RESEARCH PAPER

Antibacterial Activity of Carbon Quantum Dots against Oral Bacteria -Lactobacilli

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ABSTRACT

Carbon quantum dots (CQDs) are a type of carbon-based nanomaterials that have recently garnered attention as emerging alternatives to conventional semiconductor quantum dots. Colloidal quantum dots (CQDs) provide several advantageous characteristics, including minimal toxicity, environmental compatibility, cost-effectiveness, photostability, favorable charge transfer properties with increased electronic conductivity, and easily reproducible manufacturing techniques.__Assessing the antibacterial properties of CQDs by testing the sensitivity of different concentrations of CQDs on Oral *Lactobacilli* then comparing with chlorhexidine 0.2% and deionized water._Agar well technique was used. No bacterial growth was measured when inhibitions zones around each well were seen. No inhibition zone means a full resistance of the bacteria to the tested agent. The results showed that all the tested concentrations of CQDs exhibited antibacterial activity against *Lactobacilli* with different inhibition zones, which increases with increasing concentration of CQDs. Low concentrations of CQDs have very high antibacterial activity against *lactobacilli*, and this could be a new effective material to be used in preventive dentistry.

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INTRODUCTION

The oral cavity has multiple locations for bacterial adhesion, a temperature of about 35–36°C, a lot of moisture, a good supply of different kinds of nutrients, and variations in oxygen tension, making it a great microbial incubator. Numerous aerobic and anaerobic microbes find growth-friendly conditions [1].

Dental caries, one of the most prevalent oral diseases that is known to be chronic and can—indirectly—be damaging to other areas of the body [2], is one of many bacterial species that are associated to many oral ailments. Due to their abundance in plaque and saliva, their capacity to produce acid, and their innate capacity to live better than most organisms in environments of high acidity. Due to their numerical dominance in plaque and saliva, their capacity to produce acid, and their innate capacity to live better than most organisms in environments of high acidity,

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Mutans Streptococci and Lactobacilli are the most cariogenic bacteria [3- 4].

Regarding dental caries, *Lactobacilli* species -Gram-positive rods- are effective producers of lactic acid, and tolerant of low pH values are known as a significant secondary invader [5], with the primary invader*, Streptococcus mutans* playing a substantial part in the early stages of cavity formation.

Both kinds of bacteria have an active part in the formation of tooth decay [6], for over three decades [7, 8], chlorhexidine has been regarded as the gold standard because it is an effective inhibitor for S. mutans. However, the dominant oral *Lactobacillus*, *Lactobacillus casei,* is comparatively resistant [9]. The primary difficulties with its usage are its brief substantivity (The ability of chlorhexidine to adhere to tissues and exhibit sustained release over an extended duration) and some occurrences of cytotoxicity that have been documented [10, 11]. So, finding new materials with a strong antibacterial action but little or no impact on human health or the environment is crucial [12].

Nanotechnology has recently grown in significance within the realm of biology [13]. The capacity to create atoms and molecules, which can then be combined to create new structures one billion times smaller than anything visible to the naked eye, is a noteworthy accomplishment. As a result, high atomic accuracy may be used to design novel materials and gadgets. In order to get special and better characteristics, nanoscience uses nanoparticles with a size between 1 and 100 nm [14–20]. The majority of the body's natural activities take place at a level that is practically invisible, making nanomedicine an incredibly helpful tool [21, 22].

Antibiotic resistance develops by the indiscriminate use of antibiotics, and they frequently trigger a variety of negative side effects [23]. The rise of bacterial resistance has further presented the scientific community with a significant hurdle. The demand for creating new, efficient, and less harmful classes of antibiotics has so grown. Nanotechnology has recently demonstrated significant possibilities for solving several of these issues.

Nano-sized materials possessed a variety of biological qualities, such as antibacterial, antifungal, and antiviral capabilities, which are distinctive and diverse and allowed them to be employed in several medical fields [24].

Researchers have recently examined the antibacterial properties of numerous Nano-sized materials, including silver, gold, zinc oxide, titanium dioxide, and others. [25, 26], however there are still some significant issues with their toxicological features that are related to dentistry, such as the toxicity of silver nanoparticles [27], the cytotoxicity of ZnO nanoparticles [28], and the extended retention of gold nanoparticles within cells [29]. Since carbon has significant antibacterial properties, it was thought that carbon nanoparticles might function well as an alternative to other materials. Nanotubes, fullerenes, and other carbon nanostructures have all been created [30, 31].

The diameters of carbon quantum dots (CQDs), which are classified as zero-dimensional nanostructures, are typically less than 10 nm in diameter. Due to their simple methods of synthesis and distinctive qualities such their tiny size, high biocompatibility, strong photostability, and chemical stability, CQDs have drawn attention from all over the world [32]. The top-down approach and the bottom-up route are the two methods used to create CQDs. Additionally; CQDs have quickly become recognized as a potent, low-toxic, affordable, and ecologically friendly nanomaterial with potential futures [33]. Carbon Dots have received the greatest attention from researchers studying antibiotic-free bactericidal materials in recent years [34]. The benefits of CDs over other antibacterial drugs include nontoxicity, photostability, simplicity of surface functionalization that might be advantageous for improved bacterial interactions, and abundance of affordable and nontoxic precursors that facilitates economical and safe synthesis [35].

This study was carried out because, as of yet, no other study has examined the antibacterial effects of Carbon Quantum Dots suspension solution on Lactobacilli bacteria.

MATERIALS AND METHODS

The Carbon Quantum dots (CQDs) suspension solution being prepared according to a published procedure [36] with little modification. The synthesized solution appears light yellow under daylight and cyan blue in color when subjected to UV light emission in a dark room, as shown in Fig. 1.

As the quantum dots Particles are very

tiny, their size and shape were tested using transmission electron microscopes (TEM) and high resolution TEM .as seen in Fig. 2. Under standardized conditions, stimulated saliva samples were collected from twenty healthy participants, to obtain *Lactobacilli* isolates. All the participants were healthy-looking, with no history of systemic diseases, aged between 20-35 years old. The stimulated saliva samples were collected under typical conditions in accordance with Tenovuo and Lagerlof 1994 [37]. The following inclusion criteria were used to choose the participants: overall good health, no systemic disorders, and willingness to engage in the study's procedures. If a subject had used an antibacterial mouthwash during the previous 12 hours or had antibiotic therapy within the previous 14 days, they were disqualified from the research. For two minutes, a vortex mixer was used to homogenize the saliva. A normal phosphate buffer solution in saline was used to make a tenfold serial dilution. The pour plate technique was used to inoculate each dilution using Rogosa agar medium in triplicate. At 37 degrees Celsius, the plates were incubated aerobically for 48 hours [38, 39]. According to Brown (2005) [40], the colony morphology, Grams

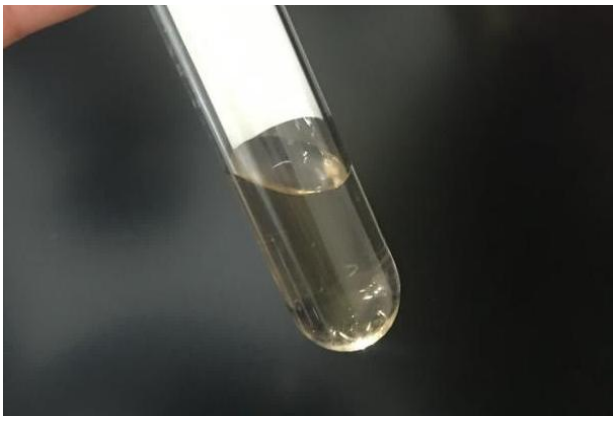

Fig. 1. CQDs dispersion in water under daylight.

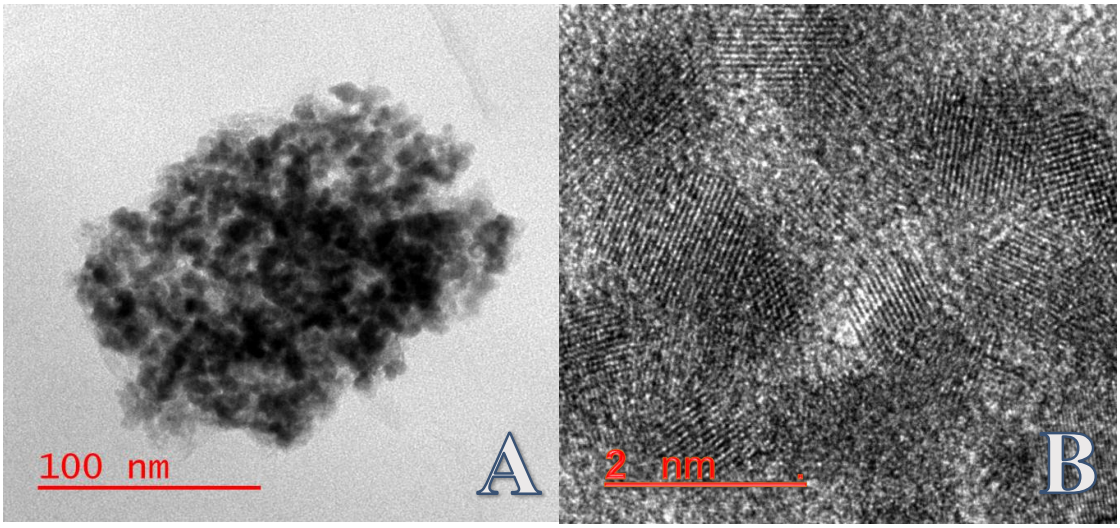

Fig. 2. TEM-images of CQDs.

stain, motility, and catalase test were used in an effort to identify the isolates. As directed by the manufacturer, the Vitek 2 compact (Biomerieux) was used to identify *lactobacilli* species.

Agar well method was used to examine the Lactobacilli for sensitivity to various Carbon Quantum Dots suspension solution concentrations. Then, as positive and negative controls, respectively, 0.2% chlorhexidine and deionized water were used to compare the results. Mueller Hinton Agar (MHA) media was made and utilized in accordance with Hi-Media's guidelines [41]. The same procedure described before was used to create CQD suspension solutions with various concentrations. The concentrations of the CQDs that were evaluated were (25 Ug/ml,

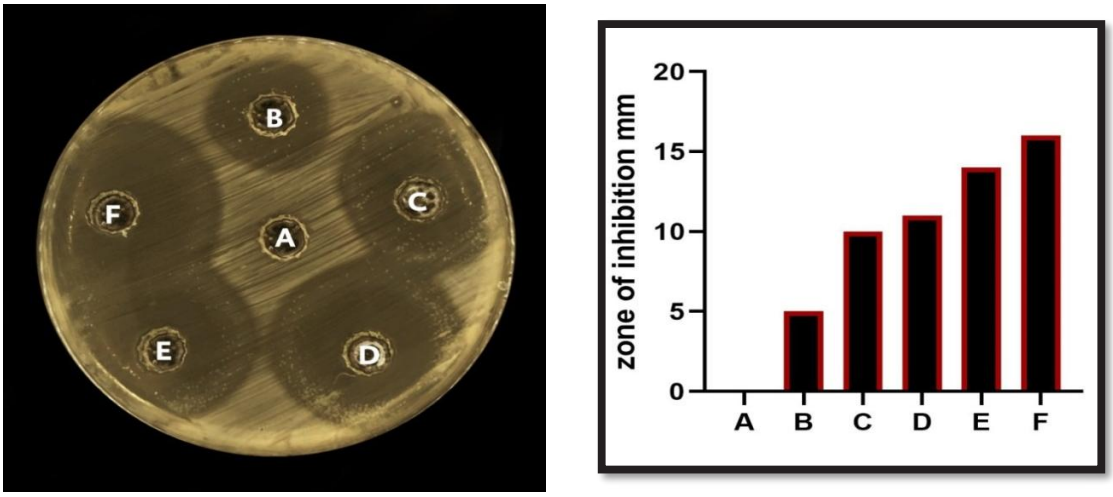

Fig. 3. Agar well diffusion method for sensitivity of different concentrations of CQDs solutions against *Lactobacilli* (Petri dish and comparison plot). A) control negative (deionized water), B) Control positive (chlorohexidine), C) 10 ug/ml CQDs, D) 15 ug/ml CQDs, E) 20 ug/ml CQDs, F. 25 ug/ml CQDs.

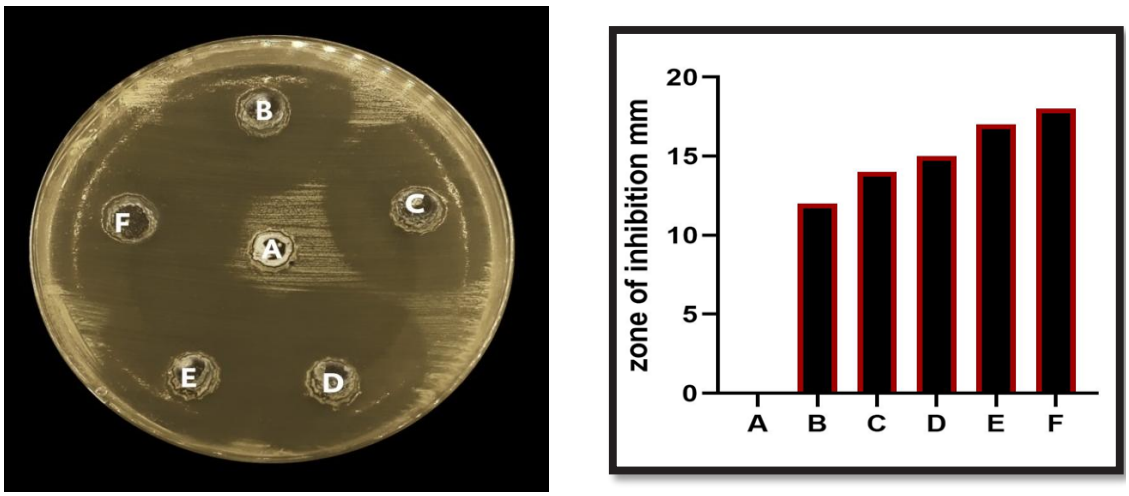

Fig. 4. Agar well diffusion method for sensitivity of different concentrations of CQDs solutions against Lactobacilli - (Petri dish and comparison plot). A) control negative (deionized water), B) 5 ug/ml CQDs, C) 10 ug/ml CQDs, D) 15 ug/ml CQDs, E) 20 ug/ml CQDs, F. 25 ug/ml CQDs.

20 Ug/ml, 15 Ug/ml, 10 Ug/ml, and 5 Ug/ml). In this experiment, the Carbon Quantum Dots suspension solution effect was tested at different concentration on the viable counts of *Lactobacilli.*

Following receipt of that acceptance (Ref. No. 564 on April 17, 2022) ethical approval was carried out at the Department of Paediatric and Preventive Dentistry, College of Dentistry, University of Baghdad.

RESULTS AND DISCUSSION

Results demonstrated that, even at the lowest tested concentrations of CQDS, separate clean zones existed with no bacterial growth. This suggests that the tested solution utilized against the chosen bacterial strains had a high level of antibacterial activity, as seen in Figs. 3 and 4. The bacterial inhibition diameter increased in line with increasing the concentrations of the tested agent, since all of the tested concentrations of CQDs suspension displayed distinct inhibition zones, with less apparent zones appearing with lower concentrations. As shown in Fig. 3, CHX demonstrated a particular inhibition zone that was less in diameter comparing to the lowest tested concentration of CQDs, while DW exhibited no inhibition zone at all.

The experimental data reveals that the size of the inhibitory halos varied between approximately 12.4 and 19 mm when different concentrations of the investigated chemical, namely CQDs, were employed. The most significant levels of growth inhibition were reported at dosages of 25 µg/ml. The findings showed that all CQD concentrations

Table 2. Multiple *pairwise* Comparisons of *Lactobacilli* between groups using Dunnett's T3.

Bacterial Species	Test agents		Mean	±SD	F	P-value
Lactobacilli	DW		0.000	0.000	477.096	0.000 **
	CQDs	5ug\mL	14.300	1.494		
		10ug\mL	15.950	1.301		
		15ug\mL	17.400	1.265		
		20ug\mL	18.600	1.075		
		25ug\mL	19.650	0.883		
	CHX 0.2%		7.700	0.823		

Table 2. Multiple *pairwise* Comparisons of *Lactobacilli* between groups using Dunnett's T3.

(I) Groups			(J) Groups					
			5ug\mL	10ug\mL	15ug\mL	20ug\mL	25ug\mL	CHX
DW		MD	-14.300	-15.950	-17.400	-18.600	-19.650	-7.700
		Sig.	.000	.000	.000	.000	.000	.000
CQDs	5ug\mL	MD		-1.650	-3.100	-4.300	-5.350	6.600
		Sig.		.307	.003	.000	.000	.000
	10ug\mL	MD			-1.450	-2.650	-3.700	8.250
		Sig.			.363	.003	.000	.000
	15ug\mL	MD				-1.200	-2.250	9.700
		Sig.				.515	.007	.000
	20ug\mL	MD					-1.050	10.900
		Sig.					.450	.000
	25ug\mL	MD						11.950
		Sig.						.000

tested had varied mean values and inhibitory zones. As concentration increased, corresponding increases in mean values were noted, as shown in Table 1. A statistically significant difference between the groups was discovered using ANOVA analysis.

The multiple comparisons of the CQDs inhibition zones across the groups revealed that the inhibition zone at (25 ug/ml) was the greatest zone and had the highest significant difference from the other lower concentrations (5, 10, and 15 ug/ml) ($p<0.01$). Since all inhibition zones increased with concentration, going from (5 ug/ml) to (25 ug/ml), with a statistically significant difference ($p<0.05$), they all followed an ascending trend. As seen in Table 2, there is a highly statistically significant difference ($p<0.01$) between deionized water, chlorhexidine, and Carbon Quantum Dots. Multiple comparisons of the inhibition area of chlorhexidine between each tested concentration of CQDs, as shown in Table 2, revealed highly significant differences ($p<0.01$). Also, a highly significant difference ($p<0.01$) was recorded when comparing the inhibition zone of all CQDs concentrations with deionized water.

The Fig. 5 showed that the Zone of inhibition of chlorhexidine was a lowest when compared to all the tested concentration of CQDs. While no inhibition zone was noticed with deionized water. Finding antibacterial alternatives that incorporate non-antibiotic items, including nanoparticles, with no bacterial resistance, simple, economical manufacturing processes, and little cytotoxicity is crucial for dental research. Since the discovery of CQDs in 2004, a variety of straightforward, inexpensive, and effective approaches for CQD synthesis have been devised. Nanotechnology has advanced quickly in its efforts to enhance health. In the context of in vivo biomedical applications, CQDs' exceptional chemical and photochemical stability combined with their chemically non-toxic composition offer a distinct benefit. [42]. This study focused on the application of CQDs against oral *Lactobacilli species.* And the data revealed high effectivity against these bacterial species with minimum applied doses. As widely recognized, bacteria are often measured in microns, which is three orders of magnitude larger than nanoparticles. Hence, the likelihood of nanoparticles interacting with bacteria increases as the size of the nanoparticles decreases; hence, Quantum Dots is smallest category which ranges from 1-10 nm only, it could be one of the most effective types of nanoparticles against different types of bacteria.

Result indicates that there was a clear antibacterial activity of all the tested concentrations of CQDs against the tested bacteria and the

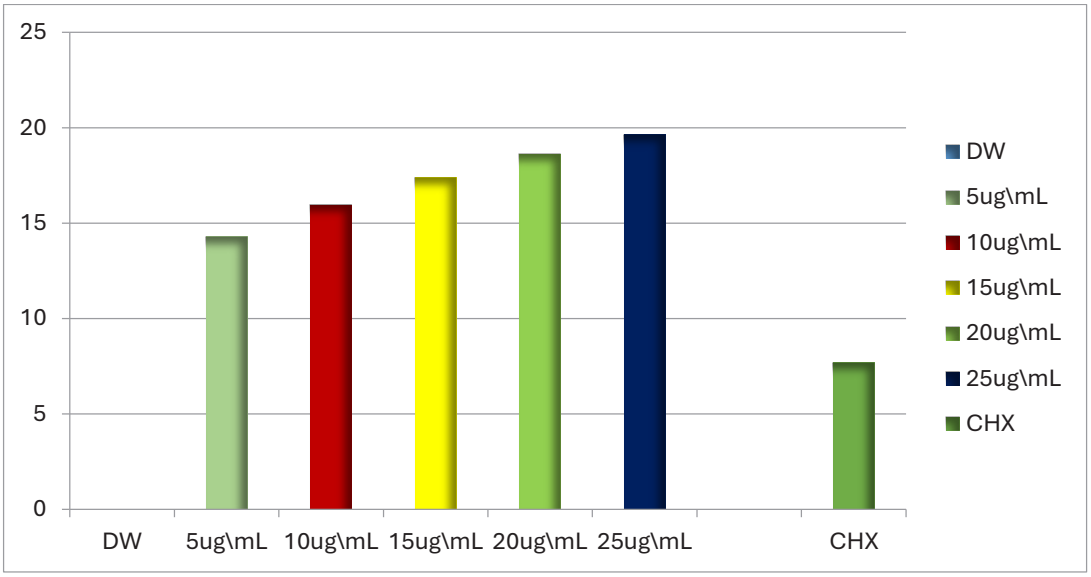

Fig. 5. Graph of inhibition zones of different concentrations of CQDs in comparison with CHX and Distilled Water.

inhibition zones' mean values were increased with increased concentration and the maximum value were recorded with the concentration of (25 ug/ml), this could be related directly to the antibacterial properties of carbon quantum dots, which are primarily because of the production of oxidative stress brought on by reactive oxygen species (ROS) [43]. When comparing the effectivity of chlorhexidine against Carbon Quantum Dots, All the concentrations revealed high significant difference with CHX, and this could be explained as the CQDs have superior antimicrobial activity at these concentrations. The exceptional efficacy of colloidal quantum dots (CQDs) may be attributed to their quantum size and form, as the dimensions of CQDs significantly influence their bactericidal potency. Bacteria are microscopic organisms, and the porins present on the bacterial membrane have nanoscale dimensions. Therefore, it has been observed that CDs with a significantly reduced size have the ability to permeate the cell walls of bacteria, resulting in the release of intracellular components due to their activity (44). The impact of the dimensions and configuration of compact discs (CDs) on their antibacterial efficacy has been documented in multiple scholarly studies [45, 46]. Zhang et al. [47] conducted a study examining the correlation between size and antibacterial efficacy, revealing that the bactericidal effects exhibit an upward trend as size increases. Furthermore, it was shown that the antibacterial activity exhibited concentration-dependent behavior. Multiple studies have demonstrated that the adsorption of proteins is positively correlated with the reduction in the local curvature of carbon nanomaterials [48, 49]. Contrary to traditional antibiotics, Carbon Quantum Dots employ an antibacterial mechanism that is sophisticated and distinct. This mechanism causes ROS to be produced, cell structure to deteriorate, and cytoplasm to leak as a result of DNA binding and gene expression regulation. The surface charge state of CDs has a significant impact on their electrostatic attraction to the microbial cell. Overall bactericidal effectiveness is also influenced by the kind of bacterial strains, CD intrinsic features, and surface modification [50]. For instance, reactive oxygen species serve as signaling molecules inside the cells during a pathogen challenge at low concentrations of CQDs. Oxidative stress will result in oxidative damage to proteins, lipids, and nucleotides, which will lead to DNA damage and lipid peroxidation, which will ultimately end in the death of bacterial cells. Additionally, it may directly oxidize lipids via free radicals on the surface of carbon quantum dots, damaging cell membranes and killing bacteria [41, 51]. Other antibacterial mechanisms that Carbon Dots possess besides ROS include DNA binding, photocatalysis, membrane destabilization, physical and mechanical damage, and blockage of bacterial metabolic pathways. [52]. The significance of CD size and shape for antimicrobial action has been discussed in several study studies, allowing the tiny carbon dots to pierce the bacterial cell wall and the internal components of the bacteria to seep through their activities. According to Zhang *et al.* [53], the bactericidal effect was stronger with increasing size, which conflicts with the findings of our investigation. It did, however, support the findings of this investigation, which indicated that the antibacterial activity was shown to be concentration-dependent.

In 2019, Zhao *et al.* [54] studied the antibacterial activity of nitrogen-doped CQD against different bacterial species that concluded that positively charged N-CQDs bind to negatively charged bacteria, leading to cell membrane rupture, and it has broad antibacterial activity against different forms of bacteria.

According to Li *et al.,* 2020 [55], the electrostatic interaction between positively charged nanoparticles and negatively charged bacteria results in bacterial membrane rupture and the CQDs have high inhibitory effects for certain bacterial species (E. coli and S. aureus). Also, Malmir *et al.,* 2020 [56] found that the antibacterial activity of CQDTiO$_2$ against E. coli was less than S. aureus, using the MIC test and Characterization of bacterial death. In 2021, Sun *et al.* [57] concentrates on the role of non-ROS pathways. Their research provided the size effect's first experimental demonstration. When compared to the other sizes, they discovered that the smaller CGCDs in these particles significantly increased antibacterial activity. This difference in antibacterial activity may be related to differences in cellular absorption and plasma membrane distribution.

CONCLUSION

It is concluded that the Carbon Quantum Dots obtained have a very good quantum size (2-10 nm), in low concentration, can be an alternative and highly-effective antibacterial for oral bacteria,

lactobacilli. This antimicrobial capability extends beyond combating dental caries, as it also aids in preventing the proliferation of pathogenic bacteria that disrupt the oral cavity's equilibrium. There exists a potential solution for mitigating the detrimental impact caused by prominent pathogens, thereby reducing the occurrence of postoperative infections. This solution also holds promise as an environmentally friendly alternative, resulting in not only cost-effective medications but also substances with reduced risks to human health and the ecosystem.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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