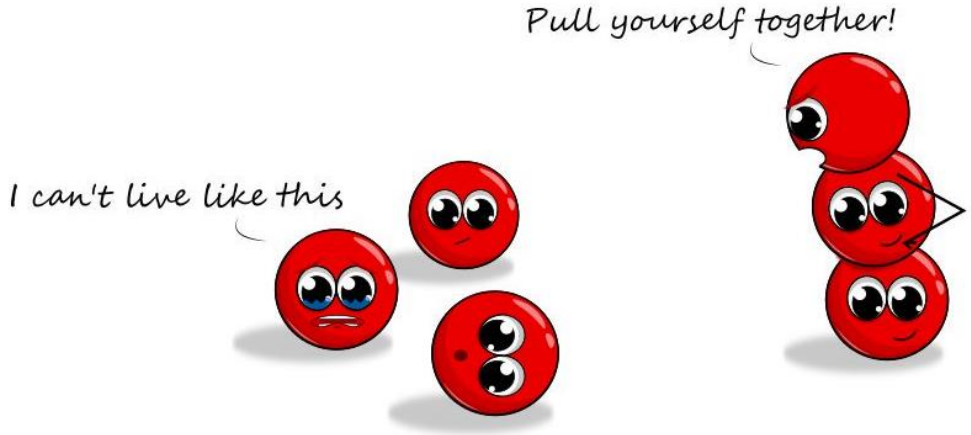
Streptococci and enterococci

Marcela Krutova

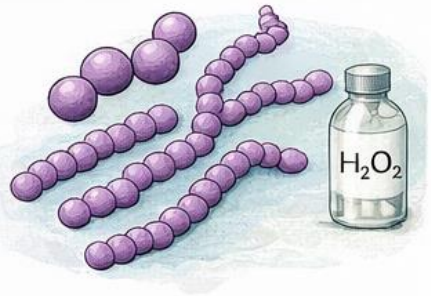


marcela.krutova@lfmotol.cuni.cz

Basic characteristics of genus *Streptococcus* spp.

Streptococcus – Basic Characteristics

- ✓ Gram-positive cocci
- ✓ Arrangement: **diplococci or chains**
- ✓ Mostly **facultative anaerobes**
- ✓ Some species: **capnophilic growth** (CO_2-enriched atmosphere)



- 🔧 Metabolism and growth:
- ✓ Carbohydrate fermentation → **production of lactic acid**
- ✓ Fastidious organisms → require **blood/serum**-enriched media
- ⚠ Key feature:

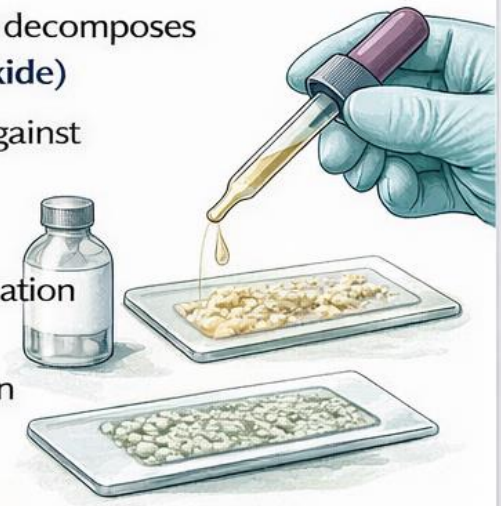
Catalase-negative → differentiation from **staphylococci**

Catalase Test

- ✓ **Catalase**: enzyme that decomposes H_2O_2 (hydrogen peroxide)
- ✓ **Function:** protection against **oxidative stress**

Test result:

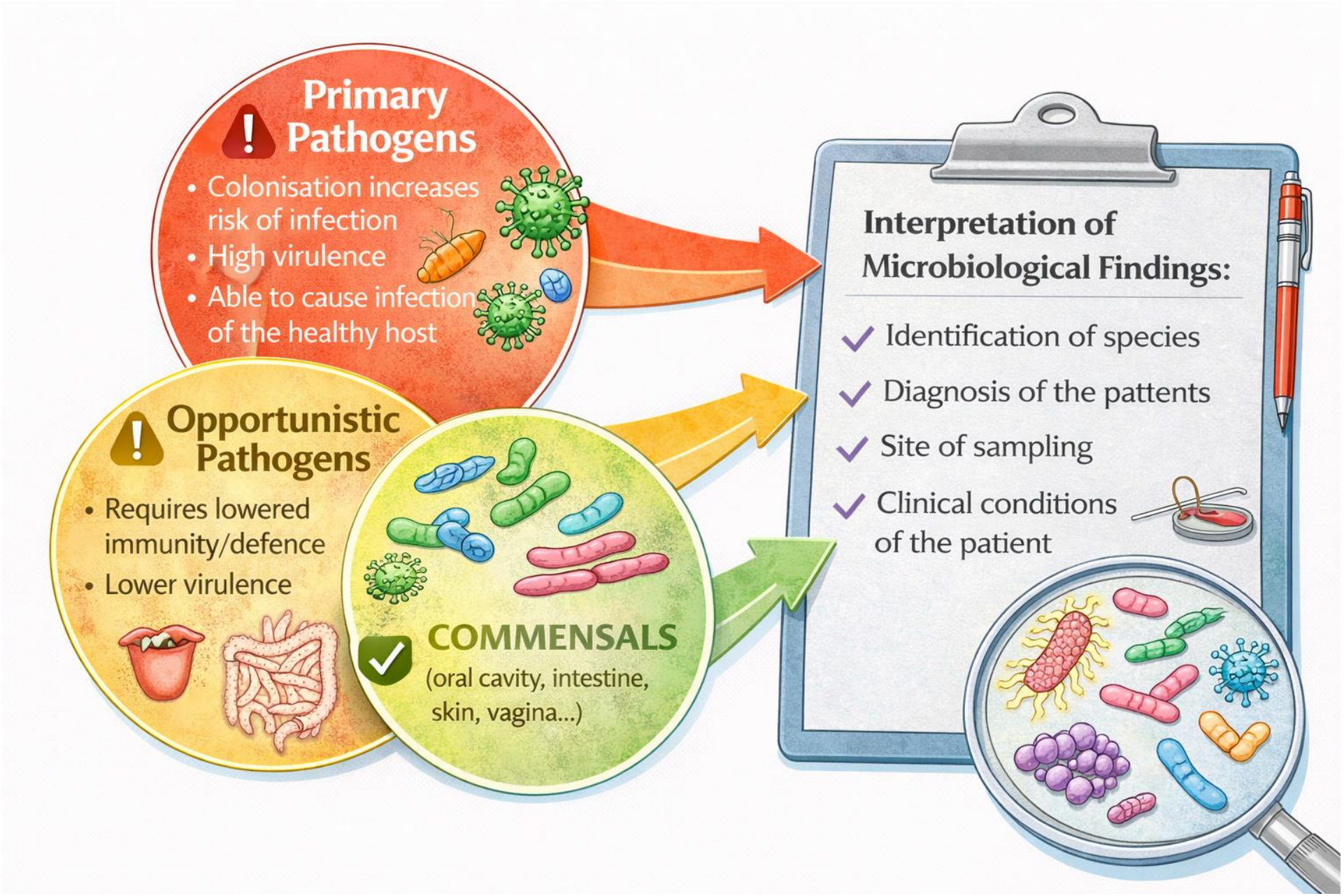
- ✓ **Positive** → bubble formation (e.g, staphylococci)
- ✗ **Negative** → no reaction (streptococci)



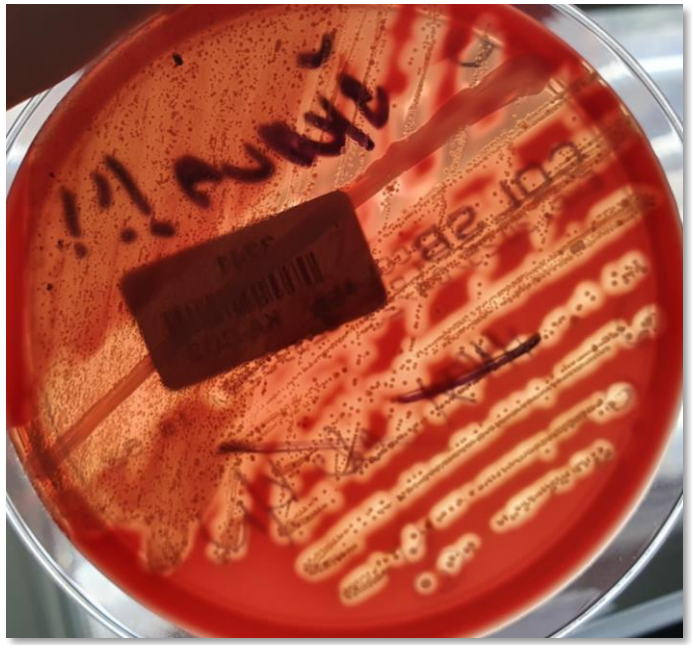
→ Alternative enzymes:

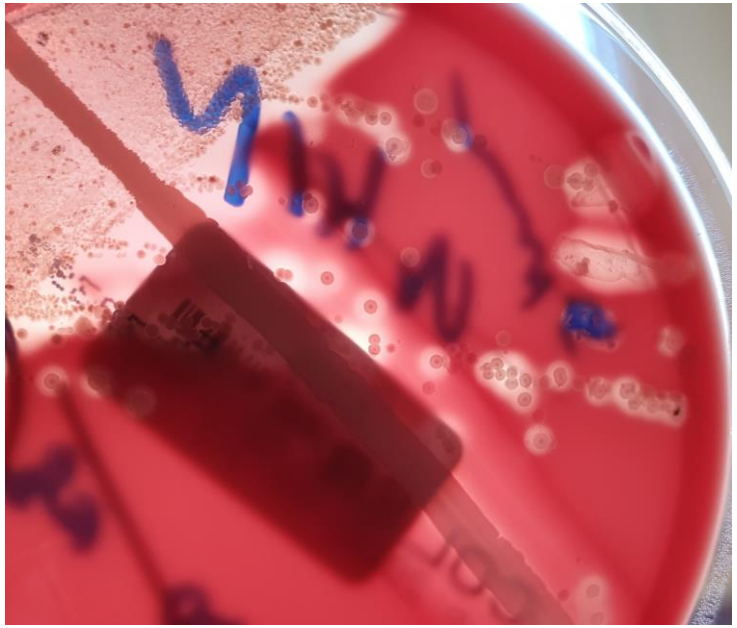
- superoxide dismutase (SodA)
- thiol peroxidase (TpxD)
- thiol peroxidase (TpxD)
- alkyl hydroperoxidaza (AhpD)

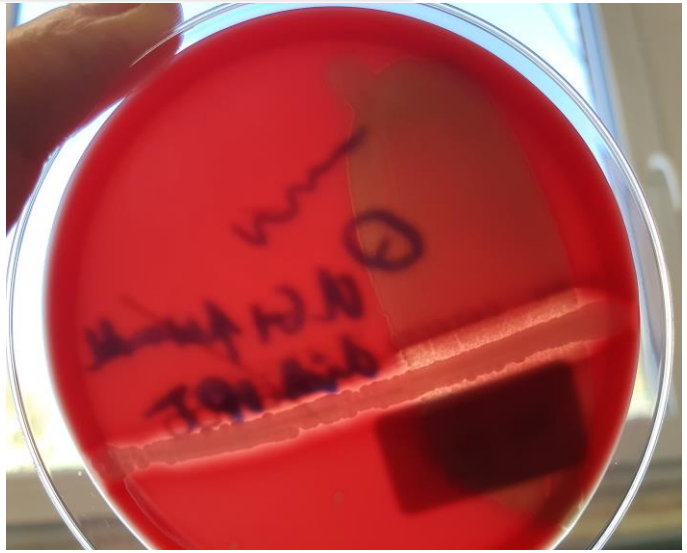
Clinical significance/interpretation



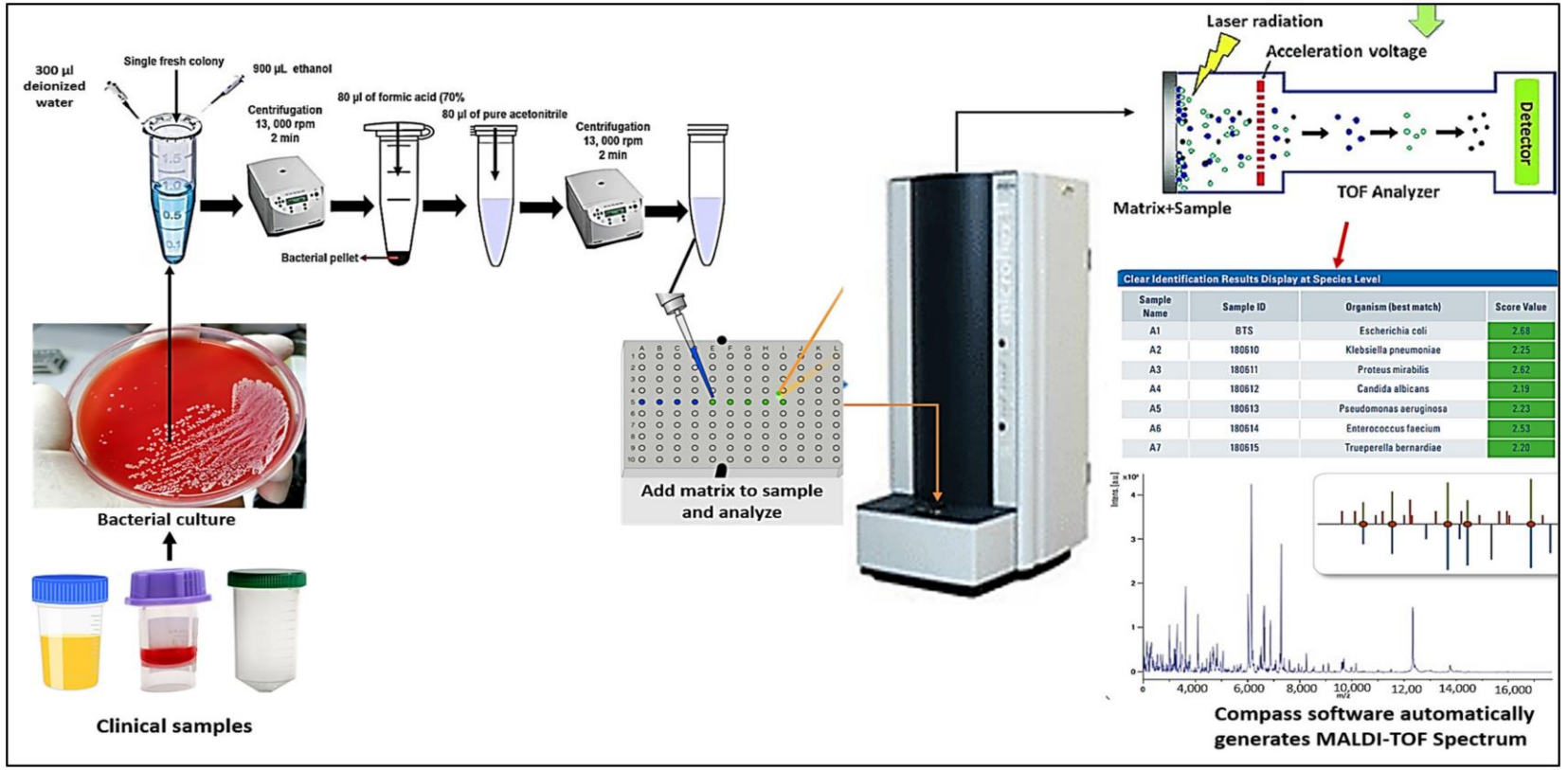
Identification of individual streptococci in clinical practice







Can MALDI-ToF mass spectrometry – distinguish streptococci?



Wan et al., 2023
3 different devices
No confusion between *S. pneumoniae* and non-pneumococci
Sequencing is required for accurate resolution (*S. mitis*, *S. bovis* group).

Workflow of MALDI-ToF, Elbehiry *et al*., 2022
Matrix: alpha-Cyano-4-hydroxycinnamic acid.

YES, except for *S. mitis* and *S. bovis*

References: Wan T, Lee T, https://doi.org/10.1128/jcm.01143-23; Elbehiry, A.; Aldubaib, M.; Abalkhail, A.; Marzouk, E.; ALbeloushi, A.; Moussa, I.; Ibrahem, M.; Albazie, H.; Alqarni, A.; https://doi.org/10.3390/vaccines10111881.

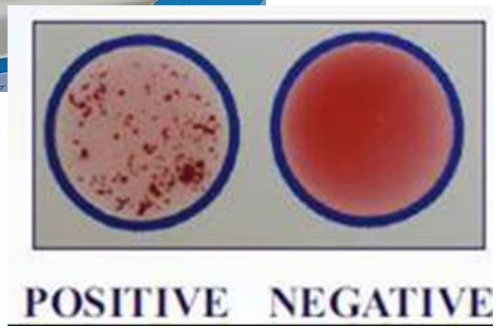
Serological classification and haemolysis patterns

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 , partial haemolysis (green)*

ϒ – no haemolysis, *non-haemolytic*

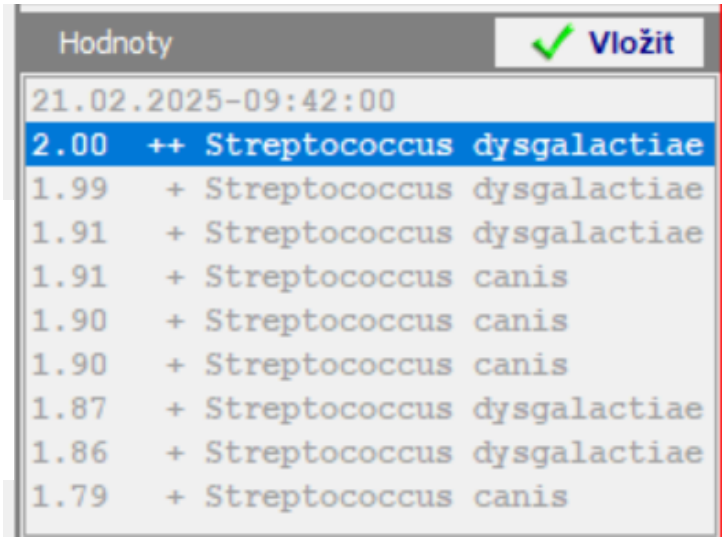


Lancefield grouping is based on the **carbohydrate composition of bacterial antigens found on their cell walls**.

The test utilizes latex particles sensitized with group-specific antibodies, which agglutinate in the presence of homologous antigens.

Streptococcus dysgalactiae or *S. pyogenes?*

Mass spectrometry



Latex agglutination

A+, B-, C-, D-, F-, G-

=*S. pyogenes*

It's an exception, but it can happen

KmerFinder-3.2-*Streptococcus dysgalactiae* subsp. *equisimilis* strain

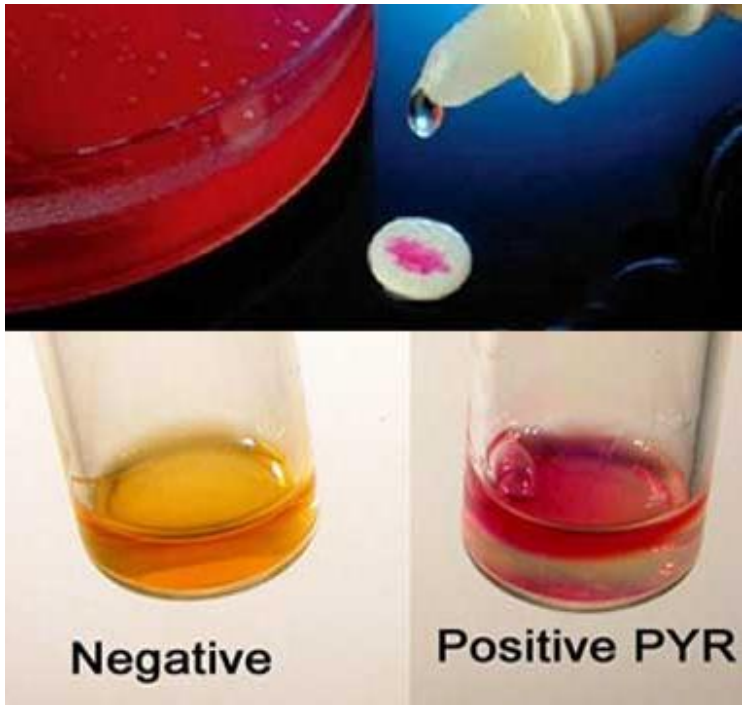
Database for *S. pyogenes*: *emm*CG: STG652.0, ST non-typable

Database for *S. dysgalactiae*: ST128

Blast M protein: *S. dysgalactiae* – deleted part of the gene = truncated protein

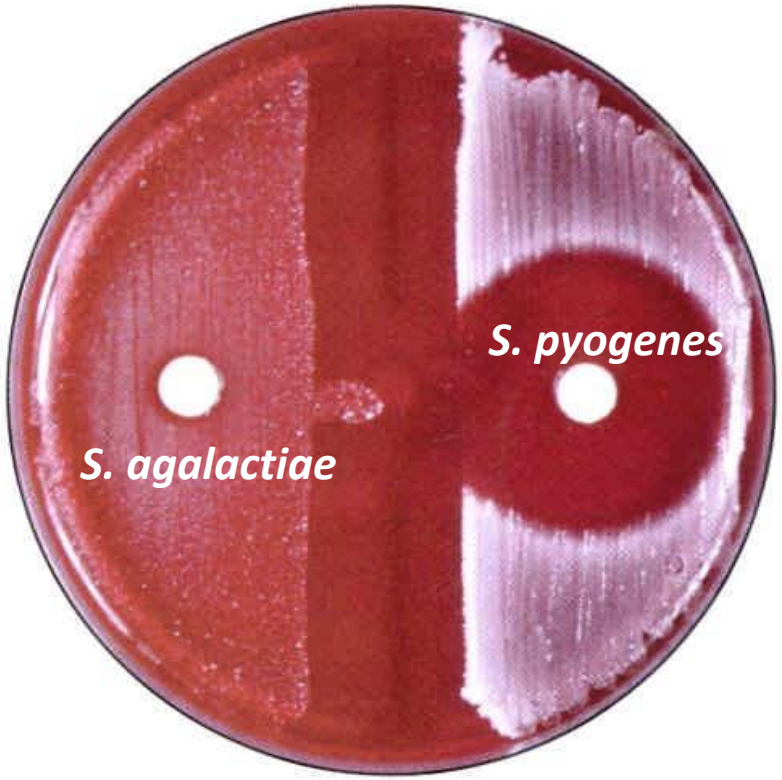
Blast 16S rDNA: *S. dysgalactiae* – 100%

Other tests for differentiation of streptococci



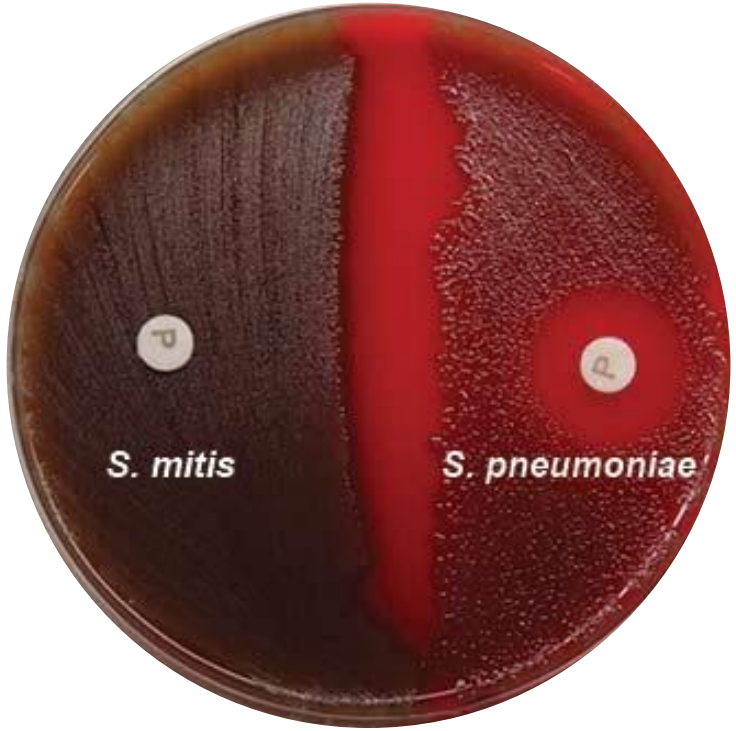
PYR Test - (Pyrrolidonyl arylamidase detection)
Identification of *Streptococcus pyogenes*, *Enterococcus* spp.

The enzyme breaks down the substrate → free β-naphthylamide is formed
Red colouration = positive reaction (PYR hydrolysis)



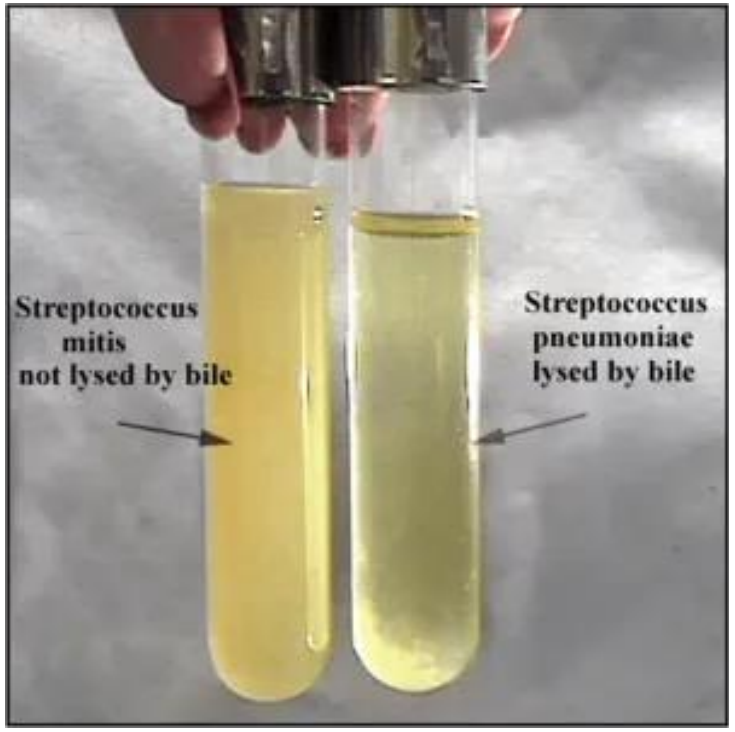
Bacitracin Susceptibility test
Bacitracin (polypeptide antibiotic)
Differentiation *Streptococcus pyogenes*-susceptible from other beta-haemolytic streptococci).

Other tests for differentiation of streptococci



Optochin susceptibility test
Optochin (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 selectively autolyse *Streptococcus pneumoniae*** when added to actively growing bacteria in agar or broth media. Deoxycholate (bile) **activates an autolysin** in the cell wall of pneumococci.

CAMP test – *S. agalactiae*

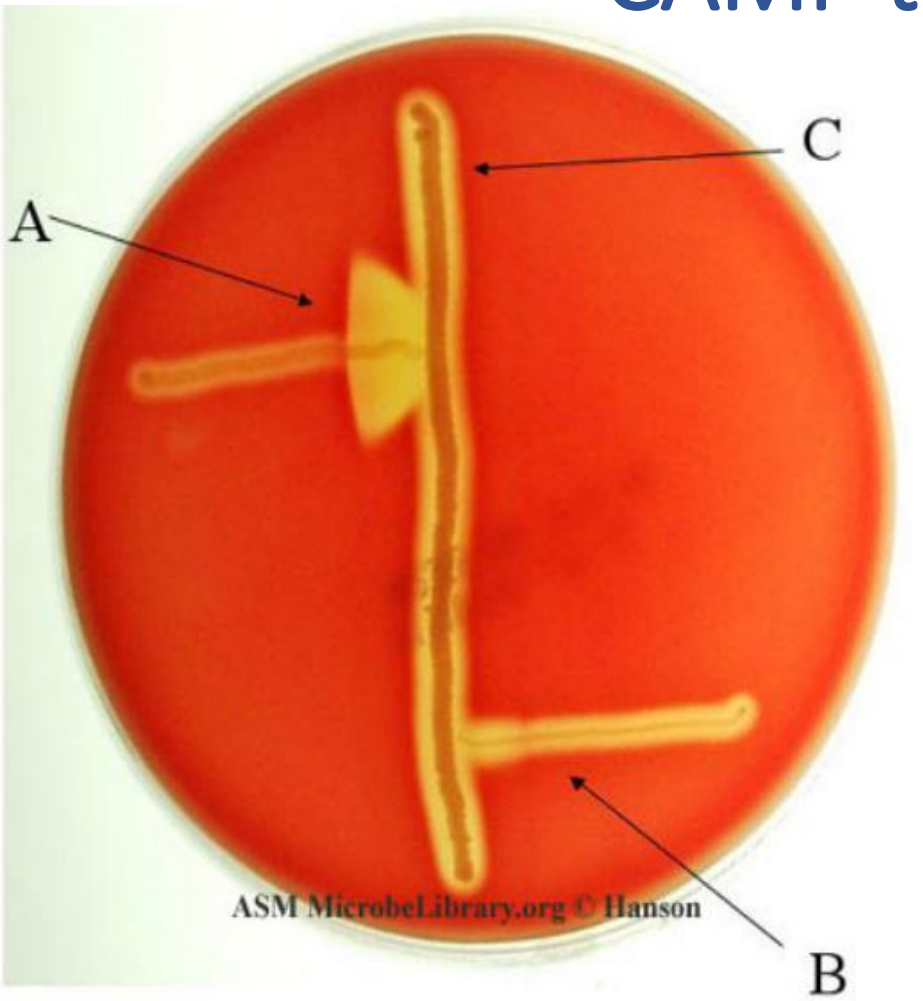


FIG. 2. CAMP test for the identification of ***Streptococcus agalactiae*** (group B). (A) ***Streptococcus***(group B) shows a positive CAMP reaction. (B) ***Streptococcus pyogenes*** (group A) shows a negative reaction when inoculated at a right angle to (C) ***Staphylococcus aureus***.

- Principle:

Synergy between:

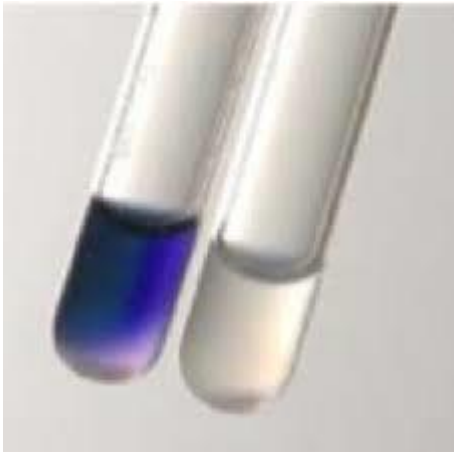
β-lysine (*Staphylococcus aureus*)

CAMP factor (*Streptococcus agalactiae*, group B)

Result:

Intensified hemolysis zone between cultures

Negative: Streptococci of groups A, C, G



Hippurate hydrolysis

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

*Christie, Atkins, Munch-Petersen

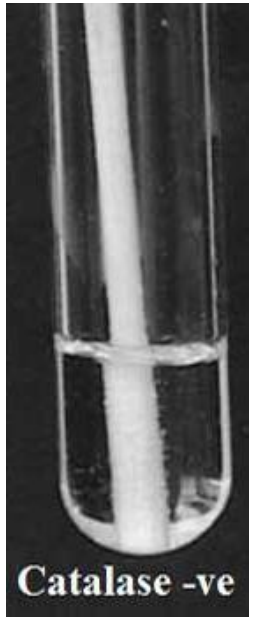
Classification of common Streptococci

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

Streptococcus pyogenes (GAS)

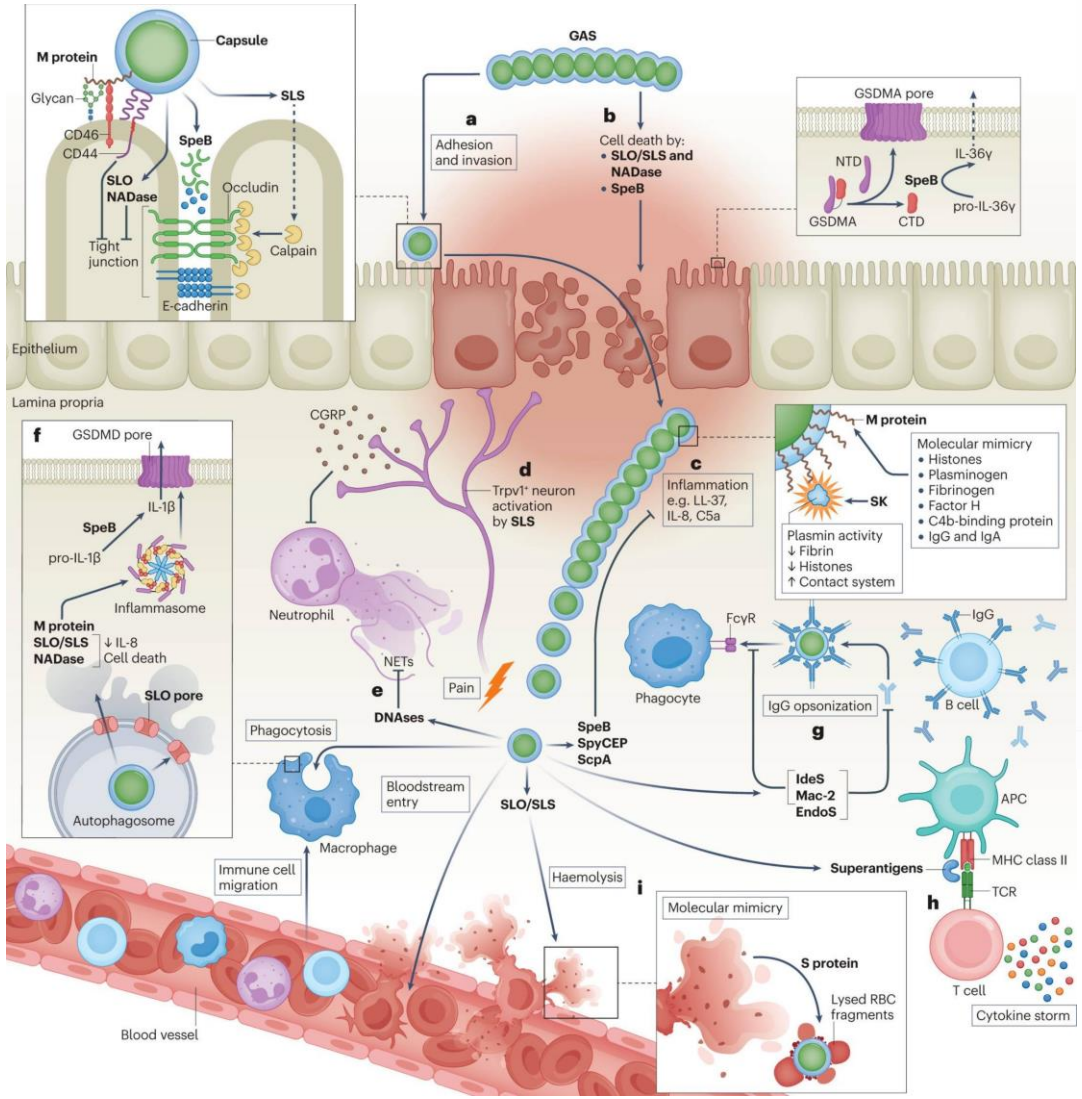


Primary human pathogen

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



Factor (Protein/Enzyme)	Category (Phase)	Target (Host)	Clinical Effect (Mechanism)
M-protein	Adhesion / Modulation	CD46 / Factor H	Firm attachment, false self identity
F-protein	Adhesion	Fibronectin	Invasion of the extracellular matrix
Hyaluronic acid capsule	Adhesion	CD44	Molecular mimicry, invisibility to the immune system
C5a peptidase	Invasion	C5a component	Impairment of neutrophil recruitment (reduced chemotaxis)
Hyaluronidase	Invasion	Hyaluronic acid	Tissue spreading (rapid local dissemination)
Streptolysins (SLO, SLS)	Invasion	Cell membranes	Cell and tissue destruction, goal of ASO diagnosis
DNases	Invasion	Neutrophil extracellular traps (NETs)	Evasion of physical immune barriers
S-protein	Modulation	Erythrocytes	Prevents phagocytosis of opsonized bacteria
SIC / DRS proteins	Modulation	Complement (C5b67, C6/7)	Inhibit pore formation by host complement
Pyrogenic exotoxins	Modulation	MHC-II and T-cell receptor (TCR)	Superantigen-mediated cytokine storm (Scarlet fever, STSS)

Each strain of *S. pyogenes* has virulence factors.
There is no single virulence factor that explains invasiveness.
A combination of strain factors and the host's immune response.

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

Streptococcus pyogenes – clinical diseases (respiratory tract infections)

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

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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 (skin and soft tissue infections)

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

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.

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

Blood cultures: fever, chills.

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

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

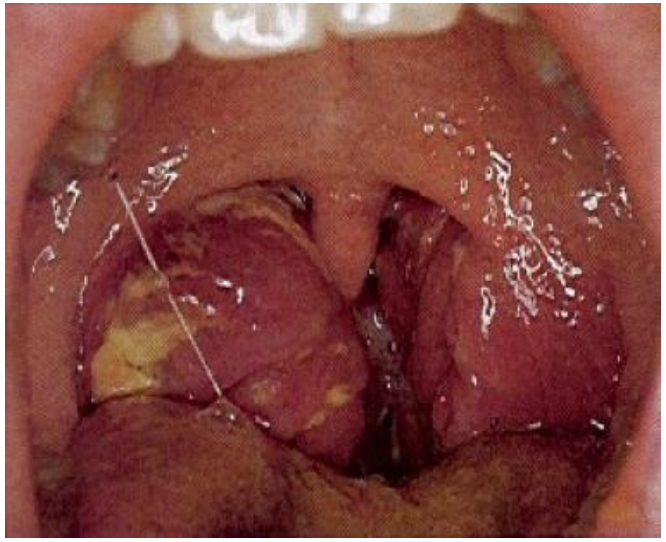
Acute glomerulonephritis – acute inflammation of the renal glomeruli with edema, hypertension, hematuria and proteinuria
Immunocomplex deposits in glomeruli - impaired renal function
After respiratory tract or skin infection - Nephritogenic types (protein M)

Detection of anti-streptolysin O (ASLO, ASO) antibodies

S. pyogenes – antimicrobial susceptibility testing

Laboratory

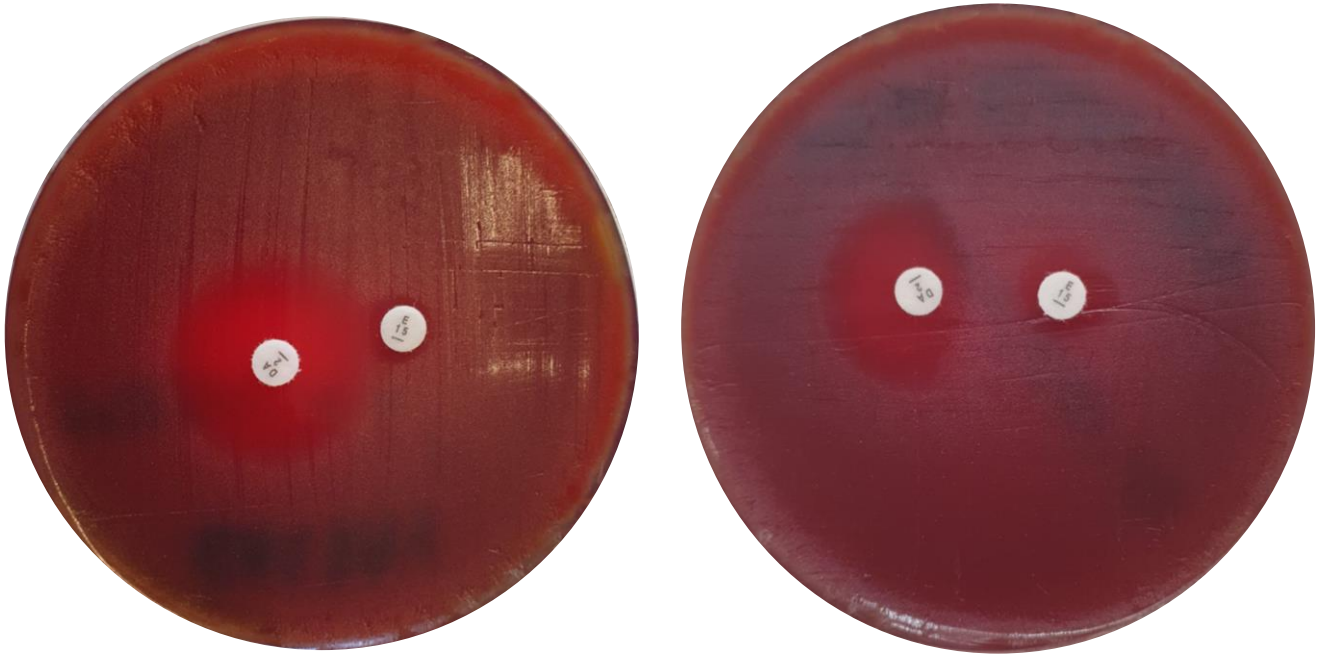
Reality



Temperature?
Nutrients?
Other microbes present?
Immune response of the host

Stress response of the pathogen

Inducibility of resistance to clindamycin



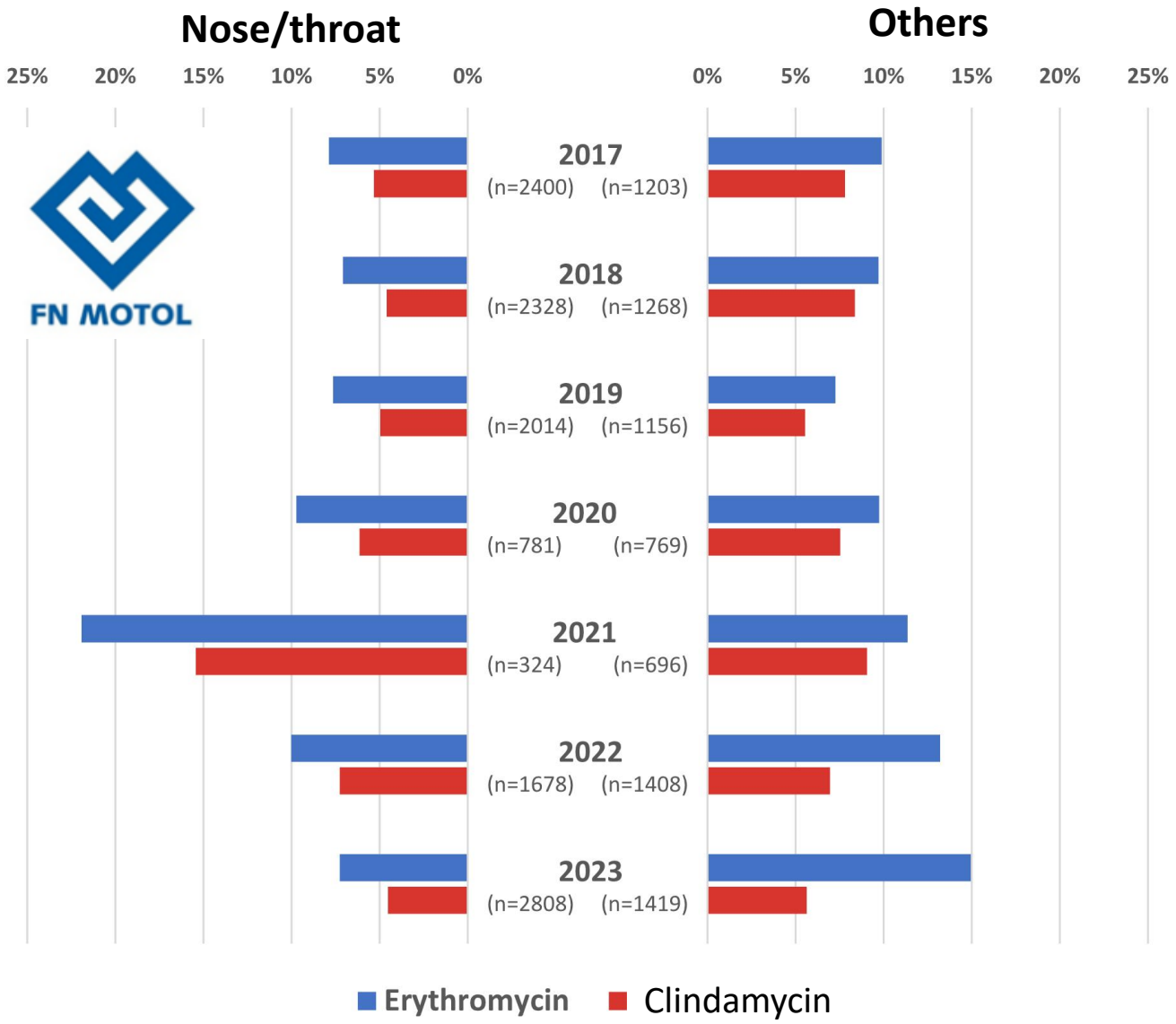
"Clindamycin can still be used for short-term therapy of less severe skin and soft tissue infections because constitutive resistance is unlikely to develop during such therapy.„

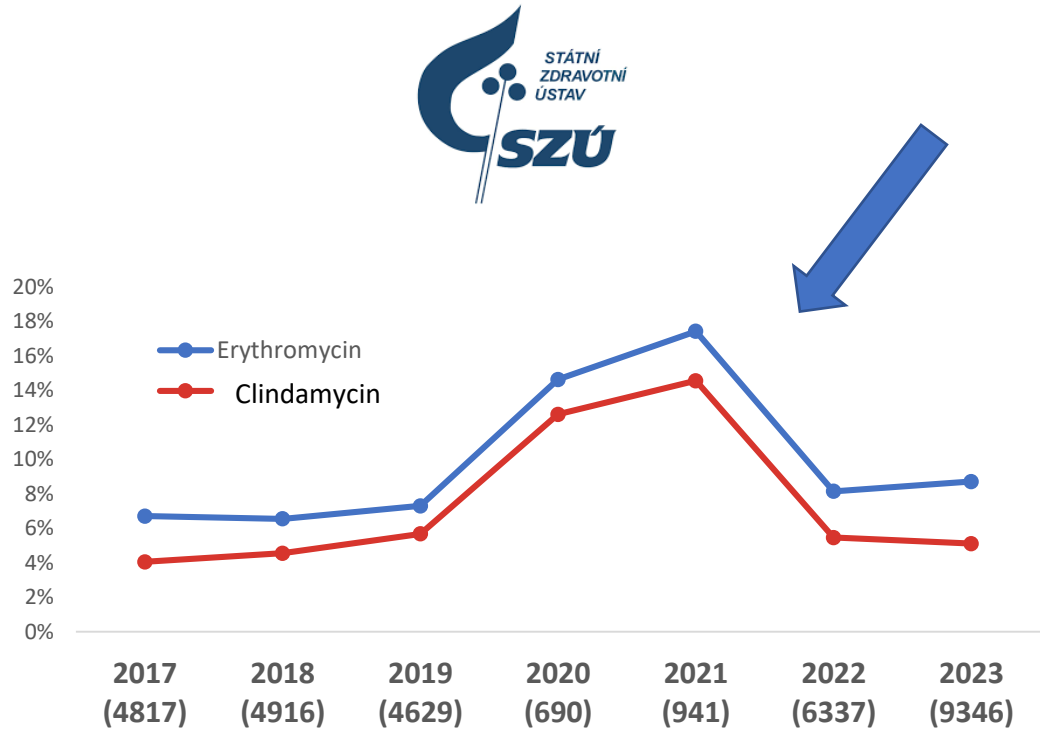
The clinical relevance of inducible clindamycin resistance in the combination treatment of severe S. pyogenes infections is unknown.

Place the discs of erythromycin and clindamycin 12-16 mm apart (from edge to edge) and look for antagonism (D phenomenon).

EUCAST v. 14.0, valid from 01/01/2024

S. pyogenes ATB susceptibility according to location



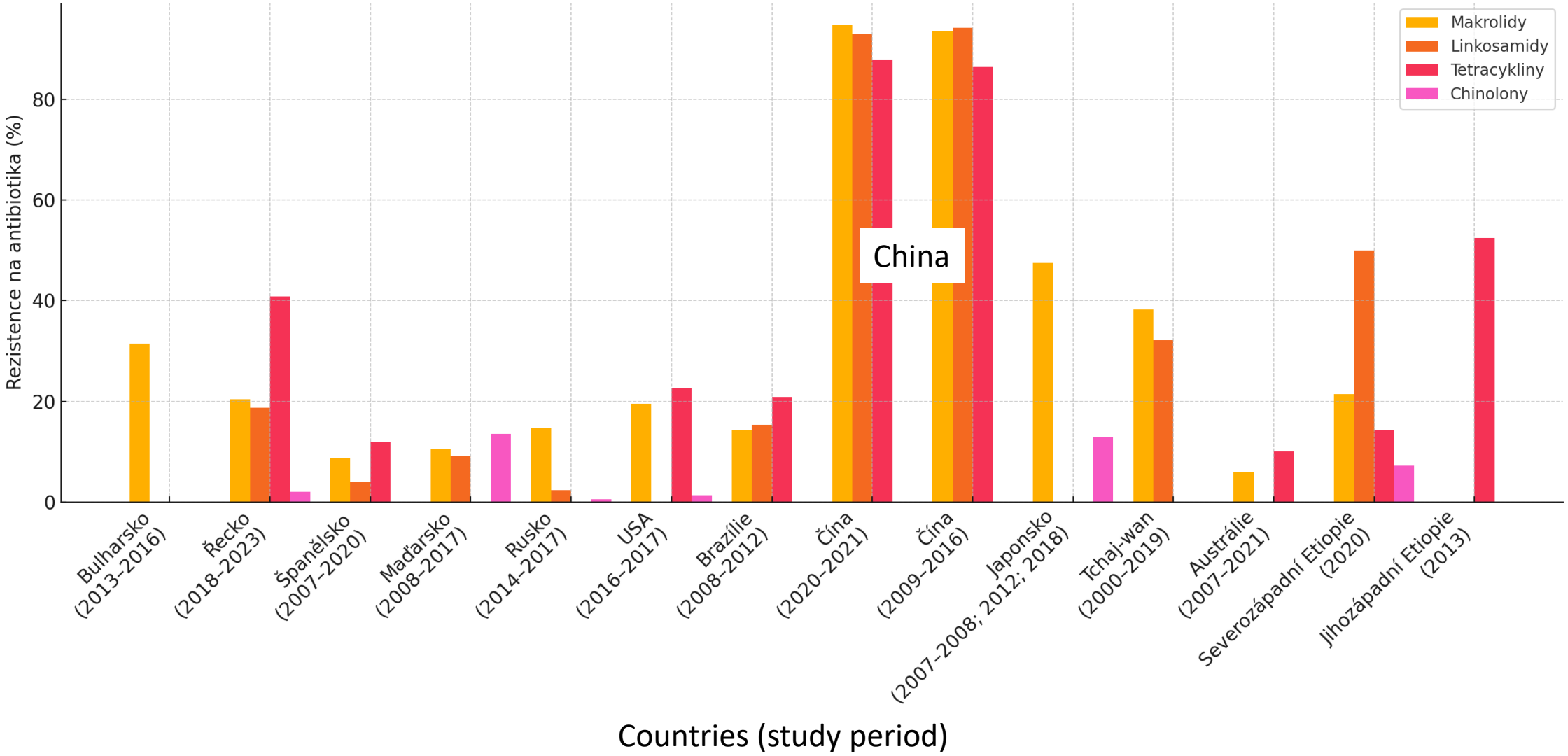


Working Group on Resistance Monitoring – PSMR

No PNC resistance

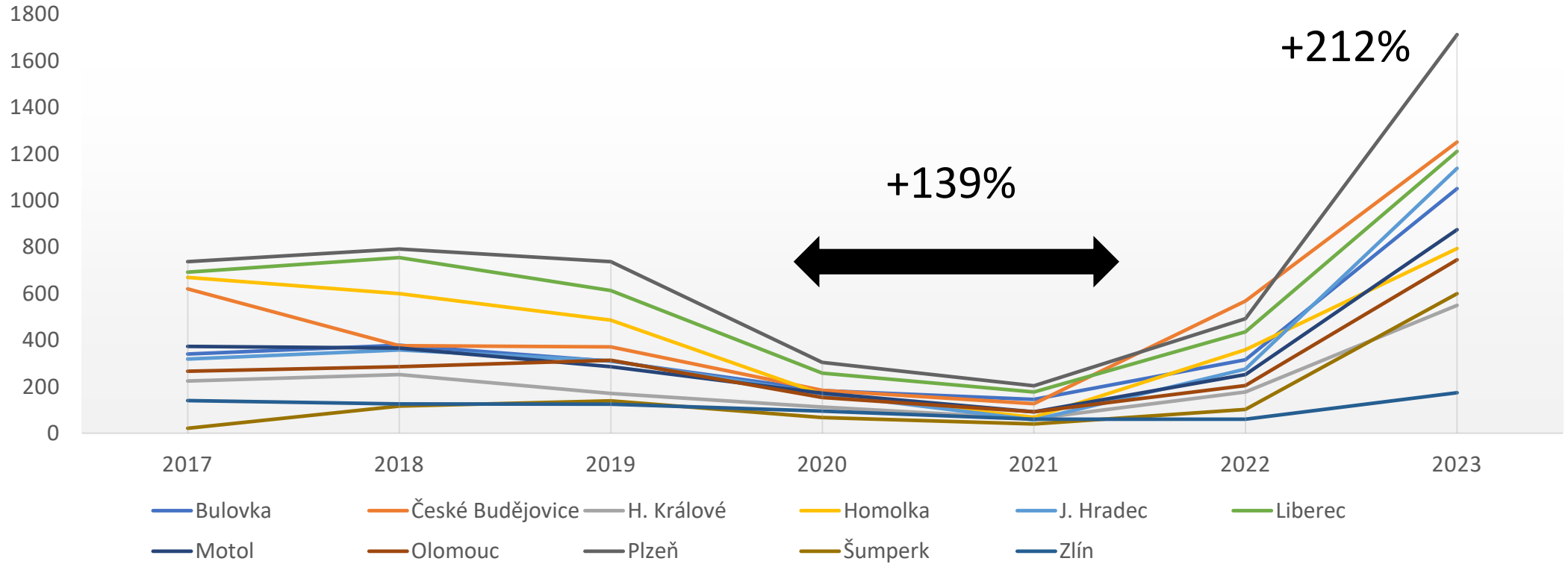
Štefan *et al.*, unpublished

S. pyogenes resistance worldwide



Gergorova *et al*., 2024, doi.org/10.3390/antibiotics13040360

Increase of *S. pyogenes*: culture positivity after COVID-19 pandemic

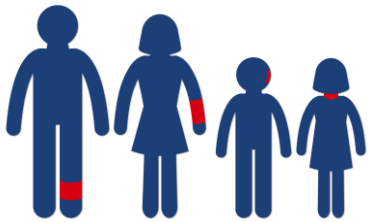


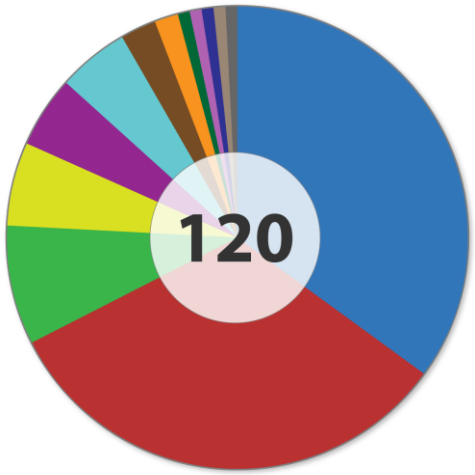
Data from 11 Czech hospitals (28978 records)

Stefan et al., 2025.

Streptococcus pyogenes – characterisation of isolates

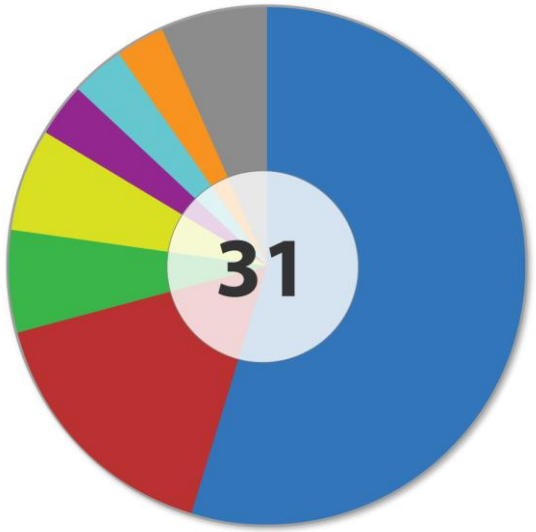
✓ *emm* typing (sequence M protein gene)



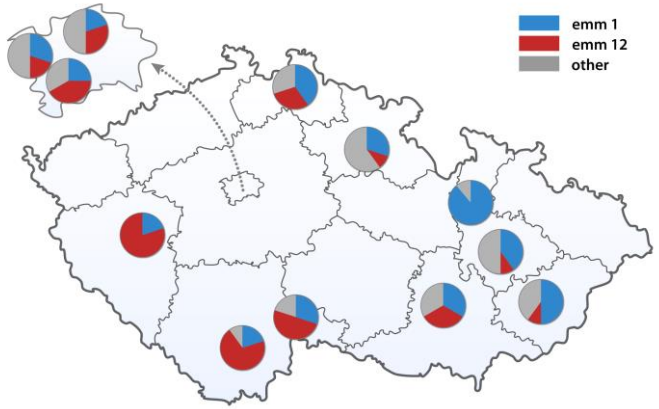


1 (35,0 %)
12 (32,5 %)
4 (8,3 %)
28 (5,8 %)
49 (5,0 %)
89 (5,0 %)
60 (2,5 %)
77 (1,7 %)
58 (0,8 %)
76 (0,8 %)
77 (0,8 %)
86 (0,8 %)
117 (0,8 %)

1 (54,8 %)
12 (16,1 %)
28 (6,5 %)
49 (6,5 %)
77 (3,2 %)
4 (3,2 %)
58 (3,2 %)
NA (6,5 %)



Dominant *emm* types have unique and shared virulence factors for adhesion and immune response evasion (*sic*/*drs* genes) plus the non-trained immune response of children due to the contact precautions measures during the COVID-19 pandemic.



FN MOTOL

Štefan et al., 2025

S. pyogenes infections (mild, moderate)- treatment

- Penicillins: resistance not reported

Allergy to beta-lactams: immunology testing recommended!!

PNC shortage- use other narrow-spectrum beta-lactams

- Macrolides (erythromycin) and lincosamides (clindamycin): second line, but growing resistance(15%).
- Mechanisms: efflux pumps (macrolides), methylase genes (lincosamides, *erm*A-ind).

- Beware of frequent carriage! Do not treat only culture positivity!

- No vaccine so far but M-protein-based vaccines are in development

CAVE! The addition of a beta-lactamase inhibitor does not constitute a clinical benefit.

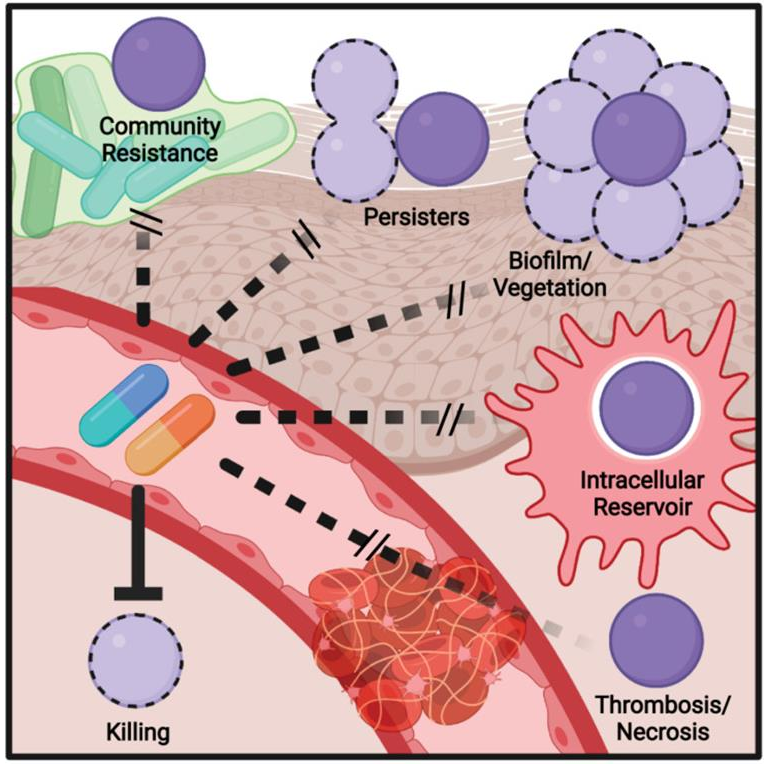
Invasive GAS and recurent GAS prevention : What to do about it therapeutically and prophylactically?

- **Surgical procedure for iGAS (removal of tissue, pus), penetration of antibiotics**
- **For iGAS: PNCs in combination with antibiotic inhibiting toxin synthesis (clindamycin, linezolid), intravenous immunoglobulins**
- **Secondary prophylaxis of depot PNC**
- **Immunological examination**
- **Decontamination of the environment**

Andreoni F, Zürcher C, Tarnutzer A, et al. Clindamycin Affects Group A Streptococcus Virulence Factors and Improves Clinical Outcome. J Infect Dis 2017; 215(2) :269-277.
Parks T, Wilson C, Curtis N, et al. Polyspecific Intravenous Immunoglobulin in Clindamycin-treated Patients With Streptococcal Toxic Shock Syndrome: A Systematic Review and Meta-analysis. Clin Infect Dis. 2018 Oct 15;67(9):1434-1436.
Thomas KS, Crook AM, Nunn AJ, et al. Penicillin to prevent recurrent leg cellulitis. N Engl J Med. 2013;368(18):1695-703.

S. pyogenes infections- treatment failure

Treatment failure?



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

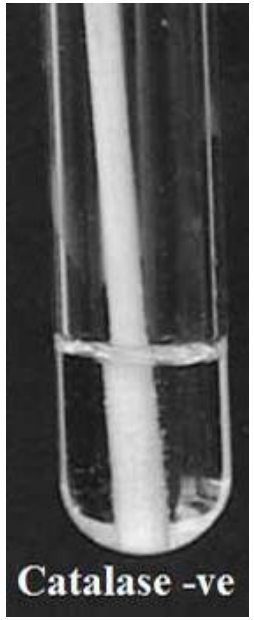
Persistence? Altered growth rate, if the cell is not dividing or is not metabolically active then ATB does not work.

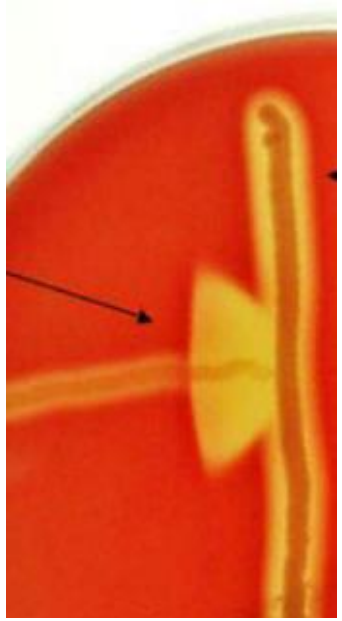
Biofilm formation, invasion of epithelial cells, survival in phagocytes?

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 agalactiae





Gram-positive coccus, long chains

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

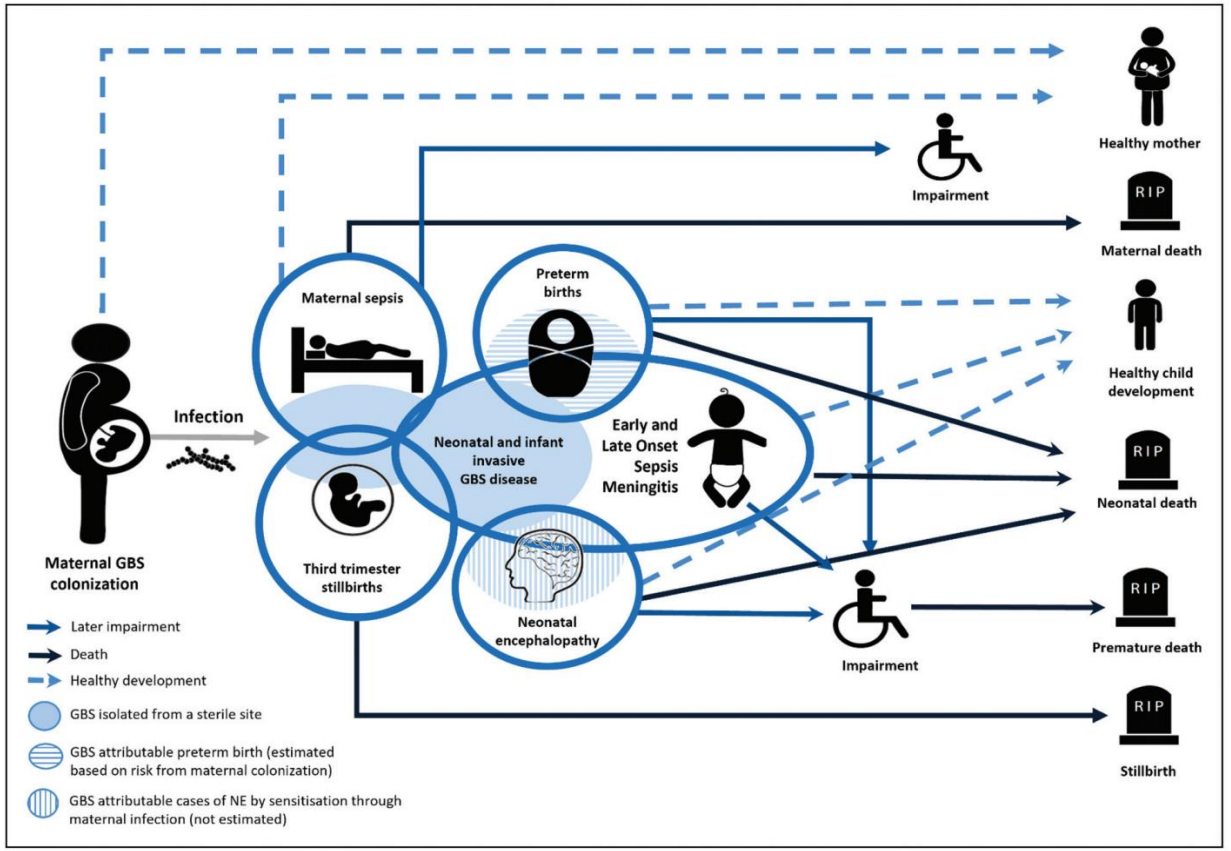
Streptococcus agalactiae

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.



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

Master of Disguises and Biological Sabotage

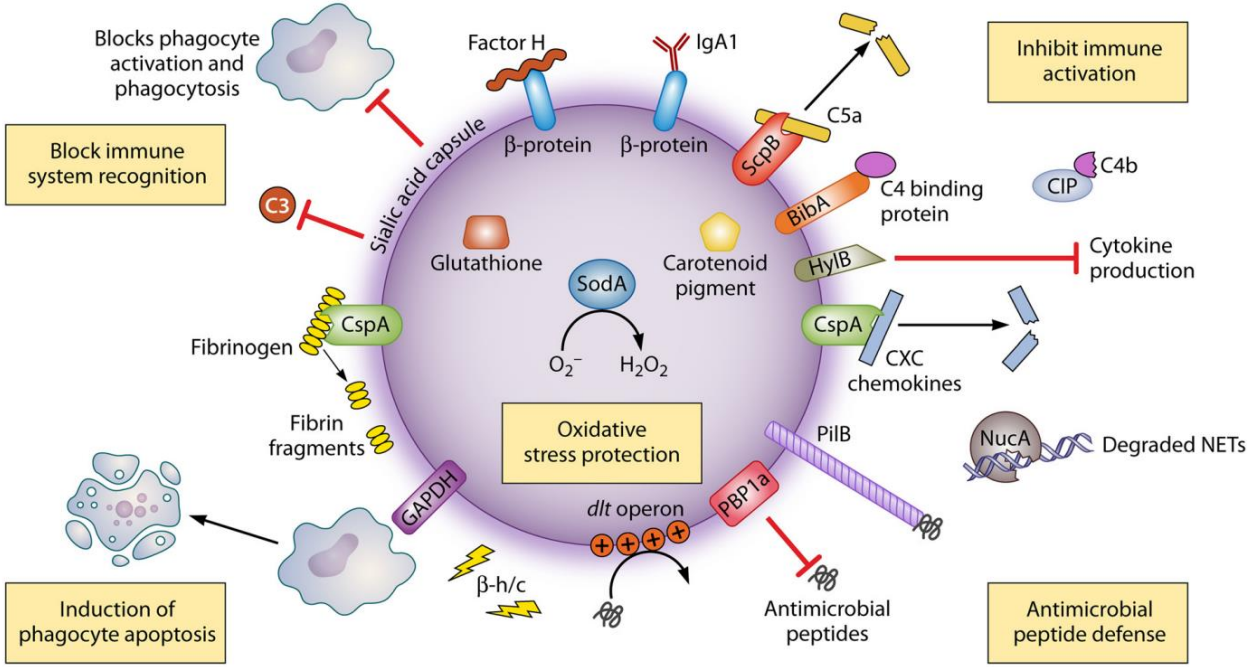


FIG 1 Mechanisms used by GBS to evade the immune system. GBS expresses many factors that help it evade the immune system and increase its survival in the host. The sialic acid capsule and fibrin fragments cleaved by CspA that coat the surface help GBS present as "self" to the immune system. The capsule 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.

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.

Sialic acid (part of capsular polysaccharide - CPS)
-Molecular mimicry
Binding to the surface of white blood cells (SigleC) – blocks phagocytosis, the formation of neutrophilic extracellular traps
Complement blocking (prevents the deposition of the C3B component and binds the H factor)

β-hemolysin/cytolysis
Osmotic lysis of red blood cells and damages host barriers, including the placenta
Activation of inflammation

The "**Trojan horse**" GBS can survive inside macrophages (the cells that are supposed to destroy it). In this way, it can be conveniently distributed around the body and overcome barriers such as the one in the brain, leading to meningitis.

Streptococcus agalactiae – disease and laboratory diagnostics

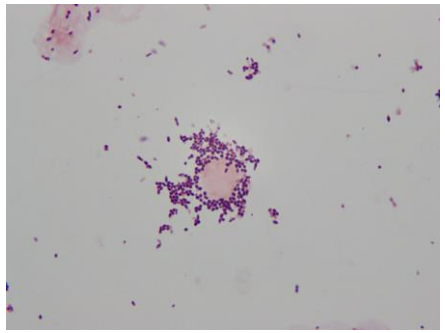
Neonatal disease (early till 7 days or late onset)
Pneumonia, meningitis, sepsis
Sample collection: CSF, blood cultures, a swab from conjunctiva, nose, throat, amniotic fluid if possible

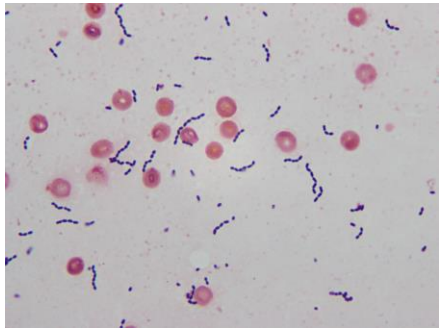
Men and non-pregnant women-bacteremia, pneumonia, bone and joint infections, skin and soft tissue infections
Sampling: blood cultures, sputum, puncture, swab?

Chromogenic medium
Screening - pregnant women

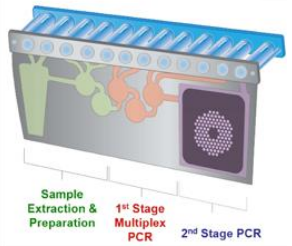
Other swabs, punctate, CSF
Blood agar, 5% CO2

Blood culture
Newborns
Fever, chills, tremor





Microscopy – CSF, positive blood cultures



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

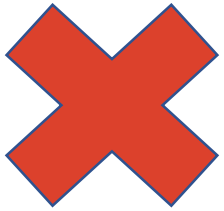
Other β haemolytic Streptococci

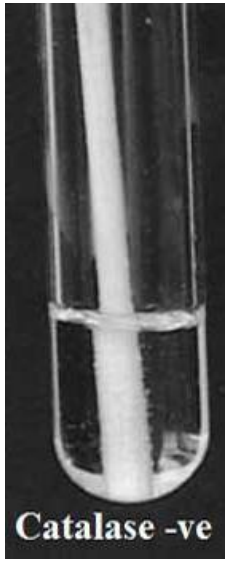
***Streptococcus dysgalactiae* (C, G, L)**

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



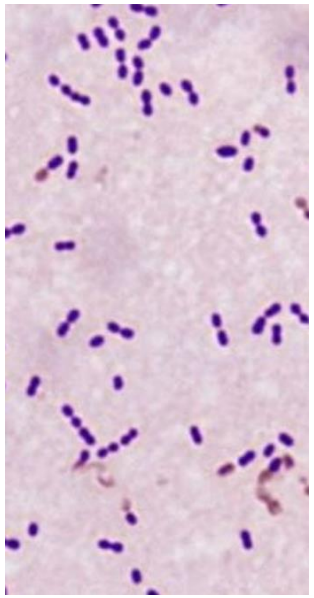


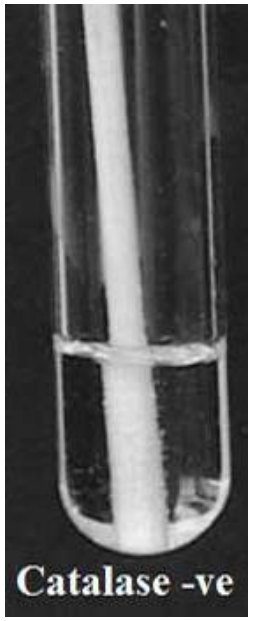
***Streptococcus anginosus* (C,F,G or non-typeable)**

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

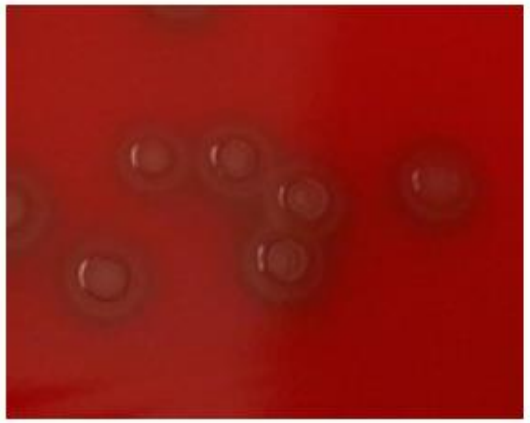




Gram-positive coccus, pairs or short chains

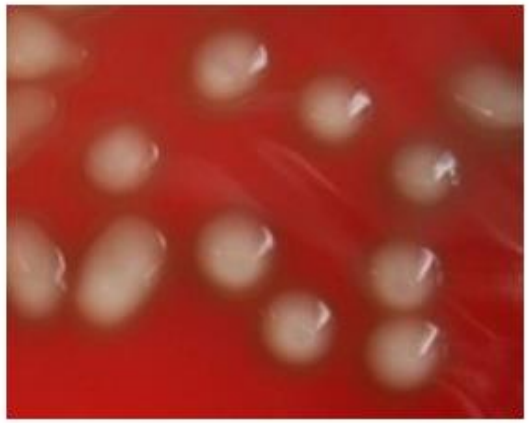
Facultative anaerobe (CO_2 thermostat), catalase-negative, α-haemolytic, non-groupable- no specific polysaccharide in the cell wall, optochin sensitive, bile salt solubility positive.

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.



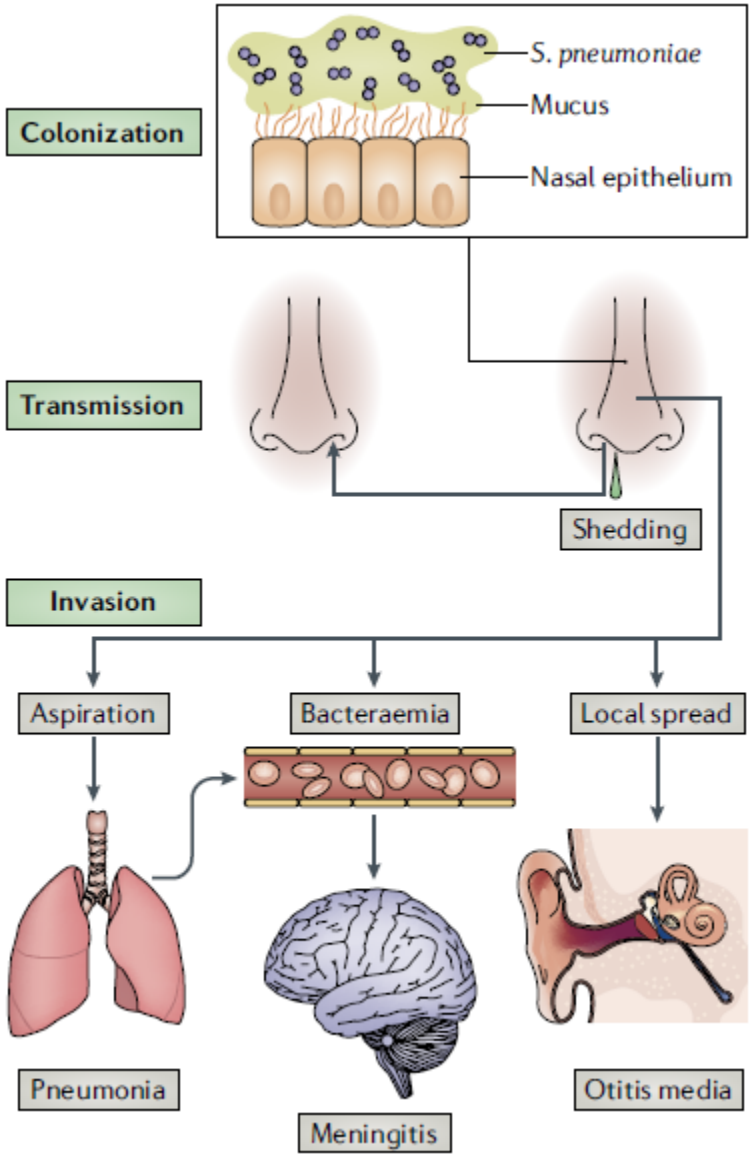
Virulent, encapsulated form **M-phase**. Cultivated on Columbia agar with 5% defibrinated sheep blood, 48 hours in an aerobic atmosphere enriched with 5% carbon dioxide, 37°C. Colonies are surrounded by a zone of alpha-hemolysis.

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.

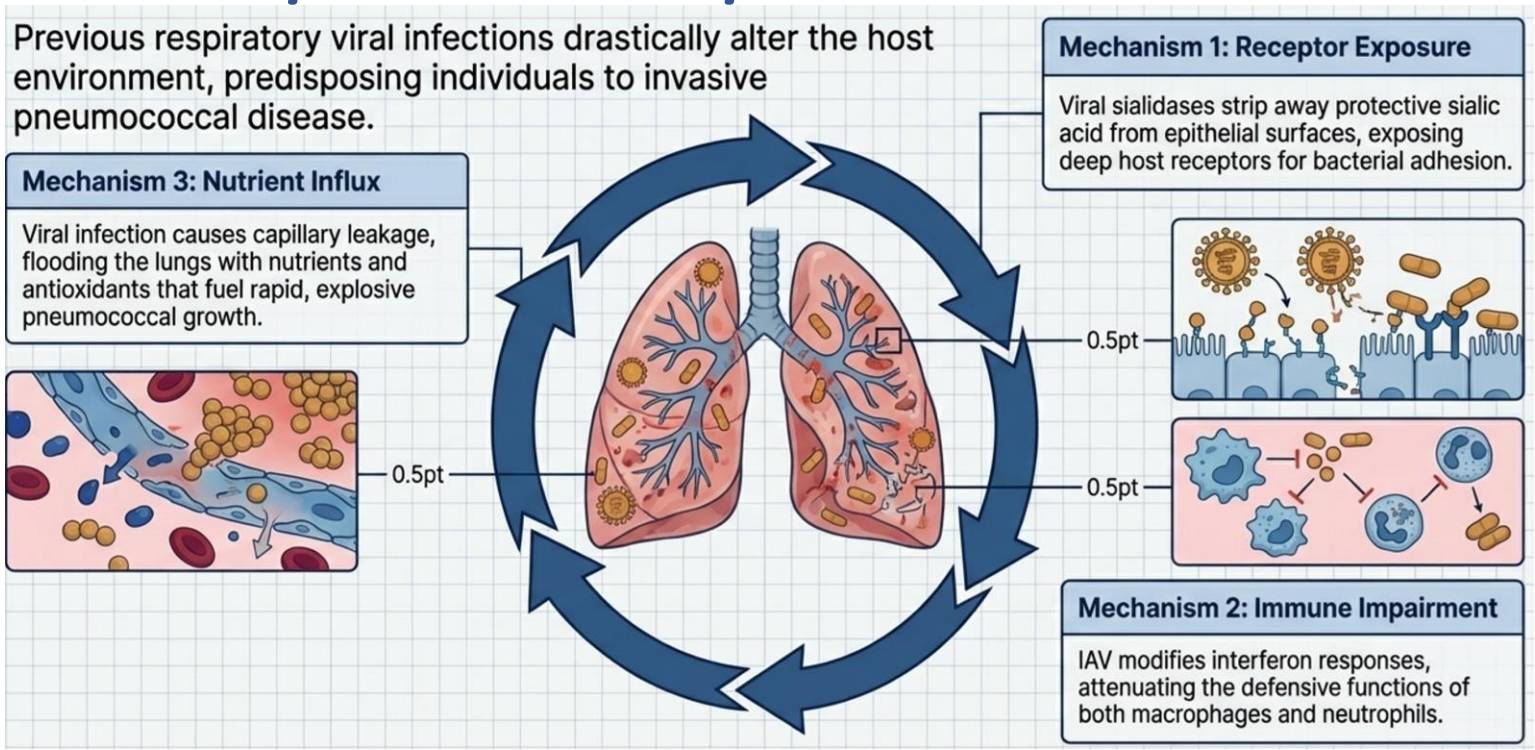
Dissemination beyond its niche along the nasal epithelium, either by aspiration, bacteraemia or local spread, can lead to invasive diseases, such as **pneumonia, meningitis and otitis media**.

Risk factors

Pneumonia: preceding viral infection, aspiration because of severe drunkenness, administration of morphine;
Meningitidis: small children, immunocompromised patients

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



Capsule

main virulence factor, prevents phagocytosis

Surface polysaccharide C

interacts with the host CRP protein – induction of inflammation

Pneumolysin (Ply)

Cell destruction, tissue damage, immune response leakage in macrophages

Disruption of mechanical barriers and mucociliary apparatus (cell damage, free radicals)

Adhesion receptor reveal: Viral neuraminidases (sialidases) remove sialic acid from the surface of host cells

Modulation and weakening of the immune system: Viruses modify the interferon responses of the host, leading to a weakening of macrophage and neutrophil function

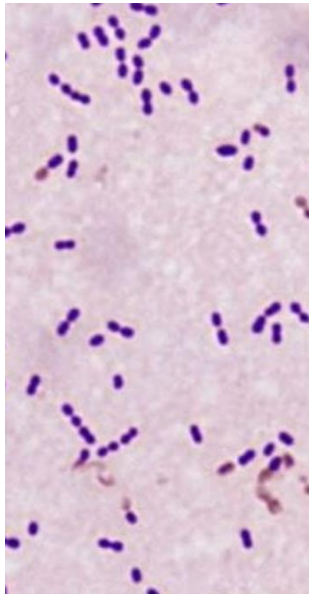
Nutritionally rich environment....

Increased bacterial load and risk of aspiration (inflammation, mucus, higher density)

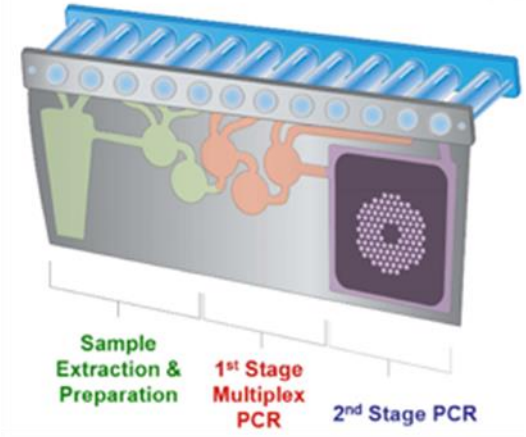
Pneumococci enter the brain from the bloodstream, but recent research shows that they can penetrate directly from the nasopharynx.

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 %

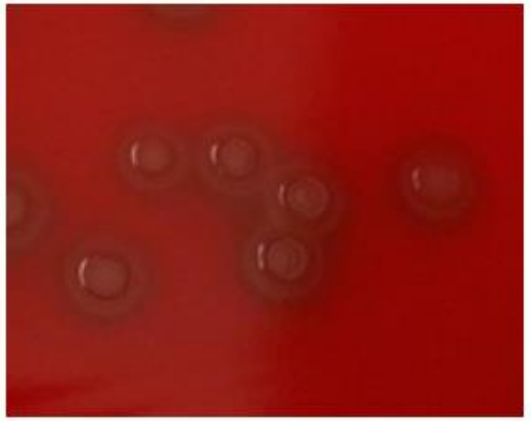


Microscopy – CSF, sputum positive blood cultures, liquid samples



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, ampicillin), cephalosporines (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.

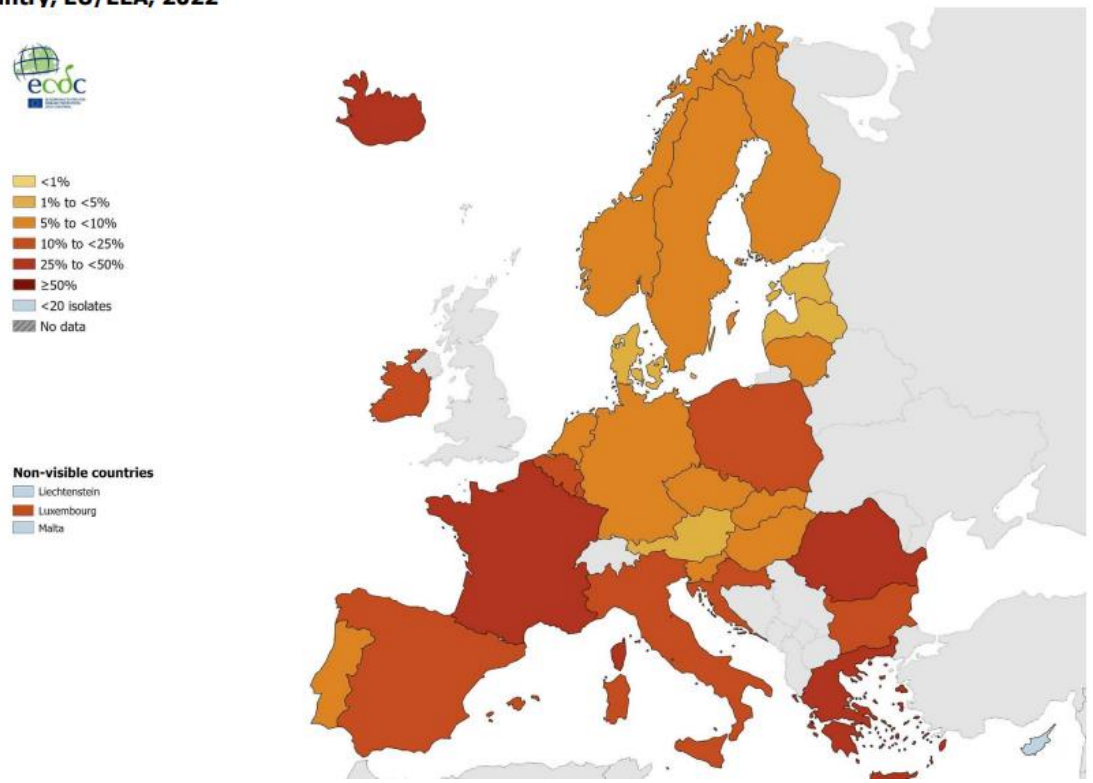
Mechanism of resistance

- **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**



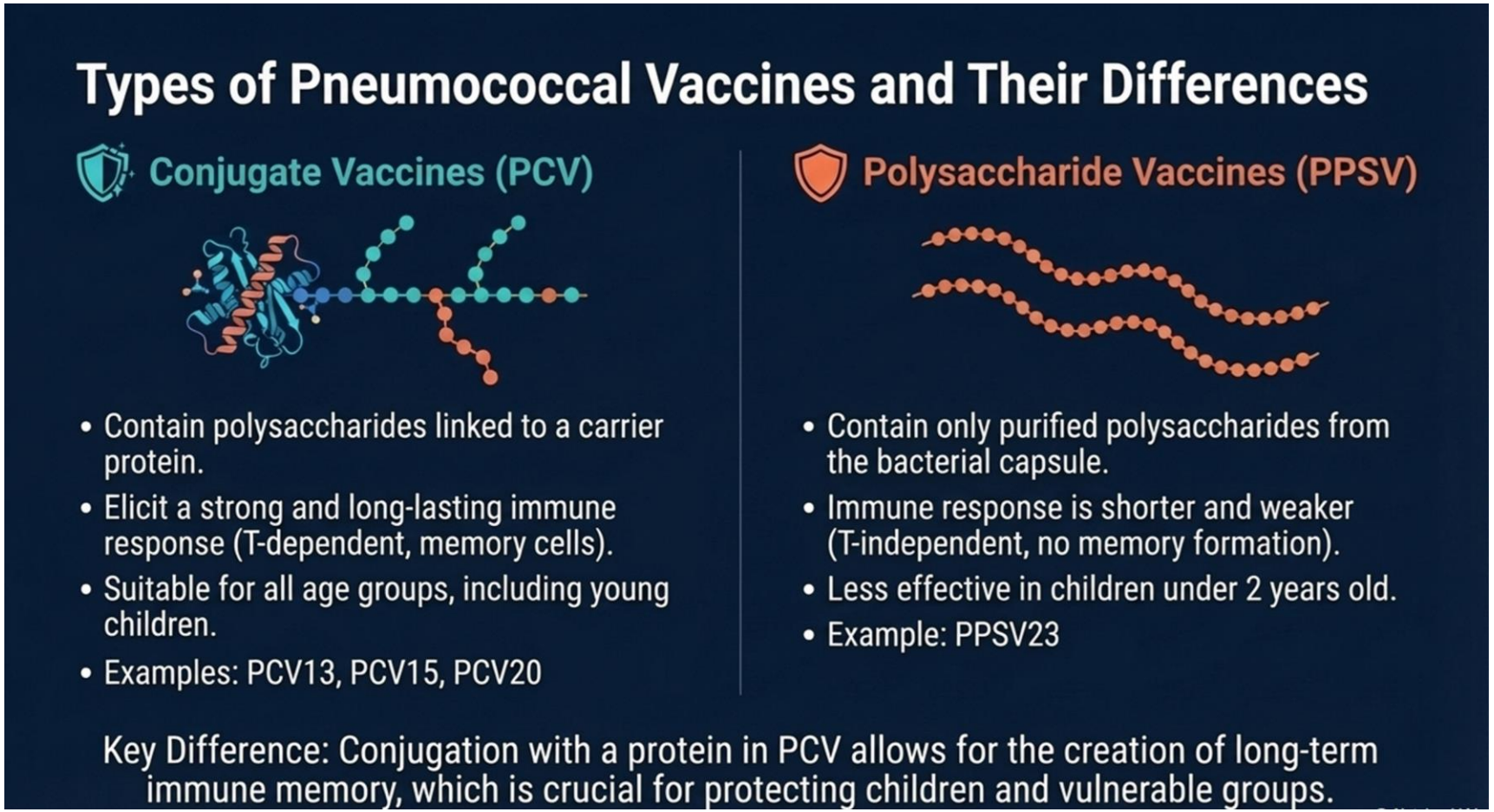
For *S. pneumoniae*, the term penicillin non-wild-type is used in this report, referring to S. *pneumoniae* isolates reported by local laboratories as **'susceptible, increased exposure' (I) or resistant (R)** to penicillin, assuming MIC for benzylpenicillin above that for wild-type isolates **(>0.06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

Travel history!

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

Vaccination against *S. pneumoniae*

Prevention of invasive infection and acute otitis media

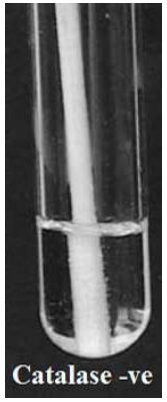


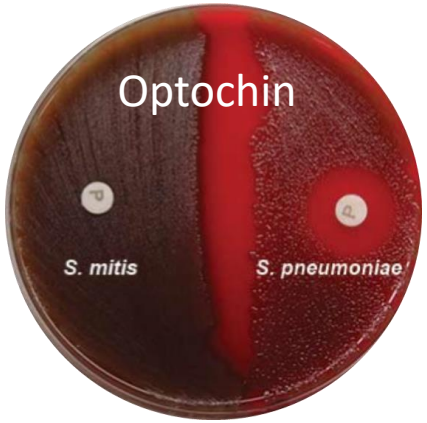
Young children, over 65 years old, and people with chronic diseases. Statistics of the Czech Republic: IPO from the age of 40. Evolutionary pressure! Serotype-independent vaccine.

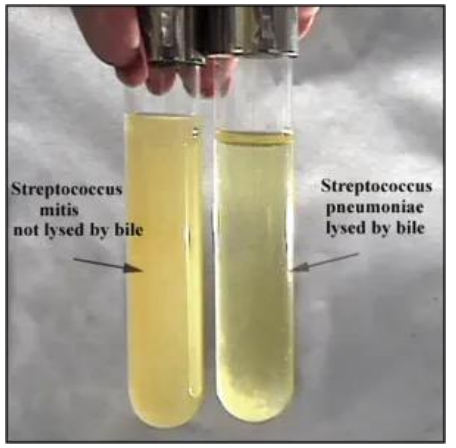
Viridans streptococci

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

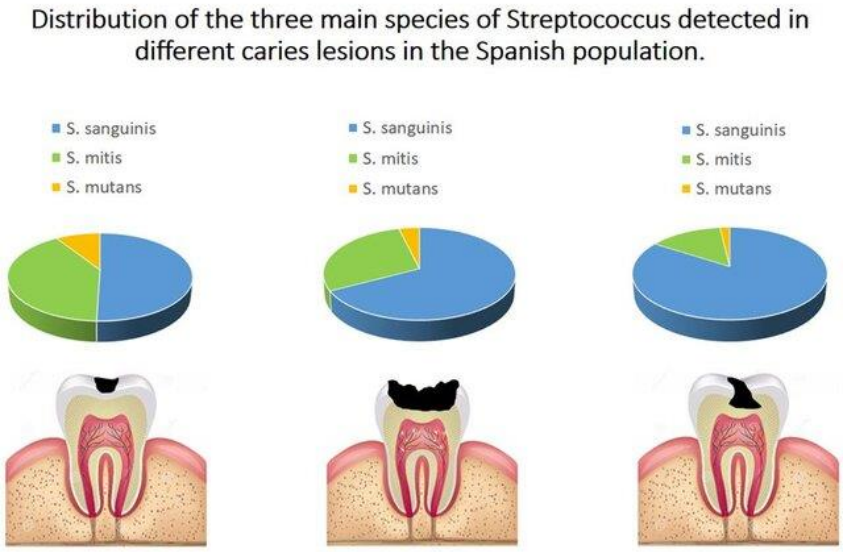
Viridans streptococci colonise the oropharynx, gastrointestinal tract and genitourinary tract.
Rarely found on the skin surface because surface fatty acids are toxic to them

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.



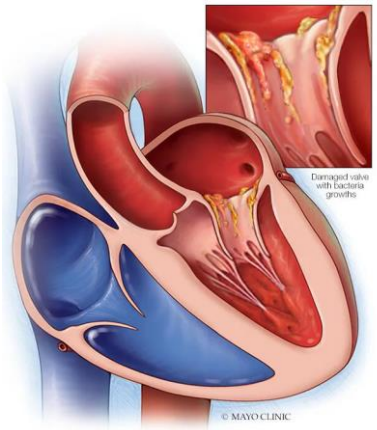
Simon Soro et al. (2014)

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.

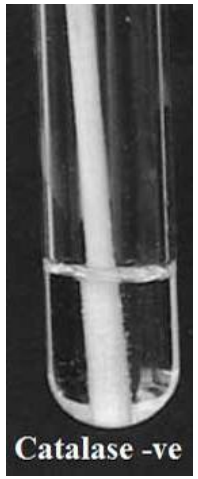


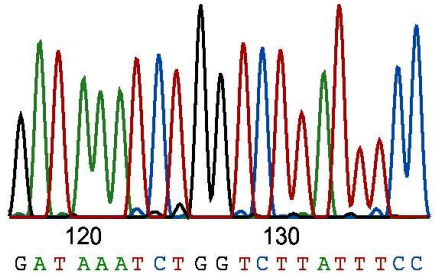
Diagnostics:
Blood cultures
Pan bacterial 16S rDNA PCR (tissue)

- **Brain abscess • Osteomyelitis • Sepsis – neutropenic patients**

Streptococcus bovis

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





16S rDNA sequencing

***mitis* or *bovis* group?**

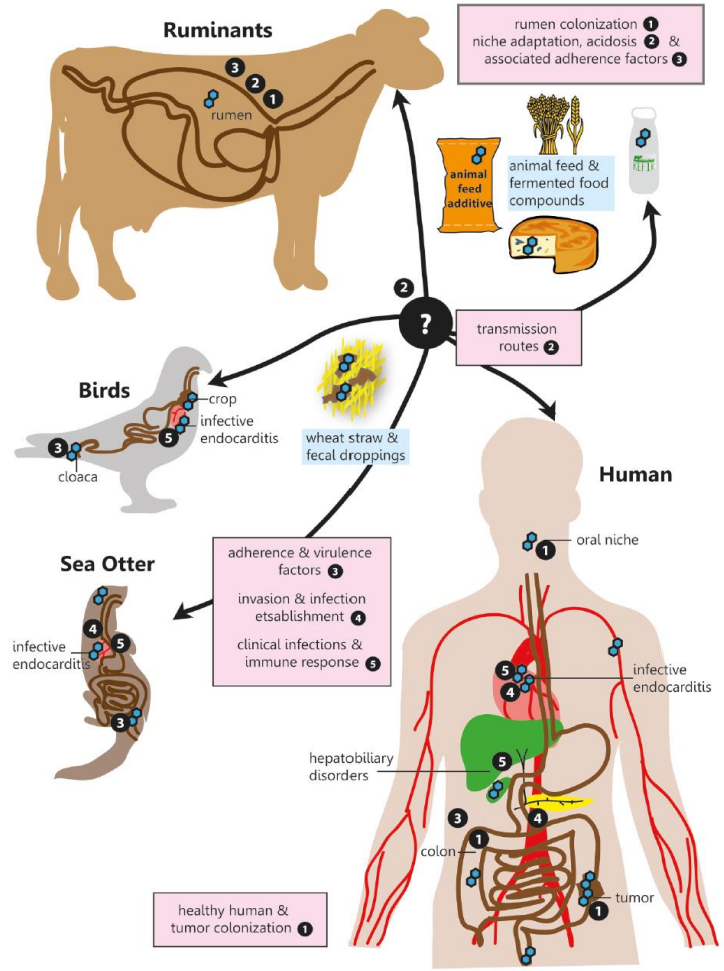
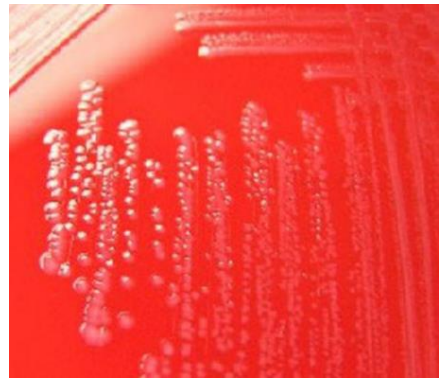


FIGURE 1 | SBSEC - the road to infection. Graphical overview of the different niches inhabited by SBSEC members as well as relevant aspects of host colonization, adherence, invasion and infection covered in the corresponding chapters indicated by bullet point numbers. 1. Prevalence and colonization of SBSEC in animals and humans, 2. Transmission and niche adaptation of SBSEC members, 3. Mechanisms and virulence factors responsible for adhesion and host colonization by SBSEC members, 4. Invasion and infection establishment, 5. Clinical infections and host-immune response due to SBSEC in animals and humans.

Jans *et al.*, doi.org/10.3389/fmicb.2018.00603

Enterococci („enteric cocci“)

*Previously classified as group D streptococci (posses group D cell wall antigen, Lancefield)
-but 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
- clinically most significant

G-positive
Short chains or pairs (microscopic morphology cannot be differentiated from S. *pneumoniae*)
Facultative anaerobic, optimal temperature 35℃
-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)

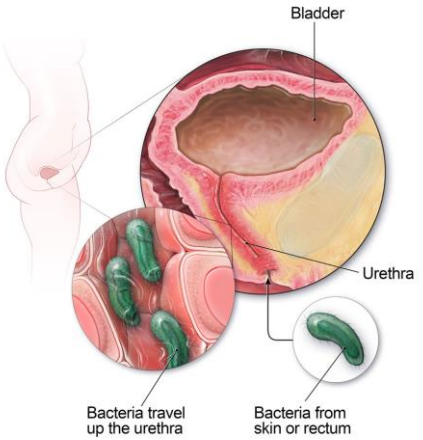
Bacteriocins: inhibition of competitive bacteria

ANTIMICROBIAL RESISTANCE

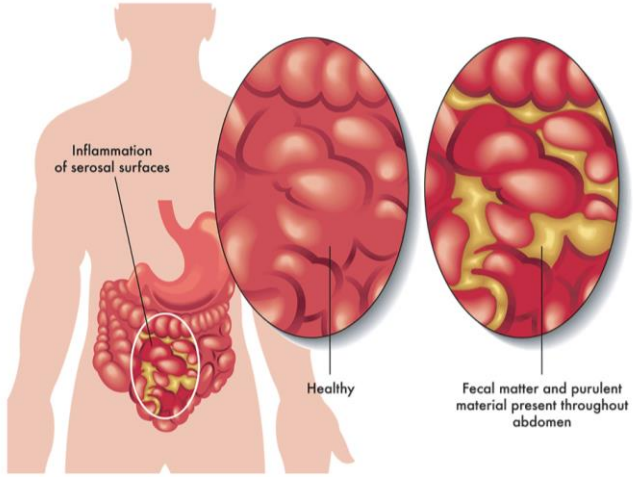
When a patient is treated with antibiotics, the enterococci that are part of normal microbial flora can proliferate and cause disease.

Enterococci („enteric cocci“)

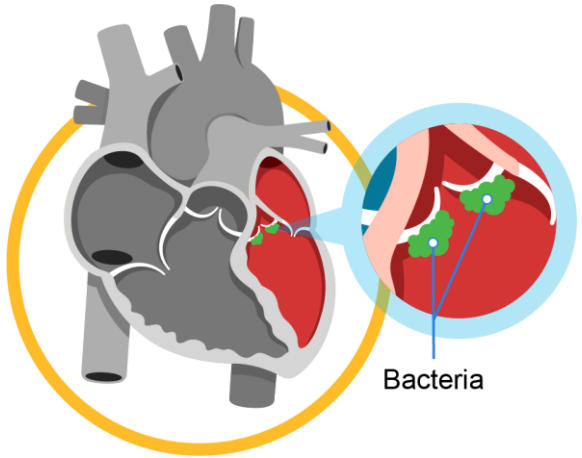
Urinary tract infection
Dysuria and pyuria – most frequently in hospitalised patients with an indwelling urinary catheter and on broad-spectrum cephalosporins



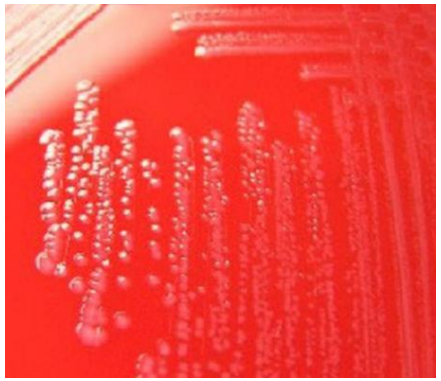
Peritonitis
Abdominal swelling and tenderness after abdominal trauma or surgery. Acutely ill patients, febrile, positive blood cultures



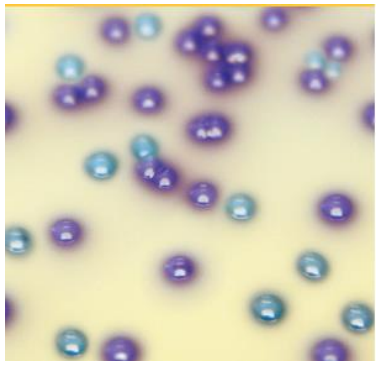
Endocarditis
Infection of heart endothelium



Enterococci – laboratory diagnostics

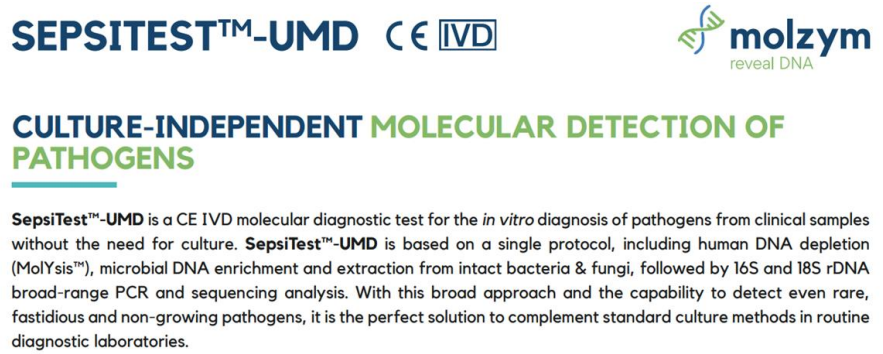


Culture* and AST



Selective culture VRE -screening

Blood cultures



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

Enterococci positive culture -identification

MALDI-ToF

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

*Culture: CAP agar (5% NaCl, aztreonam, colistin), selective for staphylococci and streptococci

Enterococci (infection)- treatment

E. faecalis – aminopenicillins (ampicilin), glycopeptides (vancomycin)

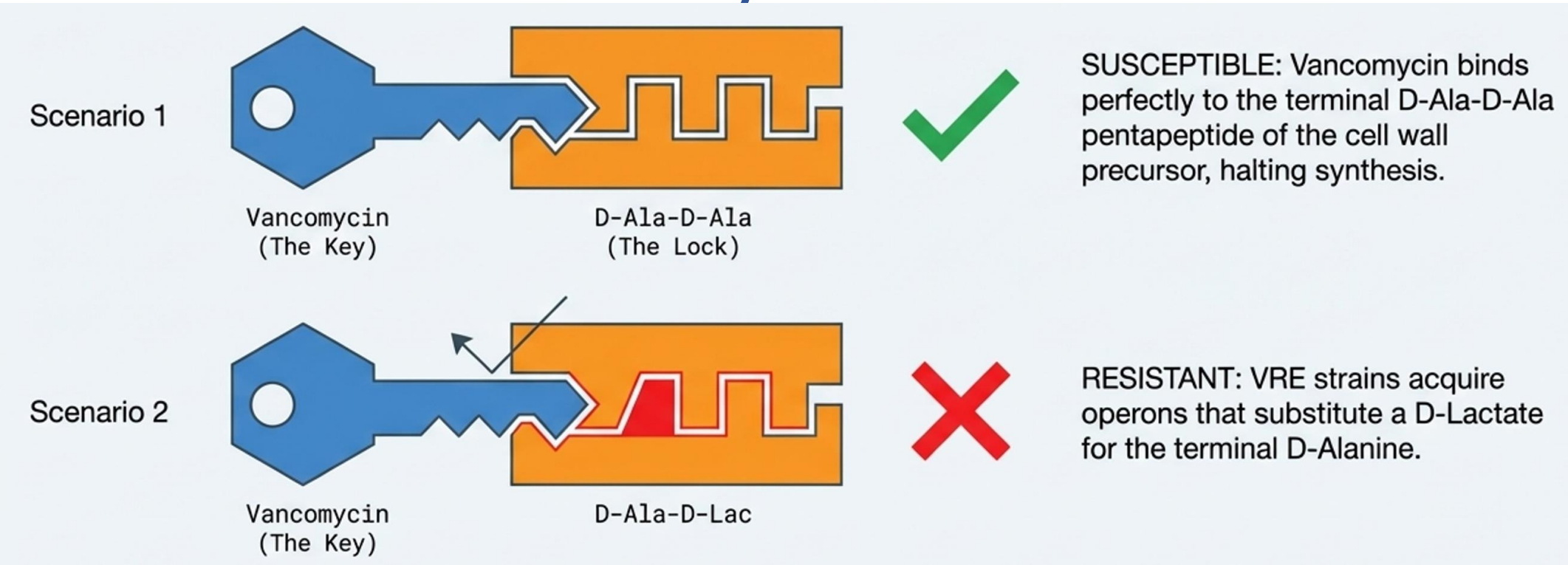
E. faecium - glycopeptides, natural resistant to aminopenicillins

Vancomycin resistant enterococci (VRE)
- oxazolidinones (linezolid), tigecycline, daptomycin

Absence of CRISPR-Cas: Most hospital isolates lack this defense mechanism against foreign DNA, which paradoxically makes it easier for them to accept new resistance and virulence genes through plasmids and transposons

Linezolid Risks:
More than two weeks: suppression of hematopoiesis
Serotonin syndrome: Patients who are taking selective serotonin reuptake inhibitors (SSRIs) concomitantly are at increased risk of developing this potentially dangerous condition

Mechanisms of vancomycin resistance in enterococci



The related *van*A or *van*B gene clusters (operon) 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).**

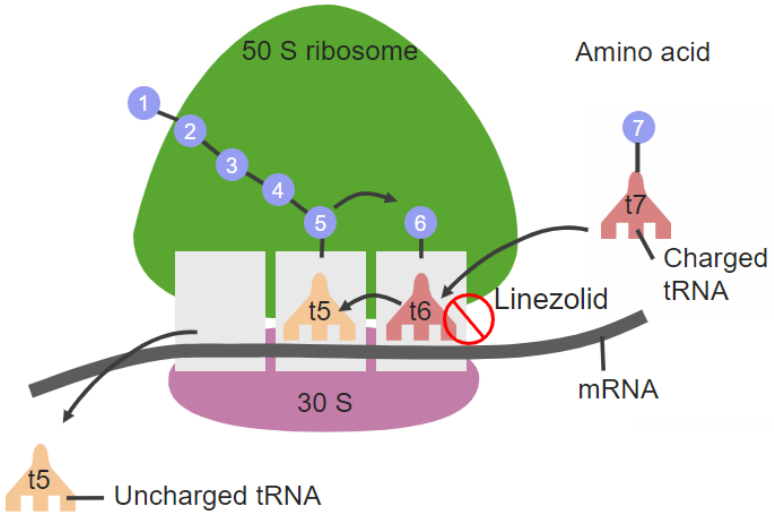
The ***van*A** gene cluster (operon) confer **high-level resistance to vancomycin and teicoplanin**, while enterococci harbouring ***van*B**-type cluster genes have a moderate level of resistance to vancomycin but remain **susceptible to teicoplanin**.

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

Mechanisms of linezolid resistance in enterococci

Effect (Mechanism of action)

- **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**

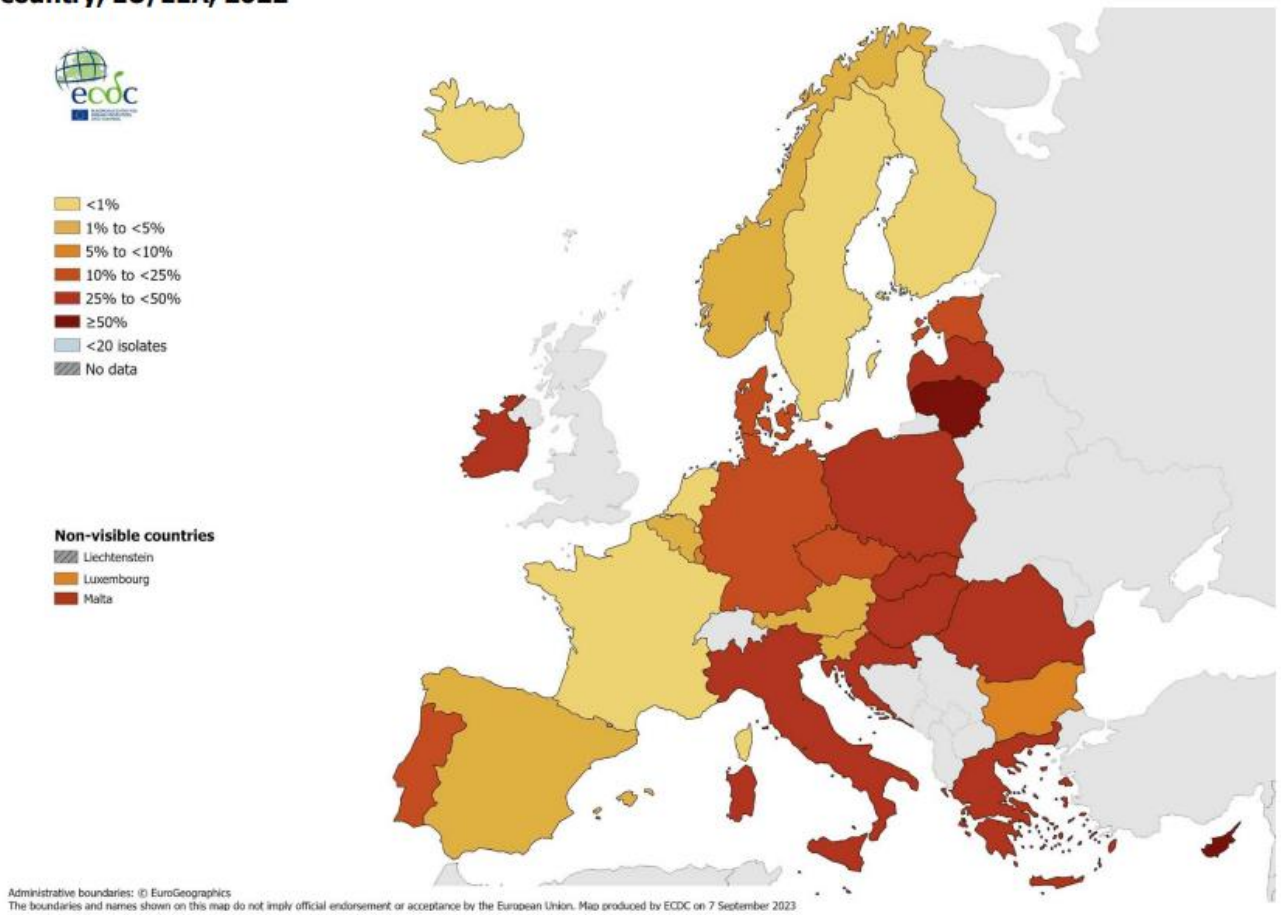


Mechanism of resistance

- 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 or other mobile genetic determinant! Other bacterial species, alsomresistence to macrolides and lincosamides**).

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**



- Resistance to tigecycline and linezolid is not yet common, but it increases.
- Surprisingly, many strains in the Czech Republic carry mutations associated with resistance to daptomycin (a new ATB) without exposition to this drug.

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

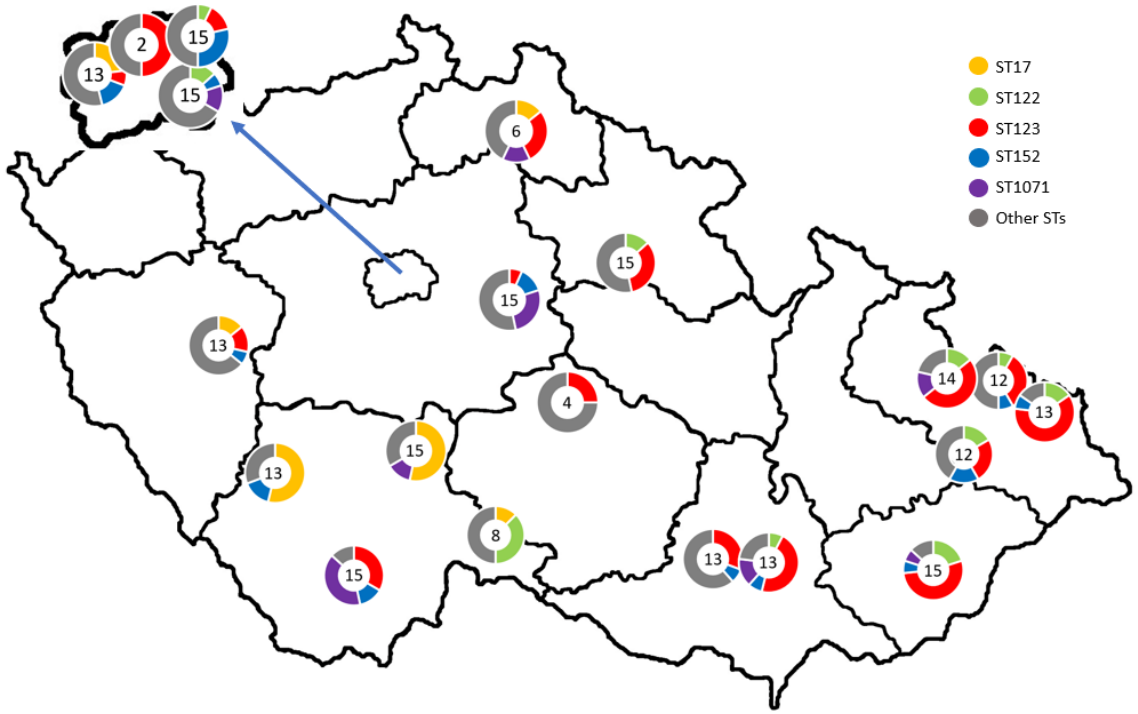


Figure 1. The distribution of participating hospitals in the study. The pie charts show the most common STs identified per hospital. The numbers in the centre represent the number of VRE and VSE isolates sent for characterization.

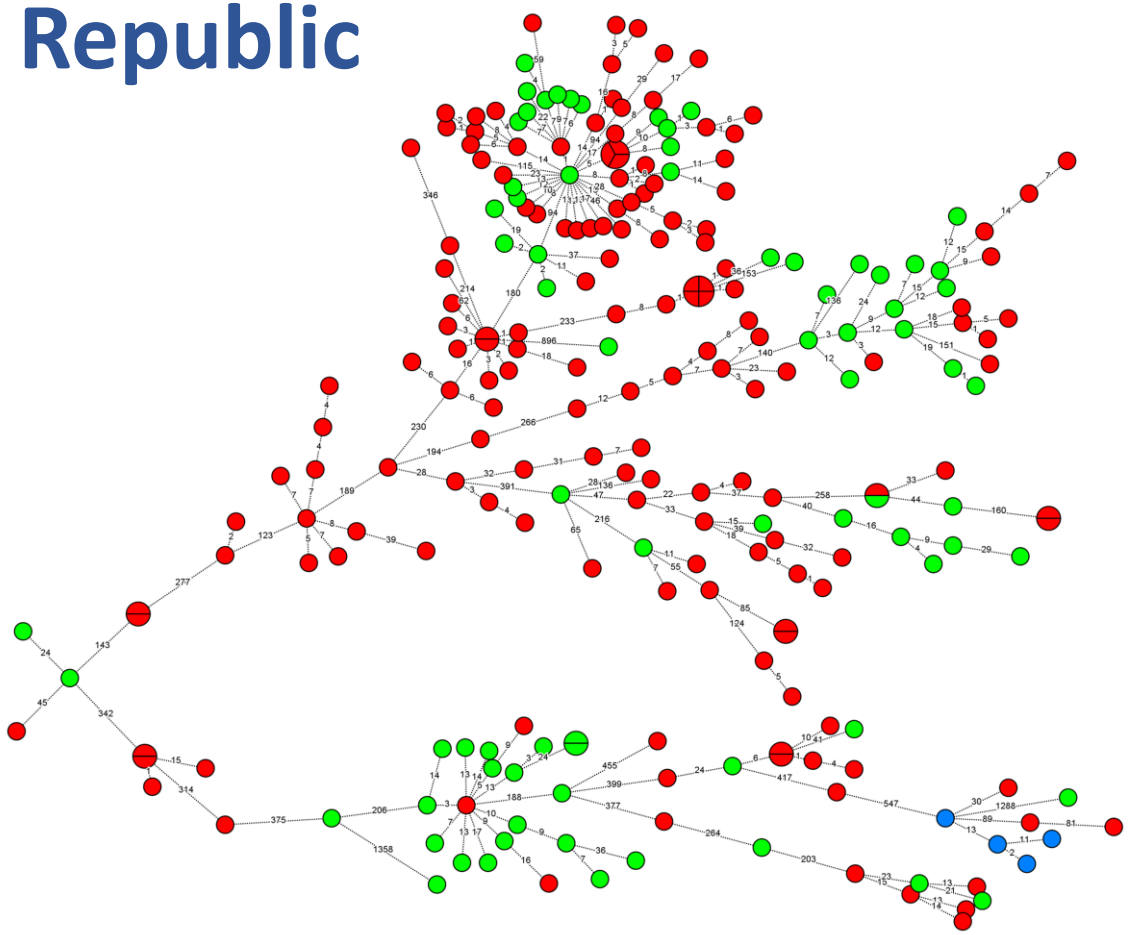


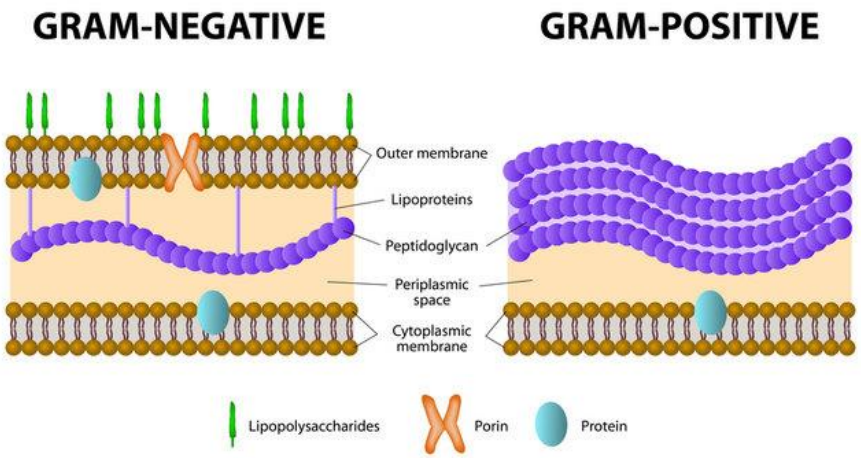
Figure 2. Minimum spanning tree generated from wgMLST analysis for *E. faecium* isolates (n=241). The colours indicate resistance to vancomycin. Red = vancomycin-resistant (n=162), Green = vancomycin-susceptible (n=75), Blue = partly deleted *van*A operon (n=4).

Marie Brajerová, PhD project)

Bacterial survival on inanimate surfaces

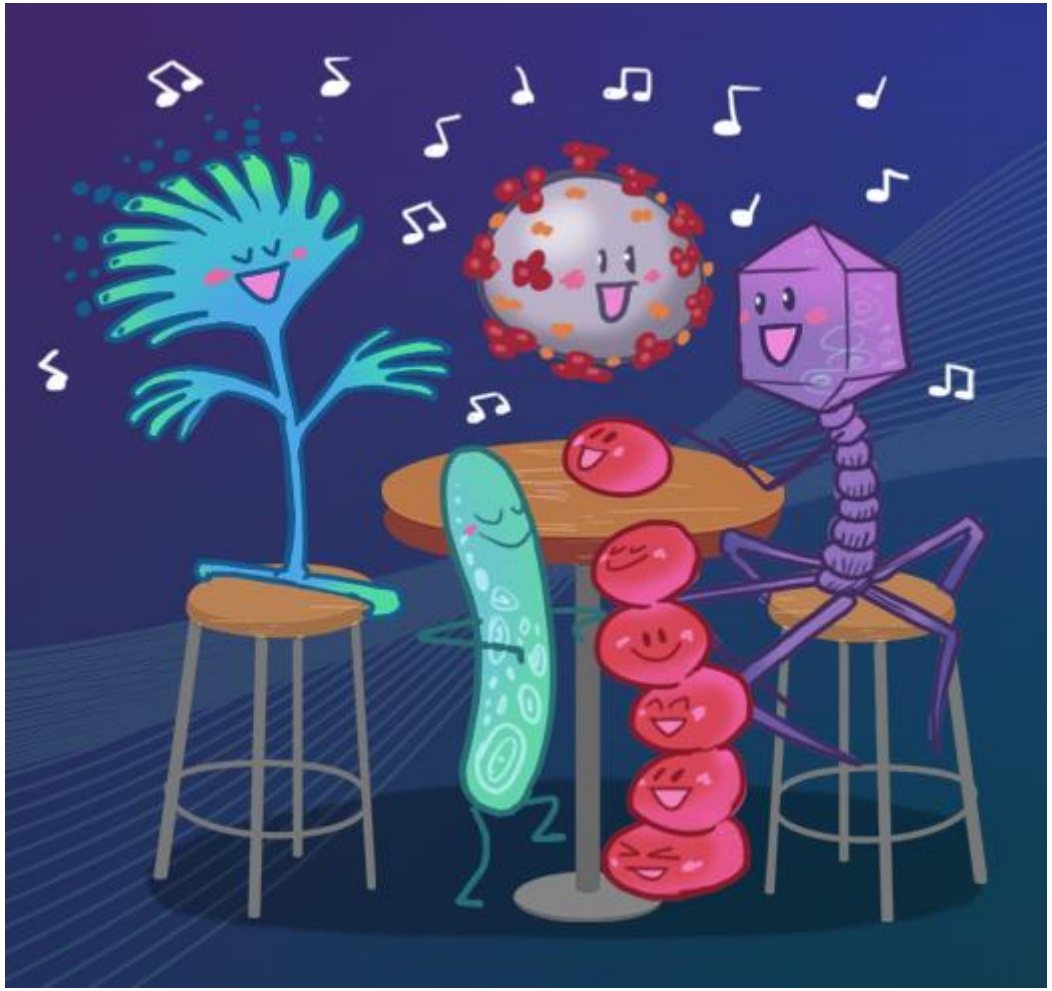
The study of Katzenberger and colleagues investigated *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *S. marcescens*, *E. faecium*, *E. coli*, and *E. cloacae*.

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.

Enjoy further exploring the wonders of microbiology



marcela.krutova@lfmotol.cuni.cz