# *Streptococcus, Enterococcus* & other catalase-negative species

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1/13/20

# *Streptococcus* spp.

- Family *Streptococcaceae*
- GPC in pairs and chains
- Most are facultative anaerobes
- Some require enhanced $CO_2$
- Grow poorly w/o blood- or serum-enriched media
- Catalase negative

# 3 Ways to Classify

1. Hemolysis on BAP
   - Beta:  complete hemolysis
   - Alpha:  partial hemolysis
   - Gamma:  no hemolysis
2. Immunologic reactions with grouping sera
   - Lancefield antisera for cell wall carbohydrates
3. Phenotypic properties
   - Biochemical reactions

# Hemolysis on blood agar

# Lancefield Serotyping

- Developed by Rebecca Lancefield in 1930s
- Based on cell wall (C) polysaccharide Ag
- Commercially available latex agglutination
- Used to distinguish Streptococcal species.
  - A, B, C, (D), F, G – commonly tested


https://assets.thermofisher.com/TFS-Assets/MBD/product-images/F103557~p.eps-650.jpg

# Streptococci Habitat

Most species are flora:

- Gastrointestinal tract
- Vagina
- Upper respiratory tract

Sometimes transient on skin, urogenital tract

# *Streptococcus pyogenes*

One of the most important bacterial pathogens of humans

-- always reported

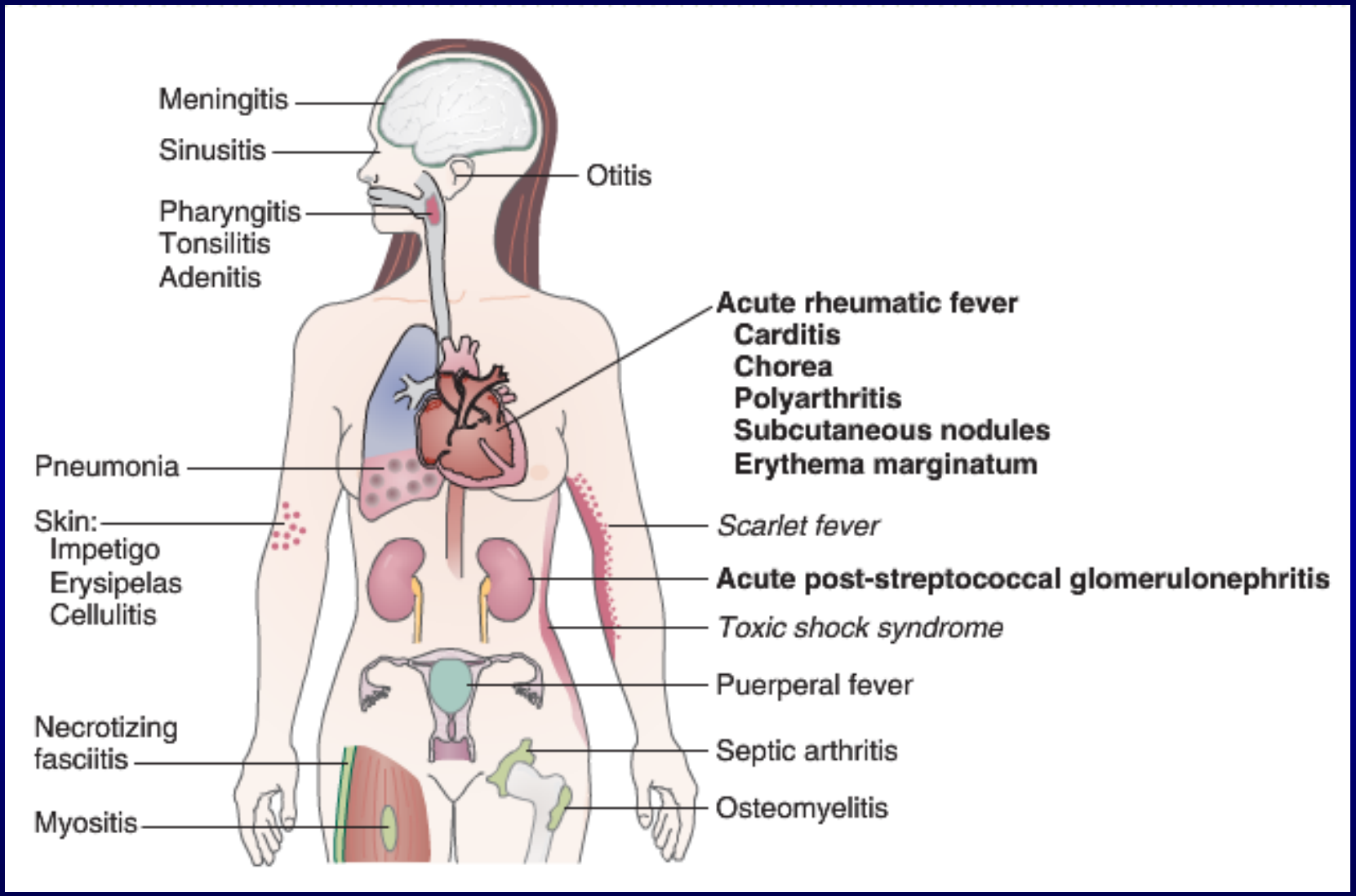
Most frequent cause of bacterial pharyngitis

Also causes variety of cutaneous and systemic infections

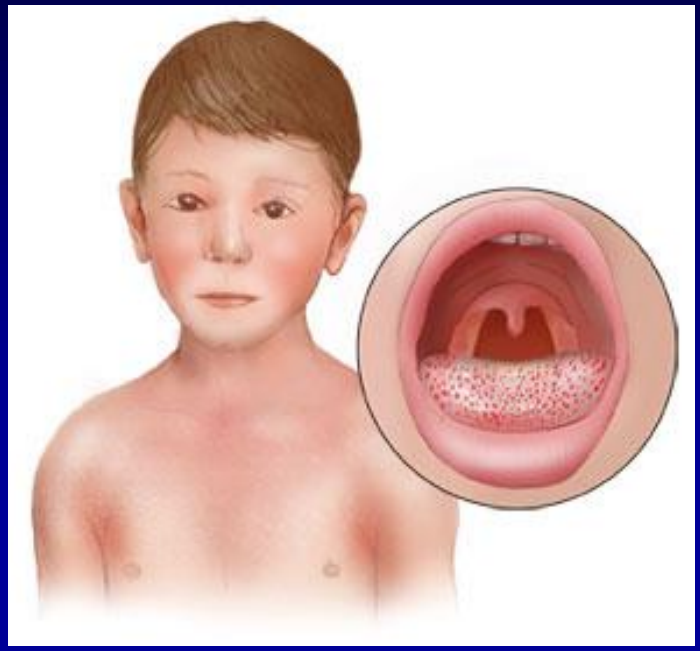
Nonsuppurative sequelae

Color Atlas of Med Bacteriology, ASM 2004

# *S. pyogenes* Disease Spectrum



# Pharyngitis & Scarlet Fever



Spread is by droplet transmission

Pharyngitis & tonsillitis; peritonsillar abscess may occur, if not resolved.

Up to 20% of school-age children carry Group A Strep in asymptomatically.

Scarlet fever –toxin-mediated associated syndrome. Usually limited disease. Most commonly follows pharyngeal infection, but may follow others.

https://www.cbsnews.com/news/scarlet-fever-makes-a-dangerous-comeback/

# *S. pyogenes* skin & soft tissue infections

- Impetigo
- Erysipelas
- Cellulitis
- Necrotizing fasciitis

Med Microbiol,
Elsevier-Mosby 2005

# Sequelae of Streptococcal Infection

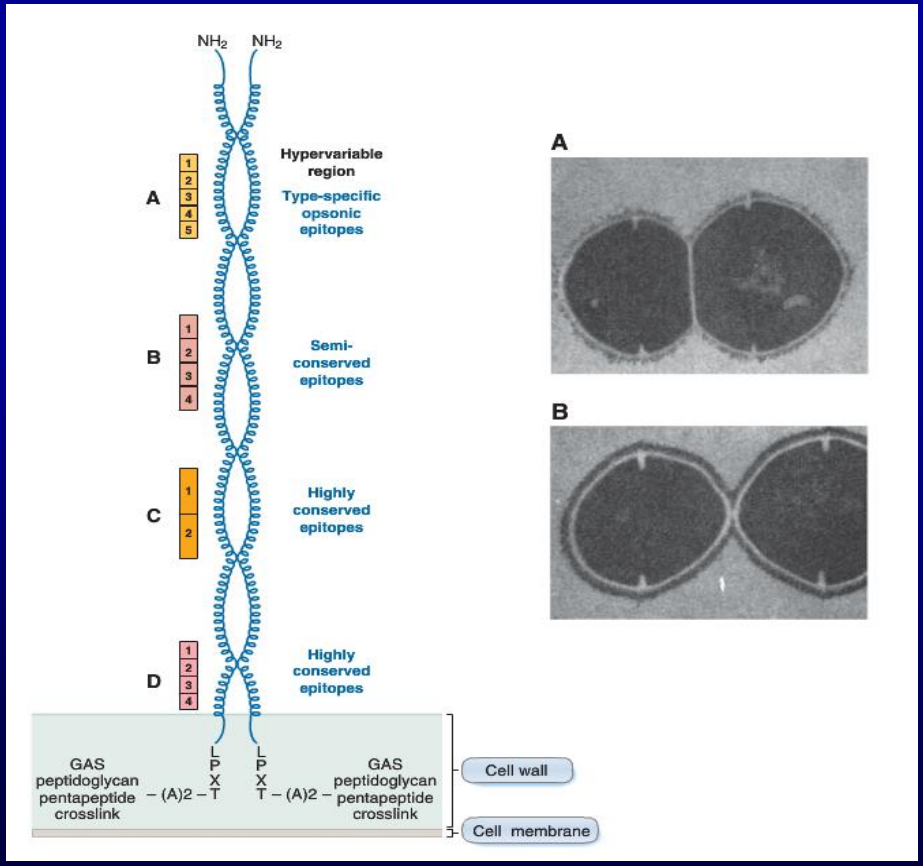
- Suppurative: cervical lymphadenitis, otitis, sinusitis, myositis, bacteremia leading to metastic infections, etc.

- Non-suppurative: AGN & rheumatic fever
  - AGN follows pharyngitis or pyoderma (skin infection)
  - Rheumatic fever follows pharyngitis

Destruction of heart valve, result of ARF

# *S. pyogenes* – virulence factors

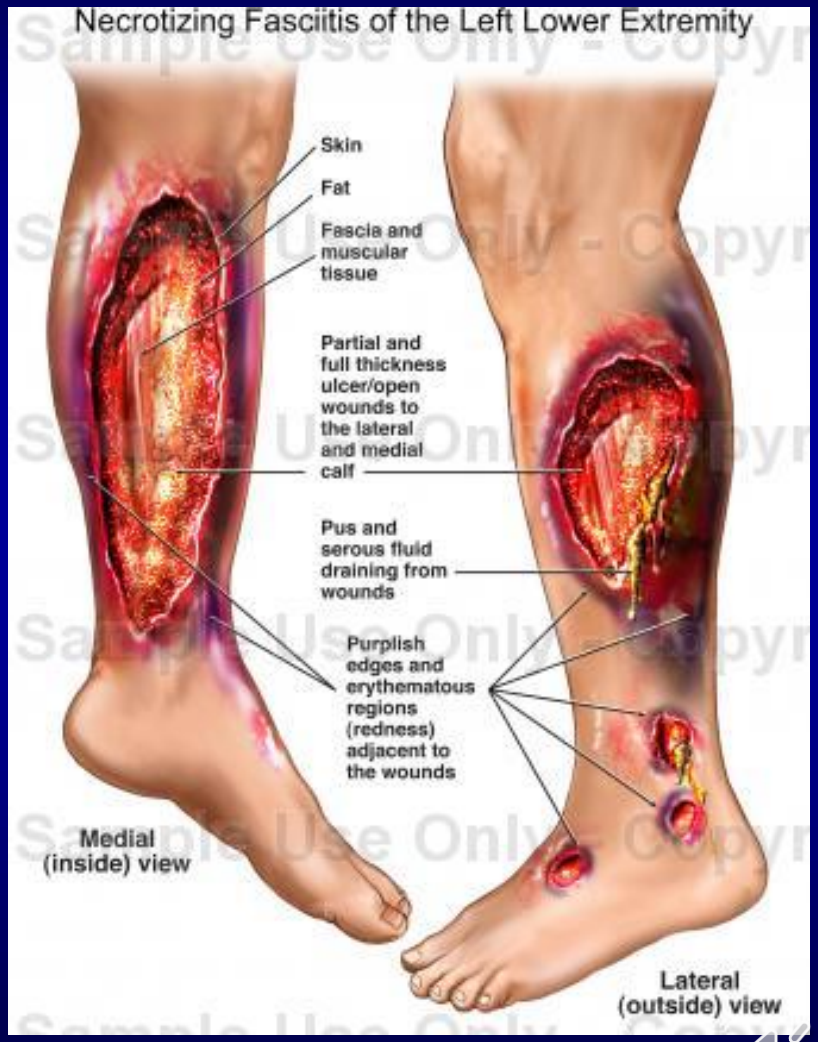
- Hyaluronic acid capsule
- Deoxyribonucleases (DNases A to D)
- M protein (emm gene, adhesin, >200 serotypes)




Schecter's 5th Ed

# *S. pyogenes* – virulence factors, cont'd

- Pyrogenic exotoxins (*Spe*)
  - Superantigens - cytokine induction
  - Syndromes:
    - necrotizing fasciitis
    - Toxic shock
    - Scarlet fever rash




http://emedical-help.com/flesh-eating-disease

# *S. pyogenes* – Virulence Factors

- Hemolysins:
  - Streptolysin S - oxygen stabile, nonimmunogenic
  - Streptolysin O - oxygen labile, immunogenic

- Anti-Streptolysin O (ASO) test:
  - Rarely requested
  - Documents recent GAS infections (pharyngitis)
  - Cholesterol in skin inhibits Ab production

- Anti-DNAse B
  - Ab made post skin infection or  pharyngitis

# Detection of Group A Strep

- Rapid Antigen Tests
  - ~ 15 min
  - Commercially available
  - Only ~80-90% sensitive; good specificity
  - Must be followed by definitive method, culture or NAAT


# Detection of Group A Strep

## Nucleic Acid Based

- Probe method
- PCR/NAAT
- <1 hr to several hours
- NAAT - very high sensitivity
- Specific for GAS
- May detect nonviable

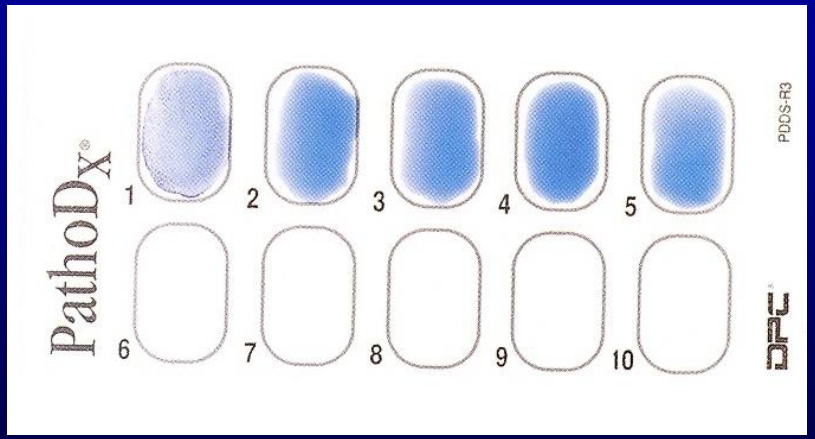
## Culture

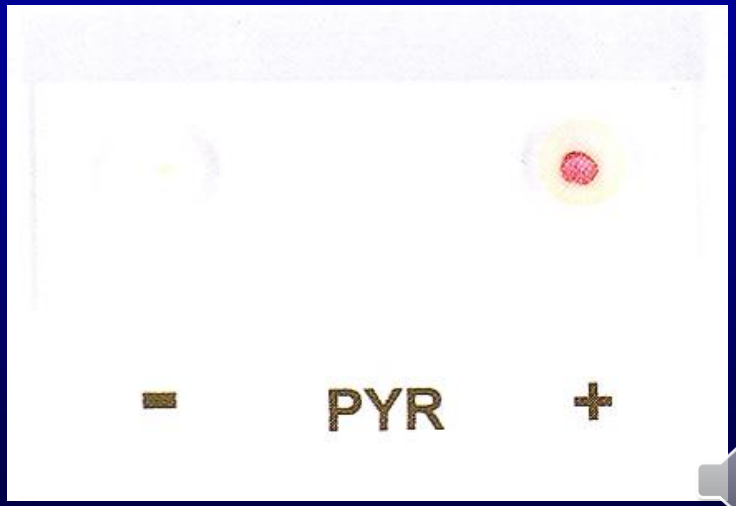
- Detects Grp A, also Grps C and G, if needed
- Highly sensitive & specific
- Incubate up to 48 hrs in room air (or anaerobic)

# *S. pyogenes* - Culture

- Catalase negative
- Large zones of β-hemolysis
- Bacitracin S
- PYR+ (L-pyrrolidonyl arylamidase)
- Lancefield Grp A Ag
- MALDI TOF


Med Microbiology, Harcourt 2000




Color Atlas of Med Bacteriology, ASM 2004

# *Streptococcus pyogenes* therapy

- Penicillin – drug of choice
- Pen allergic
  - Erythromycin
  - 1st gen oral cephalosporins
- Serious soft tissue infections
  - Pen + clindamycin (disrupts protein synthesis of toxin)

# *S. agalactiae*

- Colonization
  - Gastrointestinal tract
  - Vagina
  - Genitourinary

  Transiently on skin, respiratory tract

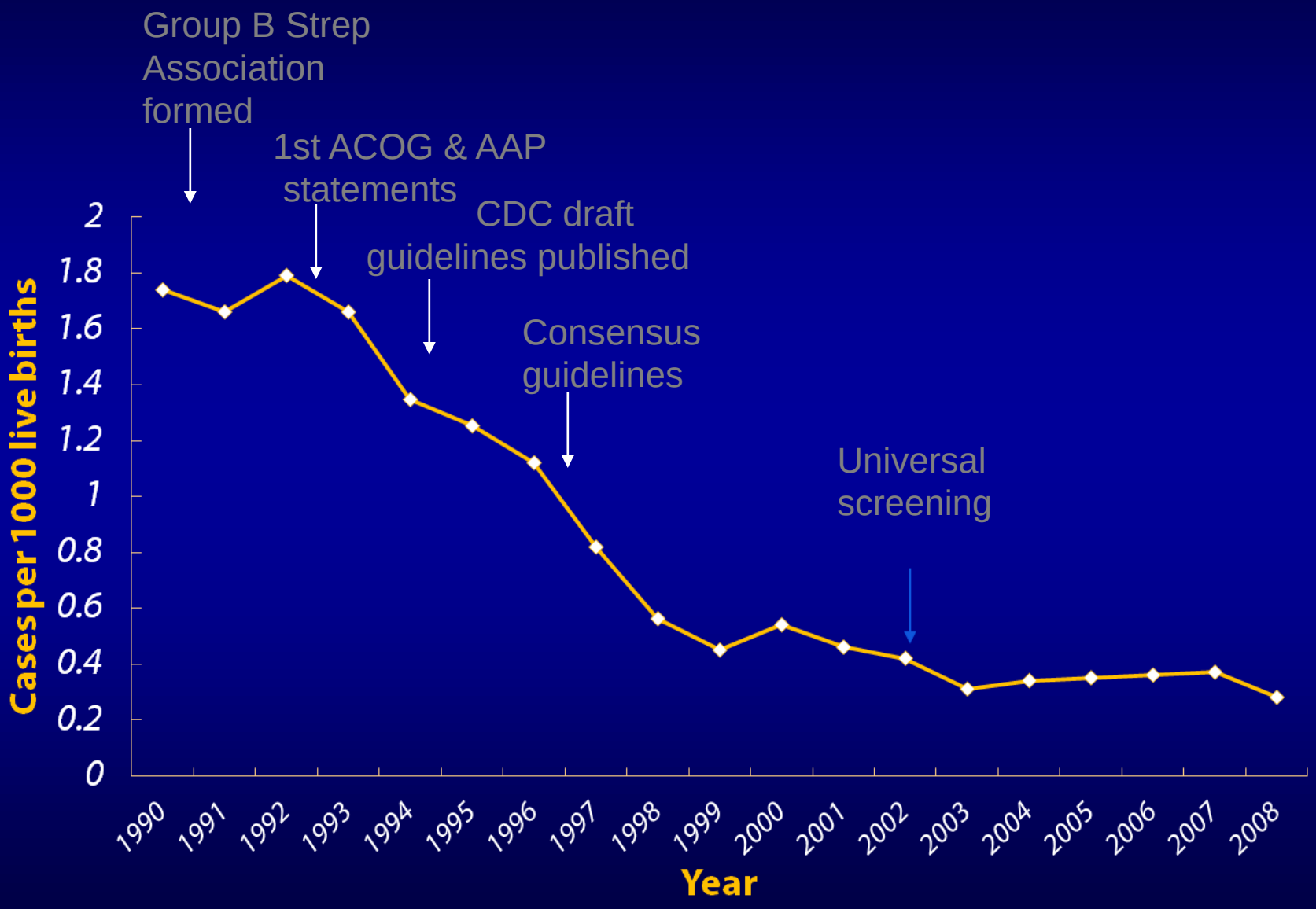
- Lancefield Group B
- Capsule is major virulence factor

ASM Image Library

# GBS, Pregnancy & Neonatal Infection

- Transient vaginal carriage in 10-30% pregnant women
- Newborns acquire during pregnancy or at delivery
  - pneumonia, bacteremia, meningitis,
- Screen at 35-37 wks gestation
  - vag/rectal swab
  - Todd-Hewitt/LIM broth cx
    - colistin/nalidixic acid or gent/nalidixic acid
  - Culture or PCR

# Early-onset GBS Disease in the U.S.



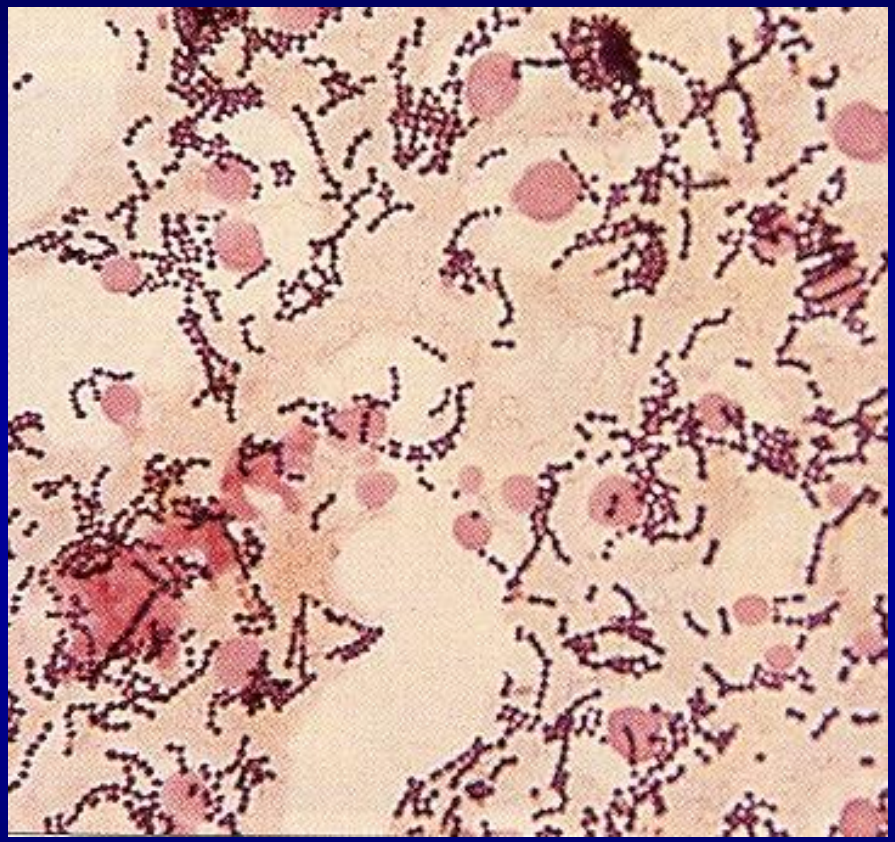

http://www.cdc.gov/groupbstrep

# *S. agalactiae* clinical disease

Late-onset neonatal
- Bacteremia
- Meningitis

Pregnant women
- UTIs
- Amnionitis, endometritis

Other adults
- Bacteremia
- Urosepsis
- SSTI
- Pneumonia
- Bone and joint infections



Medical Microbiology, Harcourt 2000

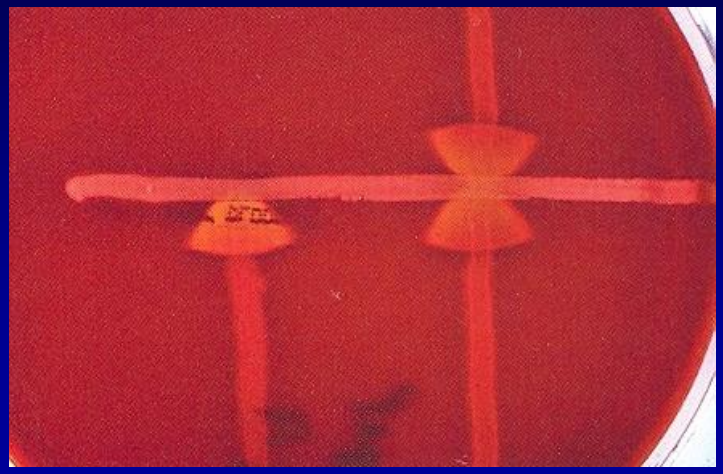
# Colony Morphology Comparison

Group A

Group B

# *S. agalactiae* - lab diagnosis

- Culture
- Lancefield gp B Ag detection
- Pos CAMP
- Hippurate hydrolysis
- MALDI TOF



Color Atlas and Textbook of Diag Microbiol, Lippincott-Raven 1997

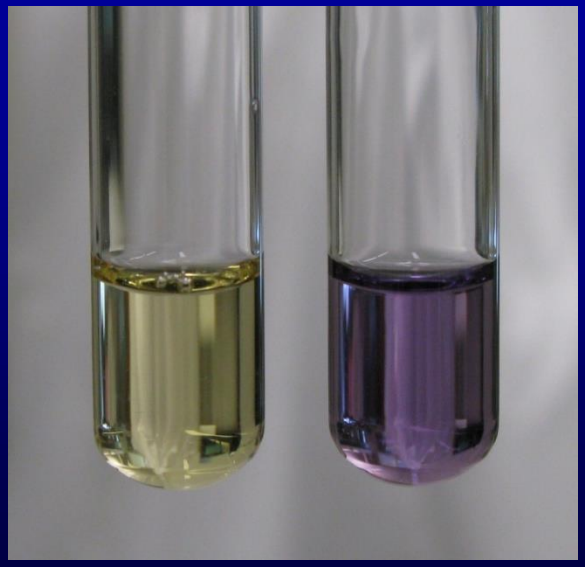


Photo courtesy of Dr. Lesley McGee, CDC

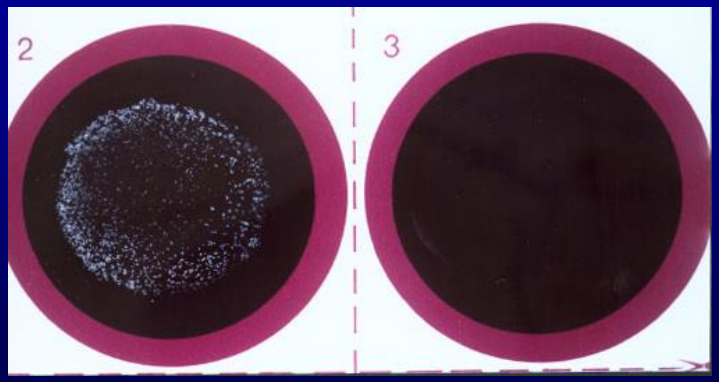



Photo courtesy of Dr. Richard Facklam, CDC

# *S. agalactiae* therapy

Infections

- Penicillin - drug of choice
- Pen + aminoglycoside for serious infections
- Vancomycin - if pen allergic

# *S. dysgalactiae subsp. equisimilis*

- Lancefield grp C or G antigen
- Large colonies
  - ≥0.5 mm with large zone of β-hemolysis
- Beta-glucuronidase pos
- Oral flora
- Wide range of pyogenic infections:
  - pharyngitis
  - skin
  - bacteremia
  - endocarditis
  - meningitis
  - septic arthritis
  - rare post-strep GN

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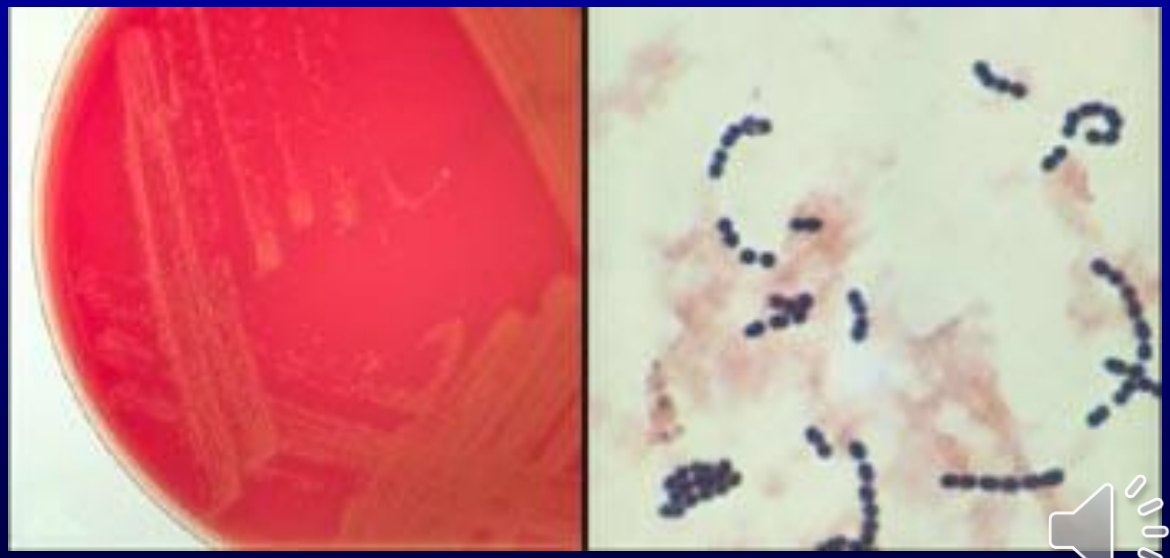
# *S. anginosus* group (*S. milleri*)

- Oral or GI flora
- May possess A, C, F, or G capsular polysaccharide
- Small colonies (<0.5 mm)
- Narrow zone of β-hemolysis (may be α or non-hemol)
- BGUR neg; Voges-Proskauer (VP) test for acetoin prod pos
- Abscess formation
- Endocarditis



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# Viridans Grp *Streptococci*

- Colonize oropharynx, GI, GU tract
- Heterogeneous collection of $\alpha$ and $\gamma$ hemolytic Strep
- Optochin R
- Bile insoluble
- >30 species
- 5 subgroups
- Precise classification problematic

Medical Microbiology, Harcourt 2000

# Viridans gp streptococci

## Anginosus ("S. milleri") gp

- Most α, γ
- Small colony β-hemolytic strains with A, C, F or G ag different from pyogenic strains
- *S. anginosus*
- *S. constellatus*
- *S. intermedius*

## Mutans gp

- *S. mutans*
- *S. sobrinus*

## Salivarius gp

- *S. salivarius*
- *S. vestibularis*
- *S. thermophilus*

## Mitis gp

- *S. mitis*
- *S. sanguis*
- *S. parasanguis*
- *S. gordonii*
- *S. cristatus*
- *S, infantis*
- *S. oralis*
- *S. peroris*
- *S. pneumoniae*

## Bovis gp

- *S. equinus*
- *S. gallolyticus subsp. gallolyticus*
- *S. gallolyticus subsp. pasteurianus*
- *S. infantarius*
- *S. alactolyticus*

# Viridans gp *Streptococcus* infections

Endocarditis
- *S. mitis* gp
- *S. salivarius* gp

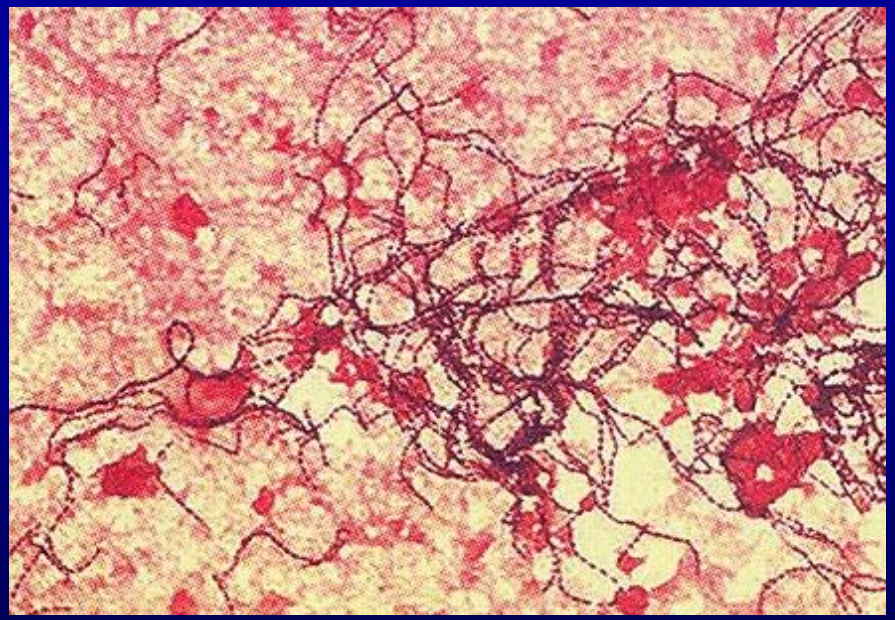
Dental caries
- *S. mutans* gp

Abscess formation
- *S. anginosus* gp

Septicemia in neutropenic pts with mucositis
- *S. mitis* gp

Malignancies of GI tract
- *S. bovis* gp

  (*S. gallolyticus* subsp *gallolyticus*)



Medical Microbiology Elsevier-Mosby 2005

# Viridans gp streptococci therapy

- Penicillin
- Penicillin + aminoglycoside
- Cephalosporins
- Vancomycin

Color Atlas of Infectious Diseases, Mosby 1974

# *Streptococcus pneumoniae*

Considered member of *S. mitis* gp

Lancet-shaped diplococcic, pairs & short chains

Colonizes nasopharynx

- 5-10% healthy adults
- 20-40% healthy children

Diseases

- Pneumonia
- Sinusitis and otitis media
- Meningitis
- Bacteremia

COPD, alcoholism, DM, CRF

Color Atlas of Med Bacteriol, ASM 2004

# Invasive Disease Prevalence

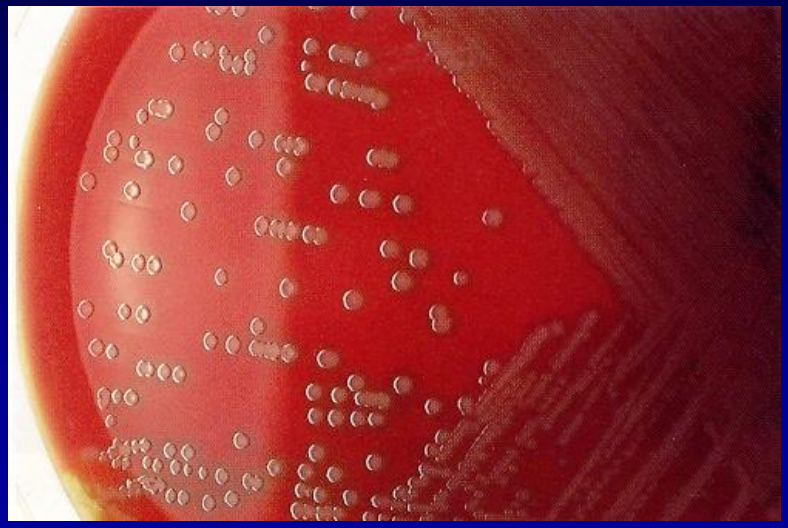
| | Cases | | Deaths | |
|---|---|---|---|---|
| **Age (years)** | **No.** | **(Rate*)** | **No.** | **(Rate*)** |
| < 1 | 48 | (11.6) | 3 | (0.72) |
| 1 | 33 | (7.9) | 2 | (0.48) |
| 2-4 | 66 | (5.2) | 4 | (0.32) |
| 5-17 | 86 | (1.5) | 6 | (0.11) |
| 18-34 | 203 | (2.5) | 14 | (0.17) |
| 35-49 | 411 | (6.1) | 30 | (0.44) |
| 50 64 | 1,116 | (16.6) | 125 | (1.86) |
| 65-74 | 618 | (20.8) | 82 | (2.76) |
| 75-84 | 407 | (29.3) | 44 | (3.16) |
| ≥ 85 | 267 | (41.8) | 61 | (9.56) |
| **Total** | **3,255** | **(9.5)** | **371** | **(1.08)** |

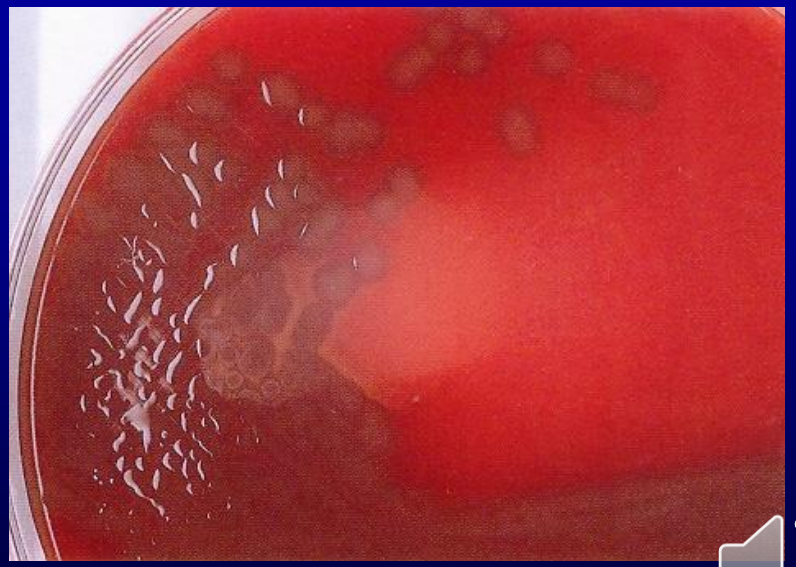
*Per 100,000 population for ABCs areas

CDC ABCs Emerg Infec Prgm 2017

# *S. pneumoniae* Identification

- Capsule (major virulence factor)
- >90 serotypes
- Autolysis
- Optochin susceptible
- Bile soluble



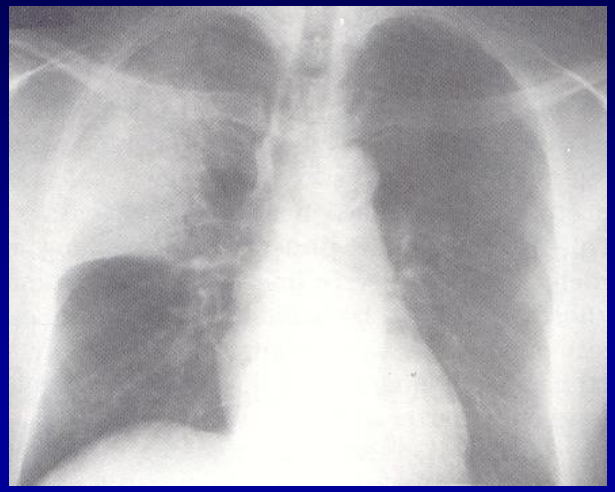
Color Atlas of Medical Bacteriology, ASM 2004

# *S. pneumoniae* Urine Antigen Test

- Direct detection in urine; non-invasive specimen collection
- May be useful if pneumonia, bacteremia, meningitis
- FP in colonized, esp children <6 yrs
- Sensitivity varies, 50 – 80%

# Pneumococcal Therapy

- 1936 - type-specific antiserum reduced pneumonia fatality from 33% to 18%
- 1941 - sulfadiazine reduced mortality to 8%
- Mid 1940s - penicillin
- WWI - 18% of soldiers w/ pneumonia died
- WWII - <1%




Alexander Fleming, Otfinoski 1992

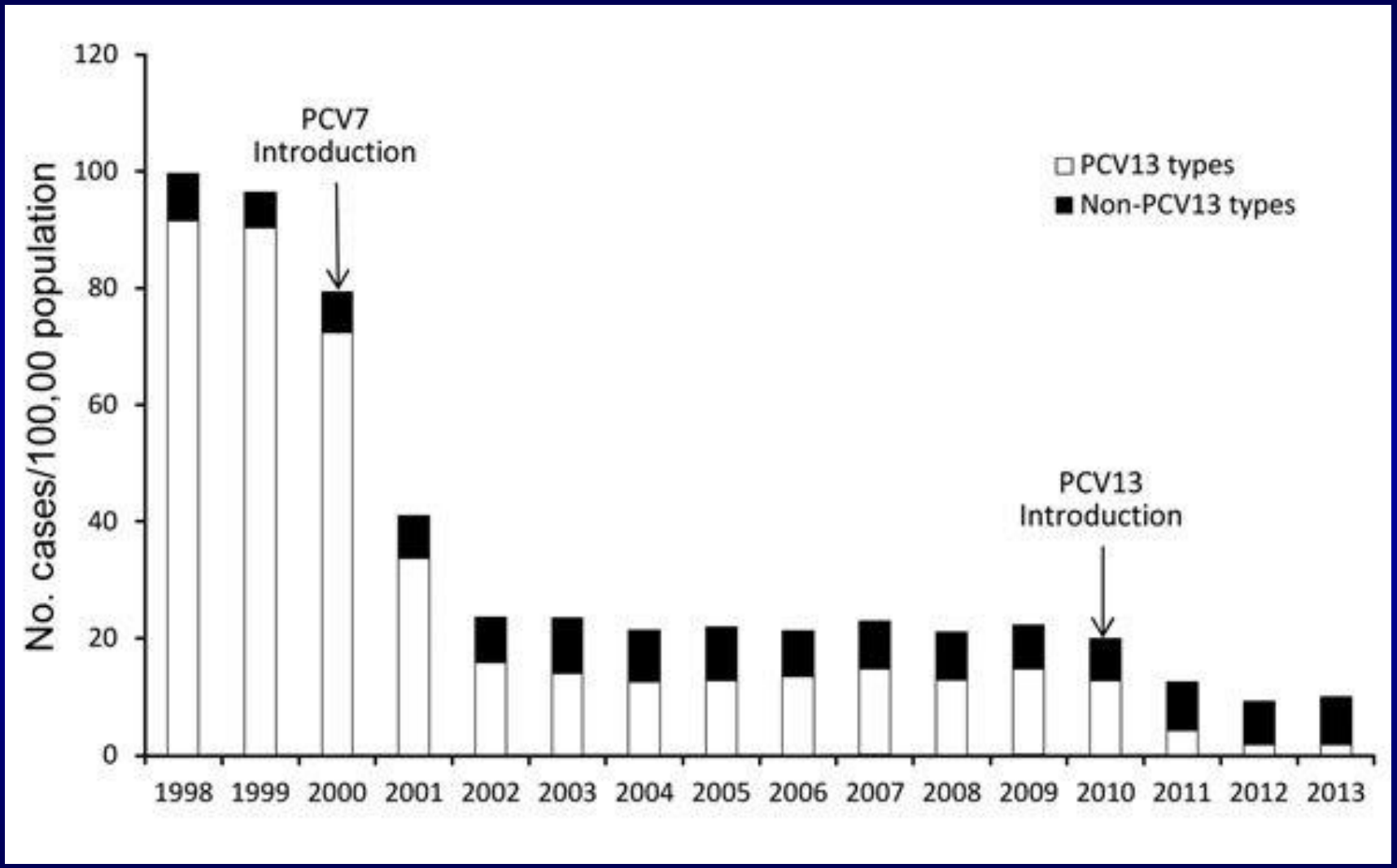
# Emergence of Antimicrobial Resistance in *S. pneumoniae*

- 1967 1st penicillin$^{R}$ strain, Australia
- 1977 1st multidrug-resistant isolates, South Africa (tet, macr, chlor)

# Vaccination against *S. pneumoniae*

- Polysaccharide vaccine
  - Available since 1977 for age 2 and older
  - 23 serotypes
  - Elderly or if increased risk of infection
  - Limited efficacy
- In 2000, 7-valent conjugate vaccine (PCV7) for kids
  - Serotypes: 4, 6B, 9V, 14, 18C, 19F, 23F
  - 78-89% of penicillin-resistant *S. pneumoniae*
- In 2010, PCV13
  - Serotypes: PCV7 types  plus 1, 3, 5, 76A, 7F, 19A
  - 4-dose series 2, 4, 6, 12-15 months

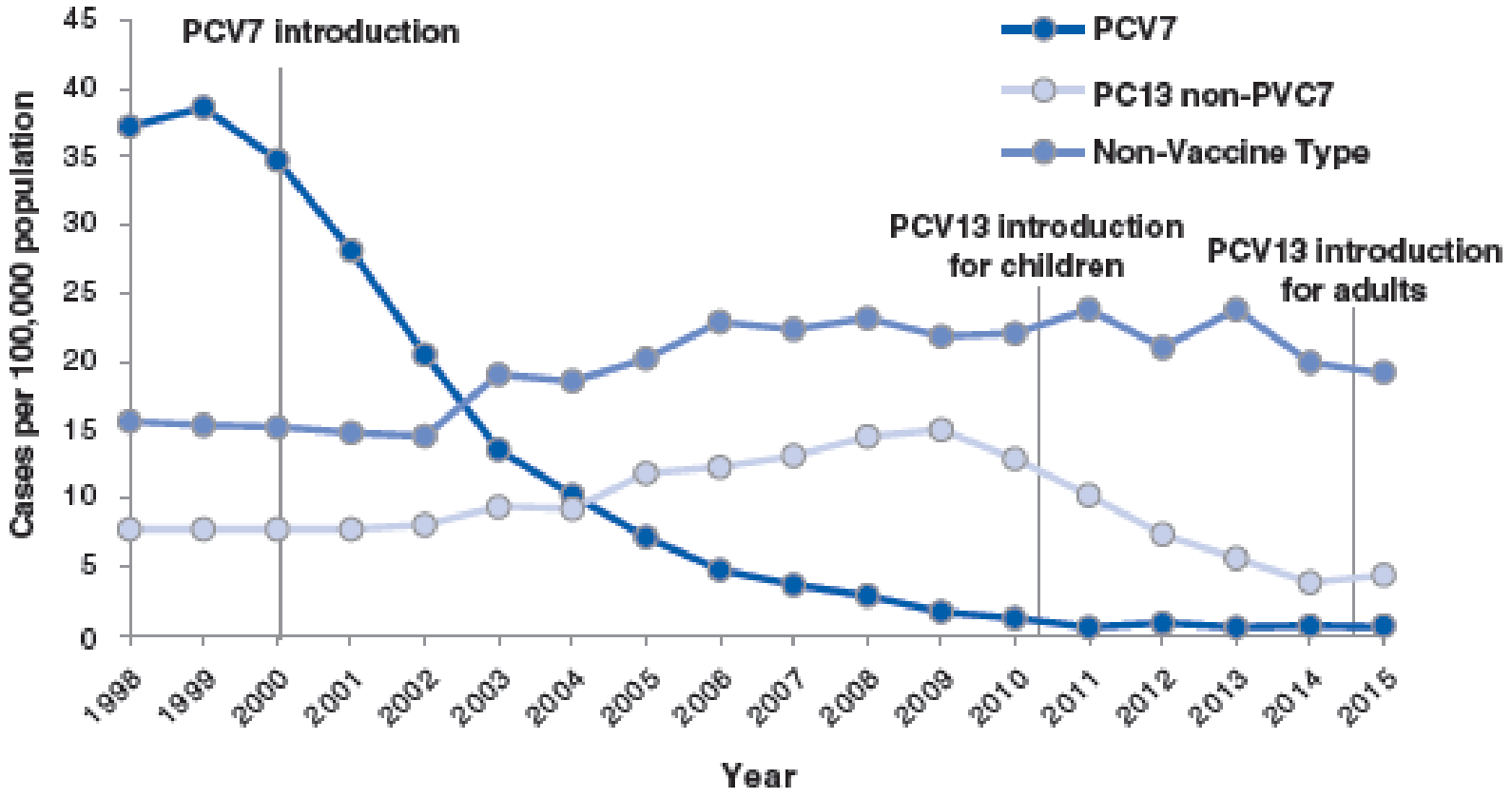
# Invasive Disease – Post PCV7




Moore MR Emerg Infect Dis 2015
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4550168/

# Pneumococcal Vaccine

**Figure 2. Rates of invasive pneumococcal disease among U.S. adults >65 years of age, 1998–2015[3]**




Vaccine Preventable Disease Surveillance Manual (https://www.cdc.gov/vaccines/pubs/surv-manual/chpt11-pneumo.pdf)

# *S. pneumoniae* Therapy

- First choice for non-invasive infection:
  - Penicillin, amoxicillin, augmentin (5-10% R)
- Macrolide e.g. erythromycin (up to 30% R)
- Quinolone e.g. levofloxacin

Meningitis or septicemia:

- Penicillin
- Ceftriaxone
- Vancomycin

# New CLSI* Penicillin Breakpoints for Pneumococci - 2008

| | Minimum Inhibitory Concentration | | |
|---|---|---|---|
| Penicillin | S | I | R |
| Oral | ≤ 0.06 | 0.12-1 | ≥ 2 |
| Parenteral Meningitis | ≤ 0.06 | - | ≥ 0.12 |
| Parenteral Nonmeningitis | ≤ 2 | 4 | ≥ 8 |

*CLSI, Clinical Laboratory Standards Institute, M100-S18

# Summary of *Streptococci*

| Species | Lancefield Antigen | Hemolysis Pttn(s) | |
|---|---|---|---|
| *S. pyogenes* | A | β | Relatively lg col |
| *S. agalactiae* | B | β (γ) | Weak hemolysis |
| *S. dysgalactiae* | C, G | β | Relatively lg col |
| *S. anginosus* gp | A, C, F, G, none | β, α, γ | β sm col, VP+ |
| *S. bovis* gp | D | α, γ | nonenterococcal |
| *S. mutans* gp | Not useful | α, γ (β) | *S. sobrinus* |
| *S. salivarius* gp | Not useful (K) | α, γ | *S. vestibularis* |
| *S. mitis* gp | Not useful (H) | α | *S. gordonii, S. sanguis, S. oralis* |
| *S. pneumoniae* | Not detectable | α | Related to mitis |

# *Enterococcus* spp.

- Gram-positive cocci in pair & short chains
- Lancefield group D antigen
  - Classified as “group D streptococci” until 1984
- Facultative anaerobe, ↑$CO_2$ not required
- 40 species
  - *E. faecalis* (most common)
  - *E. faecium*

ASM Image Library

# *Enterococcus* spp.- Epidemiology

- Colonizes GI tract of humans, animals (less common GU, oral flora)
- Widespread in nature; able to survive in harsh environments for extended periods
- Opportunistic pathogen (10% of nosocomial infections)
  - Most acquired from pt's intestinal flora
  - Some patient to patient transmission

# *Enterococcus* spp.

- Commensal organisms
- Limited potential for causing disease
- Virulence factors
  - Surface adhesin proteins
  - Cytolysin
  - Extracellular protease
  - Gelatinase

# *Enterococcus* spp.- Clinical disease

Increased risk

- Prolonged hospitalization
- Broad spectrum abx rx (cephalosporins – natural R)
- Urinary, IV catheters

- Urinary tract infections
- Bacteremia
- Endocarditis
- Peritonitis (post trauma or surgery)
- Wound infections and intra-abdominal abscesses (usually polymicrobic)

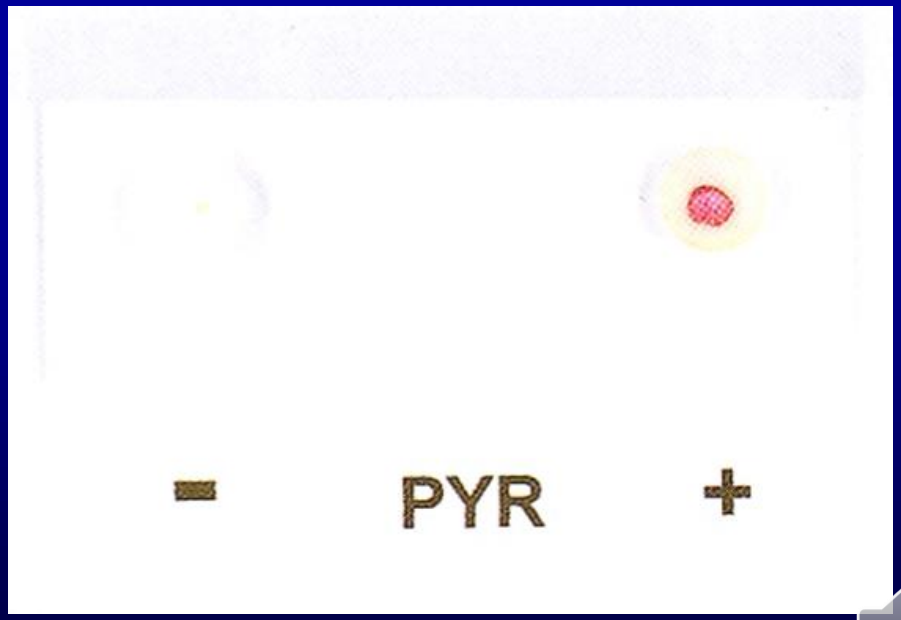
# *Enterococcus* spp.- Lab diagnosis

- Grows readily on nonselective media
- Nonhemolytic or α-hemolytic (rarely β) colonies on BAP

*E. faecalis* and *E. faecium*

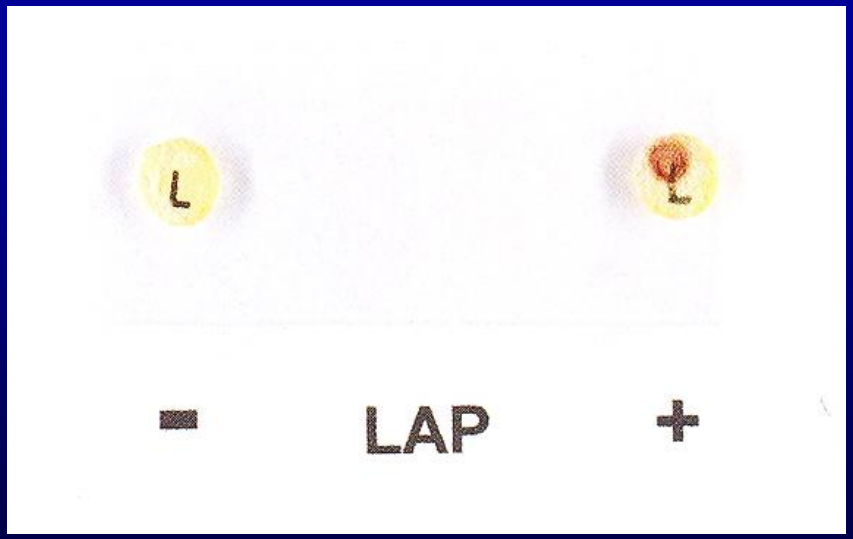
# *Enterococcus* Identification

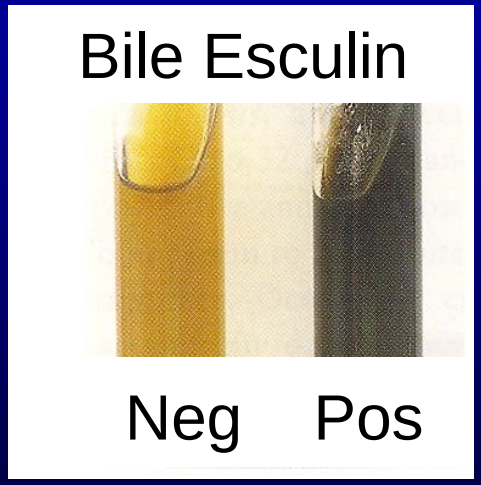
- Catalase negative
- PYR (L-pyrrolidonyl arylamidase) positive
- Bile resistant
- Optochin resistant



# *Enterococcus* spp.

- Grow in 6.5% NaCl
- Growth in 40% bile an hydrolyze esculin
- PYR positive
- Leucine arylamidase positive

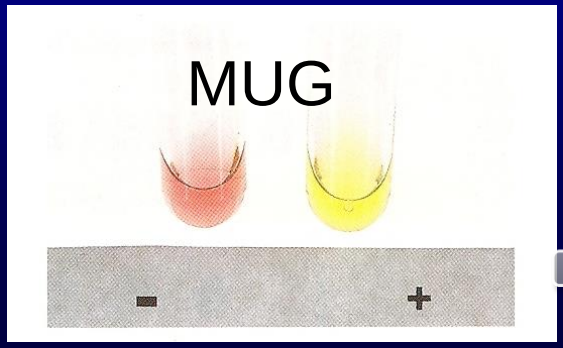



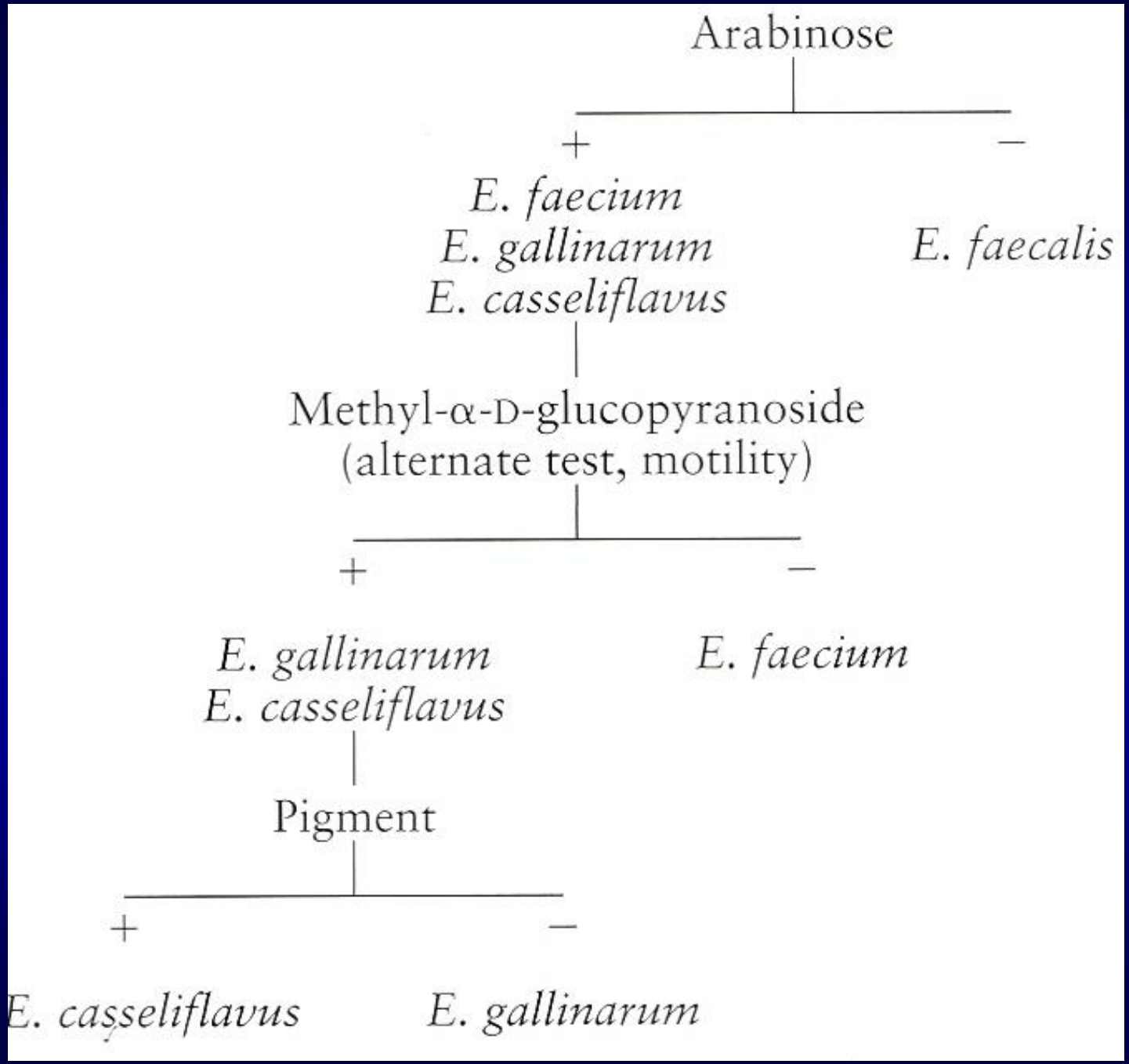



# *Enterococcus* spp.

- Arabinose utilization
  - *E. faecium* pos (yellow)
  - *E. gallinarum/E. casseliflavus* pos
  - *E. faecalis* neg

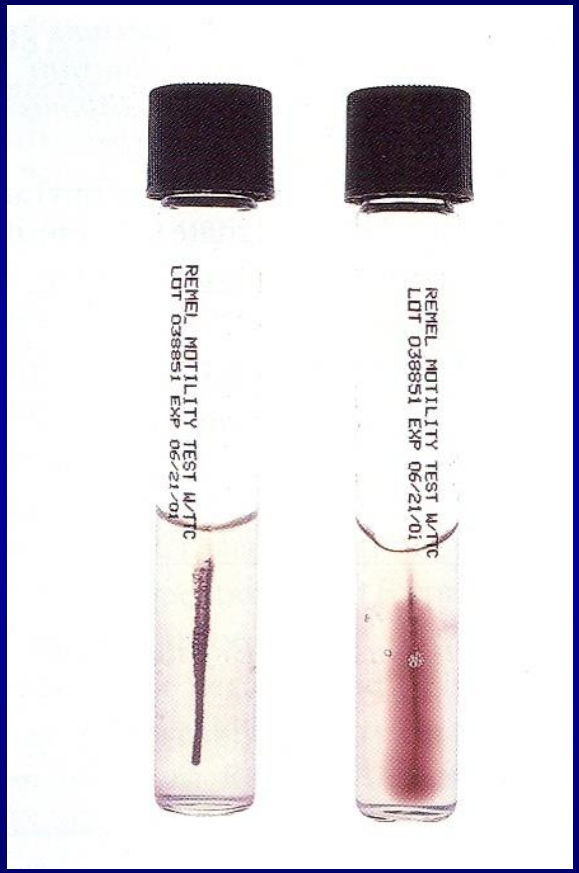
- Acidification of 1% methyl-$\alpha$-D-glucopyranoside (MGP)
  - *E. faecium* neg
  - *E. gallinarum/E. casseliflavus* pos


Arabinose
+
–
E. faecium
E. gallinarum
E. casseliflavus
E. faecalis
Methyl-α-D-glucopyranoside
(alternate test, motility)
+
–
E. gallinarum
E. casseliflavus
E. faecium
Pigment
+
–
E. casseliflavus
E. gallinarum

# *E. casseliflavus vs. E. gallinarum*

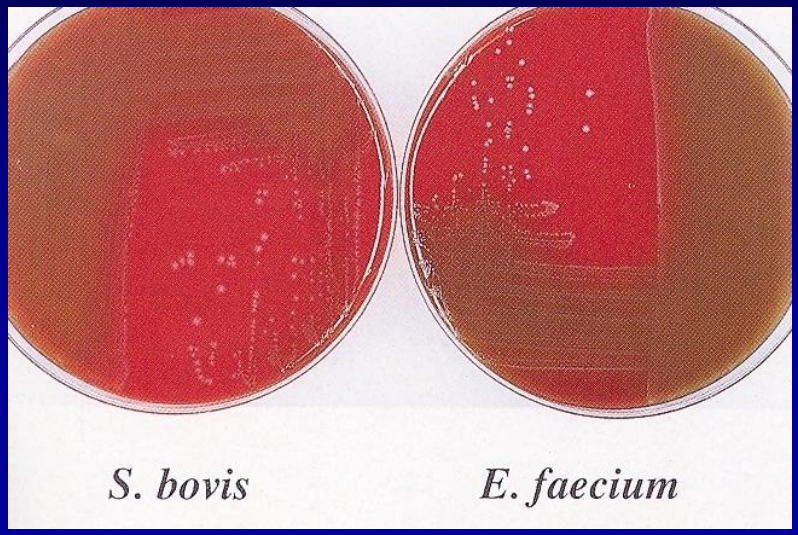
- Both motile
- *E. casseliflavus* is yellow
- Intrinsic low level vanc R (constitutive, chromosomal, *vanC*)




Motility Test
Neg Pos

# *S. bovis* vs. *Enterococcus*

- Both Group D Antigen pos
- Both $\alpha$ or non-hemolytic colonies
- Both grow in 40% bile and hydrolyze esculin
- Only *Enterococcus* can grow in 6.5% NaCl




# *Enterococcus* spp. - therapy

- Pen or amp for UTI, peritonitis, wound infection (if S)
- Serious infection: amp/pen + vancomycin
- Resistance
  - Most *E. faecium* ampicillin R; *E. faecalis* amp S
  - Most *E. faecium* vancomycin R (termed VRE)
  - *E. faecalis* R to Synercid (Quinuprisitin/Dalfopristin)
- Newer agents
  - Linezolid
  - Daptomycin
- Not active: cephalosporins, Bactrim, clindamycin, macrolides, aminoglycosides

# *Enterococcus* - Vancomycin R genes

*vanA*

- high level R to vanc (often ≥128)
- teicoplanin R
- Transferable (usually plasmid)

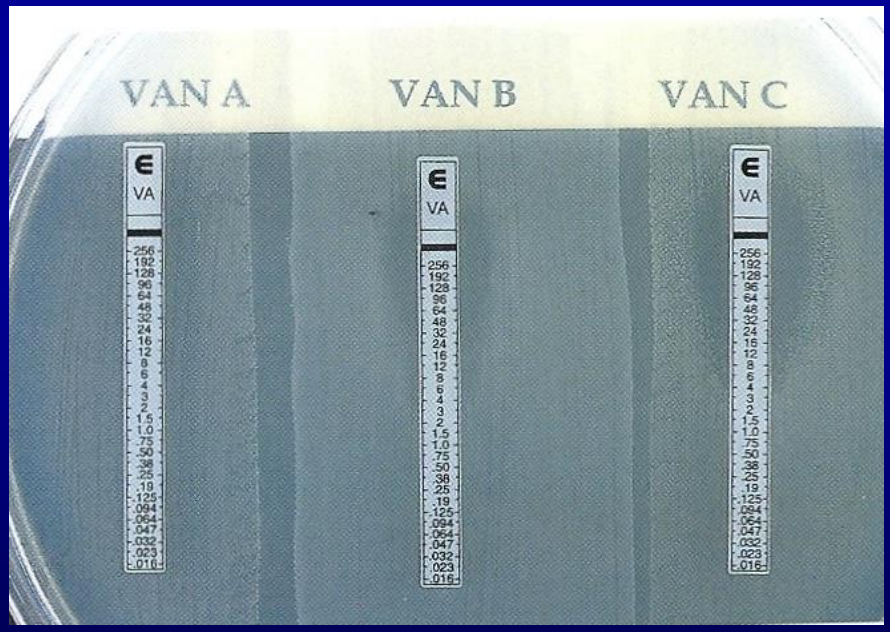
*vanB*

- mod to high level vanc R (≥32)
- teicoplanin S
- transferable

*vanC*

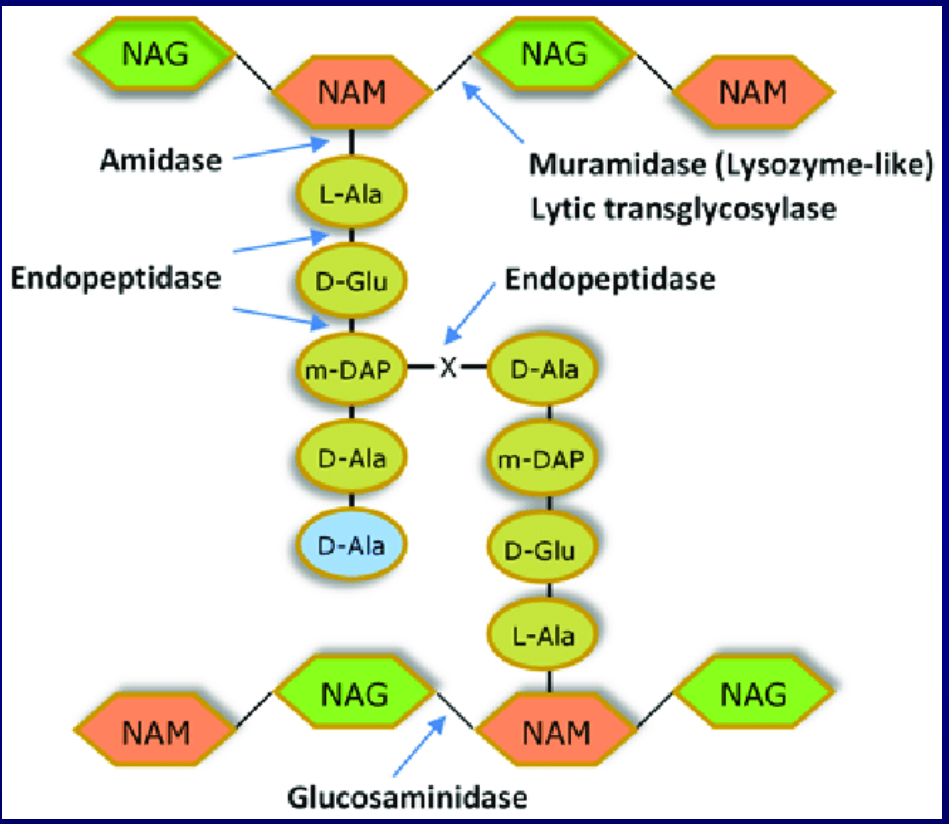
- low level vanc R (2-16)
- NOT transferable

E test for Susceptibility



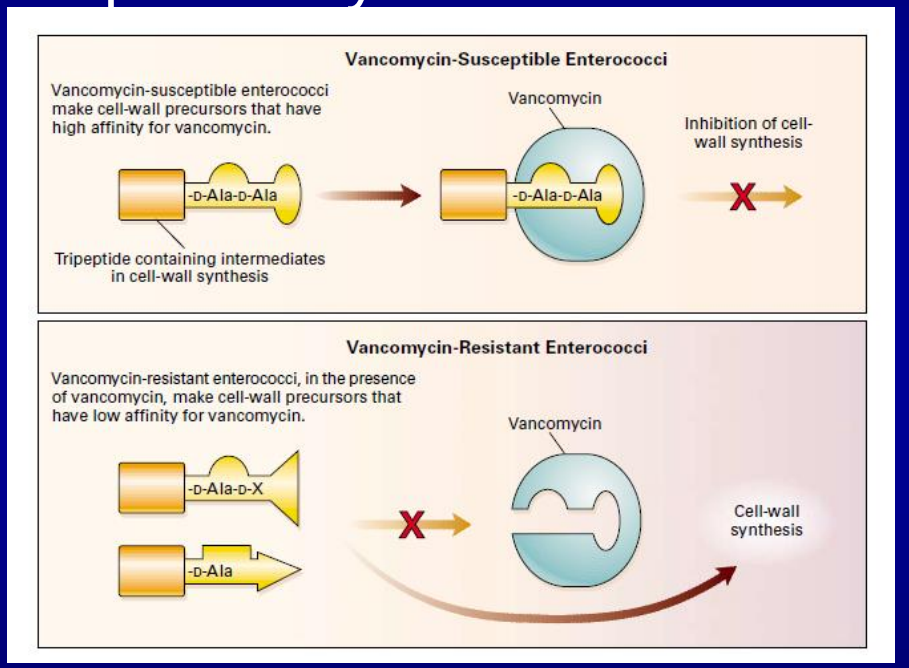
# Mechanism - VRE

## Peptidoglycan Cross-linking




https://www.researchgate.net/figure/Basic-structure-of-the-bacterial-cell-wall-peptidoglycan-The-possible-enzymatic_fig1_323953149

## VRE: D-Ala-D-Ala replaced by D-Ala-D-Lac




Murray, BE NEJM (2000) 342:10

# Controlling spread of VRE

- Rapid ID of colonized pts
- Placement of colonized pts in separate room or room with another VRE pt
- If patient contact – wear gloves and gown
- Handwashing
- Terminal cleaning and disinfection of room
- Avoid inappropriate vancomycin use

# Other Catalase-Negative GP Cocci:
## (relatively avirulent, opportunistic pathogens)

- *Abiotrophia* and *Granulicatella*
  - Nutritionally variant streptococci
  - Require pyridoxal
- *Leuconostoc* and *Pediococcus*
  - Resistant to vancomycin
- *Aerococcus*
- *Gemella*
- *Lactococcus*

# *Abiotrophia & Granulicatella*

- “Nutritionally variant streptococci” (NVS)
- Require either pyridoxal or L-cysteine for growth
- May display pleomorphic cell morphologies under suboptimal growth conditions

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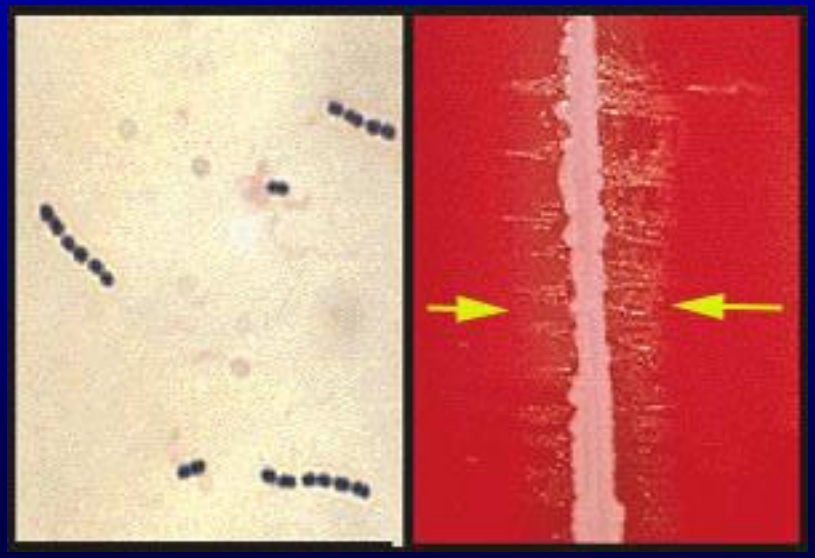
# *Abiotrophia & Granulicatella*



## Growth in Culture:

Grow on most Choc formulations, but not BAP

Supplement media with pyridoxal

BAP near Staph streak

Identify w/MALDI TOF; some commercial systems

ASM Image Library

# *Abiotrophia & Granulicatella*

- Flora of upper respiratory tract
- 4-8% of endocarditis
- Rare cases of meningitis, osteomyelitis, brain abscess…other
- Therapy:
  - Penicillin
  - Ceftriaxone
  - Vancomycin

# *Lactococcus* spp.

- GPC pairs and chains,
- Catalase neg
- PYR and LAP positive
- Grow in presence of 6.5% NaCl
- Non-motile
- Produce lactic acid from fermentation of carbohydrates.
- Formerly lactic group of streptococci (*Streptococcus lactis, S. garvieae, S. cremoris*) – transferred in 1985

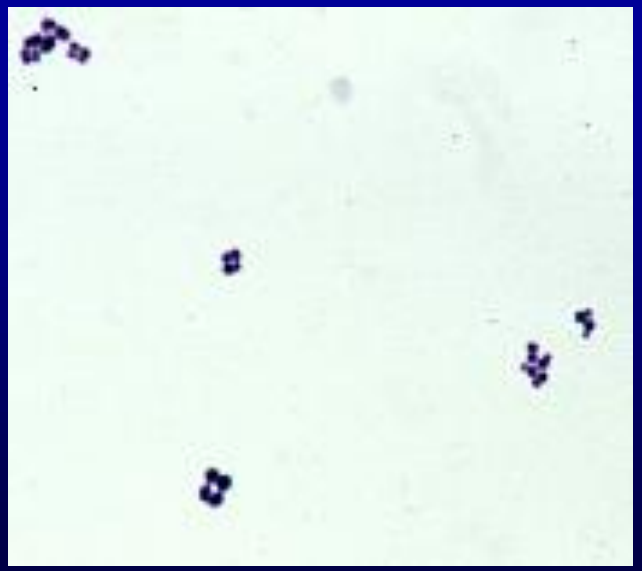
* MALDI-TOF MS FDA claimed organism

# *Lactococcus spp.*

- Human infections rare
- *L. garvieae* fish pathogen (zoonotic infections)
- *L. lactis* used in dairy industry
- Human infection examples (first report in 1991)
  - Infective endocarditis
  - UTI
  - Bacteremia
  - Osteomyelitis
  - Prosthetic joint infection

# *Aerococcus* spp.

- Gram-positive cocci in tetrads & clusters
- Catalase negative
- Alpha hemolytic
- PYR, LAP, 6.5% NaCl, β-GUR may help ID
- MALDI TOF, 16S sequencing



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# *Aerococcus* spp. Clinical Significance

- Vaginal & urogenital commensals; transient on skin
- Common contaminants in cultures
- Reported as urinary pathogen if in significant amounts
- *A. urinae & A. sanguinicola* – UTI, bacteremia, endocarditis
- *A. viridans* – bacteremia, endocarditis

# *Leuconostoc, Pediococcus & Weisella* spp.

- Catalase neg
- Alpha or non-hemolytic
- Vancomycin resistant
- *Leuconostoc & Weisella* are cocci in pairs, chains
- *Pediococcus* – cocci in tetrads, clusters
- PYR neg
- Distinguishing biochemical: PYR, LAP, esculin hydrolysis, 6.5% NaCl, gas from glucose, growth at 45ºC.

# *Leuconostoc, Pediococcus & Weisella* spp.

- Leuconostoc found in nature on plants, foods
- Commensal of human GI tract
- Transient on human skin, mucous membranes.
- Rare cause of invasive infection in immunocompromised

# *Gemella* spp.

- Alpha or non-hemolytic
- Catalase neg
- Gram-positive cocci in pairs, tetrads, clusters
- Oral flora, transient on skin
- Infections:  Bacteremia, endocarditis, brain abscess, osteomyelitis, joint infections,etc.
- Isolation from wounds, abscesses of uncertain significance.

# Acknowledgements


- Dr. Sandra Richter
- Jessica Alban, MLS(ASCP)$^{CM}$