ABSTRACT

The Effects of Blue Light on the Biofilm Formation and Disruption of *Staphylococcus aureus*

Kayla L. Fricke

Director: Dr. Tamarah Adair, PhD.

The formation of biofilm complicates the treatment of *Staphylococcus aureus* infections. This experiment measured the effect of 470 nm blue light, a possible antimicrobial agent, on biofilm formation using a biofilm assay. Blue light irradiation for 2 hours on dilute cultures inhibited biofilm formation, and irradiation on saturated cultures after 48 hours of growth resulted in strain-specific changes in the amount of biofilm. There was a positive correlation between cell count and biofilm formation and no correlation between cell count and biofilm disruption. Strain specific variation is observed for the formation of biofilm per cell on blue light treated cultures. It is hypothesized that molecular and genetic variability among strains led to these results. Blue light has the potential to serve as a preventative for infections in a clinical setting, but further investigation into the variation seen in this study is needed to make progress in treating disease.

APPROVED BY DIRECTOR OF HONORS THESIS:

____

Dr. Tamarah Adair, Department of Biology

APPROVED BY THE HONORS PROGRAM:

____

Dr. Andrew Wisely, Director

DATE: ______________________

THE EFFECTS OF BLUE LIGHT ON THE BIOFILM FORMATION AND DISRUPTION OF *STAPHYLOCOCCUS AUREUS*

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

Kayla L. Fricke

Waco, Texas

May 2013

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

Introduction

Review of Literature

Overview

Staphylococcus aureus is an opportunistic bacterium that has posed a serious challenge to both science and medicine. *S. aureus* is a gram-positive coccus bacterium that is associated with asymptomatic colonization of the nasal cavity and skin, and is known for its evolving antibiotic resistance and virulence. Horizontal gene distribution has introduced virulent genes such as the staphylococcal chromosomal cassette *mec* present in methicillin-resistant *S. aureus* strains (Fitzgerald, 2001). Resistant *Staphylococcus aureus* strains serve as the cause of bloodstream and catheter-related infections as well as serious primary skin infections and necrotizing pneumonia. The U.S. Centers for Disease Control and Prevention calculated 89,785 cases of methicillin-resistant *S. aureus* infections with 15,249 cases resulting in death in 2008 (WHO, 2011). In 2009, blood infections caused by methicillin-resistant *S. aureus* were the single most expensive condition treated nationally reaching nearly $15.4 billion (Elixhauser, 2011).

The increased incidence in healthcare-associated infections results from complications in the administration of implants and intubation and dialysis tubing due to the ability of *S. aureus* to form biofilm on medical devices (Rubinstein, 2011). The National Institutes of Health state that 80% of all chronic infections are due to formation of biofilm (Krivit, 2011). Chronic infections of the respiratory tract such as pneumonia and other infections are caused by the formation of biofilm. The only available therapy to treat *S. aureus* pneumonia derived from intubation tube contamination is antibiotics, which are becoming increasingly insufficient due to antimicrobial resistance (Ragle, 2010). In addition, the excess cost incurred by a MRSA case of ventilator-associated pneumonia is approximately $8000 per case (Shorr, 2006). Therefore, the discovery of alternative methods to eradicate pathogenic *Staphylococcus aureus* is crucial for the prevention of increased morbidity and health care costs.

Background on Staphylococcus aureus

Staphylococcus aureus is a non-motile, facultative anaerobic, Gram-positive coccus that can be identified by its spherical clusters, production of coagulase, distinctive golden colony color, and fermentation on Mannitol Salt Agar (MSA) plates. *S. aureus* is a commensal bacteria that is usually found on the skin and mucosal surfaces of humans and colonizes the anterior nares (Foster, 2004). As of 2002, approximately 30% of the general population (89.4 million persons) are consistently colonized carriers of *S. aureus*, and 0.8% (2.3 million persons) are colonized carriers of MRSA (Kuehnert, 2004). These statistics demonstrate the widespread dissemination of *S. aureus* and specifically methicillin-resistant *S. aureus* (MRSA) strains containing the penicillin-cleaving enzyme penicillinase. Methicillin is a beta-lactamase resistant antibiotic developed to combat *S. aureus* strains expressing bifunctional penicillin-binding protein 2 (PBP2). The transpeptidase domain of PBP2 is required for cell growth. PBPs normally bind penicillins or beta-lactams, preventing their function, which is to disrupt the transpeptidation step of cell wall synthesis. The binding affinity of PBP2 produced by methicillin-resistant organisms is insufficient for disrupting this function, allowing cell growth. The gene that encodes for PBP2 *mecA* is found on the staphylococcal cassette chromosome SSC*mec* (Keski, 2005).

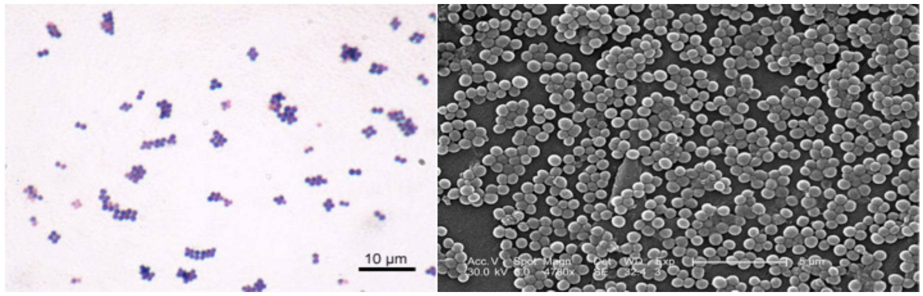


Figure 1. (left) Gram stain of *S. aureus* cells which typically occur in clusters. The cell wall readily absorbs the crystal violet stain. (Tambe, 2005). Microscopic image of *Staphylococcus aureus*. Gram staining. (right) SEM of methicillin-resistant *Staphylococcus aureus* showing grape-like clusters. (Carr, 2005).

Background on S. aureus Biofilm

Staphylococcus aureus is also capable of altering its physical characteristics as a defensive mechanism through the formation of biofilm (Beenken, 2004). The peptidoglycan cell wall is covered with a polysaccharide capsule that secretes a water-soluble film or biofilm that attaches bacteria to tissues and foreign bodies for increased survival. Biofilm consists of an extracellular matrix of polymeric substances that is formed by the initial adhesion of planktonic microorganisms to a surface (Karaolis, 2005). Microbial surface components recognize adhesive matrix molecules and polysaccharide adhesins and increase the biofilm formation. Biofilm formation of *S. aureus* is unique to other invasive bacterial types such as *Escherichia coli* and *Pseudomonas aeruginosa* due to the negative correlation between quorum sensing and biofilm formation (Lopez, 2010). In this case, the *agr* system turns on genes for protease production that at a high concentration cause biofilm dispersal. Adhesion of cells must occur for the initiation of the extracellular matrix of polysaccharide biopolymer formation, but this adhesion is only favored in the absence of proteases when quorum sensing is inhibited.

The density and impenetrability of the extracellular matrix allows for increased bacterial resistance to antibiotics. Also, biofilm-associated bacteria in a low-oxygen and high nutrient environment have a significantly reduced growth rate, which limits the efficacy of antibiotics targeting cell wall biosynthesis (Beenken, 2004). This environment also changes the access of antibiotics to molecular targets. Therefore, biofilm formation greatly inhibits antimicrobial therapy and usually requires surgical means to remove infected tissues or medical devices.

Background on Genetics of S. aureus Biofilm

Beenken (2004) identified 580 genes alternatively expressed in biofilms in comparison with the planktonic cell cultures of *S. aureus*. Although biofilm produces a new environment optimal for genetic exchange, the initial formation of biofilm relies on the requisite expression of specific genes. The polysaccharide intercellular adhesin production necessary for biofilm formation is dependent on 3 main genes including the *ica* operon, the staphylococcal accessory gene regulator *agr*, and the *sarA* regulator.

The expression of the *ica* (intracellular adhesion) operon is subject to the environment and is induced by anaerobic metabolism and a stress response transcription factor. Variation in the regulation of the *ica* operon provides for the activation of specific biofilm forming genes that induce changes to the cell wall. The *ica* operon is responsible for the genetic formation of N-acetylglucosamine, which composes the polysaccharide intercellular adhesin necessary for biofilm formation (Diemond-Hernandez, 2010). The expression of the *icaA* and *icaD* genes significantly increase polysaccharide activity in the formation of a biofilm. Although it has been found that in the absence of the *ica* operon biofilm can still form, it serves as a key genetic component that is common among *S. aureus* strains. Zmantar (2010) also found that the presence of glucose induced maximal *ica* operon function, which correlates to the finding that biofilm formation is increased when in the presence of glucose.

The *agr* signal transduction system is both a cell-density and growth-phase dependent regulator of gene expression for secreted proteins. The accessory gene regulator, *agr*, is responsible for toxin production and enzyme secretion that contributes to increased *S. aureus* virulence. RNAII and RNAIII are two transcriptional units on the *agr* locus. RNA II encodes for a cell-density sensing signal transduction system that activates transcription of four polypeptides. Although the *agr* system is conserved for all staphylococcal species, the domain of *Agr*C, one of the four polypeptides, serves as the sensor of the system and establishes specificity for four distinct groups, of which each have their own autoinducer peptide, AIP (Arvidson, 2004). The AIP encoded by the *Agr*D gene is detected and activated by *Agr*C to positively regulate the gene transcription of the proteases necessary for biofilm formation (Lopez, 2010). RNA III, a non-coding RNA, is almost completely conserved among staphylococcal species and functions in gene regulation.

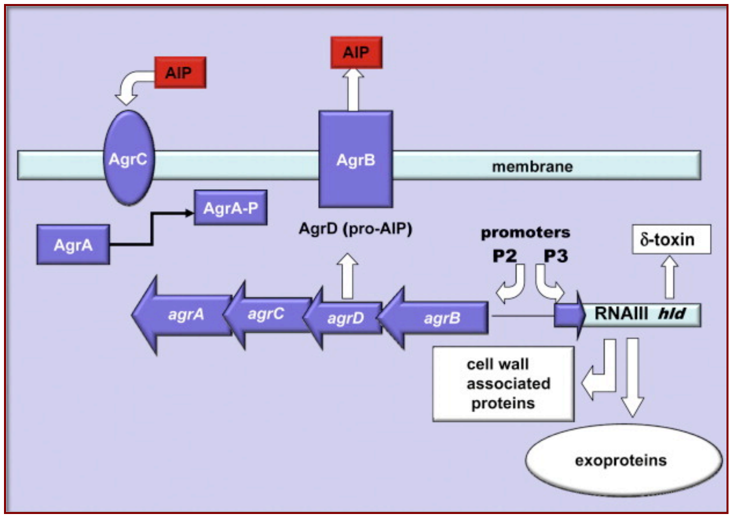


Figure 2. The *agr* signal transduction system is composed of 4 genes, *agr*A, B, C, and D. *Agr*D encodes the autoinducer peptide responsible for detecting cell density that send signals to regulate transcription for proteases and other cell wall associated proteins and exotoxins (Arciola, 2012).

The *sarA* staphylococcal transcription factor acts on the RNAIII locus expression and leads to reduced biofilm formation with the repression of transcription of extracellular proteases and with the production of gamma-toxin. The *sarA* also regulates genes critical for transcription of the *ica* operon and for the production of polysaccharide intercellular adhesin required for biofilm formation. Beenken (2004) concluded that a mutation of the *sarA* results in a reduced ability to form biofilm regardless of the *ica* operon function.

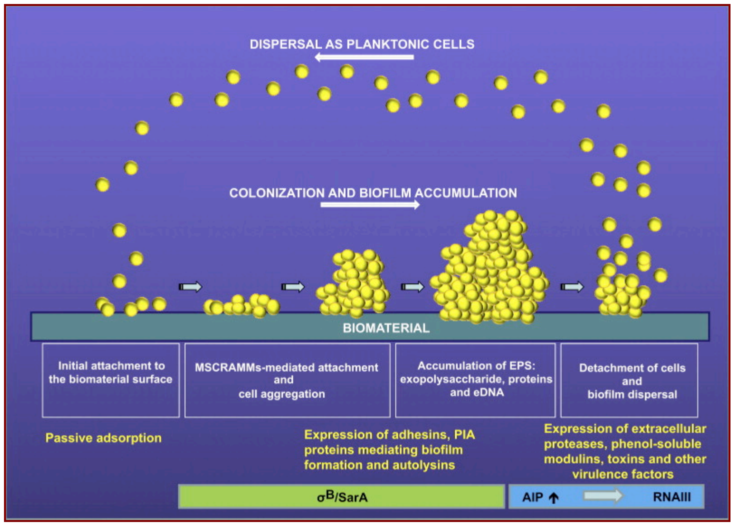


Figure 3. Biofilm formation as regulated by the *SarA* gene and *agr* loci. Initial attachment leads to cell aggregation, biofilm accumulation and initial detachment and dispersal (Arciola, 2012).

Background on Cellular Components of S. aureus Biofilm

It has also been found that within the biofilm matrix are persister cells, which are genetically identical to the surrounding cells but have the unique ability to express toxins that block the target of antibiotics and inhibits the disruption of the biofilm. Persister cells are present in both planktonic culture and in biofilm, and can survive exposure to a high concentration of antibiotics (Harrison, 2005). These cells are not necessarily antibiotic resistant because they do not grow in the presence of antibiotics, but they are extremely tolerant of and do not die in the presence of antibiotics and other antimicrobial treatments. It has been found that these persister cells make up a greater portion of biofilm and growth-suspended cells in comparison to bacterial cells in an exponential growth phase (Harrison, 2005). Another cellular component of biofilm and bacterial cells are intracellular porphyrin molecules (Gold, 2009). These are endogenous photosensitizers within bacteria that under illumination or excitation cause an energy transfer that may produce highly toxic singlet oxygen atoms.

Background on Reactive Oxygen Species

Reactive oxygen species can be formed through exposure to an ionizing radiation, such as blue light, on biological molecules. These species can include such molecules as hydrogen peroxide and hydroxyl radicals, which strongly interact with the biofilm matrix (Arce, 2011). The unstable outer electron shell of an atom emits high-energy radicals when under oxidative stress that can lead to damage of cell structure and function by affecting intracellular signaling. Environmental agents, such as blue light, can lead to the overproduction of oxygen radicals that can attack the polyunsaturated fatty acids found in membranes and disrupt membrane-bound proteins. These radicals can also affect DNA stability. Oxidative stress induces the activation of DNA repair enzymes in cells that are in the stationary phase of a biofilm. Oxidative stress also induces the *oxyR* gene that encodes for enzymes and proteins to protect the DNA, and this response leads to an increased resistance of the cell to oxidative agents (Cabiscol, 2000). It has also been shown that *Staphylococcus* strains had a greater reactive oxygen species generation in comparison to other species at a wavelength of 400 nm. Stimulation of superoxide dismutase, an antioxidant enzyme, also increases under irradiation with light of a 400 nm wavelength for *Staphylococcus* strains (Santos, 2013). Therefore, the release of oxygen radicals by blue light irradiation may disrupt a biofilm as well as lead to genetic modulation that causes cellular resistance against irradiation.

Oxidative stress in *Staphylococcus aureus* was observed to have an effect on cellular stress and on the formation of biofilm (Arce, 2011). Cellular stress within a bacterial cell matrix was tested under different conditions to determine the effects of reactive oxygen species production on biofilm. This study used an accepted biofilm assay and crystal violet stain to detect biofilm formation by OD at 595 nm on a concentration of 1×10^9 CFU/mL in TSB. The production of extracellular reactive oxygen species was detected by the amount of reduction of nitro-blue tetrazolium. Reactive oxygen species production resulted in the decreased amount of biofilm (Arce, 2011).

Background on Phototherapy

The health-care industry and scientists alike have proposed the use of phototherapy as a promising alternative to antibiotics. Photodynamic therapy is a method that uses a nontoxic dye or "photosensitizer" that is activated by a localized dosage of visible light to form singlet oxygen and free radicals that are cytotoxic to target cells (Dougherty, 1989). Metal chelators such as tetrasodium EDTA and the cationic phenothizaine dye Toluidine blue O were used to increase the sensitivity of bacteria in biofilms to antimicrobial agents (Sharma, 2008). The effects of these drugs were studied on *S. epidermidis* and methicillin-resistant *S. aureus* strains by diluting them 1:200 and 1:50 respectively in TSB with 0.25% glucose. The diluted bacterial culture was aliquoted into a 96-well flat-bottomed sterile polystyrene microplate and incubated for 16 hours at 37 °C. The microplates were coated with 20% human plasma in carbonate buffer to increase *S. aureus* biofilm formation. Formed biofilms were washed twice with phosphate-buffered saline for the removal of planktonic cells. The biofilm was then fixed to the plate with 95% ethanol and stained with 0.1 % crystal violet. After washing the plate and allowing the wells to air dry, the crystal violet was resolubilized with 10% glacial acetic acid and the absorbance of the dye was determined at 590 nm in a microplate reader. The effects of Toluidine Blue O were tested by adding 100 μL to each well and exposing the plate to 640 nm wavelength light at an intensity of 42 mW/cm^2. Each sample was plated on TSB agar plates and the cell survival rate was determined by the amount of CFU of bacteria treated with TBO and light in comparison to the samples treated with TBO alone. Sharma et al concluded that photodynamic therapy induced a destabilization of the biofilm and made the *S. aureus* cells more accessible to antibiotic penetration. Toluidine Blue O coupled with light serves as a potential photosensitizer of staphylococcal biofilms on device-related infections (Sharma, 2008).

Further studies have found that *Propionibacterium acnes* bacteria under the irradiation of blue light results in the photosensitization of intracellular porphyrins and leads to cell death (Gold, 2009). In a clinical study, patients with mild to moderate acne were asked to use a 414 nm blue light-emitting diode for six minutes a day for eight weeks. M. H. Gold et al demonstrated that a clinical blue light treatment killed antibiotic resistant, gram-positive *Propionibacterium acnes* bacteria and concluded that maximum eradication occurred between 9.2 and 8.4 minutes of irradiation at an intensity of 55 J/cm^2. Enwemeka et al displayed that the photo-irradiation treatment of methicillin-resistant *S. aureus* and *Propionibacterium acnes* strains using 405 nm visible blue light irradiation on 35 mm plates of Tryptic Soy Agar was shown to significantly reduce the number and aggregate area of colonies formed by each strain. The light source included a 5.0 cm^2 area covered with 36 SLDs placed at a distance of 1-2 mm above each open plate for direct irradiation. These studies suggest that endogenous porphyrin molecules may be the possible mechanism for this bactericidal effect.

The success of phototherapy is not directly associated with a bacterium's antibiotic resistance and genetic alterations, but it has been shown that the bacterial growth mode greatly influences bacterial susceptibility to light-activated disinfection (Gad, 2004). Bacteria in planktonic form are more sensitive to light-activated disinfection than its biofilm form made by coaggregation and coadhesion between planktonic cells. This resistance to antimicrobial agents is amplified by 1000 in biofilm in comparison to the resistance capabilities of planktonic cells (Upadya, 2000). Photosensitization decreases the CFU/mL in planktonic culture and was unrelated to the antibiotic resistance of each strain. Studies exhibited a plateau effect in the killing of bacterial cells at an increased exposure, and a higher intensity was required to produce the same killing effect in biofilm-based cultures (Taraszkiewicz, 2013).

Background on Blue Light Studies

Studies have demonstrated that exposure to visible light as a potential method of decontamination inhibits *Staphylococcus aureus* growth (Lipovsky, 2008). It was concluded that blue light of a 405 nm wavelength resulted in *S. aureus* inactivation (Maclean, 2009). Bactericidal effects were exhibited on a concentration of 2.0 x 10^5 CFU/mL suspended in phosphate-buffered saline exposed to 3.27 mW/cm for 2 hours. The photo-excitation of intracellular porphyrin molecules was the proposed mechanism for the production of highly toxic singlet oxygen atoms causing eradication. A 405 nm wavelength does not as bactericidal as ultraviolet wavelengths, but is favored due to its inability to cause lethal DNA breakage and dimerization (Lipovsky, 2010). It has also been demonstrated that *Staphylococcus* strains are more susceptible at a wavelength of 320-400 nm in comparison to shorter ultraviolet wavelengths of 300 nm or shorter (Santos, 2013).

Blue light is also favored due to its greater percent inhibition in comparison to the wavelengths of red, green, and yellow light. Previous experimentation has shown that red light exhibited a 0.0% inhibition, yellow light exhibited a 8.5% inhibition and green light exhibited a 65.0% inhibition in comparison to blue light (or white light) with a 89.0% inhibition of *S. aureus* at a concentration of 1 x 10^3 CFU/mL (Meredith Rosenthal, personal communication). Therefore, blue light is the most efficient and yet safe wavelength used to inhibit *S. aureus* growth.

Purpose of Study

The purpose of this study was to address the growing public health concerns associated with *S. aureus*. Phototherapy is one area of interest that explores alternative treatments for the inhibition of the formation and the disruption of *S. aureus* biofilms separate from the development of more potent drug-based therapies (Nakonechny, 2005). In addition, the effects of blue light on bacteria have been studied for the potential sterilization of implants and intubation and dialysis tubing prior to medical procedures to prevent secondary infections (Biel, 2010). Previous studies involving phototherapy with blue light have led to the overall hypothesis that blue light irradiation may serve as an inhibitor of *S. aureus* biofilm. This study will demonstrate the effects of blue light on *S. aureus* in both a low and high cell concentration. Results from this experiment provide support for the potential clinical use of blue light as an inhibitor and disruptor of biofilm of *Staphylococcus aureus*. Overall, the main objective of this study is to determine if blue light affects the amount of formation of a biofilm at a low or high concentration of planktonic culture.

CHAPTER TWO

Methods and Materials

Materials

Collection of Strains and Light Box

Staphylococcus aureus strains used in this experiment were obtained from a library collected between 2007-2011 from healthy undergraduate students at Baylor University, Waco, Texas. The identity of each strain was confirmed by fermentation on Mannitol Salt Agar plates, Gram stain, catalase test and coagulase production. These strains were also tested on Mueller Hinton with an antibiogram test utilizing the Kirby-Bauer disc diffusion procedure for the detection of antibiotic resistance. Cryopreservation of each strain was utilized to store the bacterial strains until inoculation in 5 ml BHI for overnight growth at 35°C.

Twenty-eight of these collected strains and the reference strain ATCC 25923 were utilized in the following experiments and exposed to blue light with a modified light box originally built by a former student (Balpreet Pamma). The light box was constructed using a prototyping circuit board (Mouser Electronics #574-8016), 430 ohm resistors (Mouser 281-430RC), standard toggle switches (Mouser 10TC405), and Kingsbright Standard LED Blue Water Clear Lights (Mouser 604WP7113PBCA). The design includes the use of a 24-well microtiter plate with the lid cut out to fit one 470 nm standard LED bulb per well, positioned 5 mm above 500 μL of broth. The circuit was attached to a power source set at 30 milliamps per well resulting in a forward power of 120 mW per well. The luminance of each bulb ranged from 3.0-5.0 Klux measured using the BK Precision Light Meter with an average luminous flux of 83 lumens (Mouser 615-615). The light box apparatus was placed inside the 35°C incubator for 2 hours.

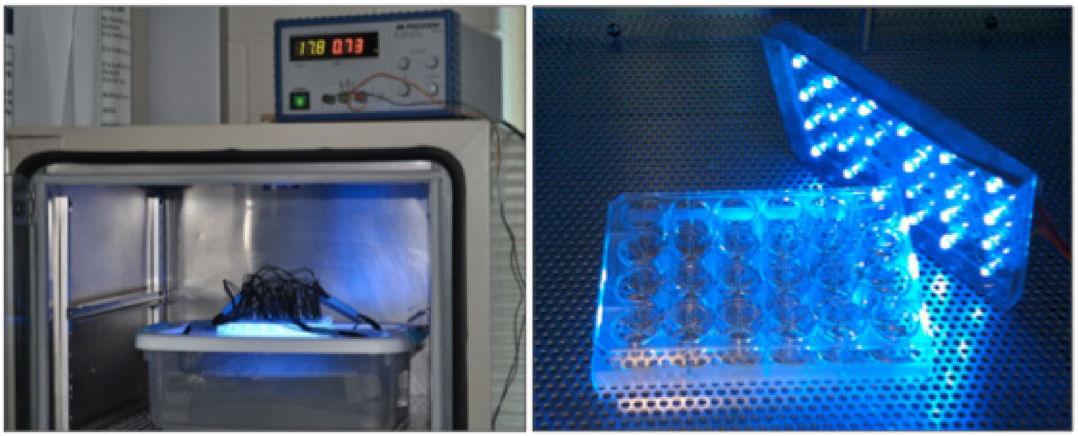


Figure 4. Image of blue light box in incubator at 35°C with external power source. Image of each 470 nm standard LED bulb per well.

Experimental Methods

Experiment 1

Each strain was grown overnight in 5 mL of BHI with 2% glucose and 100 μL from this overnight culture was transferred to a fresh 5 mL of BHI for a two-hour growth period. Next, each strain was serially diluted 10^{-4} in BHI supplemented with 2% glucose. Then, 500 μL aliquots of each strain were transferred to a 24-well microtiter plate in quadruplicate and each trial was replicated. A broth control of 500 μL aliquots of only BHI in quadruplicate was also present for each plate. To compare the same strains under the exposure to blue light versus no exposure to blue light, two microtiter plates were utilized for each experiment. The cultures were plated on Tryptic Soy Agar plates for the colony forming unit count at time zero. Both microtiter plates were incubated at 35°C while only one microtiter plate was exposed to 470 nm blue light at a luminous flux of 83 lumens per light for each well for 2 hours, and a second control plate was covered to avoid any exposure to blue light. After the irradiation time was complete, CFU/mL counts were determined by plating 50 μL of the diluted culture after exposure to blue light to display the immediate effects of irradiation on planktonic cells. The microtiter plates were then incubated for 48 hours at 35°C after which optical density was measured at 600 nm before completion of the biofilm assay.

Experiment 2

Each strain was grown overnight in 5 mL of BHI with 2% glucose and 100 μL from this overnight culture was transferred to a fresh 5 mL of BHI for a two-hour growth period. Next, each strain was serially diluted 10^{-4} in BHI supplemented with 2% glucose. Then, 500 μL aliquots of each strain were transferred to a 24-well microtiter plate in quadruplicate and each trial was replicated. A broth control of 500 μL aliquots of only BHI in quadruplicate was also present for each plate. To compare the same strains under the exposure to blue light versus no exposure to blue light, two microtiter plates were utilized for each experiment. The cultures were plated on Tryptic Soy Agar plates for the colony forming unit count at time zero. Both microtiter plates were incubated at 35°C for 48 hours to allow for the formation of a biofilm. After this 48-hour period, only one microtiter plate was exposed to 470 nm blue light at a luminous flux of 83 lumens for each well for 2 hours, and a second control plate was covered to avoid any exposure to blue light. After the blue light irradiation time was complete, optical density of 600 nm was measured to display the effects of irradiation on cells associated with a biofilm before completion of a biofilm assay.

Biofilm Assay

In order to determine the direct effect of the blue light irradiation on either the planktonic culture or the biofilm-associated bacteria, the same biofilm assay was completed for every trial. The liquid contents of the two twenty-four well microtiter plates were removed and the plates were rinsed of any remaining planktonic culture with deionized water. The biofilm-associated bacteria were fixed to the plate by adding 3 drops of 99% ethanol into each well for two minutes. The microtiter plates were then rinsed of any remaining ethanol and emptied by tapping the plates of excess water. Next, the wells were stained for 10 minutes with 100 μL of 0.33% crystal violet, which adheres to the peptidoglycan cell wall of gram-positive *Staphylococcus aureus* bacterial cells. Then, the microtiter plate was washed by submerging in water twice and left to air dry. Once the plates were dry, the cells were resolubilized with 200 μL of 33% glacial acetic acid and shaken for 20 minutes. The microtiter plate wells stained with crystal violet were read at an optical density of 540 nm using the Biotek plate reader.

The use of a 33% glacial acetic acid solution (that resolubilized crystal violet dye bound to the adherent cells on the surface of the microtiter plate wells) allowed for the specific detection of biofilm with an O.D. at 540 nm, which calculates the concentration of crystal violet present in the well (Stepanovic, 2000). These methods correlate with the methods of previous studies in order to accurately quantify *S. aureus* biofilm formation (Merritt, 2005).

Statistical Analysis

Raw data was analyzed by a two-sample t-test using SAS Statistical Software. Data was analyzed for the effects of blue light on *S. aureus* strains in planktonic culture in comparison to a no light control group as well as the effects of blue light on *S. aureus* strains in biofilm in comparison to a no light group. The data including the optical density at 600 nm was analyzed for significant differences in cell count that may contribute to the blue light effects among strains. Data was also analyzed for strain specific effects of blue light on both planktonic culture and biofilm by using the Wilcoxon/Kruskal-Wallis Tests by comparing the no light and blue light results for each strain.

CHAPTER THREE

Results

Previous Experiment

Previous data collected in 2010 established the effect of blue light irradiation on planktonic culture (Balpreet Pamma, unpublished data). This data on blue light irradiation for 2 hours on a concentration of 10^3 CFU/mL resulted in almost 100% inhibition of cell growth. The average number of colonies at time 0 for both the MRSA and MSSA strains was 162 with a range of 73 to 332 colonies. The average number of colonies after 2 hours of growth without blue light irradiation for all strains was 480 with a range of 217 to 827 colonies. In 12 out of 13 strains irradiated with blue light for 2 hours there was no colony growth 24 hours after plating, and the colony count of the only colony-forming strain was 15 in comparison to 566 colony-forming units of the same strain without blue light irradiation. These results served as a foundation that blue light irradiation at 2 hours inhibits planktonic growth. Figure 1 is a summary of T.A. Adair and Balpreet Pamma's unpublished results.

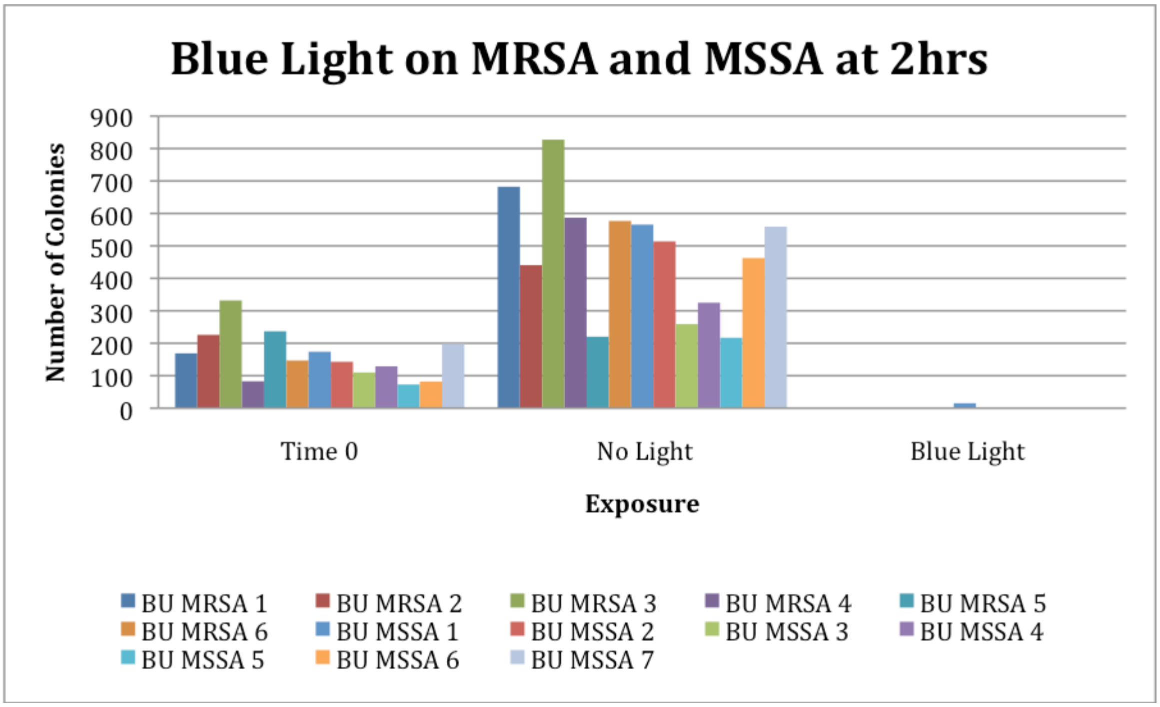


Figure 5. Thirteen *Staphylococcus aureus* strains were either exposed to blue light for 2 hours or incubated without light. The results display the direct effect of blue light on planktonic culture with a decrease in the number of colonies.

Experiment 1

Inhibitory Effect of Blue Light on a Low Concentration of Planktonic Culture

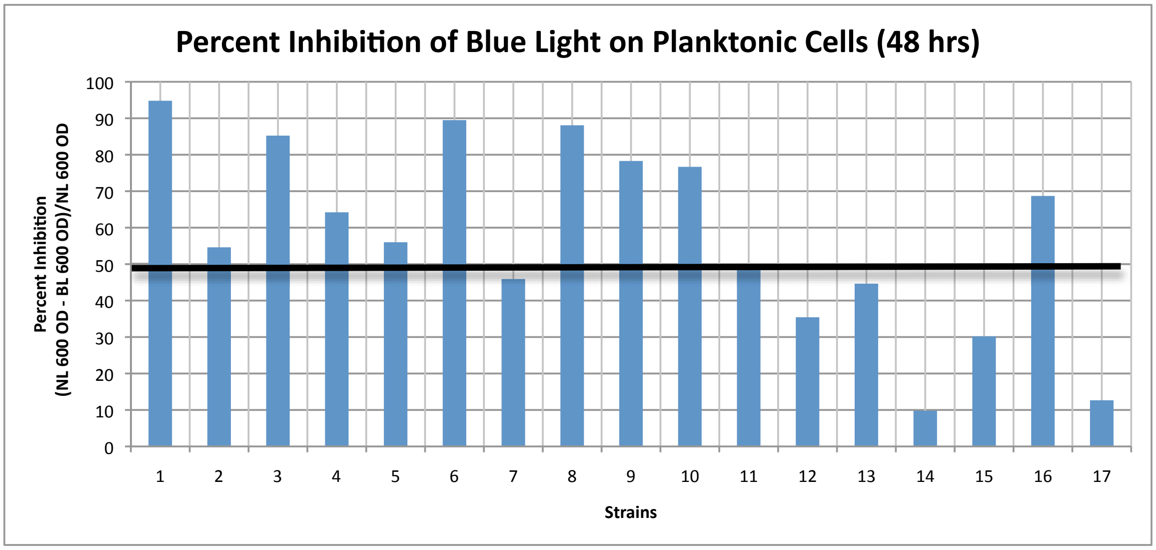
In this experiment, we are continuing to measure the effect of blue light on planktonic cell growth with the addition of measuring the effect on biofilm formation after 2 hours of irradiation given a 48-hour recovery period. The recovery of cells in planktonic culture after a 48-hour period was measured with an optical density of 600 nm in experiment 1. The percent inhibition of blue light on planktonic growth showed that 11 of the 17 strains with a measurement of 0 colony forming units per milliliter (CFU/mL) at 2 hours of growth resulted in a 50% inhibition after a 48-hour growth period. The remaining 6 strains displayed an average 30% inhibition after a 48-hour growth period and had an average measurement of 22 CFU/mL at 2 hours of growth (Table 1).

Strain	BL 2 hr CFU/mL	Percent Inhibition
1	0	94.8
2	0	54.6
3	0	85.2
4	80	64.2
5	7	56.0
6	0	89.5
7	30	45.9
8	10	88.1
9	20	78.3
10	10	76.7
11	0	50.0
12	0	35.4
13	0	44.6
14	0	9.8
15	0	30.2
16	0	68.7
17	0	12.7

Table 1. Strain specific percent inhibition of biofilm formation at 48 hours after blue light irradiation.

Therefore, the effect of blue light on a low concentration of planktonic cells demonstrated an immediate reduction of cells at 2 hours and had a continual effect on the recovery of a culture to form biofilm given a 48-hour period. The cell count was measured at an OD of 600 nm at 48 hours and was analyzed for any correlation between the effects of blue light irradiation on the number of recovered cells in planktonic culture per strain (Figure 6a). Each analysis was based on data collected in quadruplicate for each strain and in duplicate for each trial.

Further analysis was used to correlate this effect of blue light inhibition of the recovered cell count to the formation of biofilm for each strain (Figure 6b). The results showed that 12 of the 17 strains exhibited an inhibitory effect of blue light irradiation on biofilm formation. The other 5 strains either displayed no apparent effect (strain 7) or displayed an enhanced effect of blue light irradiation on the formation of biofilm (strains 2, 4, 5 and 17). These strains with a higher amount of biofilm formation for the culture irradiated with blue light compared to the culture not irradiated with blue light were among the strains that showed a lower inhibitory effect of blue light on the planktonic cells of these strains. Therefore, it can be concluded that these 5 strains displayed a resistance to blue light irradiation and demonstrated an increased recovery of cells to form a greater amount of biofilm given a 48 hour period. It was also found that the strains with a 70% inhibition or higher for planktonic cells were the only strains that exhibited a 50% inhibition or higher for biofilm formation (strains 1, 3, 6, 8, 9, 10 and 16). It can be concluded that *S. aureus* strains displayed a variation to blue light irradiation.



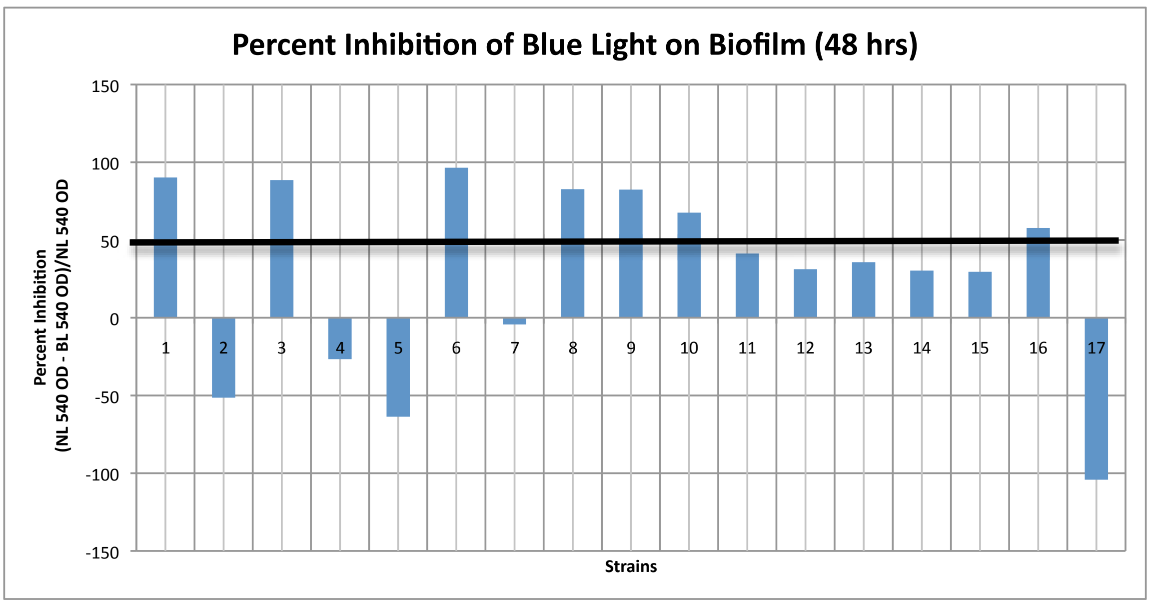


Figure 6a. Percent inhibition of planktonic culture for each strain 48 hours after a 2-hour blue light irradiation period. **Figure 6b.** Percent inhibition of biofilm formation for each strain 48 hours after a 2-hour blue light irradiation period.

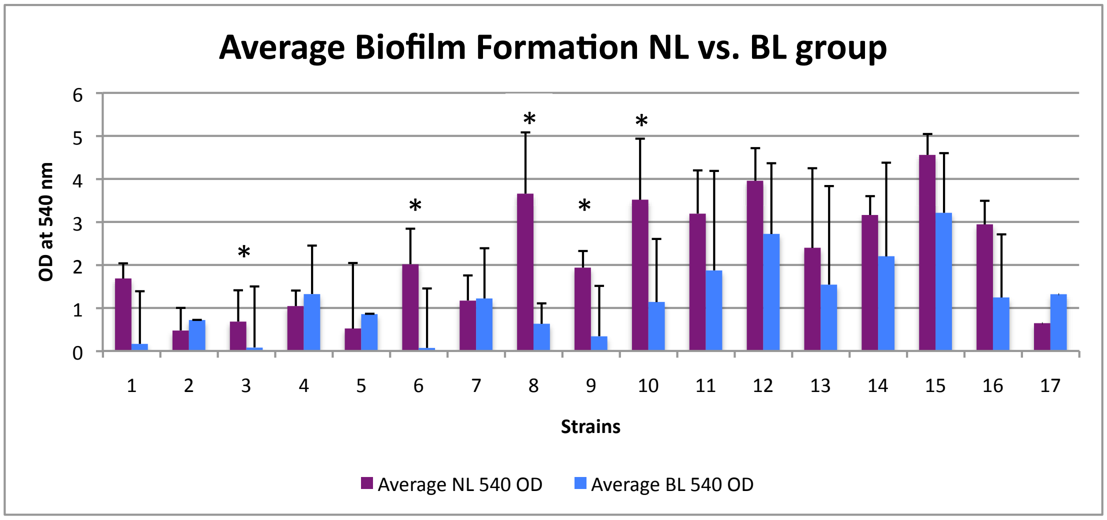
Effect of Blue Light on Biofilm Formation at a Low Concentration of Planktonic Culture

The results of the two-sample t-test analysis between the OD at 540 nm of the blue light (0.60) and no light groups (1.90) indicated that blue light significantly inhibited the formation of biofilm in comparison to the strains not exposed to blue light (t

= 4.77 and $p < 0.0002$) (Figure 7a). The graphs display the average of 2 trials each performed in quadruplicate on *S. aureus* cultures with an initial concentration of 2.5 x 10^3 CFU/ml. This significant difference was directly related to the number of cells remaining in the culture 48 hours after blue light irradiation as shown in Figure 7b.

The results of experiment 1 demonstrated that the strains not exposed to blue light had a 3-4 fold increase in biofilm formation and an average 120% difference in comparison to the strains that were irradiated with blue light. Also, regression analysis (REG Procedure, SAS) verified the data analysis and calculated a p-value = 0.0007, showing the significance of the effect of blue light versus no light on CFU/mL.

One-way data analysis using the Wilcoxon/Kruskal-Wallis Tests also test the statistical significance of blue light irradiation for each individual strain, confirming the variation and significant difference in 5 strains (strains 3, 6, 8, 9 and 10). The results showed that the biofilm formation of 5 of the 17 strains was significantly reduced by blue light irradiation in comparison to the no light group. One strain (strain 1) served as a standard for each trial and was excluded due to the difference in the number of replicates. Two of the 17 strains (strains 16 and 17) were excluded from the statistical analysis due to a lower number of replicates available for analysis.



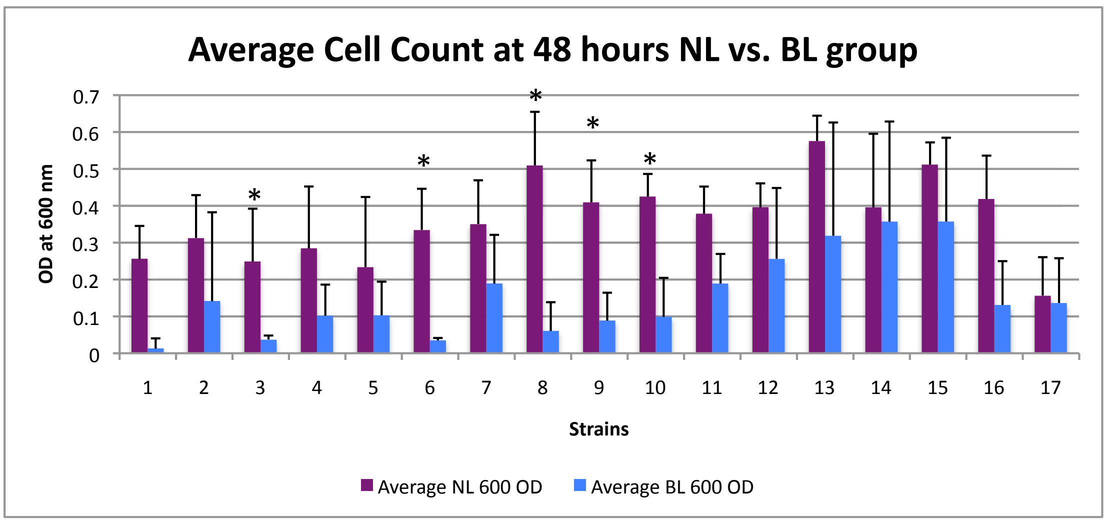


Figure 7a. This figure shows the average biofilm formation (OD of 540 nm) for each strain exposed (BL) and not exposed (NL) to blue light. **Figure 7b.** This figure shows the average cell count measured at an OD of 600 nm at 48 hours after blue light exposure.

Experiment 1: NL vs. BL	
Strain	P value
2	0.1033
3	**0.0009**
4	0.6650
5	0.2601
6	**0.0009**
7	0.3720
8	**0.0028**
9	**0.0074**
10	**0.0009**
11	0.0831
12	0.1559
13	0.3184
14	0.1036
15	0.4948

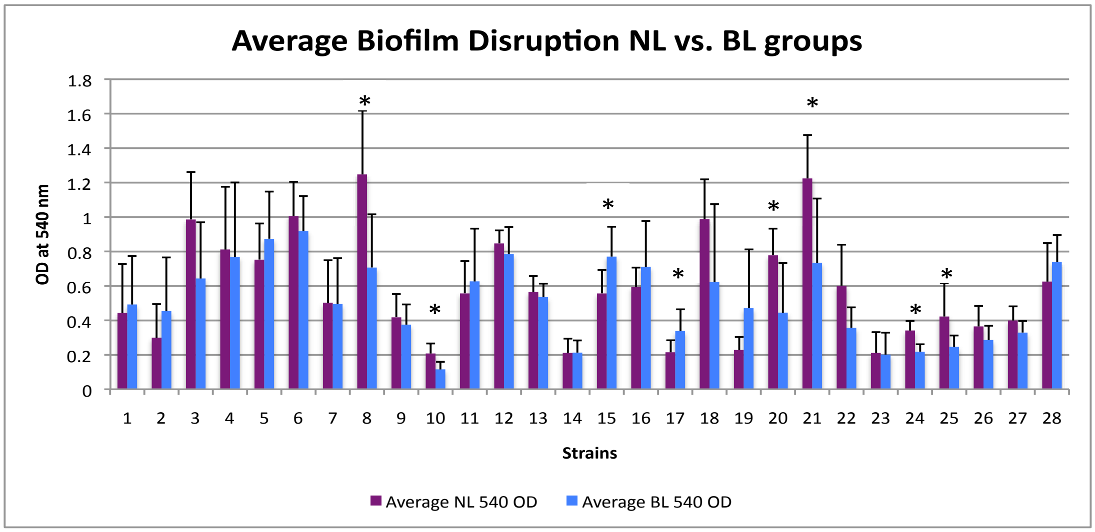
Table 2. The Wilcoxon/Kruskal-Wallis Tests calculated the statistical significance of the effect of blue light irradiation on biofilm formation per strain. Blue light inhibited the formation of biofilm in 5 out of the 17 strains ($p < 0.05$). Significance of irradiation was found to be strain specific and related to the number of cells remaining after 2 hours of blue light irradiation.

Experiment 2

Effect of Blue Light on Biofilm Disruption at a High Concentration of Planktonic Culture

In contrast to the low concentration of planktonic culture in experiment 1, experiment 2 analyzed the effects of blue light on a high concentration of planktonic culture grown for 48 hours in a 24 well plate. This experiment also measured the effect of blue light on the biofilm disruption (OD at 540 nm) during this 48-hour period in correlation with the number of cells in completely saturated culture (OD at 600 nm). The results of the two-sample t-test showed that blue light does disrupt a previously formed biofilm in comparison to biofilm with no exposure to blue light ($t = -9.07$ and $p < 0.001$) (Figure 8a). The disruption of biofilm did not display a direct correlation with the cell number measured at an OD of 600 nm, as shown by experiment 1. The average OD at 540 nm was 0.56 for the no light group and 0.46 for the blue light group. The graphs display the average of 2 trials in quadruplicate on *S. aureus* cultures with an initial average concentration of 1.3×10^3 CFU/mL. After a 2-hour illumination period on the 48-hour culture, there was an average 43.4% difference between the biofilm (540 nm OD) for the no light and blue light groups for the 28 strains.

Since the light was exposed to the plates when the biofilm was already established after 48 hours, the amount of biofilm remaining (OD at 540 nm) was correlated to biofilm disruption. The Wilcoxon/Kruskal-Wallis Tests measured the difference between the effect of blue light irradiation for each individual strain (Table 3). The results showed that the OD at 540 nm of 8 of the 28 strains was significantly different in comparison to the no light group. Of these strains, strains 8, 10, 20, 21, 24 and 25 were significantly reduced by blue light irradiation while strains 15 and 17 were significantly enhanced by blue light irradiation.



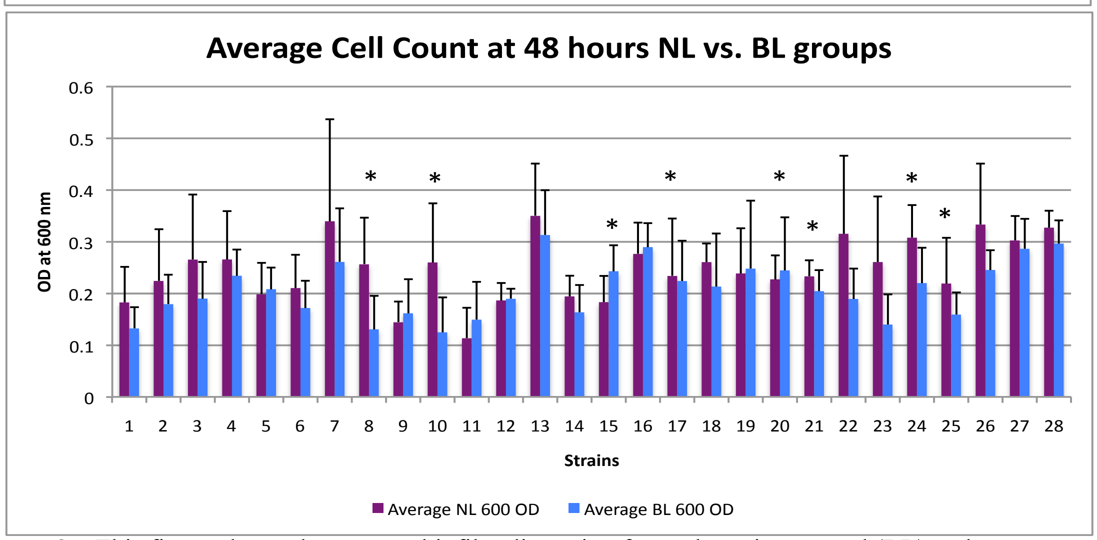


Figure 8a. This figure shows the average biofilm disruption for each strain exposed (BL) and not exposed (NL) to blue light. **Figure 8b.** This figure shows the average cell count measured at an OD of 600 nm for each strain exposed (BL) and not exposed (NL) to blue light.

Experiment 2: NL vs. BL	
Strain	P value
2	0.1871
3	0.0831
4	0.8950
5	0.1178
6	0.4948
7	0.9549
8	**0.0239**
9	0.6365
10	**0.0100**
11	0.9581
12	0.4309
13	0.7132
14	0.9581
15	**0.0180**
16	0.3720
17	**0.0406**
18	0.1563
19	0.4306
20	**0.0313**
21	**0.0181**
22	0.0661
23	0.7132
24	**0.0014**
25	**0.0312**
26	0.1563
27	0.1560
28	0.4005

Table 3. The statistical significance of the effect of blue light irradiation on biofilm disruption. Blue light affected the biofilm of 8 of the 28 strains ($p < 0.05$). Significance of irradiation was found to be strain specific.

Comparison of Experiment 1 and 2

A final component of our analysis was the overall and strain-specific correlation between the cell count (600 OD) and the amount of biofilm (540 OD) for each experiment (Figure 9). For experiment 1, both the no light controls and blue light irradiated strains displayed an overall positive correlation between cell count and biofilm formation. Therefore, our hypothesis was that the greater the number of planktonic cells present in culture remaining after irradiation, the greater the amount of biofilm able to be formed post-treatment. For experiment 2, there was no overall correlation between the cell count and biofilm formation for both the no light controls and blue light irradiated strains. Therefore, the analysis displayed the cell saturation at which biofilm formation plateaus at a pre-treatment time of 48 hours. The inconsistency among the cell count as measured by an OD at 600 nm after 48 hours and 2 hour blue light irradiation may be due to the formation of biofilm occluding the accurate detection by the Bioteck plate reader.

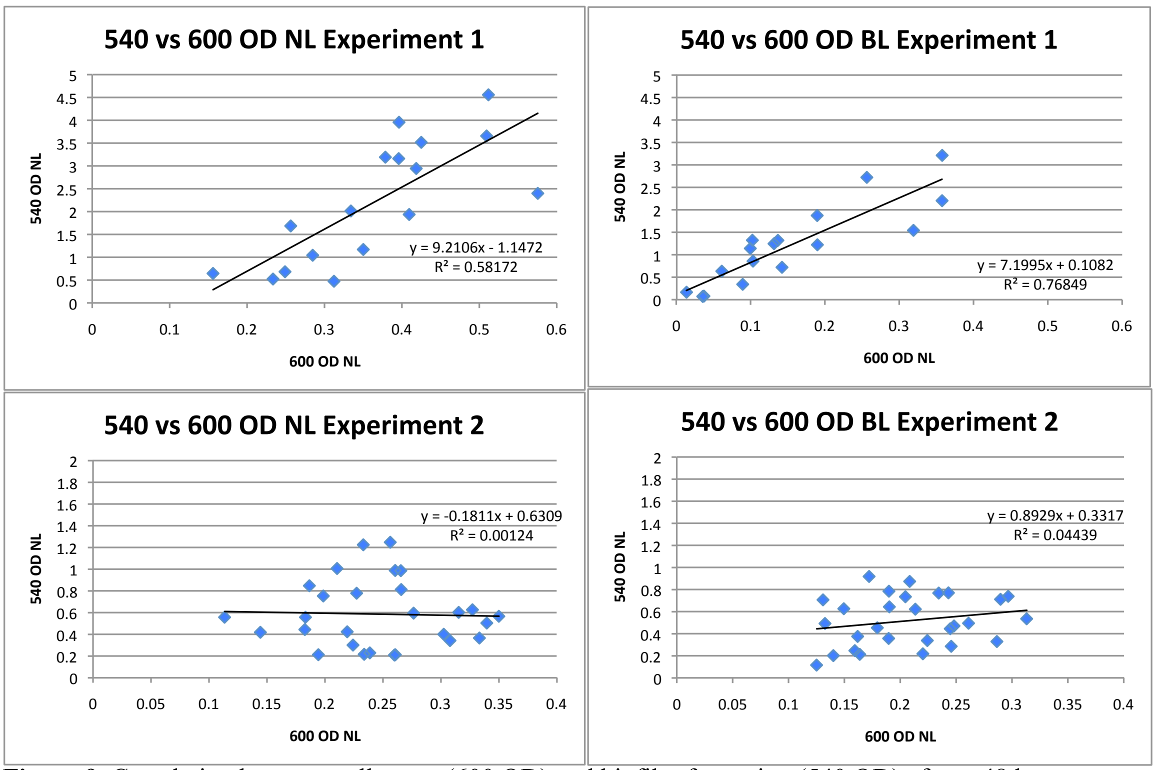


Figure 9. Correlation between cell count (600 OD) and biofilm formation (540 OD) after a 48 hour recovery was positive for experiment 1 and displayed no correlation for experiment 2. The difference between these experiments is that OD 600 was recorded directly after 2 hours of blue light irradiation at 2 hours for experiment 1 and at 48 hours for experiment 2.

Each of the strains from experiment 1 and experiment 2 were analyzed based on a ratio of the OD of 540 nm to OD of 600 nm that defined the amount of biofilm formed per cell (Table 4). We were interested in this ratio to identify the intrinsic variations in strains to form biofilm. These ratios specifically identify the strains that have an altered ability to form biofilm or a greater sensitivity to disrupt biofilm per cell due to blue light irradiation.

For experiment 1, the average ratio for the blue light group in comparison to the no light group was significantly higher, indicating that blue light increased the amount of biofilm per cell (simple T-test $p < 0.05$). The overall ratio between the BL group to NL group ((540 BL/600 BL)/(540 NL/600 NL)) displayed 3 strains (strains 2, 4 and 5) with a high (Ratio >3) or enhanced amount of biofilm in proportion to the cell number. These strains were the same strains that exhibited a negative percent inhibition on the previous analysis and were therefore enhanced by the effects of blue light irradiation. There were also 3 strains that exhibited a medium (Ratio = 1.6-3.0) enhanced effect of blue light irradiation for experiment 1 (strains 1, 7 and 17). The remaining strains (0.5-1.6) showed low amounts of variation between the two groups with an average ratio of 2.5 biofilm/cell for both groups.

Experiment 2 (strains 2, 19 and 23). There was also one strain per experiment (strain 6 for exp. 1 and strain 20 for exp. 2) that displayed an inhibition of the biofilm amount per cell for the blue light group (Ratio = 0.0-0.5). It can be concluded that blue light may have a significant inhibitory and enhancing effect or no effect at all on the amount of biofilm formed per cell depending on the strain.

Experiment 1: Biofilm/Cell Count Ratios			
Strain	540/600 NL	540/600 BL	BL/NL Ratio
1	6.58	12.38	1.88
2	1.52	5.08	3.34
3	2.74	2.13	0.78
4	3.67	12.97	3.53
5	2.24	8.34	3.72
6	6.03	2.02	0.33
7	3.34	6.45	1.93
8	7.18	10.43	1.45
9	4.73	3.84	0.81
10	8.28	11.5	1.39
11	8.43	9.9	1.17
12	9.99	10.64	1.07
13	4.17	4.84	1.16
14	7.99	6.17	0.77
15	8.91	8.99	1.01
16	7.04	9.5	1.35
17	4.14	9.69	2.34
Average:	5.704705882	7.933529412	1.65

Experiment 2: Biofilm/Cell Count Ratios			
Strains	540/600 NL	540/600 BL	BL/NL Ratio
1	2.43	3.72	1.53
2	1.34	2.53	1.89
3	3.71	3.38	0.91
4	3.05	3.28	1.08
5	3.79	4.19	1.11
6	4.78	5.34	1.12
7	1.48	1.9	1.28
8	4.86	5.4	1.11
9	2.89	2.32	0.80
10	0.8	0.93	1.16
11	4.9	4.19	0.86
12	4.54	4.14	0.91
13	1.62	1.71	1.06
14	1.09	1.3	1.19
15	3.04	3.17	1.04
16	2.15	2.46	1.14
17	0.92	1.51	1.64
18	3.79	2.92	0.77
19	0.96	1.9	1.98
20	3.42	1.82	0.53
21	5.25	3.59	0.68
22	1.91	1.89	0.99
23	0.81	1.44	1.78
24	1.11	0.99	0.89
25	1.93	1.55	0.80
26	1.1	1.17	1.06
27	1.32	1.15	0.87
28	1.91	2.49	1.30
Average	2.532142857	2.585	1.12

Scale for Ratios:

0.0 - 0.5	Inhibition (-)
0.5 – 1.5	No Difference
1.6 – 3.0	Medium (+)
> 3.0	High (+)

Table 4. 540 OD/ 600 OD ratio displays correlation between biofilm and cell count. Experiment 1 has a significantly higher ratio for the blue light group in comparison to the no light group ($p < 0.05$). Experiment 2 displays no significant difference between the average ratio of the 2 groups, although 4 strains are significantly different.

CHAPTER FOUR

Discussion and Conclusion

This study hypothesized that blue light irradiation would inhibit planktonic growth and biofilm formation, as well as disrupt an existing biofilm. The experimental data demonstrated that overall blue light does inhibit planktonic growth and biofilm formation of *S. aureus,* but does not consistently disrupt biofilm that was well established before blue light irradiation. Although internal controls and trials performed in quadruplicate controlled for the variation in this procedure, the experimental variables as well as the molecular and genetic response of the *S. aureus* strains to the blue light irradiation may contribute to the variation in the experimental results.

The mechanism of photoinactivation of cells is thought to involve reactive oxygen species (ROS), which lead to membrane damage and cytotoxic changes. The variation seen among the 28 tested strains indicates that other characteristics may also play a role. This study tested a total of 28 strains in quadruplicate and repeated each trial while using a no light control for each strain. The effect of light on both low concentration and high concentration of *S. aureus* cells were tested. Utilizing many replicates made it possible to statistically analyze the results.

Experimental Variables

One experimental variable in the development of the biofilm assay was the presence of glucose in BHI, which enhanced the growth and adherence of the *S. aureus* strains. The use of glucose to increase the amount of biofilm was suggested by the studies of Zmantar (2010), which confirmed that the presence of glucose induced greater *ica* operon function and therefore increased biofilm formation. Of the 17 strains tested in experiment 1, the average OD at 540 nm was 0.32 ± 0.10. Of the 28 strains tested in experiment 2, the average OD at 540 nm was 0.56 ± 0.34. Variation in the *ica* operon may produce variation in the strain's response to biofilm formation, unrelated to the effects of blue light.

Although the majority of published studies include a photosensitizer, such as Toluidine blue O, our study focused on the direct effect of visible light at 470 nm without the influence of a photosenstizer on the irradiation of bacteria (Upadya, 2010). We irradiated cultures grown and diluted in Brain Heart Infusion broth. The broth used for the experiment is known to play a role in the production of a hydroxyl radical in the BHI broth (Jeanne Samake, personal communication). Since this is a complex media, without known qualities for each component, there may be some variation produced between broth batches. We used no light controls for each strain to negate this variable. The differences in these averages for experiment 1 and 2 also indicate the variation intrinsic to this protocol. The effect of blue light irradiation was also predicted to be variable based on the known susceptibility of the various growth modes (Merritt, 2005).

Experiment 1

Based on the results of experiment 1, the percent inhibition of strains is due to the concentration of culture and the strain-specific sensitivity or resistance to blue light irradiation. Although most strains produce 0 CFU/mL at time 0 after a 2 hour blue light irradiation period (Table 1), the recovery of planktonic cells is variable among strains (Figure 6a). The 50% inhibition or greater seen in 65% of strains demonstrated the potential long-term inhibitory effect of blue light irradiation on the ability of planktonic cells to recover and form a biofilm. Of the 17 strains, 14 strains were statistically tested and only 5 of these strains exhibited an inhibited formation of biofilm after a 48-hour recovery period. Also, 5 strains appeared to be significantly enhanced but were not found statically significant. This variation may be from the activation of the *oxyR* gene that encodes for enzymes that protect the DNA and increase resistance to oxidative stress caused by blue light irradiation (Cabiscol, 2000).

Overall, it can be concluded that 2 hours of blue light on *S. aureus* does inhibit biofilm formation as confirmed by the two-sample t-test ($t = 4.77$ and $p < 0.0002$). This inhibition is due to the effect of blue light irradiation killing the planktonic cells in culture, and therefore lowering the number of viable cells able to produce a biofilm. An additional test measuring the same correlation, the regression procedure, demonstrated a $p < 0.0007$ for the difference between no light and blue light groups, which confirmed the significant difference between the groups. The regression test was also utilized to determine if the difference in the biofilm formation was due only to the effects of blue light or if the biofilm formation was a reflection of the number of cells in culture. This analysis displayed that the significant difference between the no light and blue light groups is due to the presence of blue light and is not dependent upon the variation in the CFU/mL at time 0.

When we investigated the effect of blue light on each strain, the one-way analysis using the Wilcoxon/Kruskal-Wallis Tests demonstrated the variability in the effects of blue light on planktonic culture. Of the 17 strains tested, 5 were significantly different ($p < 0.05$) between the blue light group and no light group (strains 3, 6, 8, 9 and 10). Of these significant strains, strains 3 and 6 were greater than 90% inhibited by blue light. For this experiment, the strains with a low p-value exhibit sensitivity to blue light, while the strains with a high p-value exhibit no difference in the no light control. The strains significantly affected by blue light in planktonic culture also produced a low amount of biofilm and appeared to be more susceptible to blue light irradiation (Figure 7a). This could be due to the strain's specific genetic and molecular components that allow for a greater sensitivity to blue light irradiation. The variation among strains may be due to experimental variables (broth, pipetting, plating or plate reader protocol) as well as to genetic and molecular variation among strains. The reference strain ATCC 25923 was used as a standard in each experiment ($n = 23$). The biofilm measurement varied with an average of 1.72 ± 1.67 at an OD of 540 nm, indicating the presence of unknown variables that can cause variation among experiments. The variables including culture age and concentration may play a role in this variation. These variables were controlled for within each experiment by performing each trial in quadruplicate with a no light control for each strain.

Further research using comparative genetic analysis of the strains that were completely inhibited by blue light in planktonic culture in comparison to those not affected by blue light is necessary for possible analysis of a mechanism. Known genes involved in biofilm formation such as the *ica* operon, the *agr* D gene or *sarA* regulator may serve as potential candidates for the exploration of blue light induced genetic alterations.

The results of experiment 1 confirmed the overall effect of blue light on the inhibition of biofilm formation and growth of planktonic culture. Variation was found among strains due to both experimental and genetic components. Experiment 2 investigates the effect of blue light irradiation on an existing biofilm.

Experiment 2

In order to determine the effect of blue light irradiation on an existing biofilm, cultures were grown to saturation for 48 hours in a 24 well plate before exposure to blue light. The results from experiment 2 demonstrated that blue light does have an overall significant effect on the previously formed *S. aureus* biofilm ($t = -9.07$ and $p < 0.0001$). Similar to the results on planktonic culture, analysis per strain displayed a significant difference ($p < 0.05$) between the blue light and no light groups for specific strains, but not for all strains (Figure 8a). The biofilm disruption analysis showed that 19 of the 28 strains were not significantly different than the controls. There was a significant difference between groups ($p < 0.05$) for strains 8, 10, 15, 17, 20, 21, 24 and 25. For the strains that were tested in both experiment 1 and 2, only 2 strains were significant in both experiments. Strains 8 and 10 demonstrated a significant reduction in biofilm formation and significant biofilm disruption. Each experiment was replicated in quadruplicate and each trial had a standard *S. aureus* strain (ATCC 25923) that was used to account for any variation in strain age and medium among trials. This data indicates that the amount of biofilm formation at a 48-hour growth period is not completely dependent on the amount of planktonic culture but rather is strain specific. It was also found that the amount of initial biofilm present is not an accurate indicator of the efficacy of blue light irradiation on biofilm disruption for a specific strain. The effects of experimental variation and the variation in age of the *S. aureus* strains may influence the amount of planktonic growth and biofilm formation after a 48-hour saturation period. Overall, this experiment demonstrated the ability for blue light to effect previously formed biofilm, but the effects were variable among strains and between trials.

The variability among trials and strains, may be due in part to experimental error, but it also leads to questions concerning the role of porphyrin molecules, oxygen radicals and genetic variation. A study by Maclean (2009) demonstrated that irradiation from blue light can induce an energy transfer within photosensitizing porphyrin molecules in the bacterial cell. This energy transfer can lead to the production of oxygen radicals that can kill the cell or cause genetic alterations. Other studies have shown that reactive oxygen species can be released in the extracellular matrix and can significantly decrease the amount of biofilm formation (Arce, 2011). Studies also demonstrated a greater decrease in CFU/mL in planktonic culture of a specific strain in comparison to that strain in biofilm from the same photosensitization (Taraszkiewicz, 2013). This result may be due to the character of the bacterial mode of biofilm, which exhibits a significantly reduced growth rate, impenetrability of the extracellular matrix, and release of toxins (Beenken, 2004). Biofilm formation is initiated by the adhesion of cells, which is favored when quorum sensing is inhibited. Therefore, it can be suggested that antibiotics that specifically do not kill the cell but alter the gene expression, or inhibit quorum sensing, can retrospectively induce greater formation of biofilm (Lopez, 2010). It has been shown that the effect of photosensitization on a strain is unrelated to the antibiotic resistance of that particular strain, but the effect of blue light has similarities to antibiotic sensitivity and resistance (Taraszkiewicz, 2013). Antibiotic resistance is one of many qualities based on the various genetic components of a strain. Variability among strains may be caused by the presence of persister cells in biofilm and the alteration of the *ica* operon, the *agr* D gene or *sarA* expression. The previously formed biofilm has a stable polysaccharide matrix that inhibits penetrance of the cell wall by diffusible molecules and may contribute to the decreased efficacy of blue light irradiation (if dependent on free radicals produced in the media) in comparison to the effect of blue light on planktonic culture.

In this experiment, the cells were exposed to light for 2 hours after a 48-hour growth period and the effect on the biofilm was determined by the amount of crystal violet detected at an OD of 540 nm. The possible mechanism for blue light to disrupt a biofilm under these conditions include the release of reactive oxygen species into the extracellular matrix to cause cell death or genetic manipulation (Goerke, 2004). The possible genetic variations that could repair the damage or protect the biofilm during this time period include mechanisms such as the cascade effect caused by reactive oxygen species that stimulate antioxidant enzymes to genetically alter the surviving cells for increased resistance to irradiation.

Further analysis of these strains may demonstrate the genetic characterization of sensitive and resistant strains to blue light effects on biofilm. Strains that displayed an effect of blue light on the disruption of biofilm should be further analyzed for the mechanism of blue light penetrance and release of oxygen radicals. Transcriptome analysis, the absence or presence of persister cells, and the amount of oxygen radicals that may be altered in these specific strains in comparison to strains not affected by blue light may lead to a better understanding of the potential mechanism of blue light efficacy.

Comparison of Experiment 1 and 2

When comparing the effect of the same strains tested in experiment 1 and experiment 2, the correlation between cell count and biofilm formation demonstrated a positive correlation for experiment 1 and no correlation for experiment 2. In experiment 1, the amount of cells present in the culture is positively correlated to the amount of biofilm that is formed (Figure 9). For experiment 2, we did not see this overall trend in the no light control experiments. This could be due to the differences in the protocol for each experiment or the ability of the Bioteck plate reader to detect the number of cells at an OD of 600 nm. A consistently lower number of cells were detected in experiment 2, which may be due to the saturated culture after 48 hours impeding full penetration of the 600 nm wavelength. This difference could also be a product of the timing of detection of the cell count at a OD of 600 nm for experiment 1 after a 48-hour recovery period after blue light irradiation in comparison to experiment 2, which had no recovery period after blue light irradiation.

We also asked whether there was a correlation between the amount of biofilm produced per cell as measured by a ratio of OD 540/600 for the blue light and no light controls (Table 4). If the amount of biofilm was only due to the amount of viable cells present, then this ratio should be the same for both the experimental and control samples. If blue light is enhancing or inhibiting the biofilm, the ratios would be higher or lower, respectively. In order to determine these effects quantitatively, the data was displayed as ratios of OD at 540 nm over OD at 600 nm, therefore, the greater the ratio, the greater amount of biofilm formed per cell (higher OD 540). In experiment 1 the concentration of culture was about 1500 CFUs/ml. At this relatively low concentration there were 3 strains (strains 3, 5 and 6) that displayed a ratio 3x higher for biofilm to cell count for the blue light group in comparison to the no light group, exhibiting an enhancing effect of blue light on the biofilm formation of irradiated planktonic culture. Although most strains exhibited a decreased amount of cells found in culture, and therefore a decreased amount of biofilm, there were some strains (strains 2, 4 and 5) with a significantly greater amount of biofilm formation per cell for the blue light group. This effect may be due to a variation in the onset of the exponential growth phase of cells where a biofilm is formed or it may be due to the variation in the sensitivity to the effects of blue light irradiation due to a variation in gene expression or other phenotypic variation in these strains.

The second experiment irradiated cells that with a concentration over 10^8 CFUs/ml and a previously formed biofilm in the wells of the microtiter plate. Under these saturated concentrations, we found that blue light does not have a consistent effect on the amount of biofilm, but rather some strains were inhibited, such as strains 8, 10, 20, 21, 24 and 25, and others were enhanced, such as strains 15 and 17 (Figure 8a). Overall, there is no correlation between the cell count and biofilm formation between groups. This may be due to the stationary phase of cells in a biofilm at a saturated cell level. Therefore, the majority of strains will remain in this stationary phase and the effects of blue light irradiation will not alter the amount of biofilm that was previously formed before irradiation in comparison to the effects of blue light irradiation on more susceptible culture in the exponential growth phase initially forming biofilm.

Further Studies

There were multiple variables in this experiment that could be further analyzed. These variables may play an important role in determining the cause of variation within the response of *S. aureus* strains to blue light. Further studies will include the variables such as the irradiation time, luminance, heating factors, type of culture media, growth phase, as well as the synergistic effects of blue light and antibiotics and the correlations between the sensitivity of strains to antibiotics and blue light inhibition of the strain's biofilm formation. Further studies will also explore why some strains appear sensitive and are inhibited to blue light exposure while other strains seem to be resistant or enhanced by blue light by analyzing different genes and gene expression. Overall, this study tested the effects of blue light on *S. aureus* in early and late planktonic culture and measured the resulting biofilm. The effect on cells is much greater in low concentrations, supporting the idea that blue light may serve as a potential preventative for infections in a clinical setting. Further studies are needed to provide supporting evidence and to test additional variables.

Conclusion

In conclusion, this study displayed a bactericidal effect of blue light on planktonic culture and an inhibitory effect on biofilm formation at a low cellular concentration. Our study hypothesized that blue light irradiation would inhibit planktonic growth and biofilm formation, as well as disrupt an existing biofilm. Although the experimental data demonstrated that blue light did inhibit planktonic growth and biofilm formation, blue light did not consistently disrupt previously formed biofilm. The resulting strain specific effects may be due to the genetic and molecular composition of each strain that contribute to the efficacy of blue light on both sensitive and resistant strains to blue light irradiation. Therefore, studies involving the genetic and molecular components of *S. aureus* strains may lead to findings on strain susceptibility to blue light and on how to address pathogenic strain eradication. Further studies pertaining to the specific variables involved in this experiment may lead to findings regarding the blue light inhibitory mechanism. Blue light has the potential to serve as a preventative for *S. aureus* biofilm-asssociated infections, but further testing must be done to make progress in the treatment of disease.

APPENDICES

APPENDIX A

Raw Data

Experiment 1

Trial 1					**CFU/mL**				
Date: 10/4/2011	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 2 hr NL**	**Time 2 hr BL**
ATCC	0.1575	0.00375	0.2375	0.0045	620	780	4920	0
1078	0.27925	0.00575	0.08325	0.008	1500	1180	18000	0
1241	0.33775	0.00725	0.17925	0.014	1460	3760	7640	0
1707	0.2715	0.05325	0.4965	0.99675	600	1380	15620	0
1109	0.15825	0	0.276	0.01225	2300	3720	3760	0
Trial 2					**CFU/mL**			
Date: 10/10/2011	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 2 hr NL**	**Time 2 hr BL**
ATCC	0.22325	0.0025	0.21825	0	1360	1100	4400	0
1707	0.285	0.08075	0.5665	0.0235	4740	3060	3340	0
1109	0.34525	0.02825	0.244	0.04325	3460	2700	6000	0
1182	0.35475	0.006	0.5515	0	2780	3660	18000	0
1175	0.3135	0.1415	0.4405	0.196	8600	6000	18000	0
Trial 3					**CFU/mL**			
Date: 10/17/2011	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 2 hr NL**	**Time 2 hr BL**
ATCC	0.22	0.0805	2.14	1.144	2840	1700	12000	0
1182	0.24375	0.00175	3.3405	0	4100	4320	12000	0
1175	0.317	0.20525	1.76375	2.086	3740	3740	8000	60
1707	0.19075	0.0435	2.57825	1.31175	2040	1920	8000	240
1109	0.1815	0.13775	0.7415	1.45725	1220	720	8000	20
Trial 4					**CFU/mL**			
Date: 10/24/2011	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 2 hr NL**	**Time 2 hr BL**
ATCC	0.3145	0.00075	3.0165	0	1380	1120	8000	0
3365	0.491	0.00175	3.509	0	1620	2140	8000	0
3407	0.38525	0.04775	0.534	0.07275	1920	1440	8000	0
3374	0.37875	0.02725	3.35875	0.98425	2340	2800	8000	0
3206	0.36125	0.096	2.03725	0.477	2160	1960	8000	0
Trial 5					**CFU/mL**			
Date: 10/31/2011	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 2 hr NL**	**Time 2 hr BL**
ATCC	0.28	0.00075	2.903	0.006	1840	1760	14520	0
3365	0.46075	0.058	3.65075	1.1305	2340	1680	9880	20
3407	0.3665	0.06825	3.186	0.47175	1800	1900	9880	40
3374	0.40425	0.13925	3.52075	1.15875	1880	3720	9880	20
3206	0.329	0.2205	4.19225	3.131	2340	1900	8000	0
Trial 6					**CFU/mL**			
Date: 11/07/2011	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 2 hr NL**	**Time 2 hr BL**
ATCC	0.4185	0.002	2.473	0	1680	1380	8000	20
349	0.39975	0.06175	2.99625	0.66675	3660	3040	8000	0
330	0.5135	0.00025	2.8575	0	3580	3340	8000	0
2860	0.18675	0.1115	1.467	0.052	3280	3080	8000	0
2344	0.43375	0.1385	4.22725	1.53425	1940	1980	8000	0
Trial 7					**CFU/mL**			
Date: 11/17/2011	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 2 hr NL**	**Time 2 hr BL**
ATCC	0.181	0.003	0.817	0	160	140	2940	0
3206	0.27775	0.0095	2.70475	1.7765	3520	3880	8000	0
2344	0.2225	0.00125	1.46775	0	2900	2380	8000	0
39	0.3865	0.1005	2.8935	1.192	640	520	4300	0
3017	0.124	0.10575	0.59525	1.267	2840	2740	8000	0

Experiment 2

Trial 1					**CFU/mL**			
Date: 1/23/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.0995	0.0595	0.24775	0.0785	100	240	TMTC	0
1705	0.2055	0.0795	0.99325	0.14325	180	420	TMTC	0
1079	0.111	0.08325	0.116	0.11275	1820	2540	TMTC	0
1127	0.156	0.1215	0.79225	0.1355	120	100	TMTC	0
1130	0.183	0.12375	1.36525	0.358	300	520	TMTC	0
Trial 2					**CFU/mL**			
Date: 1/30/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.111	0.176	0.5615	0.63	340	500	TMTC	TMTC
1705	0.2145	0.24925	0.862	0.9835	280	200	TMTC	440
1079	0.26525	0.31475	0.22125	0.711	2520	2680	TMTC	TMTC
1127	0.1975	0.26975	0.64375	0.63725	20	40	TMTC	440
1130	0.182	0.18725	0.96325	0.994	20	20	TMTC	TMTC
Trial 3					**CFU/mL**			
Date: 2/06/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.14625	0.0875	0.2225	0.0515	540	600	400	0
1020	0.19675	0.10625	0.4625	0.2375	920	820	400	0
1024	0.246	0.092	0.054	0.04425	1740	1700	400	0
1025	0.2325	0.13975	0.245	0.14725	1860	2180	400	0
1026	0.124	0.1	0.21225	0.161	2040	1460	400	0
Trial 4					**CFU/mL**			
Date: 2/13/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.1705	0.096	0.3065	0.09175	600	420	TMTC	TMTC
1020	0.3375	0.1905	0.64125	0.3795	700	580	TMTC	TMTC
1024	0.179	0.10575	0.26975	0.262	380	900	TMTC	2480
1025	0.28675	0.21825	0.33925	0.19175	840	60	TMTC	TMTC
1026	0.218	0.13625	0.53425	0.23525	260	480	TMTC	TMTC
Trial 5-1					**CFU/mL**			
Date: 2/22/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.254	0.1685	0.179	0.21325	480	240	TMTC	TMTC
3206	0.06	0.0785	0.413	0.4145	20	0	TMTC	TMTC
2344	0.16175	0.21525	0.4425	0.5805	600	560	TMTC	TMTC
39	0.23025	0.249	0.55625	0.538	40	40	TMTC	TMTC
3017	0.2675	0.1755	0.21475	0.3315	140	160	TMTC	TMTC
Trial 5-2					**CFU/mL**			
Date: 2/22/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.17025	0.1805	0.2655	0.22475	800	500	TMTC	TMTC
3206	0.081	0.1315	0.59975	0.7465	20	40	TMTC	TMTC
2344	0.11875	0.182	0.57	0.868	460	380	TMTC	TMTC
39	0.23675	0.2415	0.53175	0.793	20	120	TMTC	TMTC
3017	0.11425	0.184	0.115	0.25325	300	140	TMTC	TMTC
Trial 6-1					**CFU/mL**			
Date: 2/13/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.31075	0.1765	1.04725	0.5265	540	320	TMTC	TMTC
1241	0.312	0.1685	1.1315	0.8505	780	920	TMTC	TMTC
1182	0.2075	0.12875	1.0745	1.03775	440	420	TMTC	2480
3365	0.26925	0.128	1.4725	0.937	860	800	TMTC	TMTC
3407	0.0685	0.11675	0.45425	0.407	180	240	TMTC	TMTC
Trial 6-2					**CFU/mL**			
Date: 2/22/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.19275	0.12225	0.714	0.4055	360	320	TMTC	TMTC
1241	0.1235	0.11825	0.72975	0.34525	1120	1080	TMTC	TMTC
1182	0.118	0.12175	0.8285	0.70825	1300	1160	TMTC	TMTC
3365	0.148	0.04	0.91175	0.38525	760	940	TMTC	TMTC
3407	0.12475	0.11325	0.27125	0.253	180	280	TMTC	TMTC

Trial 7-1					**CFU/mL**			
Date: 2/22/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.1685	0.19375	0.67325	0.5495	380	200	TMTC	TMTC
1175	0.1775	0.22975	0.39225	0.7645	880	700	TMTC	TMTC
1707	0.236	0.22125	1.18775	1.06875	260	260	TMTC	TMTC
1109	0.115	0.17275	0.61375	0.88375	40	60	TMTC	TMTC
1078	0.25375	0.14725	0.19625	0.565	480	500	TMTC	TMTC
Trial 7-2					**CFU/mL**			
Date: 2/22/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.122	0.11125	0.745	0.898	500	940	TMTC	TMTC
1175	0.4215	0.19775	0.1825	0.514	920	840	TMTC	TMTC
1707	0.11575	0.17625	0.9815	1.141	300	200	TMTC	TMTC
1109	0.10475	0.1425	0.49625	0.98775	160	100	TMTC	TMTC
1078	0.184	0.15775	0.333	0.77925	440	960	TMTC	TMTC
Trial 8-1					**CFU/mL**			
Date: 2/13/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.18175	0.1095	0.541	0.27375	1740	1380	TMTC	TMTC
1175	0.29275	0.18725	0.71	0.22175	2840	1940	TMTC	TMTC
1214	0.288	0.17125	0.304	0.207	4620	3760	TMTC	TMTC
239	0.23575	0.208	0.3675	0.2995	2600	2360	TMTC	TMTC
227	0.2695	0.2275	0.771	0.73775	2760	3680	TMTC	TMTC
Trial 8-2					**CFU/mL**			
Date: 3/06/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.19675	0.10925	0.55	0.25475	1160	1920	TMTC	TMTC
1175	0.2815	0.24875	0.525	0.28425	3480	1760	TMTC	TMTC
1214	0.28025	0.232	0.323	0.262	3400	3640	TMTC	TMTC
239	0.271	0.277	0.32875	0.256	2100	1960	TMTC	TMTC
227	0.28675	0.27775	0.3765	0.6365	3500	3800	TMTC	TMTC
Trial 9-1					**CFU/mL**			
Date: 3/13/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.3345	0.13975	0.326	0.1765	3820	640	TMTC	TMTC
349	0.1517	0.14	0.768	0.625	1880	3660	TMTC	0
330	0.267	0.26675	0.528	0.51775	680	880	TMTC	0
1707	0.2965	0.14675	0.33625	0.17725	580	720	TMTC	TMTC
1109	0.208	0.17	0.8405	0.47975	280	420	TMTC	1260
Trial 9-2					**CFU/mL**			
Date: 3/13/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.21125	0.17875	0.56275	0.4485	880	1620	TMTC	TMTC
349	0.11475	0.1475	0.829	0.85	2800	3080	TMTC	TMTC
330	0.32625	0.26725	0.50625	0.45775	560	140	TMTC	0
1707	0.22125	0.20825	0.546	0.49875	600	680	TMTC	TMTC
1109	0.173	0.16325	0.864	0.956	500	240	TMTC	0
Trial 10-1					**CFU/mL**			
Date: 3/20/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.10025	0.101	0.0435	0.062	2600	3240	TMTC	TMTC
1078	0.1185	0.1285	0.0705	0.10975	1940	1780	TMTC	TMTC
2860	0.147	0.0875	0.13	0.11525	4800	4140	TMTC	TMTC
3374	0.14725	0.08375	0.164	0.09375	3240	5340	TMTC	TMTC
Trial 10-2					**CFU/mL**			
Date: 3/20/2012	**Average NL 600 OD**	**Average BL 600 OD**	**Average NL 540 OD**	**Average BL 540 OD**	**Time 0 NL**	**Time 0 BL**	**Time 48 hr NL**	**Time 48 hr BL**
ATCC	0.1555	0.1125	0.111	0.044	1620	1260	TMTC	TMTC
1078	0.156	0.103	0.39775	0.159	1540	640	TMTC	TMTC
2860	0.144	0.1515	0.18925	0.201	3620	1180	TMTC	TMTC
3374	0.275	0.078	0.1485	0.02825	1960	1800	TMTC	TMTC

Strain Categorization and Reference

Strain Label Correlation		
Strain Number	Strain Name	Strain Category
1	ATCC	MSSA
2	1078	MSSA
3	1241	**MRSA**
4	1707	MSSA
5	1109	MSSA
6	1182	MSSA
7	1175	**MRSA**
8	3365	MSSA
9	3407	MSSA
10	3374	MSSA
11	3206	MSSA
12	349	MSSA
13	330	MSSA
14	2860	MSSA
15	2344	MSSA
16	39	MSSA
17	3017	MSSA
18	1705	MSSA
19	1079	MSSA
20	1127	MSSA
21	1130	MSSA
22	1020	**MRSA**
23	1024	MSSA
24	1025	MSSA
25	1026	MSSA
26	1214	**MRSA**
27	239	**MRSA**
28	227	**MRSA**

APPENDIX B

Statistical Data Analysis

Experiment 1 Group Analysis

Kayla Fricke Data

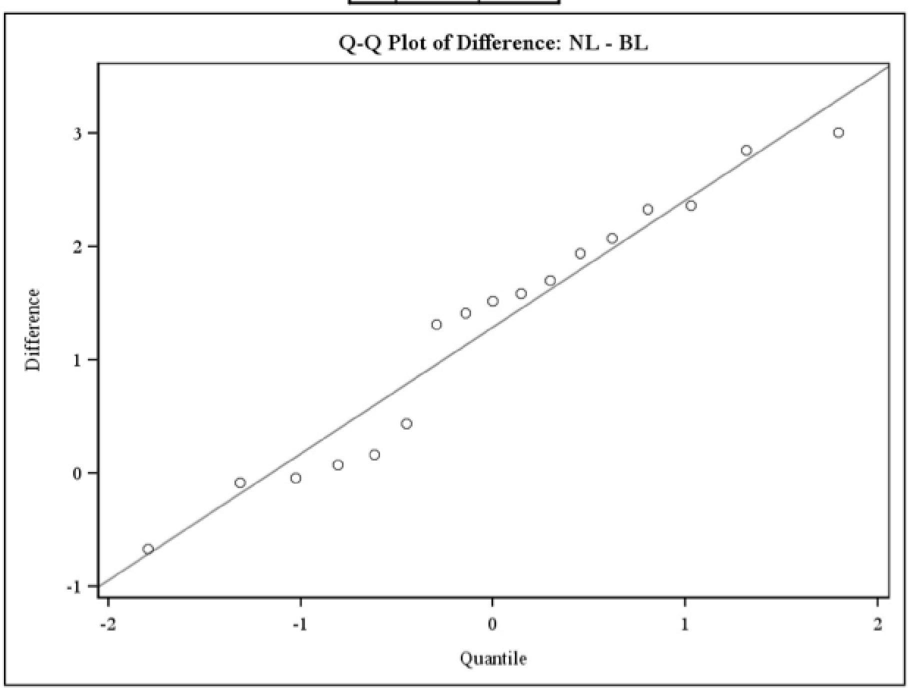
The TTEST Procedure

Difference: NL - BL

N	Mean	Std Dev	Std Err	Minimum	Maximum
17	1.2956	1.1188	0.2714	-0.6718	3.0146

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
1.2956	0.7203	1.8708	1.1188	0.8333	1.7028

DF	t Value	Pr > |t|
16	4.77	0.0002



Kayla Fricke Data

The REG Procedure
Model: MODEL1
Dependent Variable: trans_result

Number of Observations Read	34
Number of Observations Used	34

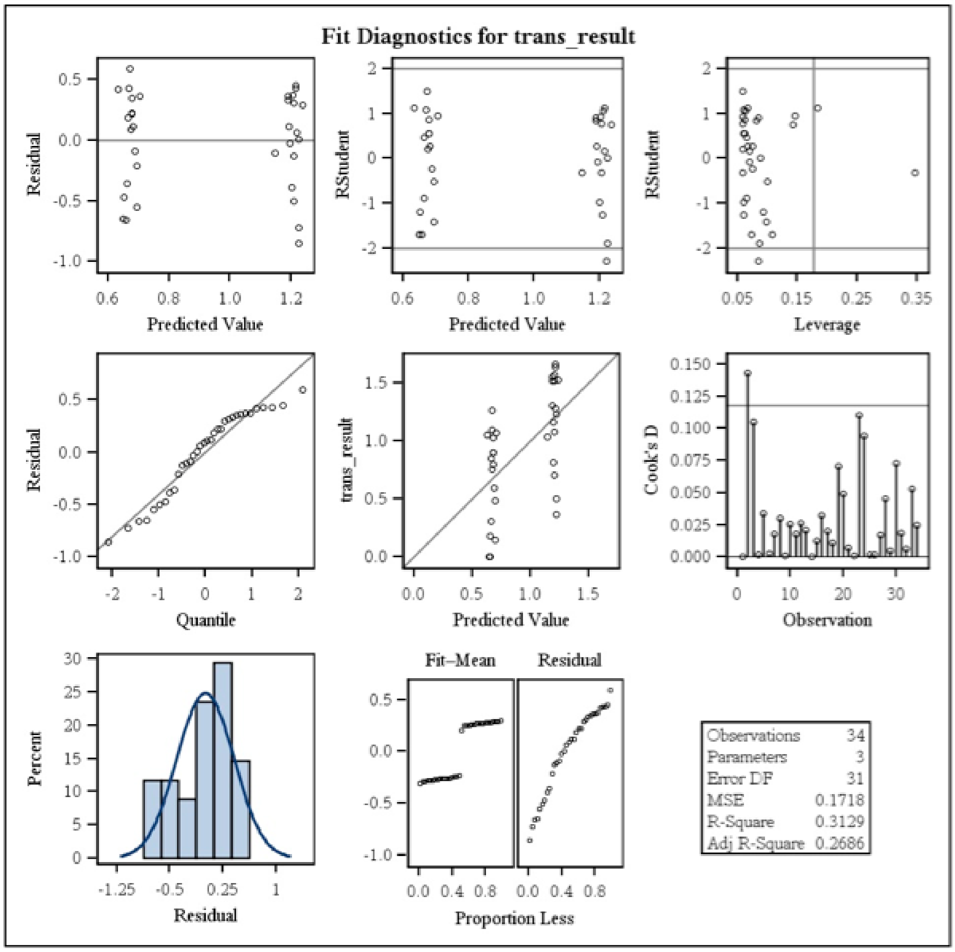
Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Value**	**Pr > F**
Model	2	2.42565	1.21283	7.06	0.0030
Error	31	5.32582	0.17180		
Corrected Total	33	7.75148			

Root MSE	0.41449	**R-Square**	0.3129
Dependent Mean	0.93946	**Adj R-Sq**	0.2686
Coeff Var	44.11997		

Parameter Estimates						
Variable	**DF**	**Parameter Estimate**	**Standard Error**	**t Value**	**Pr > |t|**	**Variance Inflation**
Intercept	1	1.24829	0.18847	6.62	<.0001	0
Light2	1	-0.53402	0.14224	-3.75	0.0007	1.00095
CFU	1	-0.00001645	0.00006186	-0.27	0.7920	1.00095

Kayla Fricke Data

The REG Procedure
Model: MODEL1
Dependent Variable: trans_result



Experiment 2 Group Analysis

Kayla Fricke Data

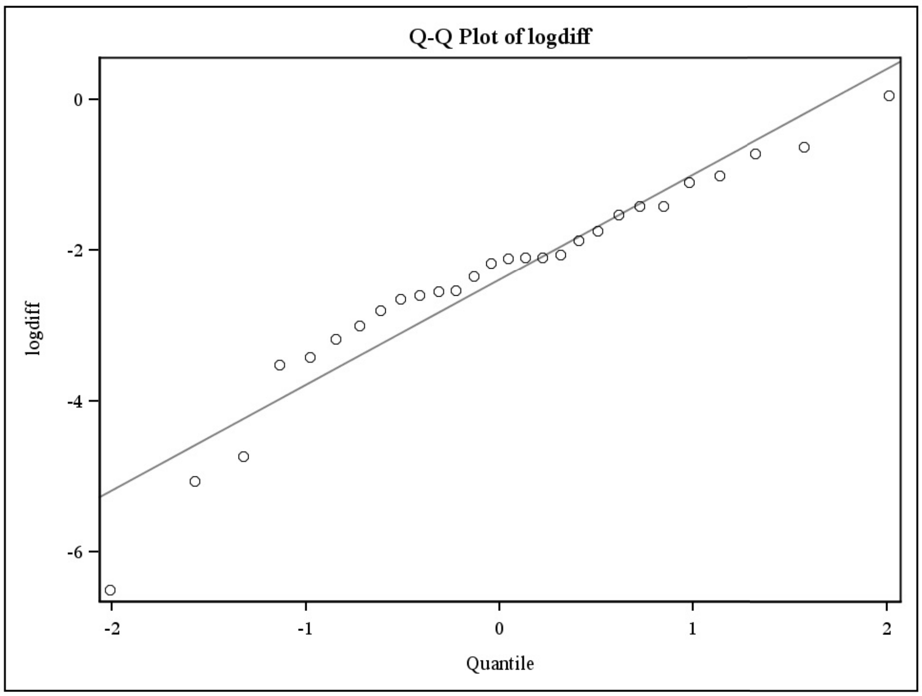
The TTEST Procedure

Variable: logdiff

N	Mean	Std Dev	Std Err	Minimum	Maximum
28	-2.3873	1.3935	0.2633	-6.5023	0.0484

Mean	95% CL Mean		Std Dev	95% CL Std Dev	
-2.3873	-2.9277	-1.8470	1.3935	1.1017	1.8968

DF	t Value	Pr > |t|
27	-9.07	<.0001



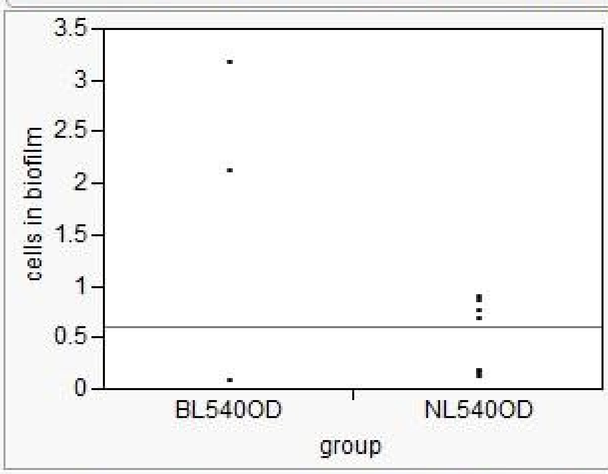
APPENDIX C

Strain-specific Analysis

Experiment 1

Strain 1078

Oneway Analysis of cells in biofilm By group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	52.000	68.000	6.5000	-1.629
NL540OD	8	84.000	68.000	10.5000	1.629

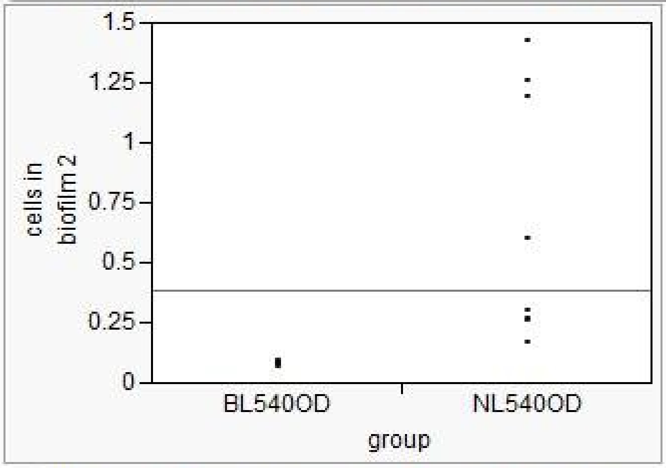
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
84	1.62902	0.1033

p-value=0.1033>0.05, there is no group effect.

Strain 1241

Oneway Analysis of cells in biofilm 2 By group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	36.000	68.000	4.5000	-3.311
NL540OD	8	100.000	68.000	12.5000	3.311

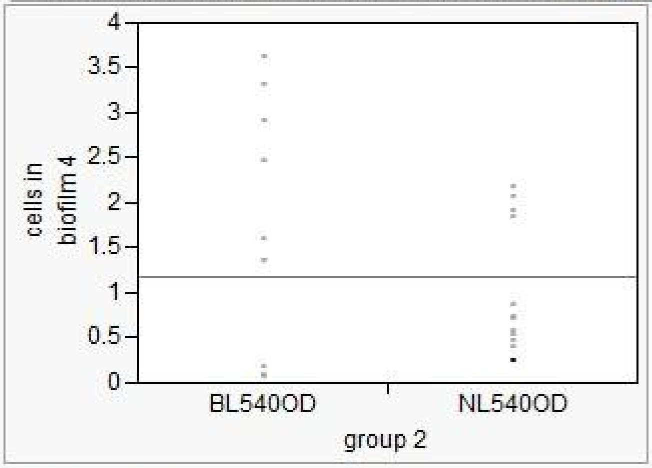
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
100	3.31060	0.0009*

p-value=0.0009<0.05, there is a group effect.

Strain 1707

Oneway Analysis of cells in biofilm 4 By group 2



Missing Rows 3

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	12	142.000	150.000	11.8333	-0.433
NL540OD	12	158.000	150.000	13.1667	0.433

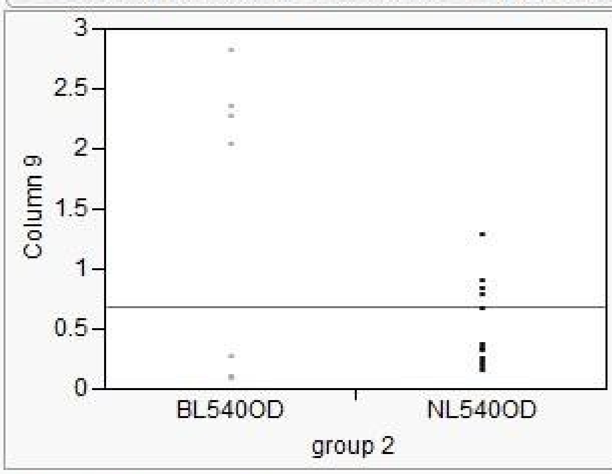
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
158	0.43301	0.6650

p-value=0.6650>0.05, there is no group effect.

Strain 1109

Oneway Analysis of Column 9 By group 2



Missing Rows 3

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	12	130.000	150.000	10.8333	-1.126
NL540OD	12	170.000	150.000	14.1667	1.126

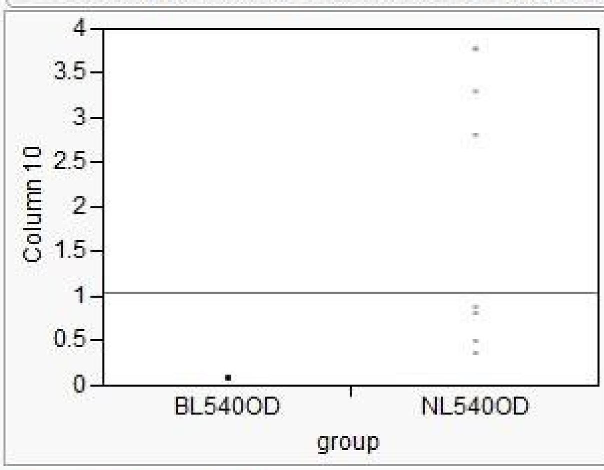
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
170	1.12608	0.2601

p-value=0.2601>0.05, there is no group effect.

Strain 1182

Oneway Analysis of Column 10 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	36.000	68.000	4.5000	-3.313
NL540OD	8	100.000	68.000	12.5000	3.313

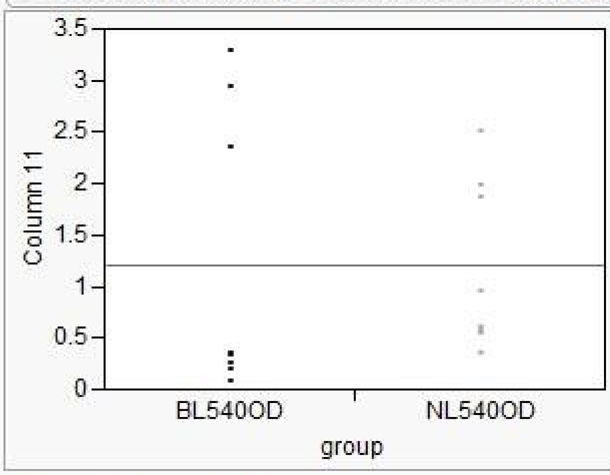
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
100	3.31304	0.0009*

p-value=0.0009<0.05, there is a group effect.

Strain 1175

Oneway Analysis of Column 11 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	59.000	68.000	7.37500	-0.893
NL540OD	8	77.000	68.000	9.62500	0.893

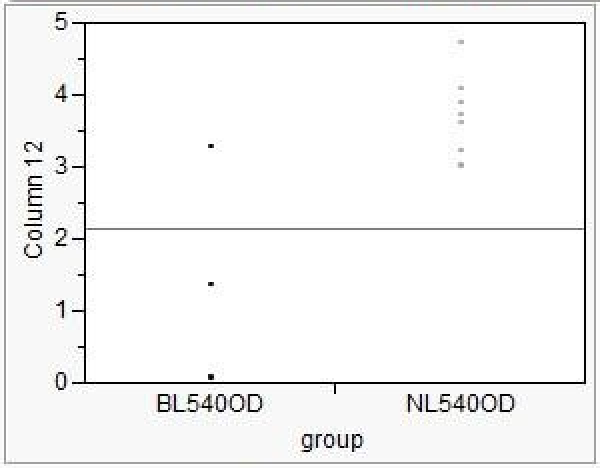
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
77	0.89268	0.3720

p-value=0.3720>0.05, there is no group effect.

Strain 3365

Oneway Analysis of Column 12 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	39.000	68.000	4.8750	-2.993
NL540OD	8	97.000	68.000	12.1250	2.993

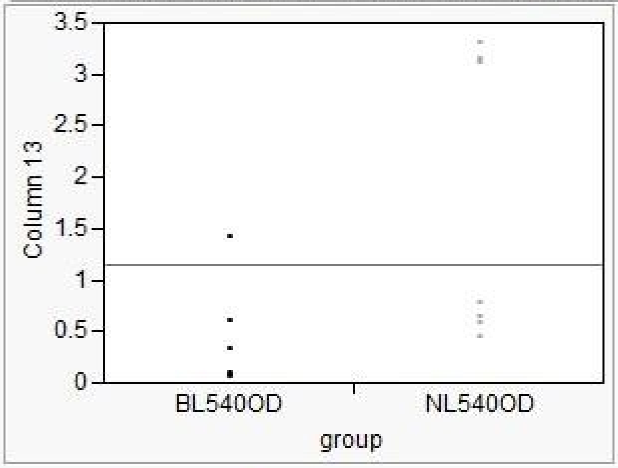
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
97	2.99310	0.0028*

p-value=0.0028<0.05, there is a group effect.

Strain 3407

Oneway Analysis of Column 13 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	42.000	68.000	5.2500	-2.678
NL540OD	8	94.000	68.000	11.7500	2.678

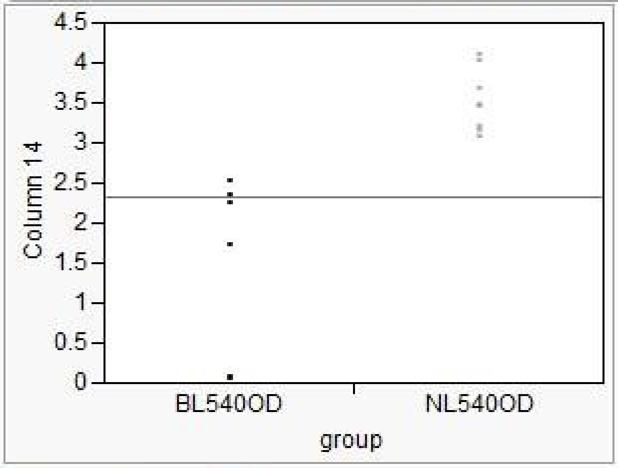
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
94	2.67804	0.0074*

p-value=0.0074<0.05, there is a group effect.

Strain 3374

Oneway Analysis of Column 14 By group



Missing Rows	11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	36.000	68.000	4.5000	-3.308
NL540OD	8	100.000	68.000	12.5000	3.308

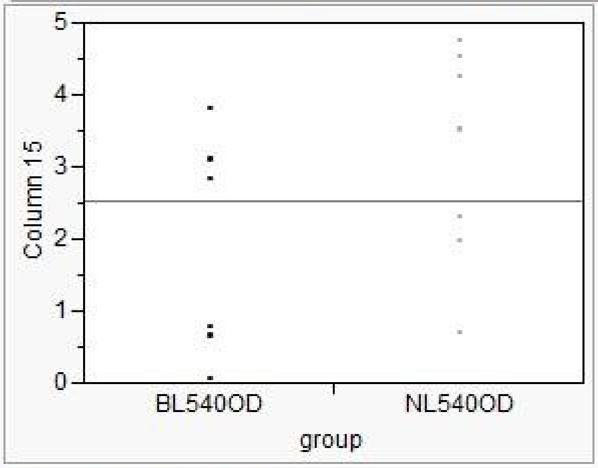
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
100	3.30816	0.0009*

p-value=0.0009<0.05, there is a group effect.

Strain 3206

Oneway Analysis of Column 15 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	51.000	68.000	6.3750	-1.733
NL540OD	8	85.000	68.000	10.6250	1.733

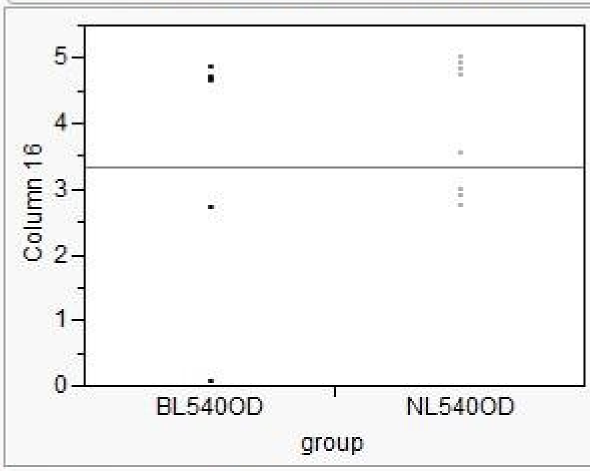
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
85	1.73285	0.0831

p-value=0.0831>0.05, there is no group effect.

Strain 349

Oneway Analysis of Column 16 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	54.000	68.000	6.7500	-1.419
NL540OD	8	82.000	68.000	10.2500	1.419

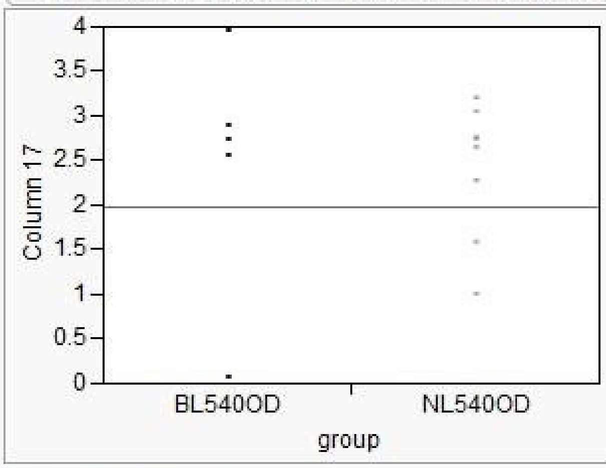
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
82	1.41883	0.1559

p-value=0.1559>0.05, there is no group effect.

Strain 330

Oneway Analysis of Column 17 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	58.000	68.000	7.25000	-0.998
NL540OD	8	78.000	68.000	9.75000	0.998

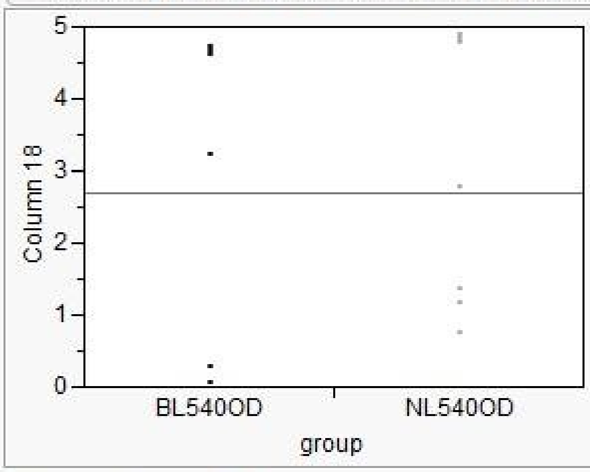
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
78	0.99770	0.3184

p-value=0.3184>0.05, there is no group effect.

Strain 2860

Oneway Analysis of Column 18 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	52.000	68.000	6.5000	-1.628
NL540OD	8	84.000	68.000	10.5000	1.628

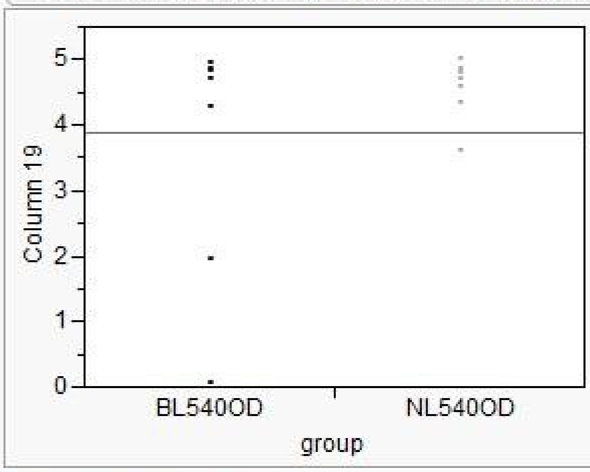
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
84	1.62783	0.1036

p-value=0.1036>0.05, there is no group effect.

Strain 2344

Oneway Analysis of Column 19 By group



Missing Rows 11

Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL540OD	8	61.000	68.000	7.62500	-0.683
NL540OD	8	75.000	68.000	9.37500	0.683

2-Sample Test, Normal Approximation

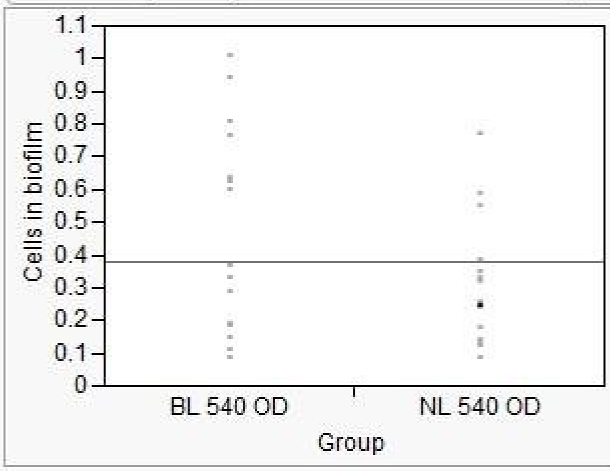
S	Z	Prob>|Z|
75	0.68264	0.4948

p-value=0.4948>0.05, there is no group effect.

Experiment 2 Strain-specific Analysis

Strain 1078

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	16	299.500	264.000	18.7188	1.319
NL 540 OD	16	228.500	264.000	14.2813	-1.319

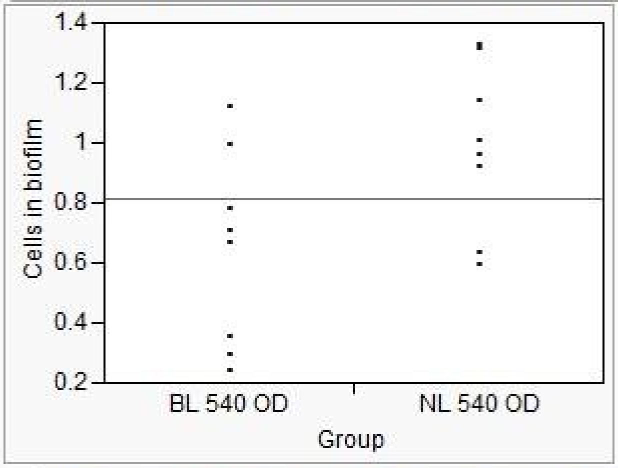
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
228.5	-1.31923	0.1871

The p-value 0.1871>0.05, so the group effect is not significant.

Strain 1241

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	51.000	68.000	6.3750	-1.733
NL 540 OD	8	85.000	68.000	10.6250	1.733

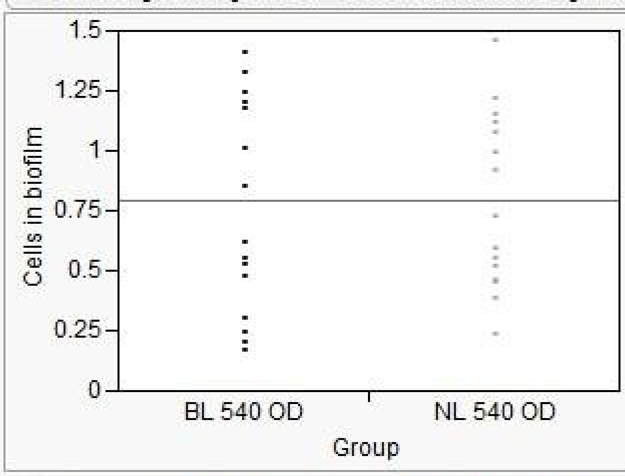
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
85	1.73285	0.0831

The p-value 0.0831>0.05, so the group effect is not significant.

Strain 1707

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	16	260.000	264.000	16.2500	-0.132
NL 540 OD	16	268.000	264.000	16.7500	0.132

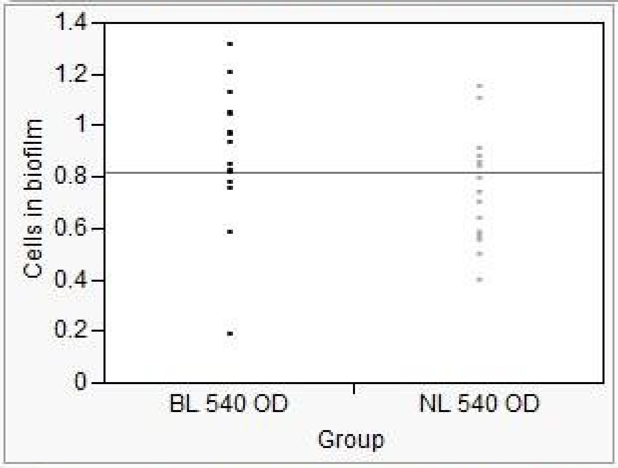
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
268	0.13192	0.8950

The p-value 0.8950>0.05, so the group effect is not significant.

Strain 1109

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	16	306.000	264.000	19.1250	1.564
NL 540 OD	16	222.000	264.000	13.8750	-1.564

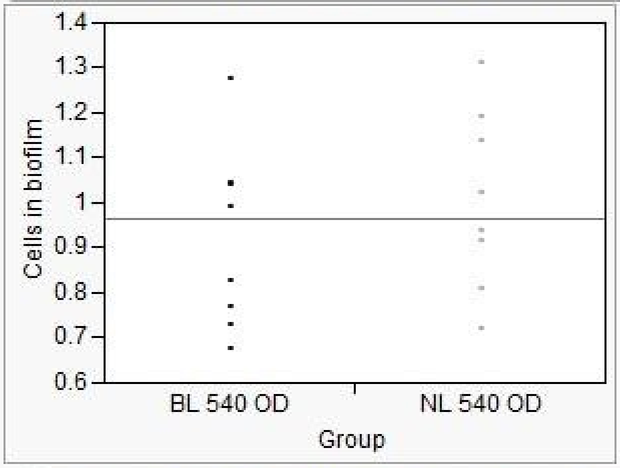
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
222	-1.56409	0.1178

The p-value 0.1178>0.05, so the group effect is not significant.

Strain 1182

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	61.000	68.000	7.62500	-0.683
NL 540 OD	8	75.000	68.000	9.37500	0.683

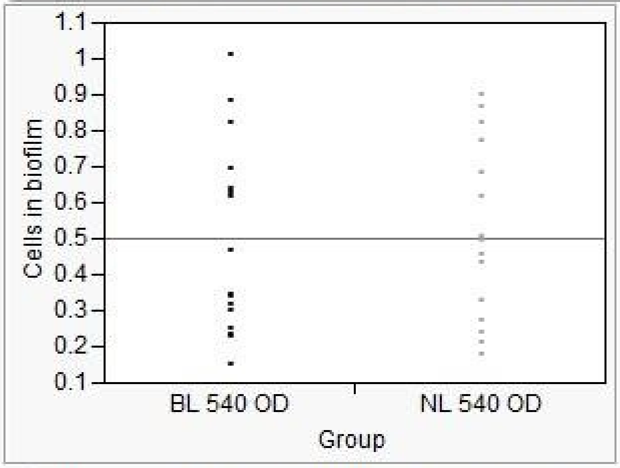
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
75	0.68264	0.4948

The p-value 0.4948>0.05, so the group effect is not significant.

Strain 1175

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	16	262.000	264.000	16.3750	-0.057
NL 540 OD	16	266.000	264.000	16.6250	0.057

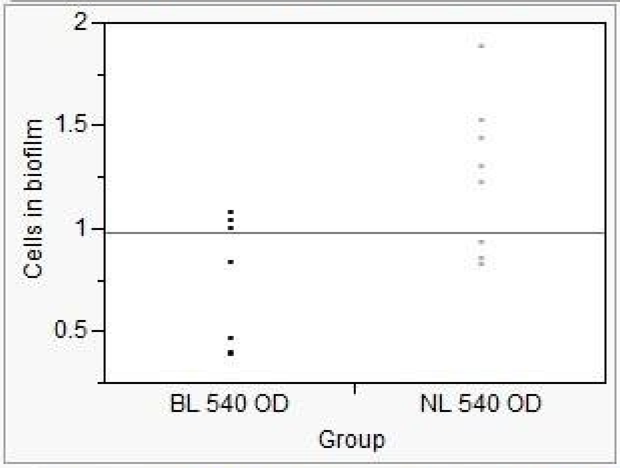
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
266	0.05653	0.9549

The p-value 0.9549>0.05, so the group effect is not significant.

Strain 3365

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	46.000	68.000	5.7500	-2.258
NL 540 OD	8	90.000	68.000	11.2500	2.258

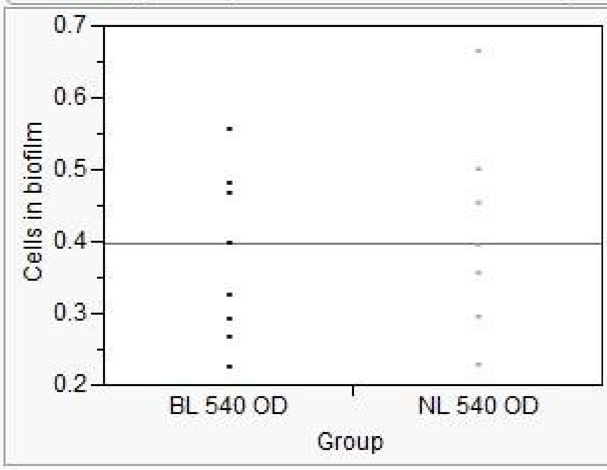
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
90	2.25795	0.0239*

The p-value 0.0239<0.05, so the group effect is significant.

Strain 3407

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	63.000	68.000	7.87500	-0.473
NL 540 OD	8	73.000	68.000	9.12500	0.473

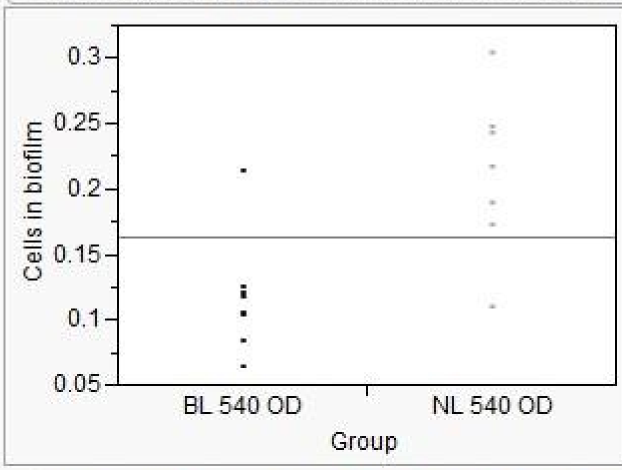
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
73	0.47259	0.6365

The p-value 0.6365>0.05, so the group effect is not significant.

Strain 3374

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	43.000	68.000	5.3750	-2.575
NL 540 OD	8	93.000	68.000	11.6250	2.575

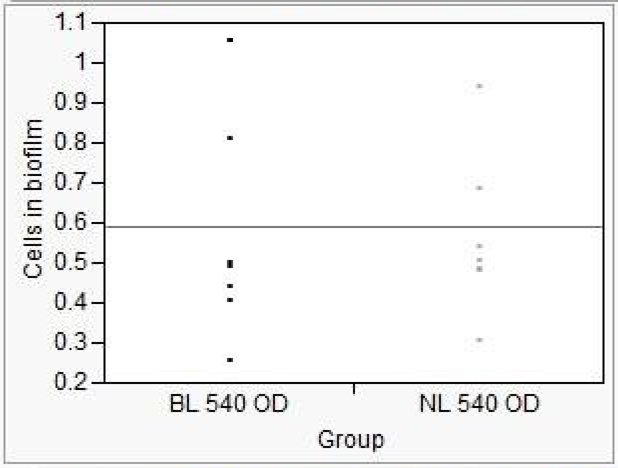
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
93	2.57491	0.0100*

The p-value 0.0100<0.05, so the group effect is significant.

Strain 3206

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	67.000	68.000	8.37500	-0.053
NL 540 OD	8	69.000	68.000	8.62500	0.053

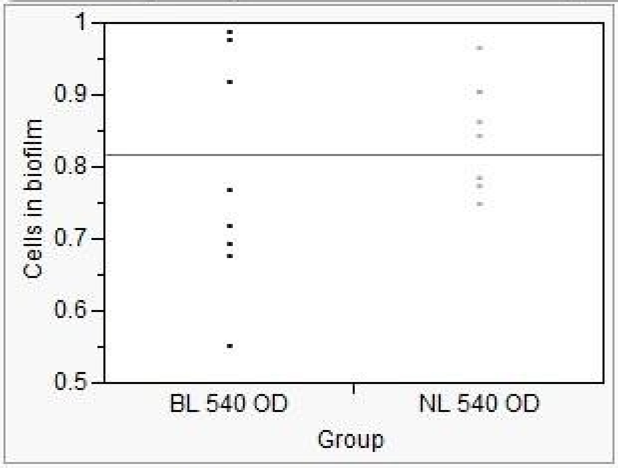
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
69	0.05251	0.9581

The p-value 0.9581>0.05, so the group effect is not significant.

Strain 349

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	60.000	68.000	7.50000	-0.788
NL 540 OD	8	76.000	68.000	9.50000	0.788

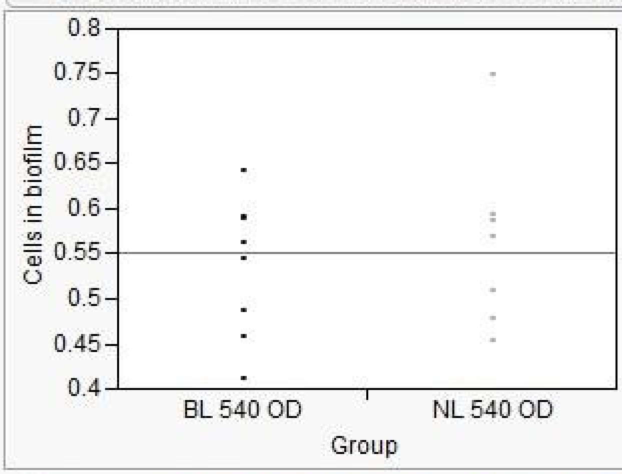
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
76	0.78766	0.4309

The p-value 0.4309>0.05, so the group effect is not significant.

Strain 330

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	64.000	68.000	8.00000	-0.368
NL 540 OD	8	72.000	68.000	9.00000	0.368

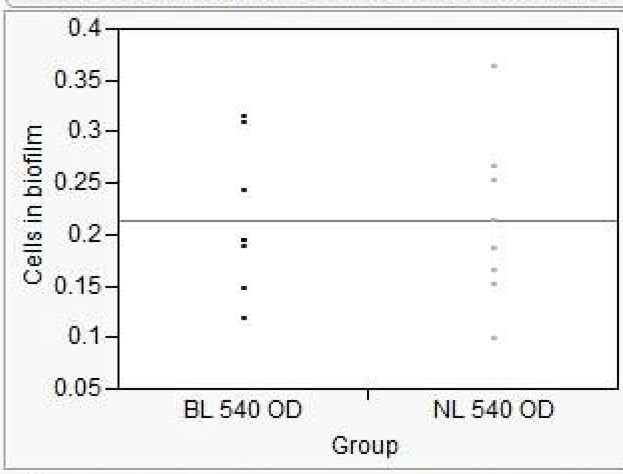
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
72	0.36757	0.7132

The p-value 0.7132>0.05, so the group effect is not significant.

Strain 2860

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	69.000	68.000	8.62500	0.053
NL 540 OD	8	67.000	68.000	8.37500	-0.053

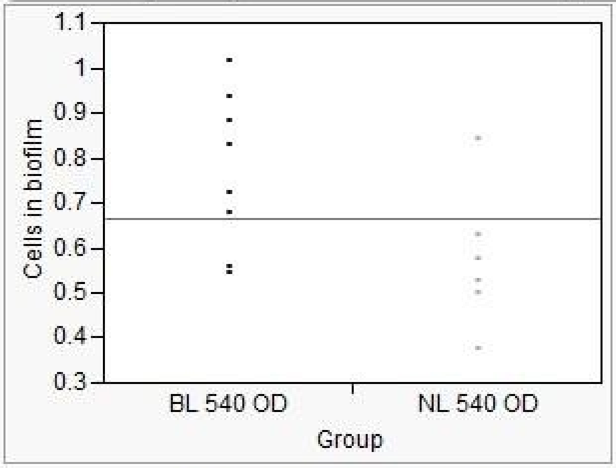
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
67	-0.05251	0.9581

The p-value 0.9581>0.05, so the group effect is not significant.

Strain 2344

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	91.000	68.000	11.3750	2.365
NL 540 OD	8	45.000	68.000	5.6250	-2.365

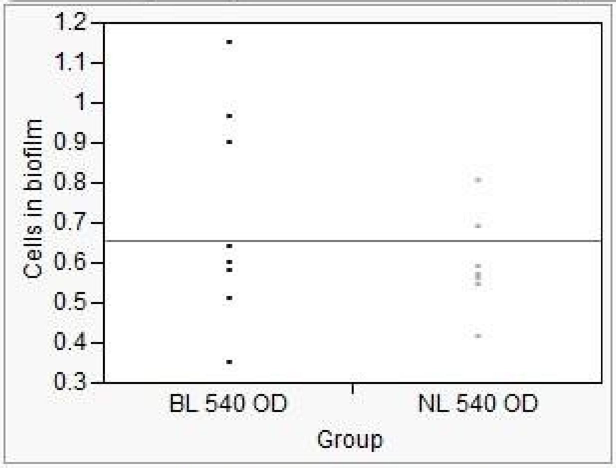
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
45	-2.36471	0.0180*

The p-value 0.0180<0.05, so the group effect is significant.

Strain 39

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	77.000	68.000	9.62500	0.893
NL 540 OD	8	59.000	68.000	7.37500	-0.893

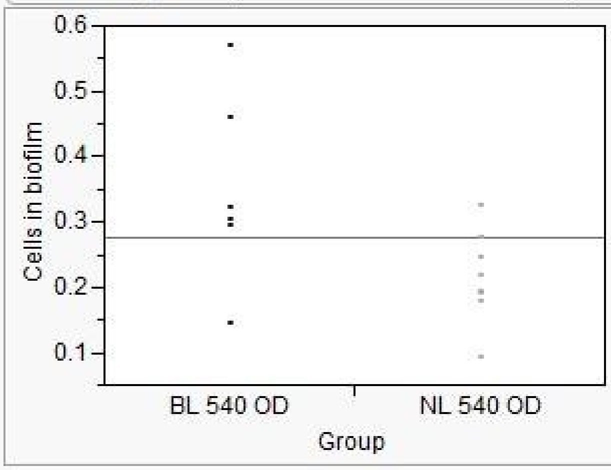
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
59	-0.89268	0.3720

The p-value 0.3720>0.05, so the group effect is not significant.

Strain 3017

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	88.000	68.000	11.0000	2.048
NL 540 OD	8	48.000	68.000	6.0000	-2.048

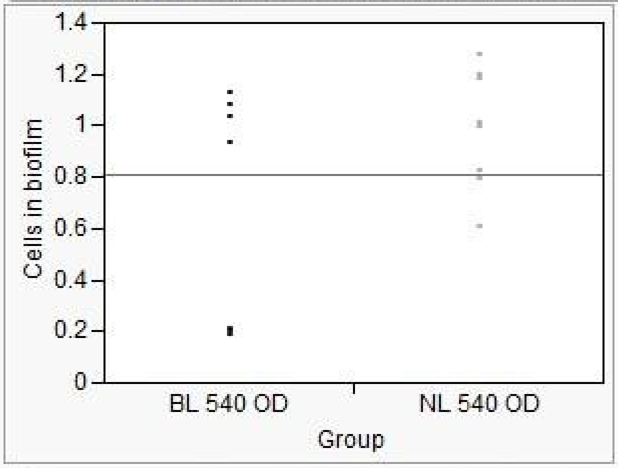
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
48	-2.04791	0.0406*

The p-value 0.0406<0.05, so the group effect is significant.

Strain 1705

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	54.000	68.000	6.7500	-1.418
NL 540 OD	8	82.000	68.000	10.2500	1.418

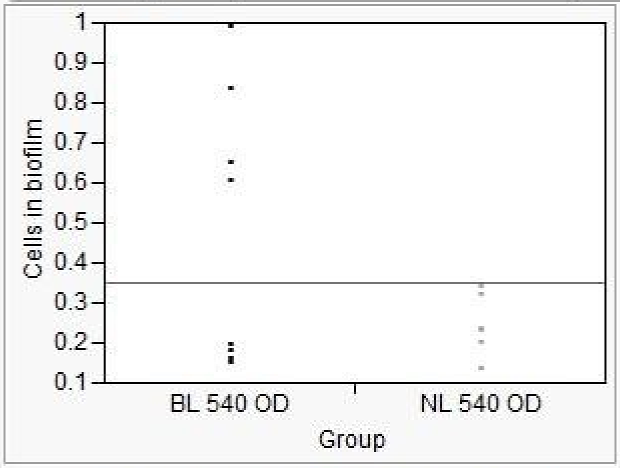
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
82	1.41778	0.1563

The p-value 0.1563>0.05, so the group effect is not significant.

Strain 1079

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	76.000	68.000	9.50000	0.788
NL 540 OD	8	60.000	68.000	7.50000	-0.788

2-Sample Test, Normal Approximation

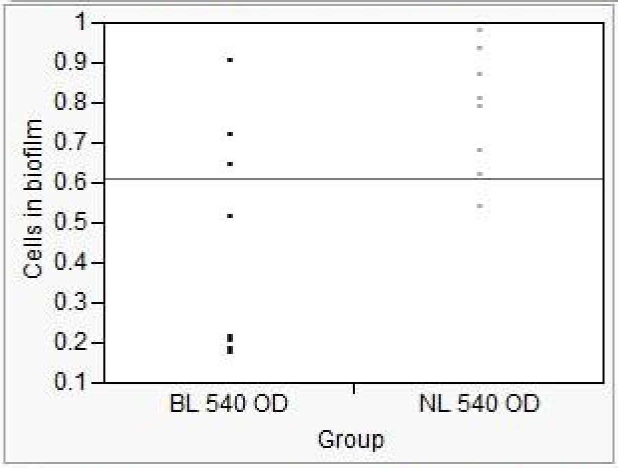
S	Z	Prob>|Z|
60	-0.78824	0.4306

The p-value 0.4306>0.05, so the group effect is not significant.

.

Strain 1127

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	47.000	68.000	5.8750	-2.153
NL 540 OD	8	89.000	68.000	11.1250	2.153

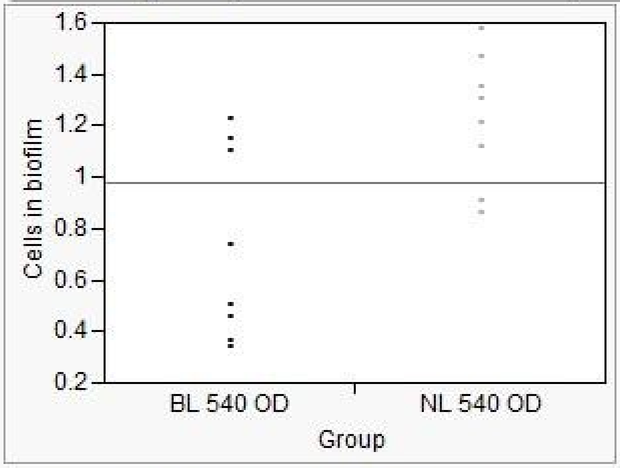
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
89	2.15293	0.0313*

The p-value 0.0313<0.05, so the group effect is significant.

Strain 1130

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	45.000	68.000	5.6250	-2.363
NL 540 OD	8	91.000	68.000	11.3750	2.363

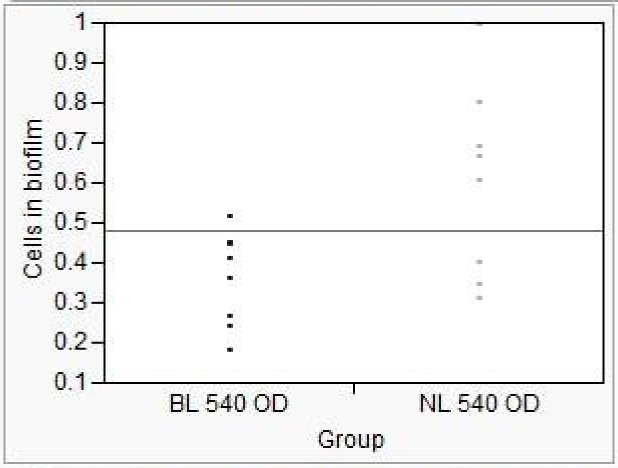
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
91	2.36297	0.0181*

The p-value 0.0181<0.05, so the group effect is significant.

Strain 1020

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	50.000	68.000	6.2500	-1.838
NL 540 OD	8	86.000	68.000	10.7500	1.838

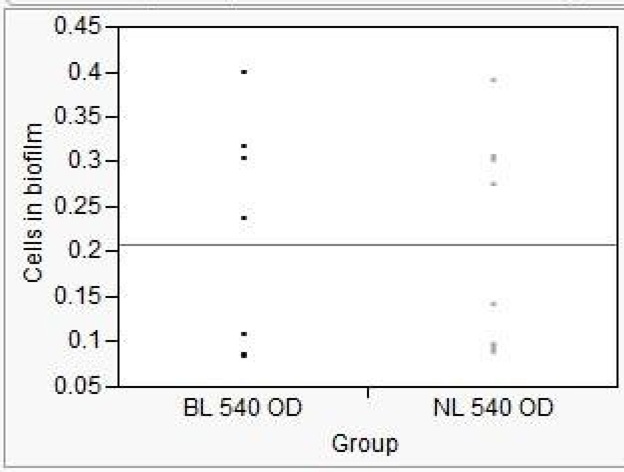
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
86	1.83787	0.0661

The p-value 0.0661>0.05, so the group effect is not significant.

Strain 1024

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	64.000	68.000	8.00000	-0.368
NL 540 OD	8	72.000	68.000	9.00000	0.368

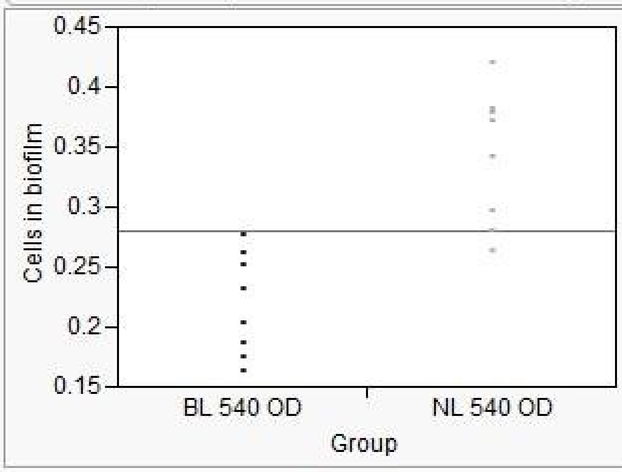
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
72	0.36757	0.7132

The p-value 0.7132>0.05, so the group effect is not significant.

Strain 1025

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	37.000	68.000	4.6250	-3.203
NL 540 OD	8	99.000	68.000	12.3750	3.203

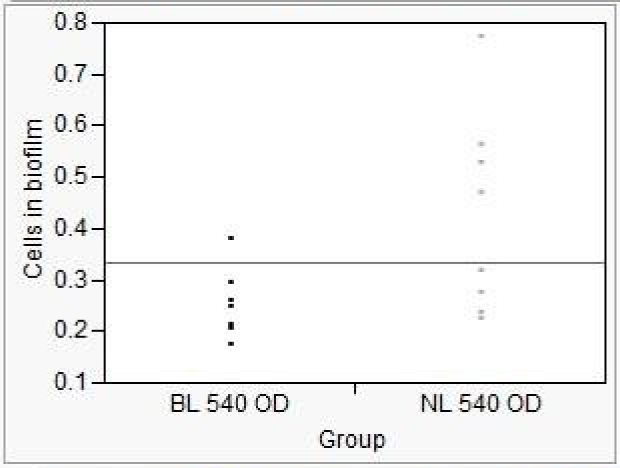
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
99	3.20314	0.0014*

The p-value 0.0014<0.05, so the group effect is significant.

Strain 1026

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	47.000	68.000	5.8750	-2.155
NL 540 OD	8	89.000	68.000	11.1250	2.155

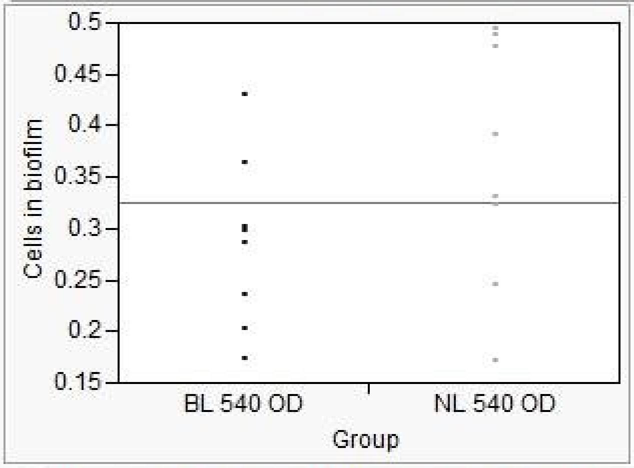
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
89	2.15452	0.0312*

The p-value $0.0312 < 0.05$, so the group effect is significant.

Strain 1214

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	54.000	68.000	6.7500	-1.418
NL 540 OD	8	82.000	68.000	10.2500	1.418

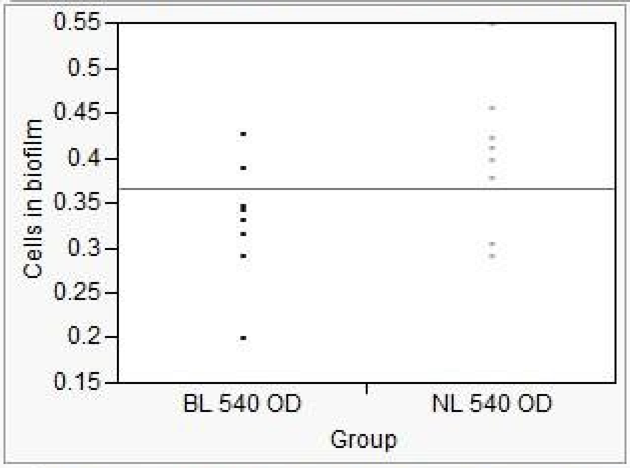
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
82	1.41778	0.1563

The p-value 0.1563>0.05, so the group effect is not significant.

Strain 239

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	54.000	68.000	6.7500	-1.418
NL 540 OD	8	82.000	68.000	10.2500	1.418

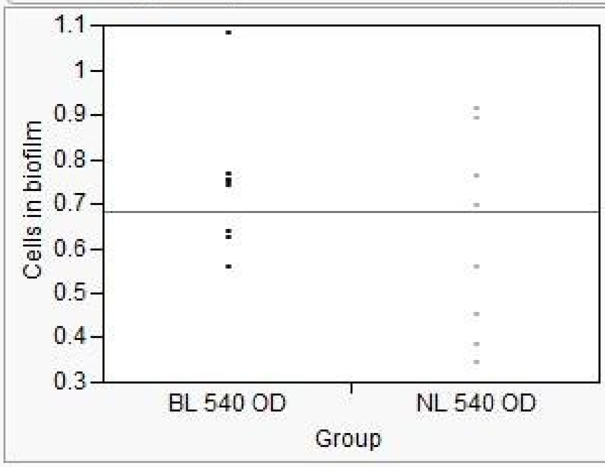
2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
82	1.41778	0.1563

The p-value 0.1563>0.05, so the group effect is not significant.

Strain 227

Oneway Analysis of Cells in biofilm By Group



Wilcoxon / Kruskal-Wallis Tests (Rank Sums)

Level	Count	Score Sum	Expected Score	Score Mean	(Mean-Mean0)/Std0
BL 540 OD	8	76.500	68.000	9.56250	0.841
NL 540 OD	8	59.500	68.000	7.43750	-0.841

2-Sample Test, Normal Approximation

S	Z	Prob>|Z|
59.5	-0.84079	0.4005

The p-value 0.4005>0.05, so the group effect is not significant.

BIBLIOGRAPY

1. Arce, M., Sotomayor, J. E., Albesa, C. E., & Paraje, M. G. (2011). Oxidative and nitrosative stress in staphylococcus aureus biofilms. *FEMS Microbiology Letters, 315*(1), 23-29.

2. Arciola, C. R., Speziale, P., & Montanaro, L. (2009). Perspectives on DNA vaccines targeting staphylococcal adhesins to prevent implant infections. *The International Journal of Artificial Organs, 32*(9), 635-641.

3. Arciola, C.R., Campoccia, D., Speziale, P., Montanaro, L. & Costerton, J.W. (2012). Biofilm formation in Staphylococcus implant infections. A review of molecular mechanisms and implications for biofilm-resistant materials. *Biomaterials, 33*(26), 5967-5982. doi:10.1016/j.biomaterials.2012.05.031.

4. Arvidson, S. (2009). Virulence Gene Regulation and their role in pathogenesis of disease. *Staphylococcus aureus: Molecular and Clinical Aspects*. Horwood Publishing Limited. p. 154-160.

5. Beenken, K. E., Dunman, P. M., McAleese, F., Macapagal, D., Murphy E., Projan, S. J., Blevins, J. S., & Smeltzer, M. S. (2004). Global gene expression in staphylococcus aureus biofilms. *Journal of Bacteriology (186).*

6. Biel, M. A. (2010). Photodynamic therapy of bacterial and fungal biofilm infections. *Methods in Molecular Biology (Clifton, N.J.), 635*, 175-194. doi:10.1007/978-1-60761-697-9_13

7. Cabiscol, E., Tamarit, J., & Ros, J. (2000). Oxidative stress in bacteria and protein damage by reactive oxygen species. *International Microbiology, 3*(3), 3-8.

8. Carr, J. & Mandhalapu, D. (2005). *SEM of MRSA.* Centers for Disease Control and Prevention's Public Health Image Library.

9. Diemond-Hernandez, B., Solorzano-Santos, F., Leanos-Miranda, B., Peregrino-Bejarano, L., & Miranda-Novales, G. (2010). Production of icaADBC-encoded polysaccharide intercellular adhesin and therapeutic failure in pediatric patients with staphylococcal device-related infections. *BMC Infectious Diseases, 10*, 68. doi:10.1186/1471-2334-10-68

10. Dougherty, T. J. & Marcus, S. L. (1989). Photodynamic therapy: status and potential. *Oncology, 3*(7), 67-73.

11. Elixhauser, A., Friedman, B., & Stranges, E. (2011). *Septicemia in U.S. hospitals, 2009*. HCUP Statistical Brief #122. Agency for Healthcare Research and Quality, Rockville, MD. http://www.hcup-us.ahrq.gov/reports/statbriefs/sb122.pdf

12. Enwemeka, C. S., Williams, D., Hollosi, S., Yens, D., & Enwemeka, S. K. (2008). Visible 405 nm SLD light photo-destroys methicillin-resistant *staphylococcus aureus* (MRSA) in vitro. *Lasers in Surgery and Medicine Journal, 40*, 734-737.

13. Fitzgerald, J. R., Sturdevant, D.D., Mackie, S. M., Gill, S. R., & Musser, J. M. (2001). Evolutionary genomics of staphylococcus aureus: Insights into the origin of methicillin-resistant strains and the toxic shock syndrome epidemic. *Proceedings of the National Academy of Sciences of the United States of America*, 98(15), 8821-8826.

14. Foster, T. J. (2004). The staphylococcus aureus "superbug." *Journal of Clinical Investigation, 144*(12), 1693-1696. doi:10.1172/jCI23825.

15. Gad, F., Zahra, T., Hasan, T., & Hamblin, M. R. (2004). Effects of growth phase and extracellular slime on photodynamic inactivation of gram-positive pathogenic bacteria. *Antimicrobial Agents and Chemotherapy, 48*(6), 2173-2178. doi:10.1128/AAC.48.6.2173-2178.2004

16. Goerke, C., & Wolz, C. (2004). Regulatory and genomic plasticity of staphylococcus aureus during persistent colonization and infection. *International Journal of Medical Microbiology, 294*(2–3), 195-202. doi:10.1016/j.ijmm.2004.06.013

17. Gold, M. H., Andriessen, A., Biron, J., & Andriessen, H. (2009). Clinical efficacy of self-applied blue light therapy for mild-to-moderate facial acne. *The Journal of Clinical and Aesthetic Dermatology, 2*(3), 44-50.

18. Harrison, J. J., Turner, R. J., & Ceri, H. (2005). Persister cells, the biofilm matrix and tolerance to metal cations in biofilm and planktonic pseudomonas aeruginosa. *Environmental Microbiology, 7*(7), 981-994.

19. Karaolis, D. K., Rashid, M. H., Chythanya, R., Luo, W., Hyodo, M., & Hayakawa, Y. (2005). c-di-GMP (3'-5'-cyclic diguanylic acid) inhibits staphylococcus aureus cell-cell interactions and biofilm formation. *Antimicrobial Agents and Chemotherapy, 49*(3), 1029-1038. doi:10.1128/AAC.49.3.1029-1038.2005

20. Keski, T. A. & Tomasz, A. (2005). Role of penicillin-binding protein 2 (PBP2) in the antibiotic susceptibility and cell wall cross-linking of Staphylococcus aureus: Evidence for the cooperative functioning of PBP2, PBP4, and PBP2A. Journal of Bacteriology, 187(5), 1815-1824. doi:10.1128/JB.187.5.1815-1824.2005

21. Krivit, B. A. & Heurertz, R. M. (2011). Bacterial biofilms and healthcare-associated infections. *Medical Laboratory Observer.* Retrieved from http://www.mlo-online.com/articles/201106/bacterial-biofilms-and-healthcare-associated-infections.php

22. Kuehnert, M. J., Kruszon-Moran, D., Hill, H. A., McQuillan, G., McAllister, S. K., Fosheim, G., McDougal, L. K., Chaitram, J., Jensen, B., Fridkin, S. K., Killgore, G. & Tenover, F. C. (2004). Prevalence of staphylococcus aureus nasal colonization in the United States, 2001-2002. *Journal of Infectious Disease, 193*(2), 172-179. doi:10.1086/499632.

23. Lipovsky, A., Nitzan, Y., Gedanken, A., & Lubart, R. (2010). Visible light-induced killing of bacteria as a function of wavelength: Implication for wound healing. *Lasers in Surgery and Medicine, 42*(6), 467-472. doi:10.1002/lsm.20948

24. Lipovsky, A., Nitzan, Y., & Lubart, R. (2008). A possible mechanism for visible light-induced wound healing. *Lasers in Surgery and Medicine, 40*(7), 509-514. doi:10.1002/lsm.20668

25. Lopez, D., Vlamakis, H., & Kolter, R. (2010). Biofilms. *Cold Spring Harbor Perspectives in Biology, 2*(7), 398.

26. Maclean, M., MacGregor, S. J., Anderson, J. G., & Woolsey, G. (2009). Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array. *Applied and Environmental Microbiology, 75*(7), 1932-1937. doi:10.1128/AEM.01892-08

27. Merritt, J. H., Kadouri, D. E., & O'Toole, G. A. (2005). Growing and analyzing static biofilms. *Current Protocols in Microbiology, Chapter 1*, Unit 1B.1. doi:10.1002/9780471729259.mc01b01s00

28. Nakonechny, F., Firer, M. A., Nitzan, Y., & Nisnevitch, M. (2010). Intracellular antimicrobial photodynamic therapy: A novel technique for efficient eradication of pathogenic bacteria. *Photochemistry and Photobiology, 86*(6), 1350-1355. doi:10.1111/j.1751-1097.2010.00804.x; 10.1111/j.1751-1097.2010.00804.x

29. Ragle, B.E. (2010). Studies in the area of pneumonia reported from B.E. Ragle and co-researchers. *Vaccine Weekly, (52).*Retrieved from http://www.newsrx.com/newsletters/Vaccine-Weekly/2010-02-17/420217201015VW.html.

30. Rubinstein, E. (2011). Pneumonia caused by methicillin-resistant *staphylococcus aureus*. *Clinical Infectious Diseases, 46*(5), 378-385.

31. Santos, A. L., Oliveira, V., Baptista, I., Henriques, I., Gomes, N. C. M., Almeida, A., et al. (2013). Wavelength dependence of biological damage induced by UV radiation on bacteria. *Arch Microbiology, 195*, 63-74.

32. Sharma, M., Visai, L., Bragheri, F., Cristiani, I., Gupta, P. K., & Speziale, P. (2008). Toluidine blue-mediated photodynamic effects on staphylococcal biofilms. *Antimicrobial Agents and Chemotherapy, 52*(1), 299-305. doi:10.1128/AAC.00988-07

33. Shorr, A. F., Tabak, Y. P., Gupta, V., Johannes, R. S., Liu, L. Z., Kollef, M. H. (2006). Morbidity and cost burden of methicillin-resistant staphylococcus aureus in early onset ventilator-associated pneumonia. *Crit Care, 10*(3): R97. doi: 10.1186/cc4934PMCID: PMC1550967

34. Stepanovic, S., Vukovic, D., Dakic, I., Savic, B., & Svabic-Vlahovic, M. (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *Journal of Microbiological Methods, 40*(2), 175-179.

35. Tambe, Y. (2005). Microscopic image of Staphylococcus aureus (ATCC 25923). Gram staining, magnification:1,000. January 2005.

36. Taraszkiewicz, A., Fila, G., Grinholc, M., & Nakonieczna, J. (2013). Innovative strategies to overcome biofilm resistance. *Biomedical Research International.*

37. Upadya, H., A. Kishen. (2010). Influence of bacterial growth modes on the susceptibility to light-activated disinfection. *International Endodontic Journal, 43*(11), 978-987. doi:10.1111/j.1265-2591.2010.01717.x

38. WHO: World Health Organization. (2011). Infectious diseases. Initiative for Vaccine Research. Retrieved from http://www.who.int/vaccine_research/development/en/.

39. Zmantar, T., Kouidhi, B., Miladi, H., Mahdouani, K., & Bakhrouf, A. (2010). A microtiter plate assay for staphylococcus aureus biofilm quantification at various pH levels and hydrogen peroxide supplementation. *The New Microbiologica, 33*(2), 137-145.