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Evaluation of microbial risk associated with the use of shared makeup applicators and the rate of hygiene practices among beauticians

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Abstract

With the increased growth in the cosmetic industries, it has become quite common to seek the services of professional beauticians thereby increasing the tendency of sharing makeup applicators. This study investigates the microbiological contamination of makeup applicators (Brushes and sponges) used by beauticians and to determine the level of hygiene practices. Microbial contamination was detected from *Staphylococcus aureus*, *Pseudomonas aeruginosa, Bacillus* and fungi with the highest percentage occurrence observed in brushes for *Pseudomonas aeruginosa* and *Bacillus* at 100% while fungi was highest in sponges with 70%. The hygiene assessment was analyzed using a structured questionnaire to ascertain the level and type of hygiene measures taken in cleaning the applicators used on their clients; the result obtained showed that less than 50% do not discard their old makeup applicators as often as they should ($2.4^{bc} \pm 0.92$) and do not clean their applicators with alcohol based agent ($1.8^{a} \pm 1.25$). The repetitive use of these applicators on clients especially over time without proper hygiene measures is the major cause of contamination. These makeup sponges and brushes have shown to be vehicle for transmission of pathogens and hygiene practices should be taken more seriously especially when dealing with multiple clients.

Keywords: Microbiology; Makeup applicators; Safety testing; Microbial contamination; Hygiene; Pathogen

1. Introduction

In recent times, the use of cosmetics by beauticians and individuals has significantly increased leading to a massive growth of the industry. The growth has seen more players in the industry with an increase in the number of brands and cosmetic products [1]. Makeup application has become more frequent and regular by a large number of the populace, especially among the younger generations. These cosmetics help to cover up imperfections, enhance appearance as well as decrease signs of aging [2]. Despite the various advantages of makeup to women, it still poses a health risk if not properly handled leading to several infectious diseases [3].

Cosmetic products are suitable environment for microbial growth owing to its moisture content, the essential minerals as well as growth factors present, providing a broad spectrum of organic and inorganic compounds [4]. A greater risk of microbial contamination and proliferation has been observed in cosmetic products without efficient preservatives which will affect the product composition as well as their organoleptic properties which may be evident in terms of changes in color, odor and texture, thereby leading to health hazards [5; 6].

Makeup products can easily be contaminated with *S. aureus, P. aeruginosa, Clostridium tetani*, yeasts and molds, especially since women have the tendency to share makeup and applicators in the company of family and friends [2; 1].

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Beauticians also play an active part in the transfer of skin and eye infections with applicators like brushes and sponges, when using a commercial makeup kits on clients [7]

Makeup applicators can be contaminated easily by sharing or repetitive use to the skin, especially since the microflora of the skin is unique and could be transferred to others thereby threatening the health of the individual [3; 8]. Although, the level of risk associated with these applicators depends on the structure, composition and configuration. Those with the highest risk of contamination transfer and pose a problem are ones capable of trapping and retaining moisture, dirt, skin cells, and microorganism [9]. There are commercially available cleaning products for reducing the level of microbial contamination in cosmetic applicators to a certain extent, but most makeup brushes and other objects can still pose a risk especially when in contact with breaks in the skin [10; 7]. Since cosmetic products are not usually known to be sterile, they must at least have a low total aerobic microbial count and be clear of microbial pathogens like *Escherichia coli*, *Staphylococcus aureus*, *P. aeruginosa* [11]. Some of the microorganisms which have been largely implicated in beauty salons in-use tools are *Streptococcus* spp., *Staphylococcus* spp., *Escherichia coli, Enterobacter* and *Pseudomonas aeruginosa* and also fungus like *Aspergillus* and *Penicillium* [7], and pose serious risk to users leading to various infections and chronic diseases. B*. cereus* can also lead to contamination of cosmetics and facial toiletries in the presence of key risk factors [12].

This research centers on the detection of human skin pathogens, *Pseudomonas aeruginosa, Staphylococcus aureus*, *Bacillus* and fungi for the assessment of microbial contamination in makeup applicators (sponge and brush), and the role of these applicators as vehicles in pathogen transmission as well as an evaluation of the hygiene practices of commercial beauticians.

2. Material and methods

2.1. Sample collection

An overall of 20 samples consisting of makeup brushes and sponges were taken from 10 different beauticians at Awka city of Anambra, Nigeria. Each makeup applicator (brushes and sponges) were wiped with sterile cotton swabs dampened with a solution of normal saline. All the samples collected were analyzed for microbial contamination at the microbiology laboratory.

2.2. Data collection and Techniques

Ten makeup artists were selected as respondents. A structured questionnaire was used for the research. The questionnaire is made up of eight (8) research questions. The respondents are expected to express the degree of their hygiene practices. The scores were based on a 5-point hedonic scale where five represented 'Always', and one represented 'Barely'.

2.3. Culture Analysis

Potato dextrose agar, Mannitol salt agar and Cetrimide agar and were used for the enumeration of fungi, *S. aureus, Pseudomonas aeruginosa, and Bacillus cerus*. Media were prepared according to manufacturers details. The culture plates were inoculated and incubated at 37 °C for 48hrs (MSA and CA), while the PDA plates were incubated at room temperature (27 ± 2 °C) for 72 h. Pure cultures were obtained by streaking distinct colonies on fresh plates and further maintained on agar slants at 4 °C.

2.4. Characterization and Identification of the Fungal Isolates

Fungal pure cultures were selected and identified by both macroscopic and microscopic observations and appearance with reference to manuals of Barnett and Hunter [13]; and also based on the microscopic fungal features like the septate, Phialides, Conidiophores, Sporangiophores, and budding, as described by [14].

2.5. Antibiotic susceptibility of the bacteria isolates

The test was performed using the method described by Babalola and Eze [14]. The commercially available antibiotic disk concentrations were tested against the bacteria isolates by inoculating a bacterial inoculum of approximately 1-2 x10^8 CFU/mL to a Mueller-Hinton agar plate using the Kirby-Bauer disk diffusion method. Culture plates were incubated for 16-24hrs at 35^{o}C preceding the results determination. The diameter of the zones of inhibition of growth around every one of the antibiotic disks are measured to the nearest millimeter, recorded and interpreted.

The following antibiotics were tested for gram positive (S. *aureus* and *Bacillus* sp) chloramphenicol, ciprofloxacin, erythromycin, levofloxacin, cephalexin, amoxicillin, streptomycin, ampicillin, rifampicin, neomycin.For gram negative (P. *aeruginosa*), cephalosporin, Gentamicin, Tetracycline, Streptomycin, pefloxacin, augmentin, ofloxacin, sulfamethoxazole, prophylaxis, cephalexin.

2.6. Statistics

Statistical Package for Social Sciences (SPSS) was used to analyze all the data. A one-way analysis of variance (ANOVA) was used to compare the means. The differences between the means were significant when $P < .05$.

3. Results

3.1. Frequency of microbial contamination

All 20 samples of makeup sponge and brush were evaluated for the contamination of fungi, *Pseudomonas*, *Staphylococcus* and *Bacillus*. The makeup sponges showed high contamination of fungi (60%) than the brush with 40%. The sponges and brushes had 100% of *Bacillus* giving the highest level of contamination when compared to other pathogens. The percentage of *Staphylococcus* was the same across all the applicators while *Pseudomonas* contamination frequency was 80% for sponges and 100% for brushes, while fungi was observed as seen in Fig. 1, to have a higher percentage (70) in sponges than in brushes(40). The fungi identified include *Candida albicans, Aspergillus niger* and *Aspergillus flavus* as observed in Table 2.

3.2. Analysis of response on the hygiene practices of beauticians

According to the responses obtained in table 1, question 1 and 6 gave the highest value with $3.9^{e} \pm 1.14$ and $3.9^{e} \pm 0.7$ respectively , which shows that an average number of the respondent often clean and air dry their makeup brushes and sponges. Question 7 with a value of $3.2^{d} \pm 0.98$ showed that not all beauticians wash their makeup bags as often as they should. Question4 ($3.2^{d} \pm 0.98$) shows that only an average of the respondents use warm water and detergent for cleaning their applicators, while question 3 with a mean rating of $2.7^{c} \pm 0.56$ showing that less than average cleans their applicators with cold water and mild soap. Questions 8, 2 and 5 shows that majority of the respondent do not discard old applicators with new ones, do not clean with wipes, do not clean with alcohol based agent as often as they should with the lowest mean ratings of $2.4^{bc} \pm 0.92$, $2.2^{ab} \pm 0.87$, $1.8^{a} \pm 1.25$ respectively.

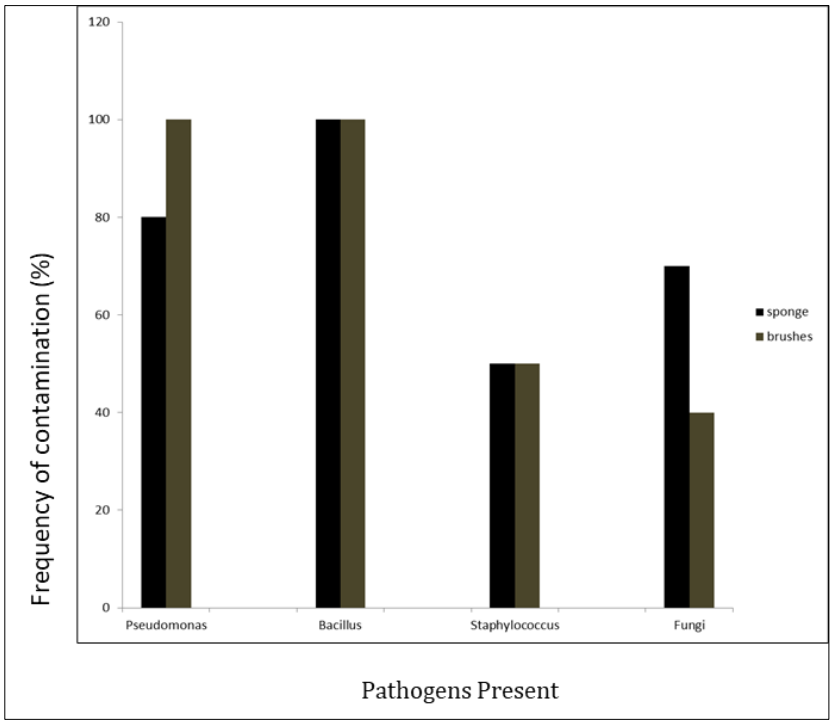


Figure 1 Percentage frequency of *Pseudomonas*, *Bacillus*, *Staphylococcus* and fungi contamination

Table 1 Mean rating of responses on the hygiene practices of makeup artists

S/N	Questions	Mean ± SD
1	How often do you clean your makeup brushes and sponges?	3.9^{e} ± 1.14
2	Do you clean makeup brushes and sponges with wipes, if yes, how often?	2.2^{ab} ± 0.87
3	Do you clean makeup brushes and sponges with cold water, mild soap or detergent, if yes how often?	2.7^{c} ± 0.56
4	Do you clean makeup brushes and sponges with warm water, mild soap or detergent, if yes how often?	3.2^{d} ± 0.98
5	Do you clean makeup brushes and sponges with alcohol based agent, if yes how often?	1.8^{a} ±1.25
6	Do you air-dry makeup brushes and sponges after cleaning, if yes how often?	3.9^{e} ±0.7
7	How often do you wash the makeup brush and sponge bags?	3.2^{d} ±0.98
8	How often do you discard old makeup brushes and sponges and replace with new ones?	2.4^{bc} ± 0.92

Samples with the same superscripts down the column are not statistically significant at P ≤ 0.05. (ANOVA: P ≤ 0.05)

Table 2 Macroscopic and microscopic morphology of Fungi isolates

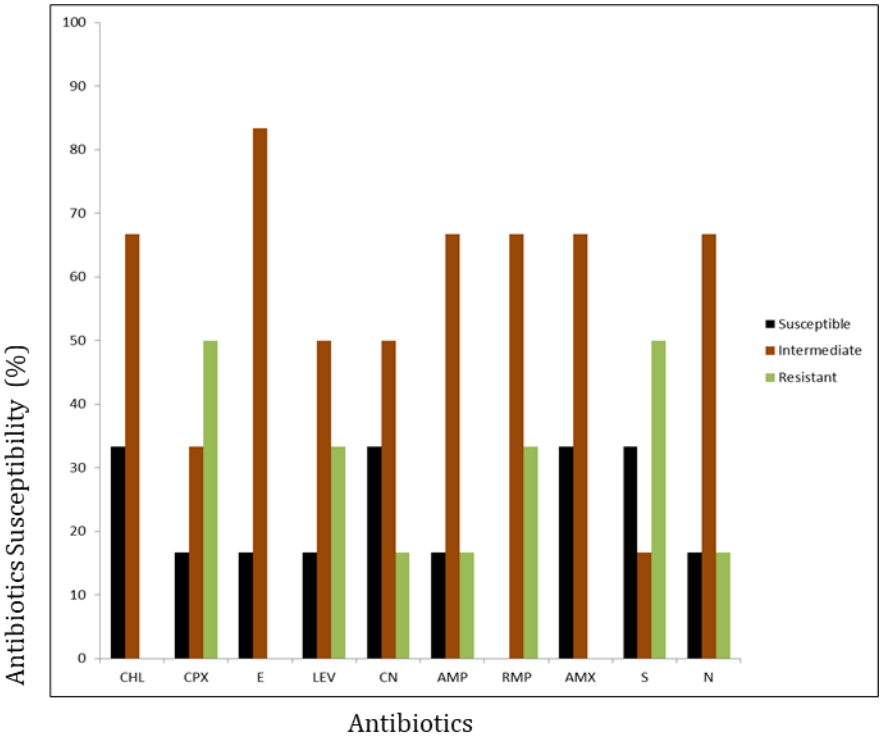
Isolate	Macroscopy	Microscopy	Identity
MBS1	Smooth, circular, creamy andshiny	Spherical budding yeast cell, presence of chlamydospores and pseudomycelia.	*Candida Albican*
MBS2	Fluffy white that changes to black with conidial heads and yellowishat the edge	Rough glubose black conidia, phialides are biserate	*Aspergillus Niger*
MBS3	Yellowish, smooth and granularwith white patches at the edge	Smooth, glubose yellow to green conidia, phialides are biserate	*Aspergillus Flavus*

3.3. Antibiotic susceptibility test of bacteria isolates

For *Staphylococcus aureus* shown in fig. 2, isolates were tested and recorded varying patterns. There was low susceptibility < 40%, with the highest resistance observed for ciprofloxacin and streptomycin (each recording 50%) and no resistance recorded for Amoxicillin, erythromycin, and chloramphenicol across all the strains. Majority of the strains were intermediate for Chloramphenicol, ampicillin, erythromycin, rifampicin, amoxicillin and neomycin (83.33-66.67%).

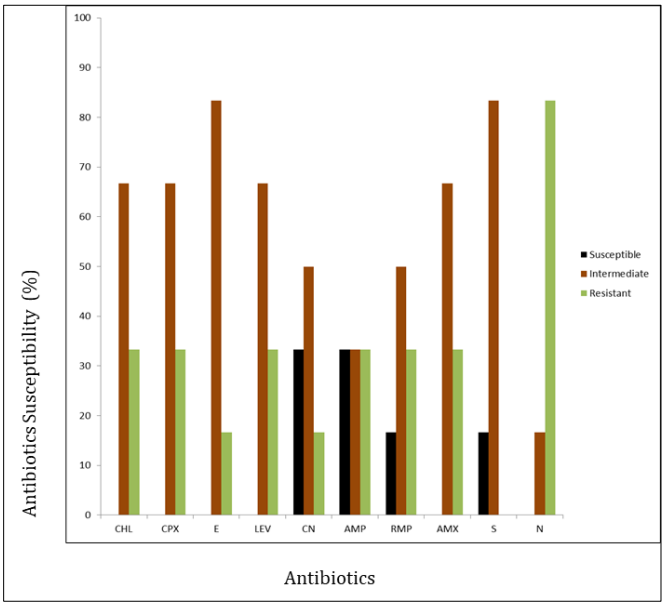
There was low susceptibility (< 40%) for *Bacillus* specie across all the antibiotics (Fig. 3), a high intermediate (66.67%) was identified for chloramphenicol, levofloxacin, amoxicillin and ciprofloxacin with the highest recorded for streptomycin and erythromycin with 83.33%. The highest resistance was observed for neomycin (83.33%)

For *Pseudomonas aeruginosa,* tetracycline was the most effective antibiotics (42.86%), with very low susceptibility (28.57%) recorded for cephalexin, pefloxacin, ofloxacin, sulfamethoxazole. while the highest resistance (42.86%) was seen in gentamycin (Fig. 4).



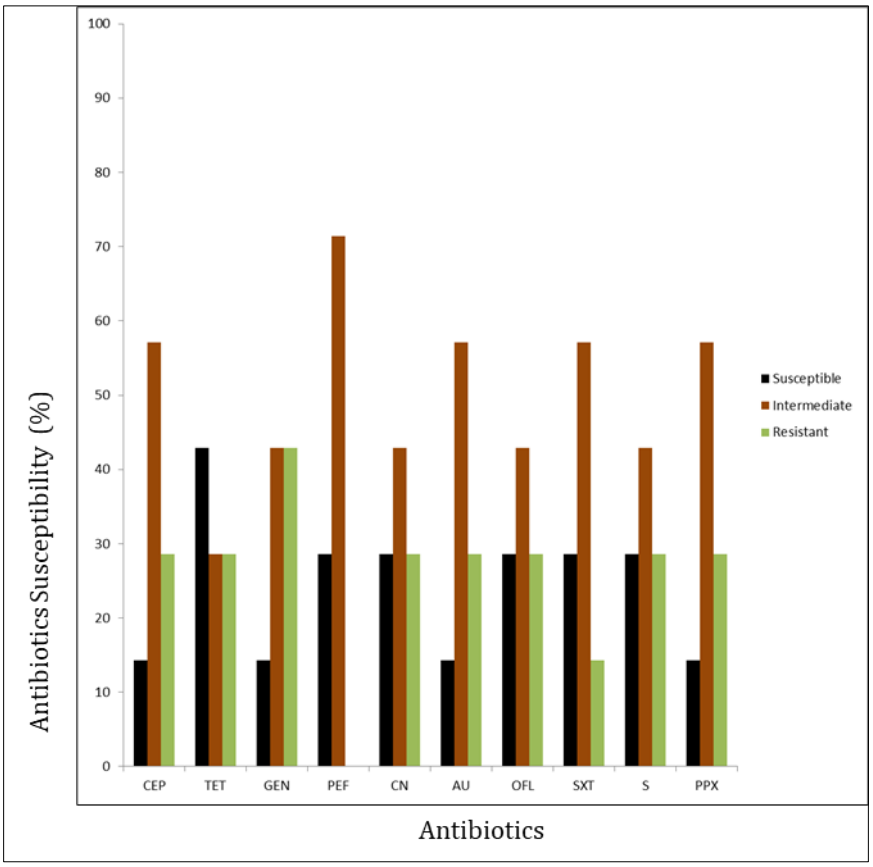
Key: CHL=chloramphenicol; CPX= ciprofloxacin; E=erythromycin; LEV=levofloxacin; CN=cephalexin; AMX=amoxicillin; S=streptomycin; AMP=ampicillin; RMP=rifampicin; N=neomycin.

Figure 2 Percentage antimicrobial susceptibility profile of S. *aureus*



Key: CHL=chloramphenicol; CPX= ciprofloxacin; E=erythromycin; LEV=levofloxacin; CN=cephalexin; AMX=amoxicillin; S=streptomycin; AMP=ampicillin; RMP=rifampicin; N=neomycin.

Figure 3 Percentage antimicrobial susceptibility profile of *Bacillus*



Key: CEP=cephalosporin; TET=tetracycline, PEF=pefloxacin; AU=augmentin; OFL=ofloxacin; SXT=sulfamethoxazole; GEN=gentamycin; S=streptomycin; PPX=prophylaxis; CN=cephalexin.

Figure 4 Percentage antimicrobial susceptibility profile of *P. aeruginosa*

4. Discussion

One of the major problems experienced is the sharing of makeup and makeup applicators and its use for a long period of time without treatment. These makeup applicators have two major risk factors, which is the ability to retain contamination and transfer contamination easily, especially when used in high sensitive areas like the eyes and mouth [9].

The study was conducted on 20 beauticians randomly selected using a questionnaire to determine their level of hygiene practices. As seen in Table I, the hygiene practices of quite a number of these beauticians is not adequate enough to eradicate pathogens present in these tools that are used repetitively on the skin of numerous clients. The responses for the treatment of applicators with warm water and detergent ($3.2^{d} \pm 0.98$); cold water and detergent ($2.7^{c} \pm 0.56$); use of wipes ($2.2^{ab} \pm 0.87$); use of alcohol-based agent ($1.8^{a} \pm 1.25$), showed that majority use warm water and soap as opposed to alcohol – based cleaning agent with the lowest response which could be due to the cost of these cleaning agents. Also these results suggest that these beauticians do not discard their old applicators for new ones as often as they should. This prolong use of applicators can lead to the survival of some microorganisms; these microorganisms can survive by forming biofilms, capsules and endospores which are difficult to eradicate [15].

In the microbiological evaluation, 20 samples of applicators (10 brushes, 10 sponges) were analyzed, and the presence of *Staphylococcus aureus* and *Bacillus* gave equal percentage occurrence in both brushes and sponges with *Staphylococcus aureus* at 50 % and *Bacillus* at 100%. This high percentage occurrence of *Bacillus* might be due to the fact that these spore formers are difficult to destroy and some of the preservatives present in cosmetic products have only succeeded in destroying the *Bacillus* species in its vegetative form, but not their spores [16; 17]. *Staphylococcus aureus*, and *Bacillus* have been considered two of the most frequently isolated microbial cosmetic contaminants, hence their presence in cosmetic tools. The presence of *Staphylococcus aureus,* and *Bacillus cereus* in applicators which could be ingested leading to gastro enteric infections, signifying a critical health risk [18].*Pseudomonas aeruginosa* was isolated with 100% and 80% occurrence of brushes and sponges respectively. *Pseudomonas aeruginosa* is being

considered an opportunistic organism since it originates from the environment [19], and its presence might be due to poor hygiene. Also, it was reported by [20] as one of the predominant bacteria from the examination of cosmetic products after microbial spoilage.

Due to the large number of clients beauticians handle, there's the tendency to use repeatedly a particular applicator on multiple people causing the spread of pathogens; especially via poly ethylene oxide PEO –coated brush which these pathogens have been reported to adhere easily to, and also due to their large surface area [21].

Yeast and fungi growth was seen at 70% for sponges and 40% for brushes, showing the highest percentage occurrence in sponges. This is in line with the work of [1], were sponges had 51.5% and brushes 30.3%. These sponges are known to trap cell debris and contaminants, while creating an ideal surrounding for growth and survival of these pathogens [9]. These fungi detected in contaminated applicators, usually have the ability to cause an opportunistic infection of the eye called mycotic keratitis which leads to ulceration and inflammation [22]. The acidic pH of the skin creates a favorable condition for the infection and invasion of fungal diseases like Aspergillosis, which is seen as the most common fungal infection of the immunocompromised patients; as well as septicemia and superficial mycotic infections caused by the Candida sp [14]. These fungal pathogens also synthesize secondary metabolites that can be harmful to the human health.

The antimicrobial susceptibility test observed resistance in some strains of *Staphylococcus aureus*, *Bacillus* and *Pseudomonas aeruginosa*. Some of the antibiotics examined did not inhibit the growth of the bacteria isolates probably due to low concentrations or it could be plasmid mediated or due to preservatives present in the products the applicators were used on.

Microbial contamination can be controlled if taken seriously, by adopting suitable preventive safety measures like disinfection and appropriate cleaning of these tools as well as discarding old applicators. The spread of opportunistic pathogens from person to person can decrease by avoiding long term use of applicators and promoting use of individual makeup kits [23].

5. Conclusion

Makeup has shown to do great in the beautification of women, but the tools used for its application can be a potential breeding ground for pathogenic bacteria and fungi. Sharing of Makeup and its applicators is very common among young ladies, which have increased the risk of contamination. Also, a lot of beauticians use same makeup applicators repeatedly on different clients without proper treatment leading to the spread of microbial contamination. The isolation of *Staphylococcus aureus, Pseudomonas aeruginosa*, *Bacillus* and fungi, which have also been previously reported, is an indication that these makeup applicators which are used in the transfer of cosmetic products to the targeted areas like skin, eyes , mouth etc, also acts as a vehicle in transferring the microorganisms into the products where they will start reproducing due to the broad spectrum of organic and inorganic metabolites which provides a suitable surrounding for microbial growth; these organisms are then transferred back to the same client or unto another client and the cycle continues.

Precautions like adequate sterilization, washing or use of alcohol based agents should be taken seriously especially by beauticians in other to ensure proper control of contamination from one person to another.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

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