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The Inhibitory Effects of a Novel Gel on Staphylococcus aureus Biofilms

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The Inhibitory Effects of a Novel Gel on *Staphylococcus aureus* Biofilms

By

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ABSTRACT

Antibiotic resistance is an ever-growing topic of concern within the medical field causing researchers to examine the mechanisms of resistance to develop new antimicrobials. Bacteria's ability to form biofilms is one mechanism which aids in antimicrobial resistance. *Staphylococcus aureus* is of special interest as it is one of the most frequent biofilm-forming bacteria found on medical devices causing infections and posing dangerous threats in a clinical setting. A recently developed antimicrobial gel has been shown to have profound effects on treating bacterial infections and wound healing. This research is centered upon examining the antimicrobial effects of this gel on the three different stages of biofilm formation in clinical and laboratory strains of *S. aureus*. Through a series of experiments examining the effects this gel has on *S. aureus* at the stages of biofilm attachment, maturation, and dispersion, the gel has shown significant levels of inhibition. These findings indicate that the novel gel disrupts biofilm forming processes of *S. aureus*, which provides useful information for fighting infections in the medical field. Further research on the uses and effects of this new gel could lead possibility using the antimicrobial compound for a variety of clinical purposes.

ACKNOWLEDGEMENTS

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TABLE OF CONTENTS

Abstract 2

Acknowledgements 3

List of Figures 5

List of Images 6

I. Introduction 7

Biofilms 7

Staphylococcus aureus 8

Previous Research 9

Antimicrobial Growth Compound 13

II. Materials and Methods 15

Attachment Assay 15

Optical Density 15

Crystal Violet 16

MTT Reductase 16

Colony Forming Units 16

Maturation Assay 17

Dispersion Assay 17

III. Results 18

IV. Discussion and Conclusion 25

References 27

LIST OF FIGURES

Figure 1: Incubation of bacteria with 4% Antimicrobial Growth Compound revealing *Staphylococcus aureus* most susceptible 13

Figure 2: Attachment Phase Optical Density 19

Figure 3: Attachment Phase Crystal Violet Staining 19

Figure 4: Attachment Phase MTT Reduction 20

Figure 5: Attachment Phase Colony Forming Units 20

Figure 6: Maturation Phase Optical Density 21

Figure 7: Maturation Phase Crystal Violet Staining 22

Figure 8: Maturation Phase MTT Reduction 22

Figure 9: Maturation Phase Colony Forming Units 23

Figure 10: Dispersion Phase Optical Density 24

Figure 11: Dispersion Phase Crystal Violet Staining 24

Figure 12: Dispersion Phase MTT Reduction 25

Figure 13: Dispersion Phase Colony Forming Units 26

LIST OF IMAGES

Image 1: Attachment Phase *S. aureus* Biofilms (clinical and lab)..... 18

Image 2: Top–Clinical *S. aureus*, Bottom-Clinical *S. aureus*+AGC)..... 20

Image 3: Dispersion Phase Crystal Violet Staining Plate (top – *S. aureus* only, bottom - *S. aureus*+AGC) 24

Image 4: Dispersion Phase MTT Reduction (top rows - *S. aureus*+AGC, bottom rows - *S. aureus* only)..... 25

I. INTRODUCTION

Biofilms

The resistance of bacteria to antibiotics continues to be a threat on human health, killing thousands of people each year as a result of infections. A major contribution to antibiotic resistance is bacteria's ability to form biofilms. Biofilms are colonies of microorganisms which are held together by an extracellular matrix, providing homeostasis and allowing them to withstand extreme and fluctuating environments (1). Biofilm development occurs in three phases: attachment, maturation, and dispersion. Through a series of reactions, free floating bacterial cells attach and adhere to a selected surface (2). Upon attachment, bacterial growth of the biofilm occurs as the cells divide and form micro colonies which cover the surface area (2). As multiple layers of bacteria are formed on the surface, the biofilm becomes mature and creates a matrix for cell signaling and distribution (2). Dispersion occurs in response to changes within the environment, such as a lack of nutrients, allowing bacterial cells to detach to preserve the biofilm (2). Dispersed cells become free floating cells that can reattach and begin the biofilm development process again (3). The maintenance of biofilms is due to their extracellular matrix of polymeric substances which compromises over 80% of the biofilm and is responsible for keeping it intact (2). The matrix has many roles within the biofilm, most importantly acting as a diffusion barrier, protecting the organism from harmful substances such as antibiotics (2). This barrier, along with several other features of the biofilm increases antibiotic resistance and allows infections to persist.

Staphylococcus aureus

Biofilms, being a leading cause of acute and chronic infections, pose a serious threat in clinical settings as they are commonly found on medical devices. The leading cause of biofilm infections is understood to be the *Staphylococcus* species, as staphylococci make up much of the normal flora and are found on the surface of the skin (4). Specifically, *Staphylococcus aureus* can persist on medical devices through interactions with the surface and human proteins which coat the device, creating an increasing infection control problem in the healthcare field (5). The regulation of several key factors has an important role in *S. aureus* biofilm formation such as the regulation of attachment factors, exopolysaccharide synthesis, and the accessory gene regulator quorum sensing system (*agr*) (4).

S. aureus contains attachment factors which contribute to the attachment of bacteria during the beginning stages of infection and allow for biofilm development (4). These attachment factors bind to human proteins like fibrinogen and fibronectin, which cover indwelling medical devices, increasing colonization of bacteria (4). As the bacterial cell density increases, an *agr* quorum-sensing system recognizes these factors are no longer needed and terminates the expression of these factors (4).

As staphylococcal biofilms mature, aggregation and structuring of the bacteria occur (4). Intercellular aggregation within staphylococci is due to the molecule polysaccharide intercellular adhesion (PIA), which combined with other molecules make up the extracellular matrix of the biofilm (4). PIA synthesis is of great importance in biofilm formation as it increases the integrity of the biofilm, resulting in increased virulence of infections (6). Research has found that anaerobic

conditions significantly increase the production of PIA in *S. aureus*, therefore increasing PIA production in biofilms as well due to decreased oxygen levels within the biofilm. (4,6).

In addition to reducing the expression of surface proteins, the *agr* quorum-sensing system of *S. aureus* produces many virulence factors which contribute to staphylococcal growth and infections (7). Among these is the expression of phenol-soluble modulins (PSM), which are involved in the development and detachment of biofilms and have fatal effects on the human immune system (8). The regulation of each of these factors, along with several others, contribute to the growth of *S. aureus* biofilms and their persistence in infections.

Previous Research

As technology in the healthcare industry has grown using more medical devices, there has been a rise of infection rates causing researchers to study biofilm function to develop anti-biofilm strategies and treatments. Several treatments have shown to prevent and inhibit biofilm growth, but there has yet to be developed a treatment which eliminates biofilm activity entirely. Previous research on current antibiotics, the resistance of biofilms to disinfectants, the antimicrobial properties of hydrogels and topical agents, and new biofilm eradication strategies has added valuable information to the discussion.

While a vast number of antibiotics exist, few are effective at targeting bacteria within biofilms. Currently, oxazolidinones and tetracyclines are being used to treat staphylococcal biofilms as they inhibit protein synthesis (9). Linezolid, the only oxazolidinone approved for clinical use, works by disrupting the assembly of ribosomes during protein synthesis (9). When combined with rifampicin, linezolid had bactericidal effects, however it was fount to only be effective against biofilm infections when administered for long periods of time (9). Research

discovered tetracycline antibiotics are most effective in preventing infections, as they prevent the binding of tRNA during protein synthesis (9). Clinical trials revealed that catheters coated with a combination of tetracyclines and additional antibiotics had only an 8% colonization, compared to a 25% colonization of those untreated (9). Despite preventing bacterial growth, gram positive bacteria have developed a resistance to tetracyclines, thus reducing their effectiveness (9).

In addition, lipopeptides and glycopeptides are antibiotics being used to target the cell membrane of biofilm bacteria (9). Daptomycin, a lipopeptide which depolarizes bacterial cell membranes and causes cell death is an alternative antibiotic as *S. aureus* is becoming resistant to vancomycin (9). Research studies revealed that daptomycin is more effective in treating staphylococcal biofilm infections than other antibiotics including clindamycin, linezolid, and vancomycin as it effectively killed 96% of biofilm bacteria (9). Although *S. aureus* resistance to vancomycin is increasing, it continues to be a commonly used antibiotic in treating such infections (9). The glycopeptide vancomycin inhibits cell wall synthesis by binding to peptidoglycan and preventing cross-linking (9). The effects of vancomycin eventually lead to cell death, but due to resistance mechanisms it does not result in complete eradication of biofilm-associated bacteria (9).

Research has also been conducted on the antimicrobial properties of hydrogels and disinfectants against biofilms as potential treatment options. A study was conducted on the inner gel of the *Aloe barbadensis* plant, commonly known as *Aloe vera*, to examine its antimicrobial properties as a possible treatment for biofilms. *Aloe vera* is a common ingredient in many medicinal products, having over 75 active ingredients found in the inner gel alone, while the leaf is known to have antibacterial and bactericidal properties (10). The study tested the effects of the *Aloe vera* inner gel on *Shigella flexneri,* a popular bacterium in gastrointestinal illnesses and *Streptococcus pyogenes* (10). Research revealed that the bacteria was susceptible to the inner gel

of *Aloe vera*, indicating that these ingredients could be vital for the future development of antimicrobial products that effectively treat biofilms (10).

Chitosan/dextran based (CD) hydrogels exhibit antimicrobial properties due to chitosan's polycationic structure which disrupts the cell membrane of bacteria (11). These hydrogels were found to lose their bactericidal activity in neutral conditions, so a modified hydrogel was used to determine how they affected varying types of bacteria (11). The modified hydrogel was created using *N*-succinyl chitosan (SC) and dextran aldehyde (DA), which allowed the chitosan to be soluble at neutral conditions (11). Results showed that the CD hydrogel had bactericidal effects against *E. coli, S. aureus, S. pyogenes,* and *C. perfringens*, and DA was found to be the more antimicrobial component than SC (11). *P. aeruginosa* and *C. albicans* were not affected by the CD hydrogel, but both were inhibited by high concentrations of the DA component (11). The study found the binding of the DA component with amino groups of bacterial cell walls was the mechanism for antimicrobial activity in the hydrogel (11). The DA component contributed to the research of biofilm treatment and further developed the field, but was not effective against all bacteria.

Biofilms increased resistance to disinfectants and other antimicrobials has prompted research to examine what properties of bacterial biofilms contribute to their resistance. Disinfectants are used on nonliving objects to destroy all bacteria, while antibiotics are used to treat internal infections and only target specific bacteria (12). While disinfectants can kill all pathogenic microorganisms, the complex structure of biofilms inhibits biocides from penetrating through the multiple layers of bacteria, reducing their efficacy (12). A study on the use of chlorine on *P. aeruginosa* and *K. pneumoniae* revealed that only 20% of the chlorine penetrated the core of the biofilm (13). Also, the components of bactericides are reactive molecules which interact

differently with varying bacteria, making them inefficient as a solution for treating all biofilms (12). The success of disinfectants requires targeting the extracellular matrix to break down these diffusion and interaction barriers in biofilms, and the use of enzymes is one possible way this could be done (12).

As the efficacy of antibiotics and hydrogels treating biofilms continues to be a challenge, new treatment options aimed at eradicating biofilms are being researched. Among the new strategies being tested is the use of enzymatic treatments to degrade the extracellular matrix and weaken biofilm structure (9). When this occurs, the bacteria disperse from the biofilm and antibiotics become more effective at targeting the bacteria (9). Several enzymes including dispersin B, DNases, and lysostaphin have properties which degrade the polysaccharide matrix, suggesting that they have the potential to be used as a treatment to prevent and clear biofilm infections (9).

Another strategy attempting to disrupt biofilms causing them to regain sensitivity to treatment options is targeting the *agr* quorum sensing system (9). The *agr* system is a cellular communication system that allows the bacteria to share information regarding cell density to regulate the formation and dispersal of staphylococcal biofilms (9). Research has shown that activation of the *agr* system has inhibitory effects on biofilm formation, resulting in increased levels of proteases and disrupting cell-cell interactions which causes cells to detach from the biofilm and return to a planktonic state (9). While this approach has proved successful in treating staphylococcal biofilm infections, it is still being studied as there is a concern that this system may cause *S. aureus* to become more invasive (9).

Antimicrobial Growth Compound

While many strategies and treatments for biofilm prevention and eradication have been studied, the complexity of their resistance has made it difficult to develop a treatment that is entirely successful. A newly developed antimicrobial growth compound (AGC), composed of antioxidants (vitamin C and E) and zinc, has shown profound effects in pain relief and wound healing. Due to its incredible results, it is believed that this novel gel could have many applications in the healthcare field, such as being used as an antimicrobial to treat bacterial infections. Previous research revealed the AGC was not susceptible to *Staphylococcus aureus*, however these studies were only performed in liquid cultures. Figure 1 shows the susceptibility of *S. aureus* to the AGC compared to other bacteria.

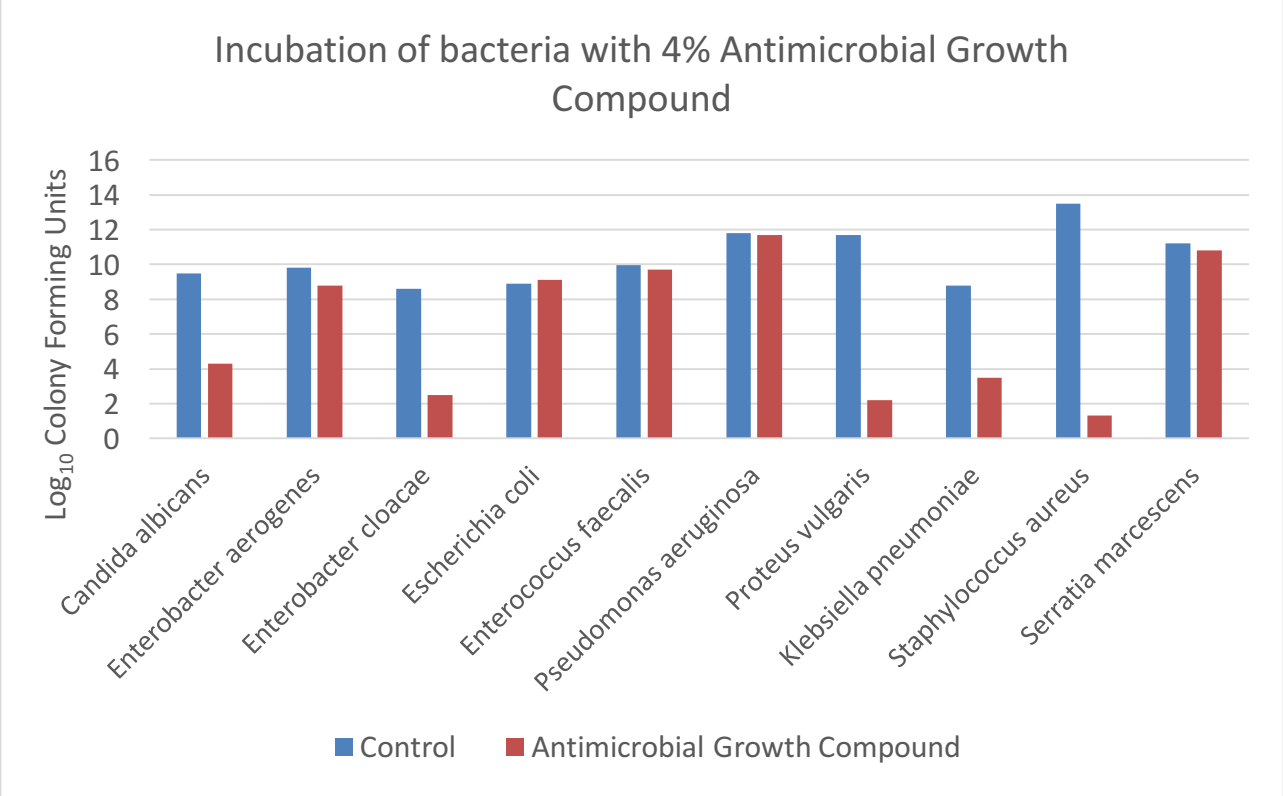

Figure 1: Incubation of bacteria with 4% Antimicrobial Growth Compound revealing *Staphylococcus aureus* most susceptible

Additional research is needed to study the effects of the AGC on biofilms. As biofilms contribute greatly to antibiotic resistance, the mechanism of action of how AGC could affect the development and maturation of biofilms is under investigation. My research is focused on studying the antimicrobial effects of the novel gel on the three stages of biofilm development, specifically in *Staphylococcus aureus*. The results of my research provide new information about this antimicrobial compound, which can lead to the progression of developing new antimicrobials to fight antibiotic resistance.

II. MATERIALS AND METHODS

The effects of the AGC were examined on the attachment, maturation, and dispersion phases of *S. aureus* biofilm development via different methods and assays by measuring the optical density, crystal violet staining, MTT reduction, and calculating colony forming units. All tests were performed in triplicate.

Attachment Assay

To observe the effects of the AGC on the attachment phase of *S. aureus* biofilm development, a 10% solution of AGC broth was initially added to a 96 well plate containing either clinical and laboratory strains of *S. aureus* in lysogeny broth (LB) at an optical density (OD600) of 0.01 which is equivalent to $1x10^6$ cells/mL. The 10% solution of AGC was prepared by weighing 10g AGC, adding it to a bottle, and raising the volume to 100mL using LB broth. Controls of clinical and lab strains of *S. aureus* in LB without AGC were also added to the well plate for comparison. The plate was placed in an incubator at 37℃ for 24 hours to allow for biofilm development. After 24 hours, the plates were removed from the incubator and assayed for OD600, OD595 after crystal violet staining, OD550 after MTT reduction, and observed for the presence of colony forming units (CFU).

Optical Density

The OD assay was used to determine the effects of the AGC on *S. aureus* biofilm density. To find the OD of the samples, 200 µl of the sample from a well was added to 800 µl of phosphate-buffered saline (PBS), and the absorbance of this solution was measured at 600 nm wavelength. LB containing 10% AGC was used as the blank.

Crystal Violet

Crystal violet staining was performed to determine the effects of the AGC on *S. aureus* biofilm mass. The liquid from each well was carefully discarded, and then the remaining biofilm in each well was washed with PBS. Upon removal of PBS, 0.01% crystal violet (200 µl) was added to each well and removed after 10 minutes. Lastly, 30% acetic acid (200 µl) was added to each well, and the OD at 595 nm wavelength was measured using a microplate reader. LB only and LB containing 10% AGC served as the controls.

MTT Reductase

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay determined the effects of the AGC on the viability of cells, comparing the *S. aureus* biofilm treated with AGC to the controls. Viable cells can reduce MTT to formazan, producing a purple color, indicating how much metabolic activity is present within the biofilm. MTT (20 µl) was added to the biofilm in each well, and the plate was placed in the incubator at 37℃ for 30 minutes. After 30 minutes, the plate was removed, and acidic isopropanol (100 µl) was added to each well to stop the reaction of MTT. A microplate reader was used to measure the OD at 550 nm wavelength of the samples, with LB only and LB containing 10% AGC as the controls.

Colony Forming Units

The number of colony forming units (CFU) was determined to assess the viability of cells, or their ability to reproduce. Viable cells, when placed on a culture under special conditions, will aggregate and reproduce to form colonies of bacteria. To determine the number of colony forming units present in the AGC treated *S. aureus* biofilm compared to the untreated, the well contents of each sample were mixed, and 100 µl of each sample was removed and added to separate mannitol

salt agar (MSA) plates. The plates were incubated at 37℃ for 24 hours. After 24 hours, the plates were removed from the incubator and examined to count the number of colonies present.

Maturation Assay

The effects of AGC on the maturation phase of *S. aureus* biofilms was examined using the previously explained technique in the attachment assay, except the 10% AGC broth was added after the clinical and laboratory strains of *S. aureus* were added to the well plate and incubated at 37℃ for 2 hours to allow for the development of biofilms. Upon addition of 10% AGC, the well plate was placed in the incubator at 37℃ for 24 hours. After 24 hours, the OD at 600 nm wavelength, crystal violet staining, MTT reduction, and colony forming unit assays were performed using the same methods as described in the attachment assay.

Dispersion Assay

The dispersion phase of *S. aureus* biofilms and their reaction to the AGC was studied using the same methods as previously mentioned, but the 10% AGC broth was added after the clinical and laboratory strains of *S. aureus* were added to the well plate and incubated at 37℃ for 24 hours. After 24 hours, the 10% AGC was added to each well and the plate was incubated for another 24 hours. The same tests were performed as described in the attachment and maturation assays.

III. RESULTS

Among all three biofilm phases, the AGC significantly reduced viability of clinical and laboratory strain *S. aureus* by assessment through biofilm density, mass, and viability. The AGC was tested on the attachment phase of biofilm formation by initially adding the gel to clinical and laboratory strains of *S. aureus* and evaluating biofilm formation after 24 hours. Image 1 shows the resulting biofilms established in the well plate after 24 hours. There is a clear visible reduction in biofilm growth of both the clinical and laboratory strains of *S. aureus* that were treated with the AGC at the attachment phase of biofilm formation. The optical density assay showed that the AGC reduced clinical *S. aureus* biofilm density by 72% and laboratory *S. aureus* biofilm density by 66% at the attachment phase, as shown in Figure 2. The crystal violet assay results shown in Figure 3 revealed that the AGC reduced clinical *S. aureus* biofilm mass by 96% and laboratory *S. aureus* biofilm mass by 87% at the attachment phase.

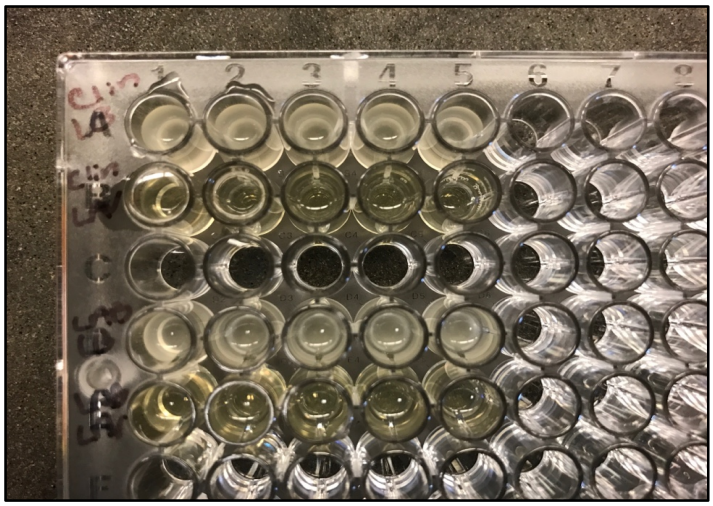

Image 1: Attachment Phase *S. aureus* Biofilms (clinical and lab)

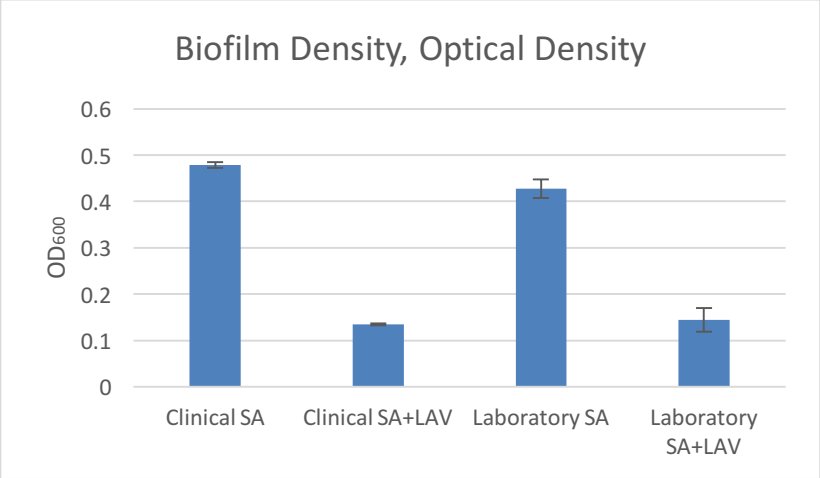

Figure 2: Attachment Phase Optical Density

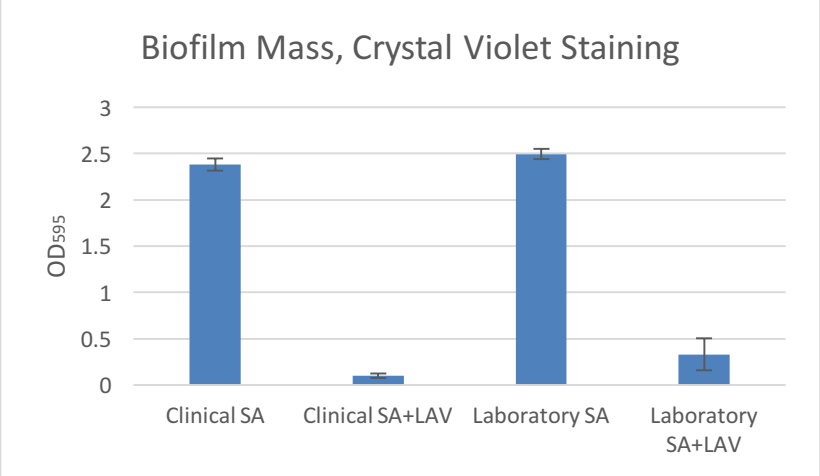

Figure 3: Attachment Phase Crystal Violet Staining

S. aureus biofilm viability was greatly reduced by the AGC at the attachment phase as shown by the MTT reduction and CFU assays in Figures 4 and 5. The MTT reduction revealed a 91% reduction of clinical *S. aureus* and a 54% reduction of laboratory strain *S. aureus*, while the amount of CFU decreased by 96% in the clinical *S. aureus* and 83% in the laboratory *S. aureus* when treated with the AGC. These results indicate that the AGC greatly prevented biofilm formation of *S. aureus* at the attachment phase.

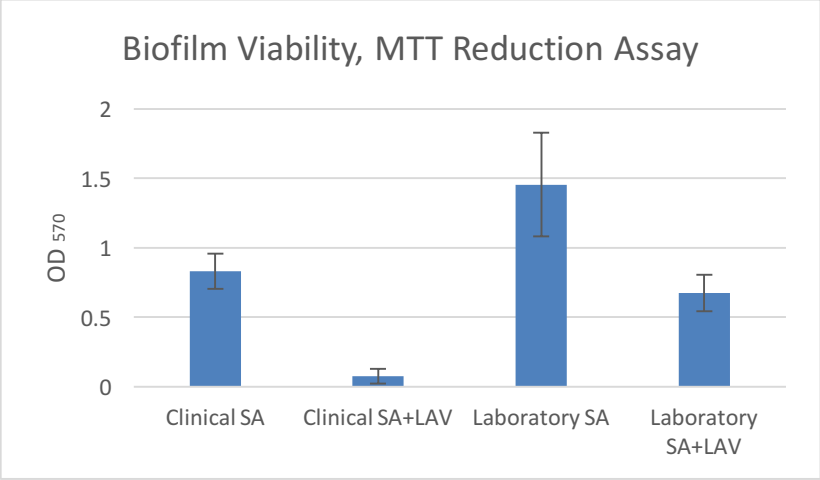

Figure 4: Attachment Phase MTT Reduction

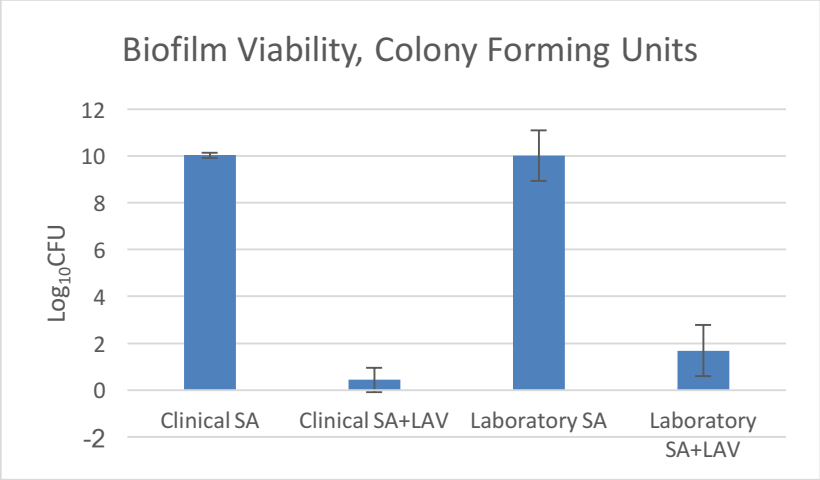

Figure 5: Attachment Phase Colony Forming Units

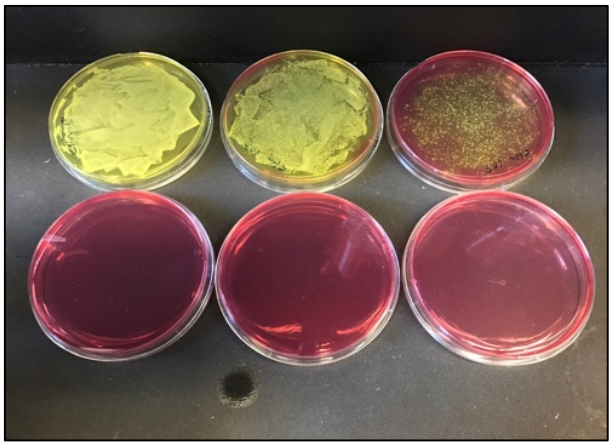

Image 2: Attachment Phase Colony Forming Units (top–Clinical *S. aureus*, bottom-Clinical *S. aureus*+AGC)

The effects of the AGC on the maturation phase of biofilm formation in *S. aureus* were tested by adding the AGC after biofilms established for two hours. The reduction effects of the AGC were not as effective on established biofilms as were preventing biofilm growth by being adding during the attachment phase, but the results still prove to be significant. The optical density assay revealed that the AGC caused a 49% reduction of clinical *S. aureus* biofilm density and 58% reduction in laboratory *S. aureus* biofilm density, as shown in Figure 6. The results of the crystal violet staining (Figure 7) show that the AGC reduced the biofilm mass of clinical *S. aureus* by 45% and laboratory *S. aureus* biofilm mass by 37%.

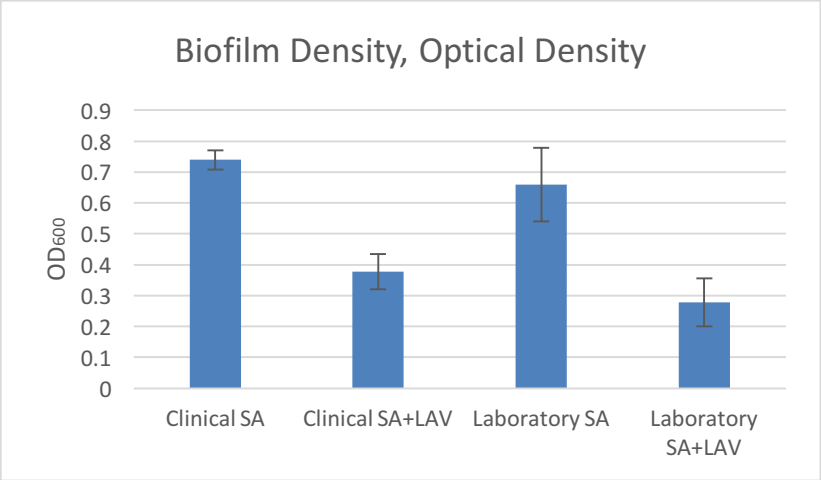

Figure 6: Maturation Phase Optical Density

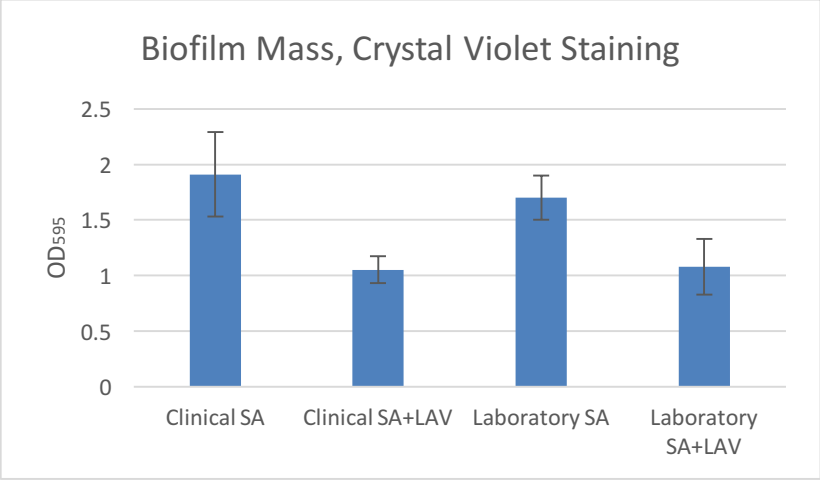

Figure 7: Maturation Phase Crystal Violet Staining

Cell viability was reduced by the AGC significantly when added during the maturation phase of biofilm formation, as shown by the results in Figures 8 and 9. The MTT reduction assay showed that the AGC had an 85% reduction in biofilm viability of clinical *S. aureus* and 86% reduction for the laboratory *S. aureus* when added to already established biofilms. When plated on MSA, the colony forming units revealed the AGC had a 46% reduction in cell viability of clinical *S. aureus* and a 37% reduction in the laboratory *S. aureus*.

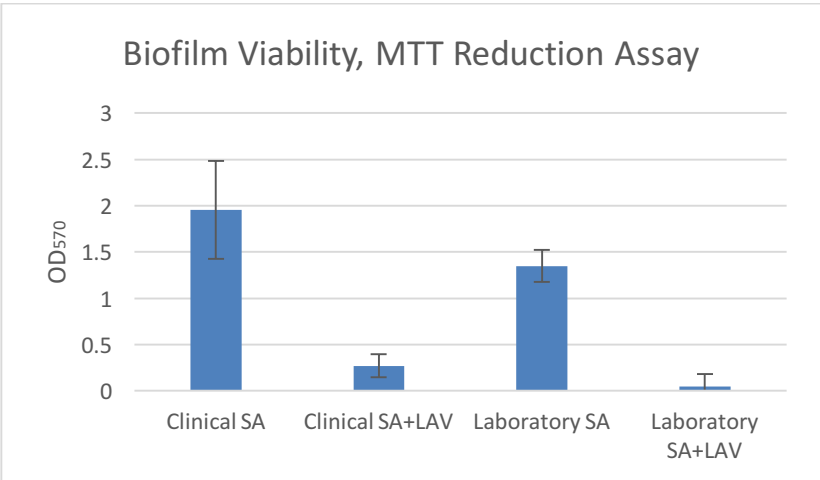

Figure 8: Maturation Phase MTT Reduction

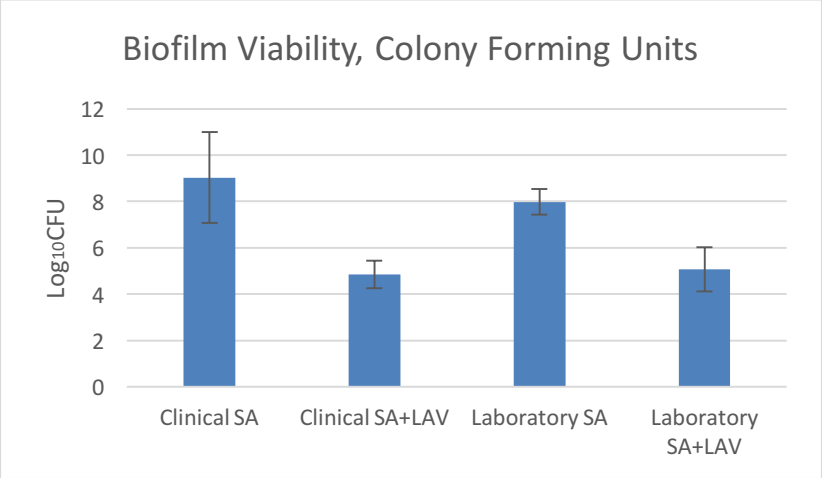

Figure 9: Maturation Phase Colony Forming Units

The effects of the AGC on the dispersion phase of biofilm formation in *S. aureus* were tested by adding the gel after biofilms were established and matured for 24 hours. While there was not a great reduction in the biofilm density of *S. aureus* after being treated with the AGC, the AGC significantly reduced the biofilm viability when added during the dispersion phase. The AGC had a 47% reduction in clinical *S. aureus* biofilm density and 32% reduction in laboratory *S. aureus* biofilm density when added during the dispersion phase of biofilm formation, shown in Figure 10. The crystal violet staining assay revealed a 66% reduction in clinical *S. aureus* biofilm mass and 60% reduction in laboratory *S. aureus*. Image 3 shows a photograph of the developed biofilms and the differences between the AGC treated and untreated, during crystal violet staining.

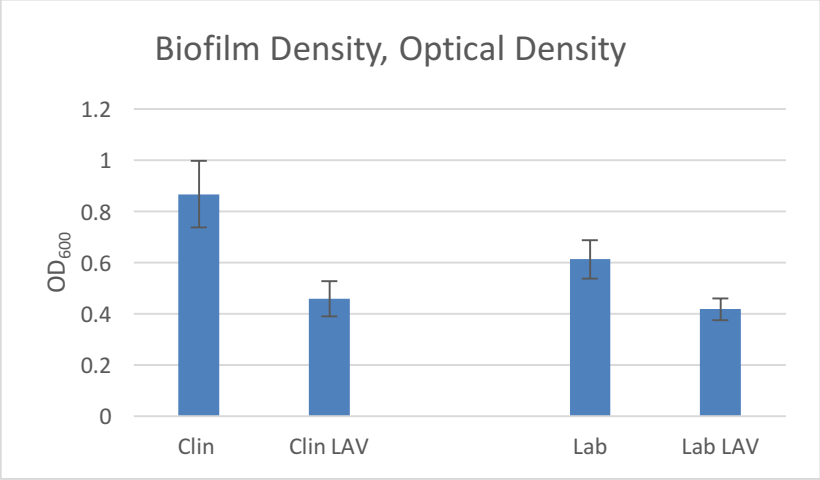

Figure 10: Dispersion Phase Optical Density

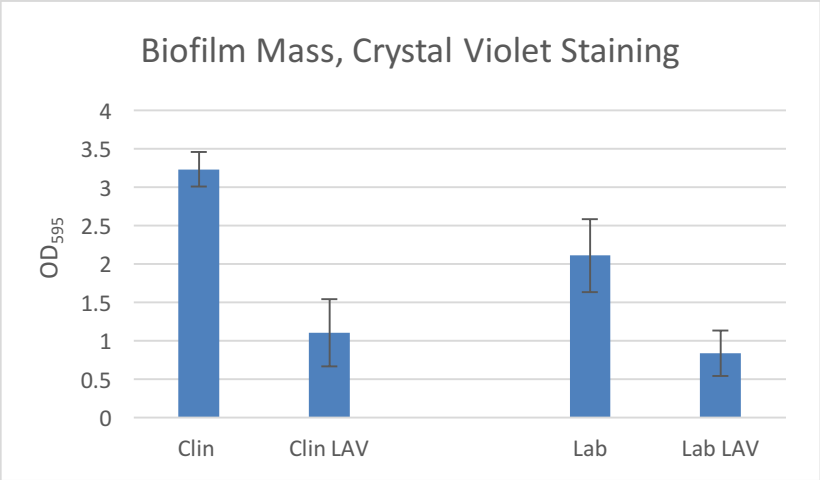

Figure 11: Dispersion Phase Crystal Violet Staining

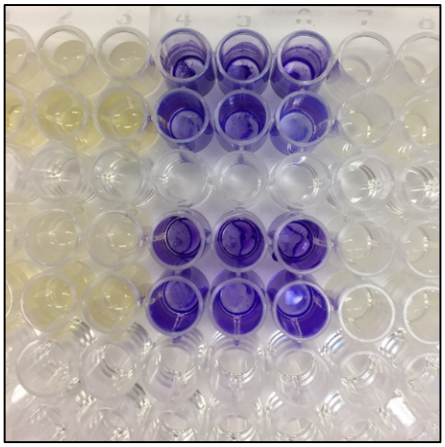

Image 3: Dispersion Phase Crystal Violet Staining Plate (top – *S. aureus* only, bottom - *S. aureus*+AGC)

The MTT reduction assay showed a significant amount of reduction in biofilm viability when the biofilms were treated with AGC during the dispersion phase of development. Figure 12 shows a 91% reduction in clinical *S. aureus* biofilm viability and 86% reduction in laboratory *S. aureus* biofilm viability. Biofilm viability was also reduced during the dispersion phase as seen in the CFU assay (Figure 13), with a 62% reduction in CFU in clinical *S. aureus* and 57% reduction in CFU in laboratory *S. aureus*.

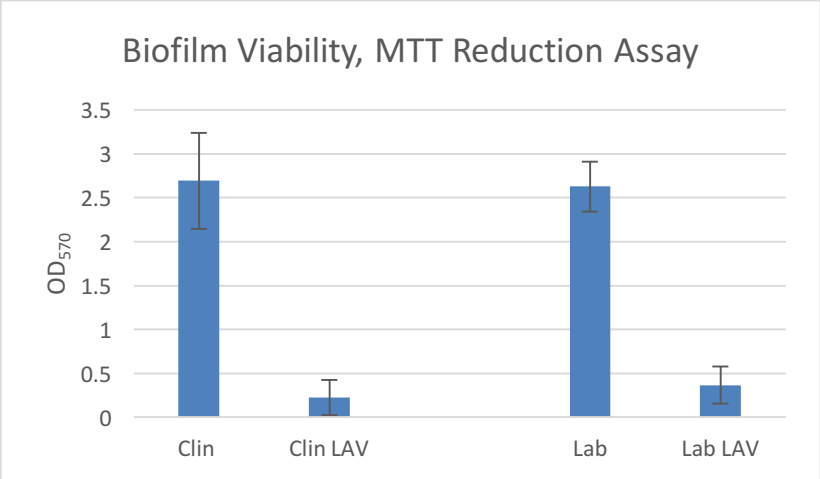

Figure 12: Dispersion Phase MTT Reduction

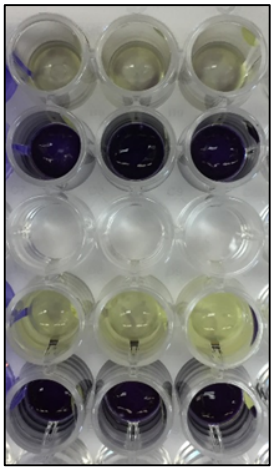

Image 4: Dispersion Phase MTT Reduction (top rows - *S. aureus*+AGC, bottom rows - *S. aureus* only)

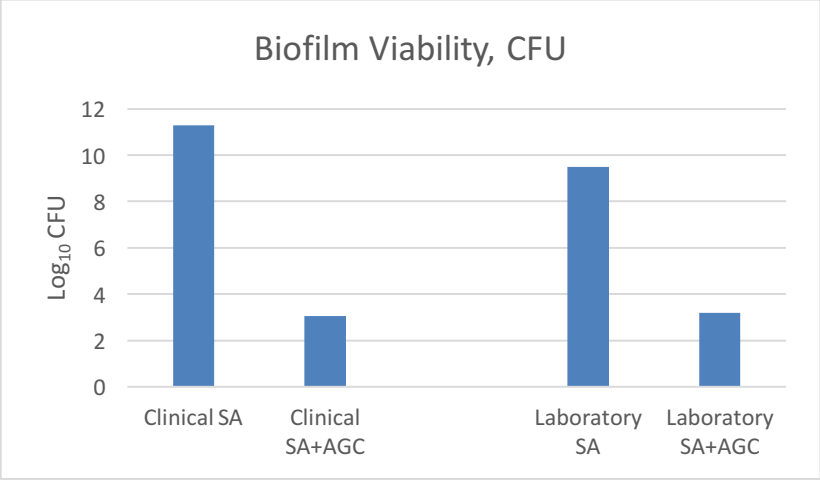

Figure 13: Dispersion Phase Colony Forming Units

IV. DISCUSSION AND CONCLUSION

As antibiotic resistance is an ongoing problem in the medical field, the attempt to develop new drugs and antibiotics that successfully fight infections is crucial. *Staphylococcus aureus* biofilms are one of the leading causes of clinical infections and are highly antibiotic resistant due to their intrinsic properties, making them difficult to treat. A recently developed AGC has had profound effects when used to treat bacterial infections and wounds, causing us to question how the AGC would react with cells of *S. aureus* biofilms. Through a series of experiments testing how the AGC effects *S. aureus* biofilm development at three different stages, we discovered the AGC significantly reduced not only the biofilm's mass and density, but also the viability of the cells present.

The AGC had the greatest effects on the attachment stage of biofilm development when added before the biofilm was able to mature. Therefore, the AGC disrupts the attachment mechanisms preventing the biofilm from growing and entering the maturation phase. The great reduction in cell viability reveals how effectively the AGC kills *S. aureus* cells before biofilm formation occurs. When the AGC was added during the maturation phase of development, it also had reducing effects on the biofilm but was not as effective as in the attachment phase. The effects of the AGC may not be as effective on already established *S. aureus* biofilms due to the complexity of the biofilm matrix preventing the AGC from reaching all the cells. The AGC also had reducing effects on the dispersion phase of development, indicating that the compound inhibited the dispersive properties. The maturation and dispersion assays which allow *S. aureus* to grow before the addition of the AGC indicate the AGC may be bacteriostatic, stopping the bacteria from reproducing, rather than bactericidal and killing the bacteria present.

These results add new information to the discussion about infectious biofilms and antibiotic resistance. *S. aureus* biofilms are one of the strongest, most threatening group of bacterial microorganisms for infection, yet this newly developed AGC has shown profound reducing effects on their biofilm activity. This leads to the need of further research to understand what mechanisms of action the AGC takes against these cells and how it is successful against such a complex biofilm structure. Researching the bacteriostatic mechanisms of the AGC, examining the effectiveness of AGC against polymicrobial biofilms, and testing the AGC in combination with medical devices are potential future areas Further research on the uses and effects of this new gel can lead to the possibility of using antimicrobial compound for a variety of clinical purposes in fighting infection and antibiotic resistance.

REFERENCES

1. **Hall-Stoodley L, Costerton JW, Stoodley P**. 2004. Bacterial biofilms: from the Natural environment to infectious diseases. Nature Reviews Microbiology **2**:95–108.
2. **Dufour D, Leung V, Lévesque CM**. 2010. Bacterial biofilm: structure, function, and antimicrobial resistance. Endodontic Topics **22**:2–16.
3. **Vickery K, Hu H, Jacombs AS, Bradshaw DA, Deva AK**. 2013. A review of bacterial biofilms and their role in device-associated infection. Healthcare infection **18**:61–66.
4. **Otto M**. 2008. Staphylococcal Biofilms. Current Topics in Microbiology and Immunology Bacterial Biofilms 207–228.
5. **Periasamy S, Joo H-S, Duong AC, Bach T-HL, Tan VY, Chatterjee SS, Cheung GYC, Otto M**. 2012. How Staphylococcus aureus biofilms develop their characteristic structure. Proceedings of the National Academy of Sciences **109**:1281–1286.
6. **Cramton SE, Ulrich M, Gotz F, Doring G**. 2001. Anaerobic Conditions Induce Expression of Polysaccharide Intercellular Adhesin in Staphylococcus aureus and Staphylococcus epidermidis. Infection and Immunity **69**:4079–4085.
7. **Yarwood JM, Schlievert PM**. 2003. Quorum sensing in Staphylococcus infections. Journal of Clinical Investigation **112**:1620–1625.
8. **Peschel A, Otto M**. 2013. Phenol-soluble modulins and staphylococcal infection. Nature Reviews Microbiology **11**:814–814.
9. **Kiedrowski MR, Horswill AR**. 2011. New approaches for treating staphylococcal biofilm infections. Annals of the New York Academy of Sciences **1241**:104–121.

10. **Ferro VA, Bradbury F, Cameron P, Shakir E, Rahman SR, Stimson WH**. 2003. In Vitro Susceptibilities of Shigella flexneri and Streptococcus pyogenes to Inner Gel of Aloe barbadensis Miller. Antimicrobial Agents and Chemotherapy **47**:1137–1139.
11. **Aziz MA, Cabral JD, Brooks HJL, Moratti SC, Hanton LR**. 2011. Antimicrobial Properties of a Chitosan Dextran-Based Hydrogel for Surgical Use. Antimicrobial Agents and Chemotherapy **56**:280–287.
12. **Bridier A, Briandet R, Thomas V, Dubois-Brissonnet F**. 2011. Resistance of bacterial biofilms to disinfectants: a review. Biofouling **27**:1017–1032.
13. **De Beer D, Srinivasan R, Stewart PS**. 1994. Direct measurement of chlorine penetration into biofilms during disinfection. Applied and Environmental Microbiology **60**:4339-4344.