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Effectiveness of Commercially-Available Cosmetic Cleaners on Cosmetics and Cosmetic Brushes

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EFFECTIVENESS OF COMMERCIALLY-AVAILABLE COSMETIC CLEANERS ON COSMETICS AND COSMETIC BRUSHES

By

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ABSTRACT

Effectiveness of commercially-available cosmetic cleaners on cosmetics and cosmetic brushes

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The complex nature of skin contributes to the microbial population present on its surface. While normal skin flora is either beneficial or has no effect on the body, there are instances where pathogenic microorganisms are present and can cause infections. Damaged skin is more susceptible to infections from these microbes. Behavioral characteristics, such as the use of cosmetics, can affect the microbial population present on the skin. *Staphylococcus aureus* is the organism most commonly isolated from cosmetics, and it can be responsible for conjunctivitis, impetigo, boils, and folliculitis. There are many ways microbial contamination of cosmetics can occur, such as ineffective preservatives and consumer habits. With the advent of commercially-available cosmetic cleaning products, consumers may have a plausible means of reducing contamination on their cosmetics and cosmetic brushes. The objectives of this study were to determine the effectiveness of commercially-available cosmetic cleaners in reducing microbial contamination on cosmetics and cosmetic brushes. Cosmetics (i.e., eyeshadow/blush and lipstick) and large and small cosmetic brushes were inoculated with a known concentration of *S. aureus*, allowed a 0-, 1-, or 5-minute contact time, and treated with commercially-available cleaning products. Isopropyl alcohol and a cotton pad were compared to commercially-available

sprays, wipes, and shampoos. Unused cosmetics and brushes were inoculated with the target organism, and culture analysis was used to determine the reduction of microbial concentration on cosmetics and cosmetic brushes after cleaning. On eyeshadows, the cotton pad exhibited a significantly greater reduction in microbial contamination compared to spray #2; 99.44% and 37.86%, respectively. For the lipsticks, both wipe #2 (99.77% reduction) and 70% isopropanol wipe (99.56%) had a significantly greater reduction in microbial concentration compared to the cotton pad (96.18%). For contact times, there were no statistically significant results. In addition, there were no statistically significant results for products used on the small brushes. On the large brushes, the wipes (98.01%) exhibited a greater percent reduction of microbial contamination compared to shampoos (89.92%). The results of this study demonstrate that cleaning products, regardless of contact time with the microorganisms, cleaning product type, or cleaning product brand, were effective in reducing microbial contamination on cosmetics and cosmetic brushes. These results may provide valuable information to consumers about the importance of regular maintenance of their cosmetics and cosmetic brushes.

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DEDICATION

This thesis is dedicated to the memory of my beautiful grandmother, Nilsa “Ita” Rodriguez.

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CHAPTER 1

BACKGROUND

The skin is an integral and complex part of the human body. While the main function of the skin is to protect the internal body from infection, the skin itself is constantly colonized with a variety of microorganisms, which include viruses, bacteria, fungi, and protozoa (Oluwole et al., 2013). The typical skin microbiota is usually mutualistic or commensal; this means that the microbial population is beneficial or has no effect on the human body (Grice et al., 2008). However, there are instances where pathogenic microorganisms are present and can cause infections. Damaged skin is most susceptible to infections from these microbes. It is in the presence of pathogenic microorganisms that we see the progression of skin infections, such as acne and dermatitis (Grice et al., 2008).

The complex nature of skin contributes to the microbial population present; these characteristics include moisture, temperature, pH, sebum content, and hair follicles (Grice et al., 2008). In addition to skin composition, there are several other factors that influence skin microbiota. These factors are host demographics, host genetics, transmission of non-resident microorganisms, environmental characteristics, and behavioral characteristics (Fredricks, 2001). Host demographics refer to characteristics, such as ethnicity, age, and gender; all of these factors are unique and vary among individuals. One study involving these factors focused on finding an association between host demographics and microbial populations on the skin (Rosenthal et al., 2011). This study found that ethnicity was “a significant predictor of skin health” (Rosenthal et al., 2011, p. 847). Host genetics largely determines the host’s immune response. Specifically, the innate, or non-specific, immune response is known to be associated with regulating the microbial

environment on epithelial surfaces. Past studies have focused on discovering variations in the human genome that may influence the microbial composition on the skin (Fredricks, 2001).

Transmission of non-resident microorganisms involves the removal, or introduction, of new species of microorganisms, and the interaction among species in the current microbial population on the skin. Direct contact with people, fomites, and the environment has the potential to introduce new microorganisms into the microbial population present on the surface of the skin. Introduction of foreign microorganisms also has the potential to cause inter-species interactions (Fredricks, 2001). For example, *Propionibacterium acnes* and *Staphylococcus aureus* have been known to work together, or synergistically, to cause significant skin lesions not seen with either of these bacteria alone (Rosenthal et al., 2011). Conversely, some bacteria have the ability to compete with other microbes present. In these instances, the microorganisms utilize antagonistic mechanisms, such as the production of toxic by-products, inhibition of adherence, and depletion of nutrients (Rosenthal et al., 2011). Environmental factors, such as ultraviolet (UV) radiation exposure and temperature, alter the structure of the skin, which can have a direct influence on the microbial population inhabiting the area. Every individual is exposed to different environments; for example, the microorganisms present in a classroom can vary significantly from that of a hospital. Therefore, the normal flora of an individual exposed to one of these environments can vary significantly from a person exposed to a different location.

Behavioral characteristics are those actions carried out by the host, such as the use of cosmetics and hand hygiene. Hand washing works by removing the top layer of oil and cutaneous microflora from the skin (Oluwole et al., 2013). Other behavioral characteristics believed to be associated with the disturbance of the natural skin microbiota include exposure to the sun, smoking, and diet (Rosenthal et al., 2011). Cosmetics can become contaminated with

various microorganisms and can disturb the normal microbial flora of the skin which can lead to skin infections.

CHAPTER 2

INTRODUCTION

Normal Skin Flora

Every individual's skin consists of intricate and diverse microbial populations (Chen et al., 2013). Each habitat on the skin has its own characteristics which dictate the microbial diversity and variability of that area. Colonizing microbes obtain nutrients for the skin in the form of proteins and fats (Fredricks, 2001). In order to colonize the skin, microbes must compete with one another for nutrients and space. On normal skin, microbes sustain an equilibrium amongst themselves to maintain their environment; this is believed to help prevent pathogens from colonizing the area (Fredricks, 2001). At the microscopic level, the skin contains uneven surfaces in the forms of groves and ridges (Kong, 2011). Structures such as nails, sebaceous glands, and hair follicles provide unique environments for microorganisms. On a macroscopic level, areas such as the back, forearm, armpit, and nose have unique characteristics that provide an ideal habitat for specific microbes. Those areas of the skin with lower exposures to the environment exhibit more stable communities of bacteria. Conversely, exposed areas, such as the palm of the hand, exhibit a higher variability of microorganisms (Chen et al., 2013).

Normal skin flora includes *Staphylococcus aureus, Staphylococcus epidermidis, Propionibacterium acnes,* and *Pseudomonas aeruginosa* (Kong, 2011). Bacterial populations are categorized as: resident (which grow and reproduce), temporary resident (non-resident, yet can colonize), and transient (contaminants that do not reproduce) flora (Kong, 2011). Researchers are working on elucidating the intricate relationship that exists between the host and microorganisms. These studies are not just focusing on pathogenic organisms, but also the consequences that occur due to imbalances of the commensal microbes present on the skin.

Skin Infections

It is estimated that at a given time, over a million bacteria can inhabit an area as small as a square centimeter on the surface of the skin (Chen et al., 2013). Microbes on the skin can cause noninfectious disorders such as rosacea, psoriasis, atopic dermatitis, and acne (Chen et al., 2013). The presence of *Staphylococcus aureus* and *Propionibacterium acnes* on the skin are the major causes of acne (Hillion et al., 2013). Skin conditions, such as folliculitis, furunculosis, cellulitis, and impetigo, have been shown to be caused by several different microorganisms; some of these microbes include *S. aureus*, *S. pyogenes*, and *Pseudomonas aeruginosa*. Most skin infections are multifactorial; for example, environmental factors combined with the presence of pathogenic microorganisms can lead to the progression of an infection. Studies have shown that over "90% of cultivable human skin bacteria" can be placed within the following groups: Firmicutes, Actinobacteria, and Proteobacteria (Hillion et al., 2013, p. 959).

Atopic dermatitis (AD) is a non-communicable, chronic skin condition that is believed to be associated with changes in the microbial population present on the skin; this condition is commonly known as eczema. AD affects 10-20% of children and 1-3% of adults; however, the prevalence of this disorder has increased three-fold within the past several years **(**Nutten, 2015**).** While AD is typically common among children and adolescents, the disorder can either resolve itself or remain throughout adulthood. In addition, adult onset of AD is also possible. Although the actual cause of AD is unknown, many hypothesize that "colonization of *S. aureus* and immune hypersensitivity" could be to blame for this disorder (Chen et al., 2013, p. 146). There are various treatments that have proven to be effective against AD and they include steroids, antibiotics, and dilute bleach baths. These treatment options function by reducing the bacterial load present on the skin thus slowing down the body's immune response. Colonization and

infection with *S. aureus* has typically been associated with AD. During AD flares, studies have found that species of *Staphylococcus* increased from 35% to 90%; interestingly, this increase was largely seen with *S. aureus* and *S. epidermidis* (Chen et al., 2013).

Acne vulgaris, a common skin condition, is characterized by blocked pores, cysts, papules, and pustules (Fredricks, 2001). Approximately 80% of adolescents are affected by acne. Some factors associated with the pathogenesis of acne include inflammation, excess sebum, and the presence of the microorganism *Propionibacterium acnes* (Numata et al., 2014). Acne is clinically diagnosed by the presence of *Propionibacterium* and *Staphylococcus* species. The causes of acne are separated into two categories: external factors and acneiform eruptions. External factors are substances that block pores, such as cosmetics. Other factors include environmental conditions (such as temperature), the presence of mites, and prolonged physical contact or friction. Acneiform eruptions are typically caused by the use of medications (such as steroids), genetics, and hormonal imbalances. Treatment of acne is difficult and varies widely depending on the severity and individual characteristics of the skin (Lovecková et al., 2002). Rosacea, another common skin disorder, typically affects the face of adults and is characterized by patchy redness, visible dilation of capillaries, and inflammation (Fredricks, 2001).

Chronic wounds were found to be less microbially diverse than healthy skin, but no specific organisms were found to be associated with this condition. On the other hand, the microbiome of follicles afflicted with acne was found to be more diverse than that of healthy follicles; however, acne follicles are colonized mainly by *P. acnes*. With psoriasis, it is still unknown whether there is a difference between the microbiome of psoriatic plaques and normal skin (Chen et al., 2013).

Overall, there is a lack of knowledge of how dermatological treatments affect the microbiome of the skin. Interestingly, the reason for the use of antibiotics for the treatment of these disorders is not fully understood. With the increased use of antibiotics, antibiotic resistance among skin flora has become a concern. It is believed that the resistant genes can be spread among the organisms of the normal flora and to transient or contaminant organisms (Lalitha et al., 2013). Elucidating the types of bacteria present on the skin with these conditions may help explain how antibiotic use is correlated with changes in the microbial population of the skin and whether this treatment option is appropriate.

Staphylococcus aureus

Staphylococcal species are among the most abundant microbial species present on the skin. The main species present on normal skin is *S. epidermidis*; it is believed that this organism protects the host from pathogenic microbes. Several species of *Staphylococcus* cause a wide range of disease, from localized skin disorders to systemic infections (Coates et al., 2014). It is believed that other microbial flora can have a huge impact on *S. epidermidis* or *S. aureus*, found on the skin (Chen et al., 2013).

Staphylococcus aureus infections range from asymptomatic to severe. *S. aureus* is commonly found in 20-30% of nasal passages of healthy individuals, but it can also cause skin infections such as impetigo or dermatitis (Kong, 2011). The increase in antibiotic resistance of *S. aureus* has led to a decrease in treatment options which makes this pathogen an important public health issue (Chen et al., 2013).

As discussed above, AD, a chronic skin disease, is commonly associated with *S. aureus* infections (Kong, 2011). In order to understand how *S. aureus* affects AD flares, it is necessary to understand how it typically functions within the normal skin flora. Some studies have shown

that *S. epidermidis* has the ability to inhibit the growth and colonization of *S. aureus*; thus the theory that *S. epidermidis* may be an antagonist to *S. aureus*. However, it is still unknown exactly how these two species interact with one another but the two main theories are: (1) the presence of *S. epidermidis* surges due to an increase in *S. aureus* present on the skin or (2) *S. aureus* and *S. epidermidis* work together to aid in the colonization of both species (Chen et al., 2013). Other, non-staphylococcal, species have been seen to increase during an AD flare. More research is needed to understand whether (1) the increased growth of staphylococcal species causes a change in other species present or (2) a change on the host's skin causes a change in the microbial composition, which leads to staphylococcal species growing in abundance (Chen et al., 2013). Discovering what role *S. aureus* plays in the fluctuation of the skin flora can lead to new treatment options, such as focusing on correcting the normal microbial balance of the skin rather than complete elimination of the pathogen. Understanding how the skin microbiota is associated with AD may also help us understand other conditions like acne, psoriasis, and chronic wounds (Chen et al., 2013).

S. aureus and *S. pyogenes* are also the cause of impetigo, a common contagious infection among children. Cellulitis, a bacterial infection of the skin marked by redness and inflammation, is also caused by these two organisms. An infection of hair follicles, or folliculitis, is mainly caused by *S. aureus*. Under normal conditions, the skin's characteristics serve as a deterrent for the proliferation of pathogenic organisms. However, when the normal flora is altered, the possibility for microbial adhesion and growth increases (Chiller et al., 2001).

Cosmetics

The Food and Drug Administration (FDA) defines cosmetics as "articles intended for beautifying, cleansing, promoting attractiveness or altering appearance" (Naz et al., 2012, p.

523). Cosmetic powders are utilized to enhance appearance, reduce the signs of aging, and cover up skin imperfections, such as dark circles or blemishes (Dashen et al., 2011). Eyeshadows, and other cosmetics, are made up of inorganic and organic materials which are ideal nutrients that aid in the proliferation of microorganisms; hence the need for preservatives and antimicrobial agents in these products (Dawson et al., 1981). A recent study found that the average person uses nine cosmetics on a daily basis, and over 25% of women use 15 or more products daily (Rastogi et al., 2015). These products have the potential to become contaminated with *P. aeruginosa, S. aureus, Clostridium tetani,* molds and yeasts (Dashen et al., 2011).

Various cosmetics are available for immediate use in makeup and department stores; these are called testers. In a study conducted on in-store testers, researchers found that 90% of organisms isolated were representative of normal skin flora, such as *S. epidermidis* (Dawson et al., 1981). In addition, *P. aeruginosa* and *S. aureus* were also commonly found. Customer observation suggests that the main culprit of cosmetic tester contamination was the use of multiple use applicators and fingers. Employee observation showed a lack of disinfection of multiple use applicators and cosmetic testers. Among the different types of multiple use applicators, sponges exhibited the greatest ability to harbor microorganisms due to their ability to accumulate oils, moisture, dead skin, cosmetics, and other materials. Many stores have the option of using disposable applicators, but often these are not easily accessible by the customer. Thus, it is suggested that testers are covered when not in use, the use of fingers is prohibited, and awareness of expiration dates are utilized to help prevent contamination of makeup testers (Dawson et al., 1981).

In addition to shared-use cosmetics, the sharing of cosmetic accessories such as makeup applicators, tweezers, and eyelash curlers have the potential for transmitting infections. Studies

have shown that cosmetic brushes used repetitively contain an increased amount of microbes that can cause skin infections (Naz et al., 2012). One study found that shared-use makeup brushes from a salon were contaminated with 2.28×10^5 colony forming units per milliliter (CFU/ ml) of *S. aureus* (Naz et al., 2012). Thus, it is suggested that proper decontamination of cosmetic tools take place to prevent transmission of infections. Few studies have looked at the microbial contamination of cosmetic brushes. One such study found that 30.3%, 81.8%, and 100% of cosmetic brushes from a beauty salon were contaminated with fungal species, *P. aeruginosa*, and *S. aureus*, respectively (Naz et al., 2012).

Cosmetics with high water content, such as cream-based products, are at a greater risk of microbial contamination (Lundov et al., 2009). Cosmetic packaging plays a major role in maintaining the integrity of the product; reducing the product's exposure to the environment will help reduce the possibility of microbial contamination (Lundov et al., 2009). There are many ways contamination can occur, and they include: manufacturer practices, ineffective preservatives, age of product, and consumer habits (Abdelaziz et al., 1989). Consumer habits, such as failure to properly disinfect cosmetics and the addition of water (to thin out the product), can lead to the likelihood of microbial contamination (Abdelaziz et al., 1989). Sharing of cosmetics can lead to the spread of infections because every individual's skin flora is different. Storage of cosmetics is also important in reducing microbial contamination. Many consumers improperly store cosmetics in the bathroom or other damp areas where microorganisms thrive (Giacomel et al., 2013). To prevent contamination in products with inadequate packaging, it is suggested that tools, such as a spatula, are used to remove products for use. The purpose of adequate packaging is to reduce the product's contact with the environment. Microorganisms in

cosmetics not only affect consumer health, they can also lead to spoilage or deterioration of the product (Birteksoz et al., 2013).

Cosmetic products have the potential to cause infections or allergic reactions. The most common reactions to cosmetics are contact allergies; this is typically due to ingredients within the product, such as fragrances and preservatives. Approximately 6% of the population has a contact allergy associated with cosmetics (Lundov et al., 2009). In addition to contact dermatitis, photosensitivity and irritation can also occur (Giacomel et al., 2013). Although it is just a minor component of cosmetics, preservatives have been shown to cause allergic responses in consumers (Herman et al., 2013). Cosmetics applied to the eye area, such as eyeshadow and mascara, have been associated with serious eye infections. *S. aureus* is the most common organism isolated from cosmetics and is responsible for the following skin infections: conjunctivitis, impetigo, boils, and folliculitis (Birteksoz et al., 2013). Opportunistic pathogens that have been isolated from cosmetic products include *Pseudomonas aeruginosa*, other *Pseudomonas* species, *Klebsiella pneumoniae, Enterobacter* species, and *Serratia* species (Birteksoz et al., 2013). Mascara, which is applied to the eyelashes, has the highest potential for contamination because it is a water-based product and is applied very close to the eye. *P. aeruginosa* is the major contaminant found on mascaras and is responsible for eye infections, such as keratitis and conjunctivitis, which can lead to vision loss (Birteksoz et al., 2013). *S. aureus* and *S. epidermidis* are also commonly found in mascaras (Giacomel et al., 2013).

Corneal infections due to cosmetics are typically exacerbated by abrasions caused by tools, such as a mascara wand. *Staphylococcus* species are the normal causes of these infections among non-contact lens wearers; *P. aeruginosa* is the common culprit among contact lens wearers. The combined use of mascara and contact lenses increases the chance of infection (Pack

et al., 2008). Studies have found that repeated use of the product by multiple individuals greatly increases the chances of pathogenic contamination; this also occurs with single use mascaras, but over a longer period of time. Clinicians recommend that cosmetics are replaced every 6 months, or 3 months for contact lens wearers, to prevent infection. In addition, it is recommended that consumers (1) avoid using old, unclean tools on new cosmetics, (2) replace cosmetics following an infection, (3) put on contact lenses prior to applying mascara and other cosmetics, and (4) avoid sharing cosmetics (Pack et al., 2008). However, most cosmetic users do not discard their makeup until the entire product is gone. A study conducted at the University of Alabama found that cosmetic users reported that a majority of their products were between 6 months to 5 years old (Pack et al., 2008).

Cosmetic Preservatives

Cosmetics are typically made up of the following ingredients: "water, emulsifiers, preservatives, thickeners, colors, fragrances, and stabilizers" (Lalitha et al., 2013, p. 61). The purpose of preservatives in cosmetics is to regulate microbial contamination during the production, storage, and use of the product (Herman et al., 2013). However, preservatives lose effectiveness over time, and prolonged misuse and inadequate storage can exacerbate microbial growth (Ashour et al., 1986). Cosmetics that lack effective preservatives are at an increased risk of microbial contamination and proliferation which can lead to health hazards for the consumer and affect the composition of the product (Ghalleb et al., 2015). Cosmetic preservatives, and other ingredients, are evaluated for safety by the Cosmetic Ingredient Review (CIR); this is an independent, non-profit agency funded by the FDA. The CIR is comprised of individuals representing consumer, industry, toxicology, and dermatology groups (Lundov et al., 2009). The CIR is concerned with labelling products with the appropriate warnings and active ingredients

(Pack et al., 2008). The FDA uses the information obtained by the CIR to help establish guidelines for cosmetics. While in the U.S. cosmetics are required to have a complete list of ingredients, many products are improperly labeled or the consumer is unable to comprehend the list (Lundov et al., 2009).

Cosmetic preservatives can remain on the skin and alter the normal flora; this is especially a concern with prolonged use of the product. The main preservatives seen in cosmetics are parabens and triclosan. Some studies have "proven that *P. aeruginosa* is highly resistant to triclosan" (Lalitha et al., 2013, p. 61). Other common preservatives found in cosmetics are organic acids, organic alcohols, isothiazolinones, and formaldehyde releasers (Birteksoz et al., 2013). The ideal preservative would be non-allergenic, non-toxic, colorless, odorless, and have the ability to inhibit the growth of a wide range of microorganisms (Lundov et al., 2009). However, there are currently no preservatives that meet all of these criteria.

Various types of parabens can be found in cosmetics, such as "methylparaben, propylparaben, butylparaben, and ethylparaben" (Lundov et al., 2009, p. 71). However, methylparaben is the most common preservative seen in cosmetics today. While methylparaben has been shown to be the most effective against fungi, studies have shown it also works well against gram-positive organisms, but it is weakest against *Pseudomonas* species (Herman et al., 2013). Although parabens are ubiquitous in cosmetic products, there is much controversy surrounding this preservative. Studies have suggested that parabens are linked to reproductive and endocrine dysfunction (Birteksoz et al., 2013). The growing controversy with parabens and other preservatives have led to the interest in natural alternative antimicrobials, such as essential oils and herbal remedies (Herman et al., 2013). Some studies have seen a greater inhibition of microbes with essential oils compared to methylparaben; however, the antimicrobial activity of

essential oils is still being studied extensively (Herman et al., 2013). In addition to preservatives, cosmetics often contain other antimicrobial agents, such as chelating agents, phenolic antioxidants, alcohol, fragrance, essential oils and extracts (Birteksoz et al., 2013).

Cosmetic Regulation in the United States

Cosmetics in the United States are regulated according to the Federal Food, Drug, and Cosmetic Act, which is under the jurisdiction of the FDA (Lundov et al., 2009). According to a 1989 FDA report, "Cosmetics are not expected to be totally free of microorganisms when first used or to remain free during consumer use" (Onurdah et al., 2010, p. 9). Because cosmetics are not required to be sterile, the United States Pharmacopoeia (USP) is responsible for articulating the requirements for non-sterile products, such as cosmetics, and has developed protocols to determine the presence of microbial contamination in these products. Specifically, the USP considers the following bacteria as indicators of microbial contamination: *S. aureus*, *P. aeruginosa*, *Escherichia coli*, and *Salmonella* species (Campana et al., 2006). Out of these bacterial indicators, *E. coli*, *P. aeruginosa*, and *S. aureus* are commonly found on cosmetic products (Di Maiuta et al., 2011). In order to prevent the contamination of cosmetics, the use of preservatives is necessary. However, as mentioned before, preservatives lose effectiveness over time.

The International Organization for Standardization (ISO) categorizes cosmetics according to their risk of contamination and details how products should be tested. ISP considers products containing more than 20% alcohol, single use products, or those with no contact with the environment as low risk products and thus do not require microbiological testing. The ISO guidelines were created to help manufacturers and regulators determine what products are potentially at risk and how to detect the risk; these policies are not strictly enforced by the FDA

(Ghalleb et al., 2015). To reduce contamination during production, Good Manufacturing Practices (GMPs) have been utilized to improve the quality of products (Campana et al., 2006). Even with these measures in place, microbial contamination can still occur; thus, the use of effective preservatives is required to prevent contamination during manufacturing, storage, and consumer use (Campana et al., 2006). Due to the use of GMPs and other quality control measures, contamination during manufacturing is no longer a major concern (Tran et al., 1994). However, consumer contamination is still a prevalent concern. With the advent of commercially-available cosmetic cleaning products, consumers may have a plausible means of reducing contamination on their cosmetics and cosmetic brushes. However, data are lacking on the effectiveness of commercially-available cleaners or the use of over-the-counter products, such as rubbing alcohol.

Consumer Concerns

As mentioned above, there is an overall lack of consistency in terms of labeling expiration dates on cosmetics. Different brands of cosmetics utilize various methods of labeling when it comes to expiration dates. These inconsistencies in labeling lead to confusion among consumers. While some brands may explicitly list the date of expiration, others use batch numbers and period after opening (PAO) labels; however, this information is not always listed on cosmetics. Batch numbers typically consist of the date in which the cosmetic was made; batch numbers vary according to manufacturer and product. PAOs are the suggested amount of time, from the moment the cosmetic is opened, before a consumer should discard the product. PAOs are represented by an open jar with a specified amount of time, such as 6M for 6 months. In order for all this information to be useful to a consumer, they must all be present on the labels of cosmetics; of the three labels, expiration date and PAO are the most informative. The expiration

date serves as the definitive date in which the cosmetic must be discarded. The PAO is important because it tells the consumer how long to keep a cosmetic once it has been opened. However, the PAO may sometimes surpass the date of expiration; if the PAO and expiration date are not provided on the label the consumer will have no knowledge of this vital information.

The cost of cosmetics and cosmetic brushes also plays a role in the prolonged use of these products. While the simple solution to the problem of microbial contamination would be to discard products, the high costs of these cosmetics and brushes does not make this prudent for the consumer. Cosmetics and brushes range from drugstore (lower priced) to luxury (higher priced) brands. Advances in the area of cosmetics have led to an increase in the quality and sophistication of cosmetic brushes. Early cosmetic applicators were disposable low quality, sponge brushes. Today, brushes are made out of a variety of materials such as natural or synthetic hair fibers. Thus, cosmetics and cosmetic brushes can cost anywhere from $1 to $200, or more, depending on the brand and material it is made of.

Objectives

The objectives of this study were to determine the effectiveness of commercially-available cosmetic cleaners in removing microbial contamination on cosmetics and cosmetic brushes.

Research Questions

1) Are commercial cleaning products effective on cosmetics such as pressed powders and cream-based products?

a) Are commercial cleaning products effective on cosmetics such as pressed powders and cream-based products at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$?
b) Will each commercial cleaning product brand be effective on cosmetics such as pressed powders and cream-based products?

2) Are commercial cleaning products effective on cosmetic brushes?
   a) Will each commercial cleaning product type be effective on cosmetics brushes?
   b) Will each commercial cleaning product brand be effective on cosmetics brushes?

Hypotheses

H^1_0: There is no difference in microbial concentration on powder-based cosmetics (e.g., eyeshadows) after the use of commercial cleaners at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$.

H^1_a: There is a difference in microbial concentration on powder-based cosmetics (e.g., eyeshadows) after the use of commercial cleaners at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$.

H^2_0: There is no difference in microbial concentration on cream-based cosmetics (e.g., lipsticks) after the use of commercial cleaners at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$.

H^2_a: There is a difference in microbial concentration on cream-based cosmetics (e.g., lipsticks) after the use of commercial cleaners at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$.

H^3_0: There is no difference in microbial concentration reduction between commercial cleaning product brands after use on powder-based cosmetics (e.g., eyeshadows).

H^3_a: There is a difference in microbial concentration reduction between commercial cleaning product brands after use on powder-based cosmetics (e.g., eyeshadows).

H^4_0: There is no difference in microbial concentration reduction between commercial cleaning product brands after use on cream-based cosmetics (e.g., lipsticks).

H^4_a: There is a difference in microbial concentration reduction between commercial cleaning product brands after use on cream-based cosmetics (e.g., lipsticks).

H^5_0: There is no difference in microbial concentration reduction between commercial cleaning product types after use on large (face) brushes.

H^5_a: There is a difference in microbial concentration reduction between commercial cleaning product types after use on large (face) brushes.

H^6_0: There is no difference in microbial concentration reduction between commercial cleaning product brands after use on large (face) brushes.

H^6_a: There is a difference in microbial concentration reduction between commercial cleaning product brands after use on large (face) brushes.

H^7_0: There is no difference in microbial concentration reduction between commercial cleaning product types after use on small (eyeshadow) brushes.

H^7_a: There is a difference in microbial concentration reduction between commercial cleaning product types after use on small (eyeshadow) brushes.

H^8_0: There is no difference in microbial concentration reduction between commercial cleaning product brands after use on small (eyeshadow) brushes.

H^8_a: There is a difference in microbial concentration reduction between commercial cleaning product brands after use on small (eyeshadow) brushes.

CHAPTER 3

MATERIALS AND METHODS

Study Design

Various brands of commercially-available cosmetic cleaning products, cosmetics (eyeshadows and lipsticks), and cosmetic brushes (small and large) were used and tested in this study; the identity of the cosmetic cleaning products will remain confidential. The study was divided into two phases: Phase 1 consisted of control experiments to determine which organism would be used as the inoculum for the tests, and Phase 2 consisted of using the organism determined from Phase 1 to inoculate unused cosmetics and brushes, which were subjected to commercial cleaning products, to determine the reduction of microbial concentrations. In Phase 1 (Figure 1), cosmetics and cosmetic brushes were inoculated with the following organisms identified from review of the scientific literature: *P. aeruginosa* ATCC #27853, *E. coli* ATCC #25922, *S. aureus* ATCC #6538, and *S. epidermidis* ATCC #12228 (American Type Culture Collection, Manassas, VA). In Phase 2 (Figures 2 and 3), unused cosmetic products and cosmetic brushes were inoculated with a known microorganism determined from the control experiments; for cosmetics, the inoculum was left in contact for 0-. 1-, and 5-minutes. The inoculated cosmetics and brushes were then subjected to the appropriate commercial cosmetic cleaners. Using traditional microbiological approaches, the inoculated products were evaluated for microbial growth after the use of cosmetic cleaners. Negative controls consisted of inoculating and processing the cosmetics and brushes without the treatment of cleaning products.

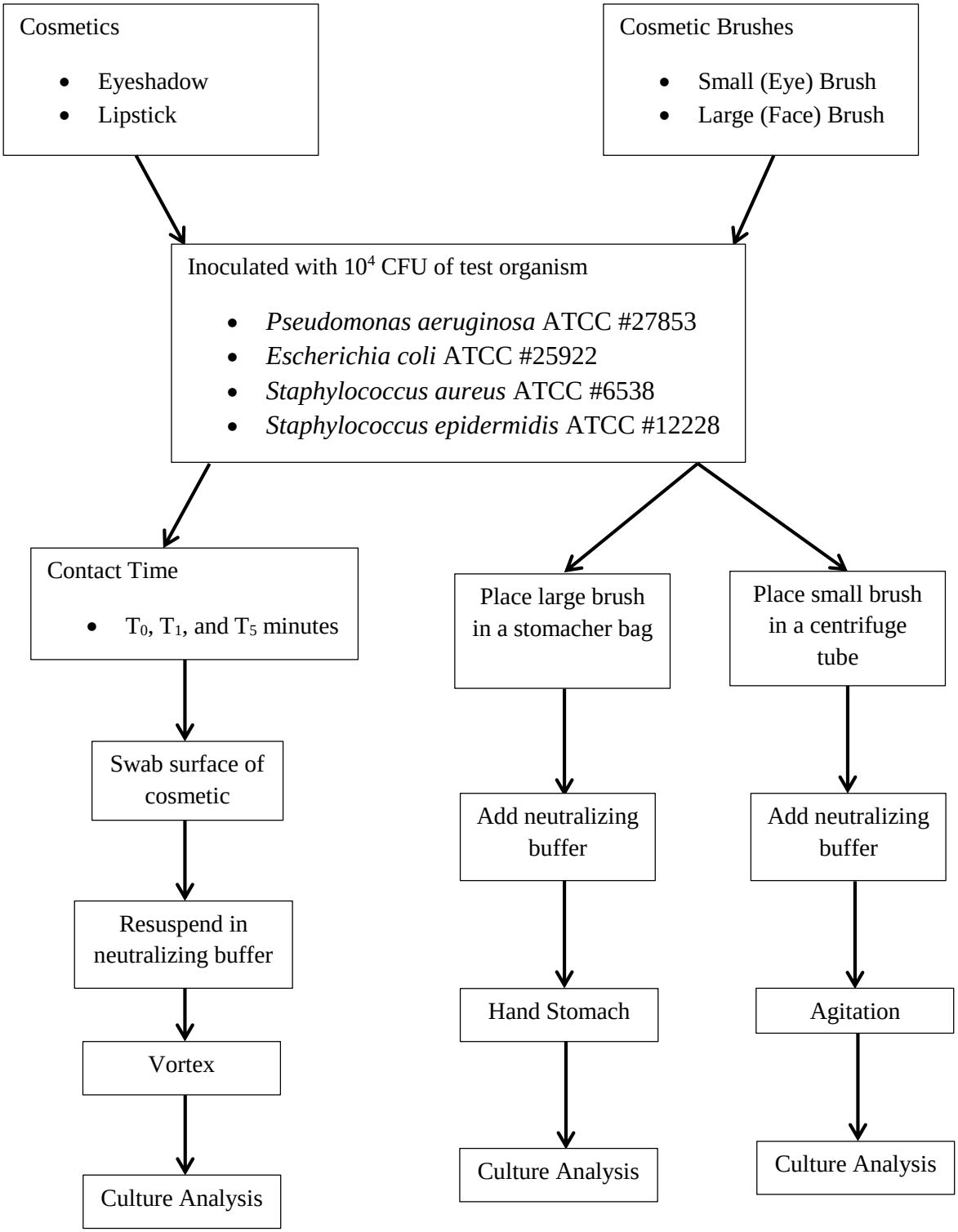

Figure 1 – Flow chart illustrating the experimental design used in Phase 1 of this study.

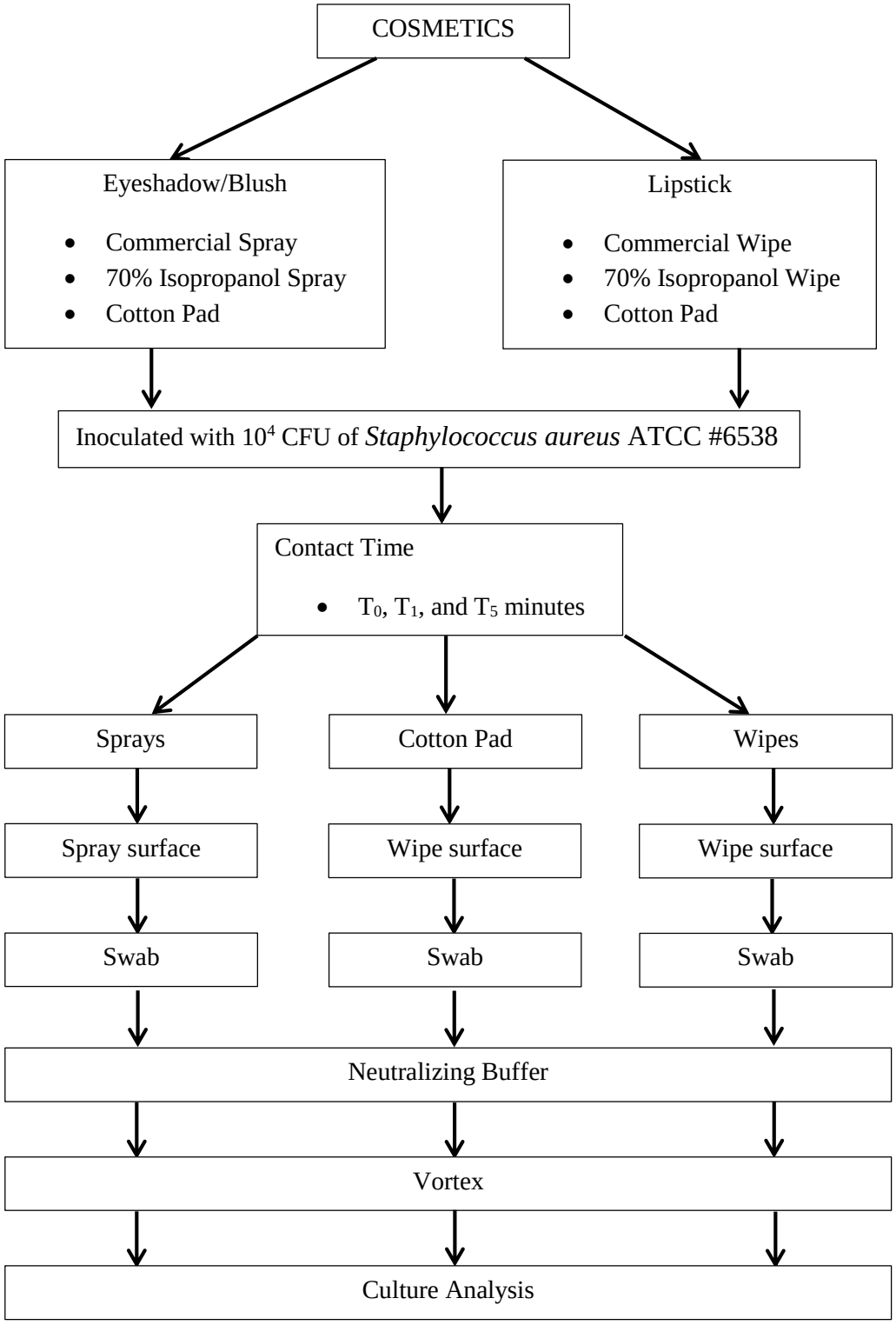

Figure 2 – Flow chart illustrating the experimental design used on cosmetics in Phase 2 of this study.

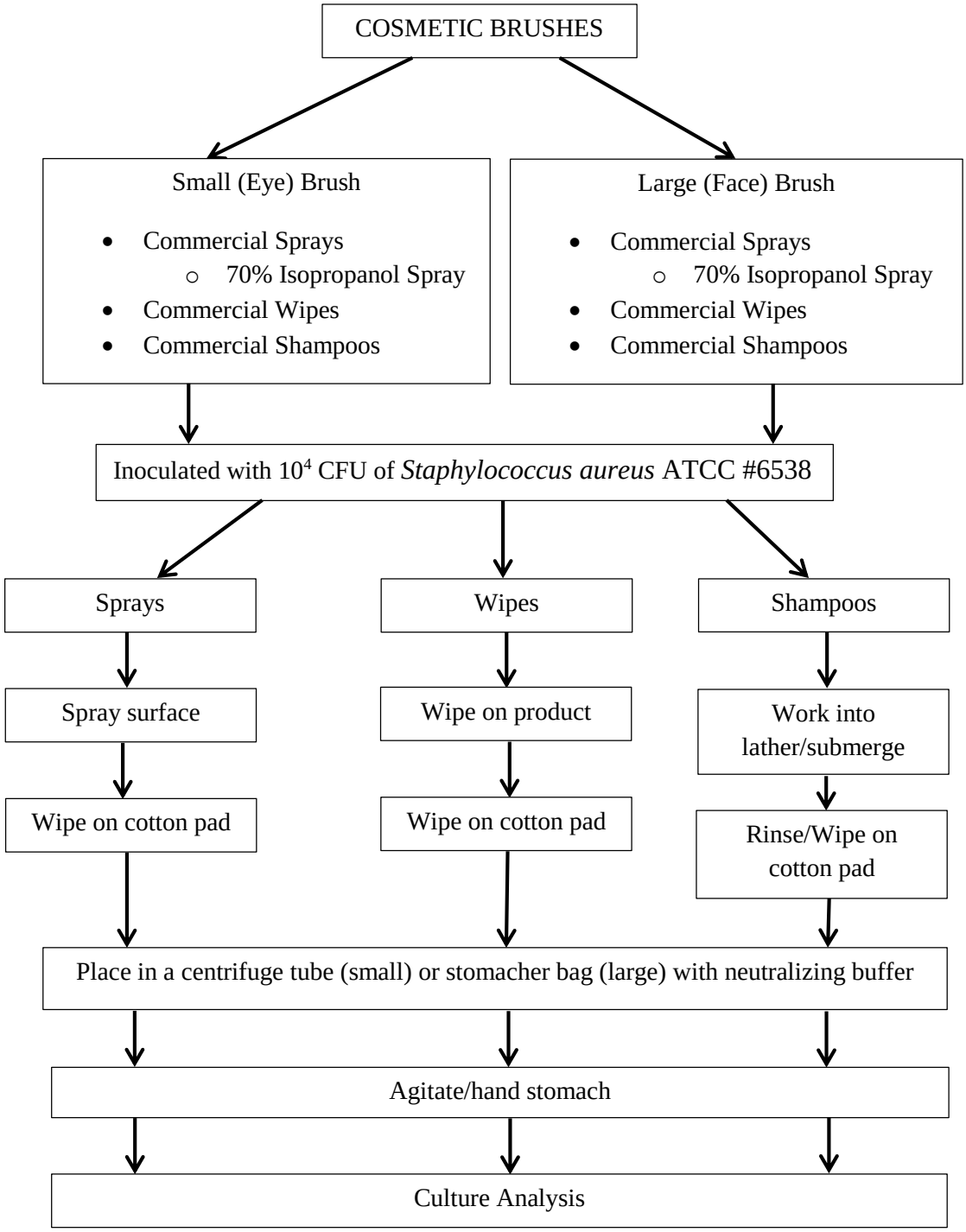

Figure 3 – Flow chart illustrating the experimental design used on cosmetic brushes in Phase 2 of this study.

Test Organisms

Unused cosmetics and brushes were inoculated with known concentrations of *Pseudomonas aeruginosa* ATCC #27853, *Escherichia coli* ATCC #25922, *Staphylococcus aureus* ATCC #6538, and *Staphylococcus epidermidis* ATCC #12228. Control tests were conducted with each organism to determine which one had better survival (i.e., the best percent recovery) across all products. *E. coli* ATCC #25922, *S. aureus* ATCC #6538, and *S. epidermidis* ATCC #12228 were used for quality control of the culture media.

Culture Media

The preparation of the inoculum required the use of an overnight cell suspension, of the test organism, cultured in tryptic soy broth (TSB, Difco Laboratories, Sparks, MD) and incubated at 35°C, 60 rpm overnight in a rotary shaking incubator. The overnight cell suspension was harvested and washed in 0.01 M phosphate buffer with 0.05% Tween (PBT; pH 7.0). The final washed cell suspension was diluted in PBT and spread plated as indicated below to determine the concentration.

Samples with and without treatment with cleaners (i.e., controls and tests, respectively) were processed in a neutralizing buffer (Difco Laboratories), serially diluted in PBT, spread plated, and incubated overnight at 35°C. Many cosmetics contain preservatives that aid in the reduction of microbial contamination, thus an appropriate neutralizing agent was necessary. The cell suspension, inoculum, and test samples were inoculated on tryptic soy agar (TSA, Difco Laboratories) and incubated overnight at 35°C.

Cosmetic Cleaning Products

Three types of commercial cosmetic cleaning products were obtained. Sprays, wipes, and shampoos were tested in this study. For the cosmetics, one brand of spray was used for the eyeshadows and one brand of wipe was used for the lipsticks (Table 1). Two different brands of

each type of cleaning product were used for the brushes. Spray-based cosmetic cleaners were used on cosmetics, specifically eyeshadows, and cosmetic brushes. For the cosmetics, the product was sprayed on and allowed to dry instantly. As for the brushes, the product was sprayed directly onto the brush and was immediately wiped off on a clean cotton pad. The wipes were used on both cosmetics, specifically lipsticks, and cosmetic brushes. Shampoo cosmetic cleaners were used on cosmetic brushes and required the use of water. In addition to the commercial products, control tests using a clean cotton pad for cosmetics and 70% isopropanol spray for brushes were conducted (Table 1). Sterile water was used to dilute 99% isopropanol to 70% (Sigma-Aldrich, St. Louis, MO). The 70% isopropanol was then placed in a Nalgene aerosol spray bottle affixed with the appropriate nozzle; in order to produce a spray similar to the commercial brand, a similar nozzle was used (Fisher Scientific, Rochester, NY).

Table 1. Commercial Cleaning Products Used on Cosmetics and Cosmetic Brushes.

Cosmetic or Brush	Product Type										
	Spray				Wipe					Shampoo	
	Brand										
	A	Alcohol	C	D	A	Alcohol	C	D	Cotton Pad	C	D
Eyeshadow	X	X							X		
Lipstick					X	X			X		
Small Brush		X	X	X			X	X		X	X
Large Brush		X	X	X			X	X		X	X

Cosmetics and Brushes

The cosmetics used in this study were pressed powder eyeshadow/blush and cream-based lipsticks. The pressed powder eyeshadow/blush used were duos and quads from Eyes Lips Face

Cosmetics (E.L.F Cosmetics, New York City, NY). The cream-based lipsticks used were from Wet N' Wild Cosmetics (Wet N' Wild, Los Angeles, CA). The brushes used in this study were small (eyeshadow) and large (face) brushes. Both brushes were from the brand E.L.F Cosmetics (New York, NY).

Cosmetic and Brush Inoculation

Cosmetics, eyeshadows and lipsticks, were inoculated with 10 µl of a 10^6 CFU/ml cell suspension applied dropwise with a pipette across the surface of the product (Figures 4 and 5). Brushes were inoculated by placing the inoculum in a petri dish and swirling the brush until the entire inoculum was absorbed (Figure 6).

Figure 4: Eyeshadow/blush inoculation.

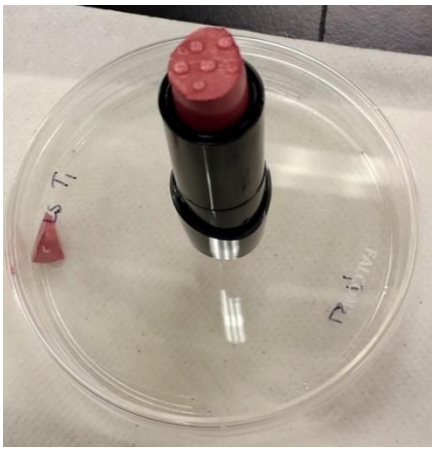

Figure 5: Lipstick inoculation.

Figure 6: Large brush inoculation.

Inoculum Preparation Methods

Freshly streaked overnight cultures of the test organisms were incubated as indicated above. Overnight suspensions were prepared as indicated above, and the liquid cultures were washed by centrifugation. Cell suspensions were centrifuged in an IEC CL31R Multispeed Centrifuge at 4516 $_{x}$ g, 4°C, for 5 minutes, resuspended, and washed in PBT three times (Thermo, Waltham, MA). Washed cell suspensions were serially diluted, plated in duplicate, incubated overnight on TSA at 35°C, and enumerated. The cell suspension used as the inoculum was made fresh on each day of testing.

Phase 1: Organism Selection

The following ATCC organisms were tested: *P. aeruginosa* ATCC #27853, *E. coli* ATCC #25922, *S. aureus* ATCC #6538, and *S. epidermidis* ATCC #12228; no cleaning products

were used for these tests. The organism with the best percent recovery from cosmetics and cosmetic brushes was chosen as the inoculum for the test experiments in Phase 2.

Phase 1: Cosmetic Sampling

Eyeshadows (n=1) and lipsticks (n= 1) were inoculated with 10 µl of the 10^6 CFU/ml suspension, for a total inoculum of 10^4 CFU. The inoculum was left in contact with the cleaning products for 0, 1, and 5 minutes (i.e., $T_{0\ minute}$, $T_{1\ minute}$, and $T_{5\ minutes}$, respectively). After each contact time, the surface of the product was swabbed with a sterile cotton swab, and the swab was placed in 3 ml of neutralizing buffer (in a 15 ml centrifuge tube). Then, the sample was vortexed on high for 1 minute and the swab was removed and discarded. The samples were then serially diluted in PBT and plated as indicated above.

Phase 1: Cosmetic Brush Sampling

Small (n=1) and large (n=1) brushes were inoculated with 10^4 CFU of the test organism. Large brushes were placed in a stomacher bag (Fisher Scientific) containing 10 ml of neutralizing buffer and hand stomached for 1 minute. Small brushes were placed in a 15 ml centrifuge tube with 3 ml of neutralizing buffer and agitated by hand for 1 minute. Samples were serially diluted in PBT and plated as indicated above.

Phase 2: Cosmetic Test Methods and Processing

These tests were conducted using the ATCC organism *S. aureus* #6538. The two types of cosmetics used for these tests were pressed powders (eyeshadows/blushes) and cream-based products (lipsticks). For the eyeshadows/blushes, one commercial spray and an isopropanol spray were tested. For the lipsticks, one commercial wipe and an isopropanol wipe were tested. In addition, a clean cotton pad was tested on both eyeshadows and lipsticks. Testing consisted of three trials, and samples were plated in duplicate; for a total of nine replicates for each test.

Phase 2: Eyeshadow/Blush [Sprays]

Eyeshadow and blushes were inoculated with 10^4 CFU of the test organism; the inoculum was placed dropwise across the surface of the cosmetic (Figure 6). The inoculum was left in contact with the cosmetics for contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$. The cleaning product was sprayed about 6 inches away from the surface of the eyeshadow. After the sample was treated with the cosmetic cleaner, the surface of the eyeshadow was sampled with a cotton swab (Fisher Scientific) which was then placed in 3 ml of neutralizing buffer (in a 15 ml centrifuge tube). The sample was vortexed on high for 1 minute, and the swab was removed and discarded. The samples were then serially diluted in PBT, plated in duplicate on TSA, incubated overnight at 35°C, and enumerated.

Phase 2: Lipstick [Wipes]

The tip of the lipstick bullet was cut and inoculated with 10^4 CFU of the test organism; the inoculum was placed dropwise across the surface (Figures 5 and 7). The inoculum was left in contact with the products for contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$. After the appropriate contact time, the surface of the lipstick was treated with the desired product wipe. After the sample had been treated with the cosmetic wipe, the surface of the lipstick was sampled with a cotton swab which was then placed in 3 ml of neutralizing buffer (in a 15 ml centrifuge tube). The sample was vortexed on high for 1 minute and the swab was removed and discarded. The samples were then serially diluted in PBT, plated in duplicate on TSA, incubated overnight at 35°C, and enumerated.

Phase 2: Eyeshadow/Blush and Lipstick [Cotton Pad]

As a control, a cotton pad was used as a cleaning product. Inoculation of the cosmetics occurred as indicated above. After the appropriate contact times, the cotton pad was used to wipe

the surface of the eyeshadow/blush and lipstick. The cosmetics were sampled and analyzed as indicated above.

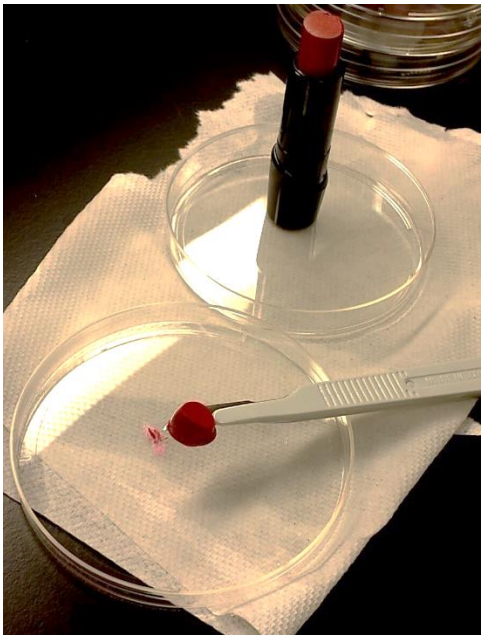

Figure 7: Aseptic cutting of lipstick bullet.

Phase 2: Cosmetic Brush Methods and Processing

These tests were conducted using *S. aureus* ATCC #6538. The two types of brushes used for these tests were small (eyeshadow) brushes and large (face) brushes. For both brushes, two brands of each product type, sprays, wipes, and shampoos, were tested. In addition, a 70% isopropanol spray was tested. All testing consisted of three trials, and the samples from each trial were plated in duplicate.

Phase 2: Small/Large Brush [Sprays]

Small and large brushes were inoculated with 10^4 CFU of the test organism; the inoculum was placed in an empty petri dish where the brush bristles were swirled. Small brushes were sprayed with the product once on each side of the bristles (front and back), for a total of two sprays. Large brushes were sprayed with the product once on each side of the bristles (front,

back, and end of bristles), for a total of three sprays. The process of spraying the brushes was conducted in a biological safety cabinet (BSC) over an empty beaker. Once treated with the cleaning spray, the brushes were wiped with back and forth motions on a clean cotton pad (VWR, Radnor, PA) for 30 seconds. Small brushes were placed in a 15 ml centrifuge tube containing 3 ml of neutralizing buffer and agitated by hand for 1 minute. Large brushes were placed in a stomacher bag containing 10 ml of neutralizing buffer and hand stomached for 1 minute. The samples were then serially diluted in PBT and analyzed as indicated in the culture analysis section below.

Phase 2: Small/Large Brush [Wipes]

Small and large brushes were inoculated with 10^4 CFU of the test organism; the inoculum was placed in an empty petri dish where the brush bristles were swirled. The inoculated brushes were wiped on the cleaning wipes using back and forth motions for 30 seconds. Then, the brushes were wiped on a clean cotton pad for an additional 30 seconds. The process of wiping the brushes was conducted in a BSC and gloves were changed between samples. Small brushes were placed in a 15 ml centrifuge tube containing 3 ml of neutralizing buffer and agitated by hand for 1 minute. Large brushes were placed in a stomacher bag containing 10 ml neutralizing buffer and hand stomached for 1 minute. The samples were then serially diluted in PBT and analyzed as indicated in the culture analysis section below.

Phase 2: Small/Large Brush [Shampoo #1]

Small and large brushes were inoculated with 10^4 CFU of the test organism; the inoculum was placed in an empty petri dish where the brush was swirled. Small brushes were submerged in 300 µl of product in a 15 ml centrifuge tube and agitated for 1 minute. Large brushes were submerged in 10 ml of product in a stomacher bag and agitated for 1 minute. Processing the samples was conducted in a BSC. Then, the brushes were wiped with back and forth motions on

a clean cotton pad for 30 seconds. Small brushes were placed in a 15 ml centrifuge tube containing 3 ml of neutralizing buffer and agitated by hand for 1 minute. Large brushes were placed in stomacher bags containing 10 ml of neutralizing buffer and hand stomached for 1 minute. The samples were then serially diluted in PBT and analyzed as indicated in the culture analysis section below.

Phase 2: Small/Large Brush [Shampoo #2]

Small and large brushes were inoculated with 10^4 CFU of the test organism; the inoculum was placed in an empty petri dish where the brush was swirled. Small brushes were dampened with 300 µl of warm sterile ultrapure (UP) water warmed to 50°C in a 15 ml centrifuge tube. Once damp, one drop of product was placed on the brush, returned to the centrifuge tube, and worked into a lather. Then, the small brush was washed three times in 300 µl of warm sterile UP water, in a 15 ml centrifuge tube. Large brushes were dampened with 10 ml of warm sterile UP water in a stomacher bag. Once damp, one pump of product was placed onto the brush and worked into a lather; this process occurred in a stomacher bag. Then, the large brushes were washed in 10 ml of sterile UP water two times, in a stomacher bag.

Once processed with the cleaning product, the brushes were wiped on a cotton pad, using back and forth motions, for 30 seconds. Processing of brush samples was conducted in a BSC. Small brushes were placed in a 15 ml centrifuge tube containing 3 ml of neutralizing buffer and agitated by hand for 1 minute. Large brushes were placed in a stomacher bag containing 10 ml of neutralizing buffer and hand stomached for 1 minute. The samples were then serially diluted in PBT and analyzed as indicated in the culture analysis section below.

Culture Analysis

After processing, the lipsticks, eyeshadows, and small brushes resulted in 3 ml of sample while the large brushes yielded 10 ml of sample. Before the samples were plated for

enumeration, they were serially diluted and 100 µl were plated, in duplicate, on TSA and incubated at 35°C overnight. Colony forming units (CFU) were enumerated and converted into CFU/sample.

Data Analysis

In total, 154 samples, including controls, were analyzed in this study. The mean CFU were calculated for controls (before cleaning) and samples (after cleaning). Using the mean CFU counts, the CFU per sample and percent reductions were calculated based on three replicates of each condition. Lower detection limits were determined based on the detection of 1 *S. aureus* #6538 CFU per milliliter which was then converted to CFU per sample. There were two detection limits determined for each sample size, 3 and 10 milliliters; both lower detection limits were $<1.00 \times 10^1$ CFU/ml.

Because this project had relatively small sample sizes across the test conditions, parametric procedures were not considered owing to the inability to verify distributional assumptions. Hence, nonparametric tests with exact or permutation-based p-values were conducted. A marginal means table was prepared for each product type and their variables based on percent reduction to gain a basic understanding of the relative differences in the results among the various test conditions. To test for potential interactions between variables (e.g., contact time and product), a rank-based ANOVA or GLM procedure-was conducted. To test for individual effects, a Kruskal-Wallis test with exact p-value was conducted. Post-hoc testing using multiple contrast and permutation-based p-values using 10,000 Monte Carlo simulations were conducted on those tests that were statistically significant.

CHAPTER 4

RESULTS

Phase 1: Organism Selection

CFU per sample and percent recovery for each organism was calculated. Of the four organisms tested (Tables 2-5), *S. aureus* ATCC #6538 and *S. epidermidis* ATCC #12228 yielded the highest recovery from all products (Tables 4 and 5, respectively). *P. aeruginosa* ATCC #27853 had the overall lowest percent recovery for eyeshadows at all contact times and for the large brush. *E. coli* ATCC #25922 resulted in the overall lowest percent recovery for lipsticks at all contact times (Table 3). *S. epidermidis* ATCC #12228 (Table 4) had a lower percent recovery for large brushes in comparison to *S. aureus* at 13.31% (Table 5). *S. aureus* ATCC #6538 was the organism chosen to inoculate all test products due to high percent recovery across all products and from supporting information from the scientific literature (Table 5) (Chen et al., 2013).

Table 2. Control experiment results with *P. aeruginosa* ATCC #27853. Inoculum used = 1.18×10^5 CFU.

Product	Contact Time (min)	CFU/Sample (n=1)	% recovery
Eyeshadow	T0	1.98×10^4	16.85%
	T1	1.59×10^4	13.53%
	T5	2.33×10^4	19.79%
Lipstick	T0	1.70×10^4	14.43%
	T1	1.82×10^4	15.45%
	T5	1.11×10^4	9.45%
Large brush	T0	8.35×10^3	7.11%
Small brush	T0	1.26×10^5	107.23%

Table 3. Control experiment results with *E. coli* ATCC #25922. Inoculum used = 1.67×10^4 CFU. Lower detection limit = 3.00×10^1 CFU/sample.

Product	Contact Time (min)	CFU/Sample (n=1)	% recovery
Eyeshadow	T0	8.06×10^3	48.23%
	T1	1.35×10^4	80.84%
	T5	1.50×10^2	0.90%
Lipstick	T0	9.00×10^2	5.39%
	T1	3.00×10^1	0.18%
	T5	$<3.00 \times 10^1$	0.18%
Large brush	T0	1.80×10^3	10.78%
Small brush	T0	5.70×10^3	34.13%

Table 4. Control experiment results with *S. epidermidis* ATCC #12228. Inoculum used = 2.78×10^4 CFU.

Product	Contact Time (min)	CFU/Sample (n=1)	% recovery
Eyeshadow	T0	2.16×10^4	77.70%
	T1	1.94×10^4	69.60%
	T5	1.38×10^4	49.64%
Lipstick	T0	1.13×10^4	40.47%
	T1	1.62×10^4	58.27%
	T5	1.23×10^3	4.42%
Large brush	T0	3.70×10^3	13.31%
Small brush	T0	1.91×10^4	68.53%

Table 5. Control experiment results with *S. aureus* ATCC #6538. Inoculum used = 2.58×10^4 CFU.

Product	Contact Time (min)	CFU/Sample (n=1)	% recovery
Eyeshadow	T0	1.86×10^4	72.09%
	T1	2.15×10^4	83.14%
	T5	1.20×10^2	0.47%
Lipstick	T0	3.87×10^3	15.00%
	T1	1.50×10^3	5.81%
	T5	6.00×10^1	0.23%
Large brush	T0	1.21×10^4	46.90%
Small brush	T0	1.43×10^4	55.23%

Phase 2: Cleaning Product Testing

Eyeshadow/Blush

The average CFU per sample and percent reduction were determined for the commercially-available cosmetic spray, the 70% isopropanol spray, and the cotton pad (Tables 6-8). The percent recovery (data not shown) indicates how much microbial contamination is still present on the product; in other words, the greater the percent recovery, the more inoculum left on the product. The percent reduction compares the percent recovery from the control to that of the test. The average percent recoveries for 70% isopropanol spray were 75.8%, 82.05%, and 55.19% at contact times T_0, $T_{1\ minute}$, and $T_{5\ minutes}$, respectively (Table 6). Cosmetic spray #2 exhibited the lowest percent recovery of 13.86% at contact time $T_{1\ minute}$ (Table 7). The clean cotton pad resulted in the highest percent recovery (99.88%) at contact time T_0 (Table 8). Overall, comparison of all three methods showed that the clean cotton pad exhibited greater percent reductions, over 98%, across all contact times (Figure 8). Ranked ANOVA testing for interaction between contact time and product type showed no interaction among these variables ($F= 0.47$, $p=0.7547$). Individual analysis of contact time showed no statistical significance ($p=0.9397$). However, individual analysis of product type was statistically significant ($p < 0.0001$). Post-hoc tests showed the cotton pad had significantly greater reduction than spray #2 ($p = 0.0034$). There was no statistically significant difference in microbial concentration on eyeshadows after the use of commercial cleaners at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$ ($p= 0.9397$). Therefore, hypothesis 1 was not rejected. However, the results show that there was a statistically significant difference in microbial concentration on eyeshadows between brands of commercial cleaners ($p<0.0001$). Therefore, hypothesis 3 was rejected.

Table 6. Eyeshadow experiment results with 70% isopropanol spray. Inoculum used = 5.25×10^4 CFU. *Inoculum used = 3.90×10^4 CFU. The asterisk (*) represents repeated trials.

Cleaning Product	Contact Time (min)	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	T0	3.48×10^4	N/A	N/A
	T1	3.15×10^4		
	T5	1.25×10^3		
None (Control)*	T5	2.43×10^4	N/A	N/A
70% Isopropanol Spray	T0	2.06×10^4	8.41×10^3	75.83%
		3.21×10^3		
		1.47×10^3		
	T1	1.41×10^4	5.66×10^3	82.05%
		2.64×10^3		
		2.25×10^2		
	T5	1.62×10^3	8.90×10^2	55.19%
		9.75×10^{2}*		
		7.50×10^{1}*		

Table 7. Eyeshadow experiment results with cosmetic spray #2. Inoculum used = 2.20×10^4 CFU.

Cleaning Product	Contact Time (min)	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	T0	1.32×10^4	N/A	N/A
	T1	1.52×10^3		
	T5	2.25×10^2		
Cosmetic Spray #2	T0	4.64×10^3	4.73×10^3	64.17%
		4.89×10^3		
		4.67×10^3		
	T1	2.45×10^3	1.31×10^3	13.86%
		6.75×10^2		
		7.95×10^2		
	T5	3.15×10^2	1.45×10^2	35.56%
		1.50×10^1		
		1.05×10^2		

Table 8. Eyeshadow experiment results with clean cotton pad. Inoculum used = 3.90×10^4 CFU. Lower detection limit (LDL) = 3.00×10^1 CFU/sample. The asterisk (*) indicates that the LDL was used to calculate the mean.

Cleaning Product	Contact Time (min)	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	T0	3.23×10^4	N/A	N/A
	T1	2.55×10^4		
	T5	2.43×10^4		
Cotton Pad	T0	$<3.00 \times 10^1$	3.50×10^1*	99.88%
		$<3.00 \times 10^1$		
		4.50×10^1		
	T1	7.95×10^2	2.80×10^2	98.90%
		1.50×10^1		
		3.00×10^1		
	T5	3.00×10^1	1.15×10^2	99.53%
		3.00×10^2		
		1.50×10^1		

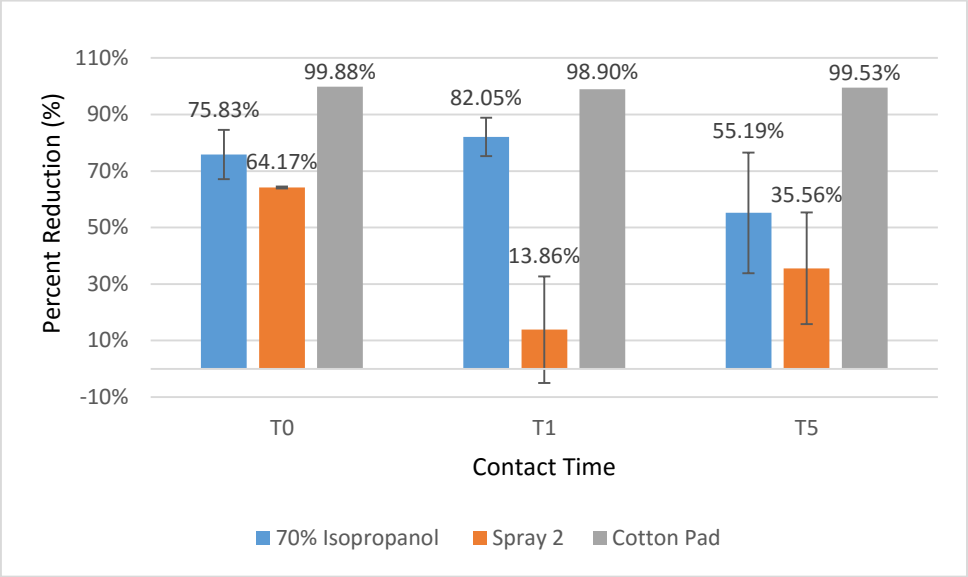

Figure 8: Percent reduction (%) of *S. aureus* ATCC #6538 on eyeshadow with three cleaning methods at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$. Bar heights represent the mean percent reduction per method with error bars representing ± 1 standard error (SE).

Lipstick

The average CFU per sample and percent reduction were determined for the commercial cosmetic wipe, the isopropanol wipe, and the cotton pad (Tables 9, 10, and 11, respectively). The average percent reductions for 70% isopropanol spray at all contact times were above 98% (Table 9). Cosmetic wipe #2 exhibited the highest average percent reductions with 99.68%, 99.86%, and 99.78% at contact times T_0, $T_{1\ minute}$, and $T_{5\ minutes}$, respectively (Table 10). The clean cotton pad resulted in the lowest percent reduction at 94.29% at contact time T_0 (Table 11). Overall, comparison of all three products showed that the clean cotton pad exhibited the lowest percent reduction across all contact times (Figure 9). Ranked ANOVA testing for interaction between contact time and product type showed no interaction among these variables ($F= 0.69$, $p=0.6108$). Individual analysis of product type was statistically significant ($p=0.0070$). Post-hoc tests showed significantly greater reduction with wipe #2 ($p = 0.0034$) and the 70% isopropanol wipe ($p=0.0013$) compared with the cotton pad. There was no statistically significant difference in microbial concentration on lipsticks after the use of commercial cleaners at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$ ($p= 0.1009$). Therefore, hypothesis 2 was not rejected. However, the results show that there was a statistically significant difference in microbial concentration on lipsticks between brands of commercial cleaners ($p=0.0070$). Therefore, hypothesis 4 was rejected.

Table 9. Lipstick experiment results with 70% isopropanol wipe. Inoculum used = 4.60×10^4 CFU. *Inoculum used = 3.90×10^4 CFU; the asterisk (*) represents repeated trials. The double asterisk (**) indicates that the LDL (3.00×10^1 CFU/sample) was used to calculate the mean.

Cleaning Product	Contact Time (min)	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	T0	3.95×10^4	N/A	N/A
	T1	3.83×10^4		
	T5	1.97×10^4		
None (Control)*	T0	5.25×10^2	N/A	N/A
	T1	1.38×10^4		
	T5	4.50×10^2		
70% Isopropanol Wipe	T0	1.50×10^1*	3.00×10^1**	98.98%
		$<3.00 \times 10^1$		
		4.50×10^1		
	T1	4.50×10^1*	3.00×10^1**	99.85%
		1.50×10^1		
		$<3.00 \times 10^1$		
	T5	$<3.00 \times 10^1$	3.00×10^1**	99.85%
		$<3.00 \times 10^1$*		
		$<3.00 \times 10^1$		

Table 10. Lipstick experiment results with cosmetic wipe #2. Inoculum used = 2.20×10^4 CFU. *Inoculum used = 3.90×10^4 CFU; the asterisk (*) represents repeated trials. The double asterisk (**) indicates that the LDL (3.00×10^1 CFU/sample) was used to calculate the mean.

Cleaning Product	Contact Time (min)	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	T0	1.61×10^4	N/A	N/A
	T1	2.09×10^4		
	T5	1.13×10^4		
None (Control)*	T0	5.25×10^2	N/A	N/A
Cosmetic Wipe #2	T0	$<3.00 \times 10^1$*	5.50×10^1**	99.68%
		$<3.00 \times 10^1$		
		1.05×10^2		
	T1	$<3.00 \times 10^1$	3.00×10^1**	99.86%
		$<3.00 \times 10^1$		
		$<3.00 \times 10^1$		
	T5	$<3.00 \times 10^1$	2.50×10^1**	99.78%
		1.50×10^1		
		$<3.00 \times 10^1$		

Table 11. Lipstick experiment results with cotton pad. Inoculum used = 3.90×10^4 CFU. LDL = 3.00×10^1 CFU/ml. The asterisk (*) indicates that the LDL (3.00×10^1 CFU/sample) was used to calculate the mean.

Cleaning Product	Contact Time (min)	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	T0	5.25×10^2	N/A	N/A
	T1	1.38×10^4		
	T5	4.50×10^2		
Cotton Pad	T0	$<3.00 \times 10^1$	3.00×10^1*	94.29%
		$<3.00 \times 10^1$		
		$<3.00 \times 10^1$		
	T1	$<3.00 \times 10^1$	2.50×10^1*	99.82%
		1.50×10^1		
		$<3.00 \times 10^1$		
	T5	$<3.00 \times 10^1$	2.50×10^1*	94.44%
		$<3.00 \times 10^1$		
		1.50×10^1		

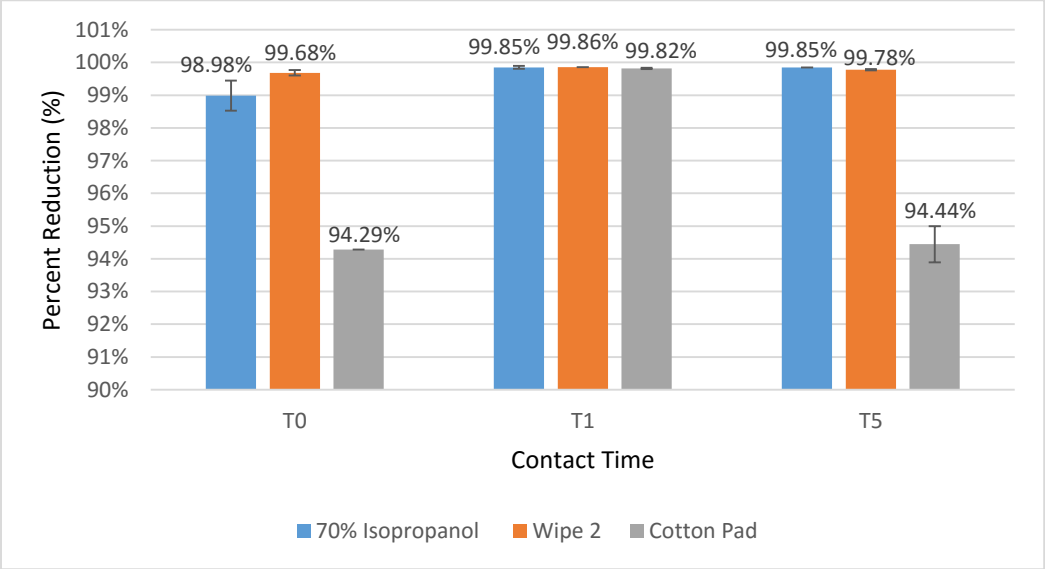

Figure 9: Percent reduction (%) of *S. aureus* ATCC #6538 on lipstick at contact times of T_0, $T_{1\ minute}$, and $T_{5\ minutes}$. Bar heights represent the mean percent reduction per product with error bars representing ± 1 standard error (SE).

Small Brush

The average CFU per sample and percent reduction were determined for cosmetic sprays, wipes, and shampoos (Tables 12-15). The average percent reductions for both brands of brush sprays were 99.32% and 99.40%, respectively (Table 12). The cosmetic wipe and shampoo from brand #1 exhibited the highest average percent reduction with 99.84% and 99.87%, respectively (Table 13 and 14). The 70% isopropanol resulted in an average percent reduction of 99.85% (Table 15). Overall, brand #1 exhibited higher percent reductions than brand #2 across all product types (Figure 10). GLM analysis testing for interaction between product type and brand showed slight interaction among these variables ($F= 3.66$, $p=0.0525$); this was seen graphically between brands of spray products. There was no statistically significant difference in microbial concentration on small brushes between types and brands of commercial cleaners ($p= 0.9833$ and $p= 0.0605$, respectively). Therefore, hypotheses 7 and 8 were not rejected.

Table 12. Small brush experiment results with brush sprays. Inoculum used = 2.63×10^4 CFU.

Cleaning Product	**CFU/Sample**	**Average CFU/Sample**	**Average % reduction**
None (Control)	2.58×10^4	N/A	N/A
Brush Spray #1	6.00×10^1	1.75×10^2	99.32%
	2.25×10^2		
	2.40×10^2		
Brush Spray #2	2.25×10^2	1.55×10^2	99.40%
	1.50×10^1		
	2.25×10^2		

Table 13. Small brush experiment results with brush wipes. Inoculum used = 2.63×10^4 CFU. *Inoculum used = 3.50×10^4 CFU; asterisk (*) represents repeated trials. The double asterisk (**) indicates that the LDL (3.00×10^1 CFU/sample) was used to calculate the mean.

Cleaning Product	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	2.58×10^4	N/A	N/A
None (Control)*	2.25×10^4		
Brush Wipe #1	$<3.00 \times 10^1$	4.50×10^1**	99.84%
	3.00×10^1		
	6.00×10^1		
Brush Wipe #2	9.00×10^1	5.00×10^2	97.82%
	1.35×10^2		
	1.28×10^3*		

Table 14. Small brush experiment results with brush shampoos. Inoculum used = 3.50×10^4 CFU. The asterisk (*) indicates that the LDL (3.00×10^1 CFU/sample) was used to calculate the mean.

Cleaning Product	CFU/Sample	Average CFU/Sample (n=3)	Average % reduction
None (Control)	2.25×10^4	N/A	N/A
Brush Shampoo #1	$<3.00 \times 10^1$	3.00×10^1*	99.87%
	$<3.00 \times 10^1$		
	$<3.00 \times 10^1$		
Brush Shampoo #2	1.35×10^2	5.00×10^2	97.8%
	1.35×10^2		
	1.23×10^3		

Table 15. Small brush experiment results with 70% isopropanol spray.
Inoculum used = 5.10×10^4 CFU. The asterisk (*) indicates that the LDL (3.00×10^1 CFU/sample) was used to calculate the mean.

Cleaning Product	CFU/Sample	Average CFU/Sample (n=3)	% reduction
None (Control)	1.64×10^4	N/A	N/A
70% Isopropanol Alcohol	1.50×10^1	2.50×10^1*	99.85%
	$<3.00 \times 10^1$		
	$<3.00 \times 10^1$		

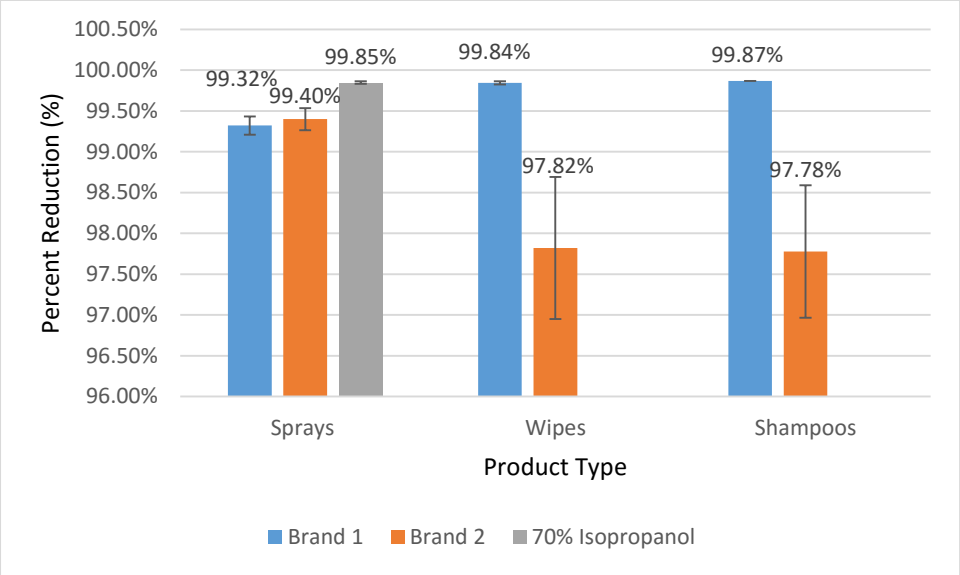

Figure 10: Percent reduction (%) of *S. aureus* ATCC #6538 on small brushes for product types sprays, wipes, and shampoos. Grey bar (70% Isopropanol Spray) serves as a control product. Bar heights represent the mean percent reduction per brand with error bars representing ± 1 standard error (SE).

Large Brush

The average CFU per sample and percent reduction were determined for cosmetic sprays, wipes, and shampoos (Tables 16-19). Brush spray brand #2 resulted in the lowest percent reduction, across all product types and brands, at 87.62% (Table 16). Brush wipe brand #1 resulted in the highest percent reduction with 99.20% (Table 17). Comparison of shampoos showed that brand #2 exhibited the highest percent reduction, at 90.74%, for this product type (Table 18). The 70% isopropanol resulted in an average percent reduction of 96.90% (Table 19). Overall, brand #1 exhibited higher percent reductions than brand #2 among two product types, sprays and wipes (Figure 11). GLM analysis testing for interaction between product type and brand showed some interaction among these variables ($F= 4.48$, $p=0.0313$). Individual analysis of product type showed statistical significance ($p=0.0004$). Post-hoc tests showed wipes were statistically greater than the shampoos ($p = 0.0051$). The results show that there was a statistically significant difference in microbial reduction on large brushes between types of

commercial cleaners (p=0.0004). Therefore, hypothesis 5 was rejected. However, there was no statistically significant difference in microbial reduction on large brushes between brands of commercial cleaners (p= 0.2157). Therefore, hypothesis 6 was not rejected.

Table 16. Large brush experiment results with brush sprays. Inoculum used = 2.27×10^4 CFU.

Cleaning Product	**CFU/Sample**	**Average CFU/Sample (n=3)**	**Average % reduction**
None (Control)	8.35×10^3	N/A	N/A
Brush Spray #1	1.00×10^2	3.50×10^2	95.81%
	4.50×10^2		
	5.00×10^2		
Brush Spray #2	9.00×10^2	1.03×10^3	87.62%
	6.00×10^2		
	1.60×10^3		

Table 17. Large brush experiment results with brush wipes. Inoculum used = 2.27×10^4 CFU. LDL = 1.00×10^2 CFU/sample. The asterisk (*) indicates that the LDL (1.00×10^2 CFU/sample) was used to calculate the mean.

Cleaning Product	**CFU/Sample**	**Average CFU/Sample (n=3)**	**Average % reduction**
None (Control)	8.35×10^3	N/A	N/A
Brush Wipe #1	5.00×10^1	6.67×10^1*	99.20%
	5.00×10^1		
	$<1.00 \times 10^2$		
Brush Wipe #2	2.50×10^2	2.67×10^2	96.81%
	3.00×10^2		
	2.50×10^2		

Table 18. Large brush experiment results with brush shampoos. Inoculum used = 3.50×10^4 CFU. The asterisk (*) indicates that the LDL (1.00×10^2 CFU/sample) was used to calculate the mean.

Cleaning Product	**CFU/Sample**	**Average CFU/Sample (n=3)**	**Average % reduction**
None (Control)	9.00×10^2	N/A	N/A
Brush Shampoo #1	$<1.00 \times 10^2$	1.00×10^2*	88.89%
	$<1.00 \times 10^2$		
	$<1.00 \times 10^2$		
Brush Shampoo #2	$<1.00 \times 10^2$	8.33×10^1*	90.74%
	$<1.00 \times 10^2$		
	5.00×10^1		

Table 19. Large brush experiment results with 70% isopropanol spray. Inoculum used = 5.10×10^4 CFU.

Cleaning Product	**CFU/Sample**	**Average CFU/Sample (n=3)**	**Average % reduction**
None (Control)	2.15×10^3	N/A	N/A
70% Isopropanol Spray	1.00×10^2	6.67×10^1	96.90%
	5.00×10^1		
	5.00×10^1		

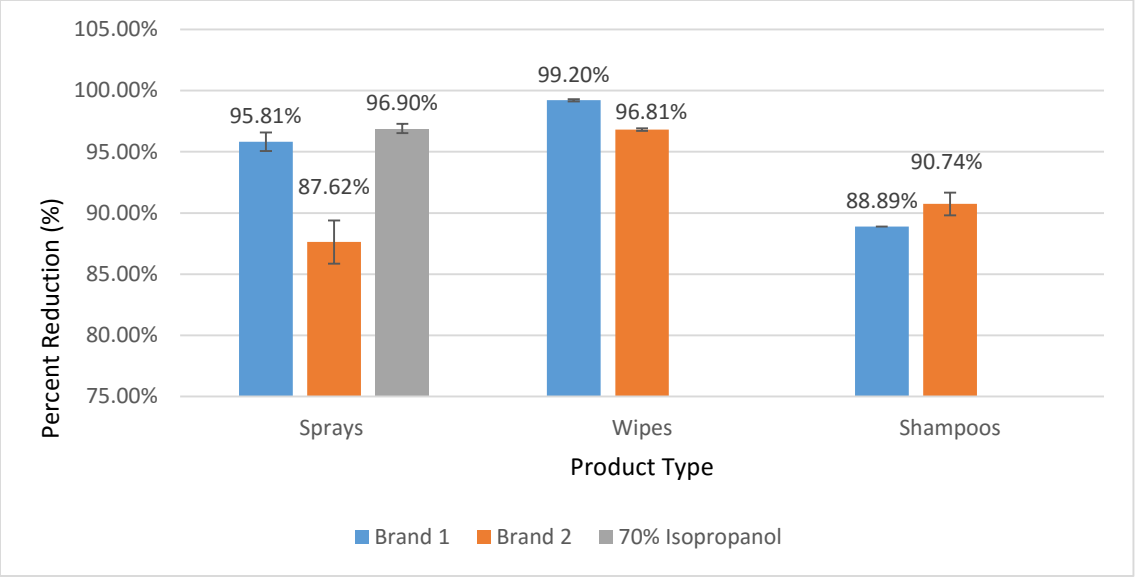

Figure 13: Percent reduction (%) of *S. aureus* ATCC #6538 on large brushes for cleaning product types sprays, wipes, and shampoos. Grey bar (70% Isopropanol Spray) serves as a control product. Bar heights represent the mean percent reduction per brand with error bars representing ± 1 standard error (SE).

CHAPTER 5

DISCUSSION

The objective of this study was to determine if commercially-available cosmetic cleaners were effective at removing microbial contamination from cosmetics and cosmetic brushes. Overall, culture analysis from all cleaning products resulted in the reduction of microbial contamination, to some degree, on cosmetics and cosmetic brushes (Eyeshadow = at least 13% reduction, Lipstick = at least 94%, Small Brush = at least 97%, Large Brush = at least 87%).

To our knowledge, this is the first study to focus on the removal of microbial contamination on cosmetics and cosmetic brushes. The main focus of cosmetic microbiology has mostly been confined to manufacturer practices and preservative efficacy. Those studies have led to the implementation of Good Manufacturing Practices (GMPs), which have decreased the amount of contamination that occurs at the manufacturer level (Campana et al., 2006). To maintain the shelf life of these products, preservatives are used to help combat microbial contamination. Many studies have been conducted to test the efficacy of preservatives in cosmetics (Lundov et al., 2009). These studies focus on determining how effective these preservatives are over time and the microbial load they can handle before they start to lose their effectiveness.

Even with the use of GMPs and preservatives, studies have shown that cosmetics still have the potential to become contaminated with microorganisms (Tran et al., 1994). These contaminated cosmetics may facilitate the progression of various infections of the skin and eyes. Birteksoz et al. (2013) analyzed microbial contamination in used cosmetics and found between 10^2 and 10^5 CFU/ml. The most commonly isolated organism from these products tested was *S.*

aureus. However, that study analyzed a wide range of unused cosmetic products, such as toothpaste and lotion, with very little focus on facial cosmetic products.

Few studies have been conducted on used cosmetics and brushes. Dawson et al. (1981) analyzed used cosmetic testers for microbial contamination. The results of the study showed a large presence of normal skin flora; other organisms found were believed to be airborne contaminants. Contamination of these products was due to: the use by multiple individuals (with either their finger or a multiple use applicator), lack of proper disinfection of multiple use applicators, lack of proper storage, and products that were past expiration dates. Naz et al. (2012) tested for microbial contamination of cosmetic brushes and sponges used in beauty salons. All of the brushes and sponges tested in the study were contaminated with *S. aureus*; the average contamination of these products was 10^5 CFU/ml. In addition, 81.8% and 69.6% of cosmetic brushes and sponges, respectively, were also contaminated with *P. aeruginosa*. Tran et al. (1994) tested in-store cosmetic testers available for consumer use. In that study, 5% of cosmetic testers had microbial loads over the acceptable limit (500 CFU/ml) established by the FDA; 50% of all cosmetics tested in the study had microbial contamination.

With the advent of commercial cosmetic cleaners, it is now possible for professionals and the general public to clean their products. There are a variety of products on the market targeted at cleaning and sanitizing cosmetics and cosmetic brushes. The aim of this project was to determine if these products are effective in reducing the amount of microbial contamination on cosmetic and cosmetic brushes.

Cosmetics are not required to be sterile, but are expected to be free of pathogenic microorganisms that could cause harm to the consumer. The USP requires that these products are free from contamination with *S. aureus*, *P. aeruginosa*, *Salmonella* spp., and *E. coli* (Di Maiuta

et al., 2011). To determine the test organism to focus on for this study, a pilot study was conducted using *P. aeruginosa*, *E.coli*, *S. aureus,* and *S. epidermidis*. These organisms were used to inoculate cosmetics and cosmetic brushes and recovered using traditional microbiological techniques; this was done without the use of cosmetic cleaners. The organism with the best percent recovery, *S. aureus* ATCC #6538, was used for the actual test experiments. *S. aureus* was also selected because it is the organism most commonly isolated from cosmetics and cosmetic brushes (Birteksoz et al., 2013).

Our first research question was whether the commercial cleaners were effective on cosmetics. There are many types of cosmetics available, but for testing purposes two categories of cosmetics were used, powder (eyeshadow or blush) and cream-based (lipstick). Cosmetic cleaners on the market for these cosmetics are sprays and wipes. The sprays were used on powder-based makeup, and the wipes were used on the cream-based cosmetics; for this study two brands of each product type were tested. Because cosmetics contain preservatives that work against microbial growth, contact times of 0, 1, and 5 minutes were tested. Allowing the inoculum to stay on the cosmetic at different contact times allowed for the determination of whether preservatives play a role in decreasing the microbial contamination present. Of all the replicates, eyeshadows exhibited the greatest variability among cosmetics and cosmetic brushes. The cosmetic spray from brand #2 resulted in the lowest percent reductions at all contact times; the lowest percent reduction was seen at contact time $T_{1\ minute}$ with a 13.86% reduction. Interestingly, of all products, the clean cotton pad resulted in at least a 98% reduction of contamination across all contact times. These results suggest that the mechanical action of wiping the surface of a cosmetic is sufficient to reduce the microbial concentration present. There was a statistically significant difference among product types for eyeshadows ($p <$

0.0001). Post-hoc tests demonstrated a significant difference between spray #2 and the cotton pad ($p = 0.0034$). Overall, comparisons of the percent reduction means showed that the cotton pad had a larger mean percent reduction (99.44%) compared to spray #2 (37.86%). Thus, the cotton pad exhibited a greater percent reduction of microbial contamination on eyeshadows compared to spray #2.

As for the lipsticks, all of the wipes tested resulted in at least a 94% reduction in *S. aureus* inoculum; lower percent reductions were seen with the cotton pad which contained no cleaning agent. For lipsticks, there was a statistically significant difference among cleaning product types ($p=0.0070$). Post-hoc tests demonstrated a significant difference between wipe #2 and the cotton pad ($p = 0.0007$). Comparisons of the percent reduction means showed that wipe #2 had a larger mean percent reduction (99.77%) compared to the cotton pad (96.18%). In addition, there was a statistically significant difference between the 70% isopropyl alcohol spray and the cotton pad ($p=0.0013$). Comparisons of the percent reduction means showed that the 70% isopropanol alcohol had a larger mean percent reduction (99.56%) compared to the cotton pad (96.18%). Thus, 70% isopropanol wipe and wipe #2 exhibited a greater percent reduction of microbial contamination on lipsticks compared to the cotton pad.

The second research question was whether cosmetic cleaners were effective on cosmetic brushes. As with cosmetics, there are many types of brushes, both in size and material. For this study, two types of brushes were tested, small and large brushes. Unlike cosmetics, there are no preservatives present to combat microbial contamination; therefore, the only contact time tested was Time zero (T_0), immediately after inoculation. The cosmetic brush cleaners tested were sprays, wipes, and shampoos; the brands tested were narrowed down to two for each product type due to the vast amount of products currently available. However, most cleaning product

manufacturers provide very little, if any, data on the efficacy of their product; some of them even make claims indicating their effectiveness against bacteria without adequate data to support these statements. For small brushes, the lowest percent reduction was seen with brand #2 spray at 87.62%; the highest percent reduction came from brand #1 wipe at 99.20%. There was not a statistically significant difference among product types ($p=0.9833$) or brands ($p=0.0605$) for small brushes. With the large brushes, the lowest percent reduction was with the brand #2 shampoo at 97.78%; the highest percent reduction was with the brand #1 shampoo at 99.87%. For large brushes, there was a statistically significant difference among product types ($p=0.0004$). Post-hoc tests demonstrated a significant difference between shampoos and wipes ($p = 0.0051$). Overall, comparisons of the percent reduction means showed that the wipes had a larger mean percent reduction (98.01%) compared to shampoos (89.82%). Thus, the wipes exhibited a greater percent reduction of microbial contamination on large brushes compared to shampoos.

Because all products produced favorable results, an additional control experiment was conducted to determine whether the mechanical action of cleaning cosmetics and brushes played a role in the removal of microbial contamination. For the cosmetics, eyeshadows and lipsticks, a clean cotton pad was used as the cleaning agent. The results from these tests showed that there was at least a 96% reduction of microbial contamination. This suggests that even the use of a clean cotton pad is sufficient to adequately clean eyeshadows and lipsticks. For the brushes, a 70% isopropanol spray was used. The tests also resulted in at least a 96% reduction of microbial contamination. However, the prolonged use of alcohol is not suggested for cosmetic brushes because they can cause damage to the bristles over time.

There are some limitations to this study. First, the small sample size and limited amount of commercially-available cosmetic cleaners tested, can affect the interpretation of these results. The second limitation is the sampling method. Swabbing the surfaces of the cosmetics, and agitating the brushes, could result in the loss of the inoculum. The third limitation is the presence of preservatives. Neutralizing buffer was used to address this issue as well as testing at different contact times. The fourth limitation is the use of only one test organism. Cosmetics and brushes can be contaminated with a number of different organisms at a given time; this may affect the efficacy of the cleaning products and should be evaluated in future studies. However, the use of *S. aureus* in this study represents a hardy (Gram positive) organism; therefore, it is assumed that if the cosmetic cleaners are effective against this microorganism, they will be as effective against more fragile (e.g., Gram negative) microorganisms. Lastly, the fifth limitation was the fact that the brushes were free of makeup. This could produce different results; however, the goal of this project was to assess and compare products given the same conditions. Brushes containing cosmetic residue should be a focus in future studies.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

Cosmetics have the potential to become contaminated with pathogenic organisms from the moment they are first opened. The chances of contamination greatly increase with consumer misuse such as sharing products, the addition of water to thin out the cosmetic, and improper storage. Even if cosmetics are not shared, the prolonged use of a product beyond its expiration date or improper usage allows for the potential of product contamination which can cause infections (Pack, et al., 2008). With the advent of commercially-available cosmetic and cosmetic brush cleaners, both professional and home cosmetic users have more options for cleaning and caring for their products. The results of this study show that cleaning both cosmetics and brushes, regardless of the cleaning product used, was effective in removing a substantial amount of microbial contamination. In addition to assuring cosmetics and brushes are cleaned regularly, consumers should also avoid sharing cosmetics, be sure to properly store products, be aware of expiration dates, and discard products used while sick or during a skin infection.

Future research should be conducted on cosmetic cleaners and used cosmetics, such as in store testers, to determine if these cleaning products are effective on higher microbial concentrations and contamination with multiple organisms. In addition, testing other organisms of significance is necessary to determine the range of effectiveness for these products. Future studies should also compare the effectiveness of cleaning products on brushes containing cosmetic residue, such as powders or cream-based products. It would also be beneficial to test different cosmetic brush materials, such as synthetic and natural fibers, to determine if there is a difference in the amount of microbial contamination that can be removed by a commercial cosmetic cleaner. The results of this study demonstrate that cleaning products, regardless of

contact time, product type, or brand, were effective in reducing microbial contamination on cosmetics and cosmetic brushes. These data can be used to inform consumers of the importance of regular maintenance of their cosmetics and cosmetic brushes.

APPENDIX A

LIST OF ACRONYMS

AD	Atopic Dermatitis
ANOVA	Analysis of Variance
ATCC	American Type Culture Collection
BSC	Biological Safety Cabinet
CFU	Colony Forming Unit
CFU/ml	Colony Forming Unit per milliliter
CIR	Cosmetic Ingredient Review
FDA	Food and Drug Administration
GLM	General Liner Model
GMP	Good Manufacturing Practices
ISO	International Organization for Standardization
LDL	Lower Detection Limit
PAO	Period after Opening
PBT	Phosphate Buffer with 0.05% Tween
TSA	Tryptic Soy Agar
TSB	Tryptic Soy Broth
USP	United States Pharmacopeia

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