Preparation and evaluation activity of silica nanoparticles against bacterial oral infections: in vitro study

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KEYWORDS

Dental infections, viridans group, multidrug resistance, antibacterial activity, silica nanoparticles.

ABSTRACT

Background: Oral infections are commonly encountered among populations and frequently result in chronic inflammatory conditions that impact the dental structures (e.g., caries), the gingival tissues surrounding the dental elements (e.g., gingivitis and endodontic lesions), as well as the supporting structures of the teeth (e.g., periodontitis). Objectives: The objective of this study is to ascertain the minimum inhibitory concentrations of silica nanoparticles in order to evaluate the antibacterial efficacy of these nanoparticles against oral pathogens. Materials and Methods: A total of 100 clinical samples were collected from individuals suffering from diverse oral infections. They were diagnosed based on the culture and microscopic characteristics, as well as the diagnosis with Vitek2 device for the purpose of diagnosis for the level of species. Molecular identification of Viridians Streptococci was performed using PCR to detect the 16sRNA gene. The study also involved the synthesis of silica nanoparticles (SiNPs) using the sol-gel technique. Characterization of the SiNPs was conducted through X-ray diffraction (XRD), transmission electron microscopy (TEM), and field emission scanning electron microscopy with energy-dispersive X-ray spectroscopy (FESEM-EDX). The minimum inhibitory concentration (MIC) of the SiNPs against viridans Streptococci was evaluated using a microdilution assay with the blue-resazurin staining method. The agar well diffusion technique was utilized to assess the antibacterial efficacy of biosynthesized silica nanoparticles against Viridans Streptococci in comparison to a standard antibiotic, utilizing the minimum inhibitory concentration (MIC) as a metric. Results: Fifty pathogenic bacterial strains were obtained from clinical specimens, with the genus Streptococcus comprising 27 of these isolates, which corresponds to 54% of the overall total, thereby constituting the highest proportion observed among all species. Molecular identification of Viridans Streptococci in this study, showed that 10/10(100%) isolates were PCR positive for 16sRNA gene has been applied to pathogenic viridians streptococcal only, which include S. mitis, S. oralis and S. sanguinis. Multidrug-resistant isolates showed significant resistance to various antibiotics, including Erythromycin, Cefotaxime, Cefepime, Ceftriaxone, and Penicillin. Clindamycin also showed resistance. Augmentin showed the highest antimicrobial activity, while Trimethoprim-sulfamethoxazole, Ampicillin, and Vancomycin showed commendable activity. The MIC for the synthesized silica nanoparticles against Viridans Streptococci was determined to be 3.13 mg/mL across all bacterial isolates. The study showed that the bio-synthesized silica nanoparticles had a higher inhibition than the standard antibiotic disc Vancomycin (VA) at a concentration of 3.13 mg/ml, as the average diameter of inhibition reached 26.2 mm, respectively. Conclusion: Silica nanoparticles demonstrated significant efficacy in combating Viridans Streptococci, which are implicated in the etiology of oral infections.

1. Introduction

The oral cavity represents a distinctive and diverse bacterial ecosystem, characterized by particular ecological niches that arise from the presence of various surfaces favorable for bacterial colonization, in conjunction with significant fluctuations in environmental factors such as temperature, pH, redox potential, and nutrient availability; these parameters, in conjunction with human behavioral determinants including oral hygiene practices, dietary choices, and tobacco consumption, as well as genetic predispositions and holistic health conditions, collectively influence the structure of the resident microbial populations [1]. The oral environmental conditions are subject to alterations under particular circumstances, which may consequently interfere with the typical commensal relationship between the host and the indigenous oral microflora; this disruption can lead to a shift in the equilibrium toward an increased vulnerability to oral diseases, especially an elevated incidence of dental caries and periodontal diseases [2]. Among the diverse array of microorganisms residing in the oral cavity, the genus Streptococcus is extensively recognized as the most significant [3], especially during the early stages of colonization [4]. Streptococci exhibit a facultative anaerobic characteristic, with specific strains demonstrating a strictly anaerobic behavior; they are categorized as Gram-positive, non-motile, non-spore-forming, and catalase-negative cocci that characteristically appear in pairs or chains, certain strains are frequently identified as components of the normal microbiota in the oral and gastrointestinal tracts, while others exhibit pathogenic properties [5]. These streptococci exhibit the ability to attach to

components of the salivary pellicle and to various other oral bacterial species through adhesin proteins located on their cellular membranes; thus, they, in conjunction with a multitude of other oral microorganisms, contribute to the establishment of multispecies biofilms [3].

In recent times, the phenomenon of antibiotic resistance has emerged as a paramount global challenge, stemming from the overuse of broad spectrum antibiotics, unwarranted prescriptions, suboptimal administration of antibiotics, and incomplete courses of antibiotic therapy. The capacity of diverse bacterial strains to endure the effects of conventional antibiotics has necessitated the exploration of various strategies for the management of antibiotic-resistant bacteria. Research has demonstrated that nanoparticles infused with antibiotics possess numerous significant benefits; indeed, these nanoparticles are increasingly regarded as nanoantibiotics due to their superior antimicrobial properties [6; 7]. Among the various nanomaterials, significant attention has been focused on silicon nanoparticles due to their remarkable capabilities in drug delivery. Furthermore, these nanoparticles are characterized by their biodegradable nature, diminished toxicity, and their ability to activate macrophages [8]. Silica has been acknowledged as an abrasive material predominantly employed in oral hygiene products; its further functional adaptability includes its role as a dentin occlusive agent utilized for the mitigation of sensitivity, enhancement of tooth whiteness, and the process of remineralization of dental structures [9].

The present study was undertaken with the objective of isolating and characterizing Streptococcus species from oral infections. Furthermore, the study aimed to evaluate the susceptibility of these isolates to a range of antibiotics as well as to synthesize silica nanoparticles and ascertain the Minimum Inhibitory Concentration (MIC) values associated with these nanoparticles. In addition, the research sought to evaluate the antibacterial activity of silica nanoparticles against Viridans Streptococci in comparison to a standard antibiotic.

Methodology

2.1. Samples collection

A total of one hundred samples were procured from diverse oral infections under the meticulous supervision of the dentist and with the informed consent of the patient, in accordance with ethical guidelines, at the Al-Kut Special Teeth Center. Based on the patient questionnaire, the ages of the participants varied from 9 to 70 years. The specimens were obtained during the timeframe extending from October 2023 to December 2023. All specimens were collected utilizing sterile swabs and subsequently placed in aseptic containers containing transport media.

2.2. Isolation and identification

Clinical specimens were cultured on blood agar (Liofilchem, Italy), followed by subculturing on selective streptococcus agar (Himedia, India) at a temperature of 37 °C for a duration of 48 hours under anaerobic conditions in candle jar. The identification of these isolates was performed utilizing both traditional and molecular methodologies, which included culture and microscopic analysis, biochemical assays, the Vitek-II system (bioMerieux, France), and was subsequently validated through 16S rRNA sequencing. The bacterial isolates were preserved in BHI broth supplemented with 20% glycerol at a temperature of -20°C [10].

2.3. 16S rRNA Based Detection of Viridians Streptococcus

The positive streptococcal isolates, identified by morphological, biochemical characteristics and Vitek2 system, were subjected to molecular identification of 16S rRNA gene sequencing. For that, DNA isolation and purification of bacterial samples were done by using DNA extraction kit provided by Geneaid presto mini gDNA . The purified genome DNA were then run on agarose electrophoresis to confirm its presence and purity. The DNA samples were then stored at -20ºC until use. For the amplification of the 16S rRNA gene, conventional polymerase chain reaction (PCR) methodology was employed to amplify the target gene utilizing specific primers (Forward, 5′-GTGAA TACGT TCCCG

GGCCT-3′ and Reverse, 5′-GGGTT YCCCC RTTCR GAAAT-3′), wherein Y represents either C or T and R signifies either A or G (Alpha DNA, Canada). The thermal cycling parameters implemented encompassed an initial denaturation step at 94ºC for a duration of 5 minutes; followed by 35 cycles comprising denaturation at 94ºC for 30 seconds, annealing at 59ºC for 40 seconds, and extension at 72ºC for 30 seconds, culminating in a final extension phase at 72ºC for 10 minutes. Subsequently, the PCR amplicons were subjected to agarose gel electrophoresis and subsequently visualized under ultraviolet (UV) light. Diagnosis using PCR technology by examining the 16S rRNA gene has been applied to pathogenic viridians streptococcal only, which include *S. mitis, S. oralis* and *S. sanguinis*.

2.4. Antibiotic susceptibility test

This test is performed by Kirby-Bauer procedure on Muller Hinton agar [11]. An inoculums isolates were prepared by emulsifying colonies from an overnight culture in sterile normal saline until a turbidity equivalent to 0.5 McFarland solution standards. The bacterial suspension was uniformly spreaded on Muller Hinton agar (Liofilchem, Italy) using sterile swab and left to dry. With sterile forceps selected antibiotic disks (Liofilchem, Italy) Penicillin G (10 IU), Ampicillin (10 µg), Tetracycline (30 µg), Vancomycin (30 µg), Erythromycin (15 µg), Cefotaxime (30 µg), Ceftriaxone (30 µg), Doxycycline (30 µg), Cefepime (30 µg), Meropenem (10 µg), Levofloxacin (5 µg), Amoxicillin-clavulanic acid (30 µg), Trimethoprim-sulfamethoxazole (25 µg), Clindamycin (2 µg)) were placed on the inoculated plates and incubated at 37°C for 48 hrs in an inverted position. After this period of incubation, the diameters of inhibition zones were noted and measured by a ruler in (mm), results were determined according to CLSI (2023).

2.5. Silica nanoparticle biosynthesis and characterization

2.5.1. Preparation of SiNPs

The SiNPs nanoparticles were prepared through sol-gel process [12]. The procedure involves 20 ml Tetraethoxysilane (TEOS) (Glentham, United Kingdom) was first added into a homogenous mixture of water (20 ml) and ethanol (80 ml) as a solvent. Followed by stirring and subsequent addition of 25 ml of urea (0.06 g dissolved in 25 ml of water) one drop for every second with stirring, then leave It for one hour, then add 1N of NaOH until the solution turns foggy (35 ml to 50 ml). Then the precipitate is washed with a decantation several times, it is then dried and burned at a temperature of 800C°.

2.5.2. Characterization of SiNPs

The crystalline structure of the samples was evaluated by X-Ray Diffraction (XRD, PW1730, Philips). The surface topology, morphological characteristics, and elemental composition of the silicon nanoparticles (SiNPs) were meticulously characterized utilizing a high-resolution field emission scanning electron microscope (FESEM-EDX) (MIRA III, Tescan). The structural integrity and particle dimensions were scrutinized through a transmission electron microscope (TEM) (Zeiss-EM10C-100 KV, Germany).

2.5.3. Determination of the MIC of Viridians Streptococci

Firstly resazurin reagent (CDH, India) was prepared by dissolving resazurin 337.5 mg in 50 ml of DW, and stored at 4C° for a maximum of 2 weeks after preparation [13]. We elucidated the minimum inhibitory concentration (MIC) of the synthesized nanoparticles against viridians Streptococci bacteria by employing the Micro-broth dilution method, which necessitated the utilization of sterile 96-well microplates [14]. Initially, serial dilutions of nanoparticles (50, 25, 12.50, 6.25, 3.13, 1.56, 0.78, 0.39, 0.20, and 0.10 mg/mL, respectively) were prepared, followed by the introduction of 100 µL of each sample into the wells of a microplate. Subsequently, 90 µL of Mueller Hinton Broth (Liofilchem, Italy) medium and 10 µL (Merck) of a microbial suspension, standardized to 0.5 McFarland, were incorporated into the microplate wells containing the nanoparticles. The maximal concentration of silica nanoparticles was situated in Column 1, whereas the minimal concentration was identified in Column 10. Column 11 served as the negative control (media only), and Column 12 functioned as the

positive control (bacterial suspensions only). Following an incubation period of 48 hours at 37 °C, resazurin was introduced into all wells (10 µL per well), and further incubation was conducted for 2–4 hours to facilitate the observation of colorimetric changes. If there is an interaction and change the color turns pink, meaning there is growth of bacteria and their ability to form biofilms, and if it does not change the color remains blue and there is no growth of bacteria, meaning it has no ability to form biofilms.

2.5.4. Minimun inhibition concentration (MIC) of silica nanoparticles

The Agar well-diffusion method was used for this experiment and in the following steps [15]:

• 10 µl of BHIB-stimulated bacterial growth was added to 5 ml of saline solution per tube .

• Use a vortex shaking device to homogenize the prepared saline solution and bacteria .

• Immersion of a cotton swab under sterile conditions inside a culture chamber (HOOD) in the diluted bacterial growth, spread it all over the dish in a spreading manner in all directions in order to plant the bacteria on the prepared Mueller-Hinton medium, and leave It for a few minutes until it dries .

• Make a hole in each dish using a cork drill with a diameter of 5 mm, then put 50 microliters of nanoparticle concentrate in addition to placing the standard antibiotic tablet Vancomycin and distilled water which represents the standard control .

• It was kept in the incubator for 48 hours at 37 C° .

• Use a graduated ruler with a millimeter unit to measure the diameters of the inhibition zones and the contents in the culture dish .

• The inhibition zones of silica nanoparticles and the antibiotic (VA) disk are measured compared to the standard control .

2.6. Statistical Analysis

The statistical analysis of all the results were done by using the system SPSS IBMOversionO20 software. For quantitative variables, the means and standard deviations (SD) were employed. Frequencies and percentages were used for qualitative variables. The means of two groups were compared using the independent T-test. P-value <0.05 was considered statistically significant.

2. Result and Discussion

The results showed that among 100 samples collected, 27 isolates had basic characteristics of *Streptococcus spp.* The microbial colonies displayed features such as translucency or opacity, ranging in color from white to gray on Streptococcus selection agar, whereas on blood agar, they were observed as small, pin-shaped, mucoid or smooth, glossy, and translucent (Figure 1, 2). The biochemical tests of *Streptococcus spp* revealed positive to Gram stain (Figure 3), negative to catalase and unable to produce hemolysin exhibiting gamma hemolysis on blood agar medium. (Table 1) and (Figure 4) shows the Vitek 2 device analysis for *Streptococcus spp* recognition.

Figure 1. *Streptococcus spp.* colonies on blood agar during 48 hours anaerobic incubation period at 37 °C

Figure 2. *Streptococcus spp.* on streptococcal selection agar after incubation at 37c for 48h under anaerobic condition

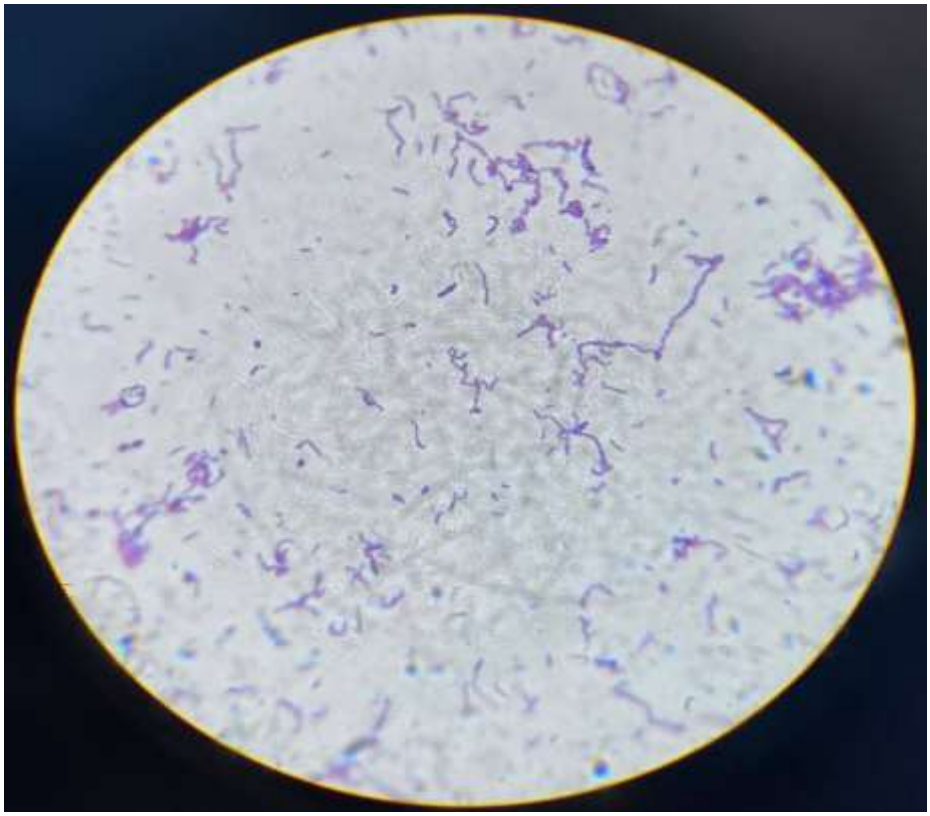

Figure 3. Gram staining results of *streptococcus spp.* isolates

Table 1. Vitek 2 device analysis for *Streptococcus spp.* recognition

Total number	Total number of isolated	Number & species of Streptococci	
50	27	6 (22.22%)	*S. sanguinis*
		2 (7.40%)	*S. mitis*
		2 (7.40%)	*S. oralis*
		3 (11.11%)	*S. salivarius*
		4 (14.81%)	*S. pluranimalium*
		5 (18.51%)	*S. pseudoporcinus*
		4 (14.81%)	*S. alactolyticus*
		1 (3.70%)	*S. parasanguinis*

Organism Quantity:
Selected Organism : Streptococcus sanguinis

Source: oral cavity **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.82 hours	**Status:**	Final
Selected Organism	99% Probability **Bionumber:**	**Streptococcus sanguinis** 001011364311531		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	+	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	+	50	NC6.5	-	52	dMAN	-	53	dMNE	+	54	MBdG	-	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Organism Quantity:
Selected Organism : Streptococcus mitis

Source: oral **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.85 hours	**Status:**	Final
Selected Organism	94% Probability **Bionumber:**	**Streptococcus mitis** 061511364315531		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	+	11	AGLU	+
13	APPA	+	14	CDEX	(-)	15	AspA	-	16	BGAR	+	17	AMAN	-	19	PHOS	+
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	+	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	+	37	dGAL	+
38	dRIB	-	39	ILATk	-	42	LAC	+	44	NAG	+	45	dMAL	+	46	BACI	-
47	NOVO	+	50	NC6.5	-	52	dMAN	-	53	dMNE	+	54	MBdG	-	56	PUL	+
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Organism Quantity:
Selected Organism : Streptococcus oralis

Source: oral cavity **Collected:**

Comments:	

Identification Information	**Analysis Time:**	5.83 hours	**Status:**	Final
Selected Organism	92% Probability **Bionumber:**	**Streptococcus oralis** 005010300000511		
ID Analysis Messages				

Biochemical Details

2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	-	9	BGAL	-	11	AGLU	-
13	APPA	+	14	CDEX	-	15	AspA	+	16	BGAR	-	17	AMAN	-	19	PHOS	-
20	LeuA	+	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	-	27	BGUR	-
28	AlaA	+	29	TyrA	+	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	-
38	dRIB	-	39	ILATk	-	42	LAC	-	44	NAG	-	45	dMAL	-	46	BACI	-
47	NOVO	-	50	NC6.5	-	52	dMAN	-	53	dMNE	-	54	MBdG	-	56	PUL	-
57	dRAF	+	58	O129R	-	59	SAL	+	60	SAC	+	62	dTRE	-	63	ADH2s	-
64	OPTO	+															

Figure 4. Vitek2 device analysis for *streptococcus spp.* recognition

16S rRNA Based Detection of *Streptococcus spp.*

Molecular identification of viridians Streptococci in the current study, relied on conventional PCR for

the amplification of a partial gene of 16S rRNA by specific primer sequences. This gene was present in 10/10 (100%) samples with a PCR product of bp (Figure 5).

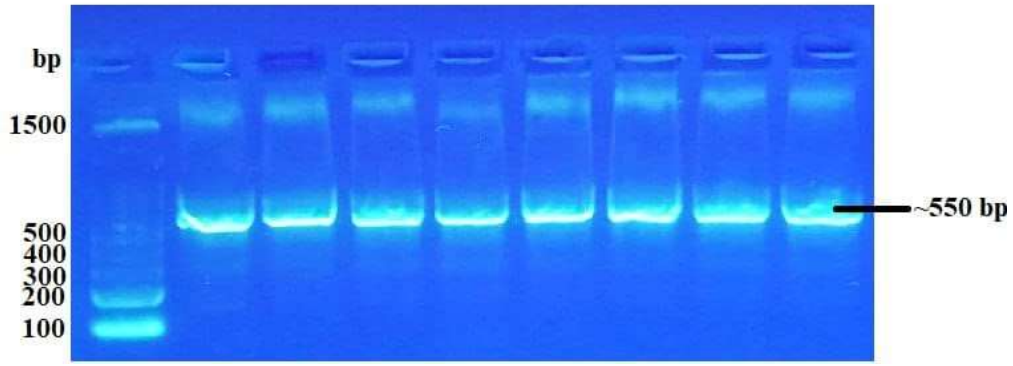

Figure 5. Agarose gel electrophoresis image shows PCR product of genes analysis of Viridians Streptococci. Lane M Marker ladder (100-1500 bp), 16S rRNA gene of Viridians Streptococci with ~550 bp.

. **Antibiotic susceptibility of *Streptococcus spp.* isolates**

Antibiotic susceptibility test revealed that *Streptococcus spp* had shown multiple resistance against the antibiotics used in this study. all the isolates exhibited resistance towards Erythromycin, Ceftriaxone, Cefepime, and Cefotaxime. With respect to Penicillin, the results indicated that 96.29% of the isolates displayed resistance, while 92.59% showed resistance to Clindamycin. On the other hand, it was shown that Augmentin was demonstrated to exhibit the highest level of activity among the molecules tested, reaching 100% efficacy against oral streptococci. Furthermore, Trimethoprim-sulfamethoxazole and Ampicillin displayed favorable activity at 100%, with Vancomycin also proving to be effective against oral streptococci at the same rate. Regarding other antibiotics Tetracycline, Doxycycline, Meropenem, Levofloxacin, the isolates showed different results between sensitive, intermediate and resistant.

Table 2. Multidrug Resistant strains of oral *Streptococcus* isolates

Streptococcus isolates	Total isolate number	No. of multi-resistant strains (%)	No. of antibiotics classes that the isolates resisted
S. sanguinis	6	4 (66%)	5
S. mitis	2	2 (100%)	6
S. oralis	2	2 (100%)	4
S. salivarius	3	3 (100%)	7
S. pluranimalium	4	3 (75%)	7
S. pseudoporcinus	5	4 (80%)	7
S. alactolyticus	4	3 (75%)	7
S. parasanguinis	1	1 (100%)	6

3.4. Characterization of the SiNPs

X-ray diffraction (XRD) represents a prevalent analytical technique employed for assessing particle dimensions and nanoparticle (NP) architecture. The XRD patterns corresponding to the NPs synthesized via the sol-gel method are illustrated in Fig. 6. The subsequent discussion elucidates the application of the Debye–Scherrer equation in the quantification of the dimensions of SiO2 NPs [16]:

$D = K \lambda / (\beta \cos\theta)$

where D is the crystal volume, λ is the wavelength of the X-ray radiation (λ = 0.15406 nm) for Cu-Kα, K is frequently taken as 0.9, and β is the line width at half-maximum height. Using Scherrer's formula, the crystal size was predicted to be around 28.93 nm. The SiO2 NPs had peaks that correspond to the planes 2θ = 21.8352◦, 28.3480◦, 31.3656◦, 36.1849◦, 42.6900◦, 44.7764◦, 46.9823◦, 48.6327◦, 57.3165◦, 60.3900◦, 62.1400◦, 65.3040◦, 69.1900◦ and 74.2542◦. The XRD spectra reveal that the SiO2 NPs produced through the sol-gel process exhibit a crystalline structure. The crystalline phase associated with this particular form of silica is characterized as tetragonal.

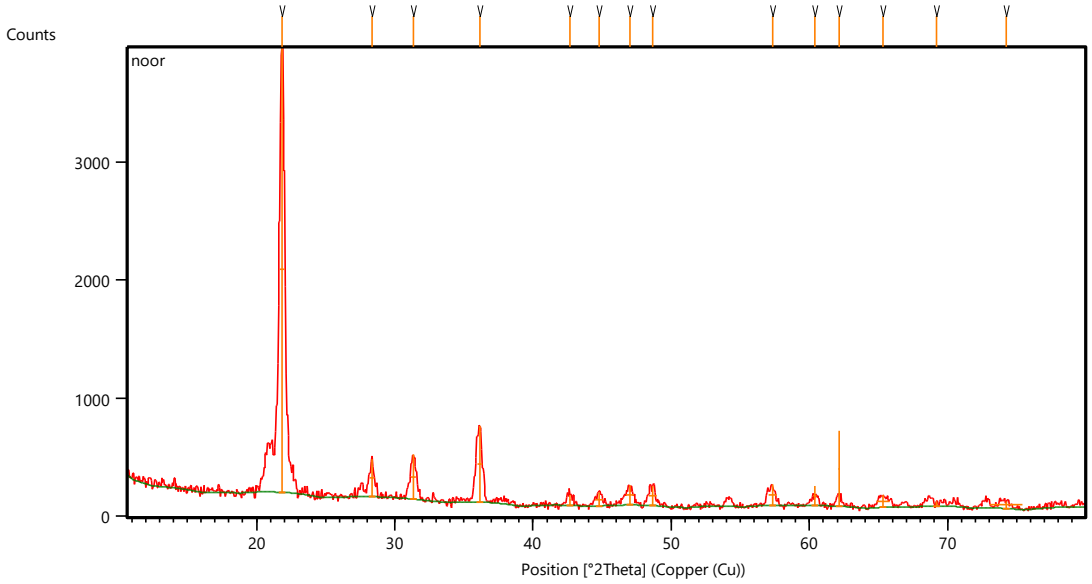

Figure 6. The XRD analysis of silica nanoparticles

Transmission electron microscopy (TEM) images facilitate a more comprehensive understanding of the internal architecture of nanoparticles (NPs). Figure 7 illustrates a TEM image depicting the SiO2 NPs synthesized via the sol-gel method. The circular formations are composed of individual entities of the distinctively produced NPs, as evidenced by the enhanced two dimensional representation of the NPs. The synthesized nanoparticles demonstrate dimensions of roughly under 100 nm, as ascertained through the transmission electron microscopy (TEM) imaging methodology. This dimension is consistent with that obtained from the X-ray diffraction (XRD) examination.

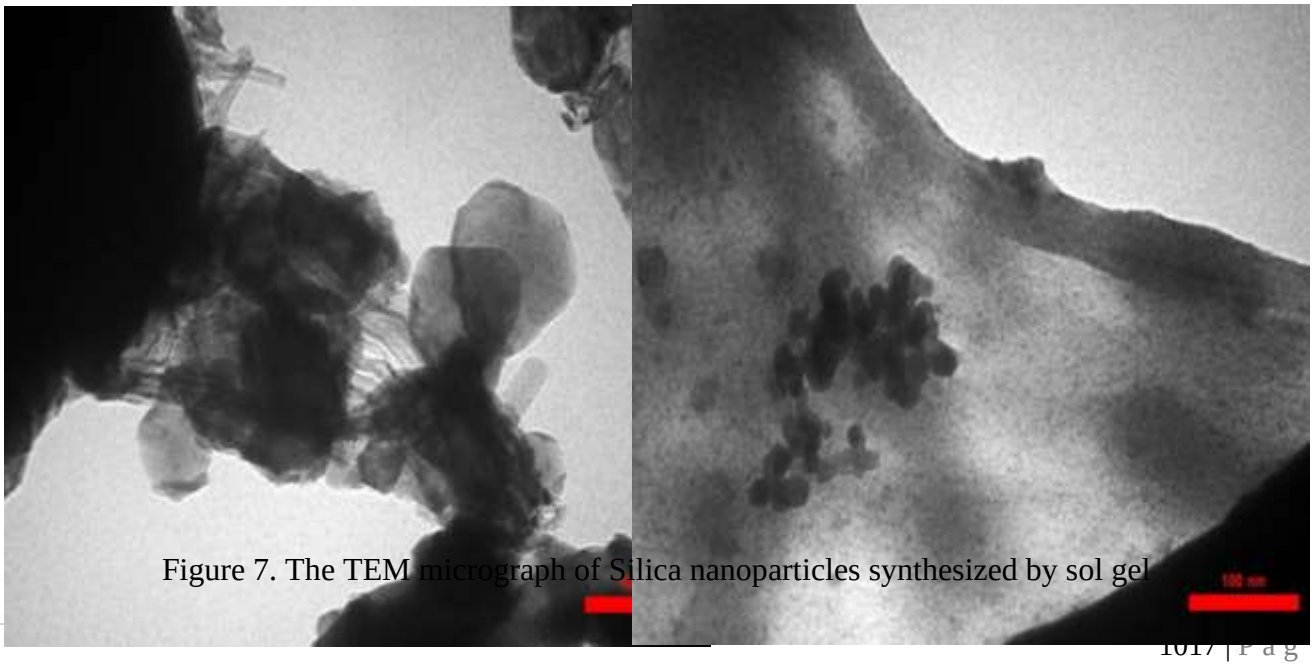

Figure 7. The TEM micrograph of Silica nanoparticles synthesized by sol gel

The morphological characteristics and elemental compositions of the silica nanoparticles (SiNPs) were analyzed employing field emission scanning electron microscopy (FESEM) and energy-dispersive X-ray spectroscopy (EDX) methodologies. The scanning electron microscopy (SEM) representation of the SiNPs is illustrated in Figure 8a. The observed structures consist of agglomerated, semi-spherical nanoparticles exhibiting diameters approximately within the range of 35.22 nm. This finding indicates that the SiNP particles possess a relatively uniform composition. Figure 8b presents the EDX spectra corresponding to the SiNPs. As indicated by the EDX analysis, silicon (Si) and oxygen (O) were identified as the predominant elemental constituents. Furthermore, in addition to Si and O, trace amounts of gold (Au) are also observable within the EDX spectrum. The quantitative analysis revealed that the respective atomic percentages of Si and O were approximately 50.43 and 49.57 weight percent.

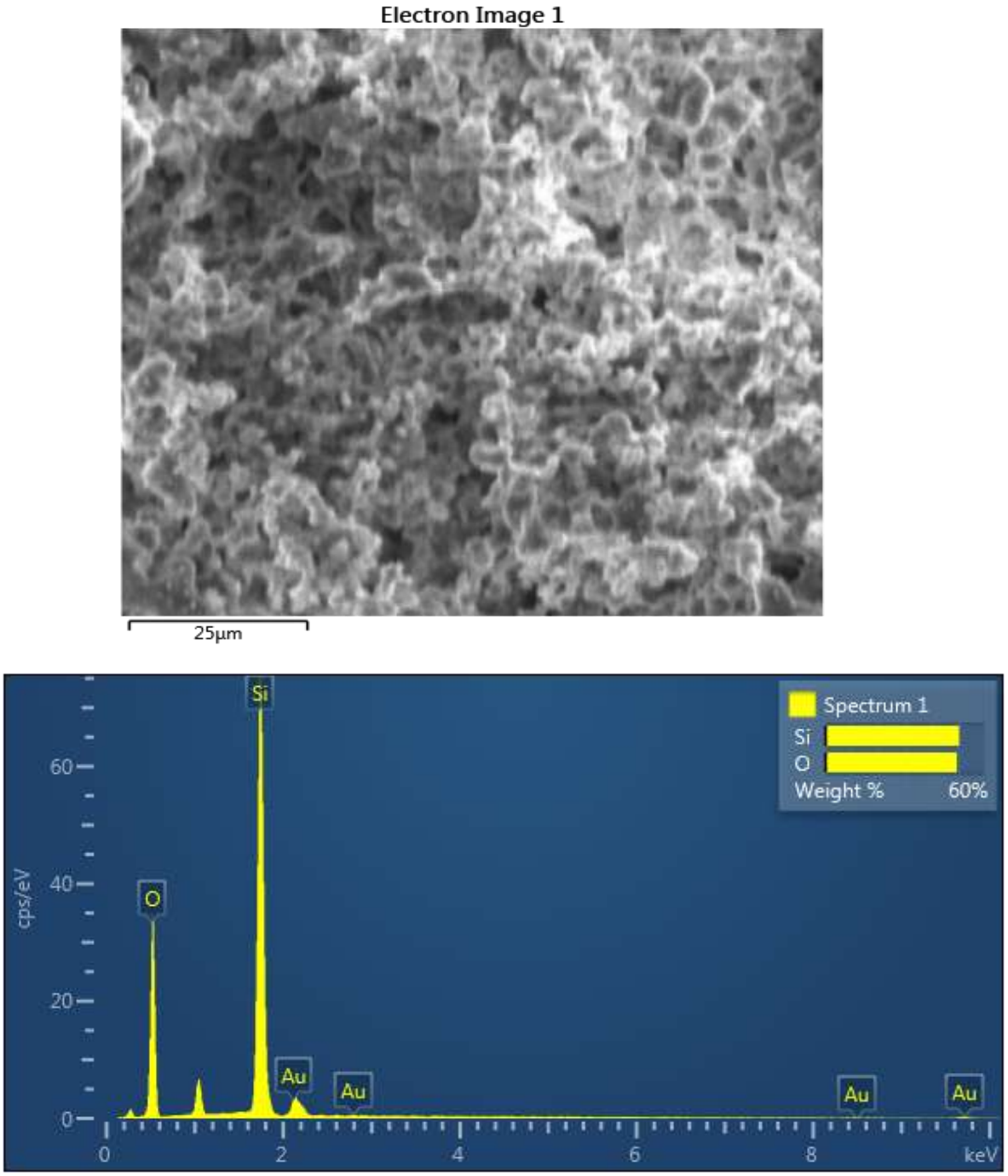

Figure 8. (a) Field emission scanning electron microscope (FESEM) image and (b) energy-dispersive X-ray spectroscopy (EDX) spectrum of silica nanoparticles

Determination of the MIC of silica nanoparticles against *Streptococcus spp.*

In this study, Si-NPs were tested against 10 isolates of *S.mitis*, *oralis* and *sanguinis*. The results have shown that the MIC concentration was 3.13mg/mL for all bacterial isolates. This experiment is based on the color change occurring in the Micro titration plate after adding Resazurin-blue stain, as the preservation of the original color of the dye (blue) means that the bacteria no longer have the ability to form biofilms, and if the color change to pink occurred, this is an indication that the bacteria it was able to form biofilms. As shown in the (figure 9). The lowest inhibitory concentration was chosen to determine the antibacterial effectiveness of biosynthesized silica nanoparticles on Streptococcus bacteria.

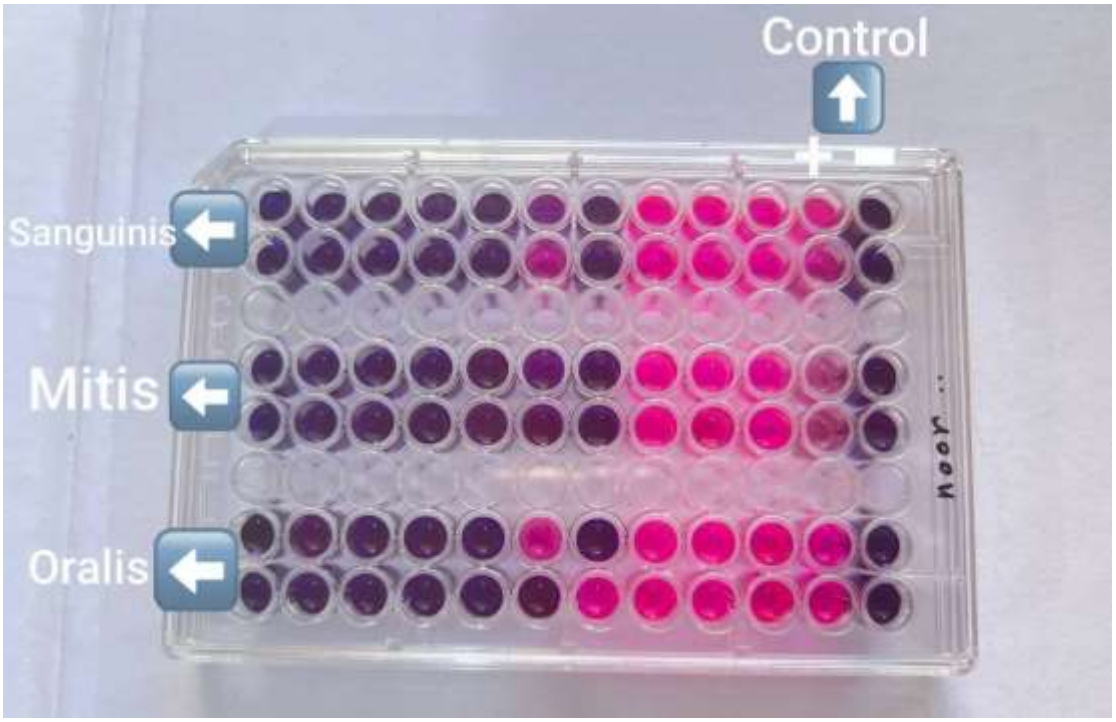

Figure 9. Microtiter plate showing MIC detection by Micro-broth dilution method using resazurin dye.

Anti-bacterial activity assy of silica nanoparticles using minimun inhibition concentration (MIC)

The results shown in Table 3 illustrate, the inhibition diameter ratio between silica nanoparticles and vancomycin was calculated to be 1.29. This indicates that the average diameter of silica nanoparticles (26.2) is 1.29 times larger than that of vancomycin (20.3). Statistical analysis illustrate, silica nanoparticles showed superior performance compared to vancomycin. Specifically, the mean efficacy score of vancomycin was 20.3 (standard deviation = 0.78, standard error = 0.24). While the mean efficacy score of silica nanoparticles was 26.2 (standard deviation = 1.25, standard error = 0.38). Statistical analysis yielded a p-value of 0.000001, indicating a highly significant difference between the two treatments. Silica nanoparticles showed a significantly higher mean efficacy score than vancomycin, indicating that silica nanoparticles are significantly more effective.

Silica nanoparticles are superior to vancomycin due to their enhanced targeting and delivery capabilities. Their small size allows the drug to be delivered precisely to specific sites, improving efficacy. Additionally, silica nanoparticles can be engineered to reduce resistance and improve safety, making them more effective than vancomycin, which has limitations in targeting and resistance issues. Figure (10) shows the effectiveness of bio-synthesized silica nanoparticles against Viridians Streptococci at 3.13 mg concentration compared to the antibiotic vancomycin disc (30μg) and distilled water (DW).

Table 3. The efficacy of biosynthesized silica nanoparticles against Viridians Streptococci compared to the standard antibiotic vancomycin tablet and distilled water, evaluated on the basis of average inhibition zones (mm).

Species of Streptococci	Control (D.W)	Vancomycin	Silica nanoparticles
S. oralis	0	20	26
S. oralis	0	20	24
S. mitis	0	22	28
S. mitis	0	20	25
S. sanguinis	0	20	27
S. sanguinis	0	19	25
S. sanguinis	0	20	27
S. sanguinis	0	21	28
S. sanguinis	0	21	26
S. sanguinis	0	20	26
Rate of inhibition diameter at the level of treatment type (mm)	0	20.3	26.2
The ratio of the average diameters	Inhibition diameter ratio = average diameter $_{\text{Silica nanoparticles}}$ / average diameter of $_{\text{Vancomycin}}$ = 26.2 / 20.3 = 1.29 mm		
SD		0.78	1.25
SE		0.24	0.38
P-Value		0.000001	

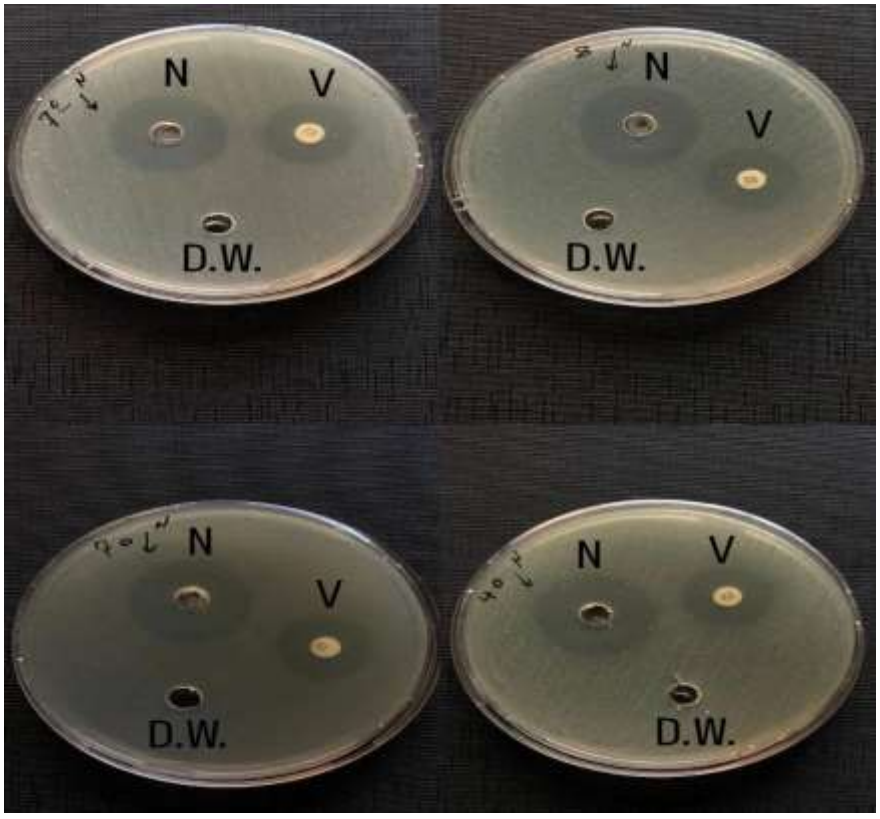

Figure 10. Shows the effectiveness of silica nanoparticles against Viridans streptococci at 3.13 mg concentration compared to the antibiotic Vancomycin disc (30μg) and distilled water (DW)

(N- concentration of silica nanoparticles, V- Vancomycin, W.D. - Distilled water)

Discussion

This result was consistent with other researcher. A study by Mohammed & Al Niaame, (2022) [17] reported that 50 pathological bacterial isolates were isolated from oral diseases, and the genus Streptococcus appeared in 16 isolates, with a percentage of 32%, which is the largest percentage among other species that support the idea that *Streptococcus spp.* are primarily responsible for oral infections. The findings of morphological characteristics were similar to those reported by another study [18]. The biochemical tests results were identical to those of others [19].

In the present study, Chen et al. (2004) [20] supported my diagnostic approach for identifying Viridians Streptococci. They employed the same primers, 13BF and 6R, to amplify VS type strains and utilized 2% agarose gel electrophoresis to separate the PCR products, consistent with my methodology.

In relation to the antibiotic susceptibility profile of the oral isolates obtained from dental patients, the present study agrees with Maripandi et al., (2011) [21] who investigated the efficacy of seven antibiotics (Clindamycin, Vancomycin, Bacitracin, Tetracycline, Penicillin, Chloroamphenicol, and Streptomycin) against dental caries strains. Their findings revealed that Streptococcus strains exhibited resistance to penicillin, while being susceptible to tetracycline.

The findings of this research demonstrate that Augmentin, Trimethoprim-sulfamethoxazole, Ampicillin, and Vancomycin exhibit significant potency and have the potential to be utilized in the efficient management of oral infections owing to their bacteriostatic/bactericidal properties against the strains, considering the notable degree of susceptibility observed. The insensitivity displayed by certain oral strains to specific antibiotics employed indicates the rise of antimicrobial resistance in these microorganisms, primarily attributed to the indiscriminate utilization and misuse of these medications. In the current study, *S. sanguinis* exhibited resistance to five distinct classes of antibiotics, namely Penicillins, Macrolides, Cephalosporins, Lincosamides, and Carbapenems, signifying a moderate degree of multi-resistance. *S. mitis* displayed resistance to six antibiotic classes, which include Penicillins, Macrolides, Cephalosporins, Lincosamides, Carbapenems, and Tetracyclines, thereby categorizing it as multi-resistant. *S. oralis* showed resistance across four antibiotic classes, specifically Penicillins, Macrolides, Cephalosporins, and Lincosamides, indicating a certain level of multi-resistance. *S. salivarius*, *S. pluranimalium*, *S. pseudoporcinus*, and *S. alactolyticus* each manifested resistance to seven antibiotic classes, including Penicillins, Macrolides, Cephalosporins, Lincosamides, Carbapenems, Tetracyclines, and Quinolones, thus identifying them as highly multi-resistant. *S. parasanguinis* exhibited resistance to six antibiotic classes, encompassing Penicillins, Macrolides, Cephalosporins, Lincosamides, Tetracyclines, and Quinolones, and is regarded as multi-resistant, notwithstanding the relatively low number of isolates. Masood et al., (2013) [22] agree with my empirical findings regarding the incidence of multidrug resistance in oral streptococci. Their investigation substantiates the elevated levels of antibiotic resistance observed in numerous species of the Viridans group, encompassing *S. sanguinis*, *S. mitis*, *S. oralis*, and *S. salivarius*, in addition to other associated species. This concurrence underscores the critical importance of multidrug resistance among oral streptococci, illuminating a persistent trend across diverse research studies. In contrast to the conclusions drawn by Enitan et al., (2023) [23] which indicated a Multidrug Antibiotic Resistance rate of zero for Streptococcus, our investigation identified a noteworthy prevalence of multidrug resistance among oral streptococci. This divergence implies that oral streptococci may manifest distinct resistance profiles or that the antibiotic panels employed in each research endeavor could influence the observed outcomes. Consequently, it is essential that empirical therapeutic approaches are guided by rigorous laboratory evaluations, particularly those pertaining to antibiotic susceptibility assessments [24; 25].

The significant determinants pertaining to the antimicrobial efficacy of silica encompass stability, adhesion, and the optimal dispersion of silica particles within an organic matrix [26]. Silica particularly in the nanoparticle form, has gained prominence in the domain of nanotechnology, as its effectiveness is chiefly attributed to its capacity to diminish bacterial resistance. The synthesis of silica nanoparticles

was accomplished through the sol-gel methodology. A comparable approach has been employed in antecedent investigations [27; 28] and this technique is regarded as one of the most prevalent methodologies for the synthesis of nanoparticles.

The XRD analysis showed that the prepared silica has a characteristic crystalline phase with an average crystallite size of about 28.93 nm. Similarly, Joni et al. (2018) [29] reported that SiO2 exhibits a tetragonal crystal phase and a crystallite size of approximately 28 nm, corroborating the findings of my study.

In this study, we observed that the silica nanoparticles synthesized via the sol-gel method exhibit circular structures with sizes consistently measured under 100 nm, as elucidated by Transmission Electron Microscopy (TEM) imaging. These findings are agree with those reported by Eissa et al. (2022) [30], who investigated SiO_2 nanoparticles produced using lemon peel extract. Eissa et al. also observed similar circular structures within the same size range, demonstrating consistency in nanoparticle size and morphology across different synthesis methods.

In our study, silica nanoparticles (SiNPs) were fabricated utilizing the sol-gel approach, and their morphological and compositional properties were analyzed using FE-SEM and EDX techniques. Our findings are generally consistent with those reported by Larkunthod et al. (2022)[31] who also investigated SiNPs using similar analytical methods. Both studies confirm that the SiNPs are predominantly semi-spherical and agglomerated. However, there are differences in particle size distribution: our measurements indicate a narrower size range of approximately 35.22 nm, whereas Larkunthod et al., reported a broader range of 60-135 nm. This discrepancy could be attributed to variations in synthesis conditions or techniques. Regarding elemental composition, both studies identify Si and O as the primary elements. Our EDX analysis found Si and O at approximately 50.43 wt% and 49.57 wt%, respectively, while Larkunthod et al., reported 52.2 wt% Si and 27.1 wt% O. Additionally, our results detected a small amount of Au, while Larkunthod et al., analysis revealed the presence of Na and Cu. These differences highlight how variations in the synthesis process, including the sol-gel method parameters, can influence the final properties of the nanoparticles.

The existence of singlet oxygen alongside other reactive oxygen species associated with silica nanoparticles culminates in oxidative impairment of bacterial membranes, subsequently resulting in bacterial mortality; this phenomenon is posited as a plausible mechanism underlying its antimicrobial efficacy [32]. Based on my results, silica nanoparticles demonstrate greater effectiveness than Vancomycin against Viridians streptococci isolated from oral infections. This finding aligns with the study by Barma et al. (2020) [33], which documented substantial antimicrobial efficacy of the synthesized silica nanoparticles in mouthwash against gram positive bacterial strains. Their research highlights the potential of silica nanoparticles in controlling biofilm formation and preventing bacterial colonization. Additionally, other studies have confirmed the antimicrobial efficacy of silica nanoparticles around implants [34]. Thus, the consistent findings across these studies support the potential of silica nanoparticles as a robust antimicrobial agent in various applications.

Of the reasonable interpretation for the Silica nanoparticles may be more effective than Vancomycin against Viridans streptococci in oral infections due to their ability to disrupt bacterial biofilms and potentially damage bacterial cell structures through mechanisms different from those targeted by Vancomycin. Additionally, silica nanoparticles may overcome specific bacterial resistance mechanisms that limit Vancomycins efficacy.

3. Conclusion and future scope

Our results might elucidate the insufficient efficacy of antibiotic treatment for infections associated with oral streptococci. In addition, the findings showed that silica nanoparticles synthesized from sol gel method have proven to be very effective against Streptococcus bacteria, which opens the door to the possibility of using them in the field of treatment oral infections.

Conflict of interest: No known conflict of Interest correlated with this publication.

Funding: This research did not receive any grant from agencies in the public, commercial, or not-for-profit sectors.

Availability of data and materials: The data used and / or analyzed throughout this study are available from the corresponding authors upon reasonable request.

Competing interest: The authors declared that they have no competing interests.

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