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

Thesis

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By

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Moataz Bellah Abd el aleem

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List of Abbreviations

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

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

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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. Theirf 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 hematopoetic 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 bacerial 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. Clinlcally 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).***

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_2 levels for adequate growth, the temperature optimum for most streptococci is around 37ºC, while some species like *S.uberis* also grow at temperatures as low as 10ºC ***(Murray et al., 2007).***

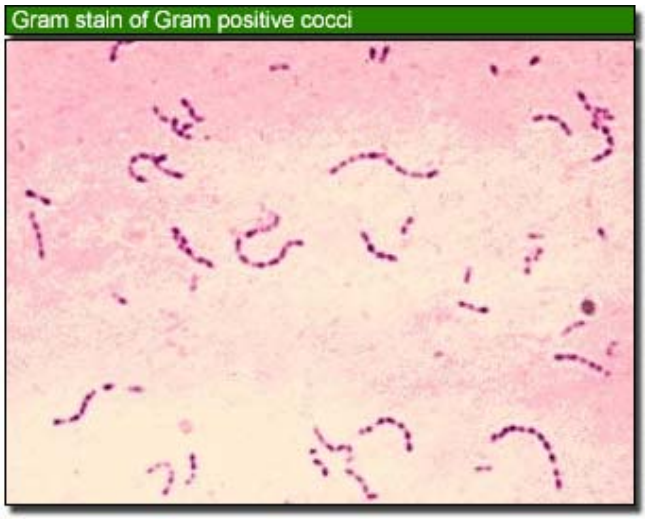

Figure (1): Streptococcus group arrangement
(courtesy of CDC PHIL/W.A. Clark)

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 [α or β] 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).***