

# What types of bacteria are in lip products and does temperature affect its growth?

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By Alice Liu

## Abstract:

Bacteria in makeup may cause severe diseases and skin infections, which makes it crucial to investigate whether heating lip products to 65°C would kill the bacteria, making it safer for consumers. This was investigated by swabbing 5 lip products under normal conditions, then swabbing them after they were heated to 65°C. Furthermore, 5 bacterial colonies were taken to test for antibiotic susceptibility to identify them. This found that most bacterial species identified were harmless to a healthy individual, except for *Staphylococcus aureus*, and were mostly killed at 65°C. However, some bacterial colonies still grew after heating, although a lot fewer colonies, so it could not entirely remove all the bacteria from the product.

## Introduction:

From the rise of the COVID-19 pandemic in the last couple years, people have emerged immensely more cautious of pathogens in their everyday lives. Bacteria are prokaryotic microorganisms invisible to the naked eye individually, however, can be spotted in colonies.<sup>1</sup> These microorganisms may or may not be harmful to human cells, which makes it important to identify.

Lip products, which includes anything applied to the lips, whether it be for cosmetic or health purposes, is something enjoyed globally by millions of people every day. Considering these products are applied closely to the mouth, any harmful bacteria from these products could cause devastating health effects, not only to the surrounding skin, but also to the body if consumed.<sup>2</sup>

Personally, before the pandemic years, I was quite severely impacted by a lip balm I had used. After applying the balm, I felt a stinging on my lips which worsened into a swelling and rashes on the surrounding skin. The doctor had declared it to be a bacterial infection and prescribed a cream for it. However, after it went down, I decided to switch to different lip balms, but strangely, each balm I used would cause another flare up. From there, I decided to just not use lip products altogether, thinking it was an allergy to a key ingredient in them, which only gave me cracked, dry lips for years. Only two years ago, I decided to test lip products again and found that they were no longer causing infections. Because of this, I have wanted to find if lip products contain harmful bacteria in them.

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<sup>1</sup> Hill, I (2018) Colonies on a Plate. <https://sitn.hms.harvard.edu/art/2018/colonies-on-a-plate/#:~:text=A%20single%20bacterial%20cell%20is.pile%20of%20bacteria%20a%20colony>

<sup>2</sup> Bashir, A (2019) Deadly bugs found in 9 out of 10 makeup bags. <https://theconversation.com/deadly-bugs-found-in-9-out-of-10-makeup-bags-127412>

This experiment will investigate the types of bacteria present in lip products, with an emphasis on whether they are harmful to people, and whether heating the products will affect the growth of bacterial colonies in any way.

**Aim:**

To identify the types of bacteria present in 5 samples of used lip products, focusing on whether they pose a risk to our health, and the effects of increasing the temperature of the lip products to 65°C on the bacterial colonies.

If the temperature of the lip products is increased to 65°C, then there will be fewer bacterial colonies, because bacteria are rapidly killed at 65°C.<sup>3</sup>

**Materials:**

- 5 x Makeup samples
- 20 x Nutrient Agar plates
- Disposable plastic loops
- Distilled water
- Marker pen
- Disinfectant spray
- Bunsen burner
- Gloves
- Incubator at 37°C
- Clear sticky tape
- Heat proof mat
- Matches
- 15 x 100mL beaker
- Water bath at 65°C
- 3 x watch glasses
- Thermometer
- 5 x test tubes
- Scissors
- Test tube rack
- 5 x pipettes
- 5 x cell spreaders
- Tweezers
- 5 x Antibiotic susceptibility disks

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<sup>3</sup> Seladi-Schulman, J (2020) What Temperature Kills Bacteria in Water and Food?  
<https://www.healthline.com/health/what-temperature-kills-bacteria>

## Method:

Letters with their corresponding lip product.

<b>R</b>	<b>T</b>	<b>N</b>	<b>P1</b>	<b>P2</b>
A red pigmented lip tint by the brand Romand, with a doe foot applicator.	A clear lip balm from a children's toy.	A clear lip balm by the brand Nivea, with a twist tube.	A lip balm, creamier than T and N, which I made in a pharmacy, stored in a glass container.	The same as P1, except I started using P2 6 months after opening P1.

## Part 1

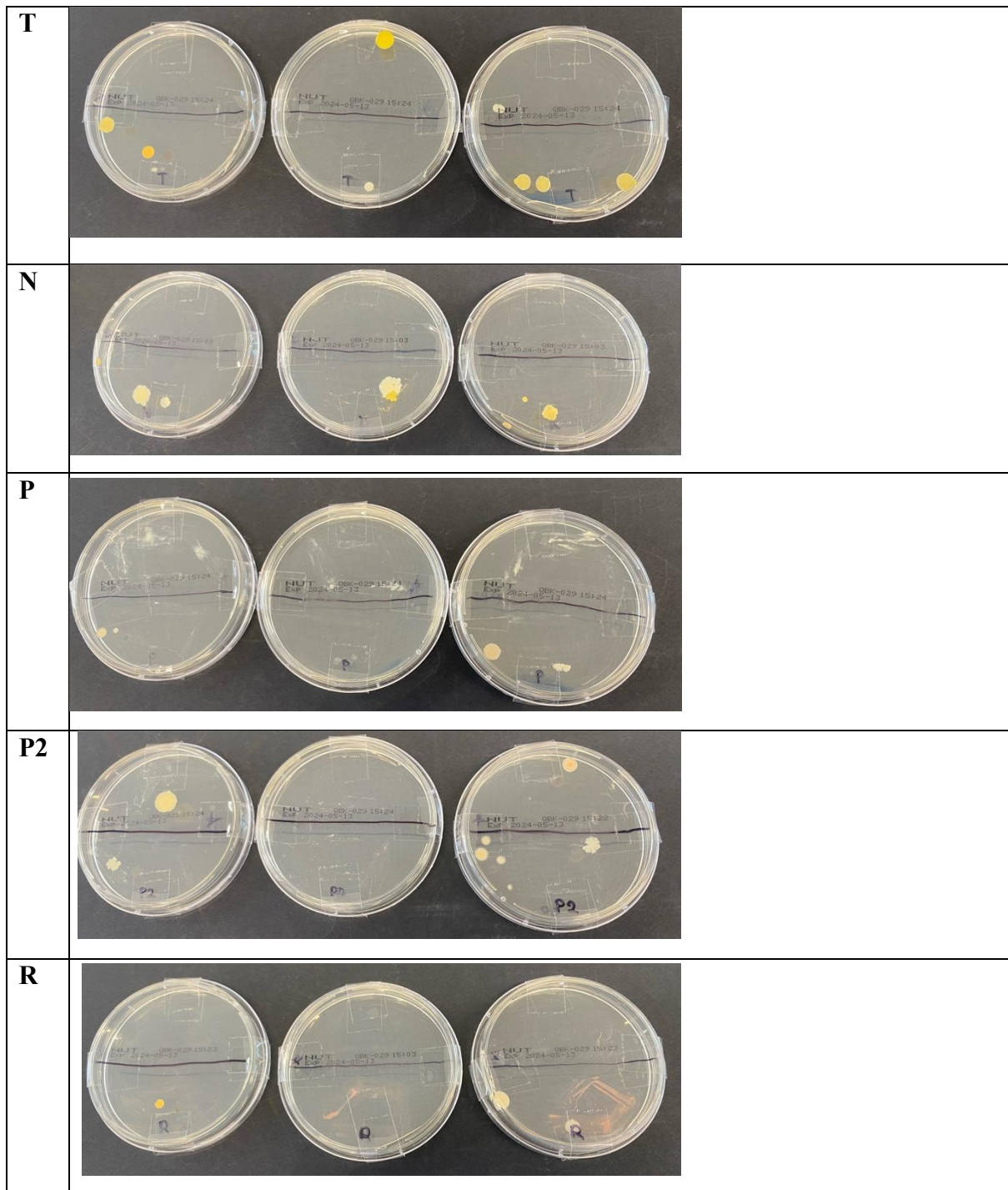
1. Gloves were put on and a Bunsen burner was set up on a heat proof mat set to an open flame to sterilise the surrounding environment.
2. A line was drawn with the marker pen across all nutrient agar plate bottoms and a star was labelled on the half intending to occupy the heated samples.
3. Nutrient agar plate lids were labelled in threes, with a letter representing the makeup product, creating 3 trials for the 5 lip products.
4. Ensuring to work under the Bunsen burner sterilisation field, approximate equal amounts of distilled water were poured into 5 100mL beakers.
5. Using a disposable, sterile loop, a thin amount was scraped from the surface of a lip product and swirled in a beaker containing distilled water to reduce its thickness.
6. The loop was streaked across an agar plate corresponding with the lip product using the quadrant streaking technique across the half without the labelled star.
7. Using the same loop and distilled water beaker, this process was repeated three times for the lip product, creating three trials.
8. For each of the 4 remaining lip products, a different loop and distilled water beaker was used per product to plate 15 agar plates.
9. Each lip product was opened and put into its individual 100mL beaker with its packaging, albeit the product with a wand was smeared on the bottom of the beaker and the lip balm in a push up tube was removed from its packaging.
10. Similar amounts of distilled water were placed into each beaker and a water bath was set to 65°C.
11. The beakers were placed into the water bath, entirely submerging the beakers in the water bath using disinfected watch glasses as weights for the beaker with the smeared product.
12. After 5 minutes, the beakers were taken out and steps 4-8 were repeated, though this time streaking on the half with the labelled star for all 15 agar plates.
13. The agar plates were taped with clear tape and placed upside down, to prevent contaminants falling into the plate, in an incubator at 37°C.
14. Results were observed and recorded every weekday after the day of plating at the same times for 8 days.

## Part 2

1. Gloves were put on and a Bunsen burner was set up on a heat proof mat set to an open flame to sterilise the surrounding environment.
2. 5 differently coloured bacterial colonies were selected from the plates used in Part 1, with each colony developed from a different lip product.
3. 5 new nutrient agar plates were labelled on the bottom with the selected bacterial colonies' respective colours.
4. All the tape was removed from the 5 agar plates containing the selected colonies.
5. 5 test tubes were filled with equal amounts distilled water and placed in a test tube rack.
6. Working under the sterile field, an agar plate was opened, and a sterile loop was used to scrape the selected bacterial colony onto the loop, which then was swirled in a test tube with distilled water.
7. After the bacterial colony had entirely dispersed into the distilled water, the loop was taken out and 1mL of the solution was drawn with a pipette.
8. Opening the new agar plate corresponding to the bacterial colony, the solution was poured onto the agar plate with the pipette.
9. The solution was spread into an even layer using a disposable, sterile cell spreader.
10. Tweezers were sterilised by holding them in the flame of the Bunsen burner and were used to place an antibiotic susceptibility disk in the centre of the agar plate.
11. The disk was pressed down into the solution by the tweezers to ensure it would not lift off the solution, and the tweezers were sterilised with the flame after this.
12. The lid was placed back on the agar plate and set aside to repeat steps 6-11 for each of the selected bacterial colonies, using different sterile loops, pipettes, and cell spreaders for each colony.
13. After plating all 5 colonies, the sides were taped up and the plates were left for half an hour for the agar to soak up the solution.
14. The agar plates were placed upside down in the incubator at 37°C.
15. Results were recorded for 3 consecutive days after the day of plating at the same times.

## Observations and results:

Figure 1: Agar plates growing bacterial colonies and fungal growth, sorted by makeup product, 10 days after plating (from Part 1).



Summary: After 10 days, plates P2, N and T had the largest number of colonies. Plates R, N and P did not have any growth on the heated sides. Plates P2, P and R all had one trial which did not have any growth on both sides, suggesting a random error. Plate P2 had the greatest number of different coloured colonies, suggesting it had the greatest number of different bacterial species present.

Figure 2: Observations for Part 1 agar plates, 8 days after plating.

<b>T</b>	<b>N</b>	<b>P</b>	<b>P2</b>	<b>R</b>
<ul style="list-style-type: none"> <li>- All colonies round but some with rough edges</li> <li>- Yellow colonies</li> <li>- White colonies</li> <li>- Orange colonies</li> <li>- Potential fungal growth (growth looked fuzzy but no blackish centre when flipped over yet)</li> </ul>	<ul style="list-style-type: none"> <li>- All colonies cloud shaped</li> <li>- White colonies</li> <li>- Yellow colonies</li> <li>- Orange colonies</li> <li>- Rough edges on colonies</li> </ul>	<ul style="list-style-type: none"> <li>- Mix of colonies that are cloud shaped and round</li> <li>- Pale orange colonies</li> <li>- Patterns on one colony</li> <li>- Fungal growth (when flipped over, a blackish centre)</li> <li>- No growth on 1 trial</li> </ul>	<ul style="list-style-type: none"> <li>- Fungal growth (when flipped over, a blackish centre)</li> <li>- Cloud shaped</li> <li>- Rough edges</li> <li>- One round, smooth edged colony</li> <li>- Peach coloured colonies</li> <li>- White colonies</li> <li>- Yellow colonies</li> <li>- No growth on 1 trial</li> </ul>	<ul style="list-style-type: none"> <li>- All colonies round with smooth edges</li> <li>- White colonies</li> <li>- Orange colonies</li> <li>- Yellow colonies</li> <li>- No growth on 1 trial</li> </ul>

Figure 3: Bacterial and fungal colonies classified by colour, present in agar plates 10 days after plating.

<b>Colony colours</b>	<b>Unheated side</b>					<b>Heated side</b>				
	<b>T</b>	<b>R</b>	<b>N</b>	<b>P</b>	<b>P2</b>	<b>T</b>	<b>R</b>	<b>N</b>	<b>P</b>	<b>P2</b>
Yellow	X	X	X			X				X
White	X	X	X	X	X	X				
Brown										X
Orange	X	X	X	X						
Fungi				X	X					

Figure 4: The bacterial/ fungal colonies selected from each product for Part 2.

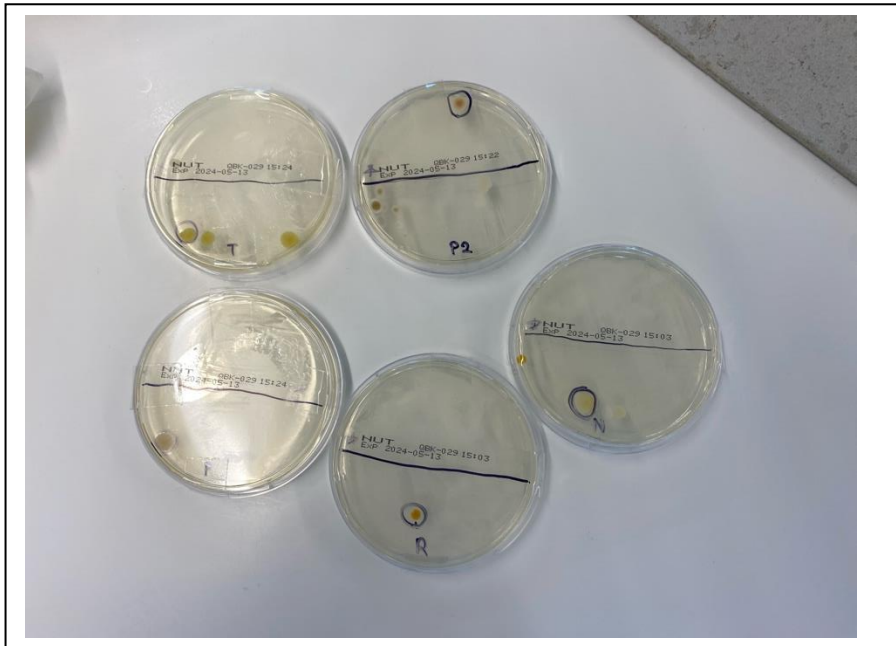


Figure 5: Part 2, 1-8 days after plating, of different colours of bacterial colonies.

Letters representing the antibiotics contained in the antibiotic susceptibility disks.

<b>AP</b>	<b>C</b>	<b>PG</b>	<b>S</b>	<b>ST</b>	<b>T</b>
Ampicillin	Chloramphenicol	Penicillin G	Streptomycin	Sulphatriad	Tetracycline

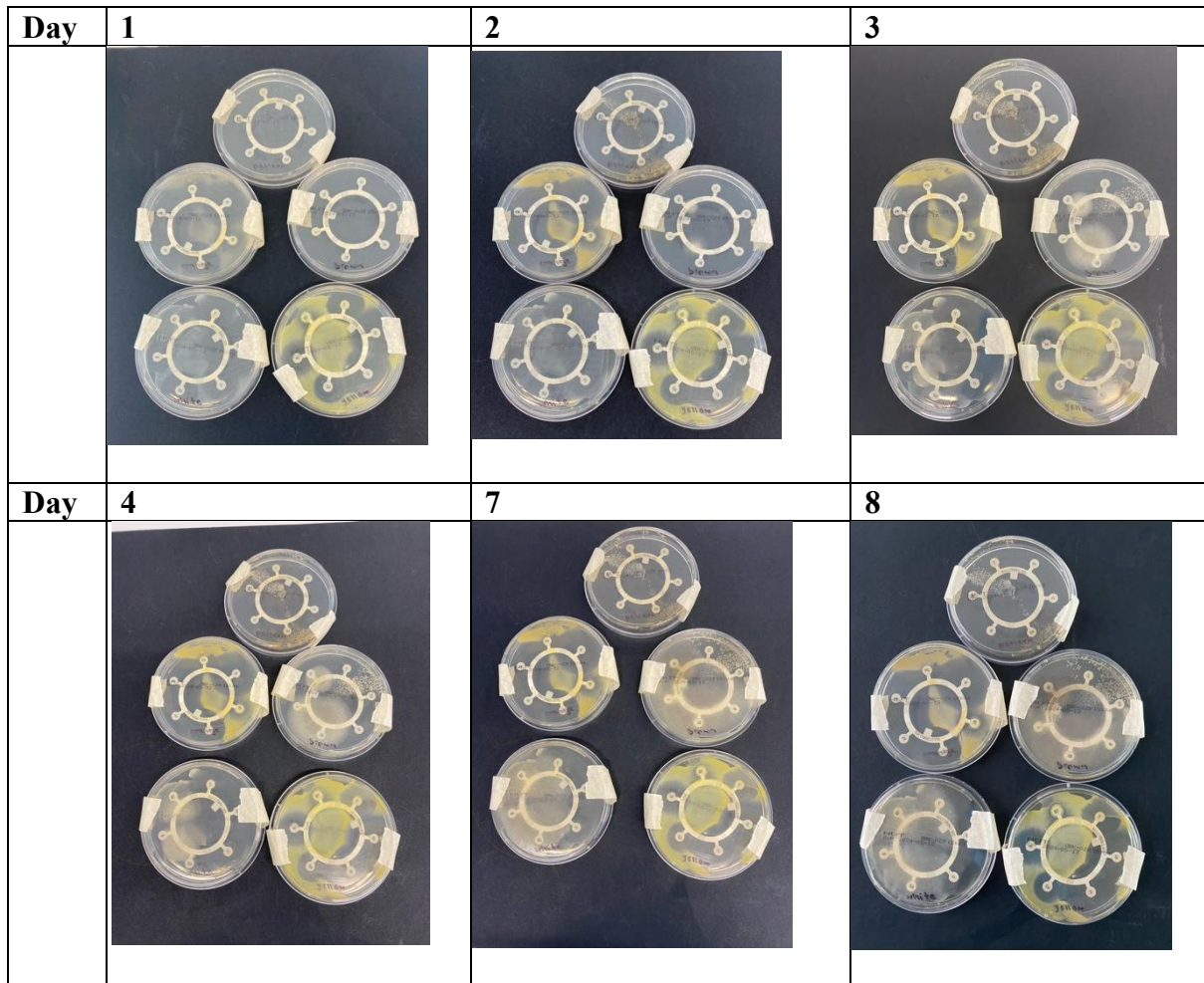
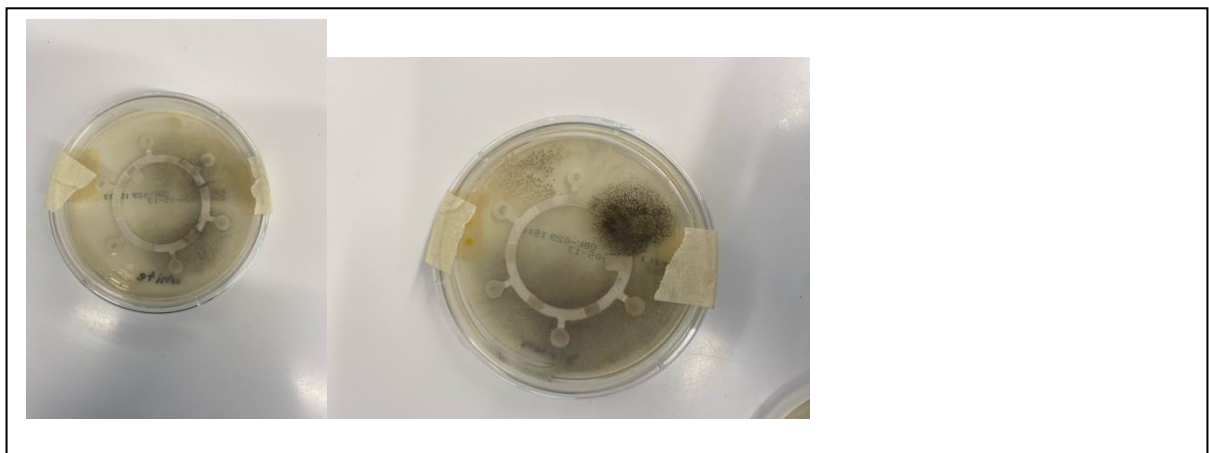


Figure 6: Pictures of plates “brown” and “white” having fungal growth.



## **Discussion:**

### Analysis of part 1

From the results gathered in Figures 1,2 and 3, my hypothesis that when the temperature is raised to 65°C, fewer bacterial colonies will develop when compared to the unheated side, is mostly supported. From Figure 3, for makeup products R, N and P, no colonies grew on all 3 trials for the heated side, despite having 2 or 3 types of differently coloured colonies growing on the unheated side. However, for one of the P and R trials, there was no growth on either side of the agar plate, which was an unexpected result. This could be due to a random error, where scraping the surface of the makeup product did not pick up any bacteria if the bacteria were not evenly distributed in the makeup. Another reason could be that the conditions of the nutrient agar plate and the incubator at 37°C was not suitable for certain types of bacteria to grow.

For the T agar plates, white and yellow colonies grew on the heated side, also seen on the unheated side, however no orange colonies developed despite being present in the unheated side. Additionally, only 2 colonies grew on the heated sides across all 3 T plates, compared to the 7 colonies on the unheated side, further supporting my hypothesis as this was the expected result.

For the P2 agar plates, there was an unexpected result as white bacterial colonies and fungal growths were present on the unheated side, however on the heated side, they did not form, but instead yellow and brown bacterial colonies formed. The brown coloured colony in P2 was not seen in any of the other plates, however the yellow colony looked like the yellow colonies growing on the T, R and N plates. However, the yellow colonies did not grow on the heated R and N sides despite being present on the unheated side. The yellow colony also did grow on the heated T side, like the P2 yellow colony, but being present on the unheated side originally. This could suggest that the yellow colonies are two different bacterial species, since some yellow colonies growing on the heated side for some plates whereas not growing on others would suggest the bacteria are killed at different temperatures. Albeit, it cannot be assumed that bacteria of the same species are killed at the same temperature, as genetic diversity within the bacterial species could cause some colonies to survive higher temperatures.<sup>4</sup> Also, the anomalies of the brown and yellow colonies growing in P2 could be due to random errors such as cross contamination when heating the makeup products. Since the products were all submerged in a water bath together, despite being in their respective beakers, bacteria from other products could have infected the water all the products were soaked in. Additionally, this anomaly could also be due to the bacteria not being evenly spread in the makeup, so when scraping the product, only some types of bacteria were picked up. Still, fewer colonies formed on the heated side, as there were only 2 colonies compared to 6 on the unheated side. However, one of the P2 agar plates had no growth whatsoever as well, perhaps due to the bacteria not being evenly distributed within the makeup product.

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<sup>4</sup> Lawrence, J (2002) Microbial Evolution: Gene Establishment and Loss. Nature Reviews Genetics. pp791-802

On a P agar plate, one of the bacterial colonies had a pattern on it, which was unusual as all the other colonies did not have patterns. It seemed this pattern developed on the bacterial colony around 7 days after plating. This could be because the colony dried up, because when scraping it for part 2 of the experiment, the colony came off in one piece and seemed to be dry compared to other colonies scraped which had a creamier consistency. However, other colonies on the same agar plate seemed to look like the other bacterial colonies which were not dry. This pattern could be a result of the bacterial colony being a different species causing a wrinkled texture, or the colony drying up due to not enough humidity. However, the other colonies on the same plate did not dry up despite all starting to grow from day 2 after plating, which could mean that colony was a different species, requiring more moisture to grow.

### Analysis of part 2

For the yellow bacterial colony, it was most susceptible to Ampicillin and Chloramphenicol, then had intermediate susceptibility to Tetracycline, Streptomycin and Penicillin G, whilst being resistant to Sulphatriad completely. From this result, *Staphylococcus aureus*, *Micrococcus luteus* or *Serratia marcescens* fit this profile.<sup>5</sup> *S. marcescens* commonly forms red colonies so it's unlikely to be this yellow bacterial species.<sup>6</sup> *S. aureus* and *M. luteus* are yellow pigmented bacteria, but with *S. aureus* being a major human pathogen, causing a wide range of skin infections and respiratory diseases.<sup>7</sup> *M. luteus* is non-pathogenic, rarely causing diseases, although still can cause infections in weaker individuals.<sup>8</sup> This plate is most likely to be *M. luteus* because referring to the original colony from plate T, it has a lighter yellow colour and not completely smooth edges, so these bacteria should be relatively safe when present in makeup.<sup>9</sup> Bacterial colonies with the same characteristics have appeared on the unheated N and R halves, so it can be assumed they are all *M. luteus*. It was again present on the heated T and P2 halves, so it is unlikely to be killed at temperatures up to 65°C, though there is insufficient results to prove this. On another T plate, there is a yellow colony on the heated side, with a bright yellow colour, different to the other yellow colonies, and a very smooth edge. This could be a different species of bacteria, possibly *S. aureus*, as its characteristics fit that profile, however this colony was not tested for its antibiotic susceptibility.

For the orange bacterial colony, it was susceptible to Streptomycin, Chloramphenicol, Ampicillin and Tetracycline, resistant to Sulphatriad, and it is unclear whether it was resistant to Penicillin G as it did not form a complete circle around it. From this result, it's most likely to be *S. aureus*, with the orange colour it was originally described as quite like the golden

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<sup>5</sup> CLSI (2021) Performance Standards for Antimicrobial Susceptibility Testing. 31st edition. Clinical and Laboratory Standards Institute. pp50-80

<sup>6</sup> Tille, P. (2017) Bailey & Scott's Diagnostic Microbiology. 14<sup>th</sup> edition. Elsevier. pp294-296

<sup>7</sup> Tong, S. et al. (2015) Staphylococcus aureus Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. PubMed Central. pp603-661

<sup>8</sup> Nunez, M. (2014) Encyclopedia of Food Microbiology. 2<sup>nd</sup> edition. Elsevier. pp627-633

<sup>9</sup> Ibid.

yellow correlated with *S. aureus*.<sup>10</sup> This colony had a relatively smooth border and small colony size of around 1-2mm, reflecting the characteristics of *S. aureus*.<sup>11</sup> This colony is more representative of *S. aureus* than the unique yellow colony on the T plate mentioned earlier. *S. aureus* leads to many skin infections such as cellulitis, furuncles and boils as well as food poisoning when ingested.<sup>12</sup> This is a severe problem when found in lip products specifically, as people easily ingest the products, causing nausea, vomiting and diarrhea.<sup>13</sup> Similar orange colonies were found on the unheated halves of the T, N and P plates, as well as the R plates which this colony was taken from. This suggests that *S. aureus* was present in 4 of the 5 makeup products, although none formed on the heated sides, implying this species of bacteria was killed at temperatures of 65°C.

For the white bacteria colony, it was susceptible to Tetracycline, Ampicillin, and Chloramphenicol for 4 days before spreading into the Tetracycline and Chloramphenicol zones. However, this growth looked like fungal growth as it appeared furry and was black when looking from underneath, which spread to the Tetracycline and Chloramphenicol zones after 4 days. It is unsure whether the bacteria is susceptible to Streptomycin and Penicillin G as there is growth right in between both of them, which is highly likely to be fungal growth. For Sulphatriad, there seems to be bacterial growth surrounding it, however the fungal growth makes it unclear. At best, it seems that the white bacteria has intermediate susceptibility to Sulphatriad from these results. The fungal growth was unexpected as the colony did not look like a fungal colony as it was not fuzzy and did not have a dark underside. This could have been from cross contamination, or the fungal colony was growing closely within the bacterial colony, making it invisible to the naked eye. However, from these results, the bacterial species is likely to be *Staphylococcus epidermidis* as the original colony was off white in colour and had a cloudlike shape.<sup>14</sup> *S. epidermidis* is less virulent than *S. aureus* however still causes infections in immunocompromised individuals.<sup>15</sup> This species is mostly harmless though, commonly found on human skin as normal flora.<sup>16</sup> This white bacteria was found across all 5 unheated makeup products, however only appearing on the heated T plate, meaning it is likely killed at temperatures of 65°C.

For the “brown” and “pattern” colony, they look to be of the same species as they have the same spotted appearance. The brown plate had mostly fungal growth across the plate, however the bacterial growth it did have was spotted, similar to many individual colonies. The brown colony chosen for this was most likely a fungal colony as it appeared slightly fuzzy, however the dotted growth is likely bacterial growth. The pattern colony had no fungal

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<sup>10</sup> Keim, K & Horswill, A. (2023) *Staphylococcus aureus*. Cell Press. pp1300-1301

<sup>11</sup> Ibid.

<sup>12</sup> Oakley, A. (2016) Boil (furunculosis).

<https://dermnetnz.org/topics/boil#:~:text=Staphylococcus%20aureus%20can%20be%20cultured,may%20cause%20fever%20and%20illness>

<sup>13</sup> Gotfried, J. (2023) Staphylococcal Food Poisoning. <https://www.msdmanuals.com/en-au/home/digestive-disorders/gastroenteritis/staphylococcal-food-poisoning>

<sup>14</sup> Lee, E & Anjum, F. (2023) *Staphylococcus epidermidis* Infection. <https://www.ncbi.nlm.nih.gov/books/NBK563240/>

<sup>15</sup> Ibid.

<sup>16</sup> Ibid.

growth, but had similar colours and susceptibility to the brown colony. From the dots, they are both susceptible to Ampicillin and Streptomycin, variable susceptibility to Chloramphenicol, Tetracycline and Sulphatriad, and both resistant to Penicillin G. With the patterned and brown colony, the susceptibility, wrinkles and colour likely align with the characteristics of the *Nocardia* species, although it cannot be determined exactly which species it is.<sup>17</sup> Bacteria of the *Nocardia* species is a pathogen, however, normally will not be able to infect people with a healthy functioning immune system.<sup>18</sup> *Nocardia* is often found in soil or water, and will typically infect areas with broken skin barriers, or people who are immunocompromised.<sup>19</sup> The bacteria would start to cause an infection on the skin, but then enter the lungs, causing more severe respiratory issues.<sup>20</sup> For people who engage in activities with soil such as gardening or hiking, there is a higher likelihood of contracting this bacteria.<sup>21</sup> *Nocardia* found in makeup is a cause for concern, however for the average person with a healthy immune system, there is a very low risk of contracting any diseases from it, even if ingested. These brown colonies were found on both heated and unheated sides, however with only 2 brown colonies, there isn't sufficient results to conclude whether temperatures of 65°C kill them.

### Experimental analysis

From Part 1, my results were mostly valid as I limited the independent variable to temperature in each of the trials for the makeup products. Control groups included using the same nutrient agar plates, same incubator temperature, and using a new sterile loop for each makeup product. The independent variable for type of makeup product used was varied to obtain 5 groups with 3 trials each, which should not have impacted the validity of the results as I was comparing the results between the unheated and heated halves within the groups.

A problem I encountered was heating the makeup, which due to the different packaging, I could not soak the products in the water bath the same way to heat them. Because of this, the R and N products were removed from its packaging into beakers, whereas the others were just put inside beakers in their opened packaging. This may have impacted results as the makeup removed from its packaging had more surface area, perhaps more effectively killing bacteria than the makeup inside its packaging. This seems to align with my results as there was no growth on the heated side for N and R, however P also had no growth on the heated side, despite being in its packaging when heating. I would improve my experimental design for this issue by taking all the products out of their packaging into beakers when heating.

For data collection in Part 2, I should have also measured the inhibition zones of the bacteria as that would provide more accurate results for whether it was susceptible, intermediate or

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<sup>17</sup> Banerjee, B. (2019) Disseminated *Nocardia asiatica* infection in an immunocompromised individual: A rare entity needs careful vigilance. Elsevier. pp167-170

<sup>18</sup> Ibid.

<sup>19</sup> Bush, L. (2023) Nocardiosis. <https://www.msmanuals.com/en-au/professional/infectious-diseases/gram-positive-bacilli/nocardiosis>

<sup>20</sup> Ibid.

<sup>21</sup> Brown-Elliot, B. et al (2006) Clinical and Laboratory Features of the *Nocardia* spp Based on Current Molecular Taxonomy. PubMed Central. pp259-282

resistant.<sup>22</sup> From just observing the inhibition zones, it becomes a bit subjective which could have impacted the identification of bacteria. To improve this, I would measure the zones with a ruler to the millimetre and compare those results to bacteria susceptibility charts with numerical data to obtain a more accurate identification.

### **Conclusion:**

This experiment identified some bacterial species in 5 lip products to be *M. luteus*, *S. aureus*, *S. epidermis* and *Nocardia*. These species are generally non-pathogenic, except for *S. aureus* which may cause food poisoning if consumed, however may cause disease in immunocompromised individuals. My hypothesis that there would be fewer bacterial colonies when the makeup was heated to 65°C is supported in all trials, since there were less bacterial colonies growing on the heated side than the unheated side. So, it can be concluded that at 65°C, the bacteria species are rapidly killed, however it does not completely eliminate all bacteria present in lip products.

### **Acknowledgments:**

Leanne Odoherly – Helped in conducting Part 1 of the experiment, gave advice on how to conduct Part 1, and edited this report.

April Dacumos-Hill – Aided in conducting Parts 1&2 of the experiment and gave advice for how to conduct Part 2, the antibiotic susceptibility test.

Angelina Rizzuto – Gave advice when recording observations and results.

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<sup>22</sup> LibreTexts (n.d) Kirby-Bauer (Antibiotic Sensitivity).

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