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Isolation and Identification of Microbial Pathogens among Eyelash Extension users in Imo State University, Owerri.

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ABSTRACT

The study on Isolation and identification of microbial pathogens among eyelash extension users in Imo State University, Owerri was carried out to detect the presence of bacteria, fungi and parasites on the eyelash extensions in use A total of 51 eyelash extension samples was collected. 30 samples for control was also collected from female students without eyelash extension. There were no fungi and parasites seen in both the test and control samples. The bacterial species isolated belong to the genera *Staphylococcus*, *Serratia*, *Bacillus and Escherichia*. Bacteria was detected in all the test samples; 51(100.00%) and the control samples too; 30(100.00%). More than one kind of bacteria was seen in all the test samples; 51(100.00%) resulting in mixed infections. While for the control samples, more than one kind of bacteria was seen in only 24 samples (80.00%). *Staphylococcus epidermidis*, *Escherichia coli* and *Serratia marcescens* were seen in all the test samples; 51(100.00%) while *Bacillus spp.* in only 38 test samples; (74.51%). *Bacillus spp.* had the highest mean bacteria count in test; 9.3×10^5 cfu/ml followed by *Staphylococcus epidermidis*; 8.35×10^5 cfu/ml, followed by *Escherichia coli*; 5.7×10^5 cfu/ml, then *Serratia marcescens*; 5.0×10^5 cfu/ml. Only *Staphylococcus epidermidis* and *Bacillus spp.* was seen in the control samples. *Staphylococcus epidermidis* was seen in all the control samples; 30(100.00%) while *Bacillus spp.* was seen in 24 samples; (80.00%). For the control, *Staphylococcus epidermidis* had the highest mean bacteria count; 5.7×10^5 cfu/ml followed by *Bacillus spp.*; 2.1×10^5 cfu/ml. The bacteria counts for the eyelash extension samples (test) were significantly higher than that of the control (natural eyelash samples). This shows that the presence of the eyelash extensions increased the colony count of these bacteria and this is dangerous to the eye. The obsession on eyelash extensions should be curbed because they can expose one to serious bacterial infections.

Keywords: pathogens, eyelash, extension, eyes.

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INTRODUCTION

The eye is a very important but delicate organ of the body; therefore, it needs to be well protected to maintain one's sight [1]. The unique structure of the human eye as well as exposure of the eye directly to the environment renders it vulnerable to a number of uncommon infectious diseases caused by bacteria, fungi and parasites. Host defenses directed against these microorganisms, once anatomical barriers are breached, are often insufficient to prevent loss of vision. Therefore, the timely identification and treatment of the involved microorganisms are paramount. The anatomy of the eye and its surrounding structures is presented with an emphasis upon the association of the anatomy with specific infection of bacteria, fungi and parasites [2]. Numerous microorganisms infect the eye either by direct introduction through trauma or surgery, by extension from infected adjacent tissues, or by hematogenous dissemination to the eye [2]. Eyelash extension involves the attachment of synthetic eyelashes made of chemical fibers or other materials individually onto natural lashes using glue [3]. They have become common practice for enhancing beauty among women on occasions such as weddings, festivities, and other social gatherings including funerals. False eyelashes can also be used for individuals who have thin or short eyelashes along with mascara to thicken the look of the natural eyelashes [1]. For eyelash follicles, the total cycle lasts 5–7 months-1–2 months, 15 days, 4–9 months for anagen, catagen, and telogen, respectively. Exogen (expulsion of the previous hair) takes place between telogen and anagen [4]. Eyelashes are shed-like other types of hair from the follicles. Each eyelash has its own growth cycle (anaphase) that lasts 6–8 weeks (excluding the telogen) so that most eyelashes are present to maintain their collective protective mechanism [4].

MATERIALS AND METHODS

Study Area

The study was done in Imo State University is located in Owerri, Imo State.

Sample Collection

The eyelash extension samples were selected randomly such that they represented different places in Imo State University, Owerri. Collection of samples for test were carried out in the school, hostels and saloons within Imo state University. A total of 51 samples were collected. They were collected aseptically and carefully by swabbing the eyelash extension using hand gloves with a sterile cotton swab stick. 30 Samples for control were also collected aseptically from female students without eyelash extensions (Appendix I). The sterile cotton swab stick was wet with sterile saline before collecting the samples for bacterial and parasites identification. It was labelled accordingly. The cotton swabs were transferred immediately to the laboratory within 2 hours of collection to prevent dryness.

Sample preparation for bacteria

The sterile cotton swab stick was wet with sterile saline before collection and stored in sterile saline.

Sample preparation for fungi

The sterile cotton swab stick was used for the sample collection.

Sample preparation for parasites

The sterile cotton swab stick was used for the sample collection and stored in sterile saline.

Preparation of serial dilutions

One ml of the washings was pipetted aseptically into 9 mls of sterile saline in tube 1 and mixed thoroughly, with the sterile diluents, then further dilutions were made up to the 10th tube.

Bacteria Culture

1ml of the serially diluted sample solutions were inoculated into the appropriate media (Chocolate agar, CLED agar, Mannitol agar and Salmonella-Shigella agar) aseptically. It was streaked with the sterile cotton swab sticks and then incubated aerobically in an incubator at 37℃ for 24 hours.

Fungi Culture

Sample was inoculated into the Sabouraud-dextrose agar plates which was mixed with 2% chloramphenicol and then incubated at room temperature (28℃) for 5days. The colonies that developed were counted and sub-cultured on fresh Sabouraud dextrose agar plates to obtain pure cultures. They were later stored on SDA slants for characterization and identification.

Gram Staining Technique

Gram staining technique was used to differentiate the Gram-positive from Gram-negative organisms according to their staining reaction.

Procedure

A smear was made on a clean grease free slide and then fixed with gentle heating by passing the slide over the Bunsen flames three times. The smear was flooded with crystal violet for a minute and washed with tap water afterwards. Lugol's iodine was applied for a minute and washed with water afterward, then the smear was decolourised with acetone for two seconds and rinsed immediately in water. The smear was counter stained with neutral red for a minute, then rinsed in water and blot dry. Finally, the smear was examined microscopically using

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Oil immersion objective (X100) after adding a drop of immersion oil. Gram positive organisms appeared purple colour while gram negative rods appeared pinkish [5].

Catalase Test

Procedure:

2ml of hydrogen peroxide was placed in a test tube. Some colonies of the organism were picked with the aid of a sterile wire loop and immersed into the hydrogen peroxide. Immediate gas bubbling indicates a positive test [6].

Coagulase Test

Procedure:

Two drops of saline were placed on a slide. Two colonies of the organism was emulsified in each of the drop of saline to make a thick suspension. A straight wire loop was dipped into the undiluted plasma and mixed into one of the bacteria suspensions and no plasma was added to other suspension as it serves as a negative control. Clumping of the mixture was observed immediately within 10 seconds. *Staphylococcus aureus* are positive for this test [5].

Oxidase Test

Procedure

A piece of filter paper was soaked with few drops of oxidase reagent (1% Tetramethyl paraphenylene diamine dihydrochloride). A colony of test organism was removed and smeared on the filter paper, the appearance of a blue purple colour within 10 seconds indicated a positive test and the absence of color was recorded as a negative test [5].

Indole Test

This test was carried out to distinguish among members of the Gram negative bacilli, i.e. the Enterobactericeae eg. *Escherichia coli*, Klebsiella, Salmonella, Shigella.

Procedure

The test organisms were grown in peptone water. The culture was shaken with equal volume of xylene and ethyl ether. 1ml of Ehrlich's reagent was added and checked for a color change. A Red rose between the layer of ether and the peptone water was observed. The presence of this indicates a positive indole production while the absence of this indicates a negative reaction [6].

Citrate Utilization Test

Procedure

Colonies on the plate were collected with the aid of sterile wire and inoculated into the Koser's citrate medium. A positive result was indicated by turbidity in the Koser's medium. *Klebsiella pneumonia* are positive for this test while *Escherichia coli* show negative result.

Motility Test:

Procedure

A small drop of suspension was placed on a slide and sealed with nail varnish or molten petroleum jelly to prevent it drying out and then covered with a cover glass. The preparation was examined microscopically for motile organisms, using the X10 and X40 objective.

Identification of Fungi

The growth of fungi on sabouraud dextrose agar was examined thoroughly after 1 week based on macroscopic and microscopic examination of cultures.

Macroscopic Examination: Macroscopic growth pattern of the fungi, their colour and opacity on plates were observed.

Microscopic Examination: (Lactophenol blue test)

A drop of sterile water was dropped on the clean grease free slide. Suspected fungal isolated colonies was picked with a sterilized wire loop and teased on the slide. A drop of Lactophenol blue reagent was then added and mixed to get a homogenous mixture. The mixture was covered with a cover slip. It was focused with x10 magnification of the objective lens and properly viewed with x40 of the microscopic objective lens.

Identification of Parasites

The swab stick used to collect the sample was dipped in a sterile saline, to wash. Two drops of the washings were placed on a clean grease free slide. Under the microscope, a positive result involved the presence of at least one adult, larva, protonymph, or nymph stage of D. folliculorum or D. brevis. The process of Demodex examination was performed under sterile conditions.

Statistical Analysis

The data were analyzed using mean as a statistical tool.

RESULTS

A total of 51 eyelash extension samples was collected. 30 samples for control was also collected from female students without eyelash extension. There were no fungi and parasites seen in both the test and control samples. Bacteria

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was detected in all the test samples; 51(100.00%) and the control samples too; 30(100.00%). More than one kind of bacteria was seen in all the test samples; 51(100.00%) resulting in mixed infections. While for the control samples, more than one kind of bacteria was seen in 24 samples (80.00%).

TABLE 1: NUMBER OF SAMPLES (TEST AND CONTROL) WITH MICROBIAL AND MIXED MICROBIAL PRESENCE

Microbes	**Samples with Microbial presence(%)**		**Mixed Microbial presence(%)**	
	Test	**Control**	**Test**	**Control**
Fungi	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Parasites	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Bacteria	51(100.00)	30(100.00)	51(100.00)	24(80.00)

Staphylococcus epidermidis, *Escherichia coli* and *Serratia marcescens* were seen in all the test samples; 51(100.00%) while *Bacillus spp.* in only 38 test samples; (74.51%). *Bacillus spp.* had the highest mean bacteria count in test; 9.3×10^5 cfu/ml followed by *Staphylococcus epidermidis*; 8.35×10^5 cfu/ml, followed by *Escherichia coli;* 5.7×10^5 cfu/ml, then *Serratia marcescens*; 5.0×10^5 cfu/ml. Only *Staphylococcus epidermidis* and *Bacillus spp.* was seen in the control samples. *Staphylococcus epidermidis* was seen in all the control samples; 30(100.00%) while *Bacillus spp.* was seen in 24 samples; (80.00%). For the control, *Staphylococcus epidermidis* had the highest mean bacteria count; 5.7×10^5 cfu/ml followed by *Bacillus spp.;* 2.1×10^5 cfu/ml.

TABLE 2: MEAN BACTERIAL COUNT OF THE BACTERIAL ISOLATES

Bacterial isolates	**Frequency(%)**		**Mean bacterial count** (cfu/ml)	
	Test	**Control**	**Test**	**Control**
Staphylococcus epidermidis	51(100.00)	30(100.00)	8.35×10^5	5.7×10^5
Bacillus spp.	38(74.51)	24(80.00)	9.3×10^5	2.1×10^5
Escherichia coli	51(100.00)	0(0.00)	5.7×10^5	0.00
Serratia marcescens	51(100.00)	0(0.00)	5.0×10^5	0.00

More than one type of bacteria was seen in all 51 test samples. *Staphylococcus epidermidis*, *Serratia marcescens*, *Bacillus spp. and Escherichia coli* were all seen in 38 samples (74.51%). Only *Bacillus spp.* out of these four bacteria was not seen in the remaining 13 samples (25.49%).

TABLE 3: FREQUENCY AND PERCENTAGE OF THE MIXED BACTERIAL INFECTIONS

Mixed bacterial infections	**Frequency**	**Percentage (%)**
Staphylococcus epidermidis+Serratia marcescens+Escherichia coli	**13**	**25.49**
Staphylococcus epidermidiss+Bacillus spp.+Serratia marcescens+Escherichia coli	**38**	**74.51**

Some of the samples were collected from participants who had used their eyelash extension less than 1 week before the experiment; 23(45.10%). Others were between 1-2 weeks old; 16(31.37%) while the rest were above 2 weeks old; 12(23.53%). The mean bacterial count for each of the individual bacterial isolates increased as the duration of eyelash application increased.

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TABLE 4: BACTERIA PRESENCE ACCORDING TO DURATION OF EYELASH EXTENSION APPLICATION

Duration of Eyelash extension application	Frequency(%)	Bacterial isolates	Mean Bacterial count (cfu/ml)
Less than 1 week	23(45.10)	*Staphylococcus epidermidis* *Serratia marcescens* *Bacillus spp.* *Escherichia coli*	7.8×10^5 4.1×10^6 2.4×10^5 4.8×10^5
1-2 weeks	16(31.37)	*Staphylococcus epidermidis* *Serratia marcescens* *Bacillus spp.* *Escherichia coli*	8.7×10^5 5.25×10^6 4.9×10^5 5.05×10^5
Above 2 weeks	12(23.53)	*Staphylococcus epidermidis* *Serratia marcescens* *Bacillus spp.* *Escherichia coli*	8.9×10^5 5.4×10^6 5.0×10^5 8.5×10^5

Three out of the four isolates are rod-shaped and the other is Cocci. The first isolate is a Gram –ve motile Rod shaped organism, which is Catalase +ve, Indole –ve, Oxidase–ve, Citrate +ve thus confirming the isolate to be *Serratia marcescens.* The second isolate is a Gram +ve, motile Rod organism, which is Catalase +ve, Indole -ve, oxidase -ve, Citrate +ve, Coagulase -ve, thus confirming the isolate to be Bacillus spp. The third isolate is a Gram -ve motile Rod shaped organism, which is Catalase +ve, Indole +ve, Oxidase -ve, Citrate –ve, thus confirming the isolate to be *Escherichia coli.* The fourth isolate is a Gram +ve non-motile Cocci shaped organism, which is Catalase +ve, Coagulase -ve, Oxidase –ve, Citrate +ve, Indole –ve thus confirming the isolate to be *Staphylococcus epidermidis.* The table is shown in Appendix VI. There were 150 respondents. Most of the participants were students from Faculty of Social Sciences 63(42.0%) and Humanities 61(24.0%). Most of the respondents, 93(62.0%) had used the eyelash extensions for less than 1 week before the experiment was conducted. Others had used theirs between 1-2 weeks and above 2 weeks before the experiment was conducted. Beauty was the most common reason for using the eyelash extensions; 135(90.0%). followed by curiosity; 8(5.30%) and peer pressure; 7(4.70%). Of the 150 respondents who use eyelashes, the most common complication was loss of natural lashes; 105(70.0%). Other complications were itching; 78(50.0%), frequent blinking of the eye; 68(45.33), Tearing; 30(20.00%), redness; 12(8.0%). Others were; Pain, casting of shadow in the field of vision, boil on the eyelid [7, 8, 9].

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TABLE 6: ANALYSIS OF QUESTIONNAIRE

Variables	Frequency	Percentage (%)
Age of eyelash application		
Less than 1 week	93	62.00
1-2 weeks	36	24.00
Above 2 weeks	21	14.00
Knowledge of predisposition to infection		
Yes	3	2.00
No	147	98.00
Reasons for usage		
Beauty	135	90.00
Curiosity	8	5.30
Peer pressure	7	4.70
Complications		
Loss of natural lashes	101	67.33
Itching	78	52.00
Frequent blinking	68	45.33
Tearing	30	20.00
Redness	12	8.00
Pain	10	6.67
Hygienic practices (Wash)		
Yes	5	3.33
No	145	96.67

DISCUSSION

The bacterial isolates from the eyelash extension samples identified were *Staphylococcus epidermidis* (100.00%), *Serratia marcescens* (100.00%), *Bacillus spp.* (78.51%), *Escherichia coli* (100.00%). The bacteria isolates from the control identified were Staphylococcus epidermidis (100.00%) and Bacillus spp. (80.00%). The bacteria counts for the eyelash extension samples (test) were significantly higher than that of the control (natural eyelash samples). The eyelash extension samples showed a wide variation in total viable count of bacteria ranging from 5.0×10^{5} to 9.3×10^{5} cfu\ml while that of control was from 2.1×10^{5} to 5.7×10^{5}. This shows that the presence of the eyelash extensions increased the colony count of these bacteria. There were no fungi and parasites detected in both the test and control samples. Coagulase positive and negative staphylococcus are the major cause of Bacterial Blepharitis, Conjuctivitis and Stye.

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The presence of Coagulase negative Staphylococcus in this study is indicative of the risk of being infected with Bacteria Blepharitis, conjuctivities or stye. Bacterial blepharitis, also known as chronic anterior blepharitis, or staphylococcal blepharitis or ulcerative blepharitis, is a chronic infection of the anterior part of the lid margin. It is a common cause of ocular discomfort and irritation. This can also be transferred from the hand, trapped from the environment or through contaminated instruments used during the fixing process. Stye is an acute suppurative inflammation of one of the Zeis's glands [5]. After the procedure, it is difficult to thoroughly wash the face, let alone the lids and lashes. This reduction in hygienic condition leads to microbial pathogens and dirt entering the eye. The bacterial load for lashes above 2 weeks was very high compared to those between 1-2 weeks followed by those less than 1 week. This confirms that the eyelash extensions trap a lot of dirt and microorganisms. It was obvious from the questionnaire that patronizers of eyelash extensions do not know about the risk of being exposed to eye infections through the process, and thus very dangerous to the population [6].

CONCLUSION

Eyelash extensions can seriously predispose patronizing females to eye microbial infections. The eye is a very important but delicate organ of the body; therefore, it needs to be well protected to maintain one's sight. Eyelash extension is inimical to the eyelid and could be detrimental to vision and therefore the obsession on eyelash extension should be curbed.

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