Original Research Article

A Bacteriological of Make-up Tools used in Calabar Metropolis, Cross River State, Nigeria

Abstract
Sharing of make-up tools by multiple users is a possible means of transfer of fungal infections, such as dermatophytoses. With abrasion on the skin, it is possible for HIV, Hepatitis B virus, Spirochaetes and other pathogens to be carried via contaminated make-up tools, from one person to another. Using sterilized swab sticks, surfaces of make-up tools (sponge and brush) were cleansed. Materials deposited on the swab sticks were cultured aerobically on Chocolate and Cysteine Lactose Electrolyte Deficient Agar plates overnight at 37^{o}C. After overnight aerobic incubation, the culture plates were read macroscopically for growth. Bacterial isolates were subjected to microscopic examination using Gram's staining technique and biochemical tests (e.g. coagulase, catalase, oxidase, and motility). Results showed that the predominant bacterial growths obtained from the make-up tools were *Staphylococcus aureus* (51.5%), Coagulase-negative Staphylococcus (25.8%), *Klebsiella pneumoniae* (6.2%), and *Pseudomonas aeruginosa* (16.5%). The different genera of bacteria were harvested from make-up brushes (66.2%) and make-up sponges (85.7%). There was no bacterial growth from 33.8% and 14.3% of make-up brushes and make-up sponges respectively. There was no statistically significant difference in the prevalence of bacterial growths on make-up sponges ($P>0.05$). This study has shown that there is a moderate possibility for the transfer of bacterial organisms (both skin flora and pathogens) from one person to another, through make-up tools, in our local communities. It is hereby recommended that health education talks should be carried out regularly among beauticians to encourage them to use disposable make-up tools with disinfectants.
Key words: Bacteria, Calabar, Make-up, Tool, Survey

1.0 INTRODUCTION

In every society, there is a standard of appearance that the female population is expected to follow. There are those that venture outside of the box, but there are general guidelines that most seem to agree as reasonable within the context of their society. In most cultures, these boundaries of behaviours are considered to be cultural norms [1].

It has often been an assumption in today's culture that if one does not take the time to groom themselves properly, there is something wrong with them. Many times those who do not keep up with these grooming habits are assumed to have a mental disease or defect, be poorly cared for, or have a low opinion of themselves [2]. This has led to the production of cosmetics and beauty enhancers for women to look beautiful, hide their acne and blemishes and boost their confidence. These products are generally termed as makeup and they include items such as foundations, powders, lipsticks, mascaras, eyeshadows, blushes, concealers etc.

If a lady thinks her appearance or beauty is far from what is obtainable, she could likely improve her appearance with the assistance of makeup [3]. These makeup products are usually applied using specific objects called makeup tools. Sponges and brushes are the most common tools used in applying makeup. While makeup can do an amazing job of enhancing beauty in women, majority of them do not know that makeup can pose a hazard to their health because it harbours bacteria and is capable of spreading infections [4]. Cosmetic contamination leads to several types of infections that range in severity from mild to serious [5].

Many women share makeup and makeup tools with family and friends, thus increasing their chances of cross-contamination. Others do not replace makeup tools despite how long ago they have been purchased [6]. The rich texture of some makeup products are mainly due to the moisture content and the presence of essential minerals that contain a wide spectrum of organic and inorganic compounds which provide a suitable environment for the growth and proliferation of microorganisms [7].

Depending on the structure and composition of these makeup tools, the risk factors associated with the intended use can be influenced. Tools that can physically trap and retain moisture, sebum, skin cells, dirt and microorganisms create the greatest problem and have the greatest probability of contributing to significantly high risk contamination transfer. An example is the makeup sponge that can trap cellular debris while providing the perfect environment for microbial survival and growth. The frequency of product application also plays a major role in the risk potential associated with the tools [8].

We live at the core of a microbial universe, microscopic organisms surround us on all sides and make their presence felt for either good or bad [9]. Humans are consistently bombarded by myriads of microorganisms that occupy the environment. Since microorganisms are ubiquitous in nature, contact on human beings is inevitable but the means of encounter varies widely [10]. Tille, [11] emphasized that human encounters with microorganisms vary widely and are unavoidable since microorganisms are found everywhere. A person's activity determined the type of microbial population a person is exposed to as well as the manner of exposure.

2.0 MATERIALS AND METHODS

2.1 Sampling

To determine the microbial contamination of shared cosmetic tools, a total of 100 samples were collected from respondents in their shops, homes and make-up studios in Calabar metropolis. Samples collection spanned between April and July 2021. The specimens from all the cosmetic tools were collected following the owner's informed consent, then samples were collected using sterile swab sticks moistened in sterile peptone water. The surface of each makeup tool was swabbed by gently rolling the swab stick on it. The swab sticks were then transported to the laboratory within an hour of collection.

2.2 Microbial survey

Samples were collected using sterile swab sticks moistened in sterile peptone water, swab sticks were then transported to the laboratory within an hour of collection. The swabs were then inoculated on chocolate agar and cysteine lactose electrolyte deficient (CLED) agar plates. The CLED agar plates were incubated aerobically at 35-37^{o}C for 18-24 hours and the chocolate agar plates were incubated in a CO_2 enriched environment using a canister at 35-37^{o}C for 18-24 hours.

2.3 Identification of bacterial isolates

Isolates were identified macroscopically, microscopically and biochemically, using the different biochemical tests available.

2.4 Determination of microbial load on makeup tools

Serial dilutions were done by putting each swab in a test tube containing 10mL of sterile peptone water and thoroughly mixed to obtain stock dilution. One milliliter (1mL) of the stock solution was diluted serially in test tubes each containing 9ml of sterile peptone water to obtain a dilution up to 10^{-5}. Spread plate technique was then employed using CLED agar plates. The plates were incubated at 37^{o}C for 18-24 hours.

Calculation of colony forming unit per milliliter (Cfu/mL) of a sample is done by multiplying the number of colonies by the dilution factor i.e.

$$\frac{\text{Number of colonies x Dilution factor}}{\text{Volume of culture plate.}}$$

The microbial load and magnitude of contamination of the makeup tools was calculated and expressed as Cfu/mL.

3.0 RESULTS

Table 1 shows the occurrence of bacterial isolates based on the type of makeup tools. Out of 65 make-up brushes collected, 43 (66.2%) had bacterial growth with 22 (33.8%) having no bacterial growth while 30 (85.7%) out of 35 make-up sponges had bacterial growth and 5 (14.3%) had no bacterial growth. There was statistically significant difference between bacterial growth by types of makeup tools (P =0.036; $P < 0.05$).

Table 2 shows the occurrence of bacterial isolates based on the location of sample collection. 20 samples each were collected from several locations which include; hostels, Watt market, Marian market, makeup studios and homes. Watt market had the most occurrence of bacterial isolates with 19 (95%) out of 20 samples, these were followed by makeup studios with 17 (85%) out of 20 samples, hostel and Marian market both had 16 (80%) out of 20 samples. The least occurrence of bacterial growth was from homes with 5 (25%) out of 20 samples. There was statistical significant difference between bacterial growth by location of sample collection (P =0.000; $P < 0.05$).

Table 3 and 4 shows the distribution of bacterial isolates based on the types of makeup tools and location of sample collection. The bacteria found on them where *Staphylococcus aureus*, *Pseudomonas aeruginosa*, coagulase-negative *Staphylococcus* and *Klebsiella pneumoniae*. *Staphylococcus aureus* (51.5%) was the most encountered bacteria isolate, followed by coagulase-negative *Staphylococcus* (25.8%), *Pseudomonas aeruginosa* (16.5%), lastly, *Klebsiella pneumoniae* (6.2%) the least isolated bacteria.

Table 5 shows the distribution of colony count of makeup tools. 14 samples had a colony count of less than 20 Cfu/mL. 24 samples had a colony count between 21 to 50 Cfu/mL. 35 samples had a colony count of more than 50 Cfu/mL.

Figure 1 shows the frequency of makeup tools use by owners. 80 (80%) of owners used their tools daily, 14 (14%) used their tools 1-3 times a week while 6 (6%) used their tools 1-5 times a month.

Figure 2 shows the frequency of cleaning of makeup tools by owners. 69 (69%) of owners cleaned their tools weekly, 18 (18%) cleaned them monthly and 13 (13%) rarely cleaned the tools.

Table 1: The Occurrence of bacterial growth based on types of makeup tools

Makeup tools	No of samples examined	No (%) with significant bacterial growth	No (%) with insignificant bacterial growth
Makeup sponge	35	30 (85.7)	5 (14.3)
Makeup brush	65	43 (66.2)	22 (33.8)
Total	**100**	**73 (73.0)**	**27 (27.0)**

(χ^2 = 4.416; *P*=0.036)

Table 2: The Occurrence of Bacterial growth based on location of sample collection

Location	No of sample examined	No (%) with significant bacteria growth	No (%) with insignificant bacterial growth
Hostel	20	16 (80.0)	4(20.0)
Watt market	20	19 (95.0)	1(5.0)
Marian market	20	16 (80.0)	4(20.0)
Makeup studios	20	17 (85.0)	3 (15.0)
Homes	20	5 (25.0)	15 (75.0)
Total	**100**	**73 (73.0)**	**27 (27.0)**

$\chi^2 = 30.746$ $P = 0.000$

Table 3: The Distribution of Bacterial Isolates Based on type of makeup tools

		No (%) of bacterial isolates				
Makeup tools	**No of sample examined**	***S. aureus***	***P. aeruginosa***	**CoNS**	***K. pneumoniae***	**Total**
Makeup brushes	65	29(58.0)	10(62.5)	13(52.0)	3(50.0)	55(56.7)
Makeup sponges	35	21(42.0)	6(37.5)	12(48.0)	3(50.0)	42(43.3)
Total	**100**	**50(51.5)**	**16(16.5)**	**25(25.8)**	**6(6.2)**	**97**

Key:
CoNS = Coagulase-Negative Staphylococci

Table 4: The Distribution of Bacterial Isolates Based on location of makeup tools

		No (%) of bacterial isolates				
Location	**No of sample examined**	***S. aureus***	***P. aeruginosa***	**CoNS**	***K. pneumoniae***	**Total**
Hostel	20	12(24.0)	3(18.7)	4(16.0)	0(0.0)	19(19.6)
Watt Market	20	14(28.0)	3(18.7)	5(20.0)	3(50.0)	25(25.8)
Marian Market	20	9(18.0)	5(31.2)	8(32.0)	2(33.3)	24(24.7)
Makeup Studio	20	12(24.0)	3(18.7)	6(24.0)	1(16.7)	22(22.7)
Homes	20	3(6.0)	2(12.5)	2(8.0)	0(0.0)	7(7.2)
Total	**100**	**50(51.5)**	**16(16.5)**	**25(25.8)**	**6(6.2)**	**97**

Key:
CoNS = Coagulase-Negative Staphylococci

Table 5: The Distribution of colony count on makeup tools

Makeup tools	No of sample examined	Colony count 21-30 Cfu/mL	Colony count 31-50 Cfu/mL	Colony count >50 Cfu/mL
Brushes	50	8	15	20
Sponges	50	6	9	15
Total	**100**	**14**	**24**	**35**

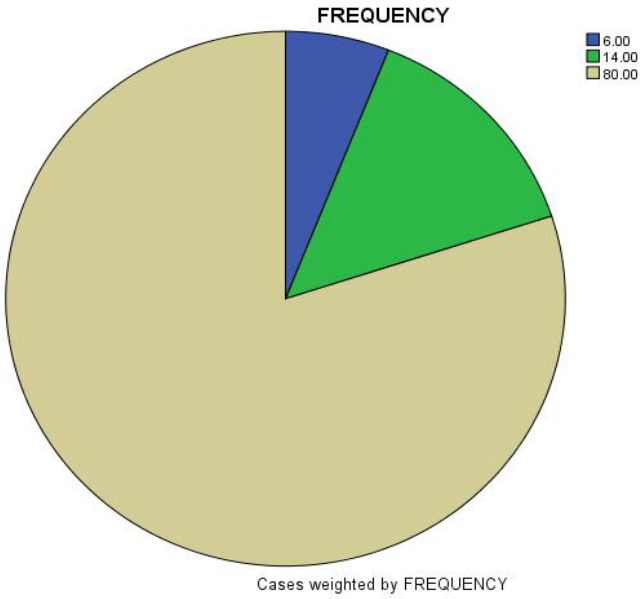

Fig. 1: Frequency of Makeup Tool Use Among Owners

KEYS: 1-5 TIMES A MONTH

1-3 TIMES A WEEK

DAILY

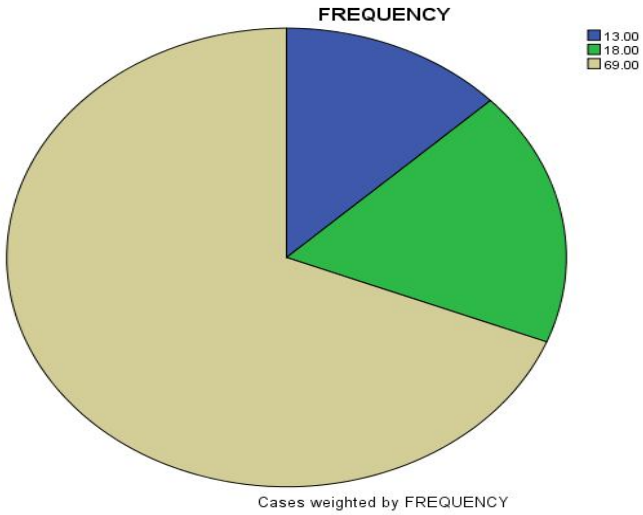

Fig. 2: Frequency of Cleaning of Makeup Tools by Owners

KEYS: WEEKLY

MONTHLY

RARELY

4.0 DISCUSSION

The objective of this study was to determine if makeup tools are contaminated with bacteria and to what level it is up to. Many cosmetics are usually not produced in a hygienic environments and probably not produced sterile and could be a source of contamination to makeup tools which are very often shared by beauticians and therefore increase microbial contamination on makeup tool [12]. Overall culture analysis from makeup sponges and brushes shows that there was significant bacteria growth with percentage of 73.0% and having a p-value of 0.036. This is in agreement with Dadashi and Dehghanzadeh [13] having culture growth of about 100%, Bashir and Lambert [14] having about 79-90% bacteria load on makeup kits in which bacteria could be transferable to tools.

Makeup brushes collected alone had 66.2% of bacterial growth while 85.7% of makeup sponges were contaminated with bacteria. Makeup sponges had higher percentage due to the fact that they are usually used damp and damp environment foster the growth of bacteria [15,16]. Cohut [17] also observed that makeup tools harbor harmful bacteria because tools are not regularly cleaned, and that beauty blenders had the worst bacteria growth [14]. Though, bacteria may be present on these makeup tools, they may not cause infection directly except when a person has open cuts or wounds on the skin and this may alongside introduce antibiotic resistant bacteria [18,19] and these may cause hard-to-treat skin infections [18,19]. Contaminants of makeup tools differ from person to person depending on how often they are used and how frequently they are cleaned. In this study, *Staphylococcus aureus* (51.5%) was the most encountered isolate on the makeup tools. This may be due to the fact that this bacterium is ubiquitous in nature and is also a part of the skin's normal flora.

The report of Almusawi [20] in his study of bacterial growth on cosmetic products reported *Staphylococcus epidermidis* (24.2%) as the most encountered isolate in comparison to *Staphylococcus aureus* (51.5%) in this study. Our findings may differ due to the geographical difference. Makeup tools from the hostels, markets and makeup studios were the most contaminated compared to makeup tools obtained from homes. This may be because of how often they are used and shared among persons. Beauty salons and markets are exposed to many irritants and allergens that may cause occupational disease. It has been estimated that 10 - 20% of beauty salon customers are affected by skin disorders [13].

It is also noteworthy that these microorganisms can be transferred from beauty salon tools to the hands and from one surface to another. Several factors have been identified to affect the transfer rate of bacteria from one surface to another. These include bacteria type, type of

surface and moisture level [21]. Since hands are important for intrapersonal and interpersonal transfer of microorganisms, as well as environmental transfer, the dynamics of hand microbial communities and factors impacting them are of considerable importance [22].

4.1 Conclusion

Study revealed that makeup tools can harbour bacteria with significantly high microbial load. Although, most microorganisms isolated from these makeup tools were skin normal flora, they have the potential of causing skin infections in individuals if the skin is compromised. These makeup tools can also serve as reservoirs and carriers of microorganisms which are transmissible from person to person. It is also noteworthy that the rate of microbial contamination in makeup tools used in beauty salons is higher than the rate reported in personal tools. The findings of this study indicate that it is important to maintain a high level of personal hygiene and environmental sanitation among individuals and beauty salons to prevent the spread of infections.

REFERENCES

1. Moriarty S., Mitchell N., & Wells W. *Advertising: Principles & practice* (8th ed.). Upper Saddle River, NJ: Pearson Prentice Hall. 2009.
2. Silverio L. *Makeup's effects on self-perception* [OTS Master's thesis]. 2010; 49.
3. Jeon Y M., & Lee MH. A study of clothing attitude, body attitude, and social values of adolescent girls. *Journal of the Korean Society of Clothing and Textile.* 2005; 29(9):1219-1229.
4. Mwambete KD., & Simon A. Microbiological quality and preservative capacity of commonly available cosmetics in Dar es Salaam, Tanzania. *East and Central African Journal of Pharmaceutical Sciences*. 2010; 9.
5. Muhammed HJ. Bacterial and fungal contamination in three brands of cosmetic marketed in Iraq. *Iraqi Journal of Pharmaceutical Science*. 2011; 20(1).
6. Hugbo PG., Onyekweli AO., & Igwe I. Microbial contamination and preservative capacity of some brands of cosmetic creams. *Tropical Journal of Pharmaceutical Research*. 2005; *2*(2): 229-234.
7. Ghalleb S., De Vaugelade S., Sella O., Lavarde M., Mielcarek C., Pense-Lheritier AM., & Pirnay S. Predictive microbiology for cosmetics based on physicals, chemicals, and concentration parameters. *International Journal of Cosmetic Science*. 2015; *37*(1): 70-7
8. Dayan N., & Kromidas L. *Formulating, packaging, and marketing of natural cosmetic products*. Hoboken, NJ: Wiley. 2011.
9. Pommerville JC. *Alcamo's fundamentals of microbiology* (8th ed.). Sudbury, MA: Jones and Bartlett. 2007.
10. Coates R., Moran J., & Horsburgh MJ. Staphylococci: Colonizers and pathogens of human skin. *Future Microbiology*. 2014; *9*(1): 75-101.
11. Tille P. *Bailey & Scott's diagnostic microbiology*. Elsevier Health Sciences. 2013.
12. Giacomel C., Dartora G., Dienfethaeler H. & Haas S. Investigation on the use of expired make-up and microbiological contamination of mascaras. *International Journal of Cosmetic Science*. 2013; *35*(4):357-380
13. Dadashi L., & Dehghanzadeh R. Investigating incidence of bacterial and fungal contamination in shared cosmetic kits available in the women beauty salons. *Health Promotion Perspective*. 2016; *6*(3):159-163
14. Bashir A., & Lambert P. Microbiological study of used cosmetic products: highlighting possible impact on consumer health. *Journal of Applied Microbiology*. 2020; *128*(2):598-605.
15. Chiu A. Bacteria can grow on your neglected makeup and brushes. Here's what to toss and what to clean. The Washington Post. https//washington post.com. 2021. Retrieved 3rd February 2022

16. Seaver M. Over 90 percent of used beauty products and makeup sponges contain deadly bacteria-here's how to keep yours germ-free. https//realsimple.com. 2019. Retrieved 3rd February 2022

17. Cohut M. Dirty makeup sponges harbor dangerous bacteria. https//medicalnewstoday.com. 2019. Retrieved 3rd February 2022

18. Naftulin J. Your makeup and sponge applicator may be teeming with dangerous bacteria and fungus. https//insider.com. 2019. Retrieved 3rd February 2022

19. Daoud E. Potentially deadly bacteria and fungi found in makeup products and beauty blenders. https//7news.com.au. 2019. Retrieved 3rd February 2022.

20. Almusawi WN. Are cosmetic products harbouring bacterial growth? *International Journal of Current* Research. 2016; *8*(3): 28563-28565.

21. Rusin P., Maxwell S., & Gerba C. Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria, and phage. *Journal of Applied Microbiology*. 2002; *93*(4): 585-592.

22. Lax S., & Smith P. Longitudinal analysis of microbial interactions between humans and the indoor environment. *Science Magazine*. 2014; 345:1048.