

# **Mechanisms of Resistance to Erythromycin among Viridans Group Streptococci (VGS) Isolated From Blood Culture**

*Thesis*

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*By*

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## List of Abbreviations

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<b>16S rRNA</b>	16 subunit ribosomal ribonucleic acid
<b>AD</b>	Agar dilution
<b>aphA-3</b>	Kanamycin resistance gene
<b>API</b>	Analytical profile index
<b>ATCC</b>	American type culture collection
<b>ATP</b>	Adenosine tri-phosphate
<b>BEA</b>	Bile esculin agar
<b>BGUR</b>	$\beta$ -glucuronidase
<b>BMD</b>	Broth micro dilution
<b>CAMP</b>	Christie, Atkins, Munch-Peterson test
<b>catQ</b>	Chloramphenicol resistant determinant
<b>CSF</b>	cerebrospinal fluid
<b>CISI</b>	Clinical and laboratory standard institute
<b>CNS</b>	Central nervous system
<b>DDL</b>	D-alanine: D-alanine ligase gene
<b>DNA</b>	Deoxyribonucleic acid
<b>E-test</b>	Epsilometer test
<b>Erm-B</b>	erythromycin ribosome methylase
<b>FbpA</b>	fibronectin-binding protein A
<b>GTF</b>	glucosyltransferase
<b>FISH</b>	Flourescence in situ hybridization
<b>GPC</b>	gram-positive cocci
<b>HACEK</b>	Haemophilus parainfluenzae, H.aphrophilus, H.paraphrophilus,H.influenzae, Actinobacillus actinomycetemcomitans,Cardiobacterium hominis, Eikenella corrodens, Kingella kingae, and K. denitrificans
<b>ICU</b>	Intensive Care Unit
<b>IE</b>	Infective Endocarditis
<b>MEGA</b>	Macrolide efflux genetic assembly
<b>MEF A/E</b>	Macrolide efflux gene
<b>MIC</b>	Minimal Inhibitory Concentration
<b>MILVA</b>	Multilocus variable number tandem repeat analysis
<b>MHA</b>	Muller Hinton agar

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<b>MLSA</b>	Multilocus sequencing analysis
<b>MLS-B</b>	Macrolide-Lincosamide-Streptogramin B phenotype.
<b>MLST</b>	Multilocus sequencing testing
<b>mRNA</b>	messenger ribonucleic acid
<b>MsrA</b>	Methionine sulfoxide reductase
<b>ORF</b>	Open Reading Frame
<b>qPCR</b>	Quantitative Polymerase chain reaction
<b>QRDRs</b>	quinolone resistance determining regions
<b>PAT</b>	Putative ATP-dependent efflux protein
<b>PCR</b>	Polymerase chain reaction
<b>PABA</b>	Para amino benzoic acid
<b>PBP</b>	penicillin binding protein
<b>PFGE</b>	pulsed field gel electrophoresis
<b>PVE</b>	Prosthetic Valve Endocarditis
<b>PYR</b>	Pyrrolidonyl- $\beta$ -naphthylamide
<b>RNA</b>	Ribonucleic acid
<b>Tn</b>	Transposon
<b>TSA</b>	<i>Trypticase soya agar</i>
<b>VGS</b>	Viridans group Streptococci
<b>VP</b>	Voges-Proskauer test
<b>VSSS</b>	Viridans related septic shock syndrome
<b>XIS</b>	excisionase

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## Introduction

The viridans group streptococci (VGS) are gram positive cocci arranged in chains, they are alpha-hemolytic, but they may be nonhemolytic. Their growth is not inhibited by Optochin and colonies are soluble in bile (deoxycholate) (*Jawetz et al., 2010*).

The VGS include *S.mitis*, *S.mutans*, *S.salivarius*, *S.sanguis*, and others. Typically they are the most prevalent members of the normal flora of the upper respiratory tract and are important for the health states of the mucous membranes there. They may reach the bloodstream as a result of trauma and are a principal cause of endocarditis on abnormal heart valves. Some viridans streptococci (eg, *S mutans*) synthesize large polysaccharides such as dextrans or levans from sucrose and contribute importantly to the genesis of dental caries (*Kohler et al., 2010*).

VGS have accounted for 25 to 30% of bacteremic episodes among patients with malignancies and are the most common cause of early bloodstream infections among hematopoietic stem cell transplantation recipients (*Bruckner et al., 2006*).

Subacute bacterial endocarditis often involves abnormal valves (congenital deformities and rheumatic or atherosclerotic lesions) where the clinical course is gradual, but the disease is

invariably fatal in untreated cases. The typical clinical picture includes fever, anemia, weakness, a heart murmur, embolic phenomena, an enlarged spleen, and renal lesions. Although any organism reaching the bloodstream may establish itself on thrombotic lesions that develop on endothelium injured as a result of circulatory stresses, subacute bacterial endocarditis is most frequently due to members of the normal flora of the respiratory or intestinal tract that have accidentally reached the blood. After dental extraction, at least 30% of patients have viridans streptococcal bacteremia (*Jawetz et al., 2010*).

Resistance to macrolides and other antibiotics among blood cultures of VGS is a major concern and could compromise currently available prophylactic and therapeutic regimens (*Nandhakumar et al., 2008*).

In streptococci, there are three well-characterized macrolide resistance mechanisms. The first mechanism is the target site modification which is mediated by methylases encoded by the *erm* (erythromycin ribosome methylation) and this methylation lead to conformational change in the ribosome result in co-resistance to macrolides, lincosamides and streptogramin B antibiotics, The second mechanism is the active efflux mechanism, encoded by the *mef* genes (macrolide efflux) causes resistance only to 14- and 15-membered ring macrolides. The third mechanism is the ribosomal mutations in the key antibiotic binding site (*MalhotraKumar et al., 2004*).

## **Aim of the Work**

The aim of this work is to evaluate the species distribution, antimicrobial susceptibility resistance mechanisms of erythromycin to VGS isolates obtained from blood cultures of adult patients with underlying diseases.

# **Streptococcus Species**

## **Historial review:**

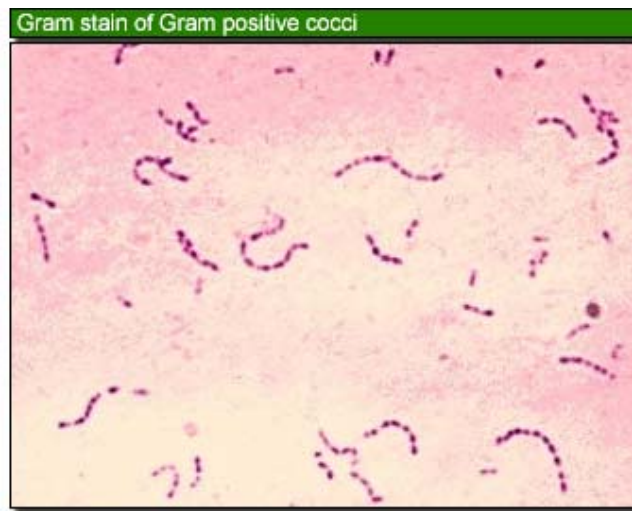
It is over 100 years since the observation by billroth (1874) of chain-forming cocci in wounds and his application of the descriptive term streptococcus to these organisms. Since then advances in our knowledge and understanding of each of the genera streptococcus and enterococcus have remained closely linked, because these bacteria were included within one genus (streptococcus) from 1906 until 1984. Most notable of these innovations were the demonstration of various types of haemolytic reaction given by different strains when cultured on whole blood-containing agar, the use of carbohydrate fermentation reactions together with physiological and morphological tests for characterization of streptococci from clinical, environmental and dairy sources, and the detection of carbohydrate antigens in acid extracts of cell walls (*Hardie et al., 1997*).

## **Genus definition:**

Streptococci constitute the main groups of medically important gram-positive cocci arranged in chains (**figure1**). Streptococci are gram-positive, nonmotile, and catalase-negative. Clinlally important genera include streptococci and enterococcus. They are ovoid to spherical in shape and occur as pairs or chains. Most are facultative anaerobes due to lack of heme compounds, streptococci are incapable of respiratory metabolism, but grow fermentatively even in the presence of oxygen. Because of their complex nutritional requirements, blood-enriched medium is generally used for their isolation (*Richard et al., 2006*).

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Most species of the genus *Streptococcus* have a low G+C content of DNA that lies in the range of 34 to 46%. Some species of viridans streptococcal group and *S.pneumoniae* require 5% CO<sub>2</sub> levels for adequate growth, the temperature optimum for most streptococci is around 37°C, while some species like *S.uberis* also grow at temperatures as low as 10°C (*Murray et al., 2007*).



**Figure (1):** Streptococcus group arrangement  
(courtesy of CDC PHIL/W.A. Clark)

Many species of streptococci, including *S pyogenes* (group A), *S agalactiae* (group B), and the enterococci (group D), are characterized by combinations of features that include: colony growth characteristics, hemolysis patterns on blood agar (hemolysis [ $\alpha$  or  $\beta$ ] or no hemolysis), antigenic composition of group-specific cell wall substances, and biochemical reactions. *S pneumoniae* (pneumococcus) types are further classified by the antigenic composition of the capsular polysaccharides (*Patel et al., 2011*).