Prevalence rate and antibiotic susceptibility of oral viridans group streptococci (VGS) in healthy children population

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Abstract

Purpose: The aim of this study was to evaluated the prevalence rate of oral viridans group streptococci (VGS) and their susceptibilities to some antibiotics in healthy children.

Material and methods: Samples of pharyngeal swabs and supragingival dental plaques for microbiological studies were collected from 206 healthy children, aged 4-18 years. Additionally, 75 samples of carious lesions from children with dental caries were included. The streptococci were isolated and identified using standard methods and commercial identification kits. For performance of antibacterial susceptibility testing of VGS strains disk diffusion and/or breakpoints procedures were used according to NCCLS standards and criteria. A total of 425 VGS strains were tested against penicillin, ampicillin, erythromycin, clindamycin, tetracycline, doxycycline, ciprofloxacin and vancomycin.

Results: A total of 239 VGS strains belonging to 8 species from pharyngeal swabs of 192 (93.2%) children were isolated. VGS strains from supragingival plaques were isolated in 149 (72.3%) healthy children ($p<0.05$), and from carious lesions in 37 (49.3%) children with dental caries. VGS strains of *S. mitis* species were isolated most frequently from 4-5 year old as compared to 12 and 18 year old children ($p<0.05$), while *S. vestibularis* strains isolated most often in 12 year old ones ($p<0.05$). Among 425 VGS strains, high level of penicillin resistance (MIC≥2.0 mg/L) was shown in 71 (16.7%) strains, 33 (46.5%) of them belonged to *S. mitis* species. VGS strains were also resistant to erythromycin (23.5%), clindamycin (23.1%), tetracyclines (T-52%, DOX-16%), gentamycin (25.9%) and ciprofloxacin (55.2%). All VGS strains were vancomycin – susceptible.

Conclusions: 1. In the oral cavities of healthy children, approximately 98% of streptococci belonged to two VGS groups, i.e. mitis and salivarius groups. Streptococci of mutans and anginosus groups were isolated sporadically (2%). 2. We observed difference in susceptibility to penicillin and other antibiotics between the various species of viridans groups streptococci. Mitis group strains (except *S. pneumoniae*) were more frequently penicillin-resistant (23%) in comparison to salivarius group of VGS strains (9%) ($p=0.0001$).

Key words: viridans group streptococci, penicillin resistance, healthy children, pharyngeal swabs, supragingival plaques, dental caries, multidrug resistance.

Introduction

The aerobic oropharyngeal or the oral cavity microbiota consists predominantly of viridans group streptococci (VGS), which play an important role in inhibiting colonization of pathogens. VGS are normal inhabitans of the oral cavity, gastrointestinal tract, and female genital tract, and they are often considered to be contaminants when isolated from blood cultures, where they may be found as transients in the bloodstream [1-3]. However, their presence may be associated with infective endocarditis, especially in patients with prosthetic heart valves, where *S. sanguis*, *S. mitis*, *S. oralis* and *S. gordonii* being frequently isolated [1,4], which together with *S. pneumoniae* are belonged to the mitis group [3,5]. Members of the mutans group streptococci are associated with dental caries in humans and animals, where *S. mutans* and *S. sobrinus* being the species most frequently isolated from carious lesions and dental plaques. *S. mutans* may also be isolated from patients with endocarditis

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Table 1. **Occurrence of viridans group streptococci (VGS) strains in throats and their resistance to penicillin (% or number of strains)**

Species \ Age (years) (No. of children)	4-5 (n=52)	6-7 (n=50)	12 (n=52)	18 (n=52)	Total (n=206)
S. anginosus	1 (1)	0	0	0	1 (1)
S. mitis	26 (38.5)	12 (41.7)	12 (16.7)	15 (13.3)	65 (29.2)
S. mutans	2 (2)	0	0	0	2 (2)
S. oralis	13 (30.8)	6 (1)	10 (20.0)	7 (0)	36 (19.4)
S. salivarius	16 (18.7)	11 (9.1)	15 (20.0)	19 (10.5)	61 (14.7)
S. sanguis	4 (0)	6 (1)	5 (0)	3 (0)	18 (5.6)
S. vestibularis	16 (6.2)	9 (0)	20 (0)	9 (1)	54 (3.7)
S. pneumoniae	2 (0)	0	0	0	2 (0)
Streptococcus spp.	80 (23.7)	44 (18.2)	62 (11.3)	53 (9.4)	239 (17.2)

[2,3]. *S. intermedius* is often isolated among the polymicrobial flora of deep abscesses, notably in the liver and brain [6]. Other member of the anginosus group may be isolated from oral abscesses, *S. anginosus* also isolated from smears of female genital tract infections [3].

VGS are the major pathogens found in non-intravenous drug users with native valve infective endocarditis [1,7,8], and are also common pathogens causing septicaemia in patients with haematological disease who receive chemotherapy and develop neutropenia [1,7-9]. The major species causing infections in neutropenic (immunocompromised) patients are *S. oralis*, *S. mitis*, *S. salivarius* and *S. anginosus* [3,10,11]. Complications associated with bacteremia in these patients include endocarditis, acute respiratory distress syndrome (ARDS) and shock [3,9,12].

In patients with haematological disease and neutropenia, oral ulcerations related to chemotherapy may develop, with the result that VGS can penetrate easily from the oral cavity into the bloodstream and cause septicaemia [8]. Antibiotic prophylaxis, especially with ciprofloxacin, has reduced the number of episodes of septicaemia caused by Gram-negative bacteria, but it has been suggested that this has increased the frequency of septicaemia caused by VGS [8,13]. Several studies have found reduced susceptibility to penicillin in VGS from such patients [8,11,13,14], with the frequency of penicillin resistance (MIC>2.0 mg/L) in isolates of VGS being as high as >40% [15].

However, recent studies have indicated that VGS are increasingly becoming resistant to many antibiotics not only to penicillin, to macrolides and others [3,5,8,11,13-16].

The aim of this study was to evaluate the prevalence of oral viridans group streptococci (VGS) and their susceptibilities to some antibiotics in healthy children.

Material and methods

Pharyngeal and supragingival dental plaque specimens were collected from 206 healthy children aged between 4 and 18 years. The specimens obtained were directly inoculated onto Mueller-Hinton agar plates with 5% defibrinated sheep blood. Plates were incubated at 37°C for 18-24h in 5% CO_2. Putative streptococcal colonies were chosen based on their morphology (β, α or γ haemolysis) and were further confirmed by compatible Gram stain and catalase negative tests [17]. These isolates further differentiated by API STREP or ID 32 STREP using API Expression automated for interpreted test strips (bioMérieux). Asignment of isolates to VGS group species was carried out according to the criteria of Facklam [5] and Ruoff et al. [3]. Additionally, samples of carious lesions from 75 children with dental caries were studied.

A total of 425 VGS strains isolated from oral cavity of 206 children were tested for antibacterial susceptibilities. For performance of antibacterial susceptibility testing of VGS strains disk diffusion and/or breakpoints procedures were used according to NCCLS (National Committee for Clinical Laboratory Standards) standards and criteria [17-20]. The VGS strains were tested against penicillin, ampicillin, erythromycin, clindamycin, tetracycline, doxycycline, ciprofloxacin and vancomycin. *S. aureus* ATCC 29 213, *S. aureus* ATCC 25 923 and *S. pneumoniae* ATCC 49 150, were used for assay control.

Statistical comparisons of the susceptibility rates and incidence of viridans group of streptococci were performed by chi-square test.

This study was approved by Ethics Committee of the Medical University of Białystok.

Results

A total of 239 VGS strains belonging to 8 species were isolated from pharyngeal swabs of 192/206 (93.2%) children (*Tab. 1*). One species of VGS strains was observed in 147 (76.6%) pharyngeal swabs, two species in 43 (22.4%) and three species in 2 (1%) pharyngeal swabs. No VGS strains isolated in 14 (6.8%) children. VGS strains belonged to 8 species were isolated most frequently from 4-5 year old children (80/239; 33.5%) as compared to 12 year old children (62/239; 25.9%) ($p<0.05$). We noticed that there was no growth of *S. anginosus*, *S. mutans* and *S. pneumoniae* species from the throat of children aged 6-7, 12 and 18 years old (*Tab. 1*). VGS strains of *S. mitis* species were isolated most frequently from 4-5 year old children (26/52; 50%) as compared to 6-7 years old (12/50; 24%) ($p<0.05$), young aged 12 (12/52; 23.1%) ($p<0.05$) and 18 year old (15/52; 28.8%) ($p<0.05$).

Table 2. **Occurrence of VGS strains in supragingival dental plaques and their resistance to penicillin (% or number of strains)**

Species \ Age (years) (No. of children)	4-5 (n=52)	6-7 (n=50)	12 (n=52)	18 (n=52)	Total (n=206)
S. intermedius	0	2 (0)	1 (0)	0	3 (0)
S. mitis	6 (4)	9 (2)	9 (2)	9 (1)	33 (27.3)
S. mutans	1 (1)	0	0	0	1 (1)
S. oralis	4 (2)	4 (1)	8 (0)	3 (0)	19 (15.8)
S. salivarius	5 (0)	4 (1)	9 (1)	13 (7.7)	31 (9.7)
S. sanguis	3 (1)	3 (0)	5 (0)	1 (0)	12 (8.3)
S. vestibularis	6 (2)	13 (0)	17 (0)	14 (0)	50 (4.0)
Streptococcus spp.	25 (40.0)	35 (11.4)	49 (6.1)	40 (5.0)	149 (12.8)

Table 3. **Occurrence of VGS strains in carious lesions and their resistance to penicillin (number of strains)**

Species \ Age (years) (No. of children)	4-5 (n=31)	6-7 (n=30)	12 (n=9)	18 (n=5)	Total (n=75)
S. intermedius	0	1 (0)	1 (0)	0	2 (0)
S. mitis	3 (3)	5 (2)	1 (0)	1 (0)	10 (5)
S. mutans	0	1 (1)	0	0	1 (1)
S. oralis	1 (1)	5 (1)	2 (0)	0	8 (2)
S. salivarius	3 (0)	1 (0)	1 (0)	1 (1)	6 (1)
S. sanguis	2 (1)	2 (0)	0	0	4 (1)
S. vestibularis	2 (1)	2 (0)	2 (0)	0	6 (1)
Streptococcus spp.	11 (6)	17 (4)	7 (0)	2 (1)	37 (11)

Table 4. **Incidence rate (%) of penicillin resistant VGS strains from oral cavity in healthy children**

Group and species (No. of strains)	Categories of susceptibility: Susceptible (S) *(≤0.06)	Intermediate (I) *(0.12-1.0)	Resistant (R) *(≥2)
Salivarius group			
S. salivarius (98)	29.6	57.1	13.3
S. vestibularis (110)	48.2	47.3	4.5
Mitis group			
S. mitis (108)	13.9	55.6	30.5
S. oralis (63)	34.9	46.0	19.1
S. sanguis (34)	32.4	28.8	8.8
S. pneumoniae (2)	**2		
Anginosus group			
S. anginosus (1)			1
S. intermedius (5)	3	0	2
Mutans group			
S. mutans (4)			4
Total (425) *Streptococcus* spp.	31.8	52.4	16.7

* MIC in mg/L according to NCCLS; ** No. of strains

Penicillin high level resistance (MIC≥2.0 mg/L) was observed in 17.2% (62/239) of VGS strains isolated from pharyngeal swabs and most frequently seen in *S. mitis* species (19/65; 29.2%) of preschool and school children (*Tab. 1*).

Similar analysis regarding the incidence rate of VGS strains from supragingival plaques of children in different ages and resistance to penicillin are shown in *Tab 2*. Compared to pharyngeal swabs no *S. anginosus* and *S. pneumoniae* species isolated from supragingival plaques, in addition 3 strains of *S. inermedius* species were isolated from supragingival plaques. In general, fews of VGS strains belonged to one species isolated from supragingival plaques (149/206; 72.3%) as compared to pharyngeal swabs ($p<0.05$). The prevalence of resistance to penicillin (19/149; 12.8%) was comparable ($p>0.05$) in both VGS strains isolated from supragingival plaques and from pharyngeal swabs (*Tab. 1* and *2*).

VGS strains from carious lesions from children with dental caries were less frequently isolated (37/75; 49.3%) (*Tab. 3*), where the highest incidence of resistance to penicillin was seen among them (29.7%). Similar to the other results (*Tab. 1* and *2*), *S. mitis* species isolated from the carious lesions was most frequent resistance to penicillin (10/37; 27%).

Tab. 4 represent the results of penicillin susceptibility among a total 425 VGS strains isolated from the oral cavity of 206 healthy children. High level resistance to penicillin (MIC≥2.0 mg/L) was shown in a total of 71 (16.7%) strains. Resistance to penicillin was observed only in 18 (8.7%) from 208 strains of salivarius group; resistance occurred more frequently in *S. salivarius* (13/98; 13.3%) than in *S. vestibularis* species (5/110; 4.5%) ($p<0.05$) (*Tab. 4*).

In 205 isolated strains of mitis group (except *S. pneumoniae*) resistance to penicillin was appeared in 48 (23.4%) of them. Highest level of penicillin resistance was observed in the *S. mitis* isolated strains (33/108; 30.5%), and the results was similar to the resistance in *S. oralis* (12/63; 19.1%) ($p>0.05$). Resistance to penicillin among *S. sanguis* species was less significant (3/34;

Table 5. **Incidence rate (%) of resistant VGS strains to penicillin and other antibiotics**

Group and species (No. of strains)	Antibiotics							
	P	AM	E	CC	T	DOX	GM	CIP
Salivarius group								
S. salivarius (98)	13.3	3.1	29.6	23.5	51.0	16.3	26.5	53.8
S. vestibularis (110)	4.5	4.5	10.0	11.8	45.5	7.3	15.5	43.7
Mitis group								
S. mitis (108)	30.5	26.9	43.5	42.6	77.8	31.5	34.3	65.1
S. oralis (63)	19.1	12.7	9.5	15.9	36.5	11.1	27.0	56.6
S. sanguis (34)	8.8	8.8	11.8	8.8	35.3	8.8	35.3	64.5
S. pneumoniae (2)	0	0	0	0	0	0	0	0
Anginosus group								
S. anginosus (1)	1*	1	1	1	1	0	1	1
S. intermedius (5)	2	1	2	2	1	0	3	1
Mutans group								
S. mutans (4)	4	4	0	0	0	0	0	4
Total (425) *Streptococcus* spp.	16.7	11.5	23.5	23.1	52.0	16.0	25.9	55.2

P – penicillin; AM – ampicillin; E – erythromycin; CC – clindamycin; T – tetracycline; DOX – doxycycline; GM – gentamycin; CIP – ciprofloxacin;
* No. of resistant strains

8.8%) compared to *S. mitis* (p=0.021). Only in 3/6 isolated strains of anginosus group resistance to penicillin was observed. Resistance to penicillin was observed also in all of *S. mutans* isolated strains (*Tab. 4*).

Resistant to erythromycin and clindamycin was seen in 98/425 (23.1%) and 100/425 (23.5%) of VGS isolated strains, respectively; most frequent in *S. mitis* and *S. salivarius* species (*Tab. 5*). Like for penicillin, particularly a significant different in resistant to erythromycin and clindamycin between mitis group (57/205; 27.8% and 59/205; 28.8%, respectively), and salivarius group (40/208; 19.2% and 36/208; 17.3%) (p=0.0399 and p=0.0056) was seen. Considerably high percent of resistance observed to tetracyclines, gentamycin and ciprofloxacin. All VGS strains were vancomycin – susceptible.

Discussion

Antimicrobial resistance among the viridans group of streptococci (VGS) has emerged as a hindrance to effective antibiotic therapy [5,8,11,14-16].

Penicillin resistance in viridans group streptococci (VGS) has been described since the 1970s [21], although the incidence has gradually increased. Penicillin resistance rates of >40% for VGS are common [11,14,15,22-24], partieularly for *S. mitis* and *S. sanguis*. Recently, Husain et al. [24] have shown that *Streptococcus mitis* accounted for 58% of invasive viridans streptococcal infections in children with malignancies of which 51% were penicillin-nonsusceptible (resistant). There was no significant association between species or penicillin susceptibility pattern and clinical presentation or outcome [24].

Rotimi et al. [25] evaluated the prevalence of antibiotic-resistant VGS in healthy children. Of the 540 VGS isolates from 102 children, 58% were from the tooth surfaces and 42% from the tongue. The most prevalent were *S. salivarius* (21.5%) and *S. sanguis* (16.3%). Resistance rate to penicillin was 15.9%. The data authors [25] showed species-related and site-related variations in the susceptibility pattern. At the species level, 26% and 23% of *S. salivarius* and 23% and 14% of *S. mutans* from the tooth and tongue, respectively were resistant to penicillin [25].

Our objective also was to evaluate the prevalence of antibiotic-resistant VGS in healthy children. Of the 425 VGS isolates 56.2% were from the throat swabs, 35.1% from the supragingival plaques, and only 8.7% from the dentine carious lesions (mainly from preschool children: 28/37; 75.7%). The most prevalent were: *S. vestibularis* (25.9%), *S. mitis* (25.4%), *S. salivarius* (23.1%) and *S. oralis* (14.8%). Among all VGS strains resistance rate to penicillin was 16.7%. Our data, similar to Rotimi et al. [25] results and show species-related and site-related variations in the susceptibility to penicillin and an emerging high prevalence of penicillin-resistant VGS. For example, at the species level, 29%, 27% and 50% of *S. mitis* and 4%, 4% and 17% of *S. vestibularis* from the pharynx, supragingival plaque and caries lesions, respectively were resistant to penicillin.

Among other classes of agents, resistance in VGS has been described for macrolides, lincosamids, tetracyclines, quinupristin-dalfopristin and fluoroquinolones [3,5,11,13,14,16,22,25,26]. The incidence of resistance to most drugs, except for the macrolides [14,16,22,23,25], usually was low. Malhotra-Kumar et al. [16] showed macrolide-resistant VGS in >70% from pharyngeal swabs of the healthy Belgian population (17-25 years old). Half macrolide-resistant isolates were *S. mitis*, while the other half were distributed among eight different VGS species. Penicillin resistance was observed in only one macrolide-resistant isolate according to early mentioned authors [16]. In contrasts with recent data on VGS where comparable levels of penicillin and macrolide resistance have been observed [14].

Our results showed that the resistance rates to erythromycin and clindamycin were 23.5% and 23.1%, respectively. Rotimi et al. [25] observed the resistance to erythromycin and clin-

damycin respectively in 32.6% and 15.4% strains of VGS from healthy children. High level resistance to tetracycline, gentamycin and ciprofloxacin was observed among VGS strains in our study (52%, 25.9% and 55.2%, respectively). All strains of VGS tested by us and Rotimi et al. [25] and others [3,5,14,22,23] were sensitive to vancomycin. Single report of resistance to vancomycin has been noted for *Streptococcus mitis*, only [27].

Conclusions

In conclusion, a high frequency of penicillin resistance in oral isolates of VGS and its co-resistance to erythromycin, clindamycin, tetracycline, gentamycin and ciprofloxacin among healthy children was observed. The data showed species-related and site-related variations in the susceptibility patterns.

There are some important reasons why clinical microbiologists should identify viridans streptococci to a level species:

1. To delineate the spectrum of disease caused by specific species; for example bacteremia caused by *Streptococcus anginosus* (known as *S. milleri*) is associated with deep visceral abscesses (see "Introduction")
2. To differentiate between therapeutic failure and reinfection. Patients with recurrent endocarditis may not have been adequately treated during a previous episode if the same strain is isolated or may present with a new infection and a new strain might be anticipated. By identifying the organism fully, this distinction can often be made.
3. Monitoring the emergence of antibiotic resistance. Strains of *Streptococcus mitis* have been noted to be more resistant to penicillin for instance. In patients with underlying neutropenia, where antibiotic-resistant VGS might itself cause life-treatening infections.
4. In healthy individuals, where antibiotic-resistant VGS might transfer the resistance determinants to pathogenic streptococci such as β-hemolytic streptococci (e.g. *S. pyogene*s, *S. agalactiae* and other) and *Streptococcus pneumoniae*. Viridans group streptococci (VGS) are gaining significance as reservoirs of resistance determinants for respiratory tract pathogens [3,5,16,23,25].

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