The Peerian Journal

Open Access | Peer Reviewed

Volume 26, January, 2024

ISSN (E): 2788-0303

Website: www.peerianjournal.com

Email: editor@peerianjournal.com

Identification of Viridans Group Streptococci's virulence factor proteins and resistance rate in dental caries patients in Al-Nasiriyah City, Iraq

Lamyaa .Y.Radhi **Fatimah.H.AL-Ataby** **Amany Shakeir Jaber**

Department of Pathological Analysis, College of Sciences, University of Thi-Qar, Iraq.

Lamyaa.radhi@sci.utq.edu.iq

Fatima.hassan@sci.utq.edu.iq

Amany_pa@sci.utq.edu.iq

Abstract

One of the most common bacterial groups in the oral bacterial ecology is the Viridans Group Streptococci (VGS). In Iraq, DC is widely used, affecting people of all ages. The purpose of this research was to identify the role of VGS bacteria in DC, their capacity to produce specific virulence factors, and their antimicrobial resistance to commercially available antibiotics . This cross-sectional study was carried out between August 2020 and June 2021 in Al-Nasiriyah City, in the province of Thi Qar, Iraq. Patients with DC provided 250 dental caries swabs in total. Patients were allocated from the Specialized Center of Dentistry and Sumar Specialized Dentistry Center in Al-Nasiriyah city. The age range was 7-59 years(male and female). Version 27 of the Statistical Package of Social Science (SPSS) was used for the statistical analysis. If the P value is less than 0.05, the difference was deemed significant. After swabbing all of the samples onto blood and MacConkey agar plates, they were all microaerobically cultured for a full day. Vitek 2 system, biochemical testing, and morphological analysis were used to isolate and identify SVG bacteria. Using the disk diffusion method on Muller-Hinton agar plates in accordance with the Kirby-Bauer method, antibiotic susceptibility was assessed for each isolate against 13 antibiotics before the MIC test. 16 sRNA gene positivity was observed in 84.3% of the isolates, while the antibiotic gene detection rate was as follow, ermB (72.0%), PBP1a (82.0%), PBP2b (90.0%), Van A (47.6%), Van C (76.0%) and tetM (80,0%).

In conclusion, the present study found that high rate of VGS were recovered from dental caries from different age groups. The isolates showed high resistant rates to a wide range of commercially available antibiotics.

Keywords: Dental Caries, VGS, virulence genes , resistance rate

Introduction

Dental caries (DC), also known as cavities or tooth decay, is the breakdown of teeth brought on by acids produced by bacteria. The color of cavities can vary from yellow to black. eating difficulties and pain are potential signs , according to Laudenbach and Simon (2014), complications can include abscess formation, tooth loss, infection, and inflammation of the surrounding tissue.

Bacteria must produce acid that erodes the tooth's hard tissues (cementum, dentin, and enamel) in order for cavities to occur. When food particles or carbohydrates that have collected on the enamel of teeth are broken down by bacteria, acid is produced. Among the risk factors are

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

conditions like diabetes mellitus and Sjögren syndrome that cause decreased saliva production, as well as specific drugs. Antihistamines and antidepressants are two medications that can reduce salivary flow. Further correlations between dental caries and poverty include receding gums that reveal the tooth roots and inadequate oral hygiene (Schwendicke *et al.* 2015, deOliveira *et al.* 2017).

Dental caries eventually increases the risk of developing new dental caries in both primary and permanent teeth, and it can also cause discomfort, infection, and difficulty chewing. These can include demineralization, loss of tooth structure, or total crown destruction (Wagle *et al.* 2018).

The bacteria most frequently linked to tooth cavity formation are the mutans streptococci, specifically Streptococcus mutans and Streptococcus sobrinus, as well as lactobacilli. In spite of the fact that cariogenic bacteria may be detected in dental plaque, their concentrations are often inadequate to create problems until a shift in the balance occurs (Marsh, 1994). A local environmental alteration, such as excessive sugar intake or inadequate biofilm removal, is the cause of this condition (tooth brushing). If left untreated, the disease may worsen, resulting in pain, tooth loss, and infection in the mouth (Marsh *et al.* 2015).

The green color of blood agar plates is caused by a vast collection of α-hemolytic Gram-positive commensal streptococcal bacteria known as the VGS. These organisms are not extremely harmful because they lack Lancefield antigens (Ryan, 2004). Apart from the fact that these bacteria can produce proteolytic enzymes, one remarkable characteristic of oral microflora is the development of a biofilm, or dental plaque, on the surface of the teeth and oral mucosa (Flemming *et al*, 2016).

The eight different kinds of streptococci used in the categorization were anginosus, mitis, sanguinus, salivarius, downei, mutans, and bovis. this make the classification more robust and reliable.(Richards *et al.* 2014).

The World Health Organization reports that there are currently 20 species of mitis living around the world. The mitis group is the largest group of bacteria that may be discovered in the oral cavity. *S. oralis* and *S. mitis* are two species of the genus Mitis that have shown to be challenging to differentiate from one another using simply 16S ribosomal RNA gene sequencing. Researchers have discovered that several strains in this group have been incorrectly classified, as a result of work done to reclassify many of the species (Jensen *et al.* 2016). the comparison of various oral microbiota strains using 16S rRNA amplicons. Researchers discovered unusual taxa and separated the salivary groups (caries-active and caries-free) as a result of the significant differences they identified between the saliva microbiota and the caries microbiome , according to Hurley *et al.* (2019), there's a chance that the existence of these taxa indicates whether or not these kids are healthy.

Specialized clones are selected from the oral niche biofilm after polymicrobial interactions between interspecies, such as recombination and horizontal gene transfer, are established inside the biofilm (Sitkiewicz, 2018). In addition to the forces exerted by bacteria communities and the host, changes in the oral environment induced by antibiotic treatment have been shown to influence the selection of bacterial species and the characterization of their virulence. the two most prevalent chronic diseases of the oral cavity, periodontitis and dental caries, are examples of oral infectious diseases that cause infections. These diseases arise when the local environment and the metabolic and physiological activities of bacteria in biofilm disturb the oral microflora's homeostasis (Struzycka, 2014).

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

Streptococci have been discovered to produce PBPs with both low and high molecular weights (Ajdic *et al.* 2002). Although both of these enzymes are necessary for the formation of cell walls, the high molecular weight PBPs are necessary for the bactericidal activity of beta-lactam antibiotics. The two kinds of high molecular weight PBPs found in VGS are PBP1 (PBP1a and PBP1b) and PBP2 (PBP2a, PBP2b, and PBP2x). This is because PBP1a and PBP1b are part of PBP1 and PBP2 respectively. (Zapun *et al.* 2008)
Beta-lactam antibiotics are effective against VGS, which contain wild-type PBPs, and are thus recommended. PBPs with a high molecular weight have a decreased affinity for beta-lactam antibiotics, which results in the development of resistance to these drugs. In order to reduce the affinity of PBPs, it is feasible to do so by replacing amino acids in the transpeptidase domain of the proteins. (Ergin *et al.* 2003).

VGS isolates from clinical settings are often found to be resistant to the antibiotic erythromycin. Erythromycin for VGS has minimum inhibitory concentrations (MIC50) of 0.25 mg/L and minimum inhibitory concentrations (MIC90) of >2 mg/L in Europe. 36.2 percent of isolates are resistant to erythromycin, the most widely used antibiotic, demonstrating the continued prevalence of antibiotic resistance,(Farrell *et al.* 2014) About 70–80% of the erythromycin-resistant VGS strains have the mef(A) gene, but only about 16–20 percent of the strains have the erm(B) gene (Gershon *et al.* 2002).

Tetracycline resistance is highly prevalent in VGS patients. According to Farrell *et al.* (2014), up to 36% of VGS strains are expected to be tetracycline resistant. furthermore resistant to the glycopeptide antibiotic vancomycin, which continues to work against it.2014; Mendes *et al.*
Iraq is thought to have a high incidence of DC, which could be attributed to factors such as patient characteristics, family history, disease-promoting environmental factors, and poor personal and public hygiene (Al- Mendalawi and Karam 2014, Joury *et al.* 2016). The study's objectives were to identify the resistance rate and virulence factor proteins of Viridans Group Streptococci that were isolated from patients with dental caries in Al-Nasiriyah City, Iraq.

Methods

1. Collection of specimens

specimens were collected between June 2021 and August 2020. A total of 250 patient swabs with dental caries were obtained from dental caries (DC) patients. The Sumar Specialized Dentistry Center and the Specialized Center of Dentistry in Al-Nasiriyah city were the two dental institutions used for this purpose. The patients were both male and female and ranged in age from 7 to 59. a sterile cotton swab that was used to clean the DC cavity or the area around it while the patient was lying down in the dentist chair with their mouth open. Once the swab was ready to be delivered to the bacteriology lab for analysis, it was promptly recapped and kept in a refrigerated box.

2.Culturing , identification by Vitek 2 and antimicrobial susceptibility testing

Swabs were used to streak blood agar and MaCconkey agar plates. The plates were then incubated for 24 hours at 37°C in a microaerophilic environment with 5% CO2 in a candle jar.after isolating

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

the bacteria and sub culturing it to purify it and diagnose it using microscopic and macroscopic characteristics, then biochemicals test according to (MacFaddin ,2000).

3.0 mL of standard sterile saline has been introduced to the test tube. To transfer a sufficient number of pure colonies and suspend the separated colonies in regular saline, sterile swabs were utilized. Densi Chek TM was utilized, and McFarland's turbidity (0.5–0.63) was changed. then, in order to determine if the samples are positive for the Streptoccoccus viridians group, the prepared bacterial suspension is transferred to the Gram Positive card (GP card), which has been sealed and placed in the VITEK system (BioMe'rieux).

Utilize the VITEK-2 system to confirm identification. The data were examined using the Vitek-2 database, which enables kinetic organism identification 180 min after the incubation period begins. The identification card is based on accepted biochemical procedures; 43 biochemical tests are available to measure resistance, enzymatic activity, and carbon source utilization. The final identification results can be accessed in less than eight hours. Following recognition by the Vitek 2 system then ,antibacterial susceptibility testing was performed for all isolates using disk diffusion method (Kirby-Bauer technique) on Muller-Hinton agar and minimum inhibitory concentration (MIC) according to (CLSL 2019).

3.Molecular identification using RT-PCR technique

The Real-Time Polymerase Chain Reaction (RT-PCR) standard technique was used to analyze 50 SVG isolates in total (Taylor *et al*. 2010). To test the isolate's viability, a single colony was injected into two milliliters of brain-heart infusion broth and cultured for twenty-four hours at 37°C. To test the study's hypothesis, the recovered bacterial pellet (10 mM TrisHCl, pH 8, 1 mM EDTA) was resuspended in TE solution after being centrifuged at 12000 x g for 5 minutes. The cells were washed and centrifuged three times with a total of 750 milliliters of triethanolamine. It was centrifuged after being cooked for 20 min in 500 milliliters TE, and the supernatant containing the DNA was kept at -20°C until it was needed.

Genomic DNA was extracted from bacterial growth using the ABIO pure Extraction technique, using the manufacturer’s protocol. using a Quantus Florometer, we were able to determine the concentration of extracted DNA and therefore the samples' suitability for usage in applications further down the line. 199 milliliters of diluted Quanty Flour Dye were mixed with each milliliter of DNA. The DNA concentrations were determined and noted following a 5-minute incubation period at room temperature.

Primers were purchased from Macrogen in Korea, which is a commercial source. This product was dissolved in nuclease-free water to provide a final concentration of 100 picomol l, When 10 microliters of stock solution of primer were added, the concentration of 10 picomol/microliter was achieved by adding 90 microliters of nuclease-free water to the stock solution of primer,(**Table 1**).

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

It was proceed according to the protocol of (Taylor *et al.* 2010) as shown in (**Table 2** and **3**). (**Fig 1) shows** RT-PCR cycle pattern and results of the assay.

Table .1 : The study used oligonucleotide primers for various genes.

GENE	OLIGONUCLEOTIDE SEQUENCE PRIMER	REFERENCE
16sRNA gene	5'-GAGTACGACCGCAAGGTTGA-3' 5'-CTGGTAAGGTTCTTCGCGTTG-3'	(Hurley *et al.* 2019)
ermB gene	5'-GAAAAGGTACTCAACCAAATA-3' 5'AGTAACGGTACTTAAATTGTTTAC-3'	(Seppala *et al.* 2003)
PBP1a gene	5'-CGGCATTCGATTTGATT-3' 5'-GATGTCTTCTCAGGCTTTTG-3.	(Dowson *et al.* 1990)
PBP2b gene	5'-GATCCTCTAAATGATTCTCAGGTGG- 3' 5'-CAATTAGCTTAGCAATAGGTGTTGG- 3'	(Dowson *et al.* 1990)
Van A gene	5'- GGGAAAACGACAATTGC-3' 5'-GTACAATGCGGCCGTTA-3'	Moosavian *et al.* 2018
Van B gene	5'-ATGGGAAGCCGATAGTC-3' 5'-GATTTCGTTCCTCGA CC-3'	Moosavian *et al.* 2018
tet M gene	5' GTGGACAAAGGTACAACGAG 3' 5' CGGTAAAGTTCGTCACACAC 3'	(Malhotra-Kumar *et al.* 2005)

Table. 2: Reaction Volume and RT-PCR Components

Master Mix Components	Stock	Unit	Final	Unit	Volume
Master Mix	2	X	1	X	1 Sample
Forward primer	10	µM	1	µM	10
Reverse primer	10	µM	1	µM	1
Nuclease Free Water					1
DNA		ng/µl		ng/µl	5
Total volume	Stock	Unit	Final	Unit	3

Table 3 Thermal cycler programming

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

STEPS	°C	M:S	CYCLE
Initial Denaturation	95	05:00	1
Denaturation	95	00:30	30
Annealing	56OR 65	00:30	
Extension	72	01:0	

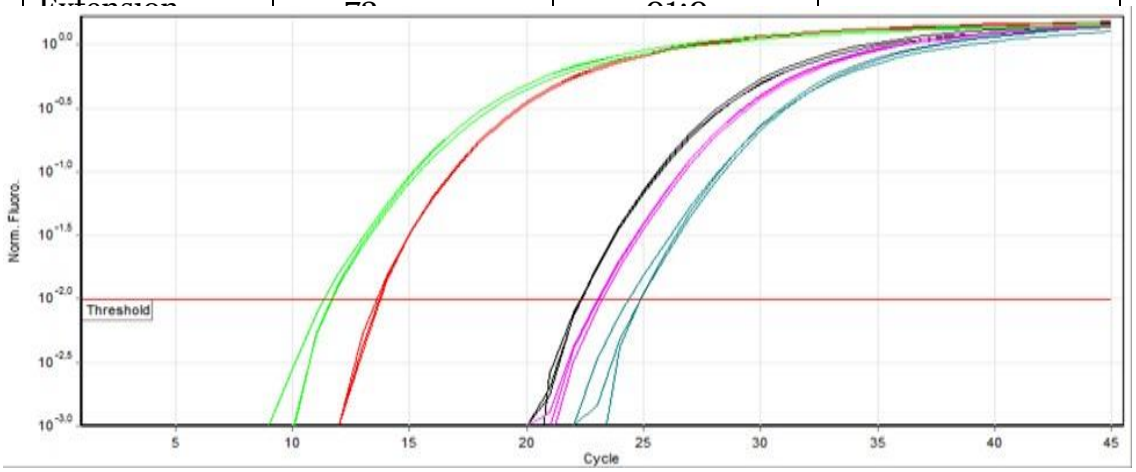

Figure 1 RT-PCR cycle pattern and results of the assay

Statistical analysis

The Students' t-test, the ANOVA test, and the statistical program SPSS-27 were used to analyze the data. Yate's adjustment or the Fisher Exact test were used to determine the significance of the difference between different percentages (qualitative data) using the Pearson Chi-square test (two-test). According to Daniel and Cross (2013), statistical significance was considered to have occurred when the p value was equal to or less than 0.05.

Results

The data collected for this study, which included 250 patients with dental caries (DC) of various ages, was statistically analyzed to produce the results that are provided in this chapter.

1.Age groups

As shown in (Table 4) ,the study include 250 patients with DC, The range of ages was 7 to 59. and the Mean ± SD of their ages was 32.17±15.02 years. The highest age group was 10-19 years (22.0%) .

AGE GROUPS	No.	%
<10years	13	5.2

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

Table 4

10---19	55	22.0
20---29	41	16.4
30---39	50	20.0
40---49	52	20.8
=>50years	39	15.6
Total	250	100
Mean ± SD (Range)	**32.17 ± 15.02**	**(7-59)**

Distribution of age groups of patients included in the study

2.Results of culture

The bacteriological culture findings were shown in (Table 5). Based on the types of isolates, 191 (76.4%) of the cultures produce a single type of growth, 23 (9.2%) produce two types of growth, 8 (3.2%) produce three types of growth, and 28 (11.2%) produce no growth at all.

Based on the type of isolates, 128 (51.2%) of the cultures produced growth of the Streptococcus viridans group (SVG), 58 (23.2%) produced growth of the lactobacillus group, and 75 (30%) produced growth of *Candida albicans*.

Table 5 Distribution of the results of culture

CULTURE RESULTS	**No.**	**%**

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

Type of growth	No growth	28	11.2
	Single growth	191	76.4
	Two growth	23	9.2
	Three growth	8	3.2
Streptococcus viridans group (SVG)	Positive	128	51.2
	Negative	122	48.8
Lactobacillus group	Positive	58	23.2
	Negative	192	76.8
Candida albicans	Positive	75	30.0
	Negative	175	70.0

3.Biofilm formation

The capacity of SVG isolates to create biofilms was shown in **(Table 6)**. It is clear that the moderately biofilm forming isolates constitute the majority of the isolates (56.2%), followed by strong biofilm former isolates (38.0%) and the least were non-biofilm former isolates (5.8%).

Table. 6 : Biofilm formation rate of the SVG isolated from DC

BIOFILM FORMATION		***STREPTOCOCCUS VIRIDANS* GROUP**	
		No	**%**
Biofilm formation	Strong	46	38.0
	Moderate	68	56.2
	None	7	5.8

5.Antibiotic susceptibility test

The antibiotic susceptibility patterns of the Streptococcus viridans group, which was isolated from dental caries, were displayed in (Table 7). according to the data, 79.2% of the SVG isolates were ampicillin-resistant**.** while 17.5% were sensitive and the remaining had intermediate susceptibility (3.3%). Regarding the Penicillin, 90.8% of the isolates were resistant, only 5.0% were sensitive and 4.2% were intermediate.

The results also found that 80.7% of the isolates were resistant to Amoxacillin, 13.4% were sensitive and only 5.9% had intermediate susceptibility. The Erythromycin had similar pattern,

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

since 85.8% of the isolates were resistant, 9.2% of the isolates were sensitive and only 5.0% had intermediate susceptibility. The lowest resistant rate (31.7%) was recorded against Azithromycin, with a sensitivity rate of 62.5%, while the remaining 5.8% of the isolates were intermediately susceptible.
Probably the highest resistant rate of the isolates was against Carbeniicillin (87.4%). While 6.7% and 5.9% of the isolates were sensitive and intermediately susceptible respectively. Regarding the Methicillin, the results found that 89.9%n of the isolates were resistant, and 5.0% were sensitive and intermediate respectively. Lastly, 84.9% of the isolates were resistant to Tetracycline, whereas, 9.2% and 5.9% of the isolates were sensitive and intermediately susceptible respectively.

Table .7: Pattern of SVG's antibiotic susceptibility found in DC.

ANTIBIOTICS	SENSI.		INTERME.		RESIST.	
	No.	%	No.	%	No.	%
Ampicillin	21	17.5	4	3.3	95	79.2
Pipracillin	30	25.0	8	6.7	82	68.3
Ciprofloxacin	43	35.8	13	10.8	64	53.3
Azithromycin	75	62.5	7	5.8	38	31.7
Erythromycin	11	9.2	6	5.0	103	85.8
Cefotaxime	58	48.3	1	0.8	61	50.8
Amoxicillin	16	13.4	7	5.9	96	80.7
Tetracycline	11	9.2	7	5.9	101	84.9
Carbencillin	8	6.7	7	5.9	104	87.4
Vancomycin	24	20.2	7	5.9	88	73.9
Methicillin	6	5.0	6	5.0	107	89.9
Penicillin	6	5.0	5	4.2	108	90.8
Clindamycin	44	37.0	11	9.2	64	53.8

6.Minimum inhibitory concentration

(Table 8) Summarize the Mean ± SD minimum inhibitory concentrations and ranges of certain antibiotics of 119 SVG isolates recovered from DC. As it is clear from the table, the Mean ± SD MIC of Penicillin (mg/l) was 5.53 ± 2.34 mg/L with a range of 0.03-9.70 mg/L. The Mean ± SD MIC of Clindamycin (mg/l) was 3.50 ± 2.96 mg/L with a range of 0.02-8.30 mg/L. regarding the Erythromycin, the results found that the Mean ± SD MIC was 8.74 ± 3.57 mg/l and the range was 0.01- 12.50 mg/L .
The results also found that the Mean ± SD MIC of Amoxicillin (mg/L) was 2.83 ± 1.47 mg/L

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

with a range of 0.12-5.90 mg/L. additionally, the mean ± SD MIC of Azithromycin (µg/ml) was 1.71 ± 2.00 µg/ml and the range was 0.10-6.20 µg/ml. Whereas, the mean ± SD MIC of Ciprofloxacin (µg/ml) was 10.05 ± 8.88 µg/m with a range of 0.20-19.70 µg/ml. while that of the Cefotaxime (mg/L) was5.02 ± 5.03 mg/ml and a range of 0.01-12.40 mg/ml and lastly, the mean ± SD MIC of Vancomycin (µg/ml) was 1.49 ± 0.81 µg/ml with a range of 0.01-12.40 µg/ml.

Table 8 Minimum inhibitory concentration of certain antibiotics of SVG

ANTIBIOTICS	***STREPTOCOCCUS VIRIDANS* GROUP**	
	Mean ± SD of MIC	**Range of MIC**
MIC Penicillin (mg/L)	5.53 ± 2.34	(0.03-9.70)
MIC Clindamycin (mg/mL)	3.50 ± 2.96	(0.02-8.30)
MIC Erythromycin(mg/L)	8.74 ± 3.57	(0.01-12.50)
MIC Amoxicillin (mg/L)	2.83 ± 1.47	(0.12-5.90)
MIC Azithromycin (µg/ml)	1.71 ± 2.00	(0.10-6.20)
MIC Ciprofloxacin (µg/ml)	10.05 ± 8.88	(0.20-19.70)
MIC Vancomycin (µg/ml)	1.49 ± 0.81	(0.11-2.50)
MIC Cefotaxime (mg/L)	5.02 ± 5.03	(0.01-12.40)

7.Gene Detection

The molecular investigations represented by detection of certain genes using PCR technique among 50 isolates of SVG was shown in **(Table 9).** The *16sRNA* gene was detected in 43(84.3) of the isolates and not detected in 7 (15.7%). The *emB* gene was detected in 36(72.0%) of the isolates but not in 14 (28.0%) of the isolates. The *PBP1a* gene was detected in 41 (82.0%) of the isolates while it was absent in 9 (18.0%) of the isolates. Whereas, the PBP2b gene was found in 45 (90.0%) of the isolates but not in 5(10.0%) of them.

The *Van A* gene was detected in 37 (74.0%) of the isolates, while it is not detected in 13 (26.0%) of the isolates. Similarly, the *Van C* gene was found in 38 (76.0%) of the isolates and it was absent in 12 (24.0%) of these isolates. Lastly the *tet M* gene was detected in 40 (80.0%) of the isolates bur it was absent in 10 (20.0%) of the isolates.

Table 9 Certain Genes detection rates of SVG isolates.

ANTIBIOTICS	***STREPTOCOCCUS VIRIDANS GROUP* (n 50)**

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

	Detected		Not detected	
	No	%	No	%
PCR 16sRNA gene	43	84.3	7	15.7
PCR *emB* gene	36	72.0	14	28.0
PCR *PBP1a* gene	41	82.0	9	18.0
PCR *PBP2b* gene	45	90.0	5	10.0
PCR *Van A* gene	37	74.0	13	26.0
PCR *Van C* gene	38	76.0	12	24.0
PCR *tet M* gene	40	80.0	10	20.0

Conclusion

For bacteriological culture, more than 80% of dental caries swabs were positive. The dental caries swab culture with the greatest positive rate is formed by the Streptococcus viridans group.the SVG isolates showed higher resistance rate against 13 commercially available antibiotics .The SVG isolates had detectable antibiotics resistant genes namely, ermB gene, PBP1a gene, PBP2b gene, Van A gene, Van C gene and tet M gene with a high rates**.**

Discussion

The importance of the current study in Al-Nasiriyah as well as in whole Iraq is emerged from different aspects, firstly, the high prevalence of dental caries affecting all ages as a result of low standard of education, insufficient dental services (Al-Mendalawi and Karam, 2014).Secondly, the availability of risk factors including bad quality of demineralized water, high rate of diabetes mellitus, obesity and other immunocompromised factors as well as certain community bad habits, more importantly, the consumption of snacks between meals and neglected mouth hygiene (Zolnikov, 2013, Joury *et al.* 2016).

The SVG detection rate was much higher (65.4%) in the 40–49 age range. It is well known that patients begin to complain of immunological compromise at this age, most likely as a result of the high prevalence of chronic illnesses (Chen *et al.* 2013). In addition to the fact that bone resorption likely began at this age because of dietary deficiencies in specific minerals, the features of the oral microbiota may also become more pathogenic at this age (Bell *et al.*, 2023).

The bulk of the obtained swabs (76.4%) had a single bacterial growth, according to the results. This clearly shows how to collect and culture dental swabs with the least amount of contamination possible.

In this regard, it is also noteworthy that the majority of culture outcomes (51.2%) result in the growth of the Streptococcus viridans group (SVG), followed by the lactobacillus group (23.2%) and the Candida albicans group (30%).These findings are in line with other research (Aas *et al.* 2008, Tanner *et al.* 2015) that confirm that SVG are mostly to blame for dental caries.

This high rate of SVG infection was consistent with research from global locations such as India (Jindal *et al.* 2020), as well as studies carried out in the Mediterranean and North African nations

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

that surround us (Elamin *et al.* 2021). Given the availability of all dental caries risk factors in Iraq, including those pertaining to patient characteristics, family history, oral hygiene, and feeding and eating behaviors, this result was actually not out of the ordinary. In addition to the general and personal health services that have been neglected in Iraq over the previous 20 years (Hotez *et al.* 2012).

Proteases might be released and hemolysin could be produced by the SVG that was isolated from DC. It was also found that these SVG isolates were capable of forming biofilms. These virulence factors seem to be essential to the pathogenesis of DC (Ardizzoni et al., 2018; Ryan and Ray, 2004). According to above results, it can be concluded that these SVG isolates recovered from DC swabs are resistant to most commercially available antibiotics.

Nutritionally variable streptococci were formerly included in SVG, but have now been transferred to the new genus Abiotrophia as a result of genetic evidence (Whiley and Beighton 1998).

In this technique, the least effective concentration in mg/mL or µg/ml were measured, The minimum inhibitory concentration (MIC) was another name for them. Our findings indicated that the Mean ± SD of MIC of above antibiotics was ranged from 1-10 mg/mL or µg/ml.

The lowest concentration at which detectable bacterial growth is inhibited is known as the minimum inhibitory concentration, or MIC, of an antibiotic or antibacterial. The bacteria determines the minimum inhibitory concentration (MIC) of an antibiotic, the human person who has been infected, and the drug itself. Other measurement units include milligrams per liter (mg/L) and micrograms per milliliter (mg/mL) (McKinnon and Davis 2004). The lowest concentration of an antibacterial agent needed to prevent visible bacterial growth is known as the minimum inhibitory concentration (MIC), whereas the lowest concentration needed to cause bacterial death is known as the minimum bactericidal concentration (MBC) of an antibacterial agent. According to Turnidge *et al.* (2003), a substance is more bactericidal the farther it is from the MIC to the MBC.

Once included in the SVG, nutritionally variable streptococci have been moved to the new genus Abiotrophia based on DNA data (Whiley and Beighton 1998).

Our findings regarding penicillin resistance were greater than those of Chun *et al.* (2015), who found that there was an uneven distribution across groups and an overall non-susceptibility to penicillin of 40.0% (resistant, 11.2%, and intermediately resistant, 28.8%).

16 antimicrobials' sensitivity to 161 viridans streptococci from the typical flora of 28 old people was tested in vitro. The double disc test and PCR gene identification were used to investigate the resistance mechanisms of erythromycin-resistant isolates. While none of the isolates shown high-level resistance (MIC > 4 mg/L), 16.8% of them were non-susceptible to penicillin (MIC ≥ 0.25 mg/L). 22.4%, 27.3%, 13.0%, 1.9%, and 1.9% of the isolates had resistance to erythromycin, tetracycline, quinupristin/dalfopristin, levofloxacin, and moxifloxacin, respectively. Thirteen percent of the isolates had both erythromycin and tetracycline resistance. Eighty-six percent of the isolates resistant to erythromycin had the M phenotype, whereas 19 percent had the MLSB (macrolide, lincosamide, streptogramin B) phenotype. The isolates with the M phenotype had the mef (A) gene, while the isolates with the MLSB phenotype contained the erm (B) gene (Seppala *et al.* 2003). These results are similar to that obtained by our study, but with lower rates, since the

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

ermB gene was detected in 72.0% and the tet M gene was detected in 80.0% of our isolates. However, combined resistant to erythromycin and Tetracycline was not detected in our study.

Our findings are somewhat in line with those of a multicenter research that assessed 156 occurrences of bacteremic streptococcal infections in individuals who were neutropenic. The most common species were *Streptococcus oralis* (26.3%), *Streptococcus pneumoniae* (26.3%), *Streptococcus agalactiae* (11.5%), *Streptococcus mitis* (9%), and *Streptococcus pyogenes* (5.8%). It was discovered that 4 (2.6%) of the strains had intermediate penicillin resistance. One (0.6%) strain had a minimum inhibitory concentration (MIC) of 8 mg/liter for penicillin. Penicillin susceptibility was shown to be lower in isolates of *S. pneumoniae* (4.9%), *S. mitis* (7.1%), and *S. oralis* (14.6%), but not in *S. agalactiae* or *S. pyogenes*. For other antimicrobials, the resistance and intermediate resistance rates were: amoxicillin, 1.3 and 3.2%; erythromycin, 16 and 2.6%; clindamycin, 5.8 and 0%; and ciprofloxacin, 1.9 and 7.7%. (Reinert *et al.* 2001).

Thirteen antimicrobial drugs' in vitro susceptibilities were found to be compatible with 207 SVG isolates that were obtained from individuals suffering from various illnesses. There were nine different species with varying susceptibilities. *Streptococcus oralis* had the highest frequency of high level penicillin resistance (MIC · 4.0 mg/L), with 35% of cases, followed by *Streptococcus mitis* (20%) and *Streptococcus salivarius* (8%). But *S. salivarius* had the lowest penicillin susceptibility percentage (50%) of all species. Additionally, the majority of *S. oralis* isolates (55%) developed macrolide resistance, whereas *Streptococcus mutans* did not exhibit any.
The most effective -lactam against isolates resistant to penicillin was imipenem. MIC 90s of 2, 1, and 0.25 mg/L for ofloxacin, vancomycin, and teicoplanin, respectively, indicated good in vitro activity against all isolates. The majority of these isolates were susceptible to chloramphenicol, and none of them showed high-level resistance to gentamicin. This suggests that susceptibility varies according on the species, particularly to penicillin, macrolides, and tetracycline. The variation in susceptibilities amongst SVG species highlights the significance of precise identification and the requirement for ongoing antibiotic resistance surveillance (Bilavsky *et al.* 2008). Apart from the site of isolation, these results are partially similar to our results.

References

1. Aas, J.A., Griffen, A.L., Dardis, S.R., Lee, A.M., Olsen, I., Dewhirst, F.E., *et al.* (2008). Bacteria of dental caries in primary and permanent teeth in children and young adults. J. Clin. Microbiol. 46:1407-1417.
2. Ajdic, D., McShan, W.M., McLaughlin, R.E., Saviæ, G., Chang, J., Carson, M.B., *et al.* (2002). Genome sequence of Streptococcus mutans UA159, a cariogenic dental pathogen. Proc. Natl. Acad. Sci. U S A. 99:14434–9.
3. Al-Mendalawi, M.D. and Karam, N.T. (2014). Risk factors associated with deciduous tooth decay in Iraqi preschool children. Avicenna J. Med. 4(1): 5–8.
4. Ardizzoni, A., Pericolini, E., Paulone, S., Orsi, C.F., Castagnoli, A., Oliva, I., *et al.* (2018) *In vitro* effects of commercial mouthwashes on several virulence traits of *Candida albicans*, viridans streptococci and *Enterococcus faecalis* colonizing the oral cavity.

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

PLoS ONE 13(11): e0207262.

5. Bell, V.; Rodrigues, A.R.; Antoniadou, M.; Peponis, M.; Varzakas, T.; Fernandes, T. (2023).An Update on Drug–Nutrient Interactions and Dental Decay in Older Adults. Nutrients 15, 4900. https: //doi. org/10.3390/nu15234900.
6. Bilavsky,AEliahou,R.etal.(2008)..Effect of benzathine penicillin treatment on antibiotic susceptibility of *viridans streptococci* in oral flora of patients receiving secondary prophylaxis after rheumatic fever. Journal of Infection 56(4):244-8DOI:10.1016 /j.jinf. 2008. 01.006.
7. Chen, X., Clark, J.J., Preisser, J.S., Naorungroj, S., and Shuman, S. (2013). Dental Caries in Older Adults in the Last Year of Life. J Am Geriatr Soc. 61(8): 1345- 50.
8. Clinical and Laboratory Standards Institute, CLSI. (2019).29th Ed. Performance standards for antimicrobial susceptibility testing. CLSI Supplement M100. Clinical and laboratory Standard Institute, Wayne, USA.
9. Daniel, W.W. and Cross, C.L. (2013). Biostatistics: A Foundation for Analysis in the Health Sciences. 10th Ed. John Wiley and Sons Inc, USA.
10. De Oliveira, K.M.H., Nemezio, M.A., Romualdo, P.C., da Silva, R.A.B., de Paula, E., Silva, F.W., *et al.* (2017). Dental Flossing and Proximal Caries in the Primary Dentition: A Systematic Review. Oral Health & Preventive Dentistry. 15 (5): 427-34.
11. Dowson, C.G., Hutchison, A., Woodford, N., Jonson, A.P., George, R.C., Spratt, B.G. (1990). Penicillin-resistant viridans streptococci have obtained altered penicillin-binding protein genes from penicillin-resistant strains of *Streptococcus pneumoniae*. Proc. Natl. Acad. Sci. USA. **87**, 5858-62.
12. Elamin, A., Garemo, M. and Mulde, A. (2021). Determinants of dental caries in children in the Middle East and North Africa region: a systematic review based on literature published from 2000 to 2019. BMC Oral Health, 21:237-67.
13. Ergin, A., Ercis, S. and Hascelik G. (2003). In vitro susceptibility, tolerance and MLS resistance phenotypes of Group C and Group G streptococci isolated in Turkey between 1995 and 2002. Int. J. Antimicrob. Agents. 22:160 –3.
14. Flemming,H.C , Wingender,J. Szewzyk,U . Steinberg,P. Rice,S.A. , Kjelleberg,S.(2016). Biofilms: an emergent form of bacterial life.Nat Rev Microbiol. 14(9) :563-75. doi: 10.1038/nrmicro.2016.94.
15. Gershon, A.S., de Azavedo, J.C., McGeer, A., Ostrowska, K.I., Church, D., Hoban, D.J., *et al.* (2002). Activities of new fluoroquinolones, ketolides, and other antimicrobials against blood culture isolates of viridans group streptococci from across Canada, 2000. Antimicrob. Agents Chemother. 46:1553–6.
16. Hotez PJ, Asojo OA, Adesina AM (2012) Nigeria: “Ground Zero" for the High Prevalence Neglected Tropical Diseases. PLoS Negl Trop Dis 6(7): e1600. https://doi.org/10.1371.
17. Hurley, E., Barrett, M.P. J., Kinirons, M., Whelton, H., Ryan, C. A., Stanton, C., et al. (2019). microbiome of children with severe-early childhood caries to the salivary microbiome of caries-free children. BMC oral Health, 19:13-33.

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

18. Jensen, A., Scholz, C.F. and Kilian, M. (2016). Re-evaluation of the taxonomy of the Mitis group of the genus Streptococcus based on whole genome phylogenetic analyses, and proposed reclassification of *Streptococcus dentisani* as Streptococcus oralis subsp. dentisani comb. nov., *Streptococcus tigurinus* as *Streptococcus oralis* subsp. tigurinus comb. nov., and *Streptococcus oligofermentans* as a later synonym of *Streptococcus cristatus*. Int. J. Syst. Evol. Microbiol. 66:4803–20.
19. Jindal, L., Dua, P., Mangla, R., Gupta, K., Vyas, D.and Gupta, P. (2020). Dental caries in relation to socioeconomic factors of 6 and 12-year-old schoolchildren of Paonta Sahib, Himachal Pradesh, India: An epidemiological study. International Journal of Clinical Pediatric Dentistry (2020): 10.5005/jp-journals-10005-1805.
20. Joury, E., Al-Kaabi, R., Tappuni, A.R. (2016). Constructing public health policies in post crisis countries: lessons to learn from the associations between free-sugars consumption and diabetes, obesity and dental caries before, during and after sanctions in Iraq. J. Public. Health 24:563-69.
21. Laudenbach, J.M. and Simon, Z. (2014). Common Dental and Periodontal Diseases: Evaluation and Management. The Medical Clinics of North America. 98 (6): 1239-60.
22. MacFaddin, J.F. (2000) Biochemical Tests for Identification of Medical Bacteria. 3rd Edition, Lippincott Williams & Wilkins, Philadelphia.
23. Malhotra-Kumar, S., Lammens, C., Piessens, J. and Goossens, H. (2005). Multiplex PCR for Simultaneous Detection of Macrolide and Tetracycline Resistance Determinants in Streptococci. Antimicrobial Agents and Chemotherapy, 49 (11): 4798–4800.
24. Marsh, P. (1994). Microbial ecology of dental plaque and its significance in health and disease. Advances in Dental Research. 8 (2): 263-71.
25. Marsh, P.D., Head, D.A. and Devine, D.A. (2015). Dental plaque as a biofilm and a microbial community-Implications for treatment. J.Oral Biosci. 57 (4): 185–191.
26. McKinnon, P.S. and Davis, S.L. (2004). Pharmacokinetic and pharmacodynamic issues in the treatment of bacterial infectious diseases. European J. Clin. Microbiol. Infect. Dis. **23** (4): 271–88.
27. Mendes, R.E., Flamm, R.K., Hogan, P.A., Ross, J.E. and Jones, R.N. (2014). Summary of linezolid activity and resistance mechanisms detected during the 2012 LEADER surveillance program for the United States. Antimicrob. Agents Chemother. 58(2):1243–7.
28. Moosavian,M. Ghadri,H. Samli,Z.(2018).Molecular detection of vanA and vanB genes among vancomycin-resistant enterococci in ICU hospitalized patients in Ahvaz in southwest of Iran. Infection and Drug Resistance.11. 2269–2275.
29. Reinert, R.R., von Eiff, C., Kresken, M., Brauers, J., Hafner, D., *et al.* (2001). Nationwide German multicenter study on the prevalence of antibiotic resistance in streptococcal blood isolates from neutropenic patients and comparative in vitro activities of quinupristin-dalfopristin and eight other antibiotics. J. Clin. Microbiol. 39:1928–31.
30. Richards, V.P., Palmer, S.R., Pavinski, B., Qin, X., Weinstock, G.M., Highlander, S.K., et al. (2014). Phylogenomics and the dynamic genome evolution of the genus

The Peerian Journal
Open Access | Peer Reviewed
Volume 26, January, 2024
ISSN (E): 2788-0303
Website: www.peerianjournal.com
Email: editor@peerianjournal.com

Streptococcus. Genome Biol. Evol. 6:741-53.
31. Ryan, K.J. and Ray, C.G. eds. (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 293-4.
32. Schwendicke, F., Tzschoppe, M. and Paris, S. (2015). Radiographic caries detection: A systematic review and meta-analysis Journal of Dentistry 48: 924-33.
33. Seppala, H., Haanpera, M., Al-Juhaish, M., Jarvinen, H., Jalava, J. and Huovinen, P. (2003). Antimicrobial susceptibility patterns and macrolide resistance genes of viridans group streptococci from normal flora. J Antimicrob. Chemother. 52:636–44.
34. Seppala, H., Haanpera, M., Al-Juhaish, M., Jarvinen, H., Jalava, J. and Huovinen, P. (2003). Antimicrobial susceptibility patterns and macrolide resistance genes of viridans group streptococci from normal flora. J Antimicrob. Chemother. 52:636–44.
35. Sitkiewicz, I. (2018). How to become a killer, or is it all accidental? Virulence strategies in oral streptococci. Mol. Oral Microbiol. 33: 1–12.
36. Struzycka, I. (2014). The oral microbiome in dental caries. Pol. J. Microbiol. 63: 127– 35.
37. Tanner, T., Pakkila, J., Karjalainen, K., *et al.* (2015). Smoking, alcohol use, socioeconomic background and oral health among young Finnish adults. Community Dentistry and Oral Epidemiology. 43(5):406-14.
38. Taylor EJ, *et al.* (2010) Fusel alcohols regulate translation initiation by inhibiting eIF2B to reduce ternary complex in a mechanism that may involve altering the integrity and dynamics of the eIF2B body. *Mol Biol Cell* 21(13):2202-16
39. Turnidge, J.D., Ferraro, M.J. and Jorgensen, J.H. (2003). Susceptibility Test Methods: General Considerations. In Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller,and Yolken, R.H. (Eds.). *Manual of Clinical Microbiology* (8th ed.). Washington: American Society of Clinical Microbiology. p. 1103.
40. Wagle, M., D'Antonio, F., Reierth, E., Basnet, P., Trovik, T.A., Orsini, G., *et al.* (2018). Dental caries and preterm birth: a systematic review and metaanalysis. BMJ Open. 8(3):e018556.
41. Whiley, R.A. and Beighton, D. (1998). Current classification of the oral streptococci. Oral Microbiol. Immunol. 13:195–216.
42. Zapun,A. Martel,C.C. Verne,T. (2008).Penicillin-binding proteins and b-lactam resistance. FEMS Microbiol Rev 32 :361–385. DOI:10.1111/j.1574-6976.2007.00095.
43. Zolnikov, T.R. (2013). The Maladies of Water and War: Addressing Poor Water Quality in Iraq. Am. J. Pub. Health 103(6): 980-90.