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Anti-Biofilm and Antimicrobial Activity of Sodium Fluoride Against Various Pathogenic Microbes

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ABSTRACT

Purpose: Many dietary supplements, dental products, few medications like antifungal and antibacterial drugs contain fluoride, typically in the form of sodium fluoride. Fluoride has been widely used for oral hygiene but the anti-biofilm and antimicrobial activity of pure sodium fluoride solutions has not been reported.

Methods: In the present study, antimicrobial properties of different concentrations of sodium fluoride against eleven clinical isolates of bacterial and two clinical isolates of fungal strains were evaluated. The effect of sodium fluoride on the biofilm formation of the most sensitive strains was also examined

Results: Sodium fluoride efficiently suppresses the growth of all pathogens in a concentration-dependent manner. 10% sodium fluoride showed the highest inhibition against *E. faecalis* among gram-positive strains and P. aeruginosa among gram-negative strains with the inhibition zone of 25±1.0 mm and 27±0.0 respectively whereas both fungal strains showed almost the same results of 15 mm of inhibition. Sodium fluoride successfully reduced biofilm formation at concentrations of 7% in dose-dependent anti-biofilm activity against S. epidermidis and *E. faecalis;* 8% against E. cloacae, P. aeruginosa, and *C.tropicalis*

Conclusions: Our results indicate that various pathogenic microbes cannot tolerate high exposure to sodium fluoride. Fluoride-containing products can help to reduce pathogens and biofilm formation. However, products containing high amounts of sodium fluoride as the active ingredient should be used carefully in order to avoid any toxicity and adverse effects.

Key-words: *Antibacterial; Antifungal; Biofilm; Sodium Fluoride*

INTRODUCTION

Fluoride often presents as sodium fluoride is widely distributed naturally in large quantities and at high concentrations [1]. It occurs naturally in varying concentrations in air, water, soil, and rocks and is a component of the earth's crust [2], and is always present in our surroundings and daily life. People are exposed to it through diet, respiration, and fluoride supplements but very high amounts can be harmful. Safe or toxic levels of fluoride concentration depend on the organism's sensitivity, fluoride concentration, and the conditions under which fluoride is delivered [3]. Low fluoride levels can prevent cavities and help certain minerals in teeth to re-mineralize whereas high fluoride levels can damage tissue by generating free radicals, and protein inhibition. ADP or a divalent metal ion interacts with fluoride to form a nonfunctional metal-fluoride complex that mimics ATP [4,5]. This complex can inhibit enolase and a wide range of metabolic enzymes, mainly phosphatases [5,6]. Fluoride increases the membrane permeability to

protons and the formation of HF directly binds and inhibits specific cellular enzymes. The toxicity of fluoride through such inhibition is found in all three domains of life i.e. Archaea, Bacteria, and Eukarya[5].

Fluoride is a powerful antibacterial agent that disrupts bacterial metabolism. Fluoride inhibits bacterial cell growth in vitro through direct inhibition of glycolysis and energy metabolism[7]. It can also induce oxidative stress and interrupt glutathione metabolism[8]. Fluoride is known to have antifungal properties which are mainly due to direct enzyme inhibition and cytoplasmic acidification in cells [9-11]. Living cells absorb the fluoride in the form of HF, which causes intracellular H^+ accumulation and inhibits plasma H^+ extrusion through ATPase[12,13]. Topical fluoride varnish has antibacterial properties against pathogenic bacteria. Generally, they contain 5% sodium fluoride and are prescribed two times a year to prevent dental caries which can be reduced by 25 to 30% and can significantly decrease the number of pathogenic bacteria in plaque [11-15].

Fluoride is frequently used as an anti-cariogenic element due to its unique properties of decreasing demineralization, increasing remineralization, and inhibiting pellicle and biofilm formation16. Biofilms are usually described as dense, highly hydrated clusters of bacterial cells which are initiated by the adhesion of bacteria to any surface [16-19]. Fluoride has anti-biofilms activity as it inhibits acid production, tolerance, and glucosyltransferase production in pathogenic bacteria. Fluoride's impact on the pathogenicity of cariogenic biofilms has also been acknowledged [20]. A single shock of 300 ppm of fluoride treatment is reported to control the biofilm of Streptococcus mutans [21]. Inhibition of adhesion to any surface by fluoride treatment is the main mechanism behind it [22]. Recently Han [20] reported a much higher rate of inhibition of biofilm growth at an early stage by fluoride treatment than at the mature stage as early-stage treatment by fluoride was very effective in controlling cariogenic biofilm development and preventing dental caries. At the mature biofilm stage, only higher concentrations of fluoride treatment are needed to inhibit biofilm accumulation [20].

Considering the above literature, the antimicrobial and anti-biofilm activity of sodium fluoride against [13] clinically isolated pathogenic microorganisms was evaluated.

MATERIAL AND METHODS

Preparation of sodium fluoride solution

Anhydrous, powder of Sodium monofluoride (NaF) was used to prepare an aqueous solution with different concentrations in the range from 2.5% to 10%. The prepared solution was directly used for the antibacterial and anti-biofilm test as mentioned below.

Microbial Strains

13 clinical isolates used in this study were Gram-positive strains of *Staphylococcus epidermidis, Enterococcus faecalis, Staphylococcus aureus, Streptococcus pneumoniae*, and *Bacillus subtilis,* Gram-negative strains of *Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Providencia stuartii, Salmonella Typhi*, and fungal strains of C*andida albicans, Candida tropicalis*. King Khalid Hospital in Riyadh provided all test strains. Prior to usage, all bacterial strains were reactivated on Mueller-Hinton Agar (MHA) plates, and all fungal strains were reactivated on Dextrose Agar (SDA) plate.

Antimicrobial activity

The well diffusion method was used to measure antibacterial activity. All bacterial strains had their overnight broth cultures adjusted to approximately 10^6 CFU/mL. 20 µL was dispersed using a sterile cotton swab onto 20 mL of sterile agar plates. For around 3 minutes, the medium's surface was allowed to dry. Six mm diameter sterile wells were placed into the plates, and 100 mL of three different concentrations (2.5%, 5% &10%) of sodium fluoride solution was poured into each well to conduct the test. Following a 24-hour incubation period at 37 °C, the diameter of the inhibitory zone (measured in mm) was used to quantify the amount of microbial growth. Each test solution will be examined three times, and the mean results are presented.

Determination of Minimum inhibitory concentration

Minimum inhibitory concentration (MIC)was carried out by broth microdilution procedure. In 96-well microliter plates, the test solutions were diluted in Mueller Hinton broth (MHB) to achieve final concentrations ranging from 1% to 10% of a sodium fluoride solution. Then, each well was added with 100 mL of log-phase bacterial culture adjusted to approximately 10^6 CFU/mL. The turbidity of media was observed for bacterial growth after 20h of incubation at 37^0 C. The MIC was taken as the lowest concentration of sodium fluoride necessary to limit bacterial growth without turbidity of the medium.

Determination of Minimum Bactericidal Concentrations

The wells showing the bacteria inhibited in the broth microdilution method mentioned above were plated on Mueller Hinton agar (MHA) and fungal inhibitions on Dextrose Agar and grown at 37^0 degrees for 20h. The Minimum Bactericidal Concentration (MBC) was taken as the lowest concentration of sodium fluoride that killed 99.9% of bacteria or fungi showing no growth on plates.

Determination of biofilm formation by Microtiter plate method.

The effect of sodium fluoride on the biofilm formation of the most sensitive strains was examined through the microdilution method. Each bacterial strain was grown in Mueller Hinton agar (MHA overnight. The culture was diluted to 10^6 CFU/mL and transferred to a 96-well microtiter plate containing different concentrations of sodium fluoride. After incubation 37^0 cells were dumped out by turning the plate over each well was washed with 200 µl sterile phosphate-buffered saline. 125 µL of a 0.1% solution of crystal violet was added to each well incubated for 15 min at room temperature. Excess stain was rinsed away under running tap water. Plates were air-dried, and stained biofilms were solubilized in 120 µl of 30% (v/v) glacial acetic acid. OD was measured with a micro ELISA automatic plate at 570 nm to quantify biofilm formation. In qualitative assays, the wells were photographed.

Statistical Analysis

Results were expressed as mean ± standard deviation (SD) for tests performed three times.

RESULTS

Antimicrobial activity

well diffusion -The antimicrobial activity of the aqueous solution of sodium fluoride at the concentration of 2.5 %, 5%, and 10% against 11 bacterial and 2 fungal strains has been assessed by using an agar well diffusion method. Sodium fluoride efficiently suppresses the growth of all the pathogens in a concentration-dependent manner and the corresponding inhibition zones are shown in Table 1 and Figure 1-2. As listed in Table 1, 10% sodium fluoride showed the highest inhibition against *E. faecalis* among gram-positive strains and *P. aeruginosa* among gram-negative strains with the inhibition zone of 25±1.0 mm and 27±0.0 respectively whereas both fungal strains showed almost the same results of 15 mm of inhibition.

MIC and MBC- Based on the antibacterial assay results four bacterial strains and one fungal strain were selected for MIC and MBC assay which include the gram-positive strain of S. *epidermidis* and *E. faecalis* gram-negative strain of E. *cloacae* and *P.aeruginosa* and fungal strain of *C.tropicalis* MIC and MBC values of sodium fluoride solution against the most sensitive strain are presented in Table 2 and Figure 3. *S. epidermidis and E. faecalis* both recorded the MIC with 3% MBC with 4% solution. *E. cloacae* and *P.aeruginosa* recorded the MIC with 4% MBC with 6% solution whereas *C.tropicalis* showed MBC with 5% solution and MIC with 7% solution (Table 2).

Antibiofilm activity

Antibiofilm activity of various concentrations of sodium fluoride was evaluated by measuring biofilm growth with crystal violet. Sodium fluoride successfully reduced biofilm formation at concentrations of 7% in dose-dependent anti-biofilm activity against *S. epidermidis* and *E. faecalis*; 8% against *E. cloacae*, *P.aeruginosa* and *C.tropicalis* (Figure 4). The positive control without sodium fluoride exhibited full growth. Notable inhibition of colony growth decreased by the

addition of sodium fluoride which goes on to decrease further with the addition of increasing sodium fluoride concentrations.

Table 1 - Zone of Inhibition (mm) of different concentrations of sodium fluoride against various microbial strains.

	Strains	Zone of inhibition		
		2.5%	5%	10%
Gram- +	*Staphylococcus epidermidis*	15±0.5	18±0.5	20±1.0
	Enterococcus faecalis	18±0.5	20±.0.0	25±1.0
	Staphylococcus aureus	10±1.0	13±1.0	20±0.5
	Streptococcus pneumoniae	9±0.5	10±0.5	12±1.0
	Bacillus subtilis	10±1.0	13±1.0	20±1.0
Gram- -	*Escherichia coli*	10±0.5	12±0.5	18±00
	Enterobacter cloacae	10±1.0	12±1.0	15±00
	Klebsiella pneumoniae	7±00	8±00	12±1.0
	Pseudomonas aeruginosa	15±1.0	20±1.0	27±00
	Providencia stuartii	8±0.5	10±0.5	18±1.0
	Salmonella Typhi	10±.00	11±.00	15±1.0
Fungi	*Candida albicans*	9±0.5	12±0.5	.5±1.00
	Candida tropicalis	9±1.00	11±1.00	15±00

Table 2 The values of MIC and MBC for sodium fluoride solution against selected microbial strains

Strains	MIC (%solution)	MBC (%solution)
Staphylococcus epidermidis	*3*	*5*
Enterococcus faecalis	*3*	*5*
Enterobacter cloacae	*4*	*6*
Pseudomonas aeruginosa	*4*	*6*
Candida tropicalis	*5*	*7*

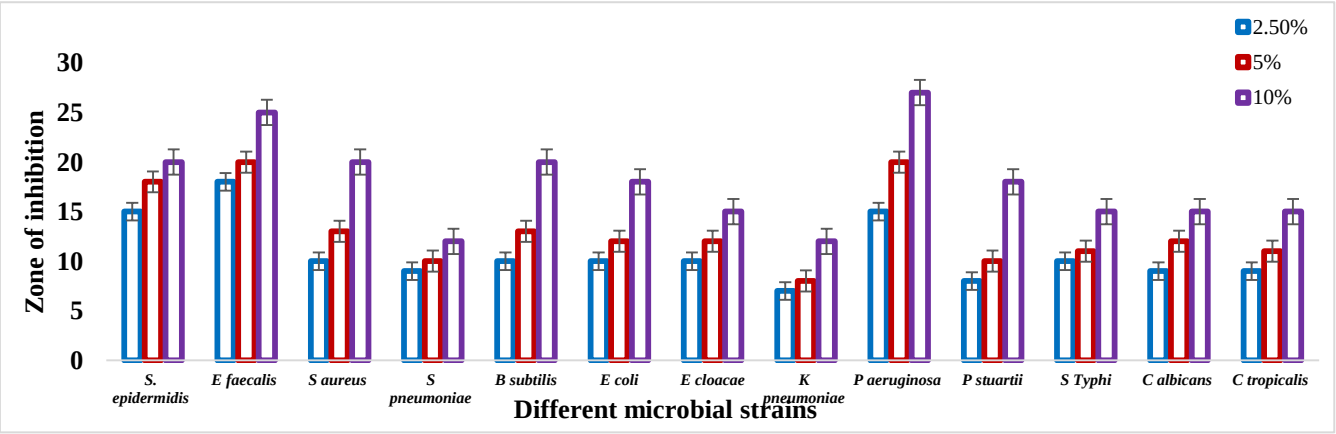

Figure 1 - Zone of Inhibition (mm) of different concentrations of sodium fluoride against various microbial strains.

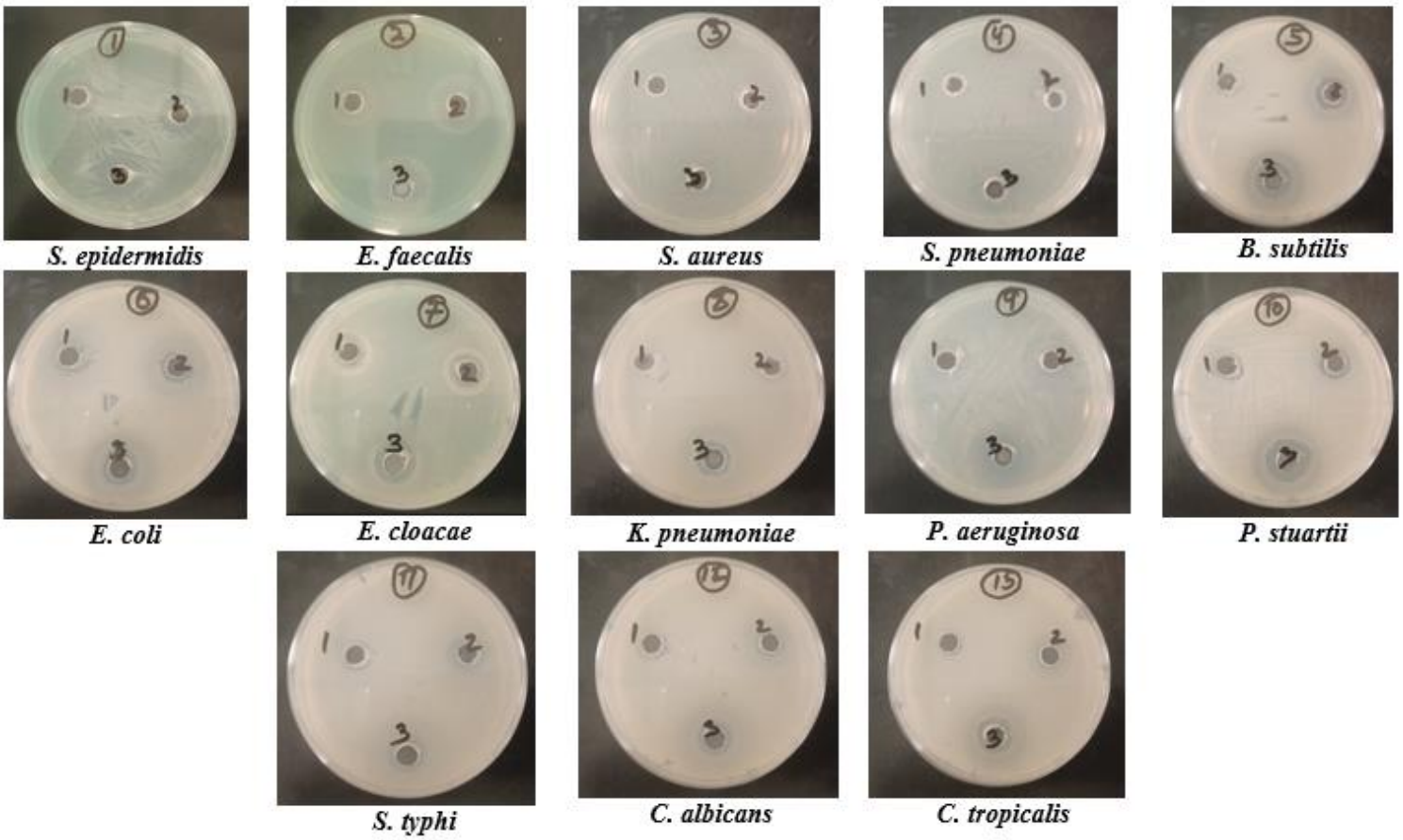

Figure 2- Well diffusion assay demonstrating the antibacterial activity of different concentrations of sodium fluoride well 1=2.5%; well 2=5%& well 3=10%.

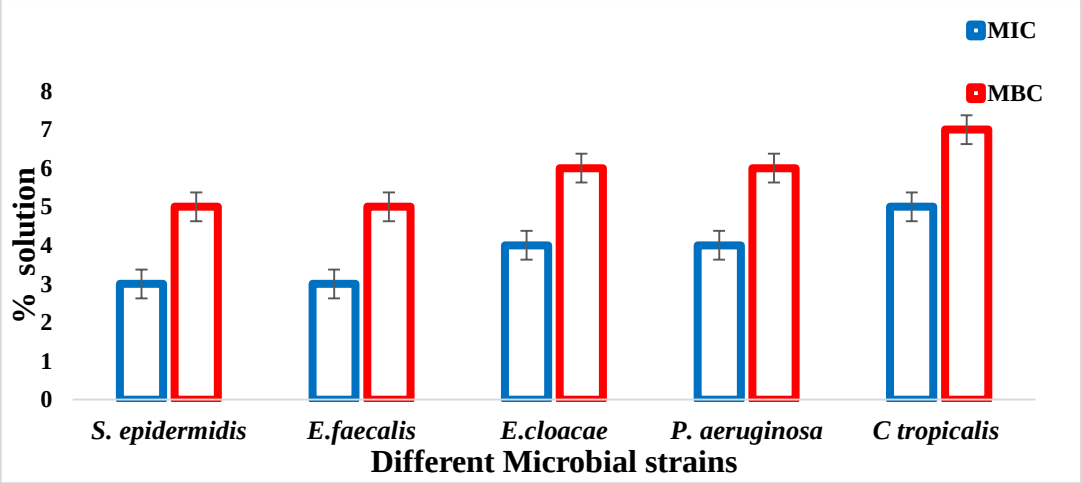

Figure 3- MIC and MBC for sodium fluoride % solution against different microbial strain

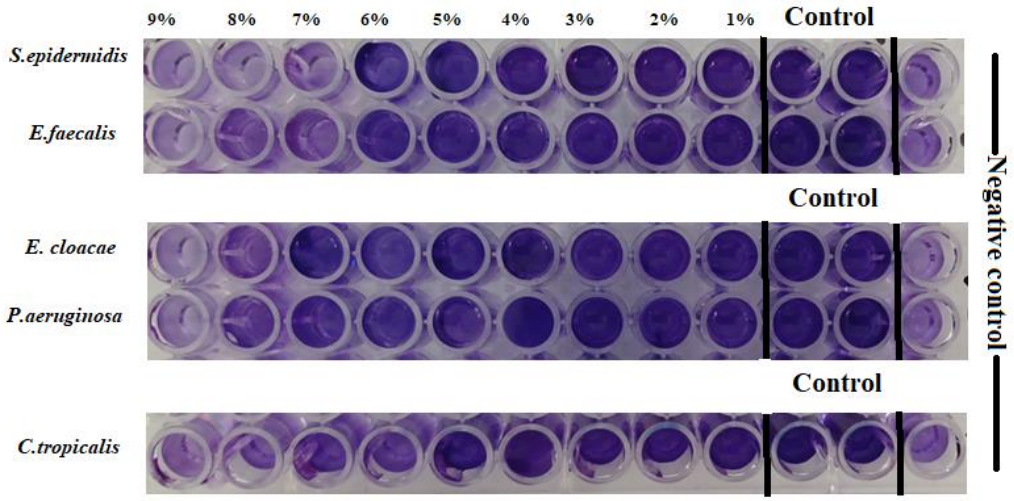

Figure 4- Microliter plates demonstrating the anti-biofilm activity of sodium fluoride solution against selected microbial strains.

DISCUSSION

Being a toxic element, a high concentration of fluoride in the environment can be harmful not only to plants[23,24] and animals [25-30] but also to microbes. vity[33]. In the present study, we tested the antibacterial ability of sodium fluoride against thirteen microbes mostly found in the oral cavity by using an agar well diffusion method. This method is widely used to measure the antimicrobial ability of any substance in vitro [34,35]. Sodium Fluoride showed antibacterial activity against all gram-positive, gram-negative, and fungal strains tested in the study confirming its potent antibacterial and antifungal ability. Our findings are in agreement with the previous report of Thomas et al [36] where sodium fluoride was reported as an excellent antimicrobial agent against both gram-positive and gram-negative florae in addition to fungal strains found in the oral cavity. Nowadays, commercially available mouth rinses and tubes of toothpaste are added with sodium fluoride for the reason of treating or preventing both dental caries and gingivitis [37-40]. Fluoride-containing dental products significantly reduce the risk of caries [41]. Places supplied with fluorine-free drinking water are recommended for brushing with fluoridated toothpaste as an effective intervention to prevent tooth decay [42,43] shows the importance of fluoride as an antimicrobial agent. We observed the anti-candidal activity of sodium fluoride against both *Candida* species with almost the same inhibition zone. These results can be supported by Yigit et al [44] where sodium fluoride-containing toothpaste was found equally effective against six oral *Candida*.

We checked the ability of a few selected strains which include bacterial strains of *S. epidermidis, E. faecalis, P. aeruginosa,* and *E. cloacae,* and a fungal strain of *C. tropicalis to* form biofilm on polystyrene and then examined the anti-biofilm activity of Sodium fluoride on it. Sodium fluoride at MIC levels endorsed biofilm detachment for all the test trains (Figure 4). *S.epidermidis* strains are commonly found in are found in dental plaque of healthy individuals[45]. *E. faecalis* is involved in persistent endodontic infections and its strains obtained from root canals, and oral cavities are able to develop a biofilm[46,47]. *P.aeruginosa* is known for its biofilm former and is a good model for biofilm studies[48]. It can colonize on different surfaces with influential binding factors like flagella and pili[49]. *E.* Fluoride-induced biochemical changes are the fundamental factors responsible for its toxicity[1]. Many studies have documented the antibacterial ability of fluoride, typically present as sodium fluoride against microbes present in soil [31], water [32], and the oral ca *cloacae* is frequently isolated from individuals with advanced gum diseases [50]. Among all *Candida* species *C. tropicalis* is the most adherent and strong biofilm producer[51]. Our results are validated through many previous studies reporting the use of sodium fluoride products as anti-biofilm agents against multi-species biofilm[16, 20,21,52,53]

CONCLUSIONS

To our knowledge, this study is the first report addressing the anti-biofilm and antibacterial activity of sodium fluoride solution. Our results indicate that various pathogenic microbes cannot tolerate high exposure to sodium fluoride. Fluoride-containing dental products with at least 2.5% sodium fluoride can help to reduce oral pathogens and biofilm formation in the oral cavity. However, products containing high amounts of sodium fluoride as the active ingredient should be used carefully in order to avoid any toxicity and adverse effects.

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CONFLICT OF INTERESTS

None

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