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Article

Antibacterial properties of household spices and toothpaste against oral bacteria

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SUMMARY

Bacteria cause tooth decay, plaque, bad breath, and other diseases. Despite being cleaned with water and toothpaste, oral bacteria live on our toothbrushes. Other bacteria also live on our toothbrushes, like *Escherichia coli*. Small particles of fecal matter are aerosolized when the toilet is flushed, carrying *E. coli* and other bacteria to our toothbrushes and surfaces. Bacterial growth has been shown to be inhibited by different toothpastes and common household spices. This study tested how different toothpastes and common household spices, including cinnamon, cumin, nutmeg, and ground white pepper, can inhibit bacteria from growing on toothbrushes. We hypothesized that three different toothpastes and all four spices would inhibit the bacterial growth from our toothbrushes. We observed the growth of bacteria isolated from toothbrushes on agar plates mixed with toothpaste, cinnamon, cumin, nutmeg, and ground white pepper and found that toothpaste did not have the strongest antibacterial properties. Instead, we discovered that cinnamon served as the best bacterial growth-suppressing spice. Our results support the claim that cinnamon has the potential to improve people's oral health, and a future experiment using cinnamon toothpaste, instead of the spice alone, could show promising antibacterial properties.

INTRODUCTION

Diverse bacteria grow everywhere around and inside of us. On average, our mouths have around 700 species of bacteria (1). In addition to protecting our teeth, some species of bacteria support human health by performing glycolysis to break down our food into simple sugars like sucrose, glucose, and fructose (2). Additionally, "good" bacteria, like Lactobacilli, have antimicrobial properties that help fight "bad" bacteria that cause tooth decay (3, 4). "Bad" bacteria, like Streptococcus mutans, cause tooth decay by releasing acids, resulting in cavities (5). Bacteria are under constant pressure to adapt to the conditions of the mouth, which change every time a person consumes food and drinks of varying temperatures, acidities, and chemical compositions (6). These stresses contribute to the diversity of bacteria in the mouth. Different species adapt to survive the ever-changing conditions of the oral environment and compete with one another for resources (7, 8).

Brushing one's teeth regularly can help prevent bacteria and cavities from spreading (1). Most toothpastes contain ingredients that improve oral health, such as triclosan (1). Triclosan is an active ingredient commonly used in toothpaste that helps destroy bacteria. It kills bacteria by tampering with the membrane of the bacteria cell, eventually destroying it (9). Using toothpaste when brushing our teeth can help prevent future problems, like tooth decay/loss, or bacteria from spreading in our mouths. However, some bacteria cannot be killed by toothpaste. For example, a study found that dental plaque, a sticky layer of film made by bacteria, protects cavity-causing bacteria from the oral environment and antimicrobial toothpastes (7).

Additionally, toothbrushes are often kept in bathrooms with toilets, where bacteria from solid waste linger (10, 11). Sixty percent of toothbrushes kept in a bathroom contain fecal bacteria (10). Toothbrush contamination can lead to the spreading of unhealthy bacteria such as Streptococcus (11). When investigators compared the bacterial content of toothbrushes stored in a room with a toilet and a room without a toilet, only toothbrushes near toilets had *Escherichia coli* growing on them (11). Regular toothbrush cleaning and replacing is often neglected, which causes bacteria to thrive in our mouths (10).

In addition to good oral hygiene, a previous study showed antibacterial properties of cinnamon and cloves (12). Thus, we were curious as to how culinary spices could fight against oral bacteria. Since many people add seasonings to their meals, we decided it was best to use common household spices in our experiment. As a result, we aimed to determine if toothpaste and the spices commonly used in our diets are sufficient to kill the microbes lingering on our toothbrushes by introducing three different toothpastes, cinnamon, cumin, nutmeg, and ground white pepper to bacteria present on our toothbrushes. We hypothesized that all four spices and all three toothpastes will inhibit the bacteria from our toothbrushes. Despite contamination of some of our experiments, our data suggest that cinnamon was most effective in inhibiting the growth of toothbrush bacteria. Thus, incorporating cinnamon into one's diet could help improve oral health by inhibiting bacterial growth.

RESULTS

We conducted three separate experiments to test the efficacy of different toothpaste and common household spices

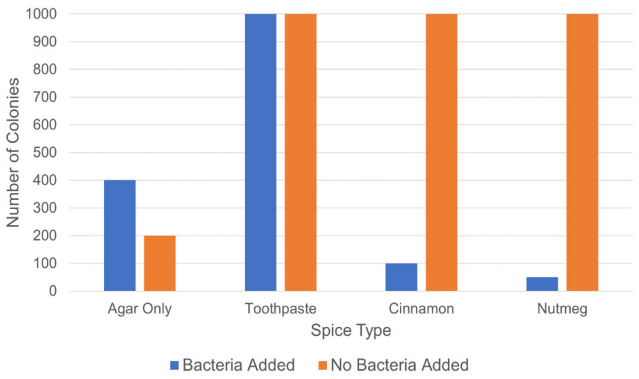

Figure 1: Colony count of Experiment A. Colony quantities of negative control (no bacteria added) and experimental (bacteria added) plates of Experiment A after 4 days of incubation at room temperature. Lawns are represented by 1000 colonies. Bacterial growth on plates with no bacteria added suggests contamination.

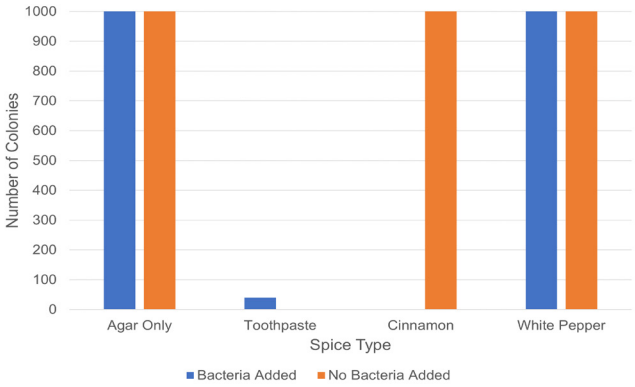

Figure 2: Colony count of Experiment B. Colony quantity of experimental and negative control plates of Experiment B after 4 days of incubation at room temperature. On this graph, 1000 represents the lawns. Bacterial growth on plates with no bacteria added suggests contamination.

in preventing the growth of bacteria from toothbrushes. After collecting bacteria from our toothbrushes, we cultured the bacteria on agar plates with a toothpaste or spice mixed into the agar at 4% volume additive per volume of agar (v/v) or no additive as a positive control for bacterial growth. Sterile water was used in place of bacterial culture for the negative. Lastly, we incubated the plates for four days and counted colonies. Each experiment was conducted by a different person in different locations, so we included cinnamon in all experiments as a measure of consistency.

Experiment A: Schmidt's Wondermint Fluoride-Free toothpaste, nutmeg, and cinnamon

We observed bacterial growth on the regular agar, ground nutmeg, and ground cinnamon negative control plates, suggesting that they were contaminated (**Figure 1**). As for the positive control and experimental plates, we observed that bacteria grew on all plates. The cinnamon plate grew between 50–100 colonies. The positive control and plates with toothpaste and nutmeg added grew into lawns, or layers of conjoined colonies (**Table 1**). Due to the contamination found on the negative control plates, we could not make any conclusions with the results of this experiment.

Test media	With bacteria added	Without bacteria added
Experiment A		
Regular Agar	Lawn About 200	Lawn About 201
Agar + Toothpaste	Lawn	Lawn
Agar + Cinnamon	Lawn 50 – 100	Lawn 50 – 101
Agar + Nutmeg	Lawn About 50	Lawn About 51
Experiment B		
Regular Agar	Lawn	Lawn
Agar + Toothpaste	Lawn 40	0
Agar + Cinnamon	Lawn	0
Agar + White Pepper	Lawn	Lawn
Experiment C		
Regular Agar	About 700	0
Agar + Toothpaste	33	0
Agar + Cinnamon	8	0
Agar + Cumin	About 560	0

Table 1: Bacterial colony counts. If bacterial growth was observed, we noted if the growth formed a lawn and approximate colony counts for all plates where bacteria grew.

Experiment B: Crest Regular Paste Cavity Protection, white pepper, and cinnamon

Similar to Experiment A, Experiment B also showed colonies in the negative control group (**Figure 2**). Agar without additive and ground white pepper were the only negative control plates that were contaminated. As for the positive and experimental control groups, bacteria grew on all plates. We discovered that the toothpaste plate was the only plate with individual colonies, counting about 40 distinct colonies. The white pepper and cinnamon plates showed bacterial growth in the form of a lawn (**Table 1**). As we did with Experiment A, we decided that no valid conclusions could be drawn from this experiment as a consequence of the contamination.

Experiment C: Crest Arctic Fresh 3D White Toothpaste, cumin, and cinnamon

In contrast to Experiments A and B, there were no signs of contamination on the negative control plates of Experiment C. Instead, we found that all positive and experimental plates of this experiment showed bacterial growth. We also discovered that the toothpaste and cinnamon experimental plates showed the strongest antibacterial properties (**Figure 3**). The toothpaste experimental plate had 33 distinct colonies, and the cinnamon experimental plate had 8 distinct colonies. The cumin experimental plate displayed about 560 countable colonies. The positive control plate had about 700 countable

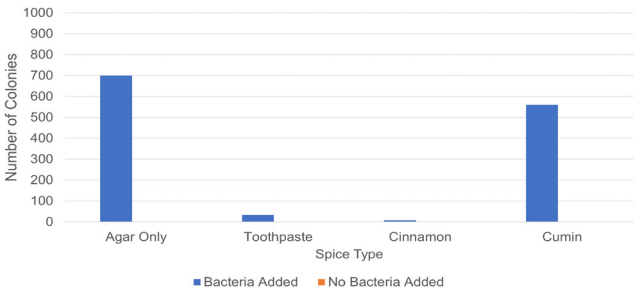

Figure 3: Colony count of Experiment C. Colony quantity of experimental and positive control plates of Experiment C. Data was recorded 4 days after bacteria was added. No growth was observed on negative control plates.

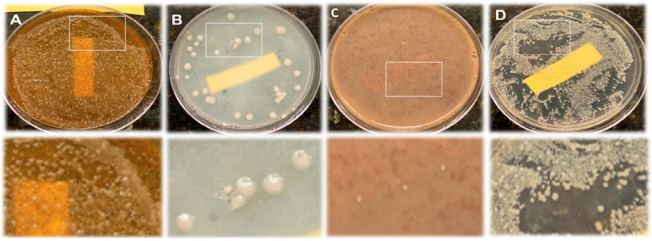

Figure 4: Photos of bacterial growth from Experiment C. These images compare the colony density and morphology from a toothbrush (Experiment C) grown on plates with (A) cumin, (B) toothpaste, and (C) cinnamon infused agars. (D) The positive control plate with no additives is shown for comparison. Images were taken 4 days after bacteria was added. The bottom row shows enlarged image of colonies in the boxed area of top row images.

colonies (**Table 1**). During this experiment, we noted that the cinnamon plate took the longest to show any signs of bacterial growth. We also noted that the toothpaste plate showed colony growth, but at a slower rate and smaller quantity than the other plates besides cinnamon.

The toothpaste experimental plate of Experiment C grew larger colonies compared to the other plates (**Figures 4**). We observed that the colonies were a solid white color and had a circular shape. In dissimilarity to the toothpaste plates, the positive control and other experimental plates showed distinctly smaller colonies. The shape of the cumin colonies on both plates were less clear due to the appearance of a bacteria lawn. We saw that all other plates had colonies with a well-defined round shape.

Low additive plates: Cumin and cinnamon

As a follow up to Experiment C, we utilized two additional experimental plates, low-additive cinnamon and low-additive cumin, to this experiment to test how a lower concentration of spice (2% v/v) would affect bacterial growth in comparison to the other experimental plates. The low-additive cumin plate displayed about 550 countable colonies. The low-additive cinnamon plate displayed about 141 distinct colonies. We concluded that the different concentrations of cumin in the plates did not massively alter the results. Additionally, there were more than 10-fold more colonies on the 2% cinnamon plate compared to 4% cinnamon plate, suggesting a dose effect (**Figure 5**). These data supported our conclusion that cinnamon demonstrates the best antibacterial properties out of all spices tested (**Figures 3–5**).

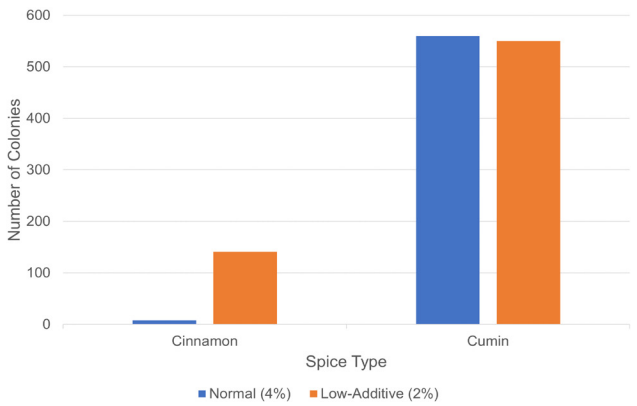

Figure 5: Low-additive cumin and cinnamon. Bacterial growth comparison between normal (4% v/v additive) and low-additive (2% v/v additive) cumin and cinnamon plates in Experiment C. Data was recorded 4 days after bacteria was added.

DISCUSSION

Three independent experiments were conducted to determine which household spices would inhibit the growth of toothbrush bacteria. Initially, we thought toothpaste would be the best way to suppress bacteria on toothbrushes; although toothpaste was somewhat effective at suppressing bacteria growth, cinnamon actually proved to be the most effective. Previous research by Gehad and Springel showed that cinnamon was effective at suppressing *E. coli* growth, while cumin and pepper were not effective (12). These results are echoed in our own experiments.

Although both Experiments B and C used a similar type of toothpaste, we noticed how different the results were, especially on the cinnamon experimental plates. This could be due to contamination from the spices or toothpaste. Experiment B's cinnamon plate contained a lawn and Experiment C's plate had 8 colonies. We concluded that these opposing results were due to the contamination in experiment B. There is the possibility that the cinnamon used in Experiment B was contaminated or an error occurred in the handling of the plate. Therefore, the results of Experiment B showing that toothpaste served as the strongest antibacterial may be false. If the contamination did not occur, we predict that the results of Experiments B and C would have aligned with cinnamon having the greatest antibacterial effect.

Contamination in Experiments A and B is a confounding factor because they contained more colonies in their negative controls than in the positive controls and experimental plates. However, there is still a possibility that the additives used would have shown greater antibacterial properties than those selected in the other experiments if no contamination occurred. We believe that the negative control plates were handled differently in their preparation. For example, rather than performing the experiments at home, where air flow and other environmental conditions could not be controlled, we could have incubated the plates in a sterile environment to reduce contamination. Using the aseptic technique is a way of reducing contamination. The aseptic technique is used to prevent contamination by pathogens through the following steps: [1] using barriers like masks and sterile gloves, [2] using contact guidelines, meaning that a sterile tool that is used on a non-sterile substance or surface it is not allowed to be used for a different substance or surface, [3] using the tool preparation method to sterilize the tool and surface using chemical or heat sterilization, and [4] using the environmental control method, which uses all of the last three methods to maintain a sterile environment (13, 14). An additional limitation is that we cannot directly compare between the three experiments because they were done by different people in different locations as part a virtual science camp and thus likely have different species of bacteria in each toothbrush culture.

We chose our methodology based on the supplies provided in our experiment kit, but other methods could have alternatively been used. For example, we could have used the disk diffusion method, whereby each bacteria-coated Petri dish is divided into even sections and a paper disk saturated

with the compounds being tested for antibacterial properties is placed in the center of each section. The compound on the disk diffuses into the agar and becomes diluted. The scientist measures the radius of the ring that forms around each disk, called the zone of inhibition. The size of the zone of inhibition indicates how strong the compound's antibacterial properties are. Compounds that are better at inhibiting bacterial growth will have a larger zone of inhibition (15). Another way to demonstrate each spice's antibacterial properties would be to present them with a dose curve. This method would illustrate how much the bacteria can resist the spice as the amount of spice added increases. For example, we could have made a plate with less spice additive than the low-additive 2% v/v plates in addition to the low-additive plates themselves and the normal 4% v/v plates. A dose curve could show an increasing rate of antibacterial effect of the spice as the additive increases.

While analyzing our results, we questioned whether naturally-flavored cinnamon toothpaste would fight against bacteria well, since toothpaste and cinnamon had the best antibacterial properties of everything we tested. A new experiment should include testing cinnamon toothpaste. An additional follow-up question would be how much of an effect the active ingredient(s), like triclosan in toothpaste, has on bacteria growth and whether the amount of an active ingredient matters. We could also improve the experiment by using fresh spices or pure extracts of the active chemicals in the spices.

One of the most interesting outcomes of this experiment came from our low-additive experiment. The cumin 2% v/v plate showed similar results as the plate with 4% v/v. We believe the colony count was almost equal due to cumin not having any strong antimicrobial properties. To go further with this claim, an experiment could be performed to test both cumin plates in different temperatures or environments to see if their colony counts continue to align. In contrast, the colony counts for the low-additive cinnamon plate was visibly greater than the normal-additive plate, demonstrating cinnamon's potential as an antimicrobial agent.

As for the colony morphology of the plates from Experiment C, the toothpaste experimental plate grew colonies with a larger appearance compared to the other plates. Although the colonies were larger, the number of total colonies was small. Since only a few colonies grew, there were more nutrients for each colony to have. We hypothesize that this led to each colony growing larger. To test this theory, another experiment should be conducted to see if plates with low colony counts have larger colonies compared to those with high colony counts. Another factor that could have contributed to this result is the possibility of different bacterial species growing on the plates. The size of the colonies on the toothpaste experimental plate were consistently large, contrasting from the positive control experimental plate, which had more variety in its smaller colony size, and the cinnamon and cumin plates, which were consistently small. The different species may have grown differently due to their individual abilities to adapt to experimental conditions, therefore having distinct colony appearances. A follow-up experiment could influence what bacterial species grew by using different nutrient agars or assess bacterial diversity via PCR or 16S sequencing (16). If this experiment were to be repeated, we recommend that more plates for each spice be observed to strengthen the statistical significance of the data and better support any conclusions made.

Our results agree with the conclusion that toothpaste does indeed fight against oral bacteria, and cinnamon could be used as a powerful antibiotic. This research can be referred to when other scientists are trying to create a new effective oral hygiene method or product.

MATERIALS AND METHODS

All supplies used for this project were pre-sterilized and included in a kit from VWR, provided by the Mini PhD Program at the Journal of Emerging Investigators. Experiments A, B, and C were each completed by a different student in a different geographical location, and the specific spices and toothpastes used in each experiment reflects items that were in use in the home at the time of the experiment. Each experimental condition was performed with a single replicate due to supply limitations.

Preparation of bacterial culture

To prepare the bacterial culture, we filled two centrifuge tubes up to 3 mL with sterile water, and we capped one of the tubes to be our negative control. In the other tube, we put a previously used toothbrush and incubated it overnight at room temperature to collect bacteria for use in our experiment. Using a pipette dropper, we added 0.5 mL of either the toothbrush water or negative control from the previous steps into a tube with 4.5 mL of tryptic soy broth. We stored these cultures overnight in the refrigerator.

Preparation of agar plates

To prepare the agar plates, the agar was heated for one minute at a time until it was fully melted. We gently swirled the agar container to test the liquidity. Next, we poured 12 mL of agar into each of eight 15 mL conical tubes. The caps were kept on the tubes when not being handled. Two of the conical tubes each received approximately 0.5 mL of one of three different types of toothpaste: Experiment A used Schmidt's Wondermint Fluoride-Free toothpaste, Experiment B used Crest regular paste cavity protection fluoride toothpaste, and Experiment C used Crest Arctic Fresh 3D White toothpaste. Each experiment was prepared according to the same protocol using different additives. Two conical tubes each received 4% v/v of each additive, and two conical tubes did not receive an additive. We added 2% v/v of cinnamon and cumin to the two low-additive tubes of Experiment C. After additives were added and lids were secured, tubes were shaken thoroughly to ensure even distribution. Each conical tube was poured onto the corresponding labeled plate. With the lids on, we tilted each plate back and forth until the agar was evenly distributed on the bottom of the plate. The plates were then placed on a level surface in the refrigerator until the next morning.

Bacterial growth and quantification

After removing the culture tubes from the refrigerator, we began by placing a single drop of either the toothbrush culture or negative control on the previously prepared agar plates. Then, using a spreader, we spread the drop evenly across the plate using a different spreader for each plate to avoid cross-contamination. We put a single drop of the negative control culture onto the negative control plate with normal agar as a

control for the sterile water and culture growth medium. We did not add any drops to the other negative control plates because these were intended to control for potential contamination from the additives. Next, we incubated the plates upside down at room temperature for 4 days. We checked on them every 12 hours and recorded bacterial growth with pictures. For the plates with bacterial growth, we carefully counted any visible colonies.

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REFERENCES

1. Deo, Priya Nimish, and Revati Deshmukh. "Oral Microbiome: Unveiling the Fundamentals." *Journal of Oral and Maxillofacial Pathology*, vol. 23, no. 1, 2019, doi:10.4103/jomfp.JOMFP_304_18.
2. Higham, Susan, et al. "Caries Process and Prevention Strategies: Demineralization/Remineralization." *Crest + OralB dentalcare.com*, 27 Aug 2021, www.dentalcare.com/en-us/ce-courses/ce372#overview.
3. van Houte, Johannes, et al. "Ecology of Human Oral Lactobacilli." *Infection and Immunity*, American Society for Microbiology, 1 Nov. 1972, doi: 10.1128/iai.6.5.723-729.1972.
4. Allaker, Robert, and Abish Stephen. "Use of Probiotics and Oral Health." *Current Oral Health Reports*, vol. 4, no. 4, 19 Oct 2017, pp.309-318, doi:10.1007/s40496-017-0159-6
5. Loesche, Walter J. "Microbiology of Dental Decay and Periodontal Disease." *Medical Microbiology*. 4th Edition., U.S. National Library of Medicine, 1 Jan. 1996, www.ncbi.nlm.nih.gov/books/NBK8259/.
6. Heng, Christine. "Tooth Decay Is the Most Prevalent Disease." *Federal Practitioner: for the Health Care Professionals of the VA, Dod, and PHS*, vol.33, no. 10, 1 Oct 2016, pp.31-33, www.ncbi.nlm.nih.gov/pmc/articles/PMC6373711/.
7. Sammons, R. L., et al. "Bacterial Survival and Biofilm Formation on Conventional and Antibacterial Toothbrushes." *Biofilms*, vol. 1, no. 2, 2004, pp. 123–130., doi:10.1017/S1479050504001334
8. Bowden, G H, and I R Hamilton. "Survival of oral bacteria." *Critical reviews in oral biology and medicine*, vol. 9, no.1, 1998, pp. 54-85. doi:10.1177/10454411980090010401
9. Weatherly, Lisa M., and Julie A. Gosse. "Triclosan Exposure, Transformation, and Human Health Effects." *Journal of Toxicology and Environmental Health*, Part B, vol. 20, no. 8, 17 Nov. 2017, pp. 447–469, doi:10.1080/10937404.2017.1399306
10. Yadav, Swati. "Toothbrushes in Bathroom- Clean before You Clean." *International Dental & Medical Journal of Advanced Research*, vol. 3, no. 5, Nov. 2015, pp. S57–S59., doi:10.15713/ins.idmjar.
11. Karibasappa, GN, et al. "Assessment of microbial contamination of toothbrush head: an in vitro study." *Indian Journal of Dental Research*, vol. 22, no. 1, 2011. doi:10.4103/0970-9290.79965
12. Gehad, Youssef, and Mark Springel. "Characterization of Antibacterial Properties of Common Spices." *Journal of Emerging Investigators,* 3 Oct. 2020, www.emerginginvestigators.org/articles/characterization-of-antibacterial-properties-of-common-spices.
13. Cherney, Kristeen. "Aseptic Technique: Uses, Benefits, and Complications." *Healthline*, 29 Sept. 2018, www.healthline.com/health/aseptic-technique#:~:text=Aseptic%20technique%20means%20using%20practices,and%20other%20health%20care%20settings.
14. "Sterilizing Practices." *Centers for Disease Control and Prevention*, Centers for Disease Control and Prevention, 18 Sept. 2016, www.cdc.gov/infectioncontrol/guidelines/disinfection/sterilization/sterilizing-practices.html.
15. Kon, Kateryna, and Mahendra Rai. "Disk Diffusion." *ScienceDirect Topics*, 2016, www.sciencedirect.com/topics/immunology-and-microbiology/disk-diffusion#:~:text=The%20disk%20diffusion%20method%20(DDM,top%20of%20an%20agar%20surface.
16. Woo, PC, et al. "Then and now: use of 16S rDNA gene sequencing for bacterial identification and discovery of novel bacteria in clinical microbiology laboratories." *Clinical Microbiology and Infection*, vol. 14, no. 10, Oct 2008, pp. 908-34. doi: 10.1111/j.1469-0691.2008.02070.x.