Make-it-Up

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URBAN BARCODE RESEARCH PROGRAM

CSH Cold Spring Harbor Laboratory DNA Learning Center

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Abstract

Cosmetic stores offer makeup testers to the public which are utilized daily by many people. Bacteria present in the skin of users can easily pass to the cosmetics, and as they are very rich environments and those testers are not being changed for several days or weeks, potential harmful bacteria can grow at ease becoming a source for illness transmission. Here, we sought to determine the species of bacteria present on the makeup testers in different makeup stores, analyzing the potential harm when using a tester for a lipgloss or mascara in NYC stores. We collected samples of three makeup brands of different quality from two different stores in midtown and uptown Manhattan. We sampled both lipgloss and mascara, which are potentially applied in skin areas close to the mucosa. The samples were Sanger sequenced for the 16S gene, which gave several high-score hits for each sample. The most interesting hits were then analyzed by microbiological methods for confirmation. We observed that most of our samples had grown strains of *Staphylococcus hominis* and other *Staphylococcus sp*. in the lipsticks and *Staphylococcus epidermidis* and other *Staphylococcus sp*. in the mascaras. Although these are resident microbiota (flora) from the skin, they have the potential to be detrimental to users with weak immune systems.

Introduction

Manhattan, with 1,644,518 inhabitants, is one of the places with the highest density population in the western world. 53% of them are women (1). Almost every woman wears makeup at some point in her life. Many stores in Manhattan provide beauty testers for people to try on before buying the actual product, and many women try on the latest lipstick without really understanding the risk they might be taking. These testers could remain open for weeks, enabling bacteria and other germs from the users to deposit and grow. Although cosmetics include preservatives in their composition to prevent bacterial growth and lengthen their lifespan, the antimicrobial preservative activity could be overwhelmed by overuse and high bacterial load (2). The contaminated makeup could therefore turn into a potential source for illness transmission to the unaware new users.

Previous studies have actually found the presence of bacterial contamination in shared-use cosmetic test kits. The US Food and Drug Administration (FDA) showed that among 35 different genera of bacteria in the samples they analyzed, there was a 2% of presence of pathogenic bacteria (*Staphylococcus*, *Klebsiella*, *Pseudomonas*, *Acinetobacter* and *Serratia*) (3). Additionally, another study from a school-project performed at Jefferson Medical College in Philadelphia identified *Staphylococcus*, *Streptococcus*, *E. coli* bacteria and herpes virus in the products (4,5). In this project, we want to analyze the potential harm that we could find in NYC when using a tester for a lipgloss or a mascara, both perfect environments for bacterial growth. We would also want to determine if this harm could be dependent on the type of preservatives present in them.

Materials & Methods

We acquired three types of samples of both lip gloss and mascara: a natural makeup brand, a luxury makeup brand, a low-cost makeup brand. This way, we intended to also analyze if the quality of the makeup, and the different preservatives in them, could relate to a better or worse preservation from the bacterial growth. As a negative control, we analyzed an unopened sample, and as positive controls we utilized swabs from our hands. We collected our samples by using sampling swabs and brushing the lip gloss brush of each lipgloss into tubes filled with Phosphate-buffered saline (PBS) solution, and securing them closed to prevent any further contamination. We performed this experiment two separate times, within two different make-up stores in Manhattan, to ensure that we had a representative sample size (50 samples total). After collecting our samples, we returned to the lab and grew each sample on 5 ml of lysogeny broth (LB) media at 37°C O/N to enrich the bacterial population. We then froze these samples in 20% glycerol and stored them at -80°C. Once we were ready for the DNA extraction, we unfroze the samples, and perform the BARCODING PCR amplification for the 16S bacteria gene using the primers 1492R and Bac27Funiv (6). We ran the PCRs in an agarose gel to observe band amplification, and send the amplified samples for Sanger Sequencing. Due to the similarity of the amplified sequence among some bacteria, we obtained several hits for each of our samples, some of them with identical high score. Therefore, we decided to analyze them by microbiological methods (gram staining followed by different tests like catalase, coagulase, antibiotic sensitivity, hemolysis and differential and selective media) for confirmation of the right hit.

Results

Figure 1

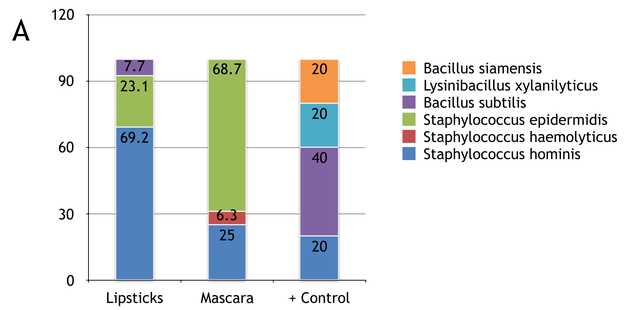

Figure 1. Prevalence of bacteria species per study group, obtained by DNA Sanger sequencing. Only main hits are represented for each sample. (A) Comparison of bacteria isolated among lipstick and mascara study groups. (B) Comparison of bacteria isolated among different quality cosmetics.

Figure 2

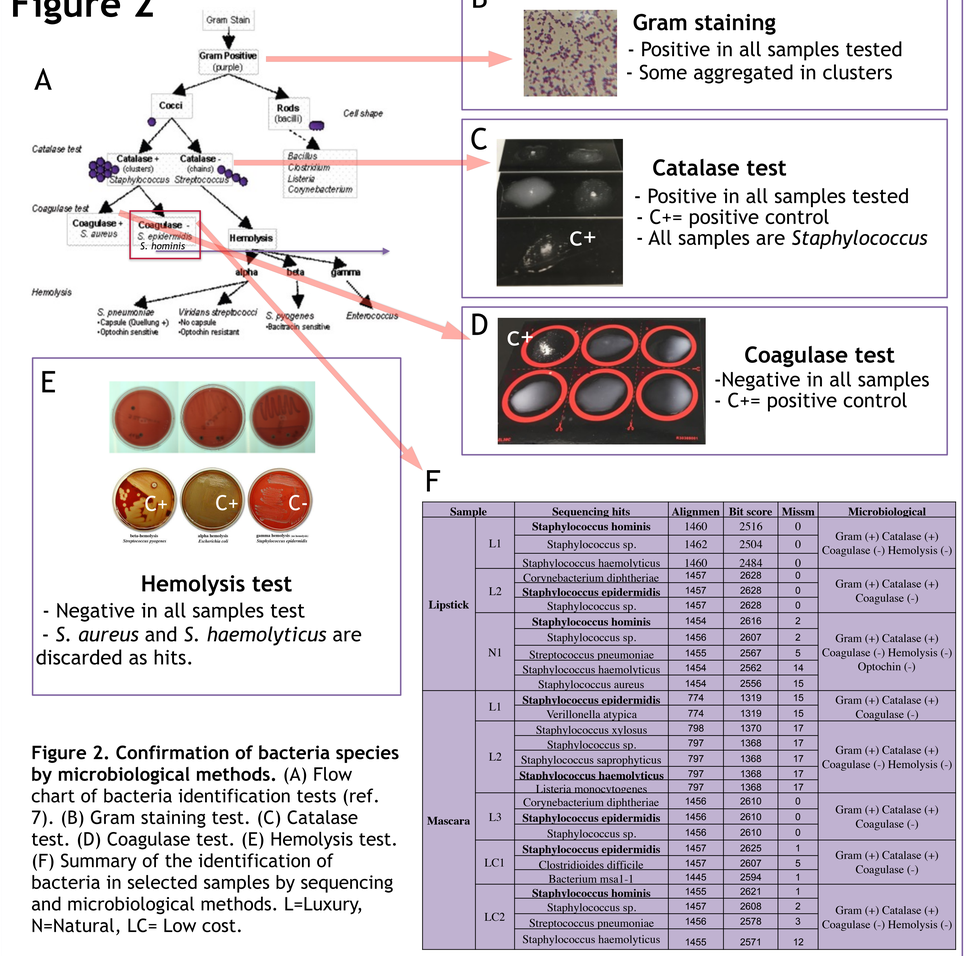

Figure 2. Confirmation of bacteria species by microbiological methods. (A) Flow chart of bacteria identification tests (ref. 7). (B) Gram staining test. (C) Catalase test. (D) Coagulase test. (E) Hemolysis test. (F) Summary of the identification of bacteria in selected samples by sequencing and microbiological methods. L=Luxury, N=Natural, LC= Low cost.

Sample		Sequencing hits	Alignmen	Bit score	Missm	Microbiological
Lipstick	L1	**Staphylococcus hominis**	1460	2516	0	Gram (+) Catalase (+) Coagulase (-) Hemolysis (-)
		Staphylococcus sp.	1462	2504	0	
		Staphylococcus haemolyticus	1460	2484	0	
	L2	Corynebacterium diphtheriae	1457	2628	0	Gram (+) Catalase (+) Coagulase (-)
		Staphylococcus epidermidis	1457	2628	0	
		Staphylococcus sp.	1457	2628	0	
	N1	**Staphylococcus hominis**	1454	2616	2	Gram (+) Catalase (+) Coagulase (-) Hemolysis (-) Optochin (-)
		Staphylococcus sp.	1456	2607	2	
		Streptococcus pneumoniae	1455	2567	5	
		Staphylococcus haemolyticus	1454	2562	14	
		Staphylococcus aureus	1454	2556	15	
Mascara	L1	**Staphylococcus epidermidis**	774	1319	15	Gram (+) Catalase (+) Coagulase (-)
		Verillonella atypica	774	1319	15	
	L2	Staphylococcus xylosus	798	1370	17	Gram (+) Catalase (+) Coagulase (-) Hemolysis (-)
		Staphylococcus sp.	797	1368	17	
		Staphylococcus saprophyticus	797	1368	17	
		Staphylococcus haemolyticus	797	1368	17	
		Listeria monocytogenes	797	1368	17	
	L3	Corynebacterium diphtheriae	1456	2610	0	Gram (+) Catalase (+) Coagulase (-)
		Staphylococcus epidermidis	1456	2610	0	
		Staphylococcus sp.	1456	2610	0	
	LC1	**Staphylococcus epidermidis**	1457	2625	1	Gram (+) Catalase (+) Coagulase (-)
		Clostridioides difficile	1457	2607	5	
		Bacterium msa1-1	1445	2594	1	
	LC2	**Staphylococcus hominis**	1455	2621	1	Gram (+) Catalase (+) Coagulase (-) Hemolysis (-)
		Staphylococcus sp.	1457	2608	2	
		Streptococcus pneumoniae	1456	2578	3	
		Staphylococcus haemolyticus	1455	2571	12	

Discussion

We had hypothesized that, due to the high transit of people testing cosmetics in make-up stores in NYC, there would be a high chance of finding potential pathogenic germs in them. We also wanted to test if different quality of cosmetics could correlate with differences in the type of bacteria allowed to grow, due to the particular preservatives that each of them contains. Our data shows that there is bacterial contamination in almost every sample tested. Although we find some differences between the species growing in lipsticks (mainly Staphylococcus hominis) with the ones in mascaras (Staphylococcus epidermidis), they all belong to the same genera and are normal flora from the skin. Nevertheless, they still have the potential to be harmful when the user is immuno-depressed. We do not find big differences between cosmetic qualities, and therefore the preservatives on them.

There are some limitations in our study that we must take into account. First, the general LB broth that was used to culture and enrich our samples might not be the optimal one to grow other fastidious microorganisms, which could have been initially there but remained undetected. Second, several species of microorganisms could be contaminating every sample, and in fact we observed different types of colonies growing in LB plates after streaking for colony isolation. Although all the colonies analyzed by microbiological methods belonged to the Staphylococcus genus, we cannot discard that there were other colonies that we missed in the process. To be able to determine all the species present in each sample, we will send them for Next Generation Sequencing (NGS) as the next step in our project. Finally, due to the relatively low number of samples tested, mainly in relation with other previous reports where some pathogenic species were found (2-5), we cannot discard that increasing the sample size we could find pathogenic bacteria within a percentage of makeup samples in the NYC stores. It would be interesting to repeat this testing by also testing samples at different times of the year, or at other locations in NYC.

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