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VIRIDANS GROUP STREPTOCOCCI SEPTICAEMIA AND ENDOCARDITIS

Molecular diagnostics, antibiotic susceptibility and clinical aspects

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To Ulf, Emelie and Victor

ABSTRACT

Viridans group streptococci (VGS) are inhabitants in the oral cavity and in the gastrointestinal tract. They cause severe infections, they are responsible for up to 39 % of the cases of septicaemia in neutropenic patients with haematological diseases and cause infective endocarditis (IE), mainly in patients with native valves and previous heart disease. Different species cause different clinical picture, therefore the identification of the species is important. The conventional methods for identification of VGS strains, Strep API and API ZYM have not been optimal. A reduced antibiotic susceptibility to penicillin in VGS has developed during the last years, primarily for patients with haematological diseases.

In the present studies we have investigated the rate of infective endocarditis and risk factors, in immunocompetent and immunocompromised patients with septicaemia.

We have identified species of VGS septicaemia with old and new diagnostic methods and analysed the antibiotic susceptibility for penicillin and other antimicrobial agents in the oral cavity and blood cultures.

In these studies we found that infective endocarditis was rare in patients with haematological diseases, in this group of patients VGS species as *Streptococcus mitis* and *Streptococcus oralis* dominated. When we used *rnpB* sequencing and PCR, it was possible to identify species of VGS that earlier has been difficult to classify.

In patients with infective endocarditis, strains for the *Streptococcus sanguinis* group dominated, when using *rnpB* sequencing we also found *Streptococcus gordonii* and *Streptococcus oralis* strains in these patients.

We found a reduced susceptibility to pencillin in 18 % (MIC $\geq$ 0.25 μg/ml) of the VGS isolates in 1998-2003, that is lower compared to studies from Canada where 37 % of the strains had a reduced susceptibility to pencillin. The antibiotic resistance to VGS was increased compared to 1992-1997, however different methods had been used. The highest rate of pencillin resistance in this study was found in oral swabs from haematological patients where 25% of the VGS isolates were resistant to penicillin (MIC $\geq$ 4.0 μg/ml), which was higher that we had expected. This is an important observation because the oral cavity has been described as a genetic reservoir for transferring resistance genes from VGS to *Streptococcus pneumoniae*.

We also found that 19% of the isolates had a reduced susceptibility to erythromycin (MIC $\geq$ 0.5 μg/ml) and 80% of these strains harbored *mefA* and 40 % *ermB*. The VGS strains in 1998-2003 had a reduced susceptibility to ciprofloxacin; which has previously been used as antibiotic prophylaxis in neutropenic patients but is not generally recommended because of emergence of resistance. Vancomycin had a high susceptibility to VGS but it should only be used as empiric therapy for severe cases and for resistant strains because of the emergence of resistance. New antimicrobial agent as linezolid seems susceptible but should be saved for cases of antibiotic resistance.

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“The cure of boredom is curiosity. There is no cure for curiosity.”

Dorothy Parker 1893-1967

LIST OF PUBLICATIONS

I. Westling K, Ljungman P, Thalme A, Julander I. Streptococcus viridans septicaemia, a comparison study in patients admitted to the Departments of Infectious diseases and Haematology from a University Hospital. Scandinavian Journal of Infectious Diseases 2002; 34(4):316-9.

II. Westling K, Julander I, Heimdahl A, Thalme A, Ljungman P and Nord CE. Reduced susceptibility of penicillin in viridans group streptococci in the oral cavity in patients with haematological diseases. Clinical Microbiology and Infection 2004; 10(10):899-903.

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IV. Westling K, Julander I, Ljungman P, Jalal S, Nord CE and Wretlind B. Viridans group streptococci in blood culture isolates, antibiotic susceptibility and identification of resistance genes. Manuscript

CONTENTS

LIST OF ABBREVIATIONS

ALL	Acute lymphoblastic leukaemia
AML	Acute myelogenous leukaemia
ARDS	Acute respiratory distress syndrome
CLL	Chronic lymphocytic leukaemia
CML	Chronic myelogenous leukaemia
CRP	C reactive protein
ermB	Erythromycin ribosome methylase
DNA	Deoxyribonucleic acid
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HACEK	*Hemophilus spp, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella kingae*
GTF	Glycosyltransferase
I	Intermediate resistant
IE	Infective endocarditis
IL 8	Interleukin 8
ITS	Intergenic spacer
IVDU	Intravenous drug user
mef	Macrolide efflux
MIC	Minimal inhibitory concentration
MLS_B	Macrolides, lincosamide, streptogramin B
NCCLS	National Committee for Clinical Laboratory Standards
NT	Not typeable
PAAP	Platelet aggregation proteins
PMP	Platelet microbiocidal proteins
RNA	Ribonucleic acid
RNase P	Endoribonuclease P
R	Resistant
S	Susceptible
S. species	*Streptococcus species*
str F	Streptococcus forward (primer)
str R	Streptococcus right (primer)
TEE	Transesophageal echocardiography
TTE	Transthoracic echocardiography
TMP/SMX	Trimethoprim-sulphametoxazole
TNF ß	Tumor necrosis factor beta
VGS	Viridans group streptococci

1 INTRODUCTION

1.1 VIRIDANS GROUP STREPTOCOCCI

1.1.1 Background

Viridans group streptococci (VGS) are inhabitants of the oral cavity, which is sterile at birth, but at the age of one year, Streptococcus species compose 70% of the cultivable oral flora and *Streptococcus salivarius*, *Streptococcus oralis* and *Streptococcus mitis* are predominant (83). VGS are also found in the gastrointestinal and urogential tracts.
The term viridans is derived from the Latin *viridis* or green, and refer to the sheen caused by haemolysis around the bacterial colonies on blood agar (117).
Severe infections caused by VGS have two dominating clinical forms; they can cause infective endocarditis (IE) in patients both with native and prosthetic valves (13, 135), (132), (123) and septicaemia in neutropenic patients with haematological diseases after anti-neoplastic therapy (11, 127). In addition VGS cause odontofacial infections (63, 72), brain abscesses (23), (26) and abdominal infections (22).

1.1.2 Species of Viridans group streptococci

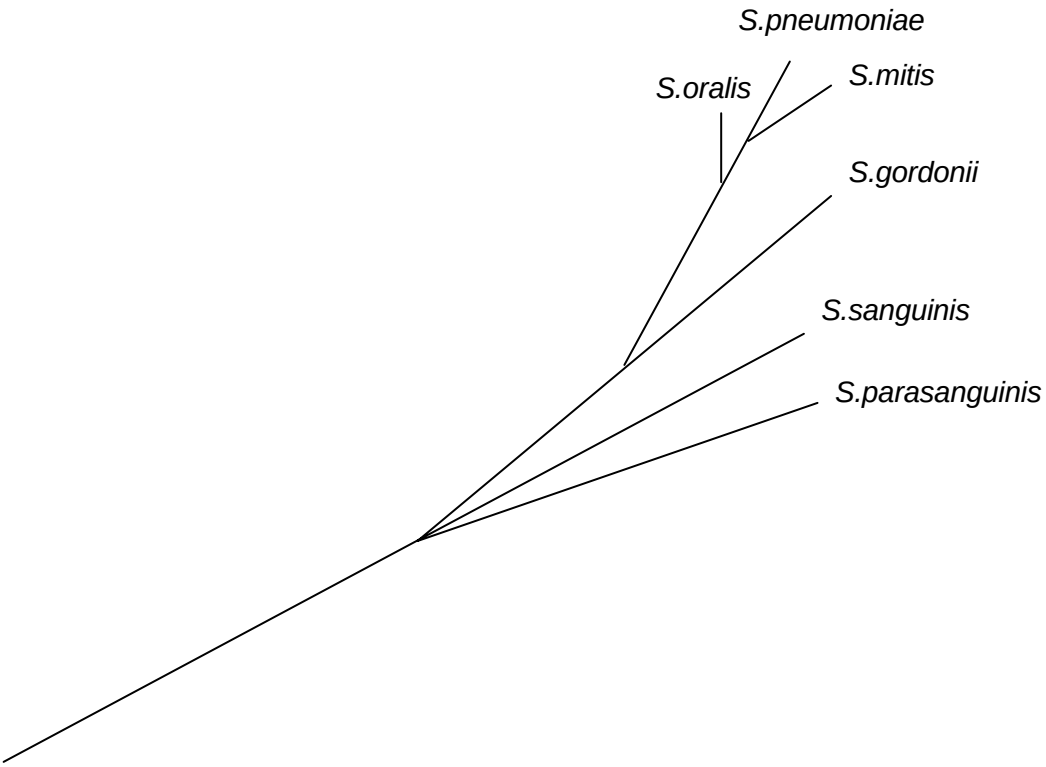
The nomenclature of VGS have been revised by Coykendall (24) in 1989, and recently by Facklam in 2002 (34) , where phenotypic characteristics were used. There are five major groups of VGS (human species); (Table 1).

Table 1. Species of Viridans group streptococci

Streptococcus mutans group	*Streptococcus mutans,* *Streptococcus sorbinus,* *Streptococcus ratti*
Streptococcus salivarius group	*Streptococcus salivarius,* *Streptococcus infantarius* *Streptococcus vestibularis*
Streptococcous mitis group	*Streptococcus mitis* *Streptococcus oralis* *Streptococcus infantis* *Streptococcus cristatus* *Streptococcus perois*
Streptococcus sanguinis group	*Streptococcus sanguinis* *Streptococcus parasanguinis* *Streptococcus gordonii*
Streptococcus anginosus group	*Streptococcus anginosus* *Streptococcus intermedius* *Streptococcus constellatus*

Different VGS species dominate in different parts of the oral cavity and the gastrointestinal tract and cause different clinical pictures, therefore the identification of the species is important *Streptococcus salivarius* is found in saliva, on the tongue and buccal surfaces, *Streptococcus mitis* and *Streptococcus mutans* are found on the tooth surfaces (63). *Streptococcus mutans* has been reported as a causative agent of caries (63, 98), where dental plaque forms a biofilm (63). *Streptococcus sanguinis* is found in the buccal mucosa (38), it has been shown that *Streptococcus sanguinis* can aggregate trombocytes (36, 55), that might predispose to infective endocarditis. *Streptococcus salivarius, Streptococcus mitis* and *Streptococcus intermedius* are found in the gastrointestinal tract as well as in the oral cavity. The members of the *Streptococcus anginosus* group (*Streptococcus intermedius, Stretococcus constellatus* and *Streptococcus anginosus*) are also found in the urogential tract (34). *Streptococcus constellatus* is most often identified in the respiratory tract and *Streptococcus intermedius* is often isolated from brain and liver abscesses (93).
The strains of the *Streptococcus bovis* group now named (*Streptococcus bovis*, *Streptococcus gallolyticus, Streptococcus infantarius Streptococcus equines* and *Streptococcus lutentiensis*) (115), are not classified as VGS but cause similar clinical infection. *Streptococcus bovis* is associated with carcinoma in the gut (73, 128).
There are different methods to differentiate and identify VGS. Biochemical, immunologic, genetic (69) and phenotyping methods have been used. Fig 1.

Figure 1. Phylogenetic tree of streptococci



Adapted from Kawamura 1995.

1.2 INFECTIVE ENDOCARDITIS

1.2.1 Background

Infective endocarditis (IE) is a severe disease that may affect one or more of the aortic, mitral, tricuspid valves but seldom the pulmonary valve. Pre-existing valve disease as mitral valve prolapse (87), rheumatic fever, mitral stenosis, aortic stenosis (45), and aortic regurgitation, bicuspid aortic valve (70), coarctation of the aortae, previous endocarditis, prosthetic heart valves, and intravenous drug use are predisposing factors (78), even if IE is described in patients without previous heart diseases.

VGS have earlier been reported as the most frequent agents in IE (8, 111, 129). In all, *Staphylococcus aureus* is now the most frequent etiological agent in IE. However, in non intravenous users, where aortic- or mitral IE dominates, VGS are still the major etiological agents (13, 123, 135). *Staphylococcus aureus* is the most frequent etiological agent in IE among intravenous drug users, where the tricuspid valve is usually affected, even if recent data have shown that the aortic or the mitral valve can be co-infected (Thalme, personal communication 2005).

1.2.2 Pathogenesis of infective endocarditis

Dental scaling and extraction leads to bacteraemia in 70-100 % (54) and after toothbrushing 40 % of the children had bacteraemia, where VGS were found in 50% of the cases (67, 110). Why VGS bacteremia in some cases leads to endocarditis depends on different factors. It might be a predisposition, i. e. a heart valvuar disease, a locus minoris; an old non-bacterial vegetation (5, 39). Bacteria may infect the non-bacterial vegetations which with adhesines attach to the endothelium of the damaged site on the valvular wall. Blood monocytes produce tissue factors, cytokines are parts of the pathogenesis (92). Bacterial components as dextran, fibronectin binding protein and teichoic acid have been described as important factors in adhesion to platelet-fibrin matrix on the valvular wall contributing to the pathogenesis of IE (14).
It is known that 60% of the *Streptococcus sanguinis* strains can induce platelet aggregation (37, 57), and this is mediated by platelet aggregation-associated proteins (PAAP) (56). Activated platelets release dense and alpha granules, which in combination with tromboxane production play a role in the later aggregation response. Alpha granulae include platelet microbicidal proteins, (PMP) that kills bacteria, they also induce production of fibrinogen and clotting factors V and VII. The later activate thrombin, which initiates the polymerisation of fibrinogen to fibrin (55). In earlier reports dextran production by *Streptococcus sanguinis* has (55) been described to play a role in the pathogenesis of IE (36, 55, 101) however one study showed that only 60 % of the *Streptococcus sanguinis* strains produced dextran (71). Studies have showed that *Streptococcus gordonii* can attach fibronectin which is adsorbed to collagen (80, 81). VGS have glucosyltransferase (GTF), an enzyme using sucrose for synthesizing extracellular polysaccharide (91). GTF from VGS has been suggested as a factor forming biofilm on the surfaces of the teeth (22, 61, 108).

1.2.3 Clinical symptoms of infective endocarditis

The estimated incidence of IE in the USA and Europe are 1.7-6.2/100.000 (60). The main symptoms of IE are fever, anorexia, weight loss, a new or changed heart murmur

is often observed, septic emboli may be present causing peripheral skin efflorescations, as Janeway phenomena (95). Other symptoms of septic emboli events are transient ischemic attacks, hemiparesis, hematuria, abdominal pain caused by spleen and/or hepatic infarctions and dyspnoea caused by septic emboli to the lungs (2). Complications as mycotic aneurysm, splenic abscesses may occur (7), and congestive heart failure (90) may force cardiac surgery.
Endocarditis caused by VGS, former called endocarditis lenta (77), is fatal without treatment.
Compared to IE caused by *Staphylococcus aureus* (79), VGS IE, cause valvular destruction in a similar degree, but with less acute onset of symptoms, and a lower mortality. The mortality in VGS endocarditis varies between 4-14 % (21, 59, 129).

1.2.4 Diagnostic criteria of infective endocarditis

The diagnosis of IE is difficult but new diagnostic criteria and echocardiography has improved the sensitivity and specificity. The diagnostic criteria of IE, previous von Reyn (137, 138), was revised by Lukes and Durack, Duke´s University in 1994 (29, 84). (See Table I and Table II). There have been several studies evaluating the new criteria (99, 125). Important factors according to the new criteria for the diagnosis of IE are echocardiographic examination, where transesophageal echocardiography (TEE) has a higher sensitivity (95%) compared to transthoracacic echocardiography (TTE) (75 %) (53, 109, 130). Blood culture isolates predictive of IE are important.

Table 2. Duke´s criteria for infective endocarditis.

Definite Infective endocarditis	
Pathologic criteria	Microorganisms demonstrated by culture or histology in a vegetation or in a vegetation that has embolized or in an intracardiac abscess or Pathologic lesions, vegetation or intracardiac abscess present, confirmed by histology showing active endocarditis
Clinical criteria using specific definitions, (Table 3)	Two major criteria or One major and three minor criteria or Five minor criteria
Possible infective endocarditis	Findings consistent with IE that fall short of Definite but not rejected
Rejected	Firm alternate diagnosis for manifestations of endocarditis or Resolution of manifestations of endocarditis with antibiotic therapy for 4 days or less or No pathologic evidence of infective endocarditis at surgery or autopsy after antibiotic therapy for 4 days or less

Adapted from Durack, Lukes et al 1994

Table 3. Definitions of terminology used in the new criteria.

Major criteria	
Positive blood culture for IE or	Typical microorganisms for IE from two separate blood cultures, VGS, Streptococcus bovis, or HACEK[1] group or community acquired Staphylococcus aureus or enterococci or
Microorganism consistent with IE from persistently positive blood cultures	Blood cultures drawn more than 12 hours apart or all of the three or a majority of four or more separated blood cultures with first and last drawn at least 1 hour apart
Evidence of endocardial involvement	Positive echocardiogram for infective endocarditis; oscillating intracardiac mass, on valve or supporting structures, or in the path of regurgitant jets or on implanted material, in the absence of an alternative anatomic explanation or Abscess or New partial dehiscence of prosthetic valve or
New valvular regurgitation	
Minor criteria	
Predisposition	Predisposing heart condition or intravenous drug use
Fever	≥38.0°C
Vascular phenomena	Major arterial emboli, septic pulmonary infarcts, mycotic aneurysm, intracranial haemorrhage, conjunctiva haemorrhages, Janeway lesions
Immunologic phenomena	Glomerulonephritis, Osler's nodes. Roth spots, rheumatoid factor
Microbiologic evidence	Positive blood culture but not meeting major criterion as noted previously or serologic evidence of active infection with organism consistent with IE
Echocardiographic findings	Consistent with infective endocarditis but not meeting major criterion as noted previously

Adapted from Durack, Lukes et al 1994

[1]HACEK: *Hemophilus spp, Actinobacillus actinomycetemcomitans, Cardiobacterium hominis, Eikenella spp, Kingella kingae*

1.3 VIRIDANS STREPTOCOCCI SEPTICAEMIA IN NEUTROPENIC PATIENTS

Viridans group streptococci (VGS) have become important pathogens in patients with haematological diseases who receive anti-neoplastic treatment and develop neutropenia. VGS septicaemia in neutropenic patients was first described in 1978 (58) (104) and has been reported in up to 39 % of the cases (11). Oral ulcers occur and predispose VGS who are inhabitants in the mouth to penetrate in from the ulcers to the blood and cause septicaemia. The use of quinolones as ciprofloxacin as antibiotic prophylaxis which had reduced the amount of gram-negative septicaemia may play a role in the development of VGS septicaemia (25, 68). Chemotherapeutic treatment by cytosinarabinoside, severe neutropenia (less then 100 cell/mm^3), and mucositis, may be other risk factors. A septic shock syndrome with acute respiratory distress syndrome (ARDS) has been described in neutropenic patients (42) with VGS septicaemia. This syndrome resembles that of *Streptococcus pyogenes* shock syndrome but exotoxines seems not to exist. TNF ß and high levels of IL-8 have been found in supernatants of clinical isolates (121), and recently an epidemic situation of toxic VGS strains has been reported from China (82). The mortality in viridans streptococci septicaemia in this group of neutropenic patients is 10-30% (11). *Streptococcus mitis* has been described as the major subtype of viridans streptococci in this group of neutropenic patients (31, 136).

1.4 ANTIBIOTIC TREATMENT OF VIRIDANS GROUP STREPTOCOCCI INFECTIONS

The standard treatment of infective endocarditis caused by VGS is penicillin G intravenously four times daily in combination with aminoglycoside for the first two weeks (49). The reason for combination therapy is to obtain synergistic effect of penicillin and aminoglycoside (30, 36, 116). In uncomplicated cases of IE with MIC ≤ 0.1 µg/ml treatment with penicillin G alone might be sufficient (134). Cephalosporins may be an alternative for patients with penicillin allergy. For treatment of endocarditis caused by VGS with a reduced susceptibility to penicillin recommendations are the same treatment as for enterococci, i e vancomycin for penicillin resistant strains (135).
VGS septicaemia in neutropenic patients is treated with penicillin G for susceptible strains and with vancomycin for resistant strains.

1.5 ANTIBIOTIC SUSCEPTIBILITY IN VIRIDANS GROUP STREPTOCOCCI

A reduced susceptibility to penicillin (MIC ≥ 0.25 µg/ml) for VGS streptococci has been described in many studies (106).
This has principally concerned patients with haematological diseases (28, 68). In studies from Spain (16, 89) 23-43 % of the isolates were resistant to penicillin (MIC ≥ 4.0 µg/l). In Canada 7% of blood culture isolates of VGS were resistant and 37 % had a reduced susceptibility to penicillin (44). Both immunocompetent and immunocompromised patients were included. The SENTRY antimicrobial surveillance program showed that 6 % of the VGS isolates were resistant to penicillin (46).

The antimicrobial susceptibility of VGS in patients with IE has been reported from the United States 1994-2002, where 4 % of the strains were resistant to penicillin (107).
Oral VGS streptococci has been described as a reservoir for transferring antimicrobial resistance genes to mainly *Streptococcus pneumoniae* (15, 51, 112, 118, 119, 126).
Resistance to erythromycin in VGS has been described in many studies particularly in immunocompromised patients. A study from Great Britain showed that 51 % of the *Streptococcus oralis* and 40% of the *Streptococcus mitis* isolates were resistant to erythromycin (120). Transfer of genes with erythromycin in the oral cavity from VGS to *Streptococcus pneumoniae* has been described.(3).
Resistance to quinolones has been recorded in many patients. In one study (68) only 18% of the isolates of VGS from neutropenic patients were susceptible to ciprofloxacin. Transfer of fluoroquinolone resistance genes from *Streptococcus pneumoniae* to VGS has also been reported. (6, 65).

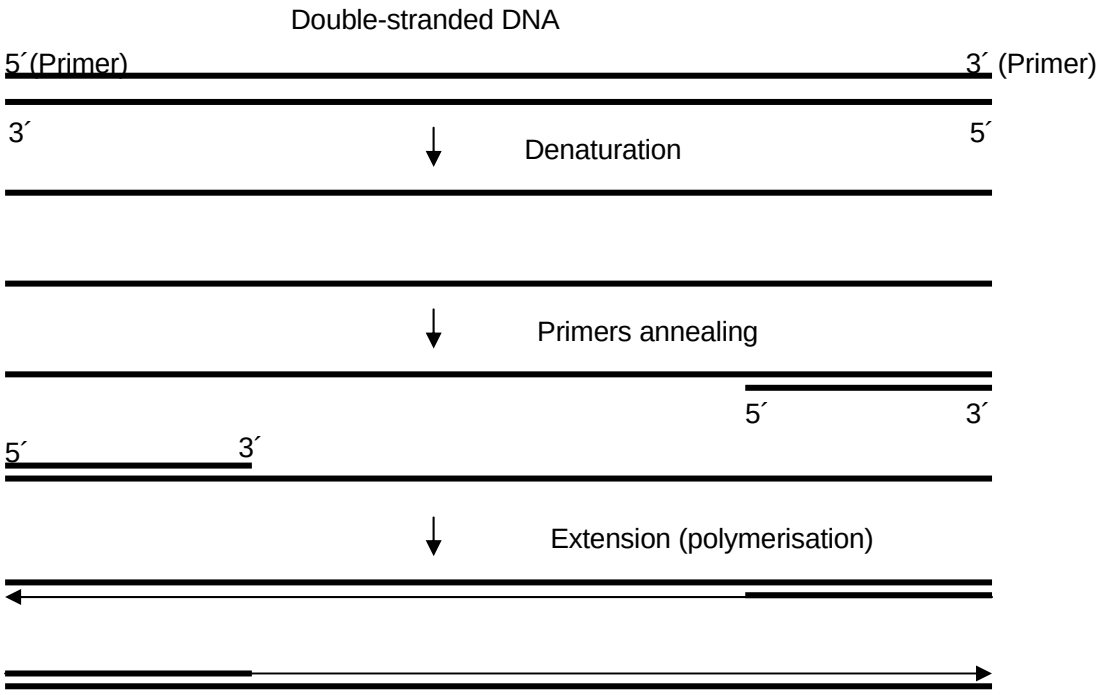
1.6 GENES FOR ANTIMICROBIAL RESISTANCE DETECTION IN VIRIDANS GROUP STREPTOCOCCI

The penicillin resistance in VGS is caused by a mosaic pattern in penicillin binding protein (PBP) genes (35).
Erytromycin resistance in VGS is transferred by posttranscriptional modification of the 23 S rRNA subunit by adenine-6-metyhyltranferases either resistance to macrolides, lincosamide and streptogramin B antibiotics (MLS_B phenotype) (72) encoded by the *ermB* (erythromycin ribosome methylase B) gene or resistance to macrolides (M phenotype), with active drug efflux protein encoded by *mefA* (macrolide efflux) gene (122) that helps to get a low intracellular concentration of antibiotics (88, 133). Resistance to quinolones in VGS is associated with mutations in topoisomerase IV (*parC*) and DNA gyrase (*gyrA*), (18, 48).

1.7 MOLECULAR BIOLOGICAL TECHNIQUES FOR IDENTIFYING BACTERIA

Polymerase chain reaction (PCR) was invented in 1985 (114). PCR based methods has been useful for identifying bacteria (1, 41, 139). PCR is a method to amplify short strains of DNA, by denaturation, polymerase hybridisation by polymerase and extension (Fig 2). Molecular diagnostic methods such as 16 S rRNA sequencing has been used for species determination and identification of bacteria in endocarditis patients in blood (113, 140) , or in valves (12, 43, 47, 105). For identifying *Streptococcus species* several methods have been used; 16 S rRNA (64, 66), tRNA gene intergenic spacer (ITS) (27), and *rnpB* (124).

Fig 2. Polymerase chain reaction



Each new double-stranded. DNA acts as target for new cycle

1.7.1 *rnpB*-sequencing

New techniques using PCR as endoribonuclease P, RNase P, *rnpB* and 16 S rRNA sequencing has been used as a diagnostic tool for determination of species of bacteria in blood cultures. RNase P RNA gene exists in many species but the catalytic activity has only been demonstrated in bacteria. There are two types of RNase P RNA genes, type A and type B, but type B exists only in Gram-positive bacteria. The RNase P RNA molecule is variable outside the conserved core. The *rnpB* gene has been shown to have a higher information value per nucleotide position than 16 S rRNA and have a short length (50, 124).

2 AIMS OF THE STUDY

1. To analyse the rate of infective endocarditis in immunocompetent and immunocompromised patients with septicaemia caused by Viridans group streptococci, and compare the causative agents of the groups of patients (Paper I and Paper III).

2. To investigate the rate of penicillin resistance in oral samples of Viridans group streptococci (Paper II) and analyse risk factors for penicillin resistance in neutropenic patients with haematological diseases (Paper II).

3. To analyse the susceptibility for Viridans group streptococci to penicillin, and other old and new antimicrobial agents in blood cultures in immunocompetent and immunocompromised patients (Paper IV).

4. To develop and use new molecular biological techniques to classify species of Viridans group streptococci and compare this with conventional methods (Paper III).

5. To identify genes for antibiotic resistance in Viridans group streptococci (Paper IV).

3 MATERIALS AND METHODS

3.1 PATIENTS

The patients were identified by charts from the Department of Bacteriology, Karolinska University Hospital/Huddinge, Stockholm, Sweden 1992-1997 (Paper I) and 1998-2003 (Paper III-IV) and the Department of Haematology (Paper II) the later study patients with either newly diagnosed acute leukaemia or bone marrow transplant recipients during the period March 1, 2000 - Jan 31, 2002 (Paper II).

3.2 STRAINS

The strains were clinical isolates (from blood cultures) (Paper I, III and IV) and oral swabs (Paper II) collected from the Departments of Infectious Diseases, and Haematology (Paper I-IV), Intensive Care and Transplantation Surgery Karolinska University Hospital/Huddinge, Stockholm, Sweden (Paper III-IV).

3.3 STUDY DESIGN AND CLINICAL METHODS

3.3.1 Paper I

Retrospective review of charts from the Departments of Infectious Diseases and Haematology, Karolinska University Hospital/Huddinge Stockholm, Sweden 1992-1997. Clinical data was analysed. Endocarditis criteria according to Dukes were used to analyse the rate of infective endocarditis in patients with VGS septicaemia (29, 84), (Table 2 and Table 3).

3.3.2 Paper II

Prospective study of patients with newly diagnosed acute leukaemia or patients with existing haematological diseases who were stem cells recipients from the Department of Haematology, Karolinska University Hospital/Huddinge March 1, 2000 - Jan 31, 2002. Samples from mouthwash were obtained once a week, within the first week of the patient's hospital stay and this procedure was repeated once a week as long as the patients stayed at hospital. From the samples cultures were performed. Clinical data, previous antibiotic prophylaxis and antibiotic treatment, within a year from the start of the study, and antibiotic treatment at actual hospital stay were registered

3.3.3 Paper III-IV

Retrospective review of charts from the Departments of Infectious Diseases, Haematology, Transplant Surgery and Intensive Care at Karolinska University Hospital/Huddinge 1998-2003. Clinical data was registered, underlying haematological and others diseases were registered and Duke´s criteria were applied (Table 2 and Table 3).

3.4 EXPERIMENTAL PROCEDURES

3.4.1 Antibiotic susceptibility testing

MICs were determined with Etest (AB Biodisk Solna). Antibiotic susceptibility testing for oral strains to penicillin was made on prepared plates with a penicillin concentration of 2.0 µg/ml. The VGS isolated resistant to penicillin (MIC ≥ 4.0 µg/ml) were also tested for susceptibility to ciprofloxacin, erythromycin, linezolid, trimethoprim-sulphametoxazole and vancomycin using the microdilution method, NCCLS 2002 (97) (Paper II). Antibiotic susceptibility testing for blood cultures isolates of VGS was made for the following agents; ciprofloxacin, clindamycin, dalbavancin , erythromycin, linezolid, penicillin, tigecyclin, trimethoprim-sulphametoxazole, and vancomycin using the microdilution method, NCCLS 2003 (96) (Paper IV).

3.4.2 Identification of species of VGS by conventional methods

Identifying species of VGS was made using Strep API (bioMérieux) (Paper I, II and III)

3.4.3 Identification of VGS by *rnpb* sequencing

3.4.3.1 Isolates

The blood culture isolates of VGS have been stored at - 70° C, the Vital (bioMérieux, Lyon) and Bactec 9240 systems had been used for cultivation.

3.4.3.2 DNA isolation

Bacterial genomic DNA was isolated directly from bacterial cultures isolates by using QIA amp DNA extraction mini kit (Qiagen) using the manufacturer's instruction.

3.4.3.3 PCR amplification and identification

Amplification of the *rnpB* gene from Streptococcus species was made by PCR with primer pair str F (5′-YGTGCAATTTTGGATAAT-3′) and
str R (5′-TTCTATAAGCCATGTTTTGT –3′). The master mix contained of 15µM of each primer str F and str R, 10 mM dNTP mix, 25 mM $MgCl_2$, 0.25 µL Ampli Taq Gold DNA polymerase. 10 µL DNA was added to the master mix to obtain a reaction volume of 50 µL. The reaction conditions consisted of heat-mediated enzyme activation at 95° for 10 minutes, followed by 5 cycles denaturation 4 s at 94°, annealing for 40 s at 58°, and extension 40 s at 72 °, 5 cycles denaturation 4 s at 94°, annealing 40 s at 54°, and extension 40 s at 72 °, and 30 cycles denaturation 4 s at 94°, annealing 40 s at 50°, and extension 40 s at 72 °(124). The PCR product was purified using Qiagen PCR purification kit (Qiagen, Santa Clarita, California) and stored for DNA sequencing analysis. The PCR product was detected using gel electrophoresis on 2% agarose gel with ethidium bromide.

3.4.3.4 RnpB sequencing:

The purified PCR product was used for *rnpB* sequencing using BigDye Terminator labelled cycle sequencing chemistry in a 310 Genetic analyzer (Applied Biosystems, Foster city, California). Both strands of the DNA were sequenced and then analysed in

BLAST nucleotide sequence analysis database (www.ncbi.nlm.nih.gov) for identification of species.

3.4.4 Determination of resistance genes

3.4.4.1 Erythromycin

Identification of *ermB* and *mefA* genes was performed in VGS with a reduced susceptibility for erythromycin (MIC ≥ 0.5 µg/ml).
PCR for identification of the *ermB* gene was done using the primer pair *ermB*1: (5´ GAA AAG GTA CTC AAC CAA ATA 3´) and *ermB*2: (5´AGT AAC GGT ACT TAA ATT GTT TAC 3´) (Inhouse method, Dept of Clinical Bacteriology, Karolinska Institutet).
The reaction mixture contained 5 µL PCR Reaction Buffert, 1.5 mM $MgCL_2$,
200 µM PCR Nucleotide Mix, 0.5 µM ermB1 and 0.5 µM *ermB2,*
0.5 U/µL Taq Polymerase Gold and 36.4 µL M and DNA-template 1 µL added.
The reaction conditions consisted of heat mediated enzyme activation for 10 min at 95°, denaturation 1 min, at 94°, annealing 1 min at 52 °, extension 2 min at 72°, and extension 7 min 72, 35 cycles. The PCR product was detected using gel electrophoresis on 1.5 % agarose gel on ethidium bromide.

Identification of *mefA*. The sequence for the primers mef A were 5´TAT GGG CAG GGC AAG CAG 3´ and 3´ATA CCC GTC CCG TTC GTC 5´ .The primers were designed from Thermo Hybrid, Premier Biosoft Internation. (www.Premier.Biosoft.com).
The reaction mixture was the same as for *ermB*, except for the primers. PCR was performed 95° 10 min, 94° 1 min, 55° 1 min, 72°2 min and 72° 7 min, 32 cycles. The PCR product was detected using gel electrophoresis as above.

3.5 STATISTICAL ANALYSIS

The chi-square test, Student's *t*-test, and Fischer's exact test were used. JMP software system; (SAS Institute, Chicago, IL, USA) were used for statistical analysis.

3.6 ETHICAL APPROVAL

The studies were approved by the local ethic committe at Karolinska University Hospital, Huddinge.

4 RESULTS

4.1 CLINICAL DATA

In this thesis, we have analysed samples strains of VGS from immunocompetent (Paper I, III-IV) and immunocompromised patients (Paper I-IV) with blood culture isolates (Paper I, III-IV) and oral swabs (Paper II). The data for the patients are described in Table 4.

Table 4. Data on patients and episodes of Viridans group streptococci septicaemia, including endocarditis

Papers	No of episodes	No of immunocompetent patients	No of patients with haematological diseases	No of solid organ transplant recipients
I	121	45	66[1]	0
II	48	0	48	0
III	131[2]	43	76[1]	5
IV	129[2]	41	75[1]	5

1 Fifteen patients in Paper I and one patient in Paper III and IV had breast cancer and were bone marrow transplant recipients

2 In the Papers III and IV, the same patients are included.

In Paper I we show that definite or possible IE was found in 39/47 isolates of VGS septicaemia in the immunocompetent patients but only in 1/74 (possible IE) of the episodes of VGS septicaemia in patients with haematological disease (Table 5). Forty-three per cent of the patients with infective endocarditis (definite or possible) had an underlying heart disease. In Paper III, 36 of the episodes of VGS septicaemia were classified as endocarditis according to Duke´s criteria, two of these episodes were from patients with haematological diseases.

Table 5. Episodes of Infective Endocarditis (IE) in patients with Streptococcus viridans septicaemia 1992-1997 (Paper I) and 1998-2003 (Paper III) according to Duke´s criteria.

Paper	I	III
Definite IE	30	25
Possible IE	9[1]	11[1]

1 One of the episodes in Paper I and two patients in Paper III with possible IE were from neutropenic patients

The majority of the patients with VGS septicaemia had an underlying haematological disease (Table 6).

Table 6. Underlying diseases in patients with neutropenia and VGS septicaemia (Paper I-IV)

Paper	**I**	**II**	**III**	**IV**
Acute myelogenous leukemia	22	24	26	26
Carcinoma of the breast	15[1]		1[1]	1[1]
Acute lymphoblastic leukaemia	9	2	5	5
Chronic lymphogenous leukaemia		1	1	1
Myeloma	11	8	19	19
Lymphoma	6	11	17	17
Myelodysplastic syndrome		2	3	3
Others	3		4	3
Total	66	48	76	75

. [1] These patients had breast cancer and were bone marrow transplant recipients

4.2 IDENTIFICATION OF SPECIES OF VIRIDANS GROUP STREPTOCOCCI

Streptococcus mitis was dominating in the group of patients with haematological disease and VGS septicemia (Table 7) (Paper I) and *Streptococcus oralis* was also found in the strains sequenced with *rnpB* (Paper III) (Table 9) *Streptococcus sanguinis* was found in immunocompetent patients with a high prevalence of IE (Paper I) (Table 7) and strains from the *Streptococcus sanguinis* group and *Streptococcus oralis* dominated in patients with IE (Paper III) when *rnpB* was use). d (Table 8). The 131 strains sequenced with *rnpB* in the study are shown in Table 10.

Table 7. Species of viridans streptococci in VGS septicaemia and endocarditis 1992-1997 in immunocompetent (Group A) and neutropenic (Group B) patients with Strep API (Paper I).

Species	Group A	Group B
S. mitis	5	51
S. sanguis	18	12
S. intermedius	7	2
S. mutans	4	0
S. salivarius	1	2
S. bovis	2	0
S. acidiominus	2	1
S. mileri	1	0
Gemella morbillorum	1	0
Not typed	6	6
Total number	47	74

Table 8. Species of VGS using the *rnpB* sequencing in 36 patients with definite or possible infective endocarditis (Paper III)

	Definite endocarditis (n=25)	**Possible endocarditis (n=11)**
Streptococcus oralis	**7**	1
Streptococcus gordonii	5	1
Streptococcus sanguinis	4	1
Streptococcus mitis	2	1
Streptococcus gallolyticus	2	0
Streptococcus mutans	2	0
Streptococcus anginosus	1	3
Streptococcus parasanguinis	1	2
Streptococcus pneumoniae	1[1]	0
Streptococcus cristatus	0	1
Streptococcus intermedius	0	1

[1]The sample was sequenced as *Streptococcus pneumoniae* but Strep API identified the strain as *Streptococcus mitis*

Table 9. Species of VGS using *rnpB* (Paper III) in 82 episodes of VGS in patients with haematological diseases and septicaemia

Species	**No (n)**
Streptococcus mitis	29
Streptococcus oralis	16
Streptococcous mitis/oralis	1
Streptococcus pneumoniae	12[1]
Streptococcus salivarius	7
Streptococcus anginousus	5
Streptococcus parasanguinis	3
Streptococcus constellatus/anginosus	1
Streptococcus sanguinis	2
Streptococcus mutans	1
Streptococcus infantis	1
Streptococcus gordonii	1
Streptococcus cristatus	1
Streptococcus gallolyticus	1
Streptococcus pneumoniae/mitis	1

1 The majority of these isolates were previously typed as *S. mitis* or *S. oralis*

Table 10. Species of 131 VGS in blood culture isolates using *rnpB* (Paper III)

Species	No
Streptococcus mitis	34
Streptococcus oralis	26
Streptococcus mitis/oralis	1
Streptococcus pneumoniae	13[1]
Streptococcus pneumoniae/mitis	1
Streptococcus salivarius	9
Streptococcus parasanguinis	8
Streptococcus anginosus	11
Streptococcus gordonii	7
Streptococcus sanguinis	8
Streptococcus gallolyticus	3
Streptococcus infantis	2
Streptococcus intermedius	1
Streptococcus cristatus	1
Streptococcus anginosus/constellatus	3
Streptococcus mutans	3

1 The majority of these isolates were previously typed as *S. mitis* or *S. oralis*

4.3 ANTIBIOTIC SUSCEPTIBILITY OF VIRIDANS GROUP STREPTOCOCCI ISOLATES

In Paper II we used oral swabs for analysing VGS, 25 % of the strains were resistant to penicillin (MIC ≥ 4.0 µg ml) (Paper II). Four of the strains with penicillin resistance were resistant to erythromycin in (MIC > 1.0 µg ml) addition. The results and the species are given in Table 11 and Table 12. All the strains from the oral cavity were *Streptococcus salivarius, Streptococcus mitis* or *Streptococcus sanguinis.* The patients with penicillin resistant VGS oral strains (MIC ≥ 4.0µg ml) had more days of treatment (at hospital stay) with trimethoprim-sulphametoxazole and more episodes of septicaemia than the patients with penicillin susceptible and penicillin intermediate resistant strains, but there were no cases of VGS septicaemia with penicillin resistant strains in this group. There were no other statistical differences between the groups.

Table 11. Isolates of oral Viridans Group Streptococci (VGS) resistant to penicillin (MIC ≥ 4.0 mg/L) in 12 patients with haematological diseases (Paper II)

*No growth of VGS in oral samples ^{R}Penicillin resistant VGS strains; MIC ≥ 4.0 µg/ml

Patient No	Week (W) 0	W 1	W 2	W 3	W 4	W 5	W 6	W 7
2	*S.salivarius* *S. mitis* *S. sanguis*	*S.salivarius* *S.mitis* *S.sanguis*	*	*	*S.mitis* *S.sanguis*^{R}	*S.mitis* *S.sanguis.*	*S.mitis*	*S.mitis*
7	*S.salivarius* *S.mitis* *S.sanguis*	*S.salivarius* *S. mitis*^{R} *S.sanguis*						
8	*S.salivarius* *S.mitis*^{R} *S. sanguis*	*	*S.mitis*^{R}					
26	*S.salivarius* *S.mitis*^{R} *S.sanguis*	*S.salivarius* *S.mitis*^{R} *S.sanguis*						
28	*S.salivarius* *S.mitis*^{R} *S.sanguis*	*S.salivarius* *S.mitis*^{R} *S. sanguis*	No sample	*S.mitis*^{R} *S.sanguis*				
30	*S.salivarius* *S.mitis*^{R} *S.sanguis*	*S.salivarius* *S.mitis*^{R} *S.sanguis*	*	*S.mitis*				
34	*S.salivarius* *S.mitis*^{R} *S.sanguis*	*S.salivarius* *S.mitis*^{R} *S.sanguis*	*S.salivarius* *S.mitis*^{R} *S.sanguis*					
35	*S.salivarius* *S.mitis* *S.sanguis*	*S.mitis*^{R}	*S.mitis*^{R} *S.sanguis*					
40	*S.salivarius* *S.mitis* *S.sanguis*	*S.salivarius* *S.mitis*^{R} *S.sanguis*						
44	*S.salivarius* *S.mitis*	*S.salivarius* *S.mitis*^{R}	*S.mitis*^{R}	*S.mitis*^{R}	*S.mitis*^{R}	*S.mitis*^{R}	*S.mitis*^{R}	
45	*S.salvarius* *S.mitis*^{R}	*S.salivarius* *S.mitis*^{R}	*S.mitis*^{R}	*	*S.mitis*			
48	*S.salivarius* *S.mitis* *S.sanguis*	*S.mitis* *S.sanguis*	*S.mitis*^{R} *S.sanguis*					

*No growth of VGS in oral samples ^{R}Penicillin resistant VGS strains; MIC ≥ 4. 0 mg/L

Table 12. Description of the samples for the 12 patients with penicillin resistant VGS. Strains with R =penicillin resistant samples (MIC ≥ 4.0 mg/L), S= penicillin sensitive or intermediate resistant samples (MIC ≤ 2.0 mg/L)

Patient No	Week (W) 0	W 1	W 2	W 3	W 4	W 5	W 6	W 7	Diagnosis	Previous antibiotic treatment
2	S	S	*	*	R	S	S	S	ALL	Yes
7	S	R							Myeloma	Yes
8	R	*	R						AML	Yes
26	R	R							AML	Yes
28	R	R	**	R					ALL	Not reported
30	R	R	*	S					Lymphoma	Yes
34	R	R	R						AML	Yes
35	S	R	R						Myeloma	Yes
40	S	R							AML	Yes
44	S	R	R	R	R	R	R		AML	Yes
45	R	R	R	*	S				AML	Yes
48	S	S	R						Lymphoma	Yes

ALL = Acute lymphoblastic leukaemia

AML= Acute myelogenous leukaemia

* No growth in oral culture of VGS

** Samples not obtained

The results of the antibiotic susceptibility testing of the VGS blood culture isolates (Paper IV) are described in Table 13 and the distributions of the VGS with a reduced susceptibility to penicillin and other antimicrobials are described in Table 14. In 18% of the strains a reduced susceptibility to penicillin was registered and 4 % of the strains were resistant to penicillin (all these resistant strains were from patients with haematological diseases). In the isolates from patients with haematological diseases, 21 % of the isolates had a reduced susceptibility to penicillin and 6 % were resistant to penicillin (MIC ≥ 4.0 µg/ml).

Two of the strains with a reduced susceptibility to penicillin were isolated from patients without immunosuppressive disease with definite or possible infective endocarditis (the strains were sequenced as *Streptococcus parasanguinis*). In the isolates from patients with definite or possible endocarditis 6% of the strains had a reduced susceptibility to penicillin.

In 19 % of the isolates, a reduced susceptibility to erythromycin was found and 17 % of the strains were resistant to erythromycin. In the strains with a reduced susceptibility to erythromycin, *mefA* and *ermB* genes were identified. *MefA* was found in 20/25 (80%) and *ermB* 10/25 (40%) (Table 15).

Table 13. Antibiotic susceptibility of 129 VGS isolates from blood cultures.

Antibiotic susceptibility for following antibiotics according to NCCLS breakpoints 2003:
Penicillin S ≤ 0.12 µg/ml; I, 0.25-2 µg/ml, R ≥ 4.0 µg/ml, Clindamycin S ≤ 0.25 µg/ml, I = 0.5 µg/ml, R ≥ 1 µg/ml, Erythromycin S ≤ 0.25 µg/ml, I = 0.5 µg/ml, R ≥ 1 µg/ml, Linezolid S ≤ 2 µg/ml, Ciprofloxacin[1] (Levofloxacin S ≤ 2 µg/ml), trimethoprim-sulphametoxazole (TMP/XMS) (S ≤ 8 mg/L, R > 16 mg/L), (Swedish Reference Group for Antibiotics; SGRA) Vancomycin S ≤ 1.0 µg/ml. (Paper IV).

	MIC Range	MIC ^{50}	MIC ^{90}	Resistance (%)
Penicillin G	≤0.016-4	0.064	0.25	4
Clindamycin	≤0.016->2	0.032	0.125	2
Dalbavancin	0.032-2	0.25	0.5	[3]
Erythromycin	≤0.016-≥16	0.125	4	17
Linezolid	0.064-2	1.0	1.0	0
Ciprofloxacin	≤0.064->8	2	>8	[1]
Tigecycline	≤0.016->2	0.125	0.5	[2]
TMP-SMX	≤0.016->4	0.25	2	0
Vancomycin	0.25-1	0.5	0.5	0

1 NCCLS 2003 has no breakpoint for ciprofloxacin in VGS, the breakpoint are identified for Levofloxacin. EUCAST (European Committee on Antimicrobial Susceptibility Testing) does not recommend ciprofloxacin for treatment of VGS, but has suggested the biological resistance to be MIC > 4 µg/ml.

2 The breakpoints for tigecyclin are suggested to be S < 2 µg/ml and R > 4 µg/ml (Wyeth 2005, personal communication).

3 No breakpoints available

Table 14. Distribution of MIC (µg/ml) for antibiotics with a reduced susceptibility to VGS against 129 VGS blood culture isolates (Paper IV)

Ciprofloxacin

MIC	≤0.64	0.12	0.25	0.5	1.0	2.0	4.0	8.0	>8
N(131)	1		2	5	15	48	35	10	13

Clindamycin

MIC	≤0.016	0.032	0.064	0.125	0.25	0.5	1	2
N(131)	36	39	40	8	2	1	0	3

Dalbavancin

MIC	0.032	0.064	0.125	0.25	0.5	1	2
N (131)	2	4	34	42	39	3	5

Erythromycin

MIC	≤0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	≥16
N (131)	2	4	46	45	7	2	7	2	5	2	7

Penicillin G

MIC	≤0.016	0.032	0.064	0.125	0.25	0.5	1.0	2	4
N (131)	22	31	42	11	11	2	3	2	5

Tigecycline

MIC	**≤ 0.016**	**0.032**	**0.064**	**0.125**	**0.25**	**0.5**	**1**	**2**	**>2**
N (131)	3	3	22	51	33	11	2	2	1

Table 15. Identification of *mefA* and *ermB* genes in VGS isolates with a reduced susceptibility to erythromycin (Paper IV).

Strain nr	Species ***rnpB***	***mefA***	***ermB***
12	*S. salivarius*	-	+
14	*S. mitis*	+	+
15	*S. pneumoniae*[1]	-	-
19	*S. parasanguinis*	+	-
20	*S. mitis*	+	-
21	*S. mitis*	+	-
22	*S. mitis*	+	+
24	*S. mitis*	+	-
45	*S. mitis/oralis*	+	+
47	*S. pneumoniae*[2]	+	+
52	*S. mitis*	+	+
60	*S. pneumoniae*[1]	+	-
61	*S. pneumoniae*[1]	+	-
69	*S. mitis*	+	-
86	*S. mitis*	-	+
93	*S. oralis*	+	-
98	*S. gordonii*	+	-
101	*S. salivarius*	-	+
109	*S. pneumoniae*[3]	+	-
115	*S. pneumoniae*[1]	+	+
126	*S. mitis*	+	-
136	*S. pneumoniae*[2]	+	+
138	*S. mitis*	+	-
140	*S. gallolyticus*	-	-
143	*S. parasanguinis*	+	-

The strains were sequenced as S. pneumoniae but API Strep identified it as S. mitis (1), S. oralis (3) or it was not typeable (2)

5 SUMMARY AND GENERAL DISCUSSION

VGS cause infective endocarditis in immunocompetent patients but seldom cause IE in immunocompromised patients with haematological diseases (Paper I and III). An explanation could be that most of the haematological patients had thrombocytopenia, and thrombocytes plays a role in the pathogenesis of IE (14). However, few of the haematological patients were investigated by TEE; but there were no relapses in this group of patients treated with antibiotics for 14 days which indicated that IE was unlikely (Paper I, Paper III). IE in solid transplant recipients is rare and only 4 % in a study of 46 IE patients were caused by VGS (102). Determination of VGS species is difficult with conventional methods. The identification of the VGS species is important because the frequency of species differ in different patients group and give disparate clinical pictures and clues to detection or portal entry for eradication. We found that *Streptococcus sanguinis* was most frequent in the immunocompetent patients with a high rate of IE, that has been reported in previous studies (32) and *Streptococcus mitis* dominated in the neutropenic group when conventional methods were used for species identification (Paper I). Molecular biological techniques using PCR have been developed for identification of bacteria (139). In this study we have sequenced VGS isolates using the *rnpB* that has been difficult to determine with conventional methods (Strep API, bioMérieux). The majority of the strains (where a previous typing was done) were sequenced within the same major group of VGS as with the conventional methods. We found that strains from the *Streptococcus sanguinis* group (*Streptococcus gordonii, Streptococcus sanguinis* and *Streptococcus parasanguinis*) and *Streptococcus oralis* dominated in the IE strains when *rnpB* was used. In the neutropenic patients with VGS septicaemia *Streptococcus mitis* and *Streptococcus oralis* dominated using *rnpB* (Paper III). The disadvantage of this method is that it might give undependable results, for examples when strains were sequenced as *Streptococcus pneumoniae* where previous methods have showed that they were VGS strains and conventional methods have determined most of them as *Streptococcus mitis* or *Streptococcus oralis*. The explanation is the close genetic relationship between *Streptococcus pneumoniae* and *Streptococcus mitis* and *Streptococcus oralis* (19), Figure 1.

We found a reduced penicillin susceptibility to VGS 18 % of all the patients, and in 21 % of the patients with haematological diseases (Paper IV). However the highest level of penicillin resistance was found in oral isolates in patients with haematological diseases, where 25 % of the strains were resistant to penicillin (MIC $\geq$ 4.0 μg/ml) (Paper II) (131). The rate of penicillin resistant VGS (MIC $\geq$ 4.0 μg/ml) in blood culture isolates was lower, 4 % in the patients from 1998-2003 (Paper IV). All the penicillin resistant strains were isolated from patients with haematological diseases. This was lower than the results of the Canadian study (44), where 7 % of the VGS were resistant to penicillin but similar to a study from Finnish hospitals where 5% of the VGS blood culture isolates were resistant to penicillin (86) . The explanation to the higher rates of penicillin resistance in the oral VGS strains compared to the blood culture isolates is unclear, maybe the oral strains are less virulent. The oral cavity has been described as a reservoir for transfer of pencillin-resistant genes from oral VGS to *Streptococcus pneumoniae*.

In 6 % of the VGS endocarditis strains (Paper IV) there was a reduced susceptibility to penicillin, but the result is lower compared to a study from the United States where 4 % of the strains were resistant to penicillin (107). There are case reports of penicillin resistant strains in VGS endocarditis (52, 74-76).

In Paper I, a majority of the patients with haematological diseases (86%) had received antibiotic prophylaxis in form of trimethoprim-sulphametoxazole (4%) or ciprofloxacin (82%). There has during the last years been a drawback to fluoroquinolones used as prophylaxis due to the emergence of resistance Gram-negative strains (25) (20, 62). There are in 2003 NCCLS no breakpoints for VGS and ciprofloxacin, although there are for levofloxacin. Quinolones are not routinely recommended for treatment of Gram-positive infections but if it is needed; levofloxacin is a better choice than ciprofloxacin (33).

The level of erythromycin resistance was 17% in blood culture isolates (Paper IV) and nearly all of these isolates are from patients with haematological diseases. In the Finnish study 27% of the VGS isolates were resistant to erythromycin (86). The use of erythromycin is higher in Finland than in other countries in Northern Europe (17) and in a recent publication from Spain, 100 % of the isolates were resistant to erythromycin (4). The role of erythromycin resistance in VGS in haematological patients is unclear; possibly it is the high antibiotic pressure and high rate of hospitalization that increases selection of erythromycin resistant strains. There are reports of erythromycin resistant VGS isolates from oral swabs in healthy children (3, 85) and adults (3, 88, 100).
The *mefA* gene was found in 80% *ermB* in 40 % of the VGS isolates (Paper IV) from the blood culture strains that had a reduced susceptibility to erythromycin.

New antimicrobial agents as linezolid, a oxazolidinone, have been in use in recent years (10) . There have been reports of the effect of linezolid for treatment of endocarditis, Tigecycline a broad-spectrum glycylcycline antibiotic (9)(103) and dalbavancin (40) (94), a new glycopeptide administrated once a week, are not yet registered.Tigecycline (GAR-936) has been showed to be active against VGS with MIC 90% 0.12 μg/ml (9), but in the present study MIC 90% was 0.5 μg/ml.

This study shows that the resistance to penicillin and erythromycin is mainly a problem in immunocompromised patients, but a reduced susceptibility to penicillin also exists in immunocompetent patients.

The susceptibility to vancomycin and linezolid was high in the blood culture isolates, and only a few strains had a reduced susceptibility to clindamycin. Because of the emergence of resistance vancomycin should only be used as empiric treatment in cases with septic shock or acute respiratory stress syndrome in neutropenic patients (127), and as for linezolid in cases of antibiotic resistance.

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