Journal Full Title: Journal of Biomedical Research & Environmental Sciences
Journal Website Link: https://www.jelsciences.com
Journal ISSN: 2766-2276
Category: Multidisciplinary
Subject Areas: Medicine Group, Biology Group, General, Environmental Sciences
Topics Summation: 133
Issue Regularity: Monthly
Review Process: Double Blind
Time to Publication: 21 Days
Indexing catalog: IndexCopernicus ICV 2020: 53.77 | GoogleScholar | View more
Publication fee catalog: Visit here

DOI: 10.37871 (CrossRef)
Plagiarism detection software: iThenticate
Managing entity: USA
Language: English
Research work collecting capability: Worldwide
Organized by: SciRes Literature LLC
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Assessment of the Microorganisms Isolated from Artificial Eyelash Users

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DOI: 10.37871/jbres1818
Submitted: 01 October 2023
Accepted: 19 October 2023
Published: 23 October 2023
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Keywords
- Eye
- Lashes
- Eyelashes
- Students
- Artificial

Abstract
Eyelashes are shed, like other types of hair, from the follicle. Each eyelash has its own growth cycle (anaphase) that lasts six to eight weeks so that most eyelashes are present to maintain their collective protective mechanisms. This project work is to identify the microorganisms isolated from eye lashes users of students in Ekpoma. Eyelashes of the students were swabbed with a sterile swab stick that was soaked with normal saline. The swab stick was immediately replaced in its casing and labeled appropriately. Each specimen was refrigerated at 4°C as soon as it was collected. The bacteria isolates are Staphylococcus aureus and Klebsiella spp while the fungi isolates were Rhizopus spp and Candida albicans. The percentage prevalence of organisms isolated from the study. Staphylococcus aureus 25 (36.2%), Klebsiella spp 14 (20.3%), Rhizopus spp 11 (15.98%) and Candida albicans 19 (27.8%). The Staph aureus in the study was sensitive to Erythromycin, Gentamycin, Ciprofloxacin and Norfloxacin while the other antibiotics were resistant. Eyelash fixing can seriously predispose patronizing females to eye injury. 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 fixing is inimical to the eyelid and could be detrimental to vision and therefore the obsession on eyelash extension should be curbed.

Introduction
The eyelids are modified folds of skin closing the front of the orbit which act to protect the anterior surface of the globe from local injury [1]. Additionally, they aid in regulation of light reaching the eye; in tear film maintenance, by dis–tributing the protective and optically important tear film over the cornea during blinking and in tear flow, by their pumping action on the conjunctival sac and lacrimal sac [2]. Eye–lashes, which are found on the margins of eyelids, serve to protect the eye from foreign objects such as sand and dust among others [3]. Eyelashes are shed, like other types of hair, from the follicle. Each eyelash has its own growth cycle (anaphase) that lasts six to eight weeks so that most eyelashes are present to maintain their collective protective mechanisms. Eyelash extension has become the latest cosmetic trend all over the world. Long eyelashes are considered a sign of femininity in many cultures, as a result some women...
seek to enhance their eyelash length artificially, using eyelash extensions [4]. False eyelashes can be used for individuals who have thin or short eyelashes along with mascara to thicken the look of eyelashes which creates a bold look [5]. According to Radeva [6], an Arizona based beautician, eyelash extension is a revolutionary way to extend the length and thickness of your eyelashes. The false eyelashes used in the extension are synthetic and tapered from thick to thin to resemble a real eyelash. It is applied on a hair-by-hair basis to one’s lashes for a complete natural look.

To the beautician, the procedure may seem simple. However, from the health care point of view, it is still important to pay close attention to its effect on vision and how safe the procedure is as the intermittent fixing of artificial lashes may interfere with the normal periodic shedding and growth of new lashes and cause some discomforts. Eyelash extension could irritate the eyelid or clog follicles in the eyelid as well as pull out eyelashes during removal [7]. The procedure does not allow frequent washing of the eyelids which could result in bacterial and/or fungal infection seen as ocular discharge with conjunctivitis and eyelid diseases [8]. The eyelashes may also be displaced when wet with water or sweat and serve as foreign body on the eye. The aim of the study therefore was to establish the comfort and safety or otherwise of eyelash extension. This will involve ascertaining the frequency of the procedure, identifying reasons why some female extends their eyelashes, finding the class of females involved in the procedure, and identifying some problems they face as a result eyelash extension [9]. This project work is to identify the microorganisms isolated from eye lashes users in among students who use artificial eyelashes.

Materials and Methods

Study area

This study was carried out in Ekpoma, Esan West Local Government Area of Edo State. Edo state lies between longitude 06° 04’E and 06°43’E and latitude 05°44’N and 07°34’N with a land mass of 17,450 sq.km located in the South geopolitical zone of Nigeria with a population of 3.1 million people. Ekpoma is a semi-urban town with the major occupation of farming, trading, civil servants and students.

Study population

The study population comprises of apparently healthy female students of visiting St. Kenny Research Consult, Ekpoma, Edo State that use artificial eyelashes and those that don’t use. The investigation was carried out on girls that use eye lashes and swab samples were obtained from forty (40) apparently healthy female students with the use of swab sticks. Microbiological investigations were carried out on the samples obtained in the Microbiology Laboratory of St. Kenny Research Consult, Ekpoma, Edo State.

Ethical approval

Ethical approval was obtained from the Health and Ethics committee of Esan West Local Government Area, Ekpoma, Edo State; and informed consent was also sought from the subjects prior to sample collection.

Sample collection

Eyelashes of the students was swabbed with a sterile swab stick that was soaked with normal saline. The swab stick was immediately replaced in its casing and labeled appropriately. Each specimen was refrigerated at 4°C as soon as it was collected.

Sample analysis/methods

Inoculation, isolation, characterization and identification: A microscopy examination was first carried out on the swab stick specimen by adding normal saline into the swab sticks and placing a drop on a glass slide for viewing under the microscope in order to check for pus cells, epithelia cells, yeast cells etc. Another few drops of the sample was placed in the nutrient broth and incubated at 37°C for 24hours. A sterile wire loop was used to inoculate from the nutrient broth on nutrient and blood agar plates. The plates were incubated at 37°C for 24hours and thereafter observed for obvious microbial growth (colonies) on the surface of the culture plate. Subsequent subculturing in selected media was carried out to further purify the isolates. Cultures were Gram-stained and morphologies of the organisms observed under the microscope. Biochemical tests were carried out on the bacterial isolates.

Gram staining: The faecal sample was carefully placed on a sterile, grease-free microscope slide and allowed to air–dry. It was fixed by passing it over the pilot flame of the Bunsen burner three times. The fixed smear was then flooded with Crystal violet for 30 seconds before washing off with tap water. Lugol’s iodine was then added and washed up after about 30 seconds and subsequently decolourised rapidly using acetone and washed off immediately. Neutral red
(Counter stain) was then added and washed off after about 60 seconds. The slides were then placed in a draining rack for the smear to air dry. After drying, a drop of immersion oil was applied on the smear and viewed microscopically using oil immersion objectives.

Biochemical screening tests: Identification of bacterial isolates involves the use of biochemical screening media are usually used. One hundred (100) bacterial isolates (100) were subjected to various biochemical tests; Motility, Indole, Urease and Citrate utilization tests.

Identification of fungi

Identification of *candida albicans*: For the identification of Candida germ tube test was carried out.

**Procedure:**
- A very small inoculum of yeast cell from an isolate was suspended in 0.5ml of human plasma in the test tube and incubated at 35°C for not longer than 3 hours.
- The suspension was removed after incubation period and a drop of the suspension was placed on glass microscope glass slide.
- It was examined under lower power magnification for the presence of pseudohyphae showing production of germ tube.

Identification of *Rhizopus* spp: Identification of *Rhizopus* was also carried out using Lactose phenol cotton blue reagent.

**Procedure:** A drop of lactose phenol cotton blue was placed on a clean grease free slide. With a sterile straight sharp needle, a small portion of the colony was picked and placed on the glass slide in which a drop of lactose phenol cotton blue has been added. It was properly teased and cover with clean glass cover-slip. The preparation was examined microscopically using low magnification.

Antibiotic susceptibility test: Susceptibility test was determined using antibiotic disc after due sub-culturing. Briefly, the isolates, adjusted to McFarland standard, were inoculated in Muller Hilton agar plates, streaked evenly using cotton swabs and allowed to set at room temperature. Antibiotic discs were placed on the set agar plates, allowed to equilibrate at room temperature for 15 minutes and finally incubated at 37°C for 24h. Thereafter, the plates were observed for obvious zone of clearing, and recorded as either susceptible or resistant, as described by Clinical and Laboratory Standards Institutes. The antibiotics tested were Amoxycillin, AMX 20μg, Augmentin (Amoxycillin/clevulanic acid), AUG 20/10μg, AmpicillinCloxacillin, AMC 10/10μg, Ceftriaxone, CRO 30μg, Gentamycin, GEN 10μg, Ciprofloxacin, CIP 5μg, Perflaxcin, PEF, Ofloxacin, OFL 5μg, Erythromycin, ERY 15μg, Trimethoprim/ Sulphamethazole, COT 1.25/23.75μg, Chloramphenicol, CHL 30μg, Cefuroxime, CXM 30 μg, Streptomycin, STR 10μg, Sparfloxacin, SPA 5μg.

**Result**

The bacteria isolates are *Staphylococcus aureus* and *Klebsiella* spp while the fungi isolates were *Rhizopus* spp and *Candida albicans* (Table 1). *Staphylococcus aureus* 25 (36.2%), *Klebsiella* spp 14 (20.3%), *Rhizopus* spp 11 (15.9%) and *Candida albicans* 19 (27.8%) (Table 2). From the eye lashes users, 33 organisms were isolated while 36 were isolated from non-eye lashes users. From the eye lashes users, *Staphylococcus aureus* 15 (45.5%), *Klebsiella* spp 7 (21.2%), *Candida albican* 7 (21.2%) and *Rhizopus* spp 4 (12.1%) while from the non-eye lashes users, *Staphylococcus aureus* 10 (27.8%), *Klebsiella* spp 7 (19.4%), *Candida albican* 12 (33.4%) and *Rhizopus* spp 7 (19.4%) (Table 3). Table 4 shows the distribution of antibiotic susceptibility pattern of isolates in the study. The *Staph aureus* in the study was sensitive to Erythromycin, Gentamycin, Ciprofloxacine and Norfloxacine while the other antibiotics were resistant. *Klebsiella* spp was sensitive to Augmentin, Tarivid, Septrim and Ampicilline while the other antibiotics were resistant.

**Discussion**

Eyelash fixing involves a number of enhancements designed to add length, thickness and fullness to natural eyelashes [10]. Eyelash fixing is gradually becoming common practice in Nigerian cities. Findings from this study showed that the following

<table>
<thead>
<tr>
<th>Organisms Isolated from Eyelashes users</th>
<th>Organisms Isolated from Non-Eyelashes users</th>
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<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td><em>Klebsiella</em></td>
<td><em>Klebsiella</em></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td><em>Candida albicans</em></td>
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**Table 1:** Organisms isolated in the study.
bacteria were isolated from the study; Staphylococcus aureus and Klebsiella spp while the fungi isolates are Rhizopus spp and Candida albicans. Although these females fix their eye-lashes to appear more attractive and to keep abreast with post-modernism. All the samples showed growth of Staphylococcus aureus which is a normal flora of the skin.

The organisms isolated from the study. The bacteria isolates are Staphylococcus aureus and Klebsiella spp while the fungi isolates were Rhizopus spp and Candida albicans. The percentage prevalence of organisms isolated from the study. Staphylococcus aureus 25 (36.2%), Klebsiella spp 14 (20.3%), Rhizopus spp 11 (15.9%) and Candida albicans 19 (27.8%).

Detrimental effects of this procedure on vision therefore could be a risk factor in socioeconomic development [11].

The percentage (%) prevalence of organisms isolated from students with and without eye lashes. From the eye lashes users, 33 organisms were isolated while 36 were isolated from non-eye lashes users. From the eye lashes users, Staphylococcus aureus (45.5%), Klebsiella spp (21.2%), Candida albicans (21.2%) and Rhizopus spp (12.1%) while from the non-eye lashes users, Staphylococcus aureus 10 (27.8%), Klebsiella spp 19.4%, Candida albican 33.4% and Rhizopus spp (19.4%). Visual disruptions can and often do interfere with reading and learning.

The Staph aureus in the study was sensitive to Erythromycin, Gentamycin, Ciprofloxacin and Norfloxacin while the other antibiotics were resistant. Klebsiella spp was sensitive to Augmentin, Tarivid, Septrim and Ampicillin while the other antibiotics were resistant (Table 4). The good news, however, is that findings of the negative implications of eyelash extension could be communicated easily to the literates with better understanding.

Almost all the participants that had Klebsiella spp, Rhizopus spp and Candida spp had various problems such as dry eyes, itchy eyelids, tearing, and burning sensation. Research has shown that dry eyes result when there is lack of sufficient lubrication and moisture in the eye [12]. Persistent dryness results in itching, burning sensation, as well as foreign body sensation and tearing [13]. This could result in dry eyes and its resultant tearing and burning sensation [14]. Occasionally, after eye lash fixing, there could be incomplete closure of the eyelids (lagophthalmos) during sleep exposing the corneal surface to air, dust and microbes among other. This may promote dry eyes syndrome and ocular bacterial or fungal infection [15-17].

Some individuals are allergic to the false eyelashes and materials (e.g. glue) used to attach the eyelashes [17,18]. The glue also causes burning sensation [5]. A participant said “when the glue melted and mixed with tears, it caused burning sensation in my eyes”. Another said “if they change the glue, we can continue doing it”, and then one also said “research should be conducted on the composition of the glue to see if it can be modified in order to minimize, if not eliminate totally, the burning sensation it causes” and also if it can lead to microbial infection of the eyes. Glue fumes could account for the watery...
eye and burning sensation. Salons should be using medical or pharmaceutical grade glue which is free of formaldehyde. Non-medical grade glue and glue with formaldehyde can irritate the eyes [19]. An experienced and highly regarded professional beautician should perform a patch test in order to determine whether or not an individual is allergic to the materials used [18]. According to the participants, most beauticians have not received formal training and therefore are not experts in eyelash extension fixation and for that matter, could end up getting a lot of glue on the eye which can block the puncta if allowed to get to the lower lids and also infections can be transmitted. All these contribute to eye irritation and watering [19].

Wearing false eyelashes to bed or for more than one day can cause bacteria to collect under the eyelash glue and on the false eyelash, causing eye infections with infections like bacteria like Staphylococcus aureus and Klebsiella spp and fungi isolates like Rhizopus spp and Candida albicans [5]. After the procedure, one cannot wash thoroughly her face, let alone the lids and lashes. This reduction in hygienic condition may lead to microbial infection and dirt entering the eye. Other problems encountered as a result of wearing eyelash are misdirection of lashes and lashes falling on their eyes of clients. Some students complained that, the artificial lashes fall on their eyes during the procedure when they or the beautician fidget. Some others also admitted that, the artificial lashes were not properly fixed (misdirection) and this made some fall on their eyes. One perceived danger is that, the artificial lashes, especially, if the base is calcified with the glue, may scratch the cornea and cause pain as well. The extended eyelashes cast a shadow on vision as some of the lashes were too many and/or too long which might affect the quality of vision.

Another area of concern is the difficulty encountered removing the eyelash extensions. Some individuals experienced pain during the removal and swelling on the upper lid after the removal. Repeatedly pulling off extensions, injure the eyelash hair follicles. The extra weight added to the lashes increasing follicle tension and solvents used to dissolve the sealant glue are potentially harmful to the follicles and irritating to the eyelids [9]. This could explain why those individuals experienced pain during the removal of the synthetic eyelashes and post-removal upper lid swelling which could have resulted in contamination of infections.

Sometimes, the lashes get stuck so hard to the lids or the natural lashes that the more they tried to pull it, the more the pain (which eventually leads to some eye-lid disorders) and the natural lashes being pulled off [18]. According to Watson & Lowe [20], the eyelashes grow and are renewed two or three times a year. This natural phenomenon, if interfered with frequent fixing of false lashes, may pose a risk of eyelash loss or "eyelash baldness" [9,17].

One would have thought that, all these problems encountered, would reduce individual interest in fixing eyelash but most students said they would not stop fixing eyelashes. Thus, so far as fixing eyelash enhances one’s appearance irrespective of the aforementioned problems, they may want to have it done again. For those that donot fix and extend their lashes linked their reason to the associated problems. Some others gave reasons such as financial constraints and peer pressure from friends to discontinue this fashion. This therefore indicates that even though there were attending problems associated with the procedure, people do not realize the imminent potentially associated hazards the procedure has on the eye and on vision. Females who patronize this procedure need to be educated on how with eyelash extension one’s vision could be affected so as to limit the frequency of involvement with the procedure.

**Conclusion**

Eyelash fixing can seriously predispose patronizing females to eye injury. 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 fixing is inimical to the eyelid and could be detrimental to vision and therefore the obsession on eyelash extension should be curbed.

i. A national survey on eyelash fixing and its effect should be conducted mostly among students. If possible, baseline vision of prospective participants could be compared with vision post eyelash extension to ascertain its effect on vision in general.

ii. The Nigeria Health Service and the Ministry of Health should be alerted on the upsurge of eyelash extension and its inimical effect on the eye and vision so that these organizations can educate eye care practitioners and the general public.

**Acknowledgement**

The authors would like to thank all the Laboratory
Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors’ contributions

The entire study procedure was conducted with the involvement of all writers.

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