

# The Effect of Publically Available Alcohol Based Colognes on Dermal Microbiota Growth

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## Abstract

Cologne application is of widespread use in American society. Consumers of these ethanol-based skin applications may not consider the effect these products could have on the dermal microbiome, which has been known to lead to pathogenic vulnerability if compromised. Conditions such as psoriasis, eczema, rosacea and acne are all known to correlate with an altered dermal biome. In this experiment, the effect of three popular colognes (Givenchy, Axe and Old Spice) were tested on microbial colonies isolated from two separate areas of the epidermis. Isolated colonies were treated with increasing dosages (5  $\mu$ L, 10  $\mu$ L, and 15  $\mu$ L) of each cologne and the effects were measured by growth inhibition. Interestingly, the different ethanol-based colognes had varying effects on the dermal microbiome. Axe and Old Spice were shown to be effective anti-bacterial agents. Givenchy, on the other hand, had less of a pronounced effect against the bacterial cultures tested. These data suggest that the human dermal microbiome may be compromised by some commercial cologne applications.

## Introduction

Skin applications are a large industry that currently with thousands of available commercial products. With the increase in focus on hygiene and personal image in American society, both men and women partake in ritual scenting of the dermal surface. The base component of most publically available colognes is a form of ethanol known as denatured alcohol, commonly labeled as specifically denatured. Alcohol is used in scented skin applications for its evaporative properties to enable the diffusion of intended scents into the air from the skin as the alcohol evaporates. However, ethanol is a well-known and widely-applied broad-spectrum disinfectant and skin antiseptic against microbes (McDonnell and Russell, 1999).

There exists more bacteria living on the dermal surface of the body than cells that make up the entire human body (Rosenthal et al., 2011). These bacteria belong to the four different phyla Actinobacteria,

Firmicutes, Proteobacteria, Bacteroidetes, Cyanobacteria, and Acidobacteria (Grice et al., 2008). The structure of the dermal microbiota communities will vary in makeup depending on the location on the body it is found (Grice et al., 2008). Many of these species have formed a symbiotic relationship with the human body. For example, certain microbiota will perform dermal protein digestion, whereas some will transform the oil secretions of the skin into a form of moisturizer (Grice et al., 2008 and Trivedi, 2012). The microbiome has been shown to have a beneficial immunological role as well. It provides a very competitive environment which makes the survival of human pathogenic bacterium unlikely (Sanford and Gallo, 2013). This symbiotic relationship also primes the human immune system through constant interaction between the host and resident bacteria and, as a result, the immune system of the host is better prepared for defense against foreign pathogens (Rosenthal et al., 2011 and Sanford and Gallo, 2013). It is becoming increasingly clear with new research that these microbiota and their overall biodiversity play a role in many dermal diseases. Skin conditions such as psoriasis, eczema, rosacea and acne have an acknowledged correlation with these shifts in the dermal microbiome (Grice et al., 2008). The diversity of dermal microbiota on persons afflicted with such conditions differs widely from that of unaffected, undiagnosed persons (Trivedi, 2012). Multiple external forces can alter the dermal biome. Temperature, radiation, moisture, and hermetic sealing such as common bandages induce a change of biodiversity on the skin (Rosenthal et al., 2011). Such disturbances of the skin microbiome will activate pathogenicity in some skin residents like *Staphylococcus aureus* (Rosenthal et al., 2011). It is not well known the effect purposefully applied applicants such as perfumes, colognes, or aftershaves have on the dermal microbiome.

In the current study, microbial communities were sampled from two areas of the author; the fingertips (Sample A), and the forehead (Sample B). Isolated colonies were exposed to three different SD-alcohol based colognes in order to expose their effects upon microbial growth. Givenchy, Axe, and Old Spice were tested due to their popularity. Isolated colonies were treated with the

cologne using disk diffusion in increasing dosages. The results show that from each epidermal area sampled, growth was inhibited with the application of the Axe and Old Spice colognes with growth inhibition increasing in dose-dependent manner. The Givenchy brand had no effect on one areas sampled, but showed similar results to the other colognes in the second area sampled. These results suggest that while different colognes may have varying effects that can depend on the specific area sampled, they may have a negative effect on overall microbiome health.

## Materials & Methods

Utilizing aseptic technique, a sterilized inoculating loop was scraped against the facial skin of a human subject. The loop was then streaked on an LB agar petri plate. The plate was sealed and incubated at 35°C for two days. After incubation, two visually different microbial colonies present were isolated in 1 mL of autoclaved, distilled water suspensions using a sterilized inoculating loop, pressed into the center of the colony to avoid contamination. After homogenizing the suspensions for 30 seconds by rolling the tubes between pressed hands, each suspension was streaked with a sterile swab upon eight LB agar petri plates for a total of 16 plates.

Disk diffusion was performed by forming sterile filter paper disks measuring 3 mm in diameter to administer the three chosen SD-alcohol colognes. Each disk was subjected to 5  $\mu$ L of cologne. To increase dosage, disks were stacked upon one another, where two disks would be the equivalent to 10  $\mu$ L, and so on. 100% ethanol was used as a positive control. Negative controls were disks that received no treatment. Each cologne was tested against the microbes isolated from each sample area (A and B) in replicate, so that each cologne had a total of four plates each containing three doses. All plates were sealed and incubated at 35°C for two days. The resulting zones of inhibition were measured and recorded from the edges of the filter paper circllet to the closest edge of the inhibition zone.

## Results

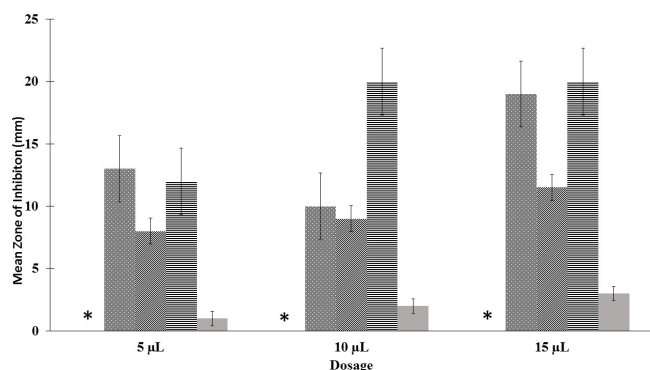


Figure 1. Effects of application from colonies isolated from human fingertips (Sample A); 100% Ethanol - grey with white crosses; Givenchy Brand - upward diagonal; Old Spice Brand - dark horizontal lines; Axe Brand - solid grey; Negative Control - \* no growth seen

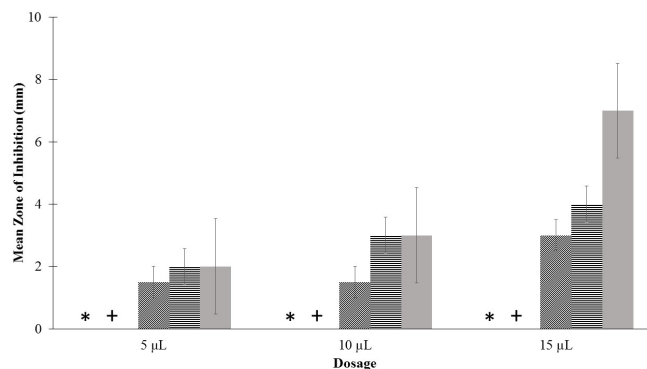


Figure 2. Effects of application from colonies isolated from human forehead (Sample B); 100% Ethanol - grey with white crosses; Givenchy Brand - + no growth seen; Old Spice Brand - dark horizontal lines; Axe Brand - solid black; Negative Control - \* no growth seen

The results of the three colognes on epidermal samples A and B are listed in Figures 1 and 2, respectively. The microbes sampled from the forehead (sample A) displayed significantly inhibited growth in the presence of all three cologne samples when compared to the negative control (Figure 1). In addition, the mean inhibition of growth for each cologne was higher than 100% ethanol at all concentrations. Growth inhibition of microbes sampled from the fingertips (sample B) showed less growth inhibition (Figure 2). Axe brand and Old Spice brand colognes had similar inhibitions of bacterial growth compared to the positive control. However, even at the highest dose administered, they showed less inhibition than the colognes administered to sample A at the lowest concentrations. The Givenchy brand showed no inhibition of growth compared to the negative control.

## Discussion

Results from this study suggest a negative correlation with the use of SD-alcohol based colognes and dermal biome health. Both samples A and B reacted negatively in the presence of the Givenchy, Axe, and Old Spice brand colognes. Sample B was the least susceptible to these colognes overall, with much smaller mean inhibition zones. It was not susceptible to the Givenchy brand cologne, having no inhibition of growth. The Old Spice and Axe brands had effects on both samples, with samples A

resulting in a larger zones of inhibition to the applicants. The mean variable inhibition zones for many colognes applied to sample A were larger than that of pure ethanol. This is indicative that this microbe is susceptible to certain compounds in all variables not found in the positive control.

Both samples react differently to the applicants, suggesting that consumer brand skin applicants based on denatured alcohol do not cause complete inhibition but do have a negative effect on biodiversity. This may have adverse public health risks as decreased dermal microbiome diversity is known to induce pathogenicity in dangerous pathogens such as *Staphylococcus aureus*, the most widespread perpetrator of *Staphylococcus* infections (Rosenthal et al., 2011). Concern for public health should also be taken considering the existing correlation between skin conditions such as rosacea, eczema, acne, and psoriasis, and an altered profile of dermal biodiversity (Grice et al., 2008). The results of this study suggest that the application of ethanol-based colognes may contribute to the weakening of the dermal biome and increase the risk for pathogenic microbes. However, it should be noted, that the scope of this study was limited to a small subset of the total dermal microbiome. For example, only microbes capable of surviving on LB media were isolated. Interestingly, microbes from both samples reacted differently depending on the applicant. This suggests that the different chemical constituents of the individual colognes may each contribute to microbe toxicity. Isolation of these compounds would be necessary to identify any other bactericidal and bacteriostatic agents in their compositions that may have had a role in the negative growth patterns. The Givenchy brand had no effect on sample B, which may be indicative of other compounds in Axe and Old Spice that are facilitating growth inhibition. Existence of an unknown, offending compound(s) other than ethanol may be determined through replication of the initial experiment, but substituting time to allow the ethanol within all three colognes to aerosolize before application to the filter paper disks. However, further testing is needed to delineate these compounds. However, further testing is needed to delineate these compounds.

The lack of inhibition observed in sample B when exposed to the Givenchy brand could be used as a means of identification of the organism. The organism isolated and tested from sample B was resistant to lower levels of ethanol not present in the Axe and Old Spice brands, if it can be determined that the luxury brand does contain less ethanol than the discount brand counterparts. The lack of inhibition could also be an indication of an overall less antiseptic cologne application. It may be that the discount cologne brands are greater culprits of decreased dermal biodiversity. What this says about all luxury brands and discount brands available cannot be stated; more testing involving all consumer brands are needed.

These results indicate that Axe and Old Spice were effective bactericides against both dermal samples. On the other hand, Givenchy was less effective as a disinfecting agent of the skin bacteria of the samples tested.

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