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The Effect of Fluorouracil on Pathogenic and Non-pathogenic Oral Microorganisms and Microbial Interactions with Preventative Measures: A Preliminary Study

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By

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Thesis

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This Thesis by Valeria Ortiz Jimenez is accepted in its present form as satisfying the thesis requirement for Honors in Biology.

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Biography

Valeria Ortiz Jimenez was born on March 13th, 2001in Puerto Rico. After 12 years of life spent on the East coast of the island, she and her family moved to Virginia. After earning her high school diploma at Robinson Secondary School, she began her Bachelor of Science at the University of Mary Washington (UMW) with intentions of graduating in May of 2023. Valeria is a member of the Honors program at UMW and has consistently been on either the Dean's or President's list through the last 7 semesters of her college career. Valeria is also a member of Phi Eta Sigma, Chi Beta Phi and Phi Beta Kappa. While at UMW, she has kept busy with extracurriculars by being President of Global Medical Brigades, Pre-Dental Chair of the Pre-Health Society and working as a dental assistant at Synergy Periodontics and Implants. Following graduation at UMW, she has committed to attend Harvard School of Dental Medicine to earn her Doctor of Dental Medicine, DMD, by 2027.

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Abstract

Cancer chemotherapy compromises the patient's oral health through dysbiosis of oral microbiota and increases the prevalence of dental cavities, gingivitis, oral mucositis, and xerostomia. This research aimed to evaluate the effect of a common chemotherapeutic agent, Fluorouracil (5-FU), on certain microorganisms that are common within the oral cavity. Varying concentrations (50 μM, 75 μM and 100μM) of 5-FU were used to simulate the dosage that reaches the oral cavity after intravenous delivery. The microorganisms tested were *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis, Streptococcus mutans, Lactobacillus rhamnosus* and *Streptococcus salivarius.* These are some of the most common ones found in the diverse oral microbiota and would, therefore, be beneficial to study. Some are associated with different oral conditions like periodontitis, the progression of cavities and lesions, and inflammation, while others are probiotics. There are topical and oral products that can be applied or consumed in order to prevent the overgrowth of certain bacteria, while also protecting the oral mucosa. In evaluating the effect of 5-FU on the microorganisms, two preventative treatments were tested in order to reduce and/or improve their effect on a patient's oral cavity: chlorhexidine (CHX) and salt water. 5-FU altered all microbial growth curves, yet it least affected *P. aeruginosa, S. mutans and S. salivarius*. CHX was successful in preventing the growth of most pathogenic bacteria, except *P. aeruginosa*, and all non-pathogenic bacteria, while salt encouraged the growth of probiotic *L. rhamnosu*s and pathogenic *P. aeruginosa* yet suppressed the growth of most pathogenic microbes.

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Introduction

Oral Microbiome

According to research conducted in 2019, the human oral cavity has the second largest and most diverse microbiome (Deo and Deshmukh, 2019). It holds over 700 species of bacteria that colonize the tooth's surface and the soft tissue of the oral mucosa. These organisms are essential in maintaining human systematic health. The present microorganisms may be non-pathogenic but can be identified as opportunistic pathogens, while others are healthy bacteria that are beneficial to our bodies. These opportunistic pathogens do not typically cause infection or harm the oral cavity, however, if the environment's conditions shift and these organisms are given an opportunity, they can cause certain diseases. As stated by Deo and Deshmukh, the bacteria become pathogenic only after they breach the barrier of commensals, causing infection and disease. The commensal populations do not cause harm and maintain a check on the pathogenic species by not allowing them to adhere to the mucosa. Each of these microorganisms have the capability of affecting the oral cavity in a variety of ways– whether they lead to an increase in inflammation and dental cavities or to periodontal disease. This negative relationship is primarily seen in patients receiving cancer treatment. The beneficial and essential microbiota diversity is disrupted through treatment.

Chemotherapy and Its Intraoral Effects

Chemotherapy used to treat cancer can affect oral microbial communities and disrupts the homeostatic balance between resident microorganisms and the adjacent mucosa (Hong et al., 2019). The chemotherapeutic agent can lead to dysbiosis of oral microbiota, and increase the prevalence of dental cavities, gingivitis, oral mucositis, and xerostomia. These conditions affect a patient's quality of life, nutrition, and treatment timeline. Specifically considering the oral

mucositis side effect, when sores and inflammation get to a level that is deteriorating the patient, chemotherapy administration is paused until side effects improve (Saito et al., 2014). In a study conducted in 2021, Wang found that the oral microflora of patients undergoing chemotherapy was less diverse and had increased differences in the microbiome composition when compared to a healthy control (Wang et al., 2021). Therefore, the prevalence of the respective side effects is associated with the depletion and augmentation of certain oral bacteria. As the oral cavity's environment shifts, so does the microorganisms' rate of growth. This shift, whether it is dry mouth or increased gingival inflammation due to decreased salivary flow, alters the concentrations of the pathogens (Taichman et al., 2018). Although the immune system significantly suffers, it triggers a domino effect that leads to an impact on the microorganism level. Overall, patients receiving chemotherapy demonstrate a compromised oral cavity (Wang et al., 2021).

Chemotherapeutic Agent: Fluorouracil

A common chemotherapeutic agent known to shift the oral microbiota is Fluorouracil (5-FU). The 5-FU drug is an anti-metabolite that is used alone or as a foundational therapeutic in combination treatment regimens for a range of cancers (Hong et al., 2019). It has been specifically correlated with reduced proliferation, increased cell death, and upregulation of pro-inflammatory mediators (Hong et al. 2019). 5-FU is a pyrimidine analog and gets incorporated into DNA and leads to DNA damage (McLeod et al. 2021). It can also be incorporated into mRNA and lead to defects in the production of proteins (McLeod et al. 2021). Hence, it causes mutations in protein synthesis in cells and bacteria that are fast growing (McLeod et al. 2021). However, McLeod mentions that the 5-FU is not bactericidal but inhibits bacterial growth. According to prior studies, changes in the oral microflora during cancer chemotherapy, using 5-

FU, reflected changes in gram-negative species coming from the Enterobacteriaceae family, *Pseudomonas sp.* and *E. coli*. Gram-positive species were also isolated, specifically *Staphylococcus sp*. and *Streptococcus sp.* (Napeñas et al., 2007). Although this study was able to isolate these organisms, it did not determine the magnitude of the alterations within the oral microflora and microbes. The results of these studies indicate that microbiota changes are primarily seen from the largely oral streptococci to a more pathogenic gram-negative anaerobic flora. Gram-negative bacteria may intensify the inflammatory processes and lead to an increase in side effects such as oral mucositis (Napeñas et al., 2007).

Preventing Chemotherapy's Intraoral Effects

There are potential ways to restore and prevent the disruption of the oral microbiome that occurs during chemotherapy. There are topical and oral products that can be applied or consumed in order to prevent the overgrowth of certain bacteria, while also protecting the tooth surface and gums. As indicated by Decker, treatments are personalized depending on the patient's side effects and their severity (Decker et al., 2018). In a study conducted in 2018, Decker said that preventative maintenance and the use of chlorhexidine are important therapeutic strategies. Chlorhexidine (CHX) mouth rinse is frequently used after a patient undergoes surgeries or procedures. CHX is known to have bactericidal and bacteriostatic activity based on its concentration and as discussed by Amoian, Omidbakhsh, & Khafri, it does not cause systemic poisoning due to its low absorption from the digestive system (Amoian et al., 2017). The typical prescribed concentration of 0.12% allows CHX to behave as a bactericidal chemical. Regarding its mechanism, the positively charged CHX molecules are attracted to the negatively charged cell wall, which leads to the CHX binding to the cell wall and causes it to rupture (Jenkins et al. 1988). On the other hand, salt water rinses, a preventative method, are frequently suggested and

encouraged for patients to reduce inflammation or the overgrowth of certain pathogenic bacteria. In Aravinth's study, salt water was successful in decreasing microbes associated with oral disease. Regarding its mechanism, it promotes human gingival fibroblasts migration while creating an alkaline oral environment that most microbes struggle to survive in (Huynh et al., 2016). This method is also easily accessible to patients and could serve as a straightforward way to prevent oral disease from further developing. These two preventative methods can serve to restore a chemotherapy patient's oral microbiome and prevent any further disturbances within their normal flora. In regards to the pathogenic bacteria, these preventative measures are expected to avoid their growth, while for non-pathogenic bacteria, they are expected to protect and/or perhaps encourage their growth. Ultimately, the protection of the microorganisms could reduce the oral effects of chemotherapy. In comparing both preventative methods, they are equally effective, yet CHX has been considered superior to salt water rinses as it primarily acts as bactericidal instead of bacteriostatic, like salt (Aravinth et al., 2017).

Ecology of Pathogenic Bacteria in The Oral Cavity

The mouth is considered an ideal environment for microorganism growth due to it being warm, moist and rich in nutrients it receives through food consumption. Pathogenic bacteria found in the oral cavity and contributing to oral disease are *Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis,* and *Streptococcus mutans. S. mutans* requires a solid, non-shedding surface for colonization and is associated with the initiation of cavities (Simon, 2007). It thrives under acidic conditions and is known to produce lactic acid. It is able to bind to the tooth surface in the presence of sugars by forming water-insoluble glucans (polysaccharide that helps the tooth and bacterium bind), which indicates its association to thrive with one's diet. As stated by Simon, dental cavities arise through the imbalance in the intricate

relationship between human host, *S. mutans* and the many microorganisms present in one's oral cavity (Simon, 2007). While *E. faecalis* is linked to the etiology of periodontitis, it is a temporary colonizer of the oral mucosa (Carrero Martinez et al., 2015). It has an ability to survive and reproduce in environments that other bacteria may find fatal. However, its source of colonization is unknown due to its low presence in a healthy oral cavity and its unclarified path towards the oral cavity (Carrero Martinez et al., 2015). *S. aureus* has been isolated from a variety of oral diseases, however, its role in the ecology of the normal oral flora has been questionable (Smith et al., 2003). As an opportunistic pathogen, its presence is increased in patients with prosthetic devices, has been isolated from supragingival plaque and is associated with infection. It produces a wide range of exotoxins which have been found to play a significant role in oral mucosal disease (Smith et al., 2003). *P. aeruginosa* is frequently isolated in immunocompromised patients and patients with periodontal disease (Rivas Caldas et al., 2015). It holds resistance to multiple antimicrobial agents giving it opportunistic characteristics. It possesses the ability to form infectious biofilm and is most commonly isolated in saliva and subgingival plaque, therefore, the oral cavity is a reservoir for this pathogen (Rivas Caldas et al., 2015). Additionally, *P. aeruginosa* is a facultative anaerobe and can adapt their metabolism in order to survive (Rivas Caldas et al., 2015).

Ecology of Non-Pathogenic Bacteria in The Oral Cavity

Streptococcus salivarius and *Lactobacillus rhamnosus* are considered probiotics and/or "good bacteria." *S. salivarius* is correlated with anti-inflammatory properties and is known to inhabit the oral cavity within a few hours after birth (Kaci et al., 2014). Besides the oral cavity, it also inhabits the stomach and jejunum and therefore, it is important in one's digestive tract ecology. *S. salivarius* is labeled as a predominant commensal inhabitant of the oral cavity

through one's lifetime (Kaci et al., 2014). On the other hand, *L. rhamnosus* is widely studied and is known to be a probiotic. In a literature review conducted in 2008, multiple studies revealed the role of lactobacilli in inhibiting some cariogenic bacteria (Badet and Thebaud, 2008). *L. rhamnosus* has a degree of antimicrobial behavior against some streptococci, like *S. mutans. L. rhamnosus* appears in early childhood and its concentration is highest in saliva, however, its presence in the oral cavity depends on various factors like the presence of orthodontic devices and erupted third molars (Badet and Thebaud, 2008).

Study's Purpose and Goals

Ultimately, patients undergoing chemotherapy already have enough side effects and issues that they struggle with. There should be treatments and measures that are accessible to them in order to reduce their likelihood of oral inflammation and diseases. In this study, direct interactions between 5-FU and the microorganisms were tested to evaluate chemotherapy's effect on their growth rate. The microorganisms tested were *P. aeruginosa, S. aureus, E. faecalis, S. mutans, L. rhamnosus* and *S. salivarius.* These were tested as *P. aeruginosa* is associated with periodontal disease and high levels of *S. mutans* and *S. aureus* are associated with cavities and inflammation. Oppositely, *L. rhamnosus* and *S. salivarius* are probiotics and considered to have healthy, commensal relationships with the oral cavity. These microorganisms are some of the most common ones found in the diverse oral microbiota and would, therefore, be beneficial to study. Preventative measures, CHX and NaCl, were also tested in order to determine ways to reduce chemotherapy therapy's side effects and increase a chemotherapy patient's quality of life. In summary, this study aimed to evaluate and determine microbial effects of 5-FU and find ways to determine ways to reduce 5-FU's negative effects on a patient's oral cavity.

Materials & Methods

Before treating and evaluating 5-FU's effect on the chosen microorganisms, different concentrations were created using a powder form of 5-FU (Alfa Aesar) dissolved into phosphate buffered saline (PBS) (8.0 g/L). PBS was created from a pre-made powder (Sigma-Aldrich) mixed with distilled water and its pH was adjusted to reach 7.4 using sodium hydroxide, NaOH (Fisher Chemicals). When chemotherapy is given to a patient, it is typically given intravenously and when it reaches the saliva, the dosage is significantly reduced. The study conducted by Hong et. al was used as a guide in determining an appropriate concentration that closely aligns with a realistic 5-FU salivary concentration. According to the study, the concentration to reach the oral cavity after administration was approximated to be 77 μM (Hong et al., 2019). The effect of three concentrations, 50 μM, 75 μM and 100μM, of 5-FU was tested on the microorganisms to corroborate 5-FU's effect on microbes.

The microorganisms were grown in a flask using 50 mL of tryptic soy broth (TSB)(BD Bacto) at 37℃ to serve as the control: *P. aeruginosa* (ATCC 15692)*, S. aureus* (ATCC 12600)*, E. faecalis* (ATCC 19433)*, S. mutans* (ATCC 15692)*, L. rhamnosus* (ATCC 53103) and *S. salivarius* (ATCC 13419). A 1.0 mL aliquot was removed from the flask every hour for a total of 12 hours and a spectrophotometer was used to measure the absorbance (Abs) at 600 nm until the readings became static. Some organisms were expected to reach stasis in less than 12 hours, therefore, once those organisms reach stable Abs values, flasks were removed from the incubator. A growth curve was created plotting time (minutes) on the x-axis versus the Abs values on the y-axis. These growth curves allowed us to determine the time frames in which the microorganism reaches optimal growth. From the final aliquot taken, a spread plate was conducted by taking 0.1 mL from the aliquot, spreading it on an agar plate (BD Difco) and

incubating it. After initial incubation a bacterial lawn formed and a streak plate was conducted to isolate colonies. A colony was taken, gram stained, and their shape/structure was analyzed with immersion oil and the 100x lens.

The same microorganisms were then grown in a flask containing 5-FU at 37℃. Based on the determined optimal growth rate for each microorganism, aliquots were taken approximately every 2 hours with some variation for slow growing microbes. Based on the control growth curve, a final measurement was taken between 24-48 hours of growth to ensure the stationary phase was reached. Since the spectrophotometer measures turbidity, not viability and 5-FU is known for the killing of cells, a spread plate was conducted to ensure there are live cells present in the samples (Wang et al., 2019). Using the spectrophotometer readings, the effects of 5-FU on each of the microbes was determined using turbidity. Only one trial was conducted per microbe and the varying 5-FU concentrations. In order to evaluate any change, if any, that may have occurred to the microbe, they were stained, and their shape/structure was re-evaluated after treatment.

The preventative measures were analyzed. These measures include chlorhexidine mouth rinses and salt water. Both materials come into direct contact with the microorganisms in the oral cavity, they were not diluted further than their initial concentration and were added directly into the TSB. The CHX rinse held a concentration of 0.12% chlorhexidine gluconate (3M ESPE). To simulate the salt water, 1.0 g sodium chloride (NaCl) (Fisher Chemicals) was dissolved into the flask containing 50 mL of TSB, the solution had a concentration of 0.02 g/mL. Once chemicals were measured and obtained, cultures were grown containing the preventative measures, NaCl and CHX, as a control. After this, cultures were grown containing the preventative measure and a final concentration of 75 μM of 5-FU to observe their growth rate. Multiple aliquots were

removed for spectrophotometric measurements at the same points that the 5-FU growth curve was measured. The microbes were only tested with 75 μM, instead of 50 and/or 100 μM, since it is the approximate amount known to reach the oral cavity upon chemotherapy infusions (Hong et al., 2019). Only one trial was conducted for each of the microbes and the preventative measures. The difference between the stationary and exponential phases of all trials and levels of treatment were used as a base for analysis. Following all trials with 5-FU, since 5-FU is a mutagen, excess and used liquid were poured and contained in a clearly marked bottle. The bottle was kept under a hood and picked-up by chemical waste personnel who properly disposed of the chemical.

All of the work listed above was conducted in the Jepson Science Center at the University of Mary Washington in the fall semester of 2022 and spring semester of 2023.

Preliminary Results

In the evaluation of 5-FU and the oral microorganisms, most microorganisms exhibited a degree of growth with varying concentrations of 5-FU. *S. aureus, E. faecalis and L. rhamnosus* exhibited minor levels of growth, while *P. aeruginosa, S. mutans and S. salivariu*s exhibited larger levels of growth. In general, as the concentration of 5-FU decreased, after 24-48 hours of incubation, the stationary phase was closer to that of the control growth curve and microbes were affected less as depicted in Figure 1. Upon microscopic analysis and gram stains, the microbe's structure and shape remained unaffected by the 5-FU and its varying concentrations.

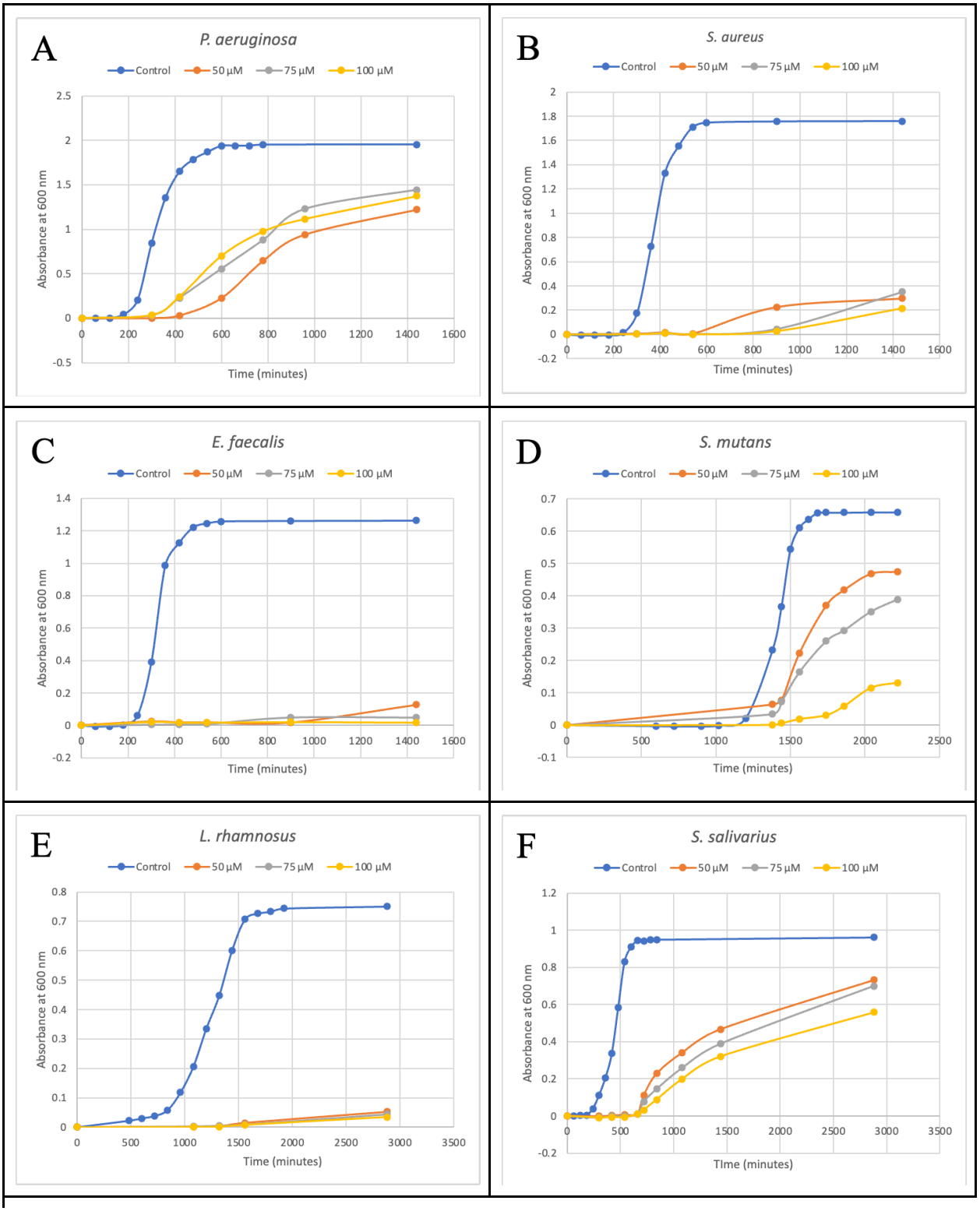


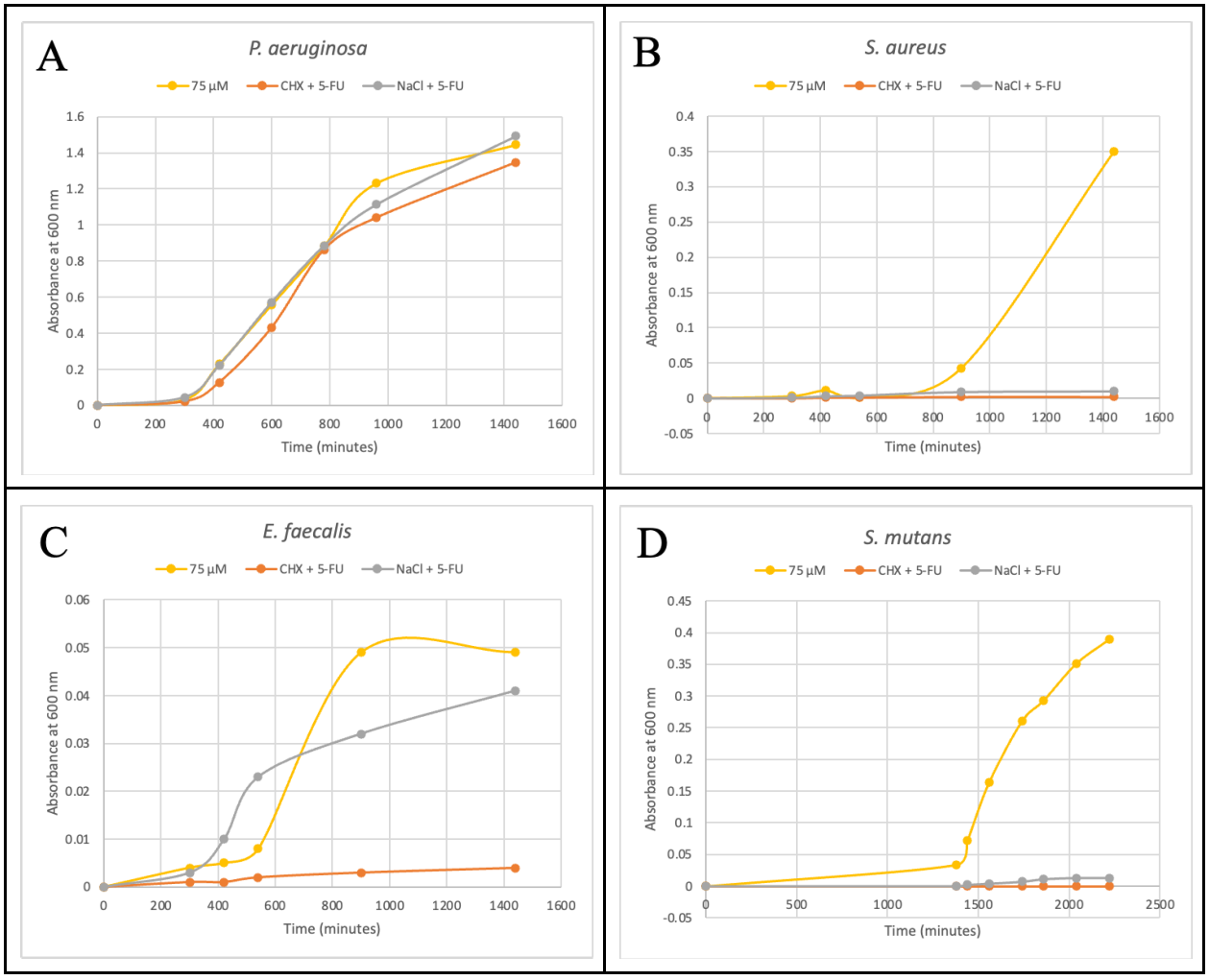
Figure 1– *The effect of varying concentrations of 5-FU on* ***(A)*** *P. aeruginosa,* ***(B)*** *S. aureus,* ***(C)*** *E. faecalis,* ***(D)*** *S. mutans,* ***(E)*** *L. rhamnosus* and ***(F)*** *S. salivarius in time (minutes) vs.*

absorbance at 600 nm. Control growth curves, depicted in blue, are depicted as reference for 5-FU's effects on the microbe's growth. The varying concentrations of 5-FU tested were 50μM (depicted in orange), 75μM (depicted in grey) and 100μM (depicted in yellow).

Regarding the controls for the preventative chemicals, the CHX inhibited nearly all microbial growth, except for *P. aeruginosa*. For *P. aeruginosa*, CHX alone allowed the stationary phase to reach a final absorbance of 1.673 yet CHX with 75 μM 5-FU held an absorbance of 1.347. The NaCl control held varying results: inhibited the growth of *S. salivarius* and *S. aureus*, encouraged the growth of *P. aeruginosa, L. rhamnosus, E. faecalis* and *S. mutans.* For the microbes that had encouraged growth, *L. rhamnosus*' NaCl alone reached a final absorbance of 0.577, while NaCl mixed with 75 μM 5-FU reached a final absorbance of 0.044. Similarly, *P. aeruginosas*' final absorbance with NaCl alone was 1.633, while its final absorbance with NaCl mixed with 75 μM 5-FU was 1.493. Although *E. faecalis* and *S. mutans*' growth was encouraged, their final absorbance did not surpass that of NaCl mixed with 75 μM 5-FU. Controls are not depicted in Figure 2 due to graph scaling and inability to differentiate and visualize minute changes in absorbance.

Regarding the preventative chemicals, CHX mixed in with 75 μM 5-FU significantly decreased the microbe's degree of growth. Although the stationary phase of the microbes was drastically lower for most organisms, *P. aeruginosa* was an exception. As pictured in Figure 2, *P. aeruginosas*'s stationary phase reached a final absorbance of 1.347 with CHX and 75 μM in comparison to the 1.445 with only 75 μM of 5-FU. Similarly, NaCl mixed with 75 μM 5-FU led to an increased absorbance of 1.493 at P. *aeruginosa'*s stationary phase. NaCl also led to a notable degree of growth in *L. rhamnosus* as its absorbance was measured at 0.121 with NaCl and 75 μM 5-FU while its absorbance was 0.044 with 75 μM 5-FU alone. For *S. aureus* and *S.*

salivarius, NaCl and CHX equally reduced the microbe's degree of growth. While *E. faecalis* and *S. mutans* exhibited minor levels of growth with NaCl, CHX nearly inhibited their growth totally. In summary, *P. aeruginosa* and *L. rhamnosus* were the only microbes to experience notable levels of growth with the preventative measures. All microorganisms showed viability at the final absorbance measured and upon microscopical analysis, their structure and shape were unaltered.



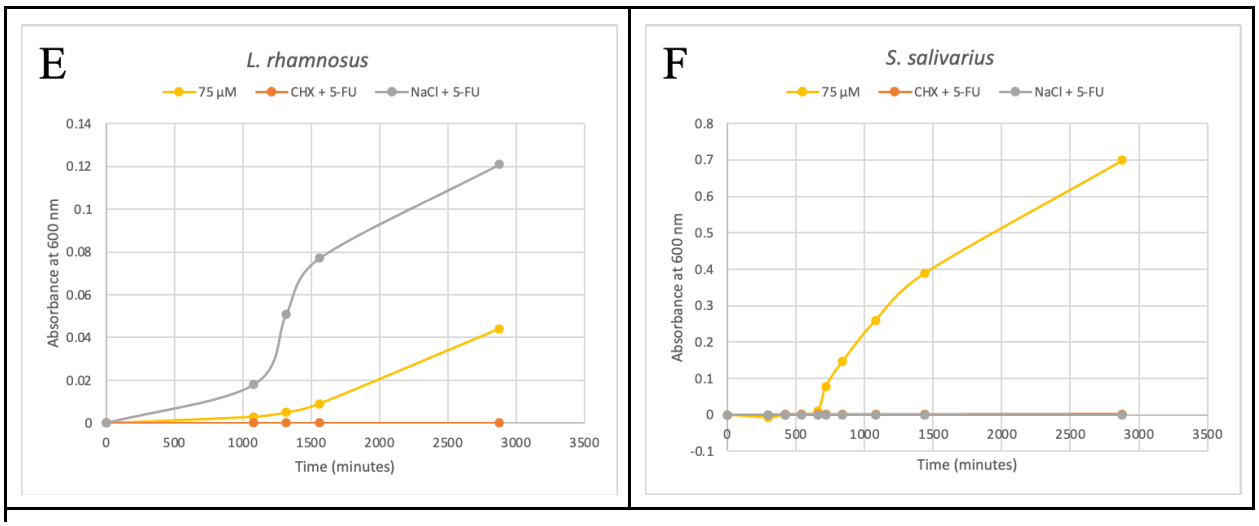


Figure 2— *The effect of CHX and NaCl mixed with 5-FU on* ***(A)*** *P. aeruginosa,* ***(B)*** *S. aureus,* ***(C)*** *E. faecalis,* ***(D)*** *S. mutans,* ***(E)*** *L. rhamnosus* and ***(F)*** *S. salivarius in time (minutes) vs. absorbance at 600 nm. The 75µM 5-FU growth curve (depicted in yellow) serves as reference and control for the preventative chemical's growth. The two preventative chemicals depicted are CHX (depicted in orange) and NaCl (depicted in grey). Controls were conducted for CHX and NaCl, however, they were not included in the graph due to scaling and inability to differentiate and visualize minute changes in absorbance.*

It is important to note that all of the results presented are based on one replicate, and for future validation and statistical analysis, this experiment requires additional replicates. Hence, the results presented are considered preliminary.

Discussion

Due to cancer patients experiencing higher levels of inflammation and diseases within their oral cavities, this study evaluated the effects of 5-FU on varying oral microorganisms associated with said symptoms. 5-FU is known to reduce proliferation and when interacting with oral microbes, it is shown to alter microbial growth curves (Hong et al., 2019). The effects were evaluated with each microbe's growth curves individually. According to prior studies, changes in the oral microflora were noted in both gram negative and gram positive microbes when mixed

with 5-FU, yet a shift is noted from a largely oral streptococci to a more pathogenic gram-negative anaerobic flora (Napeñas et al., 2007). Contrary to this, in this study, *S. mutans*, a streptococcus, was impacted less by 5-FU and grew more in comparison to *P. aeruginosa*, a gram-negative, facultative anaerobe. Although the streptococcus was affected less than the gram-negative facultative anaerobe, both of these microbes are still associated with oral disease, *S. mutans* with cavities and *P. aeruginosa* with periodontal disease (Simon, 2007; Rivas Caldas et al., 2015). *P. aeruginosas*'s resistance to 5-FU can also be attributed to its facultative behavior. It can shift its metabolism from aerobic to anaerobic which could result in slower growth under anaerobic conditions, as seen in this study. Additionally, in Moradali's study, *P. aeruginosa's* persistent nature was further explored. The study revealed the multi-layered physiological adaptations correlated to the microbe's growth behavior while responding to harsh environmental conditions. *P. aeruginosa*'*s* ability to adapt and survive relies of regulatory or controlling factors involved in complex signaling pathways, which allow it to develop mutations that could protect it against agents like 5-FU (Moradali et al.,2017). The other pathogenic bacteria, *E. faecalis* and *S. aureus*, were notably affected by 5-FU, which was unexpected since 5-FU is not bactericidal (McLeod et al. 2021). *E. faecalis* is known for its ability to survive and reproduce in environments that other bacteria may find fatal, yet it barely grew in the presence of 5-FU Carrero Martinez et al., 2015). *S. aureus* is an opportunistic pathogen and is associated with supragingival plaque, and its lack of resistance to 5-FU is not questionable due to this study analyzing it in an isolated manner (Smith et al., 2003). Additionally, 5-FU is known to partially inhibit *S. aureus'* peptidoglycan biosynthesis, hence, preventing the gram positive microbe from growing (Thomson and Lamont, 2019). Overall, although an overgrowth of pathogenic bacteria was expected but not observed, 5-FUs small impact on *P. aeruginosa* and *S. mutans*' growth,

still corroborates chemotherapy's effects on a patient's oral cavity beginning at the microbial level. *P. aeruginosa* and *S. mutans,* in comparison to the other microbes, had an increased resistance to 5-FU.

Moreover, as noted earlier, chemotherapy is known to wipe out the natural flora and eliminate healthy and good bacteria that would otherwise counteract the growth of opportunistic pathogens. As expected, *L. rhamnosus* did not exhibit notable levels of growth and was non-resistant to 5-FU. *L. rhamnosus* is a probiotic, and probiotics tend to be more susceptible to harsh drugs than pathogenic microbes (Badet and Thebaud, 2008). Its presence is highly dependent on its surroundings and the environment in which it is inoculated, and therefore, a minor disturbance would drastically reduce its ability to grow. In contrast, *S. salivarius* was the microbe that was least altered out of all of the microbes tested and was rather resistant to 5-FU. However, *S. salivarius* has been found to inhabit the stomach and jejunum where stomach acids are present, hence they may be able to tolerate harsher conditions. Although unexpected, *S. salivarius'* reaction to 5-FU is a positive one probably due to its anti-inflammatory properties and role within the oral cavity (Kaci et al., 2014). Since it still grows even in the presence of 5-FU, it may reduce pathogenic microbe's effects in a chemotherapy patient's oral cavity.

In this study, preventative measures were also tested in conjunction with the 5-FU in order to determine a possible solution for these patients. CHX can behave as bacteriostatic and bactericidal, yet its concentration of 0.12% is most associated with bactericidal activity. However, it behaved as a bacteriostatic chemical for *P. aeruginosa*; the microbe's growth was slightly suppressed with CHX and 5-FU when compared to 5-FU alone. *P. aeruginosa* was the only microbe to demonstrate notable degrees of growth, while CHX inhibited *S. aureus, E. faecalis, S. mutans, L. rhamnosus* and *S. salivarius'* growth curves. As expected, CHX seemingly

behaved like a bactericidal chemical for those microbes, since CHX binds to the cell wall of bacteria and leads to a disruption in cell membrane (Bednarek et al., 2022). Notably, *P. aeruginosa's* resistance to 5-FU could be correlated to the fact that it is a gram negative bacterium; its outer membrane protects the bacterial cell and prevents the CHX from disrupting it. Regarding CHX's efficiency in reducing oral diseases for chemotherapy patients, it was unable to target *P. aeruginosa* which is able to withstand the harsh effects of 5-FU and must be targeted in preventative care. Arguably, it did prevent *S. mutans'* growth and could, therefore, reduce cavities associated with chemotherapy drugs. Microbes like *S. aureus* and *E. faecalis* are not of primary concern as they are non-resistant to 5-FU and have an inability to maintain sustainable levels of growth upon contact with 5-FU.

The practice of salt water rinses as a preventative measure was also tested in this study. Salt water is frequently used through medicine and is known to reduce inflammation or the growth of pathogenic microbes. Many dentists encourage warm salt water rinses following a surgery in order to encourage healing and avoid potential infections. As expected, due to its bacteriostatic behavior, salt reduced most microbe's growth but not as drastically as CHX. However, salt encouraged *P. aeruginosa* to grow and it reached a higher stationary phase than with 5-FU alone– making this microbe resistant to salt. According to Havasi's study, strains of *P. aeruginosa* can growth with salt is dependent on the solution's salt concentration. At a higher concentration, ≥70 g/L, P. *aeruginosa*'s growth is inhibited, however, at lower concentrations, <20 g/L, it is still able to grow (Havasi et al., 2008). Hence, this study's concentration was unable to inhibit its growth and reflected Havasi's result. The rest of the pathogenic microbes grew less when salt was present in conjunction to 5-FU. Regarding the non-pathogenic microbes, salt encouraged *L. rhamnosus* to grow and nearly tripled in growth when compared to 5-FU

alone, while it fully inhibited the growth of *S. salivarius*. Similar to *P. aeruginosa*, certain strains of *L. rhamnosus* can withstand low concentrations, <20 g/L, of salt water (Reale et al., 2015). In previous studies, salt water was established as "effective in reducing oral disease-causing microbes" (Aravinth et al., 2017) and how it behaved through these trials generally affirms this. Although *P. aeruginosa*'s growth was increased with salt, the resistance and positive growth exhibited by *L. rhamnosus* is rather notable. Salt water rinses may have mixed effects on chemotherapy patients. They may reduce inflammation and promote the growth of probiotics, like *L. rhamnosus,* but if used extremely often, they may encourage periodontal disease-causing bacteria, like *P. aeruginosa*.

This study, due to its nature, serves as a preliminary study and creates grounds for future research. Through its methods and results, the effect of 5-FU on varying microbes could be broadened and replicates would need to be created. Other gram-negative microbes associated with oral disease could be further explored in order to identify if 5-FU's effects, or lack thereof, are closely associated to a microbe's membrane structure. Gram-negatives are hard to target and would be crucial in understanding the oral microflora's reaction to 5-FU and the side effects patients experience. Microbes are not isolated within the oral cavity and the study could be repeated with microbial cocktails to see how they affect each other within a 5-FU solution. Relationships previously stated, like the role of lactobacilli in inhibiting some cariogenic bacteria, could be analyzed if the study is conducted with the cocktails (Badet and Thebaud, 2008). Alternatively, to increase the study's reliability, it could be reproduced on an extracted tooth's surface. Microbes could be inoculated and swabbed on the tooth's surface and the effects evaluated. Some microbes tested do not directly affect the tooth's enamel, therefore, an environment that more closely resembles that of the oral cavity which considers saliva's pH,

gums and teeth could be utilized. Moreover, this study confirmed that both preventative methods, CHX and salt, were successful in varying manners. CHX may have completely eradicated the growth of all microbes except *P. aeruginosa*, but salt encouraged the growth of a probiotic while moderately suppressing the growth of most pathogenic microbes. These relationships and effects could be further tested with microbial cocktails in order to account for microbial interactions. Lastly, to accurately assess if 5-FU and the preventative chemicals caused any disruptions to the bacterium's cell wall, especially in gram negative bacteria, an electron microscope could be used. A regular microscope has limited magnification capabilities and cannot assess the cell wall on a molecular and textural level like the electron microscope would have.

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