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Isolation and Identification of Microorganisms from Makeup Brushes in Rivers State University Nigeria and Its Environs

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Components of makeups and cosmetic supply may serve as source and medium for the growth of microorganisms. A vast majority of females nowadays apply makeups in order to enhance their facial looks. Repetitive use and sharing of the makeup brushes by different customers may become a potential pathway for microbial pathogens spread. The major goal of this study was to isolate and identify microbial contaminants of makeup brushes from students within Rivers State University and its environs. And also determine the antimicrobial sensitivity of the isolated microorganisms to different antimicrobial agents. Eighty (80) samples of makeup brushes were collected from different hostels, personal houses, and beauty salons and were cultured by inoculating them into different culture media as Nutrient, Blood and Sabouraud Dextrose agar to isolate bacteria and fungi respectively. The identification of isolated bacteria was confirmed by using pure culture of the

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isolates, Gram stain, and biochemical tests which were catalase, coagulase, and others including germ tube tests. The sensitivity test of the isolated bacteria to different antibiotics was carried out using the Optodisc diffusion method. The microorganisms isolated and identified were *Staphylococcus epidermidis* (55%), *Staphylococcus aureus* (22.5%), and *Candida albicans* (22.5%). The isolated bacteria were susceptible to Ciprofloxacin, Levofloxacin and Gentamycin. The findings of this study have shown that both *Staphylococcus epidermidis* and *Staphylococcus aureus* were isolated as bacteria, while *Candida albicans* as fungi from makeup brushes. *Staphylococcus epidermidis* are considered as normal skin flora whereas, *Staphylococcus aureus* could be pathogenic and present as source of infecting bacteria. It could be concluded that it is possible to isolate bacteria and fungi that may be pathogenic from makeup brushes, that may act as source of infecting pathogens therefore increases the risk of infection and public health concern.

Keywords: Microorganisms; makeup; brushes; pathogens; antimicrobial agents.

1. INTRODUCTION

"Cosmetics have become an important part of everyday life and are widely used for beauty purposes, sun protection, and clearing extraneous matter" [1]. "Cosmetic products contain essential minerals and chemical compounds in water that provide favorable medium for microbial growth" [2]. "The majority of the people who use and shared tools in the beauty shops are unaware that makeups can harbor variety of microorganisms that cause risk for exposure to potentially infectious microorganisms" [3]. "It is believed that cosmetics can be associated with skin or eye infections which can be transmitted to and between clients if not handled properly" [4,5]. "Cosmetic testers in the beauty shops are often contaminated with microorganisms due to the sharing of makeup and repeated use of the same applicators as well as poor handling during the showcase of the product" [6,7]. Risk of skin infection to consumers can occur as a result of use of skin products such as powder and cream, eye products like mascara and eyeliner [2] and hairdressing [3]. "Some pathogenic microorganisms including *Staphylococcus aureus* and *Pseudomonas aeruginosa* have been detected in beauty products" [8,9]. "*Staphylococcus aureus* and *Staphylococcus epidermidis* have been implicated as the most important bacteria that cause diseases to humans such as skin infection, boils, bullous impetigo, hair follicles, and scalded-skin syndrome in hairdressing and beauty salons in India and Nigeria" [3,8,10]. Another study has showed that *Bacillus, Staphylococcus, Pseudomonas, Enterobacter, Escherichia coli,* and *Klebsiella* were the common bacteria isolated from cosmetic products [11]. "Skincare products, hair preparations, and facial makeups were responsible for the majority of allergic contact dermatitis in some regions of the Middle East" [12,13]. "Herpes causes blisters on the lips and around the mouth from shared make-up tools. Lipsticks and powder brushes that touch these parts of the face can then spread the allergic contact dermatitis infection to other people" [14]. Studies have revealed that "the most common bacteria such as *Pseudomonas aeruginosa* and *Pseudomonas putida* are present in the eye makeup mascara and eyeliner. *Pseudomonas aeruginosa* can cause irritation, conjunctivitis, pink eye, redness, and watery discharge, which could lead to irreversible blindness" [15]. Makeup brushes also have the potential to act as suitable homes for bacteria to thrive.

"Although the microbial standards of cosmetics have been progressively improved by strict law; their contamination has been frequently reported and even in some cases, has generated serious problems for consumers. Unfortunately, cosmetic contamination awareness and health risks are very poor among the users of all age groups. There is no established law, guidelines, and best practices for many public make-up testers. This study is designed to assess the bacterial and fungal contamination from different cosmetic testers in Port Harcourt and evaluate customers by using cross sectional study to examine if customers are conscious of the beauty products that are used" [15].

"The beauty salon's aim is glamour, and it is a business that makes used of a variety of tools and devices to improve the appearance of one's hair, skin and body. Beauty products are mostly blends of chemical compounds from natural (such as coconut oil) or engineered sources. Salons provide a wide range of services including hairdressing, nail care (manicures and pedicures), hair removal by waxing and

threading, mud baths, and many other services. However, they are also considered a major health concern. The health risks associated with beauty salons vary depending on the products and tools used, the nature of the business, and the service providers themselves" [16]. Salons can contribute to and cause the spread of viral, fungal, and bacterial infections.

"The Food and Drugs Administration (FDA) defines a cosmetic as a product (excluding pure soap) intended to be applied to the human body for cleansing, beautifying, promoting attractiveness, or altering the appearance. Cosmetic items and tools are favorable environments for the reproduction of viral, parasitic, and bacterial organisms, which contribute to and cause the spread of infections. Various components contribute to this issue. First, the components of most cosmetic products, such as organic and inorganic compounds, moisturizers, basic minerals, and growth factor such as some vitamins can provide environment conducive to the reproduction of organisms. Secondly, the dates of production and expiration are, for the most part, not checked for beauty care products; thus, the decrease in effectiveness of the preservatives within the makeup over time is not noticed. Thirdly, makeup is not produced under sterile conditions and is habitually shared in beauty salons. And fourthly, the customary apparatuses used in nail salons such as clippers, scissors, and nail care instruments can inadvertently pierce the skin, which may lead to health issues ranging from inflamed skin to hepatitis. Service providers themselves are vulnerable to transmitting diseases among their customers" [17].

"The ingredients in cosmetic products and the materials used in the tools make the salon an ideal environment for the proliferation of microbes, thus contributing to the spread of various diseases" [3]. "Cosmetics fulfill all of the requirements for microbial growth; the ingredients of most beauty products include sugar, starch, protein, amino acids, organic acids, acids, alkalis, salts, paraffin, fatty acids, alcohols, esters, moisturizers, colors and dyes, preservatives, antioxidants fragrances, and essential oils. In addition, most of these ingredients are water soluble, which is an essential factor for the growth of microorganisms" [18]. "Cosmetics that contain a high-water content are at a higher risk of contamination, which can lead to an alteration in the composition of the product and may constitute a threat to the health of the consumer" [19].

"A few bacterial species of the *Streptococcus, Staphylococcus*, and *Pseudomonas* genera are considered a major concern because they are related to numerous common infections and can cause respiratory issues and anti-microbial resistant infections owing to their pathogenic nature" [20]. The age range of female makeup users has increased widely across the decades. The age that females begin wearing makeup gets younger with every new generation. If consumers are aware about the proper ways of handling the products, they should be able to have a low incidence of infection. The aim of this study is to identify different microorganisms in makeup brushes of students of Rivers State University, Port Harcourt.

2. MATERIALS AND METHODS

2.1 Study Area

This is a cross-sectional study that was conducted in the different hostels in Rivers State University, Port Harcourt, and it is located in the Diobu (Mile III) Area of Port Harcourt, Rivers State, Nigeria. It is the first technological University in Nigeria and also the first University to be situated within the Niger Delta.

2.2 Study Population

The study population comprised of students that reside in the respective female hostels, in Rivers State University, Port Harcourt. It also included those that stay off campus, in their personal houses, and those that carry their make-up kits to classrooms in the various Departments in Rivers State University.

2.3 Sample Size

A total of 80 makeup brushes were collected from students that reside in the hostel, and also from those who stay within the University environment, from makeup artists that share their brushes, and individuals that do their makeup by themselves.

2.4 Eligibility

2.4.1 Inclusion criteria

Only those willing to provide at least oral consent will be included in the study. Only students that have used their makeup brush recently will be included.

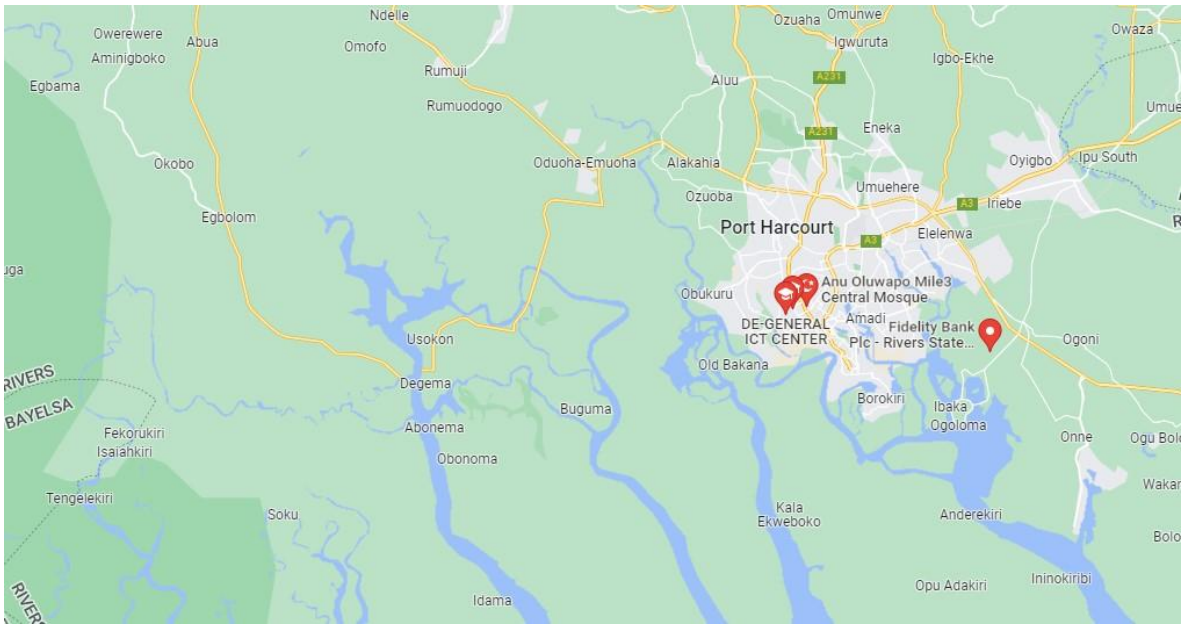

Fig. 1. Map of Port Harcourt showing Rivers State University

2.4.2 Exclusion criteria

Individuals who did not consent to the study will be excluded.

2.5 Ethical Considerations

The ethical approval for this study was obtained from the ethical committee of Rivers State University Teaching Hospital, Port Harcourt.

2.6 Specimen Collection and Preparation

A structured questionnaire was given to all participants to obtain their information. The laboratory test results for participants were anonymously linked to their questionnaire information through unique identifiers.

The samples were collected with the approval of the makeup owners using moistened sterile swabs, and transferred into transport media and sent to the Laboratory through Cold Chain. Then, the samples were carried to the laboratory and stored at room temperature until the time of analysis.

2.7 Media Preparation

Three different media used were Nutrient, Blood and Sabouraud Dextrose agar) to cultivate the fastidious bacteria, coliforms, and fungi, respectively. The culture media were prepared according to the manufacturer's instructions of weighing and dissolving in known amount of water, sterilizing and pouring in petri dishes.

2.7.1 Nutrient agar preparation

Twenty-eight (28g) grams of the nutrient agar powder was weighed and suspended in 1 litre of distilled water. The solution was mixed properly and allowed to dissolve completely. It was then sterilized by autoclaving at 121°C for 15 minutes. The molten liquid was poured into the petri dish and allowed to solidify. The plates were stored in a refrigerator.

2.7.2 Blood agar preparation

Twenty-eight (28g) grams of the nutrient agar powder was suspended in 1 litre of distilled water. The solution was mixed properly and allowed to dissolve completely. It was then sterilized by autoclaving at 121°C for 15 minutes. The liquid was poured into the petri dish and allowed to cool to 45ºC, but not solidify. When the agar had cooled to 45-50ºC, 5% sterile defibrinated blood was added and mixed gently, but well, avoiding air bubbles. The mixture was dispensed into sterile plates while liquid, and then allowed to solidify.

2.7.3 Sabouraud dextrose agar (SDA) preparation

Sixty-five (65g) grams of the powdered medium was weighed and suspended in one litre of

distilled water. It was heated with frequent agitation and boiled for one minute to completely dissolve the medium. The mixture was autoclaved at 121ºC for 15 minutes. It was allowed to cool to 45ºC and poured into petri dishes. The poured plates were allowed to solidify and were used to culture the samples after drying.

2.8 Isolation

Eighty (80) swabs of the samples were collected, and each sample was inoculated on the three (3) different solidified and dried media mentioned earlier. The isolate were incubated for 24 h at 37ºC for bacteria while SDA plates for fungi identification were incubated at 28ºC and observed daily for 7 days. All plates were observed for the growth of microbial colonies after incubation to identify the colonial morphology.

2.9 Identification of Microorganisms

2.9.1 Gram staining

Gram stains were carried out to determine if bacteria were Gram-positive bacteria or Gram-negative bacteria. The Principle of Gram Staining is that when bacteria are stained with primary stain Crystal Violet and fixed by the mordant, some of the bacteria are able to retain the primary stain and some are decolorized by alcohol. A smear was made on a clean grease free glass slide with a small picked portion of bacteria colony from a24hours pure culture. It was allowed to air dry and then heat fixed over a Bunsen burner flame. Crystal violet reagent was poured and kept for about 30 seconds to 1 minutes and rinsed with distilled water. The slide was flooded with Gram's iodine for 1 minute and washed with water. It was then decolorized with acetone for about 10-20 seconds and rinsed with water. Safranin the counterstain was added and allowed for about 1 minute and washed with water. It was allowed to air dry, blotted, and observed under the microscope.

2.9.2 Catalase test

Catalase mediates the breakdown of hydrogen peroxide (H_2O_2) into oxygen and water. To find out if a particular bacterial isolate can produce catalase enzyme, a small inoculum of a bacterial isolate is mixed into hydrogen peroxide solution (3%). It is observed for the rapid elaboration of A small amount of the bacterial colony was transferred to a surface of a clean, dry glass slide using a plastic wire loop or sterile wooden stick. A drop of 3% H_2O_2 was placed onto the slide and mixed. It was then observed for the results. A positive result showed the rapid evolution of oxygen (within 5-10 seconds), as evidenced by bubbling. A negative result showed no bubbles or only a few scattered bubbles.

2.9.3 Coagulase test

A coagulase test is a biochemical test that is used to differentiate *Staphylococcus aureus* from other Staphylococci species like *S. epidermidis* and *S. saprophyticus* on the basis of the ability to produce the coagulase enzyme. Coagulase is an enzymatic protein that is a thermostable thrombin-like substance, which converts fibrinogen into fibrin resulting in clotting or clumping.

About 10 µl of normal saline was added to a slide. Several colonies from a fresh culture were collected with an inoculating loop and emulsified into the normal saline to obtain a smooth milk-colored suspension. A drop of a rabbit or human plasma was added to the slide, and the clumping was observed immediately, not to exceed 10 seconds.

2.9.4 Germ tube test

Germ tubes are short outgrowths, non-septate germinating hyphae. They are ½ the width and 3 – 4 times the length of the cell from which they arise. When cells of *Candida* are incubated in serum at 37ºC for 2-4 hours, *Candida albicans* produce short, slender, tube-like structures called germ tubes. A germ tube appears as a short lateral extension from the yeast cell and does not have a constriction (septum) where it meets the yeast cell or any constriction at the septum along the tube. The formation of germ tubes is associated with increased synthesis of protein and ribonucleic acid. Various media like fetal bovine serum may be used as a substitute for human pooled serum. A yeast colony was lightly touched with an applicator stick. The yeast cells were suspended in an appropriately labeled tube of fetal bovine serum. The tube was then incubated for 2-3 hours in a 35 – 37ºC incubator. A drop of the suspension was placed on a slide using a Pasteur pipette and a coverslip was placed over the suspension. The wet mount was examined microscopically (at 40X) for the presence or absence of germ tubes.

2.10 Sensitivity Testing

Sensitivity analysis was done to determine the effectiveness of antibiotics against the bacteria that have been isolated from the cultures. The sensitivity test of studied bacterial isolates was done using Optodisc method. The antibiotics used were Ciprofloxacin (CPX 10mcg), Gentamycin (CN 10mcg), Norfloxacin (NB 10mcg), Amoxil (AML 10mcg), Streptomycin (S 10mcg), Rifampicin (RD 10mcg), Erythromycin (E 10mcg), Chloramphenicol (CH 10mcg), Ampiclox (APX 10mcg) and Levofloxacin (LEV 10 mcg). The susceptibility of the bacteria was examined by diffusion method and were read after 24 hours of the incubation at $37^{O}C$ in aerobic conditions. The results were investigated after measuring the inhibition zones around each disc and compared with standard inhibition zones fixed in sheets of the National Committee for Clinical Laboratory Standards [21].

3. RESULTS

3.1 Socio-Demographic Characteristics of Participants

The demographic details showed 75% of the participants are students, while 22.5% were makeup artists, with the majority of the population aged between 26-30 years. The administered questionnaire that was given to the participants showed that 50% of those that had skin irritation suspected that the skin irritation was from the makeup brushes. Ninety-three point seventy five (93.75%) percent of the population owned their personal makeup packs, while 37.5% applied makeup once per week, whereas 25% applied makeup every day. In 62.5% of the participants, the makeup lasts for 4-6 hours, and 2-4 hours in 22.5%.

Table 1. Socio-demographic characteristics of participants

Characteristics of the Population	Number of Participants	Percentage of the Population
Age Range		
16-20	16	20
21-25	31	38.75
26-30	33	41.25
Occupation		
Student	60	75
Make Up Artist	18	22.5
Others	2	2.5
Frequency of applying makeup		
Everyday	20	25
Twice per week	10	12.5
Once per week	30	37.5
Occasionally	20	25
Duration of makeup when applied		
2-4 hours	18	22.5
4-6 hours	50	62.5
>6 hours	12	15
Duration of use of current brush		
1-3 months	5	6.25
4-6 months	20	25
Over 6 months	55	68.75
Frequency of washing makeup brushes		
Everyday	3	3.75
Twice a week	2	2.5
Once a week	5	6.25
Once a month	40	50.0
Never	30	37.5
Equipment used for washing brushes		
Normal water	5	6.25
Warm water	20	25
Detergents/soaps	55	68.75

Characteristics of the Population	Number of Participants	Percentage of the Population
Had skin irritation before		
Yes	10	12.5
No	70	87.5
Suspected skin irritation was from makeup brush		
Yes	5	50
No	5	50
Visited a dermatologist before		
Yes	10	12.5
No	0	0

Table 2. Microorganisms isolated from the makeup brushes

Organism	Number Isolated	Frequency (%)
Staphylococcus epidermis	44	55
Staphylococcus aureus	18	22.5
Candida albicans	18	22.5

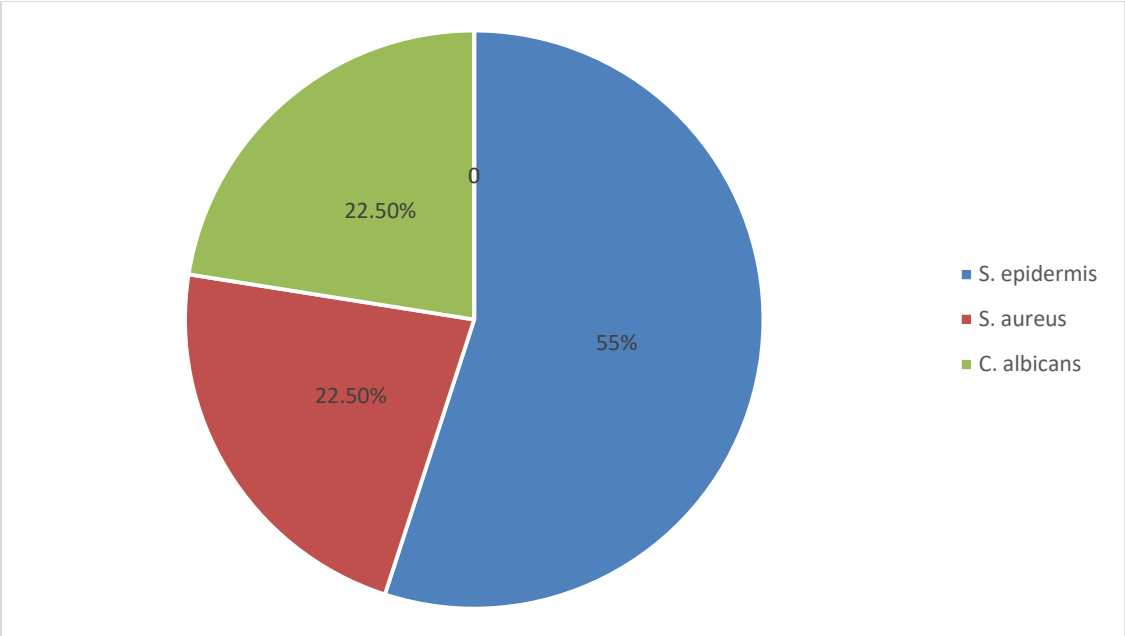

Fig. 2. Percentage of each microbial growth

3.2 Microorganisms Isolated from the Makeup Brushes

The analysis of the makeup brushes showed that all the samples examined were contaminated with microorganisms. It was observed that 77.5% of the brushes were contaminated with bacteria and 22.5% was contaminated with fungi. Using morphological characterization and biochemical tests, it showed that the most predominant isolated bacteria was *Staphylococcus epidermis* which was found in 55% of samples. *Staphylococcus aureus* and *Candida albicans* were both isolated from 22.5% of the samples each.

3.3 Antibiotic Susceptibility Testing

The result for the antibiotic susceptibility pattern of the bacterial isolates from the makeup brushes is given in Table 3. The results showed that both *Staphylococcus epidermis* and *Staphylococcus aureus* were resistant to Erythromycin, Ampiclox, Rifampicin, Amoxil, Streptomycin, Norfloxacin, and Chloramphenicol; while both organisms were susceptible to Ciprofloxacin, Levofloxacin and Gentamycin.

Table 3. Antibiotics susceptibility pattern

Isolate	Antibiotics									
	CPX	E	LEV	CN	APX	RD	AML	S	NB	CH
Staphylococcus epidermis	S	R	S	S	R	R	R	R	R	R
Staphylococcus aureus	S	R	S	S	R	R	R	R	R	R

R= Resistant, S= Sensitive; CPX = Ciproflox; E = Erythromycin; LEV= Levofloxacin; CN= Gentamycin; APX= Ampiclox; RD= Rifampicin; AML= Amoxil; S= Streptomycin; NB= Norfloxacin; CH= Chloramphenicol

4. DISCUSSION AND CONCLUSION

4.1 Discussion

In the study, microorganisms were isolated and identified from makeup brushes collected from Rivers State University and it environs and it also showed that bacterial contamination was more than fungal contamination. This may be possible as a result of the use of non-sterile makeup brushes that are always in contact with air and are being shared between costumers and even friends which has spread infection from one person to another and can cause infection of the skin and eye. Our results showed that 77.5% of the makeup brushes were contaminated with bacteria and 22.5% was contaminated with fungi. This finding corresponds with the finding from the study by Noor et al. [15]. However, while the bacterial contamination corresponds with other previous work, the fungal contamination did not correspond with the study by Dadashi and Dehghanzadeh, [2] who had a low prevalence of fungal contamination and explained that the low fungal contamination was due to the fact that some beauty products have a protective material that prevents fungal growth.

In this study, *Staphylococcus aureus* and *Staphylococcus epidermidis* are the bacteria isolated and this is in agreement with other studies that reported *Staphylococcus aureus* and *Staphylococcus epidermidis* as some of the that the most Gram-positive isolated bacteria of cosmetic products [22,23].

In the current study, *Staphylococcus epidermis* was the most commonly isolated bacteria (55%). This finding also agrees with the work by Noor et al. [15] who isolated 57% of *S. epidermis*. The reason for this high prevalence of *S. epidermis* is that it is considered to be a normal flora of the skin. Furthermore, *Staphylococcus aureus* was also isolated from the makeup brushes (22.5%). *Staphylococcus. aureus* is among the most important bacteria that cause disease in human including skin infections and abscesses. These symptoms could occur due to the impairment of the human immune system, skin structure, and wounded epithelium [24]. Contrary to this work, other studies have commonly isolated bacterial isolates such as *Pseudomonas aeruginosa* and *Escherichia coli* from cosmetic products [25]. Another study in Pakistan reported *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolated from face sponge and brush and *Streptococcus pyogenes, Pseudomonas aeruginosa, Bacillus spp, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Klebsiella pneumoniae* isolated from skin powder and cream [23]. The inability to isolate these bacteria could be as a result of the type of cosmetic products used.

Based on the outcome of the study, the only fungal species isolated was *Candida albicans* (22.5%). Candida albicans exist as a dimorphic mold in the environment and a yeast in the body [26,27,28]. It has been implicated in cutaneous and systemic infections including folliculitis, oral thrush, vaginal thrush and Candidaemia [29,30]. This percentage of Candida albicans isolated in this study is slightly lower than the percentage observed in the work by Noor et al. [15] who isolated 27% of fungi.

The result for the antibiotic susceptibility pattern of the bacterial isolates from the makeup brushes as shown in Table 3 showed that both *Staphylococcus epidermis and Staphylococcus aureus* were resistant to Erythromycin, Ampiclox, Rifampicin, Amoxil, Streptomycin, Norfloxacin and Chloramphenicol; while both organisms were susceptible to Ciprofloxacin, Levofloxacin and Gentamycin.

4.2 Conclusion

This study provided an insightful look at the significance of not sharing cosmetic brushes in order to avoid skin infections. This study showed that it is possible to isolate bacteria and fungi from makeup brushes and the sharing of makeup brushes between costumers and even friends.

In order to reduce the health hazards, public health groups have advised against using

makeup brushes properly together with other cosmetic products. Costumers must avoid sharing makeup brushes and always use sterilized instruments. Additionally, it is recommended not to use a make up brush for too long. If people are informed about the dangers of using infected makeup brushes, they will change their behavior and assist to slow the spread of these groups. It is therefore recommended that more microbial analysis be done in future studies in order to find other potential organisms that might possibly be disseminated by cosmetics brushes.

CONSENT

As per international standard or university standard, patient(s) written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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