The Saudi Dental Journal (2023) **35**, 883–890

King Saud University

The Saudi Dental Journal

www.ksu.edu.sa
www.sciencedirect.com

Antibacterial effectiveness of different zinc salts on *Streptococcus mutans* and *Streptococcus sobrinus*: An in-vitro study

Manal Mohamed Almoudi [a], **Alaa Sabah Hussein** [b,*], **Nurul Izzah Mohd Sarmin** [c], **Mohamed Ibrahim Abu Hassan** [d]

[a] *Centre of Paediatric and Preventive Dentistry, Faculty of Dentistry, AL Zawiah University, AL Zawiah, Libya*
[b] *Centre of Paediatric Dentistry and Orthodontics Studies, Faculty of Dentistry, Universiti Teknologi MARA, Selangor, Malaysia*
[c] *Centre of PreClinical Science Studies, Faculty of Dentistry, Universiti Teknologi MARA, Selangor, Malaysia*
[d] *Centre of Restorative Dentistry Studies, Faculty of Dentistry, Universiti Teknologi MARA, Selangor, Malaysia*

Received 31 December 2022; revised 18 June 2023; accepted 2 July 2023
Available online 6 July 2023

KEYWORDS

Zinc;
Zinc salts;
Streptococcus mutans;
Streptococcus sobrinus;
Oral bacteria

Abstract *Objectives:* This in-vitro study aimed to evaluate the antibacterial effects of four zinc salts namely zinc chloride, zinc sulfate, zinc citrate and zinc acetate against *Streptococcus mutans (S. mutans)* and *Streptococcus sobrinus (S. sobrinus)*.

Methods: Antibacterial susceptibility assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were undertaken to evaluate the inhibitory activities of different zinc salts against the tested bacteria. A scanning electron microscope (SEM) was used to evaluate the morphological changes of bacterial cells following exposure to zinc salts. Kruskal-Wallis and Mann-Whitney tests were used to compare the inhibitory effect of the different zinc salts.

Results: All zinc salts tested against *S. mutans* and *S. sobrinus* had a statistically and significantly smaller inhibition zone when compared to chlorhexidine, ($P < 0.001$). However, zinc chloride had the largest inhibition zone (20 mm ± 5.5) against *S. sobrinus*, which was comparable to chlorhexidine (22 mm ± 4) ($P > 0.05$). Zinc chloride, zinc sulfate and zinc acetate demonstrated higher MIC and MBC values against *S. mutans* compared to *S. sobrinus*. However, zinc citrate revealed the highest MIC and MBC values of 1 mg/mL and > 8 mg/mL for *S. sobrinus* and > 8 mg/mL for *S. mutans*, respectively.

* Corresponding author at: Centre of Paediatric Dentistry and Orthodontics Studies, Faculty of Dentistry, Universiti Teknologi MARA, Selangor, Malaysia.
E-mail addresses: aa_sa680@yahoo.com (M.M. Almoudi), dr_alaasabah@yahoo.com (A.S. Hussein), izzahsarmin@uitm.edu.my (N.I. Mohd Sarmin), mibrahim@uitm.edu.my (M.I. Abu Hassan).
Peer review under responsibility of King Saud University. Production and hosting by Elsevier

Production and hosting by Elsevier

https://doi.org/10.1016/j.sdentj.2023.07.003

Conclusion: Different zinc salts have displayed inhibitory growth effects against the common oral bacteria at very low concentrations except for zinc citrate which showed no inhibitory effect against these bacteria in vitro.

1. Introduction

Dental caries is a common disease among all age groups and is considered a major health issue with adverse effects on human health, psychologically and physically by affecting normal growth and reducing the quality of life (Hussein et al., 2021).

Mutans streptococci, mainly *Streptococcus mutans* (*S. mutans*) and *Streptococcus sobrinus* (*S. sobrinus*), are the major causative bacteria responsible for initiating dental caries (Conrads et al., 2014; Meyer and Enax, 2018). Both *S. mutans* and *S. sobrinus* are considered as equally virulent with regard to dental caries development (Conrads et al., 2014). Both pathogens can produce large quantities of extracellular glucans from sucrose fermentation, produce acid exceeding the salivary buffering capacities, bind strongly to teeth and can survive in an acidic environment (Almoudi et al., 2018). In addition, studies have indicated that individuals harbouring both *S. mutans* and *S. sobrinus* had a significantly higher incidence of dental caries compared to those with *S. mutans* alone (Oda et al., 2015).

Zinc is an essential trace element and an important nutrient for maintaining human health (Fatima et al., 2016). It is present in all enzymes as a constituent to perform their essential roles, especially protein synthesis, induction, regulation of the immune system, and DNA and RNA replication (Uwitonze et al., 2020).

In the oral cavity, zinc is present naturally in dental plaque, dental enamel and saliva (Uwitonze et al., 2020). Zinc has been incorporated into oral healthcare products to inhibit dental plaque, control calculus formation and reduce halitosis (Lynch, 2011). It was also added to dental materials given the ability of zinc ions to inhibit the growth of cariogenic bacteria (Almoudi et al., 2018).

The growth inhibition of oral mutans streptococci by zinc salts has been well documented (Almoudi et al., 2018). A significant reduction in *S. mutans* counts was indicated after rinsing either with zinc sulfate, acetate solutions (Burguera-Pascu et al., 2007) or zinc chloride solution (Dobl and Nossek, 1990). Zinc ion has several effects on oral bacteria and can inhibit numerous enzymes in bacterial cells (Phan et al., 2004; Fatima et al., 2016). It can enhance the proton permeabilities of bacterial cells and inhibits the glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenases, pyruvate kinase and phosphoenolpyruvate. Additionally, zinc reduces ATP synthesis in glycolysing cells and diminishes F-ATPase activity leading to bacterial starvation (Koo et al., 2006).

Several factors are responsible for the antibacterial activities of zinc, including its concentration, methods of application and type of zinc salts used (Watson et al., 1991; He et al., 2002). Although free zinc ions (Zn^{2+}) were indicated as the most active zinc species and were responsible for their antibacterial effectiveness (Watson et al., 1991; He et al., 2002). However, other inorganic zinc species have exhibited inhibitory effects even at lower concentrations (Watson et al., 1991). Zinc speciation has a strong effect on the adsorption of zinc to bacterial cells and on the zinc inhibition effect (Watson et al., 1991; Lavaee et al., 2018). Different zinc salts have the capacity to inhibit acid production from glucose by *S. mutans* and a significant amount of zinc adsorption was indicated (Watson et al., 1991; Lavaee et al., 2018).

To prevent dental caries, it is very important to find new treatment that have superior antimicrobial abilities with minimal side effects and capable of eliminating the causative cariogenic bacteria. Hence, despite all the information provided in the literature regarding zinc, limited data are available regarding the antibacterial activities of different zinc salts against *S. mutans* and *S. sobrinus*. Therefore, this study aims to compare the antibacterial effects of different zinc salts against *S. mutans* and *S. sobrinus* in vitro. Furthermore, we investigated the ultrastructure morphological changes of *S. mutans* and *S. sobrinus* following different zinc applications using a scanning electron microscope (SEM).

2. Material and methods

2.1. Chemicals

Four zinc salts were used namely zinc chloride, zinc sulfate, zinc acetate and zinc citrate. All zinc salts were analytic grade, 97–100% pure and were obtained from Sigma Chemical (Sigma-Aldrich, USA). Stock solutions were prepared by dissolving zinc salts in sterile deionised water to obtain a concentration of 16 mg/mL each. Then, the stock solutions were vortexed to ensure complete solubility and were sterilised with a 0.22 μm syringe filter.

2.2. Bacterial strains and growth conditions

Bacteria strains were sub-cultured from −80 °C. *S. mutans* (ATCC 25,175 American Type Culture Collection, USA) and *S. sobrinus* (DSM 20,742 obtained from the German Collection of Microorganisms and Cell Cultures, Germany), were grown on Brain Hearth Infusion broth (BHI) at 37 °C under an aerobic condition for 18–24 h. Microbiological media was obtained from Sigma-Aldrich (St. Louis, MO, USA and Oxoid Ltd, Basingstoke, UK) and prepared according to the manufacturer's instructions.

2.3. Antibacterial susceptibility assay

The antibacterial susceptibility of different zinc salts was investigated by the disc diffusion method on Mueller-Hinton agar plates (Sigma-Aldrich, St. Louis, MO, USA). Briefly, agar plates were inoculated with bacterial suspensions at a concentration of 1×10^8 CFU/mL. Then sterile blank discs (6-mm

diameter) were placed onto the agar surface and 20 µl of 16 mg/mL of different zinc solutions were applied to each disc to give a final concentration of 0.32 mg/disc together with 20 µl of chlorhexidine 0.12% (positive control) and 20 µl of distilled water (negative control). After 24 h of incubation at 37.5 °C, the inhibition zones were measured in millimetres. All experiments were done in triplicate and repeated on different days.

2.4. Minimum inhibitory concentration and minimum bactericidal concentration

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method following the Clinical Laboratory Standards (CLSI, 2012). Briefly, 100 µl serial dilutions of each zinc salt solution were done in 100 µl BHI (Oxoid Ltd, Basingstoke, UK) in a sterile 96-well plate to yield final concentrations ranging from 8 mg/mL to 0.015625 mg/mL. Then, 100 µl of bacterial inoculum (a final concentration of 1×10^6 CFU / mL) was added to each well. Each experiment was tested in triplicate along with a growth control (containing bacterial cells in BHI broth) and negative controls of (uninoculated BHI broth) and (two-fold serial dilutions of zinc solutions in BHI broth) used as sterility control and to evaluate the absorbance changes due to the different zinc salts concentrations respectively were included. Thereafter, the microtiter plates were incubated aerobically at 37 °C for 18–24 h. The growth of bacteria was estimated at 600 nm using a microplate Spectrophotometer (Infinite M200 Pro, Tecan). The MIC was assessed by subtracting OD 600 values of incubated test medium from those of incubated negative control of zinc solutions in BHI broth. The MIC was considered the lowest concentration of tested zinc at which the OD 600 absorbance falls below 0.05 for the negative control (Almoudi et al., 2021). The minimum bactericidal concentration (MBC) was determined by taking a 10 µl aliquot from the clear wells and plated on BHI agar plates and incubated at 37 °C for 24 h. The MBC was defined as the lowest concentration of tested zinc that did not show any bacterial growth on BHI plates following the incubation period.

2.5. Scanning electron microscope (SEM) analysis

The morphological changes appearing after the application of zinc salts on *S. mutans* and *S. sobrinus* bacteria were identified using SEM.

In this experiment, three types of zinc salts namely zinc chloride, zinc sulfate and zinc acetate were used. Meanwhile, zinc citrate was excluded as it was less effective against the tested bacteria and demonstrated higher MIC results compared to the other zinc salts. Briefly, an overnight culture of *S. mutans* and *S. sobrinus* bacteria were treated with MIC concentration of zinc chloride, zinc sulfate and zinc acetate. All the cultures of *S. mutans* and *S. sobrinus* were incubated for 24 h at 37 °C along with non-treated bacteria culture, which served as a negative control. Treated bacteria were fixed in 2.5% glutaraldehyde for 4–6 h and washed with 0.1 M sodium phosphate buffer (pH 7.2) and post-fixed in 1% osmium tetroxide again for two hours. After washing with 0.1 M sodium phosphate buffer (pH 7.2), the samples were dehydrated using graded alcohol series. The specimens were coated with a thin layer of titanium and were observed under SEM.

2.6. Statistical analysis

The inhibition zones of different zinc salts after proper incubation were measured and tabulated. The diameters of the inhibition zone were expressed in millimetres as median and interquartile (IQR). Statistical analyses were performed by comparing the antibacterial activities of the four zinc salts and the controls using Kruskall–Wallis and Mann–Whitney U tests. All the data analysis was performed using the Statistical Package for Social Sciences (SPSS Version 25, IBM Corp, New York). Results were considered significant at $P < 0.05$.

3. Results

3.1. Antibacterial susceptibility assay

The median inhibition zone for zinc chloride against *S. mutans* (13 mm ± 3.5) was higher than that of zinc acetate (12 mm ± 6.5) and zinc sulfate (10 mm ± 5). All the zinc salts tested against *S. mutans* exhibited less inhibition zone compared to chlorhexidine, which was statistically significant ($P < 0.001$).

Regarding *S. sobrinus*, zinc chloride demonstrated the greatest inhibitory effect (20 mm ± 5.5), which was not significantly different ($P > 0.05$) compared to that of chlorhexidine (22 mm ± 4), followed by zinc acetate (18 mm ± 5), and zinc sulfate (16 mm ± 3) as presented in Table 1.

The diameter of the zone of inhibition was not statistically and significantly different ($P > 0.05$) between zinc chloride, zinc sulfate and zinc acetate. However, all zinc salts disclosed statistically and significantly larger inhibition zone compared to the negative control ($P < 0.001$) except *S. mutans* and *S. sobrinus* that was resistant towards zinc citrate. The median diameters of the inhibition zone (in millimetres) of different zinc salts are shown in Table 1 and Fig. 1.

3.2. Minimum inhibitory concentration and minimum bactericidal concentration

Table 2 depicts the MIC and MBC values of different zinc salts against *S. mutans* and *S. sobrinus*. For *S. mutans,* zinc chloride recorded a MIC value of 1 mg/mL whereas both zinc sulfate and zinc acetate had MIC values of 2 mg/mL. MBC values for zinc chloride were 2 mg/mL, followed by 4 mg/mL for both zinc sulfate and zinc acetate. For *S. sobrinus,* zinc chloride, zinc sulfate and zinc acetate recorded MIC values of 0.125 mg/mL and MBC values of 4 mg/mL. Nevertheless, zinc citrate exhibited higher MIC and MBC values respectively of 1 mg/mL and > 8 mg/mL for *S. sobrinus* and 8 mg/mL and > 8 mg/mL for *S. mutans*.

3.3. Scanning electron microscope (SEM)

The results of SEM revealed significant changes in the morphology of zinc-treated *S. mutans* and *S. sobrinus* bacteria compared to the non-treated bacterial cells. The untreated *S. mutans* and *S. sobrinus* exhibited the typical streptococcal appearance as ovoidal (elongated) cells with smooth uniform shapes and intact cell membranes (Fig. 2, A, AI, AII, AIII and Fig. 3, A, AI, AII, AIII). Treated *S. mutans* cells depicted

Table 1 Zone of Inhibition (median ± IQR) of different zinc salts against *S. mutans* and *S. sobrinus*.

	Tested agents Median mm (IQR)								
Bacteria	CHX	DH_2O	Zn citrate	Zn chloride	Zn sulfate	Zn acetate	X^2	P-value[a]	Intergroup comparison P[b]
S. mutans	25 (7)	0	0	13 (3.5)	10 (5)	12 (6.5)	46.53	< 0.001	- Zn chloride, Zn sulfate, Zn acetate VS DH_2O, Zn citrate P < 0.001. -CHX VS Zn chloride, Zn citrate, Zn sulfate, Zn acetate P < 0.001. -Zn chloride VS Zn sulfate VS Zn acetate P > 0.05.
S. sobrinus	22 (4)	0	0	20 (5.5)	16 (3)	18 (5)	44.69	< 0.001	-Zn chloride, Zn sulfate, Zn acetate VS DH_2O and Zn citrate P < 0.001. -CHX VS Zn citrate, Zn sulfate, Zn acetate P < 0.001. -CHX VS Zn chloride P > 0.05. -Zn chloride VS Zn sulfate VS Zn acetate P > 0.05.

[a] Kruskal Wallis test; [b] Mann-Whitney test; (IQR): interquartile; Zn: zinc; CHX: chlorhexidine; DH_2O: distilled water.

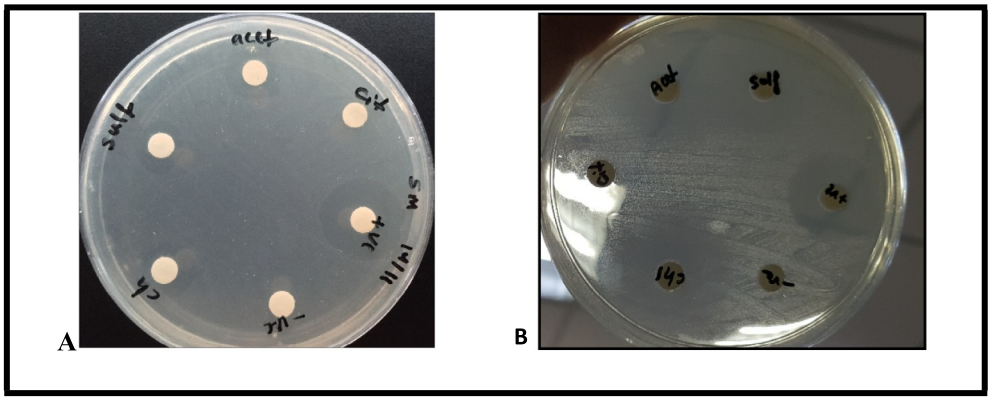

Fig. 1 Zone of Inhibition. A; four zinc salts against *S. mutans*. B, four zinc salts against *S. sobrinus*.

Table 2 MIC and MBC of different zinc salts against *S. mutans* and *S. sobrinus*.

	MIC (mg/mL)			
Tested bacteria	Zinc chloride	Zinc sulfate	Zinc citrate	Zinc acetate
S. mutans	1	2	> 8	2
S. sobrinus	0.125	0.125	1	0.125
Tested bacteria		**MBC (mg/mL)**		
	Zinc chloride	**Zinc sulfate**	**Zinc citrate**	**Zinc acetate**
S. mutans	2	4	> 8	4
S. sobrinus	4	4	> 8	4

(MIC): minimum inhibitory concentration; (MBC): minimum bactericidal concentration.

distinct surface alternations of the formation of cell membrane blebs (Fig. 2, B, BI). In addition, treated *S. mutans* had a thicker biofilm (Fig. 2, C, CI, CII, CIII, D, DI, DII, DIII), and both treated bacteria were agglomerated together. Additionally, both treated *S. mutans* and *S. sobrinus* bacterial cells showed distinct cell membrane damage/rupture (Fig. 3, C, D, BI, BII), wrinkled and rough cell membrane (Fig. 2, CI, DI and Fig. 3, CI) and appeared as misshapen and swollen (Fig. 3, DII, DIII). There were lots of materials attached to the bacterial surfaces (Fig. 2, B, BI, BII, BIII, CI and Fig. 3, B). These results indicated that zinc salts can cause damage to *S. mutans* and *S. sobrinus* cells.

4. Discussion

The present study aimed to investigate and compare the antibacterial activities of different zinc salts namely zinc chloride, zinc sulfate, zinc citrate and zinc acetate on the growth of *S. mutans* and *S. sobrinus* bacteria in vitro. Moreover, limited

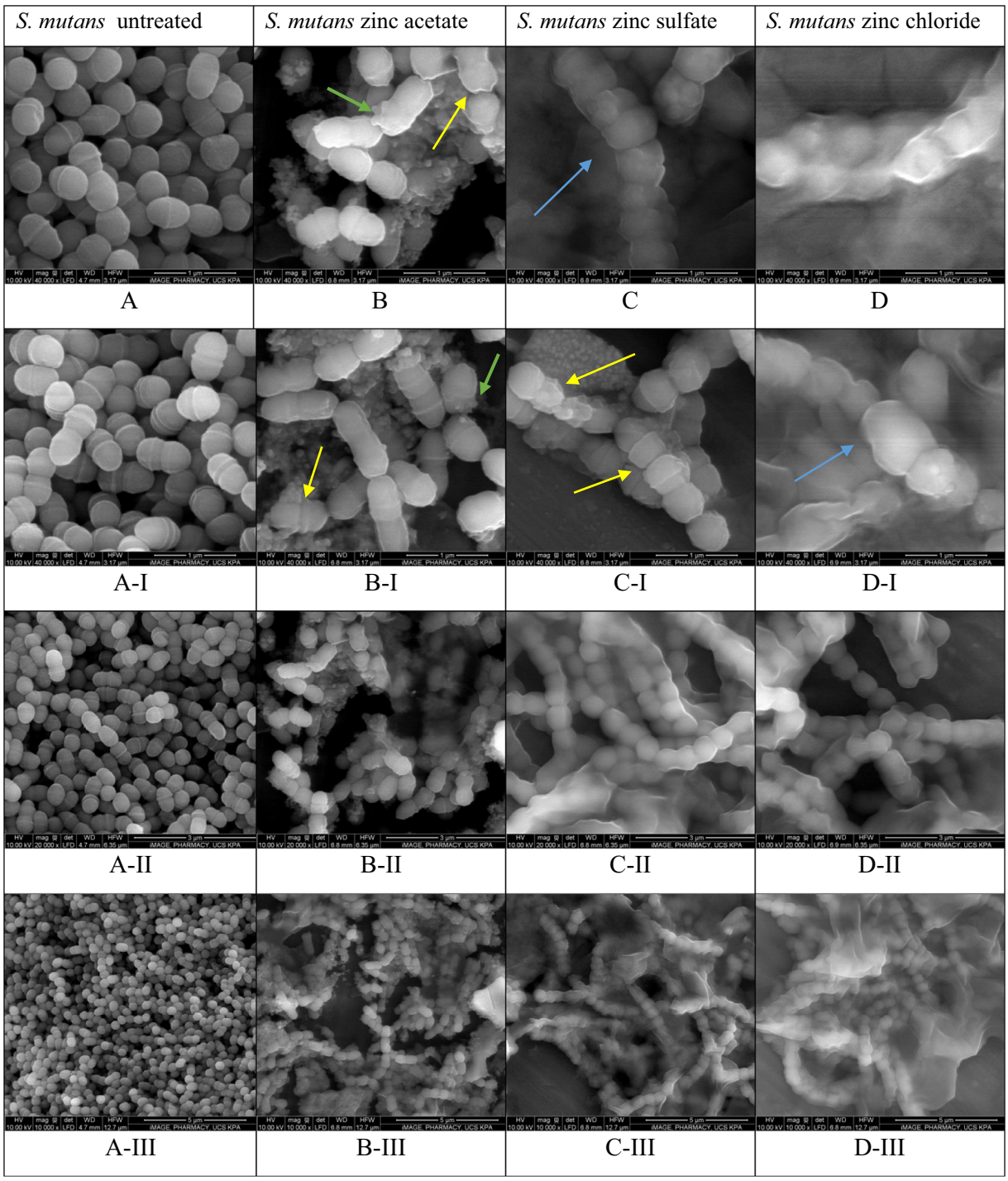

Fig. 2 Scanning electron microscope of untreated *S. mutans* (A-I,II,III)). *S. mutans* treated with zinc acetate (B-I,II,III). *S. mutans* treated with zinc sulfate (C-I,II,III). *S. mutans* treated with zinc chloride (D-I,II,III). Treated bacteria showed bleb like formation (green arrows), wrinkled and rough cell membrane (yellow arrows), and swollen and misshaped cells (blue arrows).

studies have assessed and compared the antibacterial properties of different zinc salts against *S. mutans* and *S. sobrinus*, as most studies in the literature focused on zinc nanoparticles (Almoudi et al., 2018).

Zinc has less toxicity and is considered an environmentally friendly material. It is present naturally in dental plaque and saliva. Furthermore, zinc has been used widely in dentistry such as the incorporation of zinc into dental materials and oral health products, including toothpaste and mouth rinse without the concern of high toxicity or unfavourable side effects (Almoudi et al., 2018). The high concentration of zinc can also persists for many hours after delivery from oral health

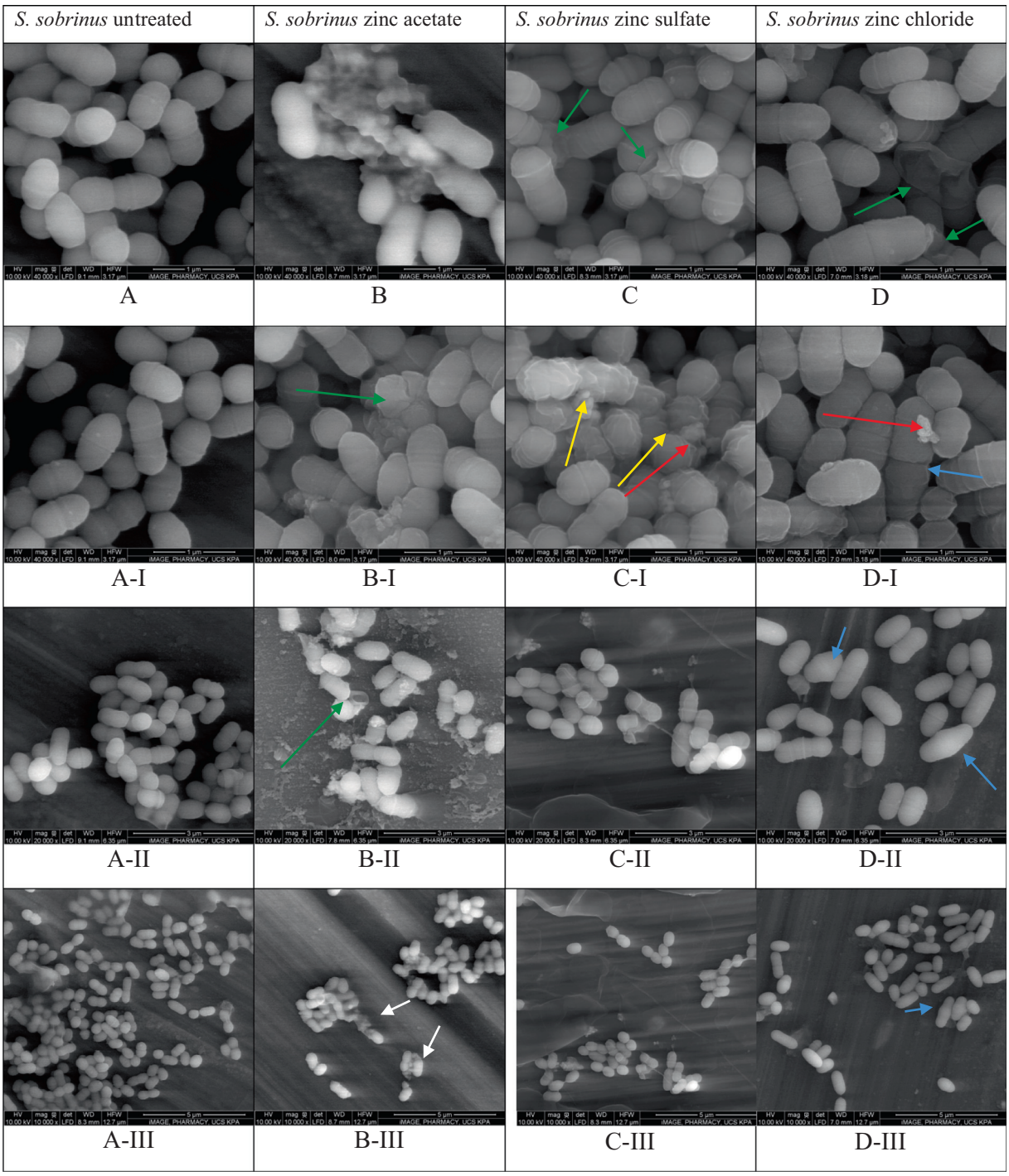

Fig. 3 Scanning electron microscope of untreated *S. sobrinus* (A-I,II,III)). *S. sobrinus* treated with zinc acetate (B-I,II,III). *S. sobrinus* treated with zinc sulfate (C-I,II,III). *S. sobrinus* treated with zinc chloride (D-I,II,III). Treated bacteria showed cell membrane damage/ruptured (green arrows), intracellular materials leakage (red arrows), wrinkled and rough cell membrane (yellow arrows), and swollen diploid cells (blue arrows).

products (Lynch, 2011). Therefore, it is vital to study this element to gain more knowledge about its different types in relation to their antibacterial effect against *S. mutans* and *S. sobrinus*.

The primary pathogenic bacteria involved in the development of dental caries are *S. mutans* and *S. sobrinus,* which have similar virulence in causing dental caries (Conrads et al., 2014). Chlorhexidine has proved to be the most effective oral antimicrobial agent compared to other agents and it is considered the gold standard. This effectiveness is linked to its broad-spectrum action against gram-positive and gram-negative bacteria (Rossi et al., 2014). It was found that *S. sobrinus* reappear in saliva and plaque more rapidly and at a higher level after chlorhexidine application than *S. mutans* (Grönroos et al., 1995). In the present study, the MIC and MBC values of

different zinc salts against *S. sobrinus* were lower than *S. mutans* indicating *S. sobrinus* is more sensitive to zinc salts compared to *S. mutans*. Hence, these results suggest that zinc has a favourable potential antibacterial effect against cariogenic bacteria, primarily *S. sobrinus,* and is a promising candidate for the management of dental caries.

The antimicrobial agent is considered bactericidal when its MBC is equal to or less than four times its MIC (de Araujo et al., 2015). The MBC result was higher than MIC, which suggests that zinc has bacteriostatic activity against tested microorganisms. Previous studies with different methods have indicated an antimicrobial effect of different zinc salts against oral bacteria, especially *S. mutans*. Nevertheless, there are limited studies to evaluate this effect against *S. sobrinus*. The antibacterial susceptibility of zinc salts was investigated using the disc agar diffusion method, which is a widely used technique to determine the antibacterial effects of an agent (Balouiri et al., 2016). In fact, all of the zinc salts tested demonstrated a zone of inhibition (ZOI) except zinc citrate. It appears that the insolubility of zinc citrate may have hindered its diffusion to the surrounding agar surface. Indeed, the antibacterial susceptibility test is used for materials which are soluble and capable of diffusing into the surrounding environment (Weiss et al., 1996; Khan et al., 2019). This may explain why zinc citrate had no ZOI in the present study. Similar results were reported by Bradshaw et al. (1993) as zinc citrate had no growth inhibition effects against *S. mutans*. Furthermore, the present results revealed larger ZOI for zinc chloride, zinc sulfate and zinc acetate differed significantly compared to the negative control (distilled water). In contrast, zinc sulfate and zinc acetate had significantly smaller ZOI compared to the positive control (chlorhexidine), whereas zinc chloride demonstrated no significant difference relative to chlorhexidine when tested against *S. sobrinus*. Similar results were reported previously in which rinsing the mouth either with zinc sulfate or zinc acetate solutions reflected a significant reduction in the total mean of *S. mutans* counts (Burguera-Pascu et al., 2007). Likewise, zinc sulfate and zinc acetate demonstrated a suitable ZOI against *S. mutans* (Lavaee et al., 2018).

The bacterial cell has a membrane that is protective and assists in maintaining its normal activities. It serves as a selective environmental barrier and contains determinants required for bacterial colonisation and survival (Azari et al., 2013). The cell wall is considered the first barrier that an antimicrobial agent must overcome when interacting with its target (Martinez de Tejada et al., 2012). The main component of the cell wall is peptidoglycan, which is found in almost all bacteria and is responsible for preserving the integrity of the cell. Gram-positive bacteria were less sensitive to antibacterial agents compared to gram-negative bacteria given the presence of a thicker peptidoglycan layer, which acts as an additional barrier for the entry of antimicrobial agents into the bacterial cells (Sinha et al., 2011). Notably, zinc ions can be transported across the bacterial cell membrane via ion channels, utilising extra energy and triggering a disturbing effect on bacteria (Chen et al., 2017).

In this study, the SEM results demonstrated the agglomeration of *S. mutans* and *S. sobrinus* which were treated with zinc salts. This may stem from the electrostatic attraction between the negatively charged cell membrane of the bacterialcells and the positively charged zinc ion+ (Yoo et al., 2021). In addition, a substantial amount of zinc materials was observed on the bacterial surfaces. The morphological changes under SEM, including the formation of blebs, wrinkled surfaces and cellular membrane damages, were identified following the treatment of tested bacteria with different zinc salts, which is considered an inactivation of the bacteria (Orasmo et al., 2013). The present results are consistent with previous studies reporting morphological changes in the surfaces of bacterial cells upon treatment with antimicrobial agents (Orasmo et al., 2013; Almoudi et al., 2021).

5. Conclusion

The results of this study indicated that zinc chloride, zinc sulfate and zinc acetate can inhibit the growth of *S. mutans* and *S. sobrinus* in vitro and alter the normal cell morphology of these bacteria. However, zinc citrate did not reflect any antibacterial effect on both tested bacteria. Future research, such as the time kill assay and mechanism of action are recommended to gain more knowledge about different zinc salts and oral bacteria.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

Special thanks to Ts. Dr. Nurhayati Mohamad Zain for sharing her precious knowledge, and valuable advice during the laboratory work.

References

Almoudi, M.M., Hussein, A.S., Abu Hassan, M.I., Zain, N.M., 2018. A systematic review on antibacterial activity of zinc against Streptococcus mutans. Saudi Dent. J. 30, 283–291.

Almoudi, M.M.M., Hussein, A.S., Abu Hassan, M.I., Al-talib, H., Khan, H.B.S., Gulam, N., Efira, S.A., Binti, N.A., 2021. The antibacterial effects of vitamin D_3 against mutans streptococci : an in vitro study. Eur. Oral Res. 55, 8–15.

Azari, F., Nyland, L., Yu, C., Radermacher, M., Mintz, K.P., Ruiz, T., 2013. Ultrastructural analysis of the rugose cell envelope of a member of the Pasteurellaceae family. J. Bacteriol. 195, 1680–1688.

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: a review. J. Pharm. Anal. 6, 71–79.

Bradshaw, D.J., Marsh, P.D., Watson, G.K., Cummins, D., 1993. The effects of triclosan and zinc citrate, alone and in combination, on a community of oral bacteria grown in vitro. J. Dent. Res. 72, 25–30.

Burguera-Pascu, M., Rodríguez-Archilla, A., Baca, P., 2007. Substantivity of zinc salts used as rinsing solutions and their effect on the inhibition of Streptococcus mutans. J. Trace Elem. Med. Biol. 21, 92–101.

Chen, J., Zhang, X., Huang, C., Cai, H., Hu, S., Wan, Q., Pei, X., Wang, J., 2017. Osteogenic activity and antibacterial effect of porous titanium modified with metal-organic framework films. J. Biomed. Mater. Res. Part A 105, 834–846.

CLSI, 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—Ninth edition. Clinical and Laboratory Standards Institute document M07-A9. Wayne, PA.

Conrads, G., de Soet, J., Song, L., Henne, K., Sztajer, H., Wagner Döbler, I., Zeng, A., 2014. Comparing the cariogenic species Streptococcus sobrinus and S. mutans on whole genome level. J. Oral Microbiol. 6, 26189.

de Araujo, A.R., Quelemes, P.V., Perfeito, M.L.G., de Lima, L.I., Sá, M.C., Nunes, P.H.M., Joanitti, G.A., Eaton, P., dos Santos Soares, M.J., de Almeida, J.R., de S.,, 2015. Antibacterial, antibiofilm and cytotoxic activities of Terminalia fagifolia Mart. extract and fractions. Ann. Clin. Microbiol. Antimicrob. 14, 25.

Dobl, P., Nossek, H., 1990. The effect of zinc chloride mouthwashes on caries-inducing plaque streptococci. 2. In vivo studies of the antibacterial effect of zinc chloride on the total streptococcal flora of the dental plaque. Zahn. Mund. Kieferheilkd. Zentralbl. 78, 393–396.

Fatima, T., Rahim, Z.B., Lin, C.W., Qamar, Z., 2016. Zinc: a precious trace element for oral health care? J. Pak. Med. Assoc. 66, 1019–1023.

Grönroos, L., Mättö, J., Saarela, M., Luoma, A.R., Luoma, H., Jousimies Somer, H., Pyhälä, L., Asikainen, S., Alaluusua, S., 1995. Chlorhexidine susceptibilities of mutans streptococcal serotypes and ribotypes. Antimicrob. Agents Chemother. 39, 894–898.

He, G., Pearce, E.I.F., Sissons, C.H., 2002. Inhibitory effect of ZnCl2 on glycolysis in human oral microbes. Arch. Oral Biol. 47, 117–129.

Hussein, A.S., Almoudi, M.M., Abu-Hassan, M.I., Schroth, R.J., Saripudin, B., Shawal, M.F., 2021. Serum and saliva 25(OH)D levels in relation to dental caries in young children. J. Clin. Pediatr. Dent. 45, 10–14.

Khan, Z.A., Siddiqui, M.F., Seungkyung, P.S., 2019. Current and emerging methods of antibiotic susceptibility testing. Diagnostics (Basel). 9 (2), 49.

Koo, H., Sheng, J., Nguyen, P.T.M., Marquis, R.E., 2006. Cooperative inhibition by fluoride and zinc of glucosyl transferase production and polysaccharide synthesis by mutans streptococci in suspension cultures and biofilms. FEMS Microbiol. Lett. 254, 134–140.

Lavaee, F., Ghapanchi, J., Motamedifar, M., Javidi, M.S., 2018. Experimental evaluation of the effect of zinc salt on inhibition of *Streptococcus mutans*. J. Dent. (Shiraz) 19, 168–173.

Lynch, R.J.M., 2011. Zinc in the mouth, its interactions with dental enamel and possible effects on caries; A review of the literature. Int. Dent. J. 61, 46–54.

Martinez de Tejada, G., Sánchez Gómez, S., Rázquin Olazaran, I., Kowalski, I., Kaconis, Y., Heinbockel, L., Andra, J., Schurholz, T., Hornef, M., Dupont, A., 2012. Bacterial cell wall compounds as promising targets of antimicrobial agents I. Antimicrobial peptides and lipopolyamines. Curr. Drug Targets 13, 1121–1130.

Meyer, F., Enax, J., 2018. Early childhood caries: epidemiology, aetiology, and prevention. Int J Dent. 2018, 1415873.

Oda, Y., Hayashi, F., Okada, M., 2015. Longitudinal study of dental caries incidence associated with Streptococcus mutans and Streptococcus sobrinus in patients with intellectual disabilities. BMC Oral Health 15, 102.

Orasmo, E.A.C., Miyakawa, W., Otani, C., Khouri, S., 2013. In vitro AFM evaluation of Streptococcus mutans membrane exposed to two mouthwashes. J. Appl. Pharm. Sci. 3, 24–28.

Phan, T.-N., Buckner, T., Sheng, J., Baldeck, J.D., Marquis, R.E., 2004. Physiologic actions of zinc related to inhibition of acid and alkali production by oral streptococci in suspensions and biofilms. Oral Microbiol. Immunol. 19, 31–38.

Rossi, A.D., Ferreira, D.C.A., da Silva, R.A.B., de Queiroz, A.M., da Silva, L.A.B., Nelson Filho, P., 2014. Antimicrobial activity of toothpastes containing natural extracts, chlorhexidine or triclosan. Braz. Dent. J. 25, 186–190.

Sinha, R., Karan, R., Sinha, A., Khare, S.K., 2011. Interaction and nanotoxic effect of ZnO and Ag nanoparticles on mesophilic and halophilic bacterial cells. Bioresour. Technol. 102, 1516–1520.

Uwitonze, A.M., Ojeh, N., Murererehe, J., Atfi, A., Razzaque, M.S., 2020. Zinc adequacy is essential for the maintenance of optimal oral health. Nutrients 12, 949.

Watson, G.K., Cummins, D., van der Ouderaa, F.J.G., 1991. Inhibition of acid production by Streptococcus mutans NCTC 10449 by zinc and the effect of metal speciation. Caries Res. 25, 431–437.

Weiss, E.I., Shalhav, M., Fuss, Z., 1996. Assessment of antibacterial activity of endodontic sealers by a direct contact test. Endod. Dent. Traumatol. 12, 179–184.

Yoo, A., Lin, M., Mustapha, A., 2021. Zinc oxide and silver nanoparticle effects on intestinal bacteria. Materials (Basel). 14, 2489.