Title: The Effect of Xylitol on *Staphylococcus epidermidis*

Research Question: How do different concentrations of Xylitol in water affect the survival and the quantity of the oral *Staphylococcus epidermidis* measured by disk diffusion method in the in vitro environment at 37°C

Subject: Biology

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Introduction

RQ: How do different concentrations of xylitol affect the survival and amount of the oral *Staphylococcus epidermidis* by disk diffusion method in the in vitro environment at 37°C.

Gum, a wildly popular sugary substance that is enjoyed especially by children and teens but is usually critiqued by parents like all candy because it has been found to feed and help the growth of opportunistic bacteria in the oral flora and cause dental caries[i]. The risk of dental caries is on the rise since sugar (glucose) consumption is getting higher and higher in teens and children[ii] leading scientists to look for sweetener alternatives to glucose. In the light of these studies, the chance of getting cavities however was found to be drastically decreased and even prevented by the usage of sugar substitutes like sorbitol and especially xylitol.[iii]

Xylitol, being a naturally occurring sugar polyol that is found in fruit and vegetables, [iv]is an extremely common ingredient in the gum industry, the top three most selling gums being xylitol gums[v]

From a young age, I observed that many of my peers were made aware of the dangers of sugar consumption including myself, therefore, people around me mostly prefer sugar-free options of food items including sugar-free xylitol gum. When I saw the xylitol in the ingredients of my gum, however, I was reminded of how xylitol is deadly even in small amounts for dogs[vi] so I was curious to find out what the effects of it were on humans and especially the oral flora containing thousands of different bacteria because I've always been interested in the mutualistic relationship between humans and bacterium. Therefore, I presumed if xylitol could kill dogs, it would do unimaginable harm to the oral bacteria and found it displayed different affects in different dosages after my research.

Background Information

The Oral Flora

The oral flora is the total of bacteria and microorganisms that exist in the oral cavity of a healthy individual. The oral flora or the oral microbiome starts forming at a young age and is essential for oral health since the oral bacteria make up the biofilm, which helps the human body defend itself from different microbes, provides mechanical protection[vii] and helps extracellular digestion by releasing different enzymes into saliva but can also cause many diseases like dental caries, periodontitis, and gingivitis if the microbial homeostasis of this ecosystem is disturbed. These bacteria among the others are beneficial to the host organism if kept under control. This microbiome is dominated by the streptococcus species[viii] like *mutans* that cause cavities, but it also includes the *Staphylococcus epidermidis* species (ATCC 1228).

Staphylococcus epidermidis

This bacterium, like mentioned, is a part of the oral microbiome, concentrated mostly in the mucosal part of the oral cavity towards the pharynx. It is an active part of producing biofilm, whose importance was mentioned in the "The Oral Flora" section.[ix] It is an opportunistic pathogen meaning it helps the organism live but could cause infections if it has the place and resources to infect. *S. epidermidis* is also an anaerobic bacterium that uses fermentation to produce energy meaning it gets effected by the usage of xylitol which will be further explained in the "Xylitol's Interaction with the Oral Flora" section.[x] It's increase in the oral microbiome, like most imbalances in the oral cavity, would cause an infection since it is, as aforementioned, an opportunistic pathogen, however its decrease would also have negative results such as the disruption of the biofilm or infections caused by other bacteria in the oral microbiome since its decrease would allow more nutrients and space for other bacteria to grow and infect the oral cavity. The aim of this study was to explore the positive or negative effects of the chemical xylitol on the bacterial species *Streptococcus epidermidis*, which is present in the epidermal layer

and oral flora, because of xylitol's extensive usage in food and skincare products which interact with the oral flora.

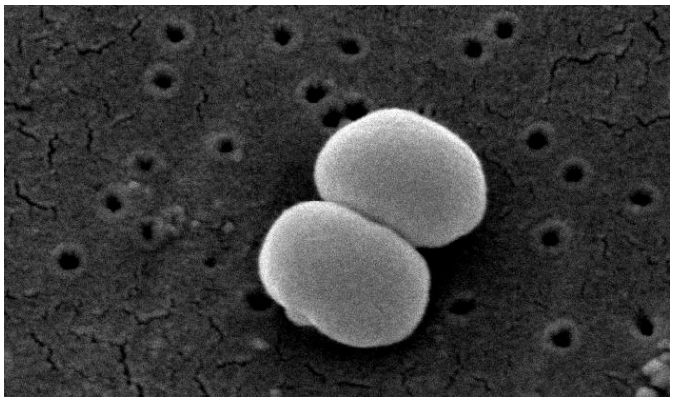


Figure 1. Electron Micrograph of Staphylococcus epidermidis[xi]

Xylitol

Xylitol is a naturally occurring sugar polyol that is found in fruit and vegetables. Xylitol is used primarily in the gum industry but also has uses in the beauty industry because it helps skin retain moisture. [xii]

Xylitol's Interaction with the Oral Flora

Over the years xylitol's benefits have surfaced such as neutralizing the pH of saliva and most importantly inhibiting the growth of Streptococcus mutans which are the species of bacterium that cause dental plaque and cavities.[xiii] Xylitol does this by disrupting their energy cycle leading them to an involuntary lysis. The *S. mutans* along with some of the other bacteria in the oral flora that use fermentation as a form of energy production[xiv] try to ferment xylitol to produce ATP but fail because xylitol is unfermentable causing the formation of intracellular vacuoles of xylitol causing the cell membrane to degrade. Since the cell cannot use xylitol for energy production, it dephosphorylates it and excretes it from the cell causing it to lose energy but not gain energy which in turn causes the cell's death.[xv]

When xylitol is present in the growing environment of the *Staphylococcus epidermidis* however, xylitol doesn't display its inhibiting properties because it was determined to have selective antibiotic properties. Furthermore, in Figure 2.[xvi] which shows the growth curve of *S. epidermidis* at varying concentrations of xylitol, no significant change is observed in the growth curve, pointing to the possibility of xylitol not displaying selectively antibiotic properties on this species. However, by the small changes observed, it was concluded that xylitol showed more inhibiting properties at the 50% concentration whereas it was more beneficial to the growth [xvii]of *S. epidermidis* at lower concentrations. However different results were obtained in this experimentation because of the different concentration usage and methodology.

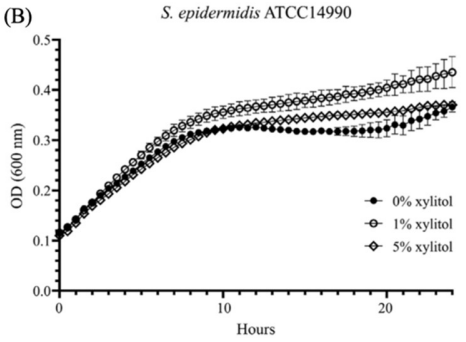


Figure 2. Growth curve, expressed as optical density (OD) values at 600nm, over a 24-h culture for S. Epidermidis ATCC4490

Hypothesis:

Null Hypothesis: Xylitol will not affect the growth of *Staphylococcus epidermidis* in the in vitro environment at 37°C.

Alternate Hypothesis: Xylitol will have a promoting effect in low concentrations and inhibiting effects on *Staphylococcus epidermidis* in high concentrations measured by disk diffusion method in the in vitro environment at 37°C.

Variables

Type of Variable	The Variable	Why It Was Chosen	How It Was Regulated
Independent Variable	Concentration of Xylitol in the Medium	Because the effect of the concentration of xylitol is being investigated	2%, 4%,10% and 20% Xylitol solutions are prepared to investigate the effect of concentration
Dependent Variable	The expansion of the disk radius in the agar	Because the length of expansion of a disk radius indicates the effect of the chemical being investigated (xylitol)	The radii of the disks were measured after the incubation period of the disks
Controlled Variables	Linezolid	This was used as a negative control to observe if the well size of linezolid matched its literature value	A 20 mL of linezolid was placed into the wells along with the xylitol solutions
	Concentration of the *S. epidermidis* in each disk	Because having close quantities of bacteria in each disk will ensure the result of the experiment has changed because of the independent variable.	The bacterial suspension is prepared according to the same standards for each repetition of the method. (Seen in methodology section)
	Length and Temperature of incubation	This is important to control because different incubation periods and temperatures can cause varying growing rates for the bacteria therefore interfering with the results	The disks were placed in the incubator at the same time for 24 hours each
	Equipment types, agar type, amount of bacteria inoculated, well sizes	These were kept constant so as not to provide reasoning for any inaccuracy caused by equipment change	The wells were made with the back of the same pipette and identical equipment was used for all disks

Table 1. Variable Types, Variables and Their Justifications

Materials

Material	Quantity	Uncertainty	Purpose
Mueller Hinton Agar (MHA)	38 grams	±0.001 grams	To incubate the bacteria
Distilled Water	≈1 L	±1 mL	To prepare the agar and the other suspensions
Digital Scale	1	N/A	To weigh xylitol when preparing the suspensions.
Autoclave	1	±1°C	To boil the agar and incubate the disks.
Graduated Cylinder	1	N/A	To measure the quantity of water.
Petri Dish and Lid	4	N/A	To perform the disk diffusion inside
Xylitol	15 g	±0.001g	To test the antibacterial effect of.
S. epidermidis Culture (ATCC 1228)	5 colonies	N/A	To test the xylitol on
pH Meter	1	±0.01 pH	To check if the pH of the agar is suitable for the bacteria culture
Spectrophotometer	1	±0.001	To test if the bacteria is at the correct concentration (0.5 MacFarland)
Sterile Cotton Swab	5	N/A	To transfer the bacteria colonies to the agar.
Sterile Pipette	5	N/A	To create wells in the agar
Millimetric Ruler	1	±0.5 mm	To measure the initial and final sizes of the wells
Inoculation Loop	1	N/A	To take out the bacteria colonies
Linezolid	125 microL	±1 microliter	To place as a negative control
Brain-Heart Broth (Mueller-Hinton)	5mL	±1 microliter	To initiate bacterial growth
Test Tube	5	N/A	To place the broth and bacteria into
Beaker	5	N/A	To prepare the xylitol suspensions

Table 2. Materials, their quantities, uncertainties, and the purpose of their usage

Procedure

Prior Tests:

Before utilizing the disk diffusion method, broth microdilution method was used for prior testing however this method showed no antibacterial effect in any concentration of xylitol (See appendix) so disk diffusion was preferred as it could provide different results or confirm the ones obtained from the broth microdilution testing.

After this testing, the experimentation which provided the most numerical data, the disk diffusion was conducted. This method is the one used in the report since the greatest number of trials were conducted utilizing this method.

After getting the same result from the disk diffusion method as the one from the broth microdilution, a modification to the disk diffusion method was used. The bacteria were isolated with different concentrations of xylitol alone and then inoculated onto sheep blood agar to ensure the *S. epidermidis* was not utilizing the agar from the disk diffusion experimentation prior for nutrition since xylitol produces an antibacterial effect on mutant types by its ingestion. However, the same result indicating no antibacterial effect because of no visible expansion in diameter was observed. (See appendix)

Justification of method

The disk diffusion method where bacteria are planted into a petri dish with agar as a medium and wells are made with the chemical whose antibacterial property is being tested is placed inside was utilized. This method was chosen because of its wide usage in microbiology, providing more resources to do research on the method, its measurability, causing more objective and accurate results[xviii], and according to the results of the prior testing (See appendix) This experiment was conducted according to the standard disk diffusion method[xix] to produce more accurate results. The concentrations 2%, 4%,10% and 20% were chosen to provide a wide range of concentrations and because 2% and 4% concentrations and 10% and 20% concentrations were

used in prior tests[xx] and they were combined to provide data for both high and low concentrations of Xylitol.

Method

Preparation of the Agar Medium

1. First, the Mueller Hinton Agar (MHA) medium is prepared inside the petri dish. This is prepared according to the manufacturer's instructions with distilled water.
2. Then the agar is heated at 100°C until it boils to dissolve and sterilize it and left to cool at room temperature.
3. The pH of the medium is checked at room temperature to ensure it is between 7.2-7.4 (the correct value for the medium to be)
4. Afterwards the agar is poured into a sterile petri dish and left to cool and solidify.

Preparation of Bacterial Suspension and Xylitol Suspensions

5. Then the *S. epidermidis* suspension is prepared to achieve 0.5 MacFarland. This is done by taking 5 colonies with an inoculation loop from the pure *S. epidermidis* culture.
6. Then the colonies are transferred to 5mL of brain-heart broth in a test tube
7. This tube is then incubated until it reaches 0.5 MacFarland which is measured using a spectrophotometer to ensure each suspension reaches the same standard.

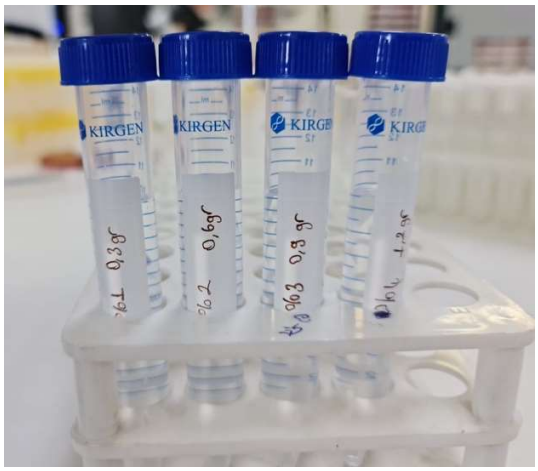


Figure 3. The Preparation of the Xylitol Suspentions

8. The xylitol suspension is prepared by measuring via electronic balance and adding 0,3g, 0,6g, 0,9g and 1,2 g of xylitol into different beakers labeled 2%, 4%, 10% and 20% Xylitol with 10mL of distilled water in each beaker.

Inoculation of the bacteria

9. The *S. epidermidis* suspension is planted into the petri dish with the pre-prepped agar medium with a clean and sterile cotton swab dipped into the test tube containing *S. epidermidis* prepared in steps 5-7.
10. Then the bacteria are inoculated by lightly streaking the agar medium with the swab diagonally, horizontally, and vertically.[xxi]

Figure 4. The Bacteria Inoculation Method Utilized

11. Allow the medium to dry for 5 minutes before proceeding.

Placing the Xylitol and Linezolid into the Disk

12. Using the back of a sterile pipette, 6 wells are created in the medium to place the xylitol solution and linezolid
13. 25 microliters of xylitol solution of 2, 4, 10 and 20 percent concentration are added into the wells along with 25 microliters of linezolid and the radii of these wells are measured.

14. The lid of the petri dish is sealed, and the wells are labeled as 2,4,10,20 and LNZ representing the concentrations of the xylitol and the linezolid.

Incubation

15. The petri dish is incubated at 37°C for 24 hours in the autoclave and taken out
16. The resulting radii of all the wells are measured and recorded.

Cleaning

17. The waste produced from the experimentation such as the post incubation residues of bacteria and xylitol are disposed of into a health hazard labeled bin while reusable items, the petri dishes, inoculation loop etc. are sterilized inside of the autoclave at the sterilization setting which sterilizes the tools using high temperature steam to kill any hazardous materials left inside. Before sterilization with autoclave, it is important to check if the lab materials being sterilized are autoclavable/heat resistant.
18. This method is repeated 5 times for 5 trials in total.

Safety and Ethical Concerns

S. epidermidis is an opportunistic pathogen and does not survive in the air[xxii], therefore the usage of *S. epidermidis* does not pose a risk to the experimenter as long as appropriate lab precautions are taken such as the usage of gloves and not having open wounds while experimenting which were applied. The petri dish is sealed thoroughly to prevent it getting contaminated by the other experimentations in the incubator and vice versa. The petri dishes with the bacteria are sterilized of by heating them up to about a 100°C to kill the bacteria inside according to standard procedure.[xxiii] Also a mask was used while experimenting to prevent from getting contaminated by the other samples in the laboratory that were being experimented on by the microbiologists utilizing the laboratory simultaneously.

The safety hazards regarding this experimentation caused by the laboratory being inside a hospital included getting possible infections from the other experimentations that were being carried out in the microbiology laboratory. This risk was minimized by conducting said experimentations on the afterhours of the students and resident and therefore experimenting in a less crowded lab with less unattended samples left in the open. Besides this precaution, proper lab attire such as gloves, a mask, a hair net and a lab coat were used to minimize the overall risk.

In this specific method[xxiv] The ICMR ethical guidelines[xxv] were considered to minimize the ethical issues that might arise from this experiment. These guidelines include Principle of Beneficence which means weighing the benefits and the harm that can be caused because of the experiment in question. The only ethical issue that arose from this experimentation in my knowledge is the usage and disposal of plastic equipment resulting in plastic waste, but minimal amounts of equipment were used, and this waste was made in the name of science although this waste could've been more justified if the experiment was conducted by more researched with higher education because of their likeliness to construct a more accurate method and achieve more reliable results. The waste of the experiment was disposed of by placing the used wire loops and pipettes in the biohazard bin inside the laboratory.

Data

Qualitative Data

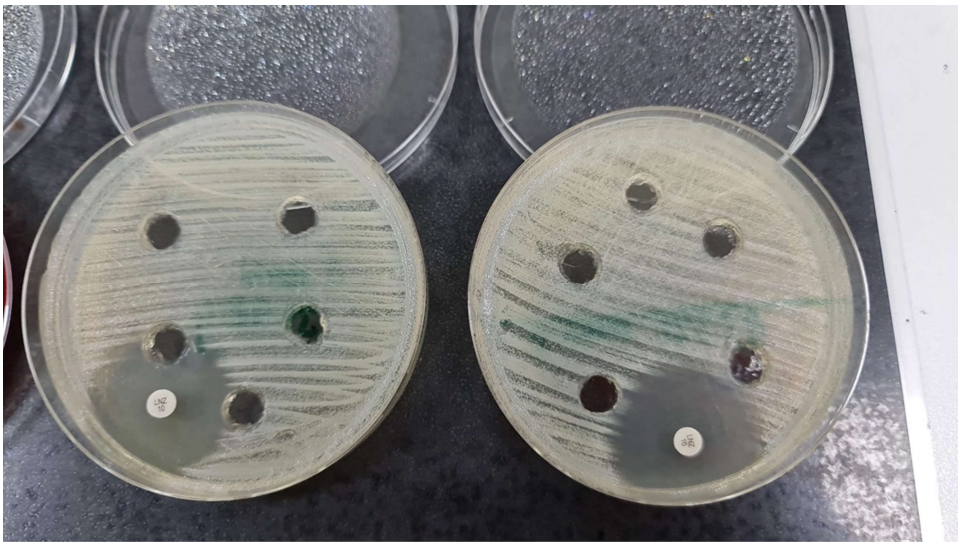


Figure 5. The Disks After the Incubation Period

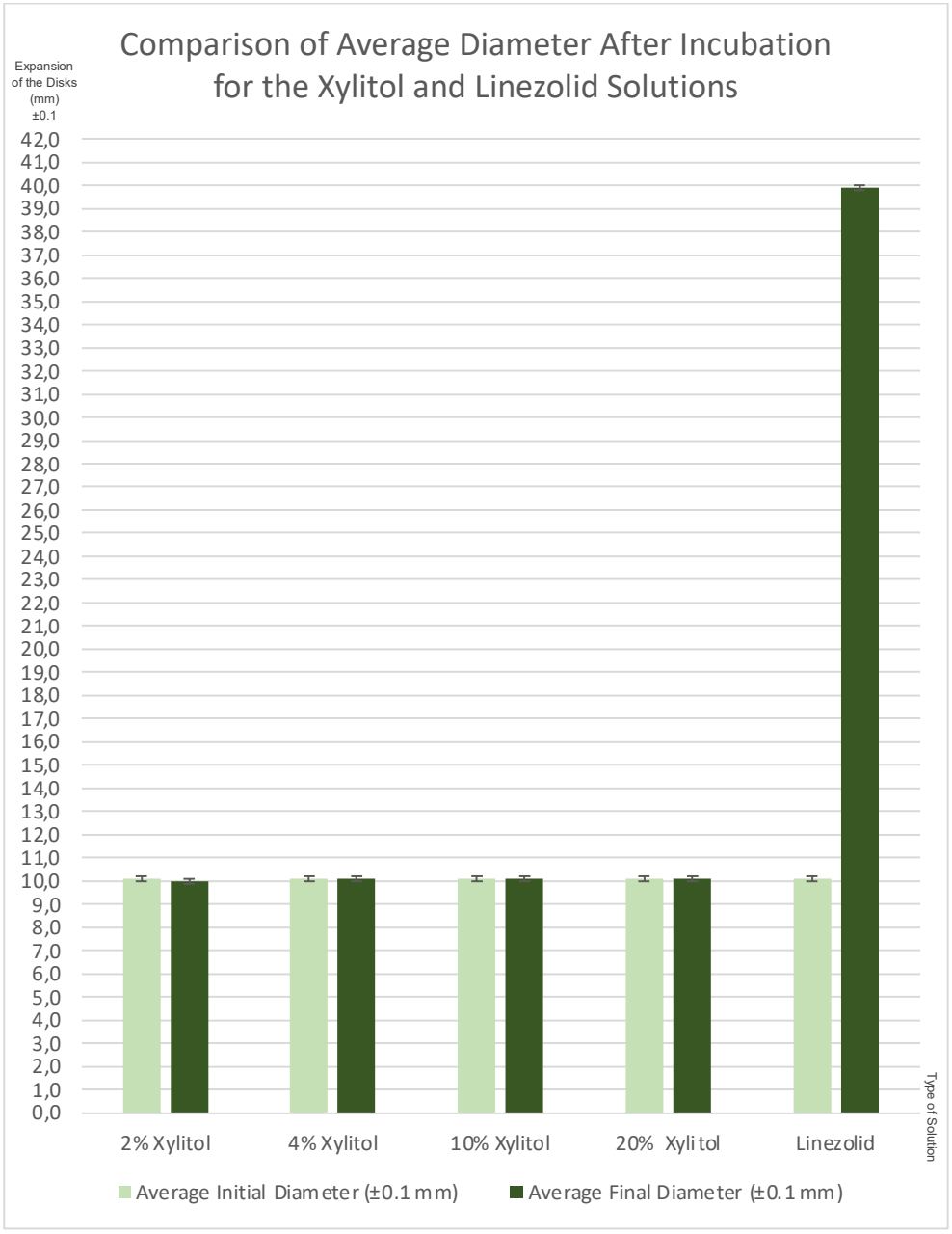
As can be seen through a visual observation of the disks after incubation, while the control group Linezolid disks display a clear dispersion displaying the antibiotic effect of linezolid on *S. epidermidis*. The other wells which were filled with different concentrations of xylitol solutions showed no signs of dispersion therefore, from qualitative observations, it can be said that they displayed no inhibiting effects on the *S. epidermidis* culture in this experiment.

Raw Data

Concentration	Trials	Initial Diameter (±0.1 mm)	Final Diameter (±0.1 mm)
2% Xylitol	1	10.1	10.0
	2	10.1	10.1
	3	10.0	10.1
	4	10.1	10.0
	5	10.0	10.0
4% Xylitol	1	10.2	10.1
	2	10.0	10.0
	3	10.1	10.1
	4	10.1	10.0
	5	10.2	10.2
10% Xylitol	1	10.1	10.1
	2	10.0	10.1
	3	10.0	10.0
	4	10.0	9.8
	5	10.2	10.1
20% Xylitol	1	10.2	10.0
	2	10.2	10.1
	3	10.0	9.9
	4	10.1	10.1
	5	10.2	10.1
Linezolid	1	10.1	38.8
	2	10.0	40.2
	3	10.1	39.2
	4	10.2	41.0
	5	10.1	40.4

Table 3. Data Obtained from Measuring the Diameters of Each Well Before and After Incubation

From a general look at the data, it can be seen that there is little to no difference in the size of the disks (inhibition cites) of the xylitol solutions while there is a change in the Linezolid inhibition cite which fits the literature range (25-31mm of expansion)[xxvi] meaning that the experimentation was applied successfully, but no inhibiting effects of xylitol can be observed in this in vitro inhibition test of xylitol.



Bar Graph 1. Comparison of Average Diameter After Incubation for the Xylitol and Linezolid Solutions

As can be seen in Bar Graph 1, there weren't any significant changes in the well diameter before and after incubation where a change is expected in the diameter when a substance displaying an antibacterial effect on *S. epidermidis* is placed in the well.[xxvii] This graph further demonstrates the fact that no visible change took place in the in vitro testing utilizing disk diffusion method.

Processed Data

T Testing on the Changes in Diameter

A t-test is a statistical test run on two groups to determine if there is a statistical difference between them[xxviii]. The t-test was run on the raw data in order to determine if there is a statistical difference between the diameters of the wells created by the expansion of the xylitol solution and the linezolid solution. A statistical difference in the diameters of the wells indicate an antibacterial effect and no statistical difference indicates no antibacterial effect in the in vitro testing of the xylitol on *S. epidermidis* via disk diffusion. The t-test was conducted through excel from its "data toolpak" for each concentration and the control group linezolid and the results can be seen in Tables 4,5,6, and 8.

t-Test for the Initial an Final Diameters for the 2% Xylitol Solution Assuming Equal Variances		
	Initial Diameter (mm)	*Final Diameter (mm)*
Mean	10.06	10.02
Variance	0.003	0.007
Trials	5	5
t Stat	0.894	
t Critical one-tail	1.86	

Table 4. t-Test for the Initial and Final Diameters for the 2% Xylitol Solution

In Table 4. The t Stat value for the 2% xylitol solution can be observed as 0.894 and the t-critical value is 1.86. A t critical value higher than a t stat value demonstrates that no statistical change is present in this group.

t-Test for the Initial an Final Diameters for the 4% Xylitol Solution Assuming Equal Variances		
	Initial Diameter (mm)	*Final Diameter (mm)*
Mean	10.1	10.1
Variance	0.001	0.007
Trials	5	5
t Stat	0.343	
t Critical one-tail	1.86	

Table 5. t-Test for the Initial and Final Diameters for the 4% Xylitol Solution

Similarly in Table 5. The t Stat value for the 4% xylitol solution can be observed as 0.343 and the t-critical value is 1.86. A t critical value higher than a t stat value demonstrates that no statistical change is present in this group.

t-Test for the Initial an Final Diameters for the 10% Xylitol Solution Assuming Equal Variances		
	Initial Diameter (mm)	*Final Diameter (mm)*
Mean	10.06	10.0
Variance	0.008	0.017
Trials	5	5
t Stat	0.566	
t Critical one-tail	1.86	

Table 6. t-Test for the Initial and Final Diameters for the 10% Xylitol Solution

Again, in Table 6. The t Stat value for the 10% xylitol solution can be observed as 0.566 and the t-critical value is 1.86. A t critical value higher than a t stat value demonstrates that no statistical change is present in this group.

t-Test for the Initial an Final Diameters for the 20% Xylitol Solution Assuming Equal Variances		
	Initial Diameter (mm)	*Final Diameter (mm)*
Mean	10.1	10.04
Variance	0.008	0.008
Trials	5	5
t Stat	1.77	
t Critical one-tail	1.86	

Table 7. t-Test for the Initial and Final Diameters for the 20% Xylitol Solution

As seen in Table 4, 5 and 6, in Table 7. The t Stat value for the 20% xylitol solution can be observed as 1.77 and the t-critical value is 1.86. A t critical value higher than a t stat value demonstrates that no statistical change is present in this group.

t-Test for the Initial an Final Diameters for Linezolid Control Assuming Equal Variances		
	Initial Diameter (mm)	*Final Diameter (mm)*
Mean	10.1	39.9
Variance	0.005	0.812
Trials	5	5
t Stat	-73.8	
t Critical one-tail	1.86	

Table 8. t-Test for the Initial and Final Diameters for the Linezolid Solution

Finally in the linezolid control groups t-test, a negative t-stat value is seen which is significantly lower than the t Critical value meaning that the linezolid increased the diameter, and a significant change was present. This confirms that the xylitol trials being constant was not caused by a systematic error caused by the incubation period, the plate or the agar since the control group demonstrated a significant change as expected.

Evaluation

As can be seen in Tables 4 through 8, the variance in the data is very small indicating the results are precise and since a systematic error is not probable because the control group displayed accurate results, the data obtained can be deemed reliable. Therefore, since the data showed there was no significant difference in the diameters of the wells after the addition of xylitol it can be said that the hypothesis is rejected and the null hypothesis "Xylitol will not affect the growth of *Staphylococcus epidermidis* measured by disk diffusion method in the in vitro environment at 37°C." is accepted.

The uncertainty taken into consideration in this experimentation is only that of the electronic measurement device which is a relatively small uncertainty considering the expected change that would be observed in the case that xylitol displayed an antibacterial effect on *S. epidermidis.* The uncertainty of the concentrations of the xylitol and linezolid were not taken into consideration because of two reasons, the data obtained in the experiment was only evaluated in the basis of length so therefore the electronic ruler's uncertainty was considered for its uncertainty and because the concentrations and amounts of the solutions were measured using an spectrophotometer therefore their values can be considered as close to precision as possible because of the machine's low uncertainty and high precision when measuring concentration.

Further Improvements

Although the standard error was low since an electronic ruler was utilized and the variance was low as well indicating the results were precise, increasing the trial number can result in more reliable results. The reason only 5 trials per concentration were conducted was the limited resources in the lab environment. A reason for the lack of resources was the many prior testing and trying different methods to determine if the lack of antibacterial effect of the xylitol was caused by the methodology. Because there was prior testing conducted prior to the development of the final experimentation, a lot of expensive lab equipment which the hospital

funded had to be used and there was limited amount of *S. epidermidis.* culture to work on. So, it can be concluded that within these limitations, the results obtained are as precise and reliable as possible.

Conclusion

The starting point of this research, the research question "How does the amount of xylitol affect the survival and amount of the oral *Staphylococcus epidermidis* in the in vitro environment at 37°C." was answered through the application of the experimentation on the concerned species, *S. epidermidis.* The results of this investigation, as mentioned in the evaluation section, demonstrate that xylitol at concentrations between 2 and 20 percent does not have an antibacterial effect on the species *S. epidermidis* in the in vitro condictiones. However, even though in vitro results usually point to what has the highest probability to happen in in vitro condictiones, it does not ensure with complete certainty that the same results will be achieved in real life condictiones in the oral environment therefore further testing in the in vivo emvironment is required to come to a full conclusion and answer all of the questions proposed by the research question.

From these results combined with the researched information in the background information section, it can be said that the usage of xylitol in oral products look to be suitable for oral usage for two main reasons, because it was proven to be effectively lethal for bacterium that cause cavities[xxix] and because it has not yet been proven to have any harmful effect toward bacterium that naturally exist in the epidermis and oral flora indicating it will most probably not be harmful to the bacterium in the oral flora and not disturb the balance within it.

Appendix

Prior Testing: 96 Well Plate Broth Microdilution

A broth microdilution was conducted utilizing a 96 well plate, utilizing a control group with no addition of any substances and only the bacteria and 1, 2, 3 and 4 percent xylitol solution concentrations. This broth microdilution was conducted on multiple species of oral bacterium such as *S.* epidermidis, Enterococcus *faecalis*, *S. aureus* and more however all of the wells showed growth indicating the xylitol did not demonstrate any antibacterial effect on the bacterium in question.

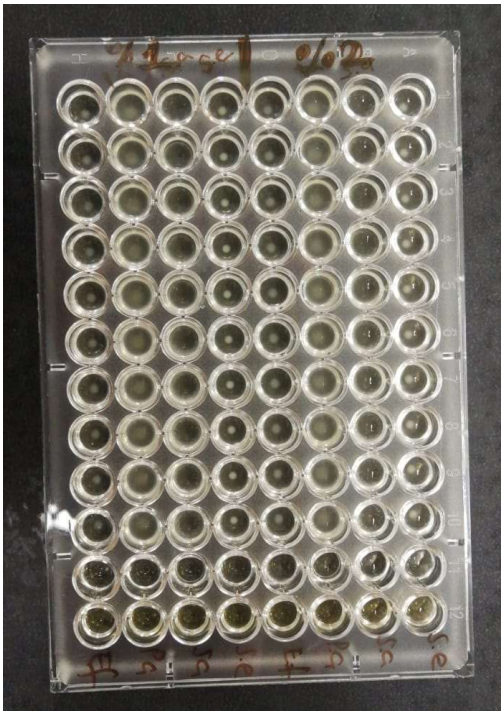


Figure 6. The 96 Well Plate After the Incubation Period for 1 and 2% Xylitol Solutions

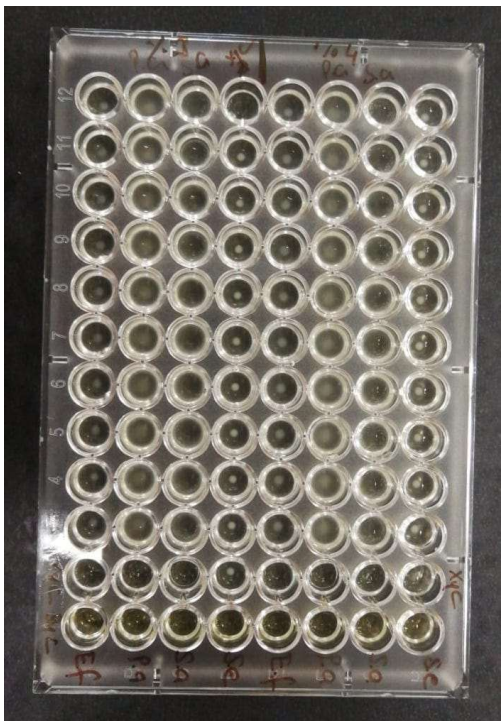


Figure 7. The 96 Well Plate After the Incubation Period for 3 and 4% Xylitol Solutions

As can be clearly seen in figures 6 and 7 the bacterium which was incubated showed signs of growth in the presence of xylitol therefore showing xylitol does not inhibit their growth and therefore does not possess antibiotic properties towards them. These signs of growth are the white areas seen in the middle of each well plate except row twelve because row twelve was left

as a control group to ensure the sterility of the well plates and make sure no outside factor is interfering with the results.

Sheep-blood Agar Disk Diffusion

This trial was conducted in order to ensure that the data were only as a result of xylitol not having an antibacterial property towards *S. epidermidis* and not because the bacterium were using the agar as a source of nutrition as opposed to the xylitol in the xylitol solution since xylitol inhibits species such as some mutans growth through their cellular respiration acting as sugar. To ensure this was not the case, the *S. epidermidis* culture was placed in the xylitol solution prior to inoculation and then inoculated in the sheep-blood agar. However, as seen in Figure 8. This method also provided the same result as there is no visible change in the diameter of the wells created in the agar. This data increases the reliability of the experimentation because a variety of methods point to the same results.

Figure 8. Staphylococcus Epidermidis in Xylitol Solution in Sheep Blood Agar After the Incubation Period

T.C.
GAZİ ÜNİVERSİTESİ REKTÖRLÜĞÜ
Tıp Fakültesi Dekanlığı
Dahili Tıp Bilimleri Bölüm Başkanlığı
Göğüs Hastalıkları Anabilim Dalı Başkanlığı

16.01.2023

To whom it may concern,

[redacted] has done her IB Diploma Program Extended Essay experiment in the Medical Microbiology Laboratory of the Gazi University Faculty of Medicine by herself with the supervision of the laboratory staff

Best regards

Prof I Kıvılcım Oğuzulgen, M D
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