

Antimicrobial susceptibility against penicillin, ampicillin and vancomycin of viridans group *Streptococcus* in oral microbiota of patients at risk of infective endocarditis

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SUMMARY

The viridans group *Streptococci* (VGS) are most abundant in the mouth; in some instances they might emerge as pathogens particularly in infective endocarditis (IE). In this study, we aimed to define and determine the susceptibility against antibiotics of VGS that are members of the oral microbiota of patients exhibiting a risk of developing IE. Forty-nine patients at risk of infective endocarditis were included in the study. Identification of the bacteria was performed using API STREP (bioMérieux, France). Gradient test strips (E-Test, France) were used to determine MIC of the bacteria against penicillin, ampicillin, and vancomycin. The distribution of the isolated VGS groups was determined as follows: *Streptococcus mitis* 32.6% and

anginosus group - 32.6%, *S. sanguinis* group - 16.3%, *S. mutans* group - 12.2%, and *S. salivarius* group - 6.1%. The rates of resistance and reduced sensitivity of the isolates for penicillin and ampicillin were determined at 61.2% and 55.1%, respectively. However, all isolates were found to be susceptible to vancomycin. We conclude that the antimicrobial resistance of VGS should be determined on a regular basis locally, and decisions on therapeutic and prophylactic interventions should be given taking this resistance into consideration.

Keywords: viridans group streptococci, oral microbiota, infective endocarditis, beta-lactam, antimicrobial resistance.

INTRODUCTION

Viridans group streptococci (VGS) are the dominant members of the oral microbiota [1]. Based on the 16S rRNA sequences, viridans group streptococci can be divided into 5 groups:

1. The *Streptococcus sanguinis* Group, which includes *Streptococcus sanguinis*, *Streptococcus parasanguinis*, *Streptococcus gordonii*, and *Streptococcus sinensis*;
2. the *Streptococcus mitis* Group, which includes *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus pneumoniae*, *Streptococcus pseudopneumoniae*, *Streptococcus cristatus*, *Streptococcus peroris*, and *Streptococcus infantis*;
3. The *Streptococcus anginosus* Group, which includes *Streptococcus anginosus*, *Streptococcus constellatus*, and *Streptococcus intermedius*;
4. The *Streptococcus mutans* Group, which in-

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cludes *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus cricetus*, *Streptococcus downei*, *Streptococcus rattii*, *Streptococcus macacae*, and *Streptococcus ferus*;

5. The *Streptococcus salivarius* Group, which includes *Streptococcus salivarius*, *Streptococcus vestibularis*, *Streptococcus infantarius*, *Streptococcus thermophilus*, and *Streptococcus hyointestinalis* [2].

Although VGS belong to some of the common elements of the oral microbiota, they can emerge as pathogens of infective endocarditis (IE). The polysaccharide component of VGS called dextran is the most important mediator of virulence and adhesion in VGS to cardiac components [3, 4]. According to the surveillance data from 13 non-member countries of the European Union affiliated to The World Health Organization, Turkey stands first in the use of β -lactam antibiotics in outpatient clinics [5].

The aim of this study was to identify the VGS isolated from the mouth swab samples of patients with potential risk of IE and to determine their antimicrobial susceptibility against β -lactam antibiotics often used in the treatment and prophylaxis of IE.

■ PATIENTS AND METHODS

Patients' selection The patients admitted to the cardiology outpatient clinic of Ankara Numune Training and Research Hospital during the period between February and June 2015 and those having prosthetic valves, valvular disease, and/or rheumatic heart disease with the risk of developing IE, and agreed to provide samples were included in the study. Patients using antibiotics in the last 15 days, immunocompromised, aged less than 18 years old were excluded from the study. Oral swab samples were taken from 49 patients meeting the inclusion criteria.

Sample collection Swab samples were obtained from the gums and buccal mucosa of patients using sterile swab sticks. The swab sticks were transferred to Amies medium (Coban, Italy) to cultivate the microorganisms.

Bacterial culture The microorganisms were cultivated in Amies medium supplemented with 5% sheep blood agar to obtain single colonies. Then, the plates were incubated in an atmosphere of 5% CO₂ at 35 ± 1°C for 18 ± 2 h.

Bacteria identification After incubation, the alpha-haemolytic colonies grown extensively in 5% sheep blood plates were evaluated. The colonies that appeared as gram-positive cocci with Gram staining were identified conclusively using API STREP (bioMérieux, France).

Antibacterial susceptibility determination Bacterial suspensions were prepared according to 0.5 McFarland standards using sterile 0.9% saline and inoculated in a medium made of 5% defibrinated horse blood agar and Mueller Hinton fastidious agar (MH-F) (Liofilchem, Italy) containing 20 mg/L β -NAD. Penicillin, ampicillin, and vancomycin gradient test strips (bioMérieux, France) were placed onto the plates, and the plates incubated in an atmosphere of 5% CO₂ at 35 ± 1°C for 18 ± 2 h. The Minimum Inhibitory Concentrations (MIC) of antibiotics were determined according to the methods of European Committee on Antimicrobial Susceptibility Testing (EUCAST) limits. *Streptococcus pneumoniae* ATCC 49619 strain was used as the control strain for the identification of bacterial and antibiotic susceptibility tests. All isolates except from susceptibility isolates were identified as resistant and reduced susceptibility isolates.

Statistical analyses The results were evaluated by the SPSS 20 program using the chi-square test.

■ RESULTS

Patients characteristics Of all the patients included in the study, 36 (73.47%) were female and 13 (26.53%) were male. The mean age of these patients was determined as 47 ± 14.7 years. Two (4.08%) patients reported to have suffered from IE earlier. Eleven patients (22.44%) included in the study had prosthetic heart valves implanted.

Identification of bacteria The distribution of the different VGS species identified in the oral microbiota of patients is presented in Table 1.

Antibiotic susceptibility of bacteria The rates of antibiotic susceptibility of the different bacterial isolates according to EUCAST clinical limits are given in Table 1.

Of the isolates, 30 (61.22%) were identified as completely resistant, or with reduced sensitivity, to penicillin and ampicillin, respectively. MIC₅₀/MIC₉₀ values of the isolates were determined to be 0.5 μ g/mL and 1.5 μ g/mL for penicillin and ampicillin.

Table 1 - Number and antibiotic susceptibility rates of VGS.

Group name	No. (%)	Penicillin		Ampicillin	
		Not susceptible* No. (%)	Susceptible No. (%)	Not susceptible* No. (%)	Susceptible No. (%)
<i>S. mitis</i> Group	16 (32.65%)	8 (50.00)	8 (50.00)	4 (25.00)	12 (75.00)
<i>S. anginosus</i> Group	16 (32.65%)	9 (54.25)	7 (43.75)	11 (68.75)	5 (31.25)
<i>S. sanguinis</i> Group	8 (16.33%)	6 (75.00)	2 (25.00)	6 (75.00)	2 (25.00)
<i>S. mutans</i> Group	6 (12.24%)	4 (66.67)	2 (33.33)	3 (50.00)	3 (50.00)
<i>S. salivarius</i> Group	3 (6.12%)	3 (100.00)	0 (0)	3 (100.00)	0 (0)

*resistant and reduced susceptibility.

DISCUSSION

Viridans group streptococci comprise the predominant members of the oral microbiota. Although taxonomical identification of these bacteria are difficult, advances in molecular techniques make possible the identification of VGS types using 16S rRNA analysis [6]. The understanding of these bacteria is significant, because they emerge as opportunistic pathogens especially in immune compromised patients, especially in patients at risk of development of infective endocarditis [7]. In particular, the adhesins of VGS play a key role in the pathogenesis of IE; VGS are often implicated as a factor, especially in patients with mitral valve prolapse [8]. Penicillin-resistant VGS was first isolated from the oral microbiota of patients who were on penicillin prophylaxis for rheumatic fever [9]. In 1995, penicillin non-susceptible isolate rates based on species were determined as *S. mitis* 41.5%, *S. sanguinis* 28.1%, *S. salivarius* 28.1%, and *S. anginosus* 14% in VGS isolated as the causative agent of bacteraemia [10]. Another study reported the susceptibility of streptococci in the oral microbiota of 550 patients; the most frequently isolated bacteria were *S. mitis*, *S. sanguis*, *S. anginosus*, *S. mutans*, and *S. salivarius*. While antibiotic resistance was determined to be 13% for penicillin and 14% for ampicillin among the bacterial species, all the isolates were found susceptible to vancomycin. In addition, penicillin MIC₅₀/MIC₉₀ values were determined to be 2 mg/mL each, in the same study [11].

Concern for serious infection due to beta lactam resistant VGS is a major factor driving empiric use of an anti-gram-positive antimicrobial in patients with febrile neutropenia, although VGS are commonly found as the members of the oral

microbiota. The β -lactam resistant VGS bloodstream infections can be life threatening for patients. A major reason for considering an empiric anti-gram-positive antimicrobial in the treatment of patients with febrile neutropenia is the concern for severe VGS infection [12]. In a study evaluating 1,448 VGS isolated from various clinical specimens was observed that the causative agents were most frequently isolated from blood cultures (28.5%), and the most common agents were *S. mitis* (40.7%) and *S. anginosus* (20%). The degree of decreased sensitivity and resistance of these isolates was 40% for penicillin and 36.2% for ampicillin [13]. The most striking similarity of this study is the identification of *S. salivarius* (79%) to be the most resistant isolate to penicillin. However, in the present study, penicillin sensitive isolates could not be determined in this group of bacteria.

In Turkey, β -lactam antibiotics are being widely prescribed for outpatients [5]. This practice could satisfactorily explain the underlying cause of high penicillin and ampicillin resistance in VGS isolates identified in this study. Overuse of antibiotics is parallel to high antibiotic resistance prevalence. Primary care physicians should be aware that increased prescribing of antibiotics may led to increased level of penicillin resistance. [7]. β -Lactam group antibiotics commonly prescribed for upper respiratory infections cause the emergence of resistant VGS that belong to the community of oral microbiota. Also these resistant VGS play a role as a reservoir for other bacteria of the oral microbiota especially *Streptococcus pneumoniae* [7, 14]. Other studies point to the fact that while resistance rates are high in Korea and Spain, they are low in Germany [13-15]. Likewise, in recent surveillance studies of the Americas, the resistance rates were

found to be lower in the USA and Canada compared to Latin America [16]. Therefore, we strongly emphasize the importance of determining the local resistance rates, and these data hold vital information necessary to determine the decisions on empirical therapy or treatment and prophylaxis protocols.

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