Viridans Streptococci: Friends or Foes?

Romina Reyes, MD, FRCPC

Medical Microbiologist, LifeLabs

Clinical Instructor, Department of Pathology and Laboratory Medicine, UBC

"There are in fact two things, science and opinion; the former begets knowledge, the latter ignorance."

— *Hippocrates*

Overview

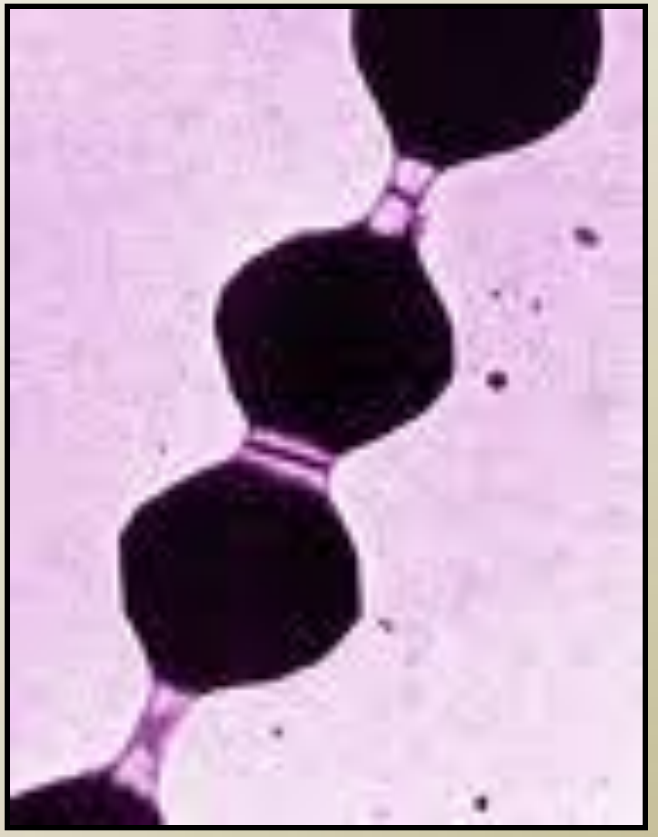
- Introduction to the group
- Current taxonomy
- Clinical significance & infections
- Emerging antibiotic resistance
- The role of the clinical microbiology laboratory

Introduction

- Viridans streptococci
  - (Latin). Viridis: green
  - (Greek). Strepto: bent or twisted like a chain
  - (Greek). Kokkos: berry

Introduction

- Viridans Group Streptococci
  - Heterogeneous group of organisms
  - Human commensals
  - Pathogens



Microbiology of the VGS

Microbiology

- VGS generally not beta-hemolytic
  - They may be non-hemolytic
  - Many produce alpha-hemolysis and a greenish discoloration on blood agar plates
- VGS generally do not react with Lancefield grouping sera

Microbiology

- Exceptions
  - *Streptococcus anginosus* are beta-hemolytic
  - S. *anginosus* react with Lancefield A, C, F or G antiserum
  - S. *mutans* has a Lancefield D reaction

VGS

- A motley crew of organisms that remain once you remove:
  - *S. pyogenes*
  - Lancefield group B organisms
  - Pneumococci
  - Enterococci
  - “Large colony” group C and G

Human Commensals

- Low pathogenic potential in immunocompetent hosts.

- Colonize:
  - Oral Cavity
  - Gastrointestinal Tract
  - Urogenital Tract

What's Really in the Mouth?

Rozkiewicz D, Daniluk T, Sciepuk M, Zaremba ML, Cylwik-Rokicka D, Luczaj-Cepowicz E, Milewska R, Marczuk-Kolada G, Stokowska W. **Prevalence rate and antibiotic susceptibility of oral viridans group streptococci (VGS) in healthy children population**. *Adv Med Sci.* 2006; 51 Suppl 1:191-5.

- 206 pharyngeal and supragingival dental plaque samples
- Healthy children aged 4-18

Results

- VGS isolated from:
  - Pharyngeal swabs in 93% of children
  - Supragingival plaques in 72%
- 4-5 year olds
  - *S. mitis*
- 12-18 year olds
  - *S. vestibularis*
- *S. mitis* and *anginosus* recovered sporadically (2%)

Taxonomy of the VGS

Taxonomy

- 1906 Andrewes and Horder
- "*Streptococcus mitis* group"
  - *S. mitis*
  - *S. salivarius*
  - *S. anginosus*

Developing Taxonomy

- 1919 Orla-Jensen: *S. bovis*
- 1924 Clarke: *S. mutans*
- 1956 Guthof: "*Streptococcus milleri*"
  - Oral nonhemolytic streptococci

"*Streptococcus milleri*" group

- 1972 Coleman & Williams
  - Included minute beta-hemolytic and non-hemolytic oral streptococci
- 1987 Coykendall (International Journal of Systematic Bacteriology)
  - *Streptococcus anginosus* group
  - Approved name for these bacteria
  - Includes *S. anginosus, S. constellatus, S. intermedius*

Historical Nomenclature

- "Streptococcus milleri"
  - Not an approved bacterial name
  - Continues to be used in European and American literature
  - Continues to carry an important message to clinicians
  - Describes streptococci that cause suppurative infections

Basis of Old Taxonomy

- Phenotypic determinations
  - Biochemical characteristics
  - Amino acid hydrolysis
  - Sugar fermentation
  - DNA:DNA hybridization studies

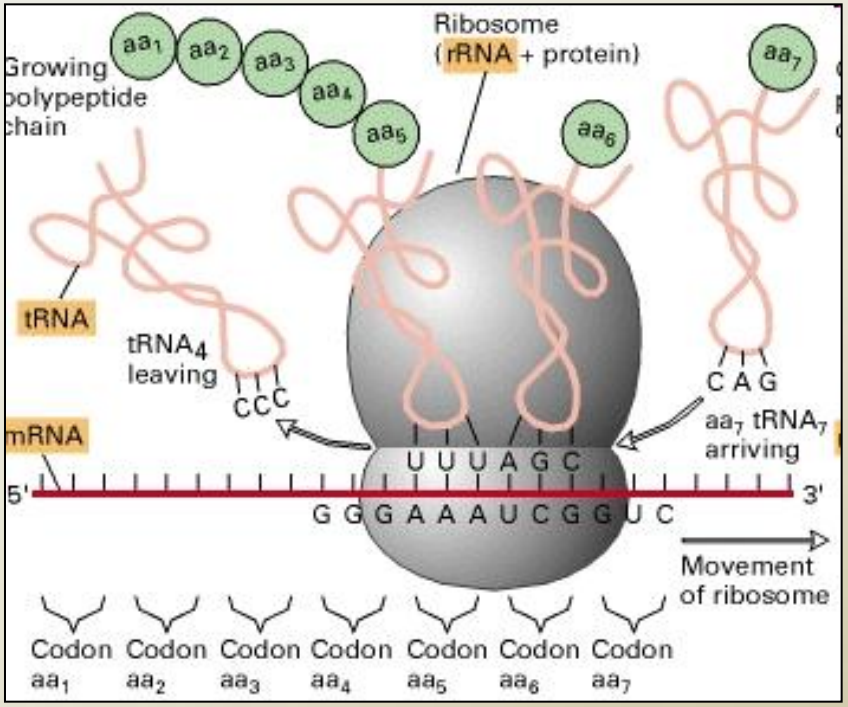
Current Taxonomy

Sequence-based identification systems

- 16S rRNA gene sequencing
  - Poor resolution to species level of VGS
  - Some of these organisms have >99% gene sequence homology
- Alternate gene target sequencing
  - *rnpB*
  - Manganese-dependent superoxide dismutase gene
  - 16S-23S intergenic spacer region
  - D-alanine-D-alanine ligase gene
  - Hyaluronate lyase gene

Lingo

- 16S rRNA
  - **16S ribosomal RNA**
  - Component of the 30S subunit of prokaryotic ribosomes
  - 1542 nucleotides in length
  - Multiple sequences can exist within a single bacterium



Taxonomy

- Classification of VGS
  - *S. mutans* group
  - *S. anginosus* group
  - *S. mitis* group
  - *S. sanguis* group*
  - *S. salivarius* group
  - *S. bovis* group

Clinical Significance
&
Infections of the VGS

Clinical Significance

- VGS infections can result in significant morbidity and mortality
- Serious infections can occur when these organisms enter body sites that are usually sterile

Clinical Significance

- Infections can occur in health hosts
- Most commonly infections occur in:
  - Immunocompromised hosts
  - People with underlying cardiac abnormalities
- Pediatric infections

Clinical Significance

- VGS account for about one half of all cases of streptococcal endocarditis.
- *S. mutans* is responsible for dental caries.
- *S. anginosus* causes abscesses in the brain, liver and joints.

VGS Infections

- Endocarditis incidence
- Endocarditis in neutropenic patients
- Pediatric infections
- Abscesses and *S. anginosus*
- VGS in wounds

Infective Endocarditis

Endocarditis is an inflammation of the inside lining of the heart chambers and heart valves (endocardium).

Vegetation on Heart Valve

- The acute inflammation caused by the infection resulted in the formation of a "vegetation" on the valve, comprised of a mixture of thrombus ("blood clot"), bacteria and inflammatory cells.

Infective Endocarditis (IE)

- Gram positive bacteria are the most frequently identified causes:
  - Adhere to heart valves
  - >80% of all IE cases are caused by *Staphylococcus aureus*, *Streptococcus* spp, and *Enterococcus* spp.

Endocarditis

- *Streptococcus bovis*
  - Patients aged >60 years
  - Causative organism in 10% of cases of IE
  - Accompanied by abnormalities of the digestive tract, particularly colon carcinoma and villous adenoma .

E. E. Hill, P. Herijgers, M-C. Herregods, and W. E. Peetermans. Evolving trends in infective endocarditis. *Clin Microbiol Infect* 2006; 12: 5–12.

Bacteremia and IE in Immunocompetent Adults & Children

- Clinical signs and symptoms
  - Upper respiratory tract symptoms
  - Lower respiratory tract symptoms
  - Post-seizure
  - Temperature >37°C
  - Hypotension

Tan LKK, Lacey S, Mandalia S, Melzer M. Hospital-based study of viridans streptococcal bacteraemia in children and adults. *J Infect.* 2008. 56:103-107.

Bacteremia and IE in Immunocompetent Adults & Children

- VGS cause of community acquired bacteremia
  - 6.9% (50/723) adult patients
  - 12.3% (13/106) pediatric patients
- Endocarditis
  - 11.6% of adults
  - No children
- 30 day mortality
  - 7.3%

Tan LKK, Lacey S, Mandalia S, Melzer M. Hospital-based study of viridans streptococcal bacteraemia in children and adults. *J Infect.* 2008. 56:103-107.

IE in Neutropenic Patients

- IE caused by VGS in this high risk population:
  - 39% of bacteremia cases are due to VGS
  - VGS are the most frequent cause of IE
- The most frequently isolated species in blood culture are:
  - *Streptococcus mitis*
  - *Streptococcus sanguis II*

Bochud PY, Calandra T, Francioli P. Bacteremia due to viridans streptococci in neutropenic patients: a review. *Am J Med.* 1994 Sep;97(3):256-64.

IE in Neutropenic Patients

- Mortality rates range from 6% to 30%.
- Case-control studies have identified the following risk factors:
  - severe neutropenia (< 100 neutrophils/mm3)
  - prophylactic antibiotic treatments with quinolone or co-trimoxazole
  - absence of intravenous antibiotics at the time of bacteremia
  - high doses of cytosine arabinoside
  - oropharyngeal mucositis
  - heavy colonization by viridans streptococci

Bochud PY, Calandra T, Francioli P. Bacteremia due to viridans streptococci in neutropenic patients: a review. *Am J Med.* 1994 Sep;97(3):256-64.

Pediatric IE Infections

- IE occurs less frequently than in adults (1 per 1000 admissions):
  - Pediatrics: 1 per 1280 pediatric admissions
- VGS is the most common cause if IE in children
  - 20-43%
- Mortality rate is low (5.3%)

Doern CD, Burnham CAD. It's not easy being gree: the viridans group streptococci with a focus on pediatric clinical manifestations. *J Clin Microbiol.* Nov 2010;48(11):3829-3835.

Pediatric IE Infections

- VGS symptoms
  - Prolonged low grade fevers
  - Arthralgias
  - Myalgias
  - Weight loss
  - Rigors
  - Fatigue
  - Weakness
- Very common for children to have continuous bacteremia

Doern CD, Burnham CAD. It's not easy being gree: the viridans group streptococci with a focus on pediatric clinical manifestations. *J Clin Microbiol.* Nov 2010;48(11):3829-3835.

Pediatric CF Infections

- *S. anginosus* may be a significant pathogen
  - Associated with colonization with *Pseudomonas aeruginosa.*
  - Patients responded clinically and microbiologically to *S. anginosus* directed therapy (that had no activity to *P. aeruginosa)*

Pediatric Cancer Patients

- IE with VGS is common
  - Infections predominantly with *S. mitis* and *S. oralis.*

- mucositis is an important risk factor as it provides a route of entry for VGS.

Infections by the Anginosus Group

- Suppurative infections (abscess formation):
  - Bacteremia
  - Endocarditis (3-15% of VGS)
  - Brain abscess
  - Pleural empyema
  - Lung abscess
  - Maxillary sinusitis
  - Intra-abdominal abscess
  - Infection of pacemaker
  - Infection of vascular graft
  - Skin and soft tissue

Infections by the Anginosus Group

- Often isolated with other organisms (such as anaerobes)
  - Japanese study of 68 hospitalized patients with *S. anginosus* group infections
    - 18% of cases were pure cultures
    - 82% cases were mixed cultures

Nagata K. Clinical significance of Streptococcus milleri isolated from various suppurative lesions. Kansenshogaku Zasshi. 1990 Apr;64(4):444-54.

Infections by the Anginosus Group

- Sites of clinical infection:
  - *S. anginosus* most frequently identified in the gastrointestinal tract and genitourinary specimens
  - *S. constellatus* most frequently identified from the respiratory tract
  - *S. intermedius* showed an association with infections of the central nervous system.

Whiley RA, Beighton D, Winstanley TG, Fraser HY, Hardie JM. *Streptococcus intermedius, Streptococcus constellatus,* and *Streptococcus anginosus.* (the *Streptococcus milleri* group): association with different body sites and clinical infections. *J Clin Microbiol* 1992, 30:243-244.

VGS Brain Abscess

- Viridans streptococci from dental procedures can seed to the heart.

- Literature reports of cases of VGS brain abscesses following dental procedures and maxillofacial trauma.

VGS Brain Abscess

- A 19-year-old male patient.
  - Diagnosed with *S. sanguinis* brain abscess
  - Unknown etiopathology
  - Subclinical endocarditis
- Highlights the importance of:
  - Prompt diagnosis
  - Initiation of antimicrobial therapy
  - Given the potential for long-term sequelae such as focal deficits and seizures

Kassis H, Marnejon T, Gemmel D, Cutrona A, Gottimukkula R. Streptococcus sanguinis brain abscess as complication of subclinical endocarditis: emphasizing the importance of prompt diagnosis *South Med J*. 2010 Jun;103(6):559-62.

S. anginosus Group Wound infections

- Intravenous drug users:
  - Septic complications occur frequently at the injection site
  - In the groin large abscesses around the femoral vessels can threaten life or limb.
  - Antecubital abscesses and bacteremia

Abscess Formation

- Marked swelling and redness is apparent just above antecubital fossa.

- This is caused by an abscess, the result of bacteria innoculated under the skin during injection drug abuse.

Antimicrobial Susceptibilities of the VGS

General AST Principles

- Often, knowledge of the taxonomic identity of bacteria causing the clinical infection can be used to predict the antimicrobial susceptibility patterns of the organism.
- But the VGS have undergone many rearrangements in taxonomy!

VGS Generalizations

- Antimicrobial resistance is substantial in the VGS as a group
- Penicillin resistance is high
  - 48% in USA strains
  - 45% in Canadian strains
  - 33% in Latin American strains.

Pfaller MA, Jones RN, Doern GV, Sader HS, Kugler KC, Beach L and the SENTRY participants group. 1999. Survey of blood stream infections attributable to gram-positive cocci: frequency of occurrence and antimicrobial susceptibility of isolates collected in 1997 in the United States, Canada, and Latin America from the SENTRY antimicrobial surveillance program. *Diagn Microbiol. Infect. Dix.* 33:238-297.

Penicillin Resistance

- *S. mitis:*
  - Was the most common species identified in clinical samples
  - Was the species most likely to be penicillin resistant
- *S. oralis:*
  - Found to be (with *S. mitis*) most common in blood cultures of cancer patients
  - Commonly resistant to beta-lactam antibiotics

Pfaller MA, Jones RN, Doern GV, Sader HS, Kugler KC, Beach L and the SENTRY participants group. 1999. Survey of blood stream infections attributable to gram-positive cocci: frequency of occurrence and antimicrobial susceptibility of isolates collected in 1997 in the United States, Canada, and Latin America from the SENTRY antimicrobial surveillance program. *Diagn Microbiol. Infect. Dix.* 33:238-297.

Penicillin Resistance

- *S. sanguis* group:
  - Resistance is also present but not as high as in the *S. mitis* group organisms.

S. mitis Group

- Among the VGS this group is most likely to become resistant to beta-lactams and macrolides.
  - Penicillin 16-34% R
  - Clindamycin 4-14% R
  - Erythromycin 40-51% R
  - Tetracycline 29-34 resistance R

Brown DFJ, Hope R, Livermore DM, Brick G, Broughton K, George RC, Reynolds R. Non-susceptibility trends among enterococci and non-pneumococcal streptococci from bacteraemias in the UK and Ireland, 2001–06. *Journal of Antimicrobial Chemotherapy* (2008) 62, Suppl. 2, ii75–ii85.

S. mitis Group Resistance

- Implications of the emergence of resistance in VGS group are serious:
  - *S.mitis/oralis* are closely associated to *S. pneumoniae*
  - Similar species can transfer genetic material
  - Development of pneumococcal resistance to penicillin

S. anginosus Group

- Penicillin:
  - Resistance to beta-lactams is emerging
- Macrolides:
  - Resistance was found in 17% of strains

Limia A, Jimenez ML, Alarcon, Lopez-Brea M. 1999. five year analysis of antimicrobial suscpeptibility of the *Streptococcus milleri* group. *Eur J Clin Microbio infect Dis.* 18:440-444.

S. anginosus Group

- Species of *S. anginosus* group were identified by 16S rRNA (*S. anginosus, S. constellatus, S. intermedius)*
  - There was no difference found in the susceptibility patterns for the three species
  - Identification to the "milleri/anginosus group" may be sufficient for patient management and it's not necessary to go to species level.

Tracy MA, Wanahita A, Shuhatovich Y, Goldsmith EA, Clarridge JE, Musher DM. 2001. Antibiotic susceptibilities of genetically characterized *Streptococcus milleri* group strains. *Antimicrob Agents Chemother.* 45:1511-1514.

Antibiotic Usage

- Antibiotic usage drives resistance of penicillin and macrolides in VGS
- In pediatric and adult populations the most at risk for developing resistant and invasive VGS infections are the immunocompromised
  - This is also a population that receives frequent antibiotic treatment.

Antibiotic Usage

- Study by Kastner *et al* 2001 found that macrolide resistance developed in VGS in children treated for URTI
  - Initial pretreatment cultures taken
  - Antibiotics: azithromycin or clarithromycin
  - 1 week post-treatment 60% of patients had at least one macrolide-resistant organism
  - 6 weeks later 87% of patients treated with azithromycin were colonized with macrolide-resistant VGS (60% in clarithromycin group)

Kastner U, Guggenbichler JP. Influence of macrolide antibiotics on promotion of resistance in the oral flora of children. *Infection.* 2001. 29:251-256.

“The Good News”

- VGS remain susceptible to a group of antibiotics:
  - Vancomycin
  - Linezolid
  - Daptomycin

Emerging Problem

- VGS have developed resistance to penicillin and macrolide (MLS) antibiotics
- There are some group-specific resistance patterns
- Resistance to antibiotics is increasing the virulence of *S. pneumoniae* (a well defined human pathogen)

The Role of the Clinical Laboratory

Sterile Body Fluids

- Culture examination
  - Examine all inoculated plates and broth for growth at 24 hours
  - Reincubate if there is no visible growth
  - Read the plates daily for 4 additional days for invasively collected specimens
- Blood cultures
  - Incubate for 5 to 7 days

Garcia L, Isenberg HD. Clinical Microbiology Procedures Handbook. ASM. 2007.

Sterile Body Fluids

- Cultures with growth on media
  - Notify your microbiologist
  - Correlate the culture results with those of the Gram stain made from the specimen
  - Identify all organisms

- The clinical picture can help direct us.

Garcia L, Isenberg HD. Clinical Microbiology Procedures Handbook. ASM. 2007.

AST and Sterile Body Fluids

- Most viridans strep tested for AST are from serious infections
  - Report MIC
  - Report S/I/R
- MIC is clinically used to treat VGS bacteremia/endocarditis

Non-sterile Sites

- Aerobic Bacteriology Section
  - **Chapter 3.13** "Wound Cultures"
    - Wound Abscesses and Soft Tissue Cultures
- **Table 3.13.1-1** Aerobic and Anaerobic Isolates from Acute and Chronic Infections
  - Lists Streptococcus spp. (viridans group)
- **Figure 3.13.1-5** Initial evaluation of positive wound cultures for organisms growing aerobically
  - A picture is worth a thousand words!!

Garcia L, Isenberg HD. Clinical Microbiology Procedures Handbook. ASM. 2007.

Non-Sterile Sites

- Note: There are microorganisms that are usually considered significant even if isolated in low numbers or with mixed flora
  - Group A Streptococci
  - Group B Streptococci
  - *Pseudomonas aeruginosa*
  - etc

Non-Sterile Sites

- Generally identify VGS if isolated with 2 other organisms in a mixed culture (3 microorganisms) IF
  - WBCs seen on direct smear
  - The specimen was collected from a normally sterile site
  - The specimen is of good quality (few epithelial cells)
  - The organism was seen on the direct smear

Non-Sterile Sites

- Minimal testing for non-invasively collected specimens IF:
  - Many epithelial cells seen in direct smear
  - No inflammatory cells seen in direct smear and no clinical information available to indicate an infection
  - >3 organisms growing

Non-Sterile Sites

- Identify VGS to the genus level
  - Surgically collected specimens (biopsy)
  - Invasively collected specimens
  - If single or predominant pathogen
  - Inflammatory cells seen on the gram stain

Non-Sterile Sites

- May not need to identify VGS to the genus level if:
  - Very mixed culture
  - Not predominant

General Principles

- A positive culture indicates infection with the organism.
- WBCs are usually present in infections of body fluids.
- FP cultures can result from contamination of the specimen with flora.
- FN can be caused by low numbers of organisms, prior antibiotics or the fastidious nature of the infective organism.

Garcia L, Isenberg HD. Clinical Microbiology Procedures Handbook. ASM. 2007.

Overview

- Introduction to the group
- Current taxonomy
- Clinical Significance & Infections
- Emerging antibiotic resistance
- The role of the clinical microbiology laboratory

Summary of the VGS

- ***S. mutans* group**
  - *S. mutans, S. sobrinus*
- ***S. anginosus* group**
  - *S. anginosus, S. constellatus, S. intermedius*
- ***S. mitis* group**
  - *S. mitis, parasanguis, gordonii, cristatus, oralis, infantus, peroris, pneumonieae*
- ***S. sanguis* group***
- ***S. salivarius***
  - *S. salivarius, S. vestibularis, S. thermophilus*
- ***S. bovis* group**
  - *I S. equinus* (used to be *S. bovis*)
  - *II S. gallolyticus*
  - *III S. infantarius*
  - *IV S. alactolyticus*

Thank you.

Happy Holidays!