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Antimicrobial Susceptibility Pattern of *Viridians Streptococci* Isolated from Saliva in Young Healthy Subjects

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Abstract

Purpose: The aim of this study was to evaluate the prevalence rate of resistant oral viridans group streptococci (VGS) to some β-lactam antibiotics in healthy young adults.

Material and methods: Saliva were collected from 33 healthy young adults, aged 19-24 years. The streptococci were isolated and identified using standard methods. For performance of antibacterial susceptibility testing of VGS isolates disk diffusion procedures were used according to NCCLS (National Committee for Clinical Laboratory Standards) standards and criteria. A total of 75 VGS strains were tested against ampicillin, ampiclox, cephalexin and carbenicillin.

Results: high rate of resistance of 33 bacterial isolates (44%) towards ampicillin was observed. Furthermore, a similar scenario was observed for cephalexin in which 51 isolates of the tested organism (68%) showed remarkable resistance against cephalosporin group of antibiotics. On the other hand, only 27 (36%) and 12 (16%) of saliva isolated viridance streptococci recorded to be antibiotic resistant to ampiclox and carpenicilin, respectively. A multiple resistance patterns of viridians streptococci against the four antibiotics was also prudent with (16%) of isolates, and (8%), (24%) were resistant to three and two isolates respectively. Cross resistanse was obvious between ampicillin and ampiclox.

Conclusion: A high prevalence of a significant of β-lactam resistant VGS in healthy adults was observed and documented.

Keywords: **Antibacterial susceptibility; Ampiclox; Viridans; Antibiotics**

Introduction

Viridans streptococci represent a group of 24 currently described *Streptococcus* species that are nutritionally fastidious and mainly alpha-hemolytic on blood agar [1]. These gram-positive cocci are commensals of the oral cavity, upper airway, and gastrointestinal and genitourinary tracts. Despite the overall low virulence, *Viridans* group streptococci continue to be the

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most common cause of both native valve endocarditis [2] and late prosthetic valve endocarditis [3]. They have also been implicated in serious pyogenic infections moreover as emerging pathogens in neonates and neutropenic patients [4], and they appear to be a serious problem in patients with hematologic malignancies receiving cytotoxic chemotherapy [5,6].

Antibiotic resistance is most prevalent in countries with a high level of antibiotic consumption, supporting the link between antibiotic use and the emergence of bacterial resistance [7]. Resistance to beta-lactams, macrolides and other antibiotics among blood cultures of VGS is a major concern and could compromise currently available prophylactic and therapeutic regimens [8]. The antibiotic resistance of oral and gut flora may be due to the selection of clones with reduced susceptibility during antibiotic treatment, but also to the transfer of resistance genes from exogenous bacteria [9].

However, recent studies have indicated that VGS are increasingly becoming resistant to many antibiotics not only to penicillin, to macrolides and others [10,11]. Since a recent study reported, among 425 VGS strains, a high level of penicillin resistance and other antibiotics was shown among *viridians Streptococci* strains reached to 16.7% [12]. The aim of the present study was to evaluate the prevalence of oral *viridans* group streptococci (VGS) and their susceptibilities to some antibiotics in healthy young adults.

Materials and Methods

Study design

During the period from January 2012 to March 2012, a total of 75 VGS isolates isolated from saliva of 33 healthy students of both sexes aged from 19 to 24 years old in Tikrit university campus. All these healthy individuals assured that they haven't taken any antibiotics during in the last three months.

Sample collection

Healthy students were asked to chew on a piece of paraffin wax for 2 min, expectorate the saliva and collect it into sterile glasstubes. Samples were immediately brought to the microbiologylaboratory for further processing. Saliva was diluted (1: 1000 (v/v)) in sterile distilled water and an aliquot (50 mL) of thedilution was manually seeded on blood agar plates (Himedia. india) incubated at 37°C for 24 h as described [13].

Identification of organisms

The organisms were all presumptively identified as *viridans* streptococci based on alpha-hemolysis, gram-positive reaction, coccus morphology in chains, negative catalase test, and exclusions of pneumococci and enterococci by routine biochemical tests (optochin test, bile solubility). Following identification 75 colonies were randomly selected and streptococcal strains in saliva were isolated [14].

Susceptibility testing for antimicrobials

For experimental purpose, the chosen isolates from healthy subjects were tested for antibacterial susceptibilities. For performance of antibacterial susceptibility testing of VGS strains disk diffusion procedures were used on Muller-Hinton medium (Himedia. india) according to CLSI standards and criteria [15]. The VGS strains were tested against ampicillin (10

mcg), cephalexin (30 mcg), ampiclox (25/5 mcg) and carbenicillin (100 mcg) (Bio analyse). *S. aureus* were used for assay control.

Statistical analysis

Statistical comparisons of the susceptibility rates and incidence of *viridans* group of streptococci were performed by chi-square test. Significant *P* values (≤ 0.05) or nearly significant ones were given.

Results

About 75 bacterial isolates from a total of 33 saliva samples were identified depending on the hemolytic activity on blood agar plates, furthermore by using different biochemical tests. The results revealed that all 75 bacterial isolates were alpha-hemolytic, catalase negative, oxidase negative, and also negative for optochin test.

TABLE 1 demonstrates the antimicrobial resistance profile of *viridians streptococci* against the tested antibiotics. The results clearly showed that there was a high rate of resistance of 33 bacterial isolates (44%) toward ampicillin. Furthermore, a similar scenario was observed for cephalexin in which 51 isolates of the tested organism (68%) showed remarkable resistance against cephalosporin group of antibiotics. On the other hand, only 27 (36%) and 12 (16%) of saliva isolated *viridance streptococci* recorded to be antibiotic resistant to ampiclox and carpenicilin, respectively.

TABLE 2 shows a multi resistance profile of viridance streptococci to the tested antimicrobials. The most important results in this study were shown in TABLE 2, which clarify the multiple resistance patterns of *viridians streptococci* against the four antibiotics. The biggest highly significant percentage of obtained in our prospective is the resistance of *viridians streptococci* to the all four antibiotics (16%), from a total of 75 isolates, 12 were found to be resistant. About 8% of isolates were found to be resistant to three of tested antimicrobials. Cross resistance was obvious between ampicillin and ampiclox (24%). Also 24% were sensitive to all four antibiotics and 28% of the isolates were recorded to be resistant to only one antibiotic.

TABLE 1. **The antimicrobial resistance profile of *viridians streptococci* against the tested antibiotics.**

	Ampicillin resistant	**Ampiclox resistant**	**Cephalexin resistant**	**Carbencillin resistant**
No. of isolates	33	27	51	12
Percentage%	44%	36%	68%	16%

TABLE 2. **Shows a multidrug resistance profile of viridance streptococci to the tested antimicrobials.**

	Sensitive to 4 antibiotics	**Resistant to 4 antibiotics**	**Resistant to 3 antibiotics**	**Resistant to 2 antibiotics**	**Resistant to one antibiotics**
Number of isolates	18	12	6	18	21
Percentage%	24%	16%	8%	24%	28%

Discussion

The using o B-lactam antibiotic in general population with high rate in treatment of different disease such as dental alveolar periodontal diseases and prophylaxix for infective endocarditis, and also they used in medicine for treatment of otitis media, sinusitis. Bronchitis is factors can promote an increase in b-lactam resistance in the oral microbiota such as *viridians streptococci* that found in mouth as a normal flora. Meanwhile, endocarditis is also important consequences after tooth extraction, and it could be implicated also with healthy individuals [16].

The results of the present study showed that the *viridians streptococci* isolates have very divers' sensitivity pattern for the antibiotics under study. A pronounced result showed that from a total of 75 isolates, 33 isolates (44%) were resistant to ampicillin. These results agrees with the results of Gary et al. [17], that observed 39% of *viridians streptococci* were resistant to ampicillin, on the other hand, our results disagree with Jarvinen et al. [18] who indicate that 68% of *viridians streptococci* were resistant to ampicillin. Regarding ampiclox, our results showed that 36% of the bacteria were resistant to this antibiotic. This result is in parallel with Al-Saady Z. [19] In this study 24% of *viridians streptococci* were resistant to ampiclox. Also Gamboa et al. [20] indicate that 27% of these bacteria were resistant to ampiclox, while it disagrees with another study [21] that recorded 66% of these bacteria were resistant to ampiclox. Concerning cephalexin, our results showed that 68% of viridance streptococci were resistant to cephalexin. This is in agreement with Teng et al. [22]. Wich showed that 61% of these bacteria were resistant to cephalexin. Moreover, Jarvinen et al. [23] observed that 72% of *viridians streptococci* were resistant to cephalexin. Also, another study showed that cephalexin with limited activity versus of *viridians streptococci* about 10-15% from isolates only was susceptible to cephalexin [24]. Regarding carbencillin, this antibiotic is not conventional to be used in large scale in the treatment, so it showed a large activity against viridians bacteria. Low levels of resistance (16%) were recorded.

The most important results in this study were shown in TABLE 2, which clarify the multiple resistance patterns of *viridians streptococci* against the four antibiotics. *Viridance streptococci* developed resistance for the all antibiotics, which could become a big problem in treating streptococcal infections. In this respect, the biggest highly significant percentage of obtained in our prospective is the resistance of *viridians streptococci* to the all four antibiotics (16%). From a total of 75 isolates, 12 were found to be resistant. About 8% of isolates were found to be resistant to three of tested antimicrobials. Cross resistanse was obvious between ampicillin and ampiclox (24%). Also 24% were sensitive to all four antibiotics and 28% of the isolates were recorded to be resistant to only one antibiotic. Approximately, half of our isolates were multiple resistant. There were remarkable cross-resistances between ampicillin and ampiclox.

In summary, more than 75% of the healthy subjects investigatedyielded oral isolates of VGS that were resistant toβ-lactam singly or in multi pattern. This percentage expresses which is real fact present among our population in all sectors. This percentage was higher than expected, possibly because the patients infected with VGS frequently receive antibiotic therapy to treat infections, subsequent to chemotherapy that may select for penicillin-resistant strains of VGS. It is important to be aware of the high level of penicillin and β-lactam resistance in oral VGS in patients with hematological diseases, and this parameter should be considered when selecting antibiotic therapy for cases of septicaemia caused by VGS in immune compromised patients and also endocarditis, as it is remarkably shown the dissemination of resistant strains of VGS in blood [25]. Therefore, for an effective management of these infections an ultimate and detailed bacteriological diagnosis and

susceptibility testing required to overcome global problem of antibiotic resistance and by encouraging greater understanding of this problem different solutions can be planned by health care providers.

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