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# Antibiogram of bacteria and fungi isolated from makeup kits in Port Harcourt metropolis

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**ABSTRACT**

Makeup kits are materials put together to enhance a person's appearance. Sharing of makeup kits is mostly found amongst women owing to getting their makeup done by professionals in beauty salons. Patrons of beauty salons share makeup items like mascara, eyeliners, lipsticks, lip glosses, and brushes. This study assesses the presence of microorganisms on some makeup kits; brushes, mascara and razor blades used in Port Harcourt metropolis. Swab samples were aseptically collected from the surfaces of makeup kits at three different locations: Iwofe, Saint John and Mile 3. Organisms were enumerated and identified using standard microbiological techniques. Kirby Bauer disc method was used for antibiotic sensitivity. Total Heterotrophic bacterial mean counts ranged from 1.5±0.7 to 4.0±1.41 CFU/ml while the total heterotrophic fungi mean counts ranged from 1.3±0.28 to 8.0±1.41 SFU/ml. Identified bacteria isolates belonging to the five genera and their percentage occurrence were; *Streptococcus* sp. 1(16.6%), *Staphylococcus* sp. 2(33.3%), *Micrococcus* sp. 1(16.6%), *Bacillus* sp. 1(16.6%), *Escherichia coli* 1(16.6%) and identified fungal isolates belonging to four genera were: *Candida* sp.(20%), *Mucor* sp.(20%), *Aspergillus* spp.(40%), and *Penicillium* sp.(20%). Sensitivity results showed that *Staphylococcus* sp. was resistant to reflacine, amplicox, zinnacef, and ciprofloxacin and was susceptible to septrin, erythromycin, gentamicin, and streptomycin. *Micrococcus* sp. was susceptible to erythromycin, gentamicin septrin, ciprofloxacin, and streptomycin and was resistant to reflacin, zinnacef, rocephin and amplicox. *Streptococcus* sp was resistant to erythromycin and streptomycin and was susceptible to septrin, gentamicin and zinnacef. *Bacillus* sp was resistant to septrin, gentamycin, amplicox, rocephin, and ciprofloxacin and was susceptible to reflacine and erythromycin. *Escherichia coli* was resistant to nalidixic acid, gentamicin, augumentin, and ceporex, and was susceptible to reflacine. The mere presence of antibiotic-resistant organisms on the surfaces of makeup kits calls for public health concern. Sharing of makeup kits has been reported to harbor potentially pathogenic microorganisms. It is therefore recommended that makeup kits should not be shared and must be cleaned before and after use to forestall any public health crisis.

**Keywords:** Makeup kits, microorganisms, antibiotic resistance.

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## INTRODUCTION

Makeup kits are products put together to enhance a person's appearance, they could be used to conceal blemishes and enhance a person's natural features. In recent years, cosmetics (makeup) have been extensively used for beauty purposes, meanwhile, beauty salons play an important role in the possible transfer of skin and eye infections due to the use of public makeup kits by women (Enemuor et al., 2013). Although the microbial standard of cosmetics has been progressively improved by stringent legislation, their contamination has been frequently reported and in some cases, has generated serious problems for consumers (Lyndon et al., 2009). Often production and expiration dates are not labeled on the products, as are the effectiveness of makeup kits.

Preservatives decrease with time. In addition, cosmetics (makeup kits) are comprised of essential mineral growth factors, organic and inorganic compounds and humidity which provides suitable conditions for the augmentation of microorganisms (Behravan et al., 2005). Skin microflora of anyone is unique and could be transferred to another person using tools such as brushes and blades, which could threaten the health of women (Noah, 1995). Therefore, it is likely that makeup kits in beauty salons have more diversity and density of microorganisms. The most common skin infections are caused by *Staphylococcus* sp., *Streptococcus* sp., *Escherichia coli, Klebsiella* and fungi like *Aspergillus* sp. and *Penicillium* sp. (Enemuor et al., 2013). Cosmetic products can be contaminated in three ways: a). Application of unsterile raw materials as ingredients, b). In the course of the production process or c). During use of these products (Charnock, 2004).

On the other hand, trafficking counterfeit cosmetics products is a problem in many countries. Microbial contamination and the occurrence of skin contamination due to cosmetics is still one of the causes of product recalls in the world (Okeke and Lamikanra, 2001). Sharing makeup kits can lead to serious breakouts, cold sores, eye infections etc. This study aimed to determine the presence of microorganisms on some makeup equipment used in Port Harcourt metropolis.

## MATERIALS AND METHOD

### Study area

The study was carried out in three locations within Port Harcourt metropolis: Iwofe, Saint John and Mile 3. These locations were selected based on the population and high economic activities around them. The location coordinates of the study areas are as follows: Iwofe, $4^{o}48'47"$ N $6^{o}56'20"$ E; Saint John, $4^{o}48'49"$ N $6^{o}56'19"$ E and Mile 3, $4^{o}48'18"$ N $6^{o}59'29"$ E.

### Sample collection

The samples were collected from different makeup kits: brushes, razor blades and mascara, by swabbing the surfaces of the makeup kits. The samples were collected using a sterile swab stick (which had been moistened with sterile normal saline). The samples were then labelled accordingly and transported in an ice pack container to the microbiology laboratory at Rivers State University for microbiological analysis.

### Enumeration of bacteria and fungi from makeup kits

The total heterotrophic bacterial and *Staphylococcal* load on the different kits were enumerated using standard plate count (Prescott et al., 2011). In this method, the swab samples were immersed in 9 mL of sterile normal saline. Ten-fold serial dilution was later carried out by transferring 1mL from the initial stock with the aid of a sterile 1mL pipette into test tubes containing sterile 9mL normal saline. This was repeated to obtain dilutions of $1{:}10^{-4}$. After which, an aliquot from the $10^{-1}$ dilution was aseptically inoculated at the centre of well-dried mannitol salt agar plates in duplicates for enumeration of *Staphylococcus*, while an aliquot from the $10^{-3}$ dilution was inoculated on a nutrient agar plate in duplicates for enumeration of the total heterotrophic bacteria. The same procedure was followed for the isolation of fungi on Sabouraud Dextrose agar (SDA).

### Identification of bacterial and fungal isolates

The colonies were sub-cultured to obtain pure isolates. The pure isolates were then characterized by Gram's staining and Biochemical tests such as catalase test, indole test, methyl red test, citrate test, coagulase test, Voges Proskauer test and sugar fermentation tests. The identity of the isolates was matched with the Bergy's Manual of Determinative Bacteriology for confirmation. The fungal isolates were identified based on macroscopic characteristics (growth characteristics, pigment formation and texture) and microscopic morphology (formation of macroconidia and microconidia or other typical elements). The microscopic identification was done by lactophenol cotton blue mounts. In this method, a drop of lactophenol cotton blue was placed on a grease-free slide, and the aerial mycelium of the investigated fungal isolates was cut and transferred into the drop of lactophenol cotton blue on the slide using a sterile inoculating needle. The slide was covered with a microscope cover slip and viewed under the x10 and x40 magnification lens of the compound microscope (Robinson et al., 2021). Characterization of fungal isolates was done by matching results with those reported by Mcdonald et al. (2000) and Elis et al. (2007).

### Antibiotics sensitivity test

The disk diffusion method of antibiotics testing was in accordance with the Clinical laboratory standard institute. First, the isolates (24 hours old) were standardized using the 0.5 McFarland standard (CLSI, 2022). This was done by matching the turbidity of the isolates in sterile 4mL normal saline to the 0.5McFarland standard. Afterwards, sterile swab sticks were dipped into the standardized isolates and swabbed uniformly on the surface of the dried Mueller-Hinton agar plates. The bacterial isolates were tested against already prepared commercial antibiotics: Ciproflox (10µg), Augmentin (30µg), Tarivid

(10µg), Streptomycin (30µg), Reflacine (10µg) Nalididixic Acid (30µg), Ceporex (10µg), Septrin (30µg), Norfloxacin (10µg), Levofloxacin (20µg), Ampiclox (20µg) Chloramphenicol (30µg), Amoxil (20µg), Rifampicin (20µg), Erythromycin (30µg) and Ampicilin (30µg). The plates were held at room temperature for 3-5mins to allow drying. The antibiotic discs were placed on the plates, and the plates were incubated for 18-24 hours at 37ºC. The diameters of the zone of inhibition were recorded to millimeters and classified as resistant (R), intermediate (I) and susceptible (S) according to a published interpretive chart (CLSI, 2022).

## RESULTS

The results of the microbial counts from the various makeup equipment are presented in Table 1. Results of the total heterotrophic bacterial counts ranged from 1.5±0.7 to 4.0±1.41 CFU/ml, while the total heterotrophic fungi count ranged from 1.3±0.28 to 8.0±1.41 SFU/ml respectively. Results also showed that the microbial counts in the different equipment varied. Despite the disparity in the total heterotrophic bacterial counts, there was no significant difference (P>0.05) recorded, while there was a significant difference (P<0.05) in the fungal counts of the various makeup equipment. Identified bacterial isolates associated with the different makeup equipment include *Streptococcus* sp., *Staphylococcus* sp., *Micrococcus* sp., *Bacillus* sp. and *Escherichia coli*. Identified fungal isolates were *Candida albicans, Mucor* sp., *Aspergillus* sp. and *Penicillum* sp.

Results showing the percentage distribution of bacterial isolates from the makeup equipment are presented in Figure 1 and are as follows: *Streptococcus* sp. (16.6%), *Staphylococcus* sp. (33.3%), *Micrococcus* sp. (16.6%), *Bacillus* sp. (16.6%), *Escherichia coli* (16.6%). Results show that *Staphylococcus* sp. was the most occurring bacterial isolate in the study, while the other isolates had the same percentage. The percentage distribution of fungal isolates is presented in Figure 2. *Aspergillus* spp. (40%) was the most predominant fungal isolate, while the other isolates had the same percentage: *Candida* sp. (20%), *Mucor* sp. (20%) and *Penicillium* sp. (20%).

Results of the gram-positive and gram-negative bacterial isolates susceptibility pattern are presented in Tables 2, 3 and 4 respectively. Sensitivity results showed that *Staphylococcus* sp was resistant to reflacine, amplicox, zinnacef, and ciprofloxacin and was susceptible to septrin, erythromycin, gentamicin, and streptomycin. *Micrococcus* sp. was susceptible to erythromycin, gentamicin septrin, ciprofloxacin, and streptomycin and was resistant to reflacin, zinnacef, rocephin and amplicox. *Streptococcus* sp was resistant to erythromycin and streptomycin and was susceptible to septrin, gentamicin and zinnacef. *Bacillus* sp was resistant to septrin, gentamycin, amplicox, rocephin, and ciprofloxacin and was susceptible to reflacine and erythromycin. *Escherichia coli* was resistant to nalidixic acid, gentamicin, augumentin, ceporex, and was susceptible to reflacine.

**Table 1.** Microbial counts (Cfu/ml) of makeup brush, razor blade and mascara.

| Samples | Total heterotrophic bacteria ($x10^3$) | fungal count ($x10^4$) |
|---|---|---|
| RA 1 | 2.0±0.14[a] | 1.3±0.28[b] |
| RA 2 | 4.0±1.41[a] | 2.3±0.57[b] |
| BR 1 | 1.5±0.7[a] | 8.0±1.41[a] |
| BR 2 | 2.2±0.14[a] | 1.6±0.28[b] |
| MA 1 | 2.8±0.42[a] | 4.0±1.41[ab] |
| MA 2 | 3.3±0.99[a] | 8.0±1.41[a] |
| P-value | 0.251 | 0.002 |

*Means with similar superscripts down the group showed no significant difference (P>0.05).
Key: RA 1: Razor 1, RA 2: Razor 2, BR 1: Brush 1, BR 2: Brush 2, MA 1 : Mascara 1, MA 2: Mascara 2.

## DISCUSSION

Makeup kits are used for many reasons, one of the major reasons being to improve self-esteem by appearing prettier or more handsome than one's normal self (Nusrat et al., 2023). The findings in the present study showed that all the makeup kits, including the brushes, razor blades and mascara, were contaminated with both bacterial and fungal isolates. The high bacterial and fungal load associated with the makeup tools could be due to contamination arising during storage or improper hygiene especially when the kits like brushes or razor blades are not properly cleaned. More so the kits could be contaminated by the microflora present on the face of the client due to regular use without properly cleaning or sterilizing the kits. This agreed with Cohut (2019), who attributed the contamination of makeup tools by harmful bacteria could be due to regular use. Furthermore, Mbah et al. (2023) observed that contaminants of makeup tools differ from person to person depending on how often they

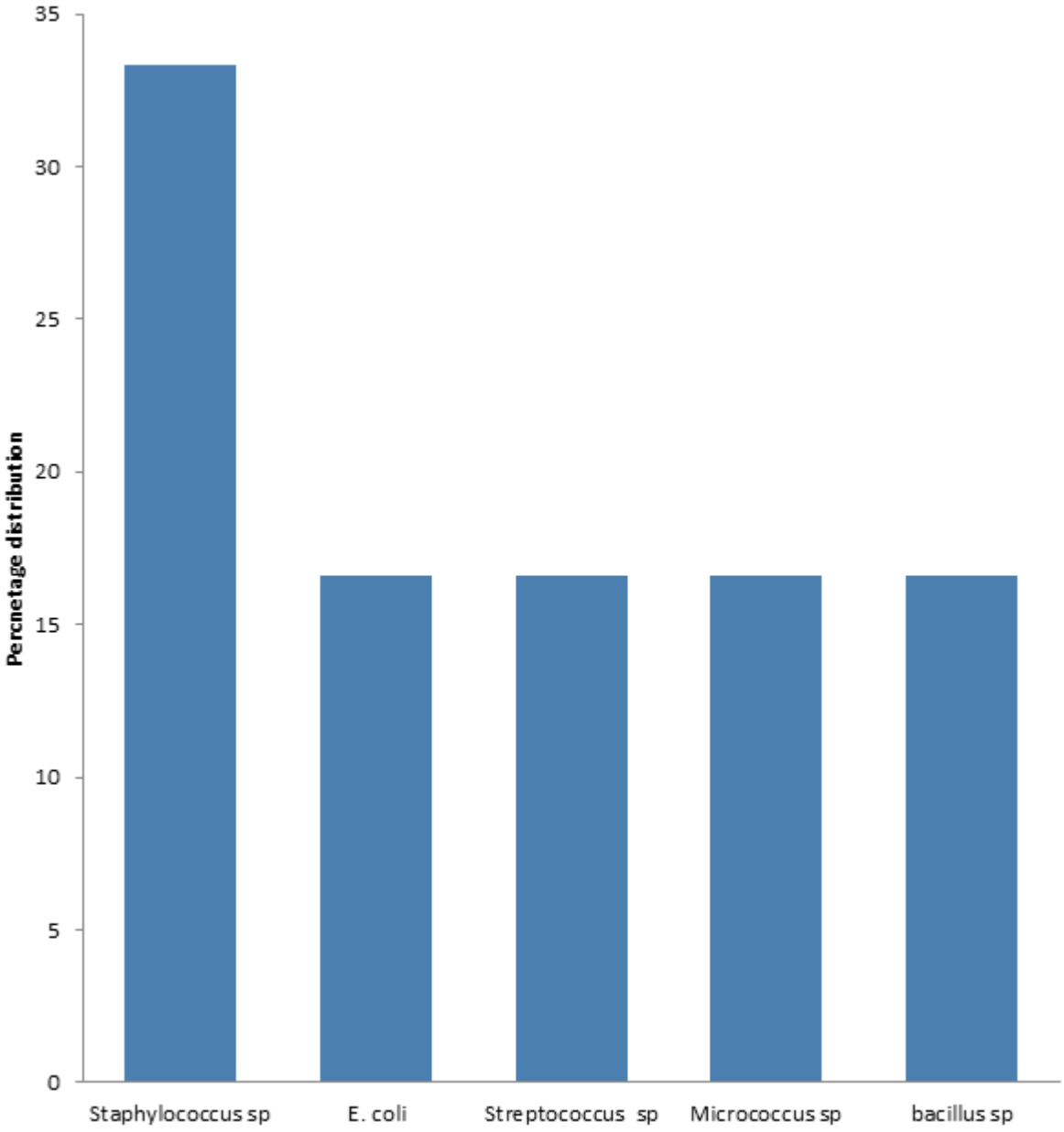


**Figure 1.** Percentage occurrence of bacteria isolates from all samples.

are used and how frequently they are cleaned.

Most of the bacterial and fungal isolates associated with makeup kits in the present study have been reported in previous studies. *Streptococcus pyogenes, Pseudomonas aeruginosa, Bacillus* spp, *Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli* and *Klebsiella pneumoniae* were isolated from skin powder and cream in a previous study by Mohammed et al. (2020). Chidimma et al. (2023) isolated *Bacillus* sp, *Pseudomonas* sp, *Staphylococcus* sp, *Candida albicans, A. niger* and *A. flavus* from different makeup kits. Although they reported that *Bacillus* sp and *Staphylococcus* sp had the highest prevalence, in the present study, *Staphylococcus* sp had the highest prevalence in the samples. *Staphylococcus* sp, *P. aeruginosa* and *Klebsiella* sp were reported by Mbah et al. (2023). Although *Staphylococcus* sp and *Bacillus* sp in the present study agreed with their study, *E. coli, Micrococcus* sp, and *Streptococcus* sp in the present study contradict their study. The disparity in the microbial or bacterial types between these studies could be attributed to the geographical location as well as how often these kits are used. The present study also showed a disparity in the bacterial and fungal contamination of the various kits. For instance, brush 1 had the least bacterial contamination and higher fungal contamination,

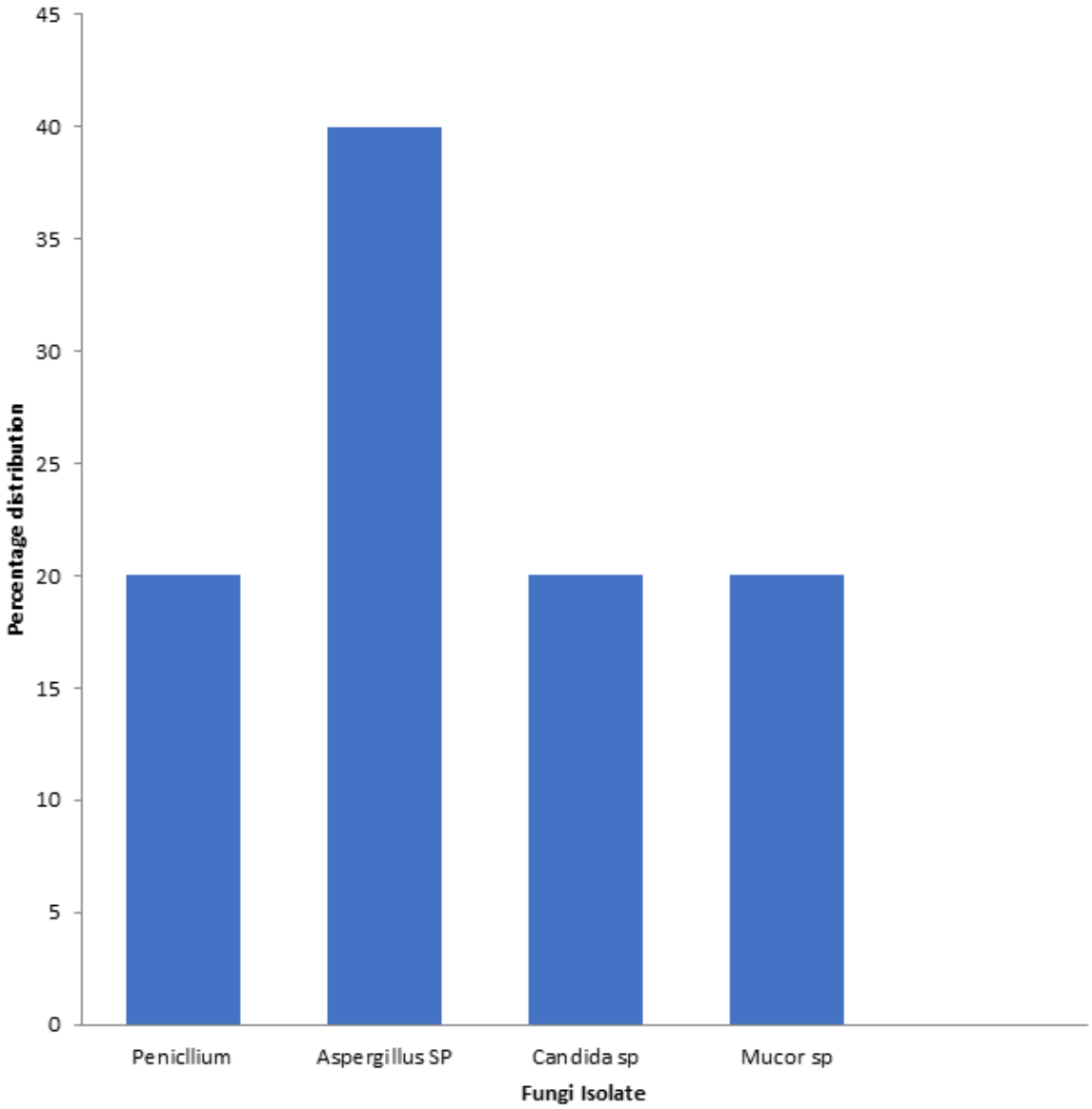


**Figure 2.** Percentage occurrence of fungal isolates from all samples.

**Table 2.** Antibiotics susceptibility pattern of *Micrococcus species and Streptococcus* species.

| Antibiotics | Conc. (µg) | *Micrococcus* sp. | | | *Streptococcus* sp. | | |
|---|---|---|---|---|---|---|---|
| | | R n(%) | I n(%) | S n(%) | R n(%) | I n(%) | S n(%) |
| Septrin | 30 | 0(0.00) | 0)0.00) | 1(100) | 0(0.00) | 0(0.00) | 1(100) |
| Erythromycin | 30 | 0( 0.00) | 0(0.00) | 1(100) | 1(100) | 0(0.00) | 0(0.00) |
| Reflacine | 10 | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 1(100) | 0(0.00) |
| Gentamicin | 10 | 0(0.00) | 0(0.00) | 1(100) | 0(0.00) | 0(0.00) | 1(100) |
| Ampiclox | 20 | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 1(100) | 0(0.00) |
| Zinnacef | 20 | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 1(100) |
| Amoxicillin | 30 | 0(0.00) | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) |
| Rocephin | 25 | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) |
| Ciprofloxacin | 10 | 0(0.00) | 0(0.00) | 1(100) | 0(0.00) | 1(100) | 0(0.00) |
| Streptomycin | 30 | 0(0.00) | 0(0.00) | 1(100) | 1(100) | 0(0.00) | 0(0.00) |

**Table 3.** Antibiotics Susceptibility Pattern of *Bacillus* species and *Staphylococcus* species.

| Antibiotics | Conc. (µg) | *Bacillus* sp. | | | *Staphylococcus* sp | | |
|---|---|---|---|---|---|---|---|
| | | R n(%) | I n(%) | S n(%) | R n(%) | I n(%) | S n(%) |
| Septrin | 30 | 1(100) | 0(0.00) | 0(0.00) | 1(50) | 0(0.00) | 1(50) |
| Erythromycin | 30 | 0(0.00) | 0(0.00) | 1(100) | 0(0.00) | 0(0.00) | 2(100) |
| Reflacine | 10 | 0(0.00) | 0(0.00) | 1(100) | 1(50) | 0(0.00) | 0(0.00) |
| Gentamicin | 10 | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 1(50) | 1(50) |
| Ampiclox | 20 | 1(100) | 0(0.00) | 0(0.00) | 1(50) | 0(0.00) | 0(0.00) |
| Zinnacef | 20 | 0(0.00) | 1(100) | 0(0.00) | 2(100) | 0(0.00) | 0(0.00) |
| Amoxicillin | 30 | 0(0.00) | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) |
| Rocephin | 25 | 1(100) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) | 0(0.00) |
| Ciprofloxacin | 10 | 1(100) | 0(0.00) | 0(0.00) | 1(50) | 0(0.00) | 0(0.00) |
| Streptomycin | 30 | 0(0.00) | 1(100) | 0(0.00) | 0(0.00) | 1(50) | 1(50) |

**Table 4.** Antibiotics susceptibility pattern of *Escherichia coli.*

| Antibiotics | Conc. (µg) | *Escherichia coli* | | |
|---|---|---|---|---|
| | | R n(%) | I n(%) | S n(%) |
| Tarivid | 10 | 0(0.00) | 1(100) | 0(0.00) |
| Nalidixic acid | 30 | 1(100) | 0(0.00) | 0(0.00) |
| Reflacin | 10 | 0(0.00) | 0(0.00) | 1(100) |
| Gentamicin | 10 | 1(100) | 0(0.00) | 0(0.00) |
| Augumentin | 30 | 1(100) | 0(0.00) | 0(0.00) |
| Ciprofloxacin | 10 | 0(0.00) | 1(100) | 0(0.00) |
| Septrin | 30 | 0(0.00) | 1(100) | 0(0.00) |
| Streptomycin | 30 | 1(100) | 0(0.00) | 0(0.00) |
| Amplicin | 10 | 0(0.00) | 1(100) | 0(0.00) |
| Ceporex | 10 | 1(100) | 0(0.00) | 0(0.00) |

while the total heterotrophic bacterial load of brush 2 from a different makeup studio was higher than that of brush 1. This observation was seen across the samples implying that microbial isolates in a particular makeup studio or environment could influence contamination of the makeup kits. The bacterial and fungal isolates in the present study could serve as opportunistic pathogens or become allergens, thereby causing ulceration and inflammation to the skin or the part of the body where the makeup is applied (Chidimma et al., 2023). *Staphylococcus* species are among the most important bacteria that cause disease in humans, including skin infections and abscesses, while *Candida* sp has been implicated in cutaneous and systemic infections including folliculitis, oral thrush, vaginal thrush and Candidaemia (Agi et al., 2023).

All the bacterial isolates: *Micrococcus* sp, *Streptococcus* sp, *Bacillus* sp, *Staphylococcus* sp and *E. coli* all displayed resistance to more than one antibiotic. Thus, there could be an emergence of multidrug-resistant isolates from makeup kits. This study agreed with Nandi and Mandal (2016), who reported that *S. aureus* and *Bacillus* spp amongst other isolates from cosmetic products (lotion and creams) showed resistance to one or more antibiotics. Nasir et al. (2023) reported high resistance of *Staphylococcus* isolates to erythromycin, while in the present study, *Staphylococcus* isolates were susceptible to erythromycin. The high antibiotic resistance of the bacterial isolates in the present study could be attributed to either the acquisition of antibiotic-resistant plasmids or the misuse of antibiotics. This agreed with a previous study (Chidimma et al., 2023).

## CONCLUSION

The study has shown that all the makeup kits were contaminated with bacterial and fungal isolates and the level of contamination varied based on the tool and the location where the makeup studio is situated. More so, the presence of *E. coli* on makeup kits could imply contamination of the kits with fecal matter. The bacterial

and fungal isolates could pose serious health challenges to patrons and handlers if proper hygiene is not strictly followed. The bacterial isolates are highly resistant to the antibiotics and this could imply the emergence of resistant bacterial isolates in makeup kits, thus, antibiotic stewardship is highly recommended. Proper cleaning, sterilization and storage of makeup kits is recommended to reduce or eliminate microbial contamination.

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