REVIEW ARTICLE

Typing Methods for Viridans Group Streptococci- A Review

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Abstract:

The "Viridans group streptococci" include a large number of diverse organisms. They are the part and parcel of the normal flora of the various body sites and especially the oral cavity in human beings. They are important pathogens causing a variety of infections in human beings. They are the most important cause of orodental infections, bacteremia, infective endocarditis and other infections involving various body sites. Attempts have been made to divide these organisms into separate species. Many typing systems have been devised to study the epidemiology and taxonomy of viridans group streptococci. These methods include two main types, namely phenotypic methods and genotypic methods. The commonly used phenotypic methods for typing purpose are biotyping, bacteriocin typing, serotyping and matrix-assisted laser desorption ionization–time of light mass spectrometry (MALDI-TOFMS) and other methods like cellular fatty acid analysis, whole cell protein analysis and multilocus enzyme electrophoresis. The various genomic typing methods commonly used for typing purpose include plasmid analysis; restriction endonuclease analysis of genomic DNA, restriction fragment length polymorphism (RFLP), ribotyping, pulsed field gel electrophoresis (PFGE) and arbitarily primed polymerase chain reaction (AP-PCR). In this review an attempt has been made to throw light on the details of these techniques used for typing of viridans group streptococci.

Key words:

Viridans group streptococci, typing methods, phenotypic methods, genotypic methods

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Introduction:

The designation "viridans streptococci" includes a large number of diverse organisms. Viridans group streptococci (VGS) are the part and parcel of the flora of the various body sites and especially the oral cavity in human beings. They are important pathogens causing a variety of infections in human beings. They are the most important cause of oro-dental infections, bacteremia, infective endocarditis and other infections involving various body sites. Gingivitis and periodontitis are among the most common human oro-dental infections.

Periodontitis have inflamed and ulcerated crevicular or pocket epithelium around the teeth, which may act as a portal of entry for oral bacteria into the blood stream causing bacteremia. Bacteremia and low-grade systemic inflammation induced by periodontal infections may confer a risk for systemic conditions including infective endocarditis and other diseases like cardiovascular diseases, stroke, and premature low birth weight delivery. Because of these infections, VGS have acquired clinical importance that makes their detailed study obligatory for the effective clinical management. In view of this, an attempt had been made to review the different typing methods used for typing of these cocci. A number of attempts have been made to divide these organisms into separate species. Many typing systems have been devised to study the epidemiology and taxonomy of viridans streptococci. These methods include phenotypic and genotypic methods. The commonly used phenotypic methods for typing purpose are biotyping,[1] bacteriocin typing,[2] serotyping[3] and matrix-assisted laser desorption ionization–time of light mass spectrometry.[4] The commonly used genotypic methods for typing purpose are ribosomal RNA gene polymorphism analysis,[5] restriction endonuclease analysis of genomic DNA,[6] polymerase chain reaction based typing methods[7] and analysis

of the 16S-23S ribosomal DNA spacer region.[8]

Phenotyping Methods:

1. Biotyping:

Biotyping method is one of the oldest and commonest methods for identification and discrimination of VGS for epidemiological purposes. The VGS are grouped into five groups and 26 different streptococcal species on the basis of phenotypic characteristics by Richard Facklam, (2002). All VGS strains were gram-positive cocci arranged in chains, catalase-negative, leucine aminopeptide positive, pyrrolidonylarylamidase negative, and showing no growth in 6.5% NaCl broth. Nearly all species, with the exception of those in the salivarius group, are bile-esculin negative. Differentiation of the VGS species was done on the basis of results of the Voges-proskauer reaction, pattern of acid formation from mannitol and sorbitol, ability to hydrolyze esculin and arginine, and production of urease. As this method is unable to identify individual species but can help to place them in one of the groups; hence, it was suggested to use molecular genetic procedures for accurate identification of VGS).[9]

Biotyping of VGS strains can also be performed using commercial kits such as Rapid Strep32 (Biomerieux) and API20-STREP biotyping profile index (Biomerieux). These biotyping systems are test kits for the identification of streptococci; these systems utilise many of the conventional streptococcal tests in addition to others that include the production of a diverse range of enzymes. The system identifies viridans streptococci on the basis of the Facklam classification, but includes additional biotypes for several species. The patterns of reactions were coded into numerical profiles where the number and its corresponding strain type were identified.[10]

Biotyping can also be done using and automated systems such as VITEK 2, this system that includes a total of 43 biochemical tests, of which 17 are enzymatic tests, which are present in the card and interpreted in a kinetic mode, for up to 8 h. The VITEK 2 database, used in conjunction with the GP (Gram positive) identification card, provides reliable results for the identification of VGS under routine laboratory conditions.[11]

2. Bacteriocin Typing:

Several strains of streptococci produce bacteriocin-like inhibitory substances (BLIS), which hamper the growth of a wide range of bacteria.[12] BLIS activity among *Streptococcus* species found in humans and animals are believed to play a role in their ability to interfere with growth of other bacteria in their local environment along with

other released inhibitory substances such as hydrogen peroxide and lactic acid.[2]

BLIS activity has been reported in several *Streptococcus* species with the highest prevalence among *S. salivarius* and other oral streptococci.[13] The commensal *S. salivarius* produces bacteriocins named as salivaricin A and salivaricin B, which have strong inhibitory activity against *S. pyogenes* strains and a variety of other bacterial pathogens.[14,15] The majority of BLIS produced by *Streptococcus* are relatively small (<5 kDa) heat-stable proteins termed lantibiotics because they contain the unusual amino acids lanthionine and/or 3-methyllanthionine.[2] It has been reported that bacteriocins in many Gram-positive bacteria are encoded by plasmid-born genes, but neither of the currently documented lantibiotic-producing strains of *S. pyogenes* and *S. salivarius* appears to contain plasmids.[16] The finding that different bacteriocin activities in *S. mutans* strains reflect distinct phylogenetic lineages support the notion that the genes have evolved over time along with the genome in general.[17]

Bacteriocin production and sensitivity have been successfully used as a fingerprinting method in epidemiological studies of streptococci before DNA techniques became available.[18]

3. Serotyping

Bratthall demonstrated five serological groups of mutans streptococci by using Ouchterlony immunodiffusion method.[19] A total of eight serotypes were subsequently identified.[20] Although serological grouping based on cell wall carbohydrates has been a valuable tool when applied to the more pathogenic streptococci, as originally intended by Lancefield (1933),[21] this approach was less successful and sometimes misleading for the viridans streptococci, in which group-specific antigens may be absent or shared by several distinct taxa.[10]

4. Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOFMS):

A number of mass spectrometry based methods have been developed in recent past for the rapid differentiation of bacteria phenotypically. Ionization of bacterial extracts by fast-atom bombardment, electrospray ionization and sample pyrolysis, followed by MS and MS/MS, have been reported.[22,23] More recently, whole-cell MALDI-TOF-MS has been used to quickly differentiate microorganisms.[24] Based on abundant peptides and small proteins, MALDI-TOF-MS generates complex spectra, which contains unique *m/z* signatures for different microorganisms due to the inherent variations

in their masses and/or the abundance of the related proteins that they express. The MALDI-TOF-MS spectra can be analyzed with respect to the detection of one or several specific peaks(25) or by analyzing the complete spectral pattern.(26) Friedrichs (2007) in their study on identification of viridians streptococci using MALDI-TOFMS found that MALDI-TOFMS, in combination with pattern analysis, may be useful for differentiation of *S. oralis* and *S. mitis*; so also is an rapid and reliable method for the identification of species of VGS from clinical samples.(4)

The other phenotypic methods include cellular fatty acid analysis, whole-cell protein analysis and multilocus enzyme electrophoresis (MEE). MEE is based on the relative electrophoretic mobility of metabolite cellular enzymes. MEE has been successfully applied in studies with many organisms, but only one report using MEE in strain identification of viridans streptococci has been published.(27)

Genotyping Methods:

For fingerprinting of isolate from clinical specimens, molecular typing methods have a higher discriminatory ability and reproducibility since these methods do not examine the gene expression but rather the DNA of the microorganisms to be studied.(28) These typing methods include plasmid analysis, restriction endonuclease analysis (REA), restriction fragment length polymorphism (RFLP) (including ribotyping), pulsed field gel electrophoresis (PFGE) and arbitrarily primed polymerase chain reaction (AP-PCR).

1. Plasmid analysis:

Plasmids are extrachromosomal elements of DNA that encode many properties, including antimicrobial resistance, many virulence traits and hydrocarbon metabolism.(29) Yagi et al. (1978) discovered a small plasmid determining inducible resistance to erythromycin, lincomycin, and vernamycin B_{α} from a strain of *Streptococcus sanguis* isolated from dental plaque.(30) Leblanc and Lee (1979) developed a rapid screening method for detection of plasmids in streptococci.(31) Plasmid analysis was the first DNA-based technique applied in epidemiological studies on mutans streptococci.(32) As plasmids are infrequently detected in viridans streptococci, plasmid analysis is not applicable to typing of these bacteria.(33)

2. Restriction Endonuclease Analysis (REA):

Restriction endonuclease analysis (REA) is a technique in which bacterial chromosomal DNA is cut with a restriction

endonuclease and separated by gel electrophoresis. The restriction endonucleases are enzymes that cut the DNA chain at specific recognition sequences. The restriction enzymes are nowadays synthetically produced, but were originally isolated from bacteria, with their original function being defense against other bacteria. After separation by gel electrophoresis, gels are stained with ethidium bromide and detected under UV light, and the banding patterns obtained for different strains are compared. This process results in fingerprints with many bands, thus the interpretation of the REA profiles can be complicated. REA has been applied for evaluation of relatedness of mutans streptococcal isolates.[34]

3. Restriction Fragment Length Polymorphism (RFLP), including Ribotyping:

In this technique, after cleaving the chromosomal DNA of the microorganisms to be studied, the separation products can be labelled with either DNA or RNA probes in the Southern blot technique.[35] Grimont and Grimont in 1986 introduced the use of a probe derived from the *Escherichia coli* ribosomal operon.[36] They had discovered that variations of the genes encoding ribosomal ribonucleic acid (rRNA) and variations in sites flanking those loci, could serve as a means of typing strains since ribosomal sequences are highly conserved. In ribotyping of an isolate, after the gel electrophoresis of the cleaved DNA, the fragments are hybridized with the rRNA probe. While detecting the hybrids, every fragment containing a ribosomal gene will be highlighted. The banding patterns obtained in ribotyping include only a small number of bands, thus rendering comparison of fingerprints among isolates easier than comparing REA patterns. The term ribotyping was introduced in 1988[37] and ribotyping of mutans streptococci has been applied since 1993[38] mainly in studies on transmission of mutans streptococci and stability of infection. Rudney and Larson in 1994 in their study evaluated restriction fragment length polymorphisms of rRNA genes (ribotyping) for genotypic identification and proposed that ribotyping can be used for genotypic identification of *S. sanguis, S. oralis* and *S. gordonii* isolates. [39]

4. Pulsed Field Gel Electrophoresis (PFGE):

In pulsed field gel electrophoresis, the orientation of the electric field across the gel is changed periodically ("pulsed"), thus larger bacterial DNA fragments can be analysed. PFGE is considered the "gold standard" of molecular typing methods, with excellent discriminatory power and reproducibility.[28]

Wisplinghoff et al. (1999) used this typing method to study epidemiologic relatedness between strains of VGS isolated from blood cultures of neutropenic cancer patients by analysis of genomic DNA by PFGE and found unique PFGE patterns of *S. mitis*, *S. oralis* and *S. salivarius* concluding that fingerprinting of viridans streptococci with PFGE produced reliable, discriminatory and reproducible typing results.(40)

5. Arbitrarily Primed Polymerase Chain Reaction (AP-PCR):

AP-PCR is perhaps the least laborious technique.(41) AP-PCR can be performed with a very small quantity of sample. For the polymerase chain reaction, the template is annealed to one or more short primers (typically 9-10 bp) at low stringency. Amplification results in an array of DNA fragments, often termed random amplified polymorphic DNA (RAPD) can be resolved by gel electrophoresis. AP-PCR requires no previous knowledge of the DNA to be analysed.(42) A limitation of the method is that it is highly sensitive to even minor variations in technical factors such as temperature, Mg^{2+} concentration and polymerase source. Interlaboratory comparison of typing results is impeded by only a fair reproducibility and only isolates processed simultaneously and fingerprints obtained concomitantly can be compared. This fair reproducibility is, however, complemented by a good discriminatory power.(28) AP-PCR typing has been shown to be most applicable method for typing of mutans streptococci.(43) RAPD fingerprints obtained by AP-PCR can be used to compare bacterial strains, including the groups of mutans streptococci and mitis streptococci.(44) Truong et al. (2000) reported that RAPD protocol could be used to distinguish *S. mutans* and *S. sobrinus* from other species of oral streptococci and their study could identify and sub-classify all oral streptococci. Their data confirmed that RAPD fingerprinting is a potentially valuable tool in bacterial epidemiology and transmission studies of viridans streptococci by virtue of its rapidity, efficiency and reproducibility in exploring the genomic polymorphism of these organisms.(45)

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