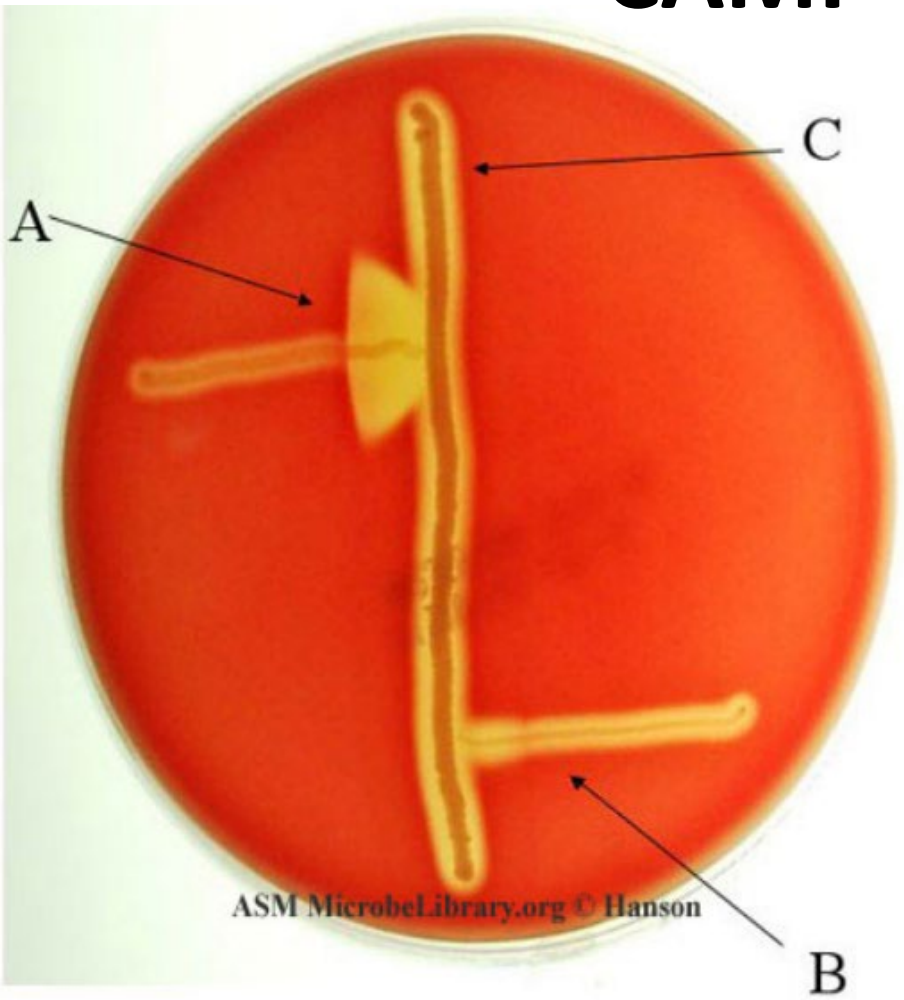
Optochin is a derivative of quinine, an antimalarial agent, but it is not used for *S. pneumoniae* treatment. Differentiation of *S. pneumoniae* among other α haemolytic *Streptococci* (viridans *Streptococci*)



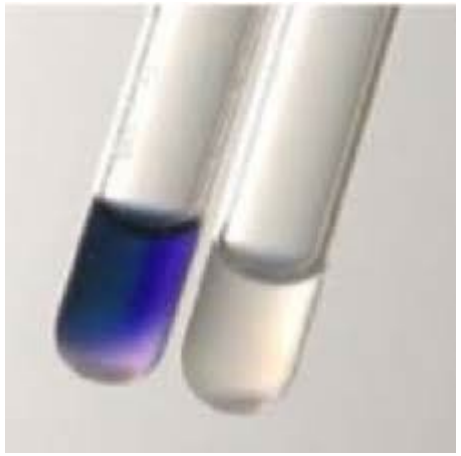
Bile salts solubility test

Bile salts, specifically sodium deoxycholate and sodium taurocholate, **can autolyse *Streptococcus pneumoniae*** selectively when added to actively growing bacteria in agar or broth media. Deoxycholate (bile) **activates autolysin** in the cell wall of pneumococci.

CAMP* test – *S. agalactiae*



The β-lysin produced by β-hemolytic *Staphylococcus aureus* acts synergistically with the CAMP factor (diffusible, heat-stable protein, a pore-forming toxin) produced by both β-haemolytic and nonhemolytic ***Streptococcus agalactiae* (group B)**. This synergistic reaction results in an enhanced and very visible zone of hemolysis in the region between the two cultures. The synergistic zone is **NOT** observed in group A, C, and G Streptococcus



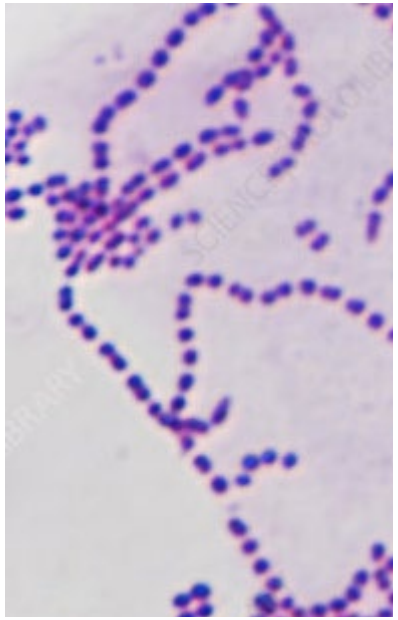
Hippurate hydrolysis

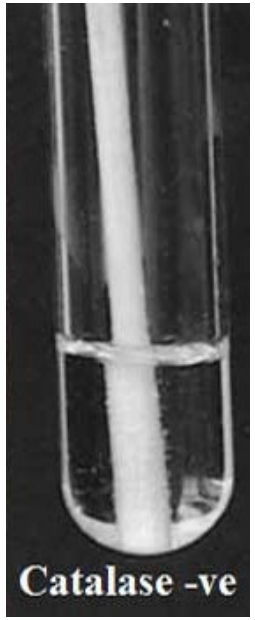
The ability of the organism
to hydrolyze sodium hippurate
to benzoic acid and glycine by
the action of the enzyme hippuricase

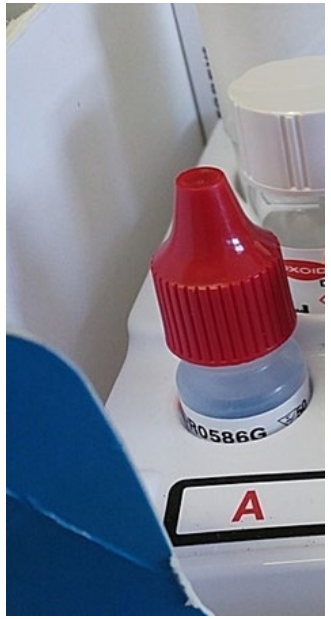
FIG. 2. CAMP test for the identification of ***Streptococcus agalactiae*** (group B). (A) ***Streptococcus***(group B) shows a positive CAMP reaction. (B) ***Streptococcus pyogenes*** (group A) shows a negative reaction when inoculated at a right angle to (C) ***Staphylococcus aureus***.

*Christie, Atkins, Munch-Petersen

Streptococcus pyogenes (GAS)



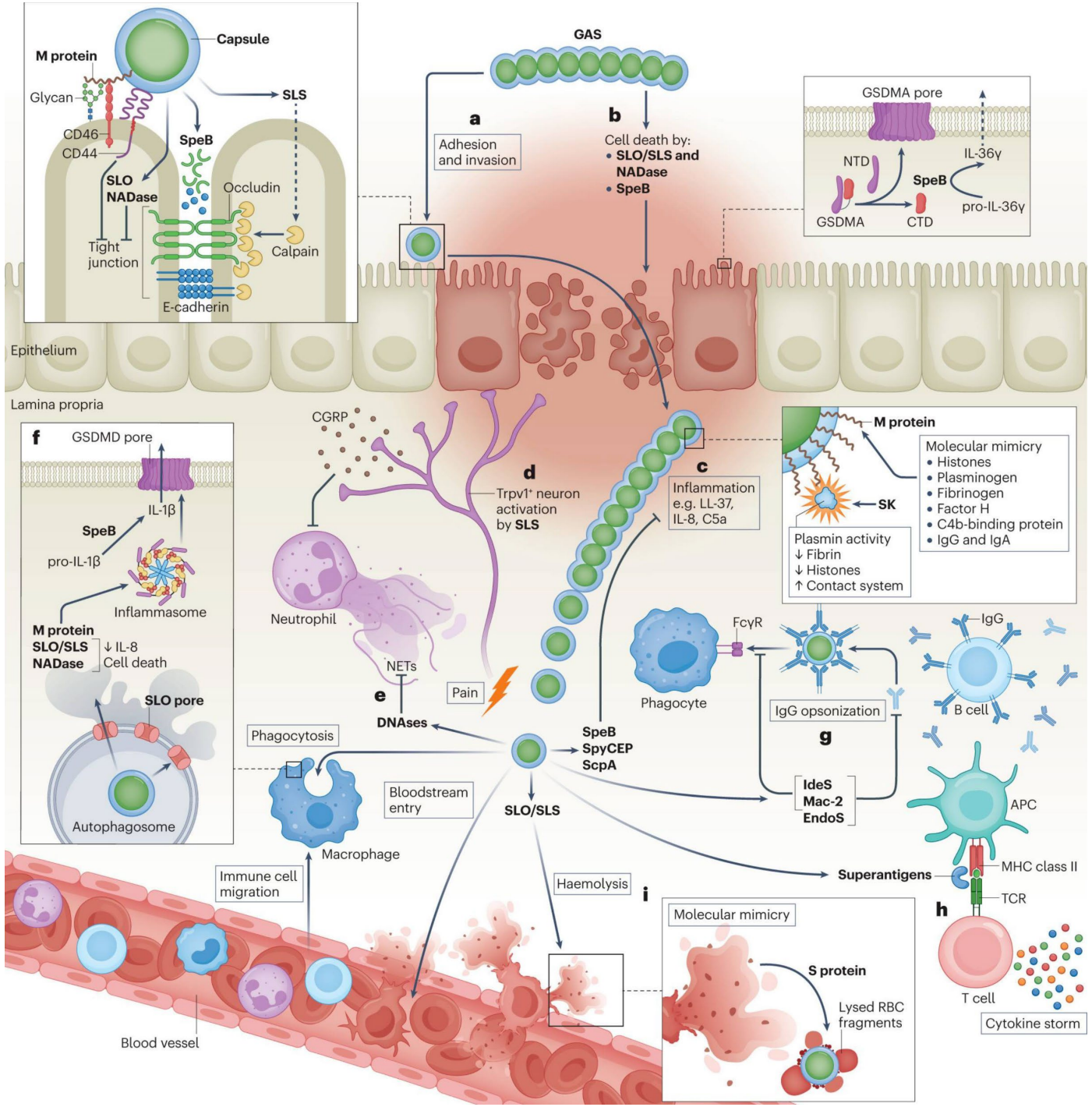




Primary human pathogen

Gram-positive coccus, arranged in pairs or long chains (liquid media)

Facultative anaerobe (CO_2 thermostat), catalase-negative, β-haemolytic, group A, PYR positive, bacitracin sensitive.



S. pyogenes: virulence factors

Adhesion (capsule and M protein)

Invasion (streptolysins (SLS and SLO, deoxyribonuclease)

Immune system escape (capsule, the same structure like human hyaluronic acid; M protein binds host factors; S protein binds the membranes of erythrocytes)

Brouwer S, et al. Nat Rev Microbiol. 2023. PMID: 36894668

S. pyogenes virulence factors

M –protein: adhesin, antiphagocytic, degradation of complement component C3b

CDC Centers for Disease Control and Prevention
CDC 24/7: Saving Lives, Protecting People™

Streptococcus Laboratory

The *emm* typing – part of the gene for M protein is used for typing in reference laboratories (more than 200 *emm* types)

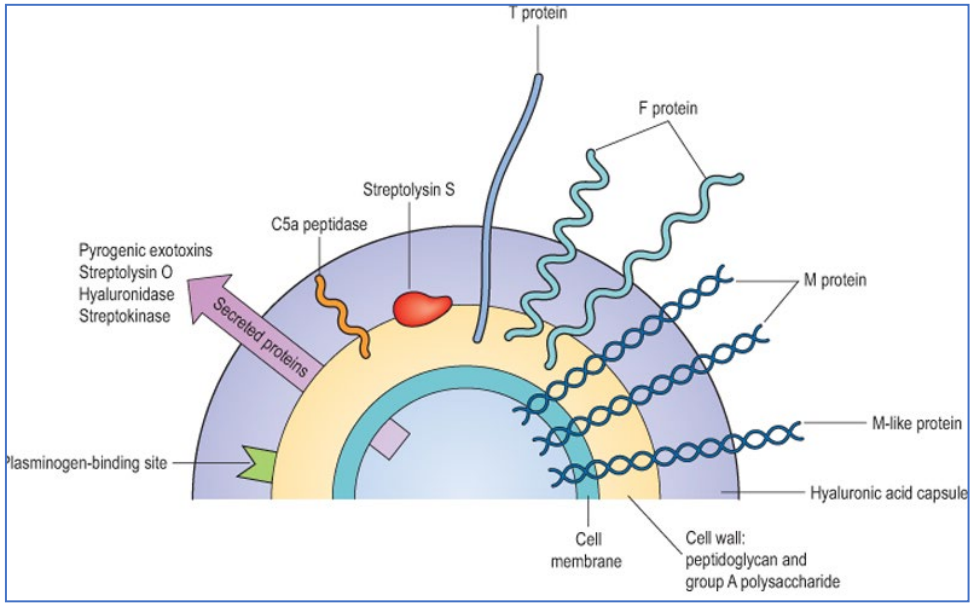
Capsule: antiphagocytic

Pyrogenic exotoxins: mediate pyrogenicity, cytotoxicity, nonspecific mitogenicity for t-cells, immunosuppression of B-cell function, production of scarlatiniform rash

Streptolysins S, O: lyses leucocytes, platelets, and erythrocytes, stimulates the release of lysosomal enzymes. S-nonimmunogenic. ASLO test: anti-streptolysin O antibodies (recent StrepA infection).

F protein: adherence to epithelial cells

C5a peptidase: degradation of complement component C5a



Streptococcus pyogenes – clinical diseases

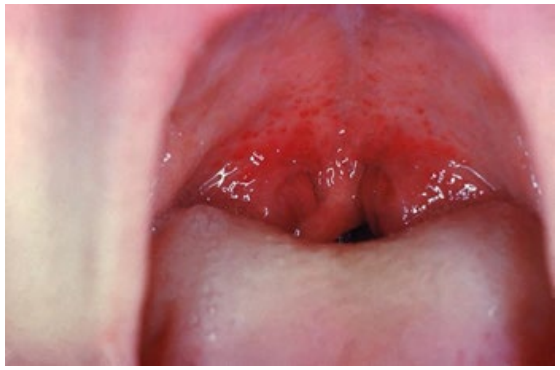
Pharyngitis – reddened pharynx with exudates generally present, cervical lymphadenopathy can be prominent

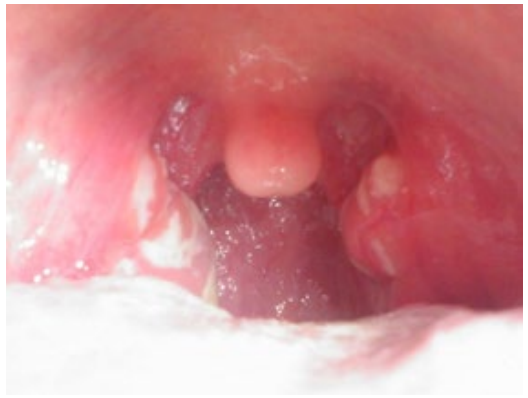
Tonsilitis- sore throat, fever and swollen lymph nodes

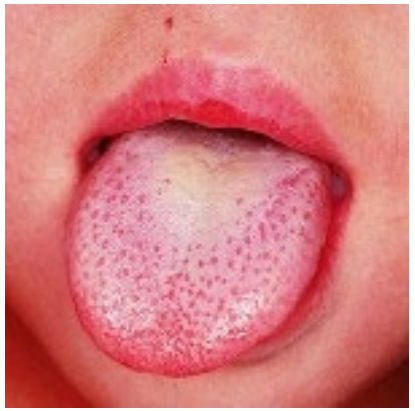
Tonsillopharyngitis

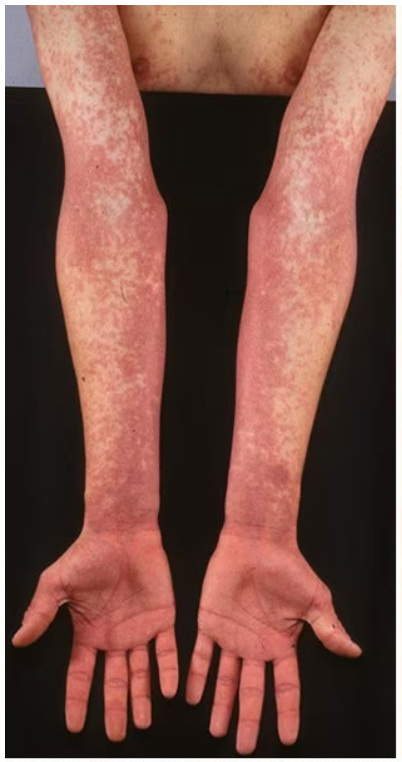
Scarlet fever - diffuse erythematous rash beginning on the chest and spreading to the extremities, strawberry tongue
- the complication of streptococcal pharyngitis

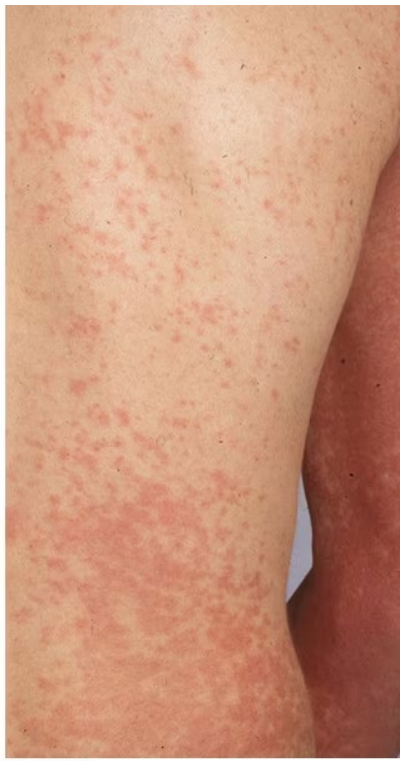
Peritonsillar abscesses



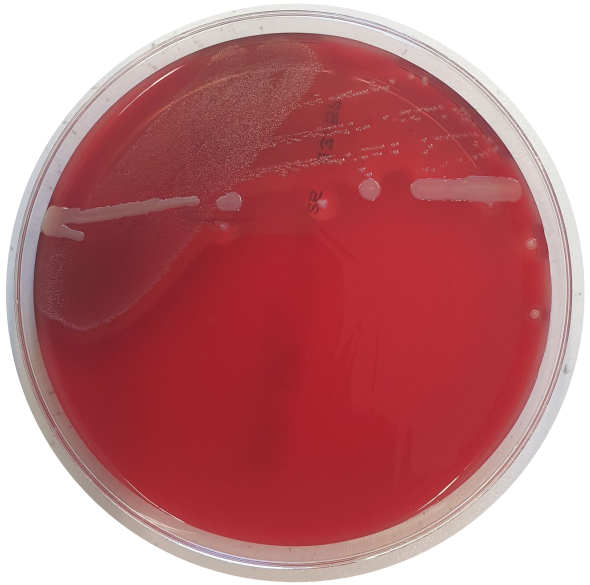








S. pyogenes respiratory infections- laboratory diagnostics



Swab: culture

Antimicrobial susceptibility testing

Antigen detection

Sensitivity and specificity 86% (95% CI 83 to 88%) and 96% (95% CI 94% to 97%)

PCR detection (expensive)

Sensitivity of 97.5% (95% CI 96.2%–98.3%) and a specificity of 95.1% (95% CI 93.6%–96.3%

Lean WL, Arnup S, Danchin M, Steer AC. Rapid diagnostic tests for group A streptococcal pharyngitis: a meta-analysis. Pediatrics. 2014 Oct;134(4):771-81. doi: 10.1542/peds.2014-1094. Dubois C, Smeesters PR, Refes Y, Levy C, Bidet P, Cohen R, Chalumeau M, Toubiana J, Cohen JF. Diagnostic accuracy of rapid nucleic acid tests for group A streptococcal pharyngitis: systematic review and meta-analysis. Clin Microbiol Infect. 2021 Dec;27(12):1736-1745. doi: 10.1016/j.cmi.2021.04.021.

Streptococcus pyogenes – clinical diseases

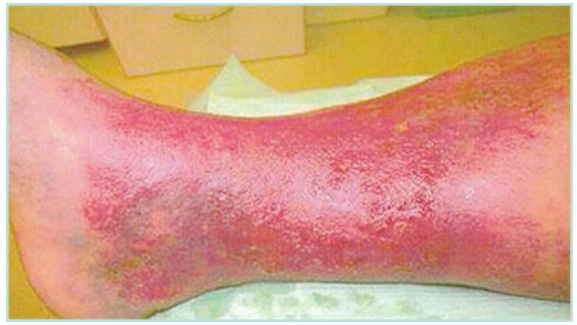
Pyoderma (impetigo) – localised skin infection with vesicles progressing to pustules; no evidence of systemic disease

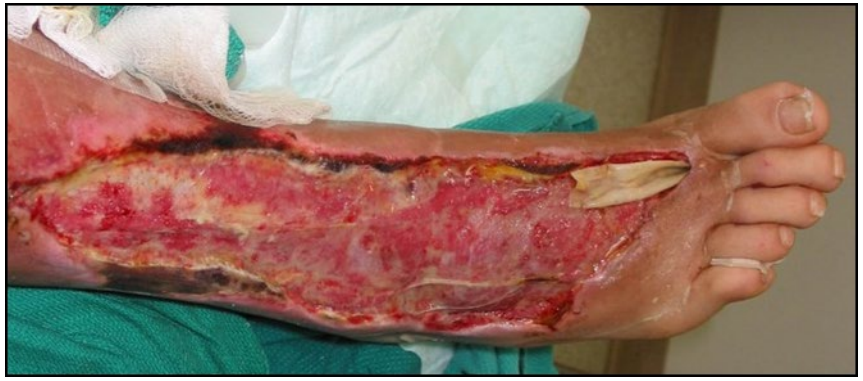
Erysipelas – localised skin infection with pain, inflammation, lymph node enlargement and systemic symptoms

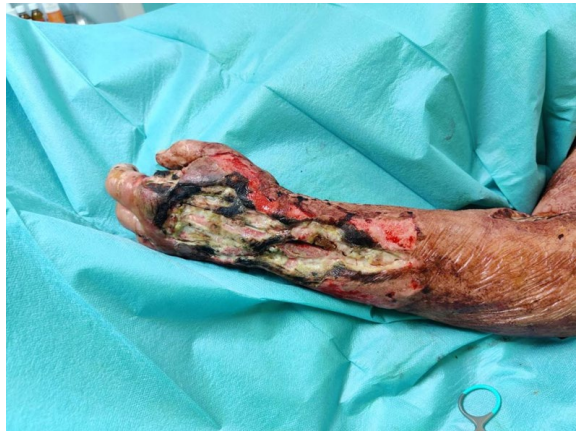
Cellulitis – infection of the skin which involves subcutaneous tissues

Necrotizing fasciitis – deep infection of the skin which involves the destruction of muscle (myositis, myonecrosis) and fat layers

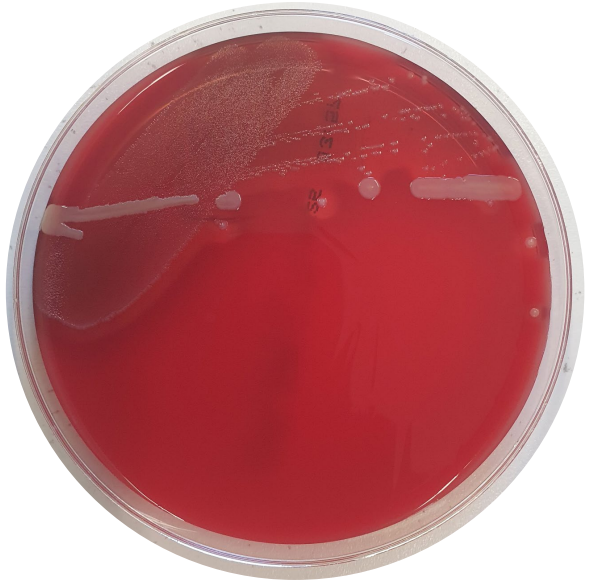
Streptococcal toxic shock syndrome – multiorgan systemic infection resembling staphylococcal toxic shock syndrome –massive activation of immune response, cytokine storm







S. pyogenes skin/soft tissue infections- diagnostics



Culture and Antimicrobial susceptibility testing: Swab, tissue, pus, purulent lesion.

Blood cultures: fever, chills.

Pan bacterial PCR (16S rDNA): necrotizing fasciitis, culture-negative tissues.

SEPSITEST™-UMD CE IVD

molzym
reveal DNA

CULTURE-INDEPENDENT MOLECULAR DETECTION OF PATHOGENS

SepsiTest™-UMD is a CE IVD molecular diagnostic test for the *in vitro* diagnosis of pathogens from clinical samples without the need for culture. **SepsiTest™-UMD** is based on a single protocol, including human DNA depletion (MolYsis™), microbial DNA enrichment and extraction from intact bacteria & fungi, followed by 16S and 18S rDNA broad-range PCR and sequencing analysis. With this broad approach and the capability to detect even rare, fastidious and non-growing pathogens, it is the perfect solution to complement standard culture methods in routine diagnostic laboratories.

Streptococcus pyogenes – non-suppurative infections Post-Streptococcal Autoimmune Sequelae

Rheumatic fever – inflammatory changes of the heart (pancarditis), joints (arthralgitis to arthritis), blood vessels and subcutaneous tissues
cross-reactivity of antibodies against some streptococcal antigens
After streptococcal pharyngitis

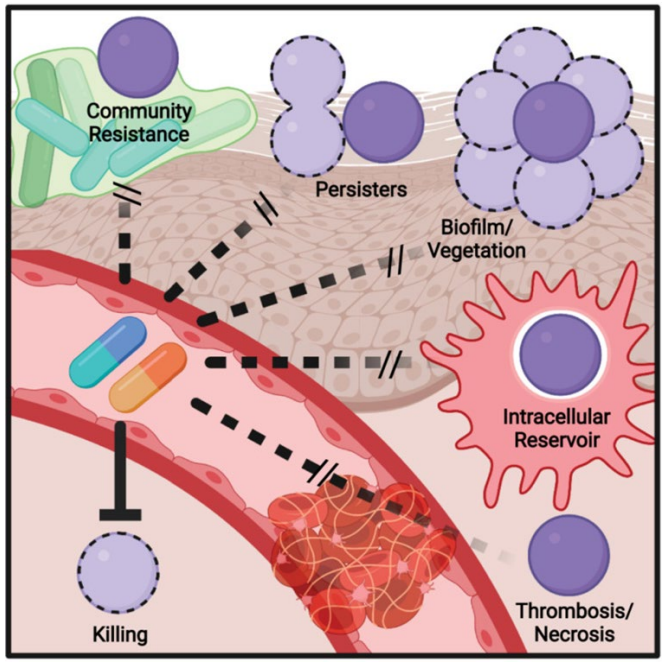
Acute glomerulonephritis – acute inflammation of the renal glomeruli with edema, hypertension, hematuria and proteinuria
Immunocomplex deposits in glomeruli - impaired renal function
After respiratory tract or skin infection - Nephritogenic types (protein M)

Detection of antistreptolysin O (ASLO, ASO) antibodies

S. pyogenes infections- treatment

- Penicillins: resistance not reported
- Macrolides (erythromycin) and lincosamides (clindamycin): second line, but growing resistance(15%)
- Severe infection: combination of penicillin and clindamycin (linezolid)
- No vaccine so far but M-protein-based vaccines are in development

Treatment failure?



Resistance mediated by other bacteria present? More likely in pharyngitis than iGAS.

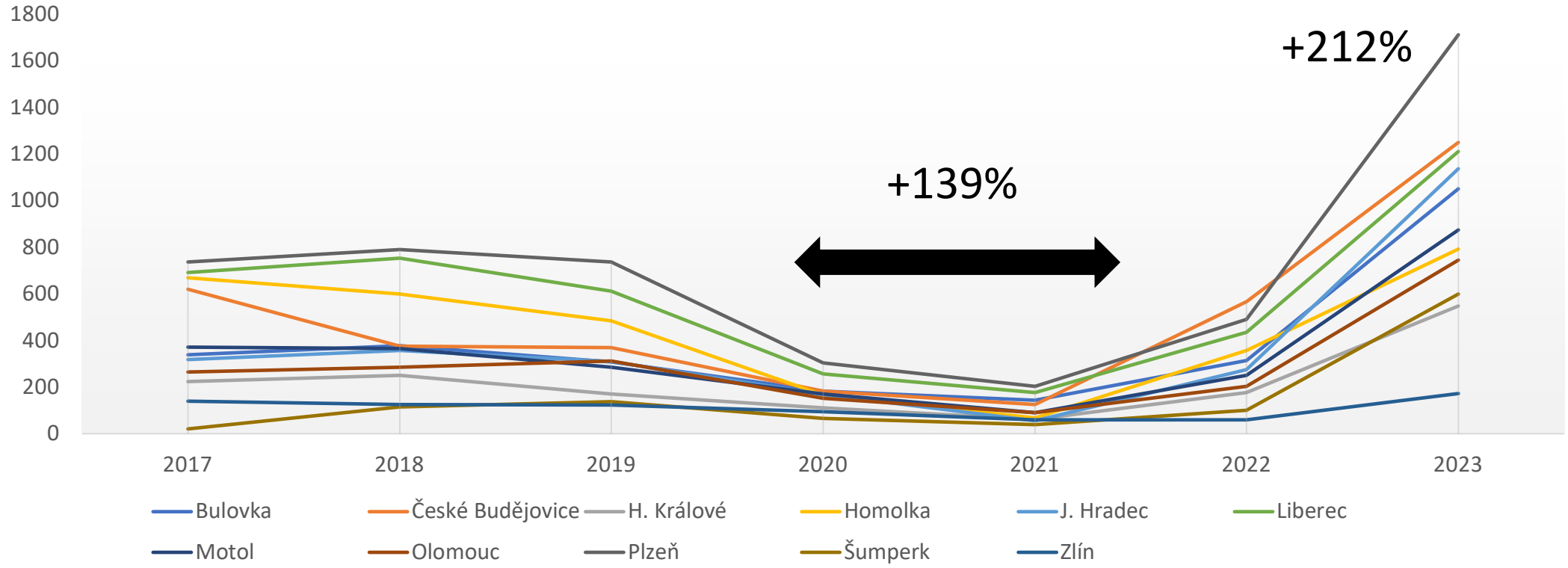
Persistence? Altered growth rate, if the cell is not dividing or is not metabolically active then ATB does not work.

Biofilm formation, invasion of epithelial cells, survival in phagocytes?

In iGAS, tissue necrosis, inflammation and thrombosis of skin vessels may interfere with antibiotic penetration, requiring repeated surgical revisions.

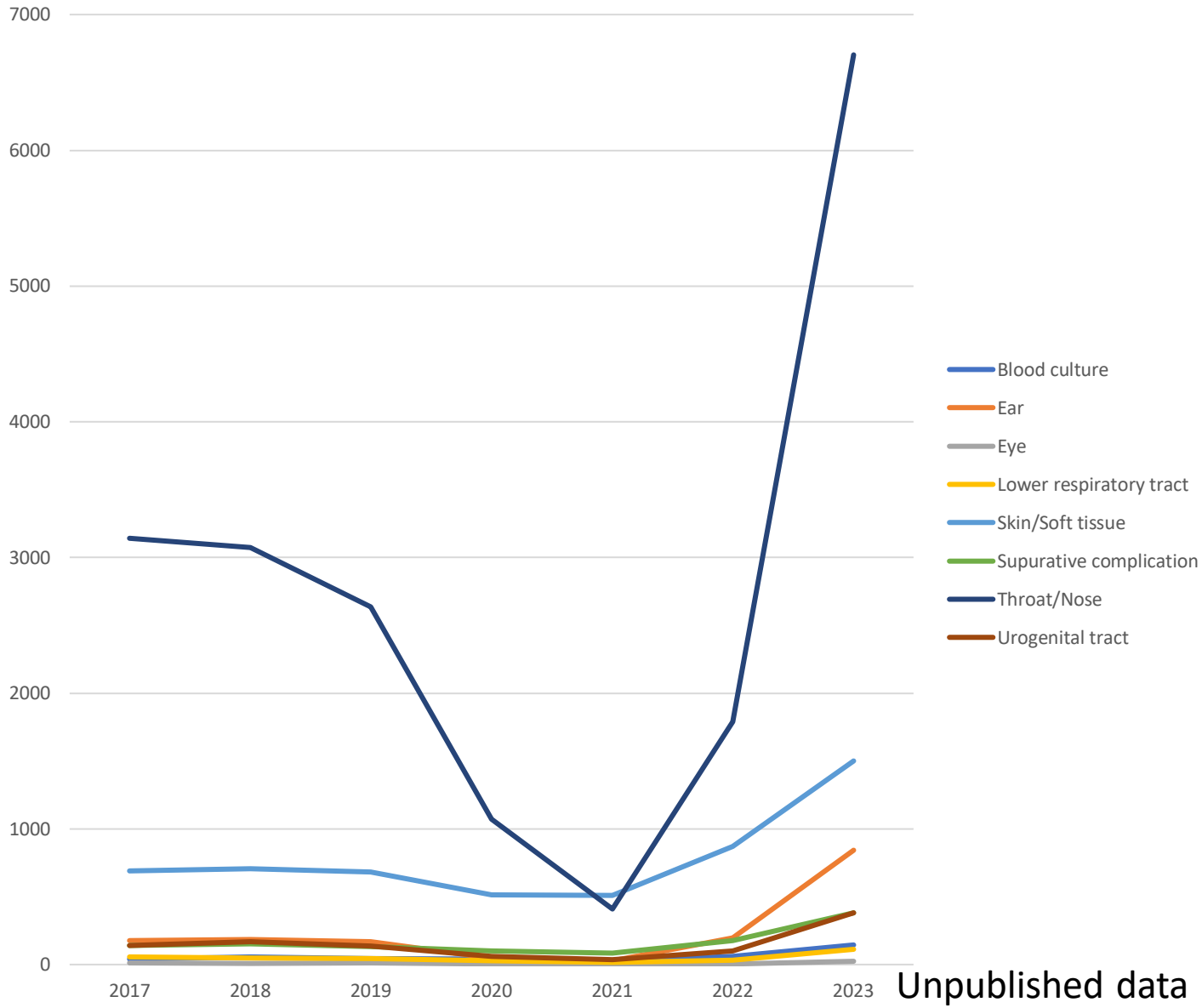
Johnson AF, LaRock CN. doi: 10.3389/fmicb.2021.760255.

Streptococcus pyogenes: culture positivity



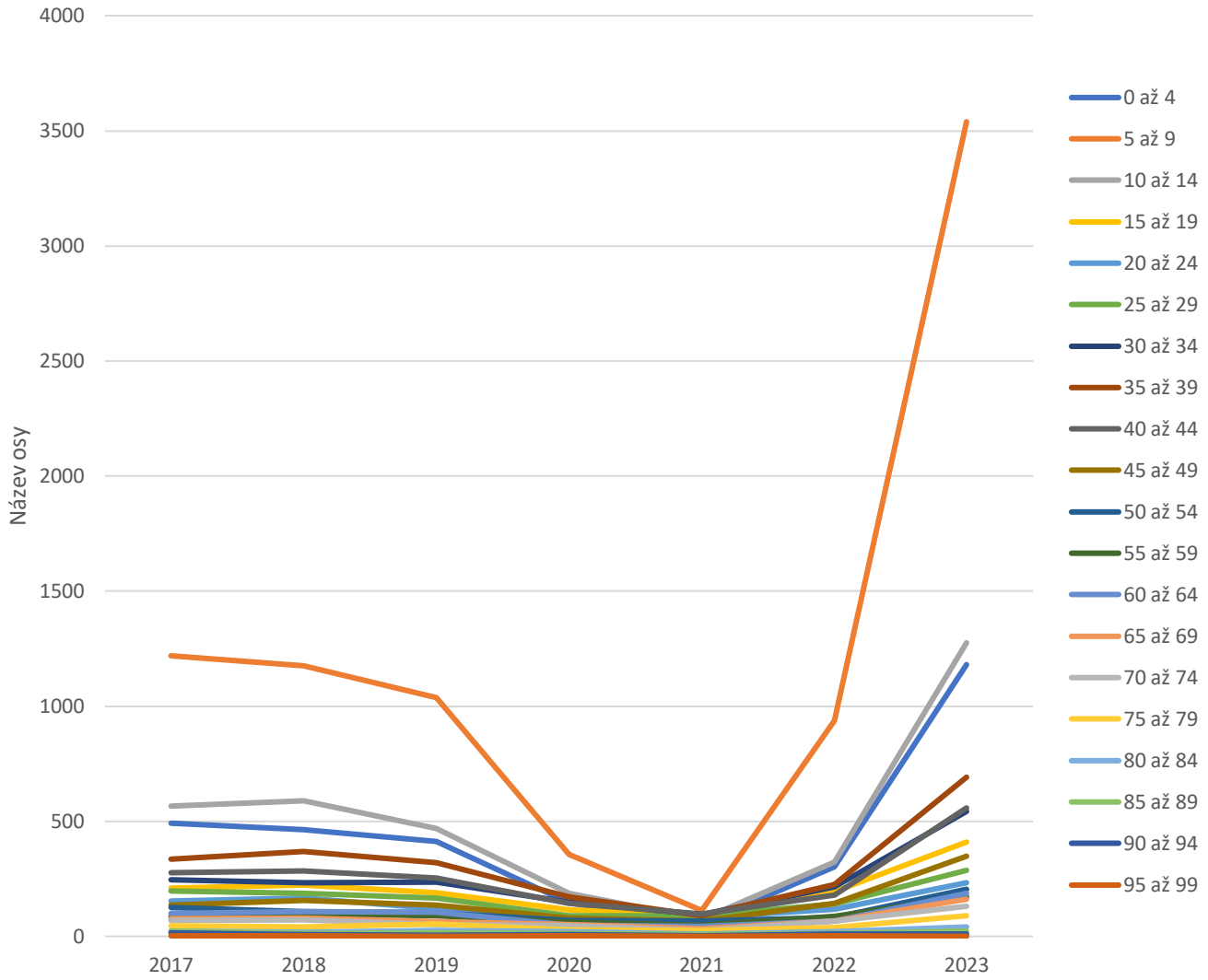
Unpublished data

Streptococcus pyogenes: culture positivity, localisation



Unpublished data

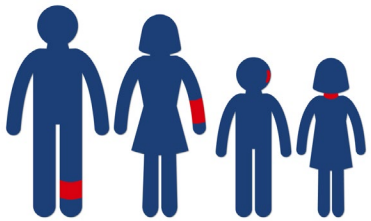
Streptococcus pyogenes: culture positivity - age

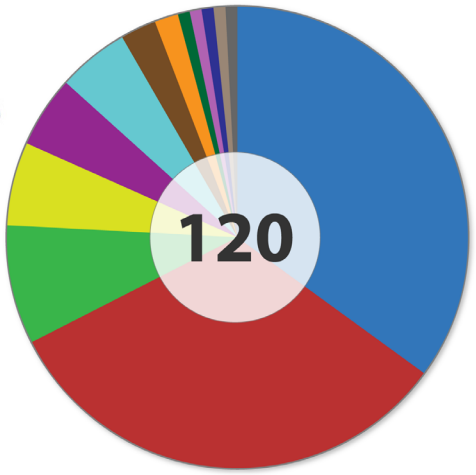


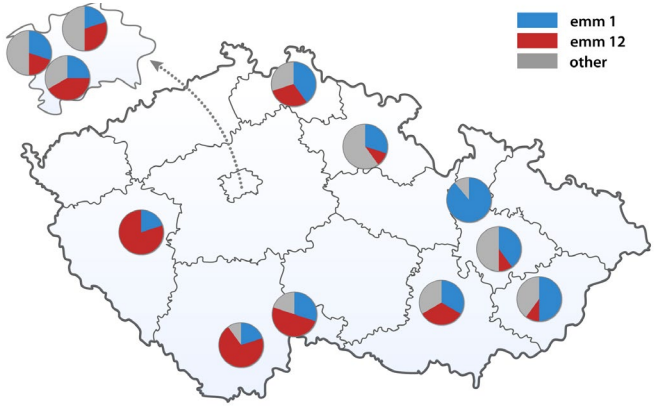
Unpublished data

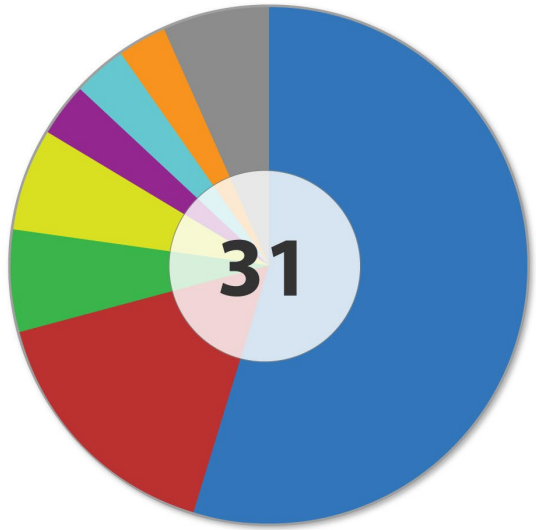
Streptococcus pyogenes – characterisation of isolates

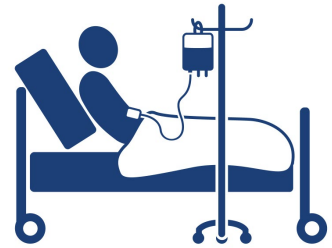
✓emm typing (sequence M protein gene)

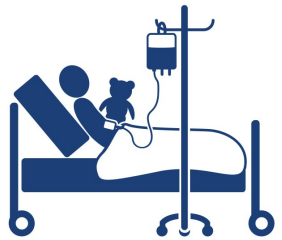








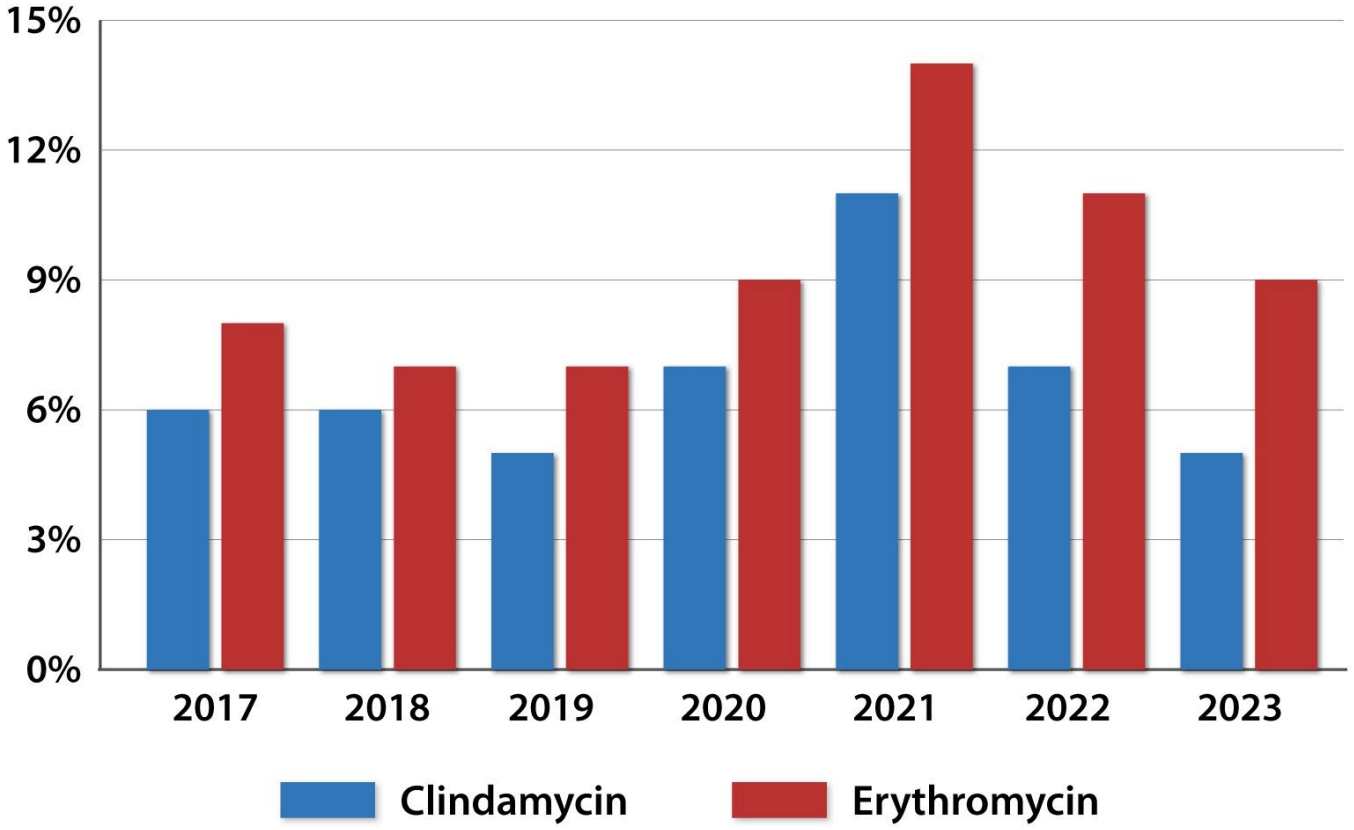




FN MOTOL

Unpublished data

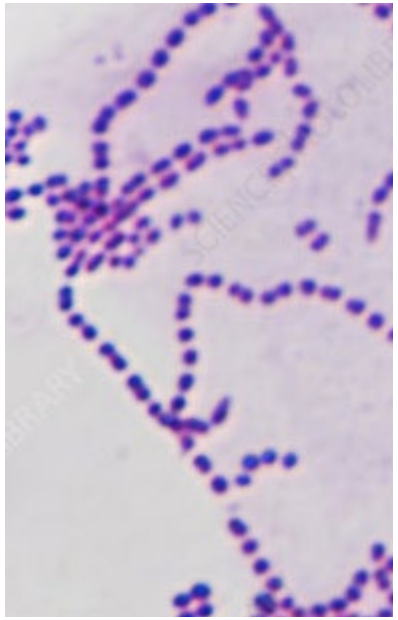
S. pyogenes resistance

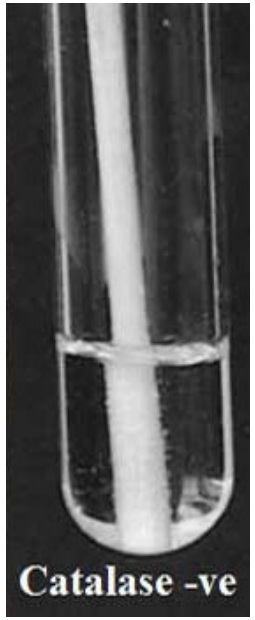


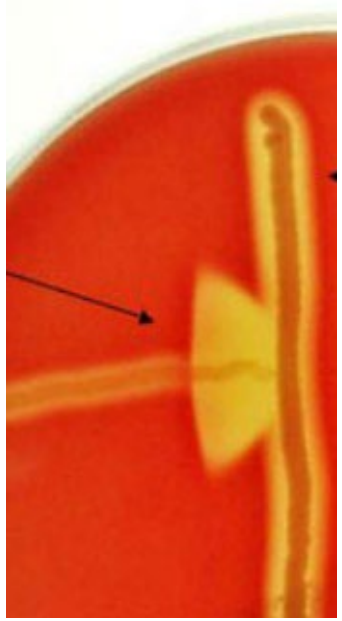
NO PNC resistance

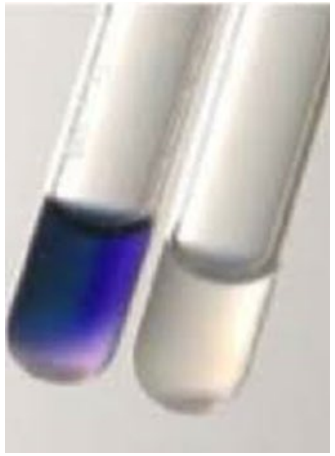
A shortage of penicillin in 2022/2023, a selective pressure of other ATB

Streptococcus agalactiae







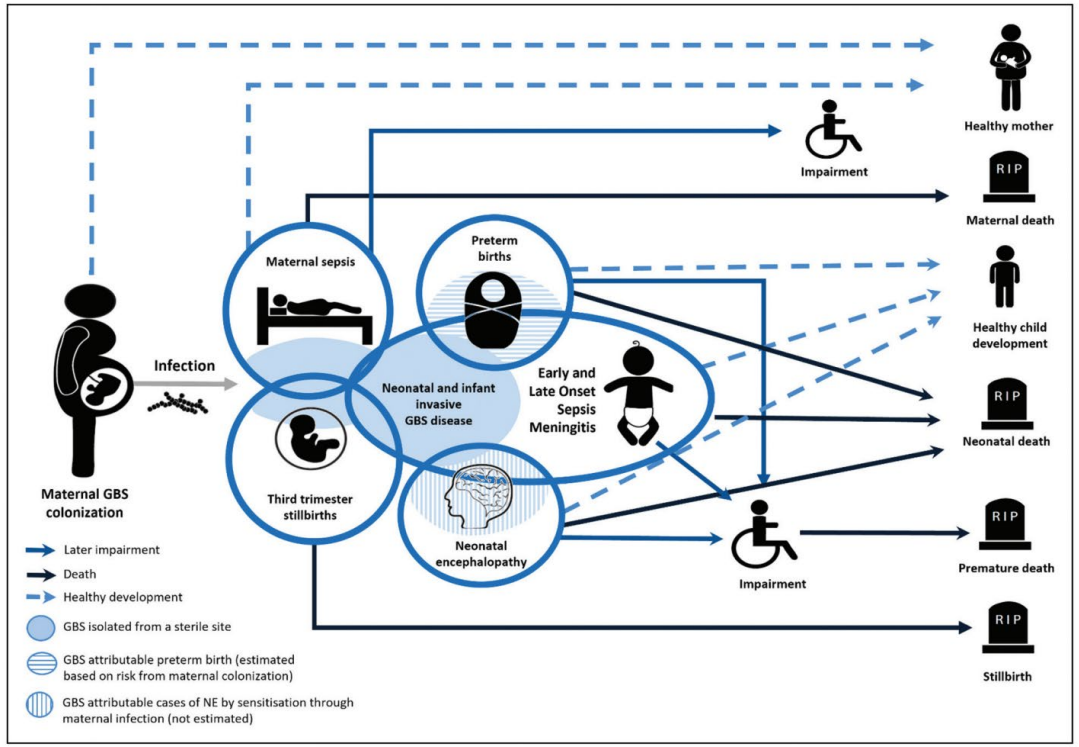


Gram-positive coccus, long chains

Facultative anaerobe (CO_2 thermostat), catalase-negative, β-haemolytic or **non-haemolytic (1-2%)**, group B, CAMP test positive, hippurate hydrolysis positive.

Streptococcus agalactiae

Group B streptococcus (GBS)
-Originally bovine mastitis
-Now, a common cause of infections in newborn infants, including sepsis and meningitis
Pregnant women – asymptomatic colonisation in vagina (10-40%).
Screening (rectal and vaginal swabs) of pregnant women is recommended at 35 to 38 weeks' gestation.
Antibiotics (penicillin, ampicillin) are routinely administered intrapartum (the period spanning childbirth, from the onset of labour through delivery of the placenta) to patients who test positive.



Neonatal disease (early till 7 days or late onset)
Pneumonia, meningitis, sepsis
Sample collection: CSF, blood cultures, a swab from conjunctiva, nose, throat, amniotic fluid if possible
Men and non-pregnant women-bacteremia, pneumonia, bone and joint infections, skin and soft tissue infections
Sampling: blood cultures, sputum, puncture, swab?

Paul P, Gonçalves BP, Le Doare K, Lawn JE. 20 million pregnant women with group B streptococcus carriage: consequences, challenges, and opportunities for prevention. Curr Opin Pediatr. 2023;35(2):223-230. doi: 10.1097/MOP.0000000000001223

Streptococcus agalactiae-virulence factors

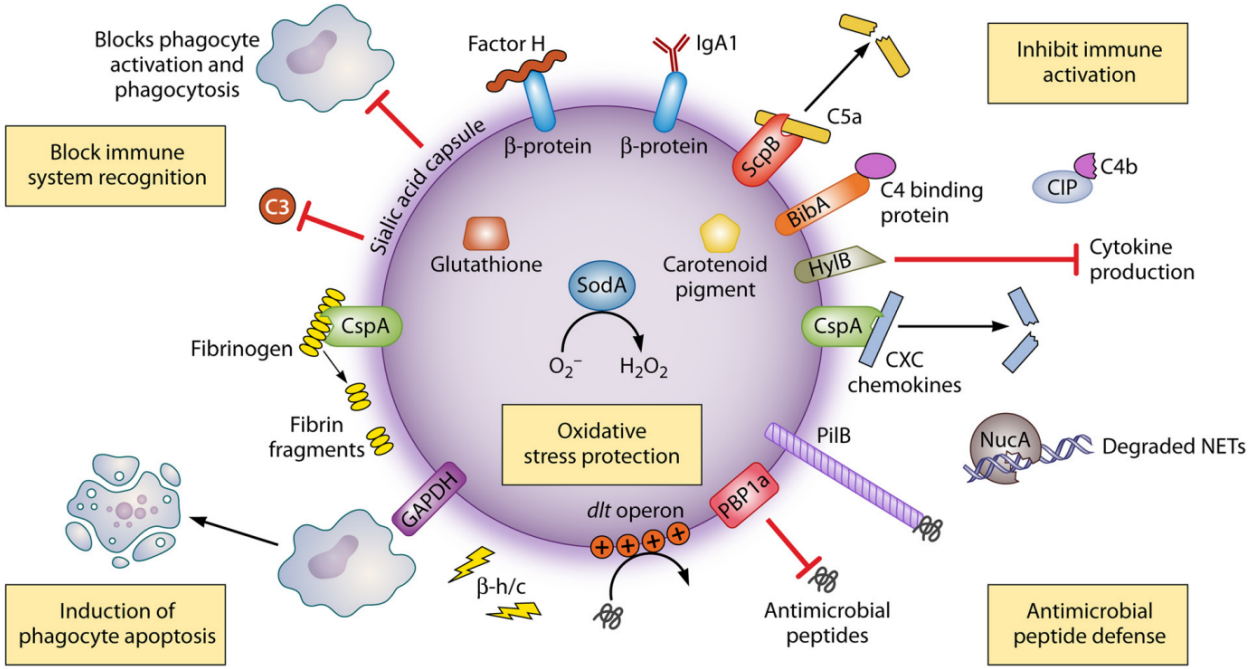


FIG 1 Mechanisms used by GBS to evade the immune system. GBS expresses many factors that help it evade the immune system and increase its survival in the host. The sialic acid capsule and fibrin fragments cleaved by CspA that coat the surface help GBS present as "self" to the immune system. The capsule also blocks C3 deposition and recognition by phagocytes. Sialic acid in the capsule, β-protein, ScpB, CIP, and BibA inhibit the complement system by binding or cleaving complement components. The GBS β-protein also binds the Fc region of IgA1 to inhibit immune activation. HylB and CspA inhibit or cleave cytokines, while PilB, PBP1a, and proteins encoded by the *dlt* operon assist in resisting antimicrobial peptides. NucA degrades the DNA matrix of neutrophil extracellular traps. Glutathione, carotenoid pigment, and SodA all aid in defense against reactive oxygen species, and both β-hemolysin/cytolysin (β-h/c) and GAPDH aid in inducing apoptosis in phagocytes.

Adherence to host epithelial surfaces and invasion

-surface expressed proteins (adherence)

-Secreted **β-haemolysin/cytolysin** (invasion)

Resistance to innate immune clearance

-**sialic acid in the capsule** and other proteins

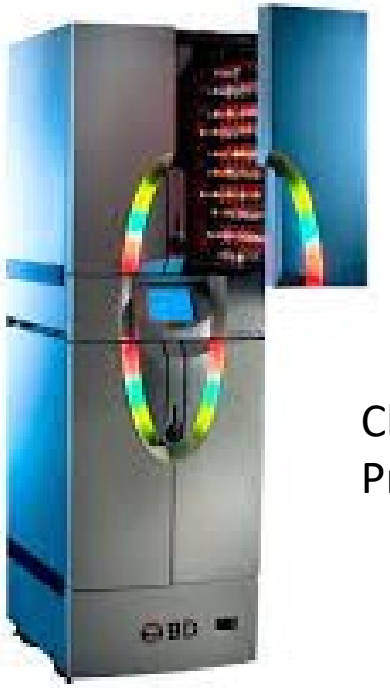
inhibits complement

blocks phagocyte activation – phagocytosis

-glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) are surface-localized enzymes that can induce apoptosis in macrophages

Korir ML, Manning SD, Davies HD. Intrinsic Maturational Neonatal Immune Deficiencies and Susceptibility to Group B Streptococcus Infection. Clin Microbiol Rev. ;30(4):973-989. doi: 10.1128/CMR.00019-17. PMID: 28814408; PMCID: PMC5608877.

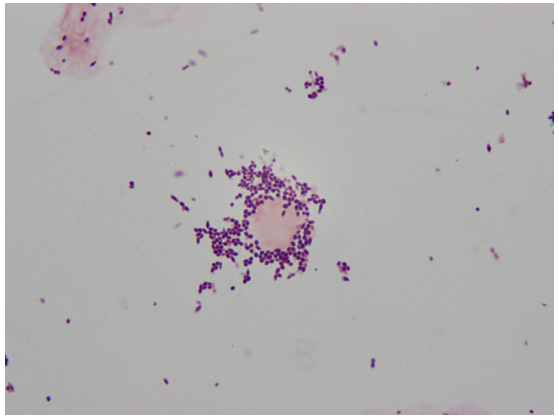
Streptococcus agalactiae - laboratory diagnostics

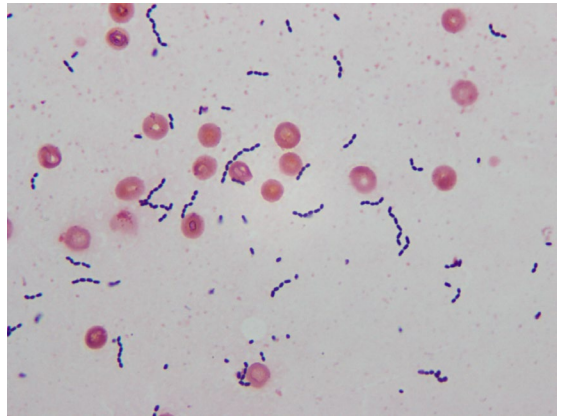


Blood culture
Newborns
Fever, chills, tremor

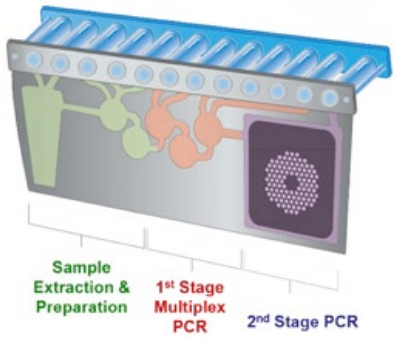
Chromogenic medium – screening
Pregnant women

Other swabs, punctate, CSF
Blood agar, 5% CO2





Microscopy – CSF, positive blood cultures



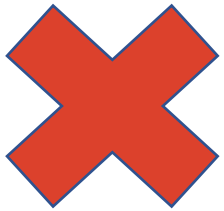
PCR-meningitis (CSF), pneumonia (sputum, BAL),*culture-negative punctate (16S), pregnant women (USA) screening

Other β haemolytic Streptococci

***Streptococcus dysgalactiae* (C, G, L)**
Coloniser of nasopharynx, rectum, vagina, skin

- Infection (similar to GAS, especially in elderly)
- Pharyngitis
- Skin and soft tissue (even necrotising fasciitis)
- Sepsis
- Pneumonia
- Post streptococcal glomerulonephritis

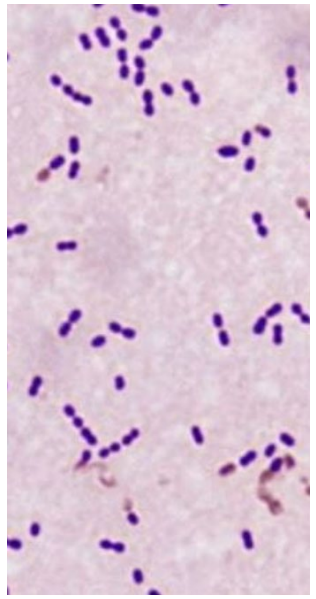
C, G, F, L

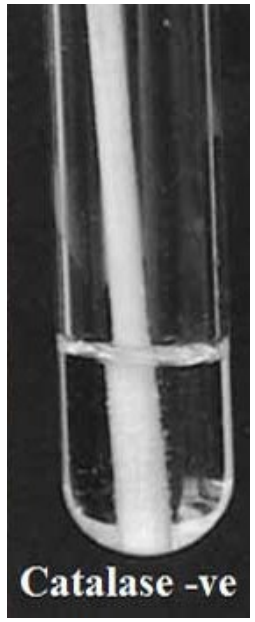


***Streptococcus anginosus* (C,F,G or non-typeable)**

More often just mucosal commensals.
Pyogenic infections in the oral cavity, gynaecological infections...Abscesses of liver, brain.... Interpretation always in relation to the clinical condition

Streptococcus pneumoniae





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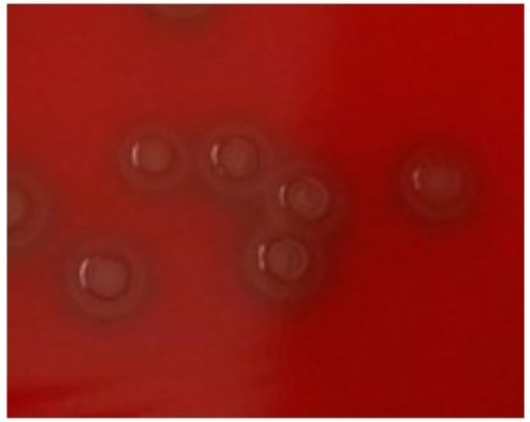
New data yes

Gram-positive coccus, pairs or short chains

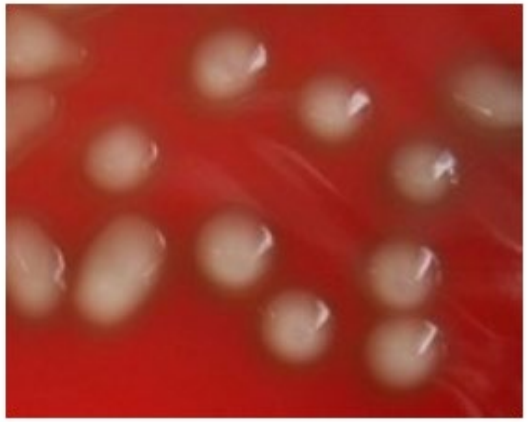
Facultative anaerobe (CO_2 thermostat), catalase-negative, α-haemolytic, non-groupable- no specific polysaccharide in the cell wall, optochin sensitive, bile salt solubility positive.

Streptococcus pneumoniae



Alpha-haemolytic colonies on sheep blood agar. Cultivation 24 hours in an aerobic atmosphere enriched with 5% CO_2, 37°C.

Craterlike appearance of colonies **R-phase**. Cultivated on Columbia agar with sheep blood, 24 hours in an aerobic atmosphere enriched with 5% carbon dioxide., 37°C.

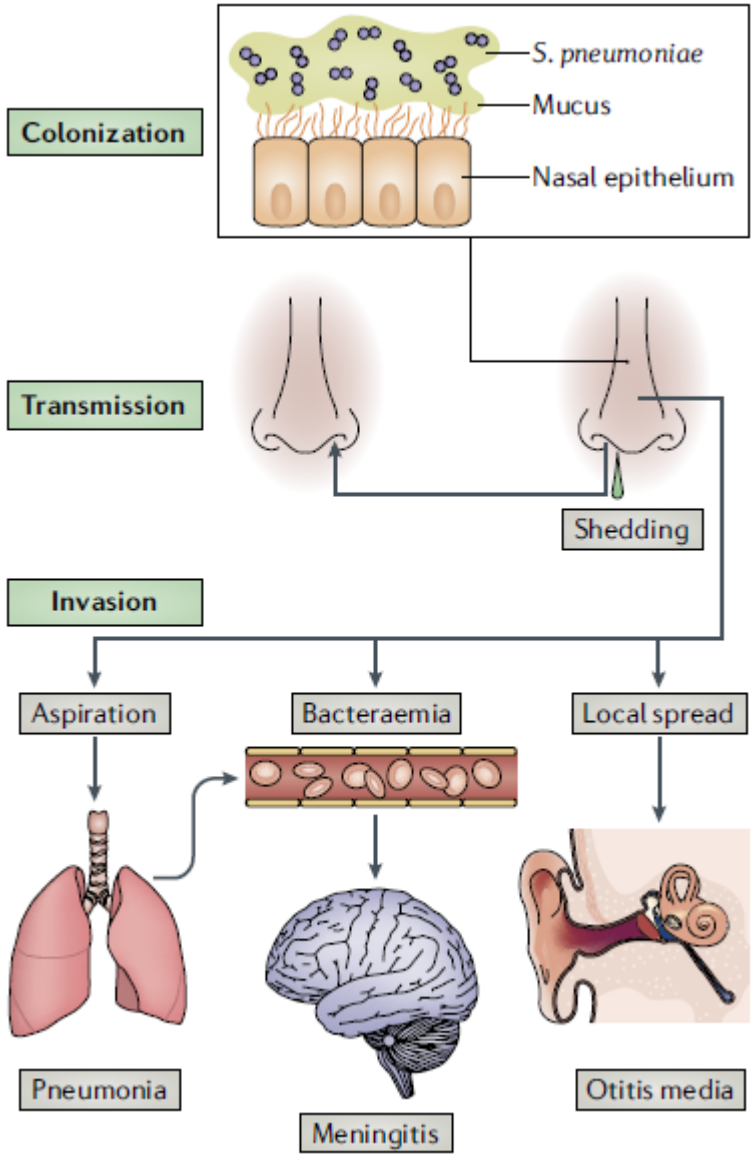


Virulent, encapsulated form **M-phase**. Cultivated on Columbia agar with 5% defibrinated sheep blood, 48 hours in an aerobic atmosphere enriched with 5% carbon dioxide, 37°C. Colonies are surrounded by a zone of alpha-hemolysis.

S-phase

Colony phase variation
M-phase – mucoid – highly virulent, high capsule expression
S-phase - smooth - most common
R –phase – rough – avirulent, lacks capsule

Streptococcus pneumoniae



Extracellular, **opportunistic pathogen** that colonizes the mucosal surfaces of the human upper respiratory tract. Up to 27–65% of children and <10% of adults.

This carriage is the prerequisite for both **transmission** to other individuals and **invasive disease** in the carrier. Carriers can shed *S. pneumoniae* in nasal secretions.

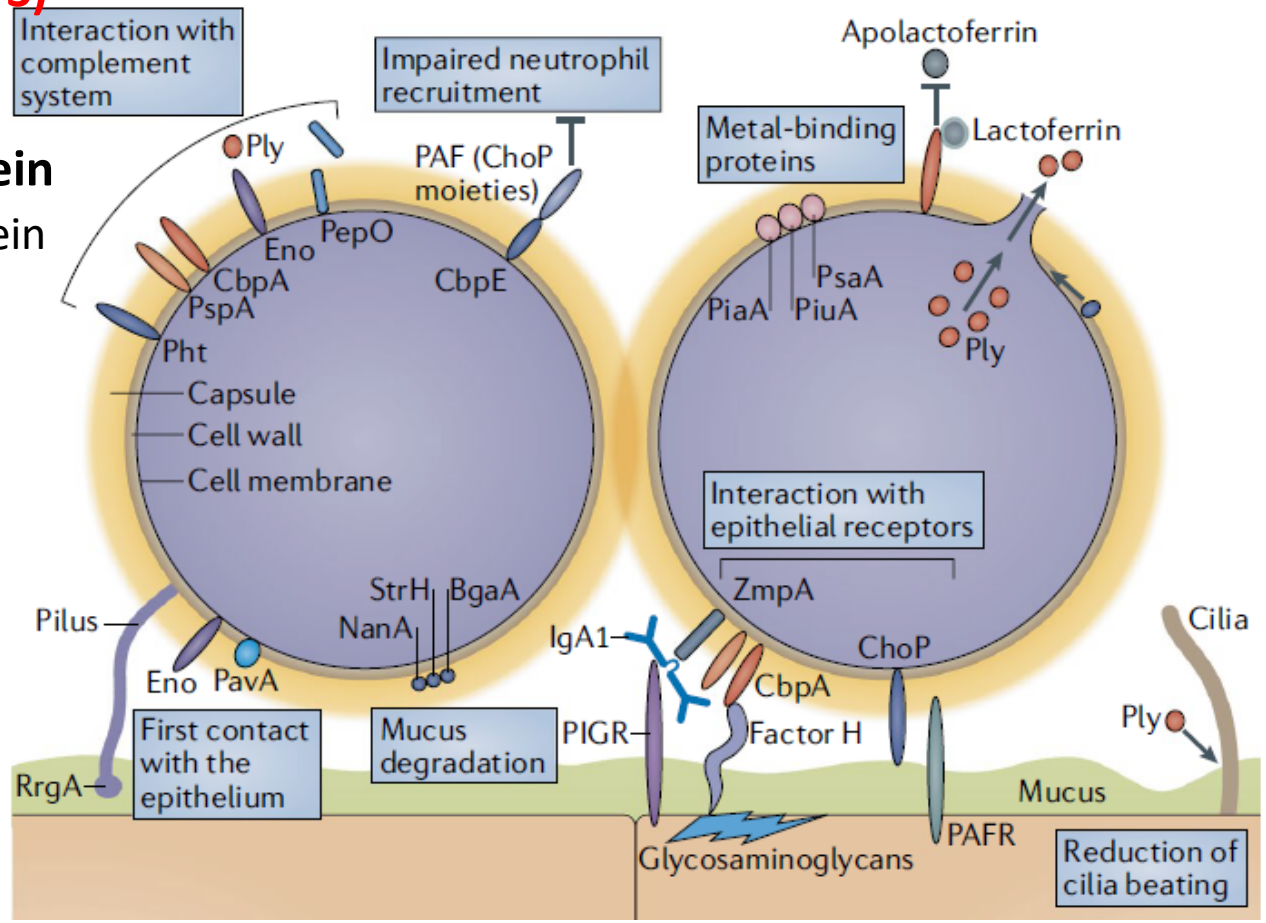
Dissemination beyond its niche along the nasal epithelium, either by aspiration, bacteraemia or local spread, can lead to invasive diseases, such as **pneumonia, meningitis and otitis media**.

Weiser JN, Ferreira DM, Paton JC. *Streptococcus pneumoniae*: transmission, colonization and invasion. Nat Rev Microbiol. 2018 Jun;16(6):355-367. doi: 10.1038/s41579-018-0001-8.

Streptococcus pneumoniae – virulence factors

Capsular polysaccharide (CPS) and several pneumococcal proteins, including **pneumococcal surface protein A** (PspA), choline- binding protein A (CbpA), enolase (Eno) and pneumococcal histidine triad protein (Pht), directly and indirectly, **block complement deposition**.

The pneumococcal enzymes **Neuraminidase A** (NanA), **β- galactosidase** (BgaA) and β- *N* acetylglucosaminidase (StrH) **degrade mucus and thereby inhibit mucociliary clearance**. **Invasion!!**



Surface C-polysacharide – interacts with host CRP protein – induction of imflamation

Pneumococcal CbpE **impairs neutrophil recruitment** by degrading platelet-activating factor (PAF), a host-derived inflammatory phospholipid.

The zinc metalloprotease ZmpA (also known as immunoglobulin A1 protease) subverts mucosal humoral immunity by **cleaving IgA1**.

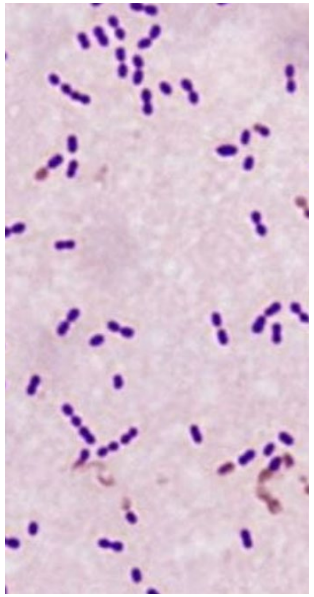
Phosphorylcholine (ChoP) on teichoic acid **mimics host platelet-activating factor (PAF)** and allows binding to its receptor.

LytA (autolysin)-facilitated release of **Ply (pneumolysin) damages the epithelium and reduces ciliary beating**.

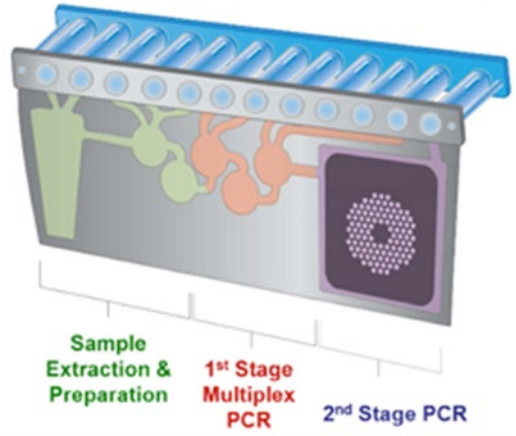
Weiser JN, Ferreira DM, Paton JC. *Streptococcus pneumoniae*: transmission, colonization and invasion. Nat Rev Microbiol. 2018 Jun;16(6):355-367. doi: 10.1038/s41579-018-0001-8.

Streptococcus pneumoniae – laboratory diagnostics

S. pneumoniae **antigen in urine** if patients have pneumonia, and **in cerebral spinal fluid CSF** sample if patients have meningitis
Sensitivity : 70 – 90 %, specificity 95 – 99 %

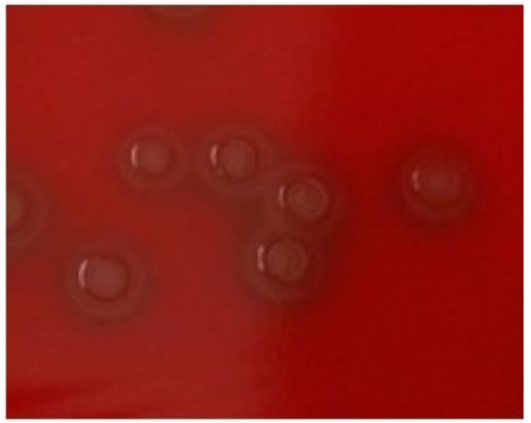


Microscopy – CSF, sputum positive blood cultures, liquid samples



Meningitis **PCR** panel CSF
Pneumonia PCR panel- sputum, BAL

Blood culture
Fever, chills, tremor



Culture, 5% CO2
Antimicrobial susceptibility testing

Treatment and resistance in *S. pneumoniae*

Treatment: Penicillin (penicillin G, ampicilin), cefalosporines (3rd gen), macrolides

Effect (Mechanism of action)

- **ß-lactams:** inhibit the final steps of peptidoglycan synthesis (cell wall) by binding to high-molecular-weight penicillin-binding proteins (PBPs).
- **Macrolides:** inhibit protein synthesis by binding 23S ribosomal target sites in bacteria.

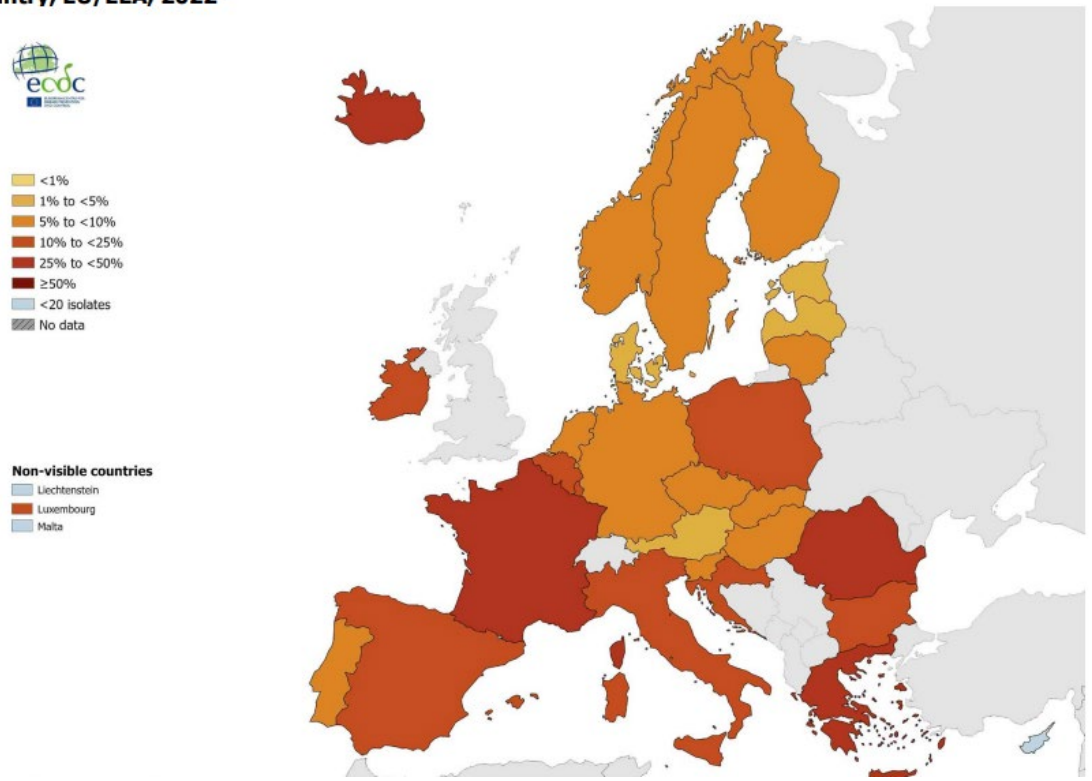
Mechanism of resistance

- **ß-lactams:** Alteration of the cell wall PBP, resulting in decreased affinity for penicillin
- **Macrolides:** Target site (ribosomal) alteration by an enzyme that methylates 23S rRNA subunits and is encoded by the *erm*B (lincosamides), high level OR Active efflux pumps encoded by the *mef*E or *mef*A gene (only macrolides, low level).

S. pneumoniae - resistance

Bacterial species	Antimicrobial group/agent resistance	2018		2019		2020		2021		2022		2022 EU/EEA country range[b]	Trend 2018–2022[c]
		n	%	n	%	n	%	n	%	n	%		
Streptococcus pneumoniae	Penicillin non-wild-type[g]	14 498	14.0	14 568	13.2	8 076	15.5	8 479	16.2	13 230	16.3	2.8–46.7	↑*
	Macrolide (azithromycin/clarithromycin/erythromycin) resistance	14 753	16.6	15 069	15.9	8 407	16.8	8 773	18.3	13 947	17.9	3.4–36.1	↑*
	Combined penicillin non-wild-type and resistance to macrolides[g]	14 016	8.6	14 102	8.0	7 782	8.9	8 155	9.8	12 694	9.7	0.8–33.3	↑*

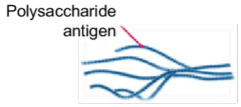
Figure 9. ***Streptococcus pneumoniae*****. Percentage of penicillin[a] non-wild type[b] invasive isolates, by country, EU/EEA, 2022**

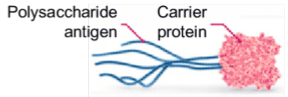


For *S. pneumoniae*, the term penicillin non-wild-type is used in this report, referring to S. *pneumoniae* isolates reported by local laboratories as **'susceptible, increased exposure' (I) or resistant (R)** to penicillin, assuming MIC for benzylpenicillin above that for wild-type isolates **(>0.06 mg/L**)

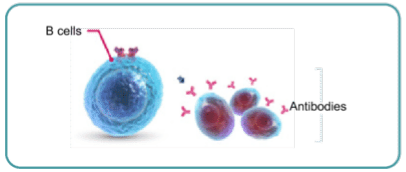
European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2022. Stockholm: ECDC; 2023.

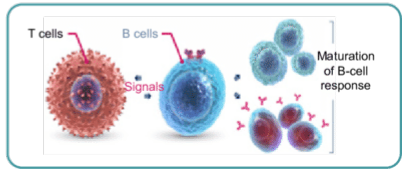
Vaccination *S. pneumoniae*



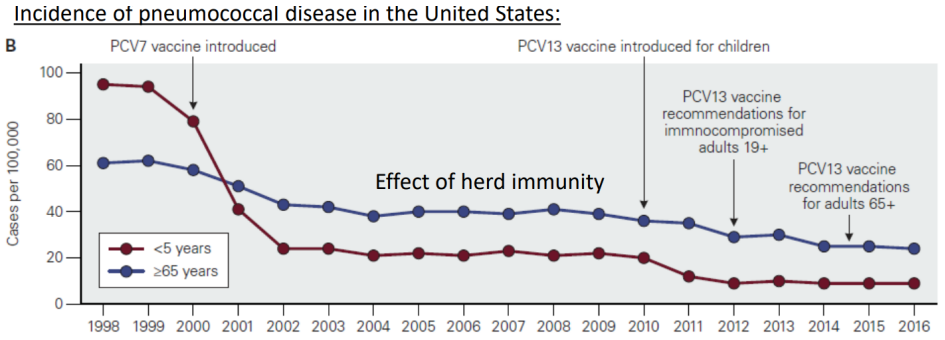


Polysaccharide vaccines	Conjugate vaccines
Contain polysaccharide antigens	Contain polysaccharide antigens covalently linked to a carrier protein
T-cell-independent immunoresponse	T-cell-dependent immunoresponse
Stimulate B cells to produce antibodies	Stimulate T cells to help B cells produce antibodies and generate immune memory Provide improved immunological responses Prevent nasopharyngeal carriage





Prevention of invasive infection and acute otitis media



Wilson et al Bacterial Pathogenesis 2019

Registered vaccines (polysaccharide (over 2years) or conjugated (over 2 months)

***PPSV23-**23 polysaccharide vaccine (polysacharide serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F)

***PCV10** (10 capsular serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F conjugated to D-protein/diphtheria and tetanus carrier and adsorbed to aluminium phosphate)

***PCV13** (13 capsular serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F conjugated to protein carrier CRM197 and adsorbed on aluminum phosphate

***PCV15** (15 capsular serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F and 33F conjugated to protein carrier CRM197 and adsorbed on aluminium phosphate

PCV20 (20 capsular serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F, 8, 10A, 11A, 12F, 15B, 22F and 33F conjugated to the protein carrier CRM197 and adsorbed on aluminium phosphate.

The advantage of conjugated pneumococcal vaccines is the **higher immunogenicity** and **absence of hypo-responsiveness** in older age groups (over 65 years of age) and persons with chronic diseases and reduced immune function.

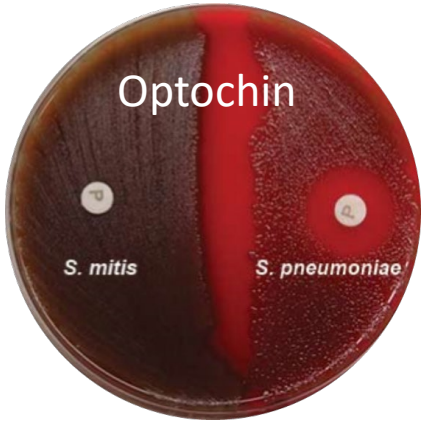
The disadvantage is the lower serotype coverage compared to PPSV23 (13-20 serotypes).

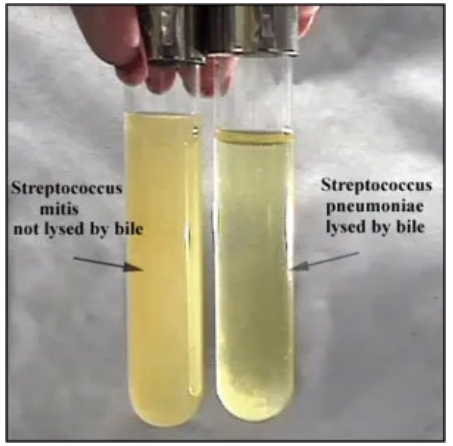
Viridans streptococci

Heterogenous collection of **α hemolytic** and non-hemolytic streptococci
„Viridis“ Latin from green (incomplete breakdown of Hgb=verdeglobin)
Non-groupable by Lancefield scheme
MALDI-ToF (still not very reliable, T. Wan et al., 2023)
Optochin R, bile-esculin test (insoluble) to differentiate from *S. pneumoniae*

S. mitis
S. mutans
S. salivarius
S. sanguinis

MALDI-ToF can distinguish *S. pneumoniae* from *S. viridans* group





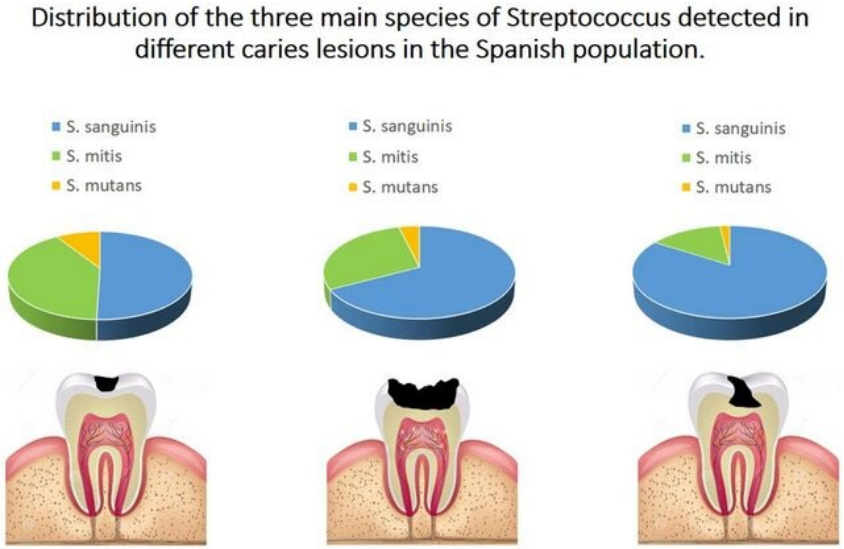
Viridans streptococci colonise the oropharynx, gastrointestinal tract and genitourinary tract.
Rarely found on the skin surface because surface fatty acids are toxic to them

Viridans streptococci

Dental caries

S. mutans….

The cariogenic capacity is determined by their ability to adhere to the tooth structure (biofilm formation=dental plaque), resistance to low pH, and their ability to produce lactic acid from the sugar in the food, destroying the hard tissues of the tooth.



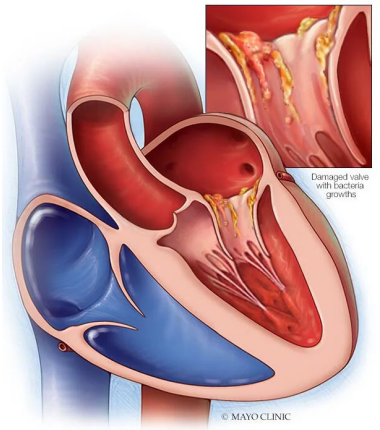
Simon Soro et al. (2014)

Subacute endocarditis

The bacteria enter the bloodstream (stomato-surgical procedure or injury) and attack the lining of the heart valves. This causes growths, called vegetations, on the heart valves.

Vegetations can cause holes in the valves and cause the infection to spread outside of the heart and blood vessels. Subacute infective endocarditis develops slowly over a period of several weeks to several months. Endocarditis is fatal without treatment.

Treatment based on AST: PNC, AMP, CEF 3. gen., VAN.



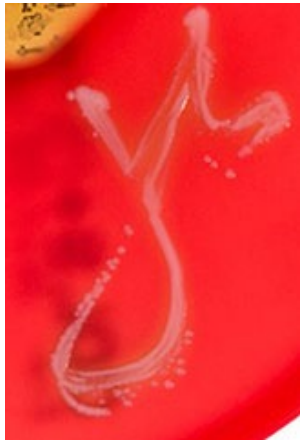
Blood cultures

Pan bacterial 16S rDNA PCR

• **Brain abscess** • **Osteomyeliti**s • **Sepsis** – neutropenic patients

Streptococcus bovis

- Gamma haemolytic
- Group D
- Gastrointestinal colonisation
- Infective endocarditis
- Penicillin sensitive, but high resistance to macrolides (60%)

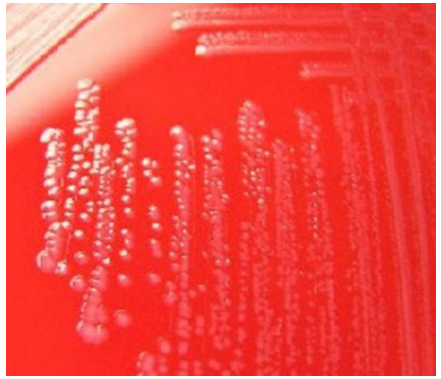


mitis or bovis group?

Enterococci („enteric cocci“)

*Previously classified as group D streptococci (posses group D cell wall antigen)
-distinct from non-enterococcal group D streptococci (e.g. *S. bovis*)
-1984-enterococci were reclassified into the new genus *Enterococcus* (29 species).

Enterococcus faecium and Enterococcus faecalis



G-positive
Short chains or pairs (microscopic morphology cannot be differentiated from S. *pneumoniae*)
Facultative anaerobic, optimal temperature 35°C
-complex nutritional needs (**sheep blood agar**)
-can tolerate exposure to harsh environmental conditions (**6.5% NaCl**, 40% bile salts)
-white colonies, non-haemolytic, α-haemolytic, rarely β-haemolytic

Enterococci („enteric cocci“)

Commensal organisms (do not produce toxins or other well-defined virulence factors)
The amount of enterococci in human intestinal contents ranges from **$1.4x10^2$ to $2.5x10^8$ cfu/g**.
Limited potential for causing disease
BUT in hospitalised patients can cause life-threatening infectious complications

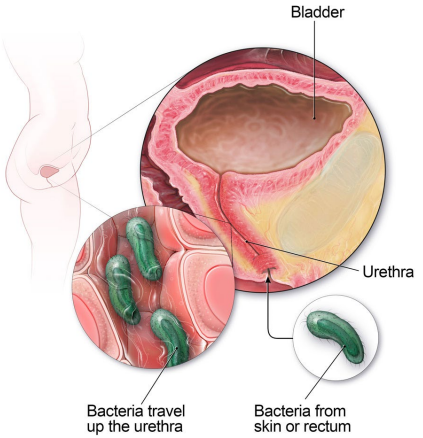
Adhesins: binding to cells lining the human intestine or vagina host tissues
Extracellular proteins: haemolytic activity (cytolysin), proteolytic activity (gelatinase, serine protease, pheromone (chemoattractant for neutrophils)
Bacteriocins: inhibition of competitive bacteria

ANTIMICROBIAL RESISTANCE

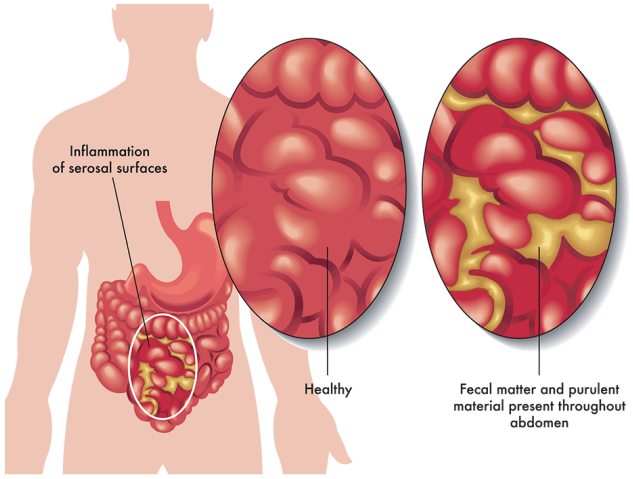
When a patient is treated with antibiotics, the enterococci that are part of normal microbial flora can proliferate and cause disease.

Enterococci („enteric cocci“)

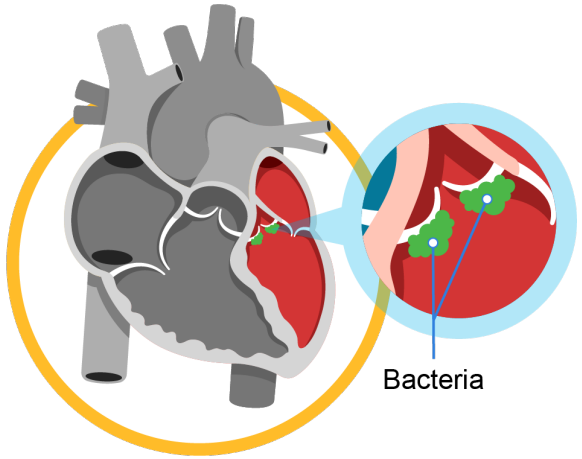
Urinary tract infection
Dysuria and pyuria – most frequently in hospitalised patients with an indwelling urinary catheter and on broad-spectrum cephalosporins



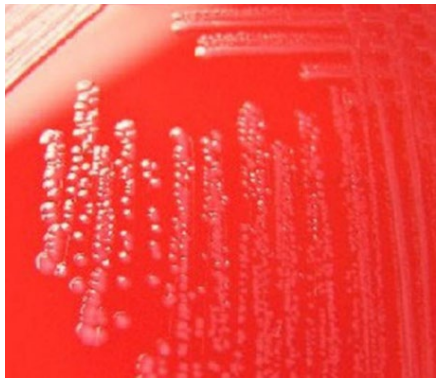
Peritonitis
Abdominal swelling and tenderness after abdominal trauma or surgery. Acutely ill patients, febrile, positive blood cultures



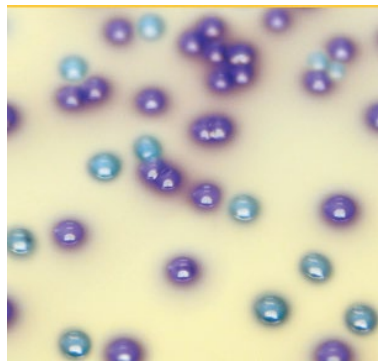
Endocarditis
Infection of heart endothelium



Enterococci – laboratory diagnostics



Culture and AST



Selective culture VRE -screening

Blood cultures

SEPSITEST™-UMD CE IVD

molzym
reveal DNA

CULTURE-INDEPENDENT MOLECULAR DETECTION OF PATHOGENS

SepsiTest™-UMD is a CE IVD molecular diagnostic test for the *in vitro* diagnosis of pathogens from clinical samples without the need for culture. **SepsiTest™-UMD** is based on a single protocol, including human DNA depletion (MolYsis™), microbial DNA enrichment and extraction from intact bacteria & fungi, followed by 16S and 18S rDNA broad-range PCR and sequencing analysis. With this broad approach and the capability to detect even rare, fastidious and non-growing pathogens, it is the perfect solution to complement standard culture methods in routine diagnostic laboratories.

Pan bacterial PCR (16S rDNA): endocarditis, culture-negative tissues.

Gram staining: similar to *S. pneumoniae*
Group D – cell wall antigen
Resistant to optochin
PYR test positive
The bile salt solubility test negative

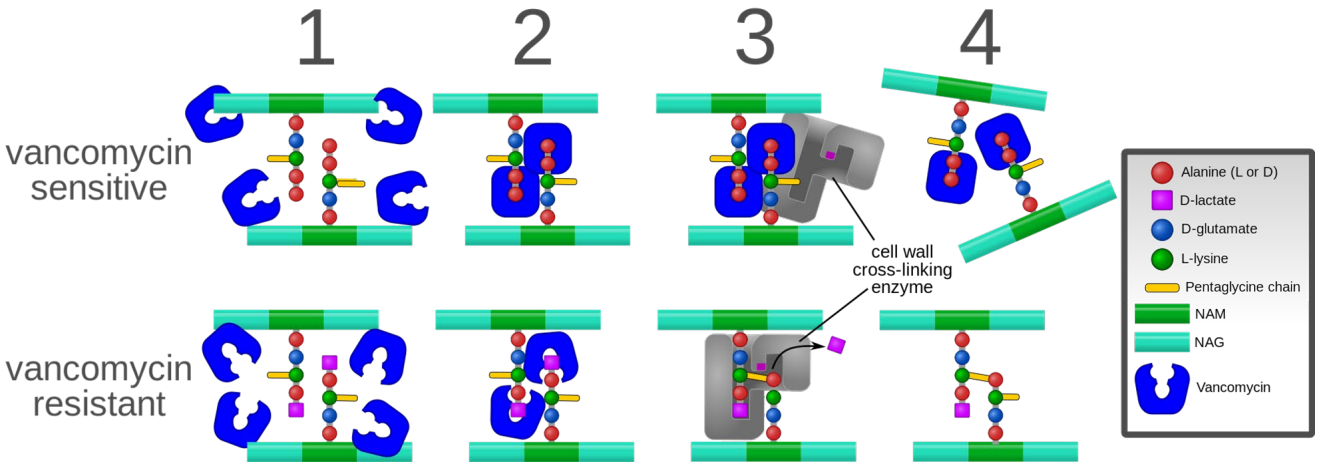
Enterococci - treatment

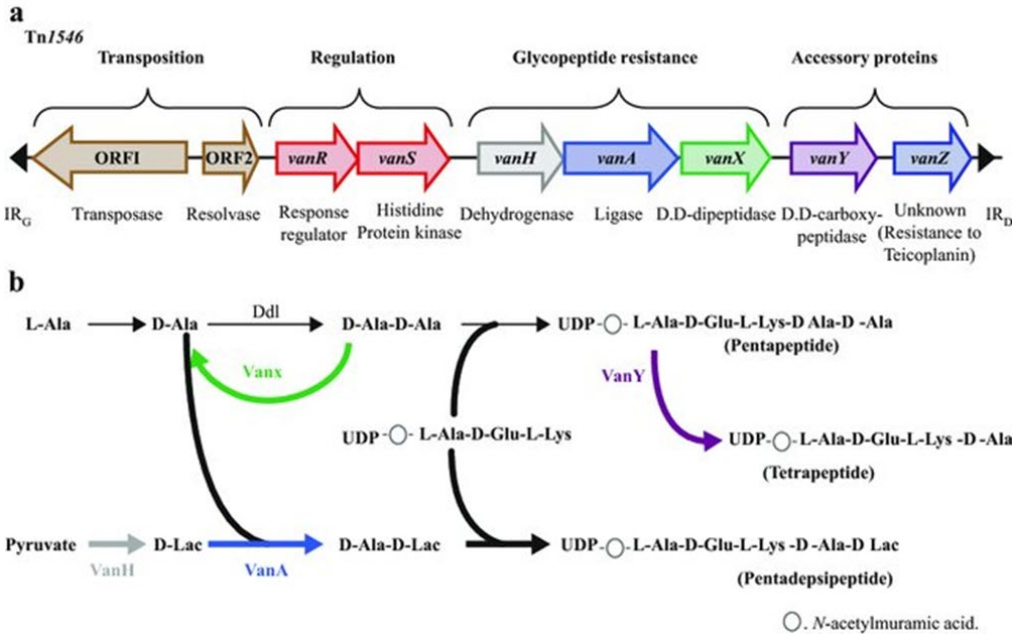
E. faecalis - aminopenicillins, glycopeptides

E. faecium - glycopeptides, natural R to aminopenicillins

VRE - oxazolidinones (linezolid), tigecycline

Mechanisms of vancomycin resistance in enterococci





The glycopeptide antibiotics (vancomycin and teicoplanin) act by **binding to the terminal d-alanyl-d-alanine (d-Ala-d-Ala) dipeptide of peptidoglycan precursors**, preventing their incorporation into the bacterial cell wall .

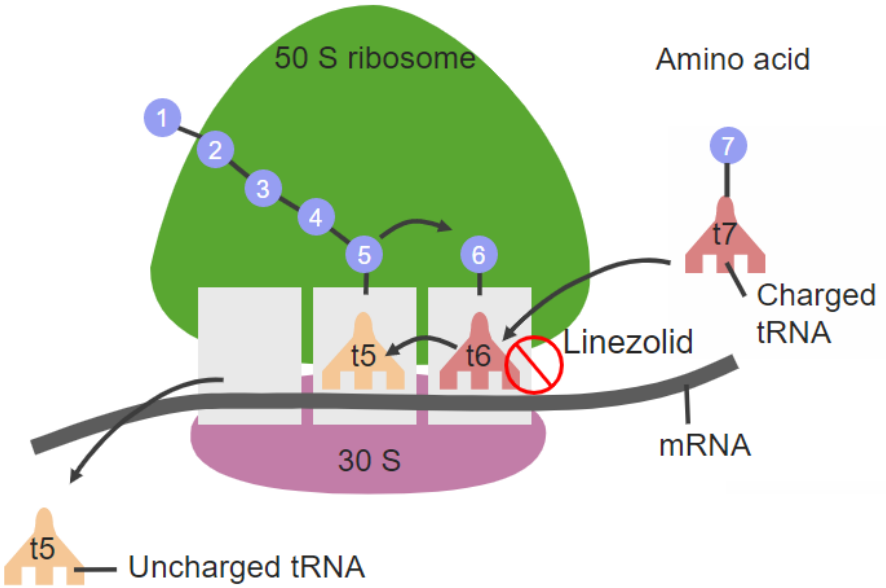
The related *van*A or *van*B gene clusters mediate acquired resistance to glycopeptides in enterococci by **remodeling the dipeptide termini of peptidoglycan precursors from d-alanyl-d-alanine (d-Ala-d-Ala) to d-alanyl-d-lactate (d-Ala-d-Lac).**

The ***van*A** gene cluster confer **high-level resistance to vancomycin and teicoplanin**, while enterococci harbouring ***van*B**-type cluster genes have a moderate level of resistance to vancomycin but remain **susceptible to teicoplanin**.

Miller WR, Munita JM, Arias CA. Mechanisms of antibiotic resistance in enterococci. Expert Rev Anti Infect Ther. 2014 Oct;12(10):1221-36. doi: 10.1586/14787210.2014.956092.

Mechanisms of linezolid resistance in enterococci

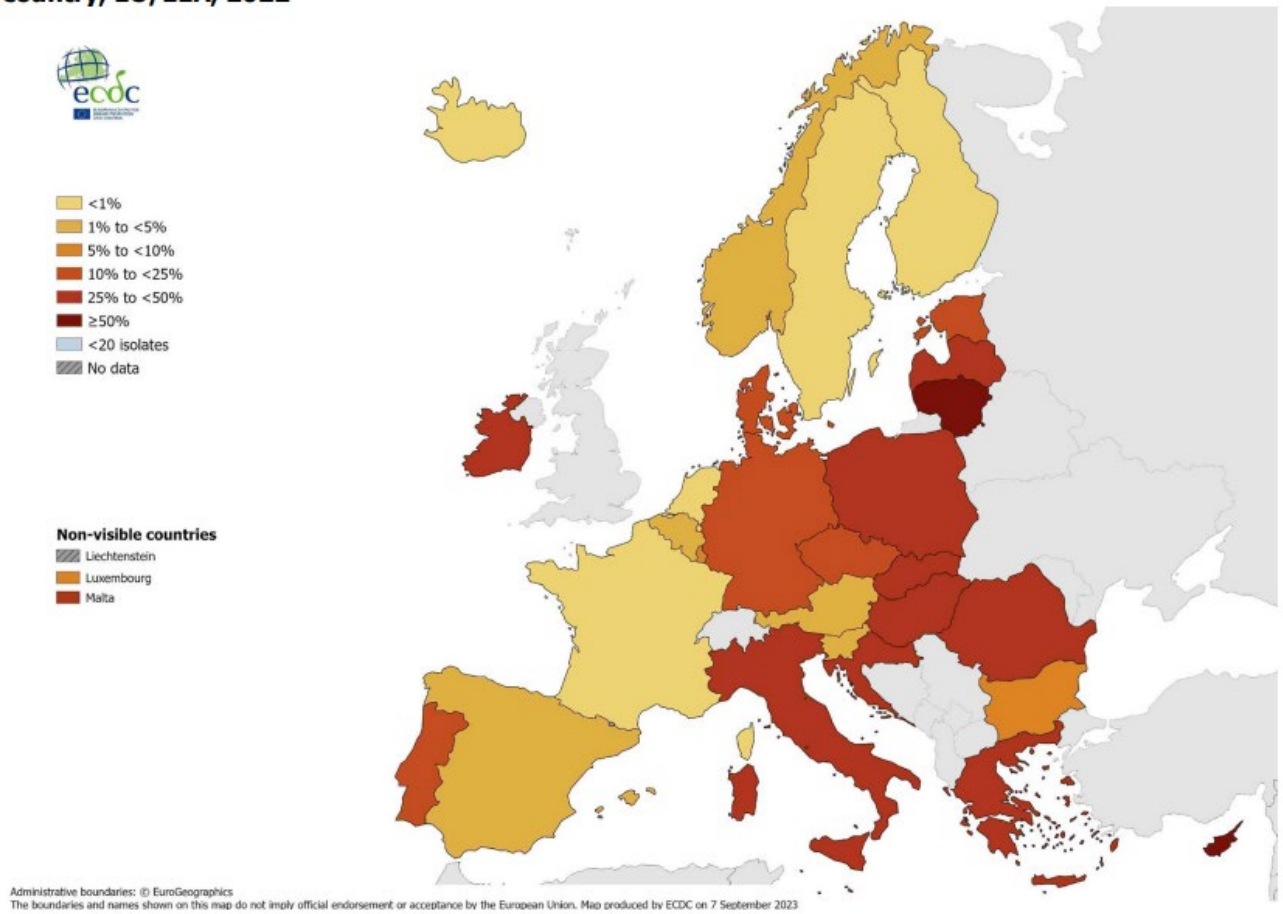
- **Linezolid:** is a bacteriostatic agent with broad activity against gram-positive bacteria. It binds to the 23S rRNA and **disrupts the docking of the aminoacyl-tRNA** in the A site of the ribosome, thus inhibiting the delivery of peptides and the subsequent **elongation of the polypeptide chain**



- Mutations in genes encoding the 23S rRNA (multiple copies of the gene)
- Mutations in the ribosomal proteins L3 and L4, which border the peptidyl-transferase centre where linezolid binds, are associated with an increase in the linezolid MIC
- Enzymatic modification of the 23S rRNA by methylation of an adenine in position 2503 by *cfr* gene (**plasmid-borne determinant!)**

Bacterial species	Antimicrobial group/agent resistance	2018		2019		2020		2021		2022		2022 EU/EEA country range[b]	Trend 2018–2022[c]
		n	%	n	%	n	%	n	%	n	%		
Enterococcus faecium	Vancomycin resistance	13 346	16.2	14 095	17.7	18 349	16.8	22 328	17.2	22 709	17.6	0.0–67.7	↑*

Figure 10. ***Enterococcus faecium*****. Percentage of invasive isolates resistant to vancomycin, by country, EU/EEA, 2022**



European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA (EARS-Net) - Annual Epidemiological Report 2022. Stockholm: ECDC; 2023.

Vancomycin-resistant *Enterococcus faecium*, epidemiology in the Czech Republic (Marie Brajerová, PhD candidate)

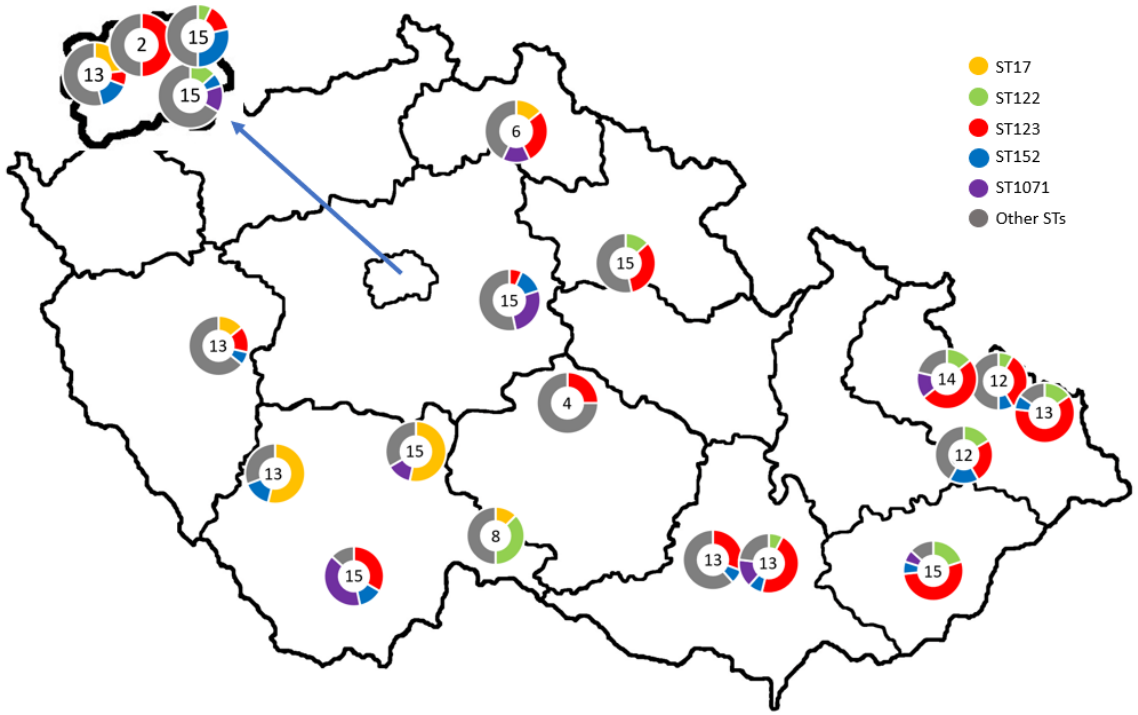


Figure 1. The distribution of participating hospitals in the study. The pie charts show the most common STs identified per hospital. The numbers in the centre represent the number of VRE and VSE isolates sent for characterization.

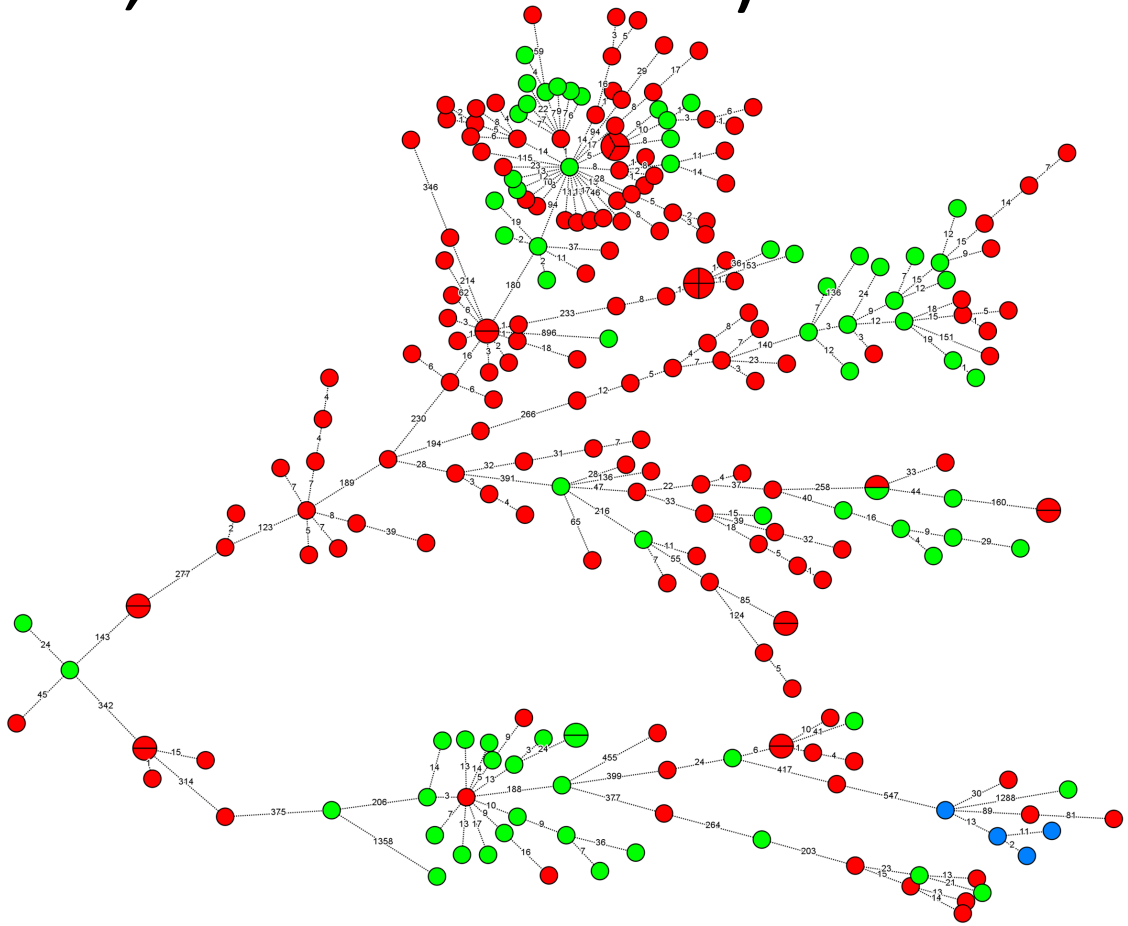
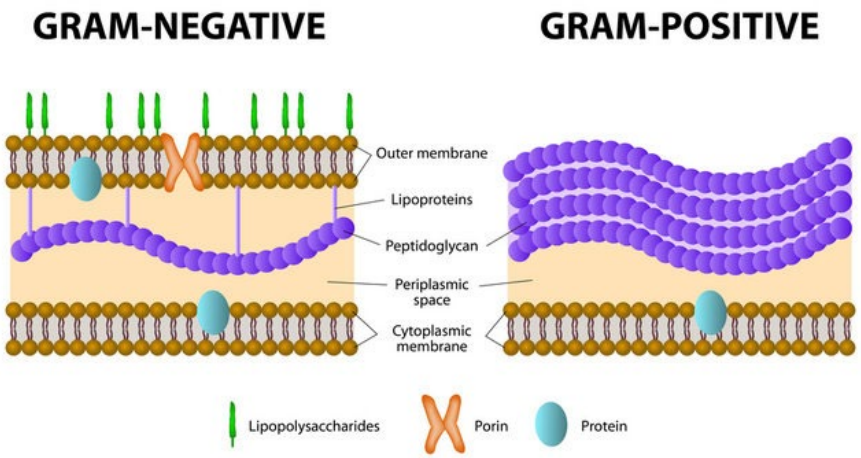


Figure 2. Minimum spanning tree generated from wgMLST analysis for *E. faecium* isolates (n=241). The colours indicate resistance to vancomycin. Red = vancomycin-resistant (n=162), Green = vancomycin-susceptible (n=75), Blue = partly deleted *van*A operon (n=4).

Bacterial survival on inanimate surfaces

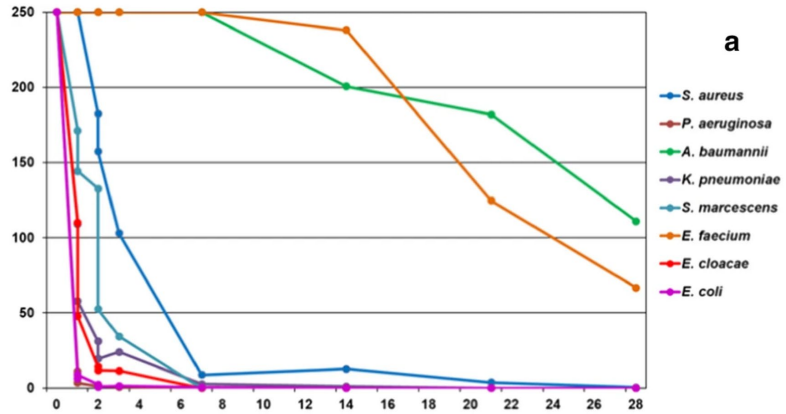
The study of Katzenberger and colleagues investigated *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *S. marcescens*, *E. faecium*, *E. coli*, and *E. cloacae*.

Bacterial suspension in 0.9% NaCl solution at a McFarland of 1. Plating via cotton swabs either on glass, polyvinyl chloride, stainless steel, or aluminium.

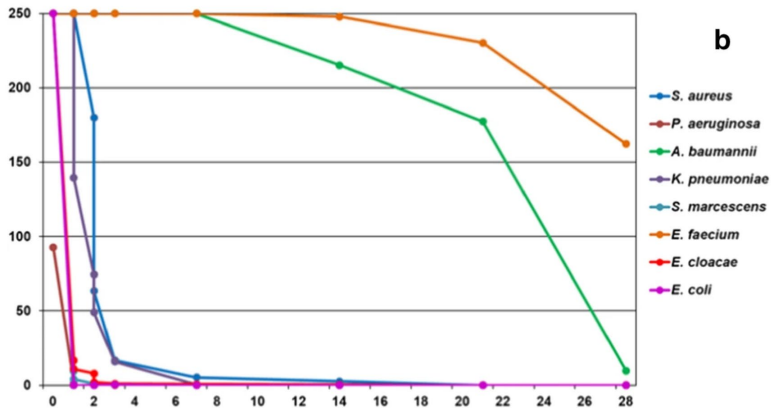


Katzenberger RH, Rösel A, Vonberg RP. Bacterial survival on inanimate surfaces: a field study. BMC Res Notes. 2021;14(1):97. doi: 10.1186/s13104-021-05492-0.

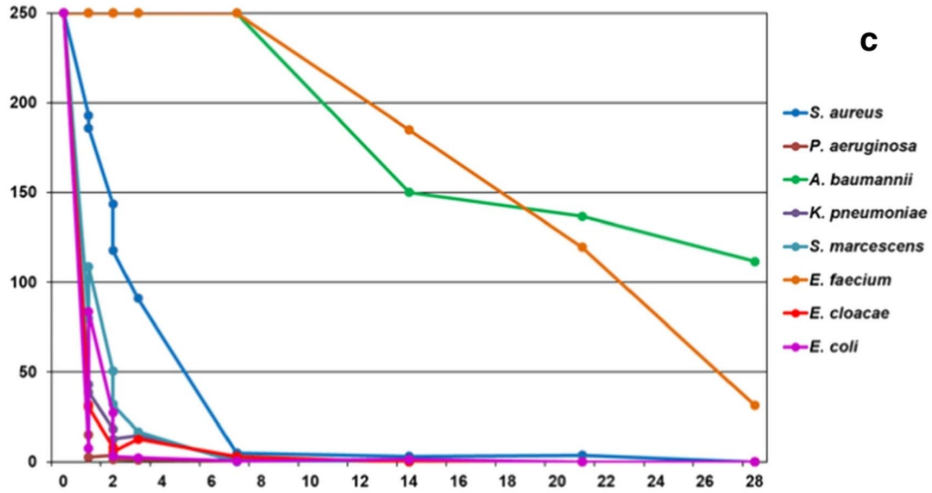
Survival of different bacterial species



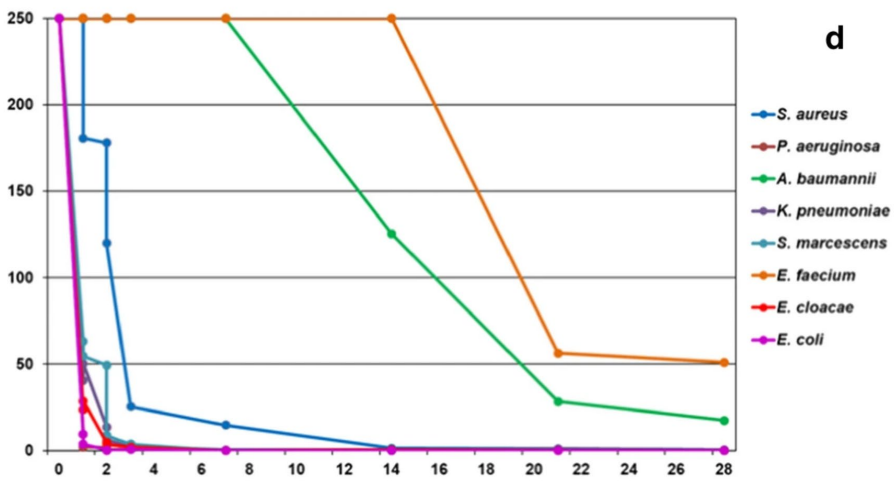
Glass



Stainless steel



Polyvinyl chloride

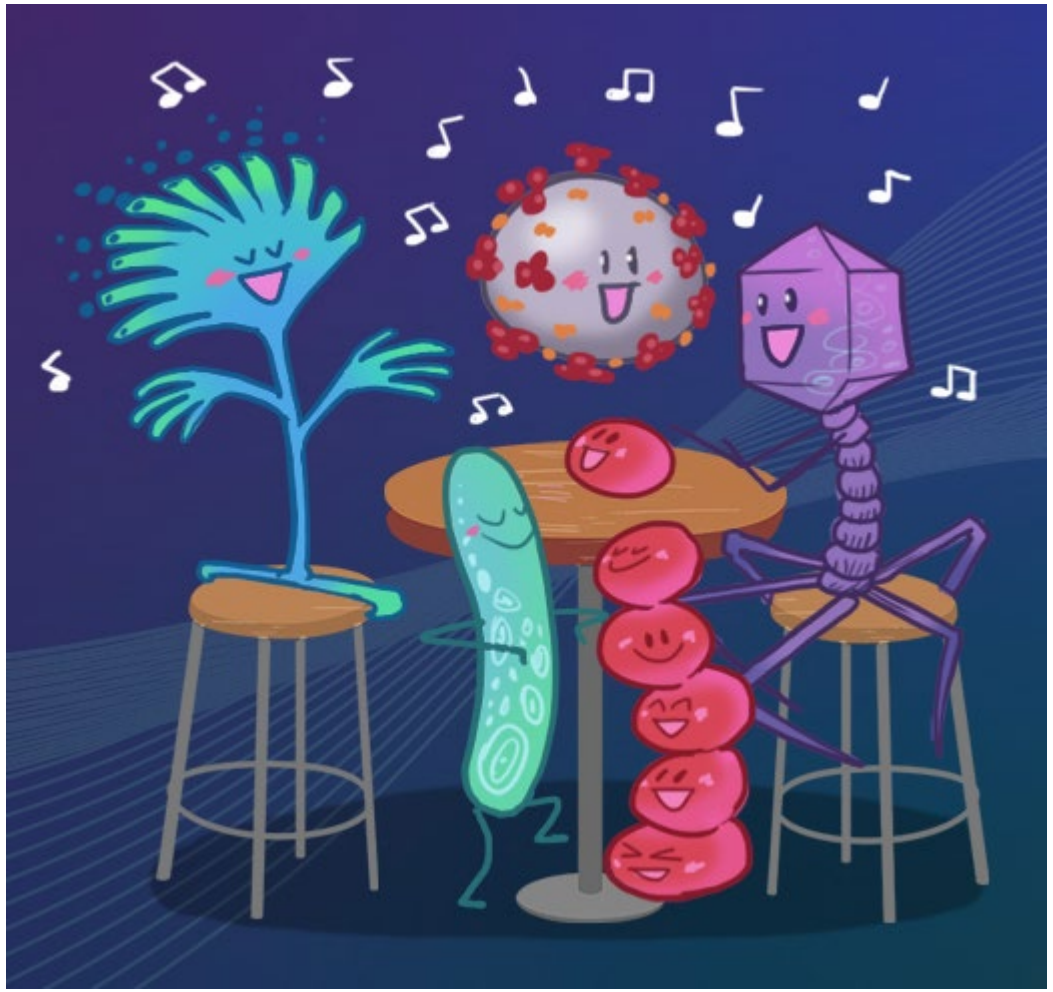


Aluminium

AND CONTAMINATED SURFACE?

Katzenberger RH, Rösel A, Vonberg RP. Bacterial survival on inanimate surfaces: a field study. BMC Res Notes. 2021;14(1):97. doi: 10.1186/s13104-021-05492-0.

Enjoy further exploring the wonders of microbiology!



marcela.krutova@lfmotol.cuni.cz