International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

# Isolation and Identification of Some Microorganisms that Cause Abnormal Hair Loss (Baldness)

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**Abstract:** ***Abnormal hair loss means the presence of an excessive hair shedding or losing batches of the hair and not like the case of the most common type of hair loss, the hair will not grow back .It is a phenomenon with a great psychological impact especially in youth. Theaim of this study is to determine the possible microbial causal agents among other factor, that can be responsible for hairloss (baldness) among students and young workers. The study was carried out among a sample of students and young workers at the University of Gezira –Sudan, during November to December 2015 in order to isolate and identify the different causal agents that can be possibly involved in causing balding together with a questionnaires to collected relevant data that can help understanding the role of these causal agents in the initiation or activation of baldness.30 samples were collected by the use of sterile small forceps from the bald area ,the sample containing hair follicle was cultured in a nourishing media and after germination , it was subjected to three tests including: purification of the isolates, grams staining and different biochemical tests(Catalase test- Oxidase test- Urease test-Indole test –Motility test and Citrate test), at the Medical laboratory, University of Gezira. The assessment of the scalp samples revealed the presence of a number of bacteria in hair, including: Bacillus 12(40%), Staphylococcus 1(3.3%), Enterobacter 4 (13.3%), and Pseudomonas 2 (6.7%), with mixture of Staphylococcus and Pseudomonas 1 (3.3%), mixture of Bacillus andEnterobacter 5 (16.7%), mixture of Bacillus and Pseudomonas 2 (6.7%), and mixture of Pseudomonas, Staphylococcus, and Enterobacter 3 (10.0%). The questionnaires survey revealed a significant association between hair loss and Age (p = 0.003), Gender (p = 0.000), Location of living (p = 0.000), History of hair loss (p = 0.000), hair cutter sterilization (p = 0.004) and use of shampoos (p = 0.000). Further studies are required to focus on the other factors associated with baldness.***

**Keywords:** Baldness-Alopecia- Microorganisms-Youth-Sudan

## 1. Introduction

Hair loss (alopecia) affects men and women of all ages and often significantly affects social and psychologic well-being (Karyn*et al*., 2003). Hair loss is a common problem that affects up to 50% of men and women throughout their lives. Male and female pattern hair loss affects 50% of men by 50 years of age and nearly 50% of women (Rogers *et al*., 2008). It can occur any where on the body, but more commonly affects just the scalp when the patient presents with concerns about the cosmetic effect. Physicians need to be able to distinguish hair loss that represents true disease from the more common age-related hair loss. Hair loss is commonly categorized into scarring and nonscarring alopecia. Scarring alopecia is rare and most cases of hair loss seen in primary care will be nonscarring. Hair loss on the scalp can be further classified as focal or diffuse. This distinction is the first step in diagnosis (Annel ,2009).

Male pattern baldness or androgenetic alopecia in men is very common. It accounts for more than 90% of all cases of alopecia of the scalp in men (Pragst andBalikova , 2006). It has the highest prevalence (45%) in the Caucasian race and the lowest prevalence (15%) in the Mongoloid race. lts prevalence in pre- and post-menopausal women is about 13% and 37% respectively (Adrianus , 1992).

Hair loss results from numerous factors such as aging, genetic pre-disposition, thyroid imbalance, eating disorders, illness, hormonal effects of birth control pills, pregnancy, or menopause and certain medications. The most common cause of hair loss is a hereditary condition known as androgenetic alopecia (AGA). Male pattern hair loss tends to run infamilies (CAPS, 2011).

Genetic or hereditary hair loss does not discriminate between sexes or races. Approximately 40 million men or 2 out of 3 men in the United States have significant hair loss. About 25% have some form of balding by age 30, and 65% begin to bald by age 60. In women, the number affected by pattern-type hair loss is slightly less: about 30 million or 1 in 4. Thinning hair can occur anytime between ages 25 to 45, but most commonly hair loss presents after age 40. Hair loss occurs in about 25% of premenopausal women and in 38% of post-menopausal women .

Diagnosis of hair loss is based on detailed clinical history, physical exam, clinical diagnostic tests, laboratory testing, and scalp biopsy, which may be necessary to confirm some diagnoses (Thiago*et al* , 2013).

Humans have been identified as the primary source of microbial contaminants within industrial clean rooms and hospital operating rooms. The majority of airborne contaminants containing bacteria has been associated with the hair, skin, and respiratory tracts of humans. Microbial contaminants evaluated in hospital operating rooms have been associated largely with humans, rather than dust and

soil particles. Human hairs may function as an air-collecting agent for micro-contaminants, because the hairs are constantly exposed to air and can readily adsorb a variety of airborne particles via electrostatic attraction, grooved surfaces, thin and long structures, and biochemical affinity (Aram *et al*., 2010).

Some of these bacteria complete their life cycle on the skin and are considered to be residents whereas others, (visitors) spend only a limited time there. The resident flora mostly consists of gram-positive rods including coagulase-negative *staphylococci*, *micrococci* and *peptococcus*, as well as *corynebacterium*, *brevibacterium*and *propionibacterium*. Any organism that inhabits non-cutaneous body sites or is found in the environment can transiently live on the skin. The most important organism in this group are the group A streptococci, which rapidly die when placed on normal skin (Vorgelegt , 2008).

**Rationales**

Throughout history, poems and folktales have acknowledged long, lushes, golden hair as a mark of beauty. The loss of hair has been reported since biblical times, resulting in the diminishment of male power. Although hair insulates the head under extremely hot conditions, as well as preventing heat loss under colder conditions, realistically it serves little aside from cosmetic purposes. Nevertheless, the partial or complete loss of one's hair can trigger profound psychological distress. This may reflect a number of beliefs, such as, that hair symbolises beauty, individuality and/or belonging to a particular social or cultural group (Thiago ,2013).

**Objectives**

The aim of this study is to determine the possible microbial causal agent(s) ,that can be associated for hairloss (baldness) among students and young workers at University of Gezira .

## 2. Materials and Method

**Sampling methodology:**

**Study area:**

The study was conducted in El nashishiba companion, University of Gezira, Wad Madani, Sudan. The companion containing four collage including: Engineering, Economic, Agriculture Sciences and Textile. The experimental work was carried out at the Medical Laboratory ,University of Gezira to identify and isolate the target organisms .

**Study population:**

The study subjects included (26) male and (4) female students and young workers (age ranged between 20 -40 year) with sings of hair loss or baldness. The student or workers undergoing treatment at the time of the study were excluded.

**Study design:**

A cross–sectional study was carried out to isolate and identify different type of bacterial genera that present in hair follicle and may cause hair loss ,during the period from November to December 2015 at University of Gezira , Sudan. The sampling method was random sampling method The sample size was 30 students and workers.

**Sample collection:**

The samples were taken using sterile small forceps from baldness area after sterilize area by using alcohol (ethanol 70%) . only the sample contain follicle was taken , sterilized the hair sample by alcohol for 30 second , then delivered to distilled water (30 second) to remove the alcohol and drying the sample in filter paper to be analyzed .

**Sample analysis:**

The collected samples to isolate and identify deferent types of bacterial genera that present in hair follicle and may cause hair loss were shipped to the Medical Laboratory, University of Gezira. The diagnosis was performed using three tests including: Purification of the isolates, grams staining technique and biochemical test.

**Purification of the isolates:**

The samples were cultured in a nutrient agar plate , then each plate was incubated for 24 hours at 37˚ C. After incubation, only the colonies that were grown around the hair follicle, were subjected to microscopical examination by using classical grams staining technique followed by the biochemical tests to identify these isolates.

**Grams staining technique:**

Using a culture loop a smear was prepared and lifted to dry. The air dried smear was then fixed by heat and covered with crystals stain for 30-60 seconds; the stain was rapidly off with clean water .then covered with lugol's iodine for 30-60 seconds. The iodine solution was washed out by clean water then the smear was decolorized rapidly with safranine for two minutes. The stain was washed off with clean water and the back of the slide was wiped clean, after that it was examined microscopically, gram positive show a purple color and gram negative show red color (Cheesebrough , 2000).

**Biochemical tests:**

**The test was done according to Cheesebrough ,( 2000).**

**Catalase test:**

The procedure has been demonstrated by having 3 ml of hydrogen peroxide poured into the tube, and few colonies of cultured organisms were immersed by a glass rod. Positive control test using cultured staphylococci species and negative control test were performed. The result is evident by the production of active bubbles.

**Motility test:**

The motility test was demonstrated by melted3.86 grams of the nutrient agar media, and then suspended in 168 ml of the distilled water and heated to boiling. They were then poured into the container and sterilized in the autoclave at 121$^{o}$ C for 15 minutes. Media were then poured into the test tubes. The test organism was inoculated with the sterile straight wire in the tubes container.

**Urease test:**

The tested organism was cultured in a medium which contains urea and the indicator phenol red, then inoculated

heavily the test organism in a bottle containing 3 ml sterile urea broth and incubated at 35-37 C to 12 hour. The color of the medium was changed to pink which mean that the strain was urease producing, that breakdown the urea to give ammonia and carbon dioxide, with the release of ammonia the medium became alkaline.

**Citrate tast:**
It based on the ability of an organism to used citrate as it's only as source of carbon. Slope of the medium was prepared in bottle using sterile straight wire. the slope was streaked with saline suspension of the test organism and then stabbed the butt , then incubated at 35 C for 48 hour After incubated the color of the medium turned into blue .

**Oxidase test (Cytchrome oxidase test):**
The oxidase test was used to assist in the identification of pseudomonas, Neisseria, Vibrio, Brucella, and Pasteurella species, all of which produce the enzyme cytochrome oxidas. The procedure had been demonstrated by using an oxidase reagent disc had moisted with a drop of sterile water, using a piece of stick or glass rod (not an oxidized wire loop). Removed a colony of the test organism and rubbed it on the strip, and looking for a red-purple color within 20 second. The red-purple color positive oxidase test.

**Indol test:**
To rehydrate the medium, 4.2 grams of the peptone water were dissolved in 168 milliliter of cold distilled water and heated to boiling to dissolve the medium completely. The medium was then sterilized in the autoclave at 15lbs (121$^{o}$ C) pressure for 15 minutes and poured into the tests tubes. Three ml/g of the peptone water in the tubes inoculated by the organism with the sterile straight wire was then incubated at 37$^{o}$c for 48 hours.One ml of kovac's reagent was added to culture for examining indoleproduction. Positive reaction was indicated by the red color in circle**.**

**CLED agar:**
CLED agar (cystine lactose electrolyte deficient medium) is a valuable non-inhibitory growth medium used in the isolation and differentiation of urinary organisms. Being electrolyte deficient, it prevents the swarming of Proteus species. Cystine promotes the formation of cystine-dependent dwarf colonies. Bromothymol blue is the indicator used in the agar, it changes to yellow in case of acid production during fermentation of lactose or changes to deep blue in case of alkalinization.

**Data collection**
The questionnaire was conducted to collected data from students and young workers, Gezira University. The questionnaire included nine closed ended questions. The data included were: gender, age location of living, location of work level, history of family, hair cutter sterilizer, uses of shampoo, signs and symptoms, and previous treatment. The questionnaire for each person was completed by students or workers who interviewed in person.

**Data analysis:**
The data collected were stored in the Microsoft excel spread sheet program, and the statistical analysis was performed using SPSS version 16.0. The data were analyzed using descriptive statistics such as frequency cross tabulation table to determine thedistribution of selected possible risk factors related to hair loss.The associations between the outcome variable and potential risk factors were analyzed using Chi-square test. A risk factor with a P-value $\leq$ 0.25 was considered significant.

## 3. Results

**Results of samples analysis:**
The samples cultured in the nutrient agar plate showed a growth of different colonies with different size, shape, color, Texture, Height, and Edge.

The results of Gram's stain is shown in Table 1. The colonies stained with Gram's stain showed Gram negative and Gram positive bacteria with different color and different shapes under the microscope, including: cocci, and bacilii.

The results of biochemical tests is shown in Table 2. From a total of 44 colonies isolated from scalp of students and workers and applied for biochemical test 21 colonies were revealed the following criteria: motility positive, catalase positive, citirate positive, urease positive and indol positive. the type of bacteria is *Bacillus spp*. Seven of isolated colonies were submitted to biochemical tests they showed the following criteria: catalase positive, oxidase positive, and non lactose fermented in CLED agar(blue colonies) .The type of bacteria is *Pseudomonus* spp. Twelve of colonies isolated showed the following criteria when submitted to biochemical tests: motility positive, catalase positive, oxidase negative, citiratepoitive, indol positive, and lactose fermented in CLED agar (yellow colonies) the type of bacteria is Enterobacter spp. A total of 4 colonies applied for biochemical test they revealed the following criteria:,catalase positive the type of bacteria is staphylococcus spp.

**Table 1:** The results of Gram's stain reaction

| Gram stain reaction | Shape | Number of colonies |
|---|---|---|
| Positive | Cocci | 4 |
| Positive | Bacilli | 21 |
| Negative | Bacilli | 19 |

**Table 2:** Identification of isolated bacteria

| No of sample | Type Of bacteria | Motility | Catalase | Oxidase | Citirate | Urease | Indol | CLED Agar |
|---|---|---|---|---|---|---|---|---|
| 1 | Bacillus | + | + | * | + | + | + | * |
| 2 | Staph | * | + | * | * | * | * | * |
| 3 | Enterobacter | + | + | - | + | * | + | LF |
| 4 | Pseudo | * | + | + | * | * | * | NLF |

Staph=staphylococcus spp , Pseudo= pseudomonas spp , LF= lactose fermented, NLF = non lactose fermented, *= not submitted to test

**The results of the questionnaire:**

The results of the questionnaire are shown in Table 3. This study included students and workers of both sexes with various ages. Twenty six were male and 4 were female. The age divided to four categories, they distributed as fallowing: age ranged from 20 – 25 were 13, age ranged from 26 – 30 were 7, age ranged from 31 – 35 were 4 and age range from 36 – 40 were 6.

The frequency of microorganism according to students and workers gender is shown in Table 4. The result showed 0 *Bacillus* organism (0.0%), 0 *Enterobacter* (0.0%), 1 *staphylococcus* (25.0%), and 1 *pseudomonas* (25.0%), 0 with a mixture of Staphylococcus and Pseudomonas (0.0%), 0 with a mixture of Bacillus and Enterobacter (0.0%), 2 with a mixture of Bacillus and Pseudomonas (50.0%), and 0 with a mixture of Pseudomonas, Staphylococcus, and Enterobacter (0.0%) among females, also the study revealed 12 *Bacillus* organism (46.2%), 4 *Enterobacter* (15.4%), 0 *Staphylococcus* (0.0%), and 1 *Pseudomonas* (3.8%),%), 1 with a mixture of Staphylococcus and Pseudomonas (3.8%), 5 with a mixture of Bacillus and Enterobacter (19.2%), 0 with a mixture of Bacillus and Pseudomonas (0.0%), and 3 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (11.5%) among males.

The frequency of microorganism according to students and workers age is shown in Table 5. The result showed 8, 4, 0, and 0 *Bacillus* organism among age ranged from 20 – 25 (61.5%), 26 – 30 (57.1%), 31- 35 (0.0%), and 36 – 40 (0.0%) respectively. The frequency of *Enterobacter* organism was 4, 0, 0, and 0 among age ranged from 20 – 25 (30.8%), 26 – 30 (0.0%), 31- 35 (0.0%), and 36 – 40 (0.0%) respectively. The frequency of *Staphylococcus* organism was 0, 0, 0, and 1 among age ranged from 20 – 25 (0.0%), 26 – 30 (0.0%), 31- 35 (0.0%), and 36 – 40 (16.7%) respectively. The frequency of *Pseudomonas* organism was 0, 0, 0, 2 among age ranged from 20 – 25 (0.0%), 26 – 30 (0.0%), 31- 35 (0.0%), and 36 – 40 (33.3%) respectively. The frequency of a mixture of Staphylococcus and Pseudomonas was 1, 0, 0, and 0 among age ranged from 20 – 25 (7.7%), 26 – 30 (0.0%), 31- 35 (0.0%), and 36 – 40 (0.0%) respectively. The frequency of a mixture of Bacillus and Enterobacter was 0, 0, 4, and 1 among age ranged from 20 – 25 (0.0%), 26 – 30 (0.0%), 31- 35 (100.0%), and 36 – 40 (16.7%) respectively. The frequency of a mixture of Bacillus and Pseudomonas was 0, 0, 0, and 2 among age ranged from 20 – 25 (0.0%), 26 – 30 (0.0%), 31- 35 (0.0%), and 36 – 40 (33.3%) respectively. The frequency of a mixture of Pseudomonas, Staphylococcus, and Enterobacter was 0, 3, 0, and 0 among age ranged from 20 – 25 (0.0%), 26 – 30 (42.9%), 31- 35 (0.0%), and 36 – 40 (0.0%) respectively.

The location according to living and working was divided to two categories: living or working inside Gezira locality and living or working outside Gezira locality. The students and workers living inside Gezira locality were 16, and students and workers living outside Gezira locality were 14, while all the 30 students and workers working inside Gezira locality.
The frequency of microorganism according to students and workers living is shown in Table 6. The result showed that the students and workers who living inside Gezira locality had 11 Bacillus (68.8%), 0 Pseudomonas (0.0%), 0 staphylococcus (0.0%), 4 Enterobacter (25.0%), 1 with a mixture of Staphylococcus and Pseudomonas (6.2%), 0 with a mixture of Bacillus and Enterobacter (0.0%), 0 with a mixture of Bacillus and Pseudomonas (0.0%), and 0 with a mixture of Pseudomonas, Staphylococcus, and Enterobacter (0.0%) while the students and workers living outside Gezira locality had 1 Bacillus (7.1%), 2 Pseudomonas (14.3%), 1 staphylococcus (7.1%), 0 Enterobacter (0.0%), 0 with a mixture of Staphylococcus and Pseudomonas (0.0%), 5 with a mixture of Bacillus and Enterobacter (35.7%), 2 with a mixture of Bacillus and Pseudomonas (14.3%), and 3 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (21.4%).

The frequency of microorganism according to students and workers working is shown in Table 7. The result showed that the all the students and workers were working inside Gezira locality. The students and workers were showing 12 Bacillus (40.0%), 2 Pseudomonas (6.4%), 1 staphylococcus (3.3%), 3 Enterobacter (13.3%), 1 with a mixture of Staphylococcus and Pseudomonas (3.3%), 5 with a mixture of Bacillus and Enterobacter (16.7%), 2 with a mixture of Bacillus and Pseudomonas (6.7) and 3 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (10.0%).

Out of 30 selected students and workers 19 answered with no, for the presence of previous history of hair loss and 11 answered with yes. Also 27 students and workers answered with no for sterilization of the hair cutter before the use and 3 students and workers answered with yes for sterilization of the hair cutter before use by their barbers.

The frequency of microorganism according to previous history hair loss is shown in Table 8. The result showed that the students and workers who had no previous history of hair loss had 12 Bacillus (63.2%), 4 Enterobacter (21.1%), 0 Staphylococcus (0.0%), 0 Pseudomonas (0.0%), 1 with a mixture of Staphylococcus and Pseudomonas (5.3%), 0 with a mixture of Bacillus and Enterobacter (0.0%), 0 with mix of Bacillus and Pseudomonas (0.0), and 2 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (10.5 %), while the students and workers who had previous history of hair loss had 0 Bacillus (0.0%), 0 Enterobacter (0.0%), 1 Staphylococcus (9.1%), 2 Pseudomonas (18.2%), 0 with a mixture of Staphylococcus and Pseudomonas (0.0%), 5 with a mixture of Bacillus and Enterobacter (45.5%), 2 with a mixture of Bacillus and Pseudomonas (18.2), and 1 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (9.1%).

The frequency of microorganism according to sterilization of the hair cutter before use is shown in Table 9. The result showed that the students and workers who not sterilized the hair cutter before use had 12 Bacillus (44.4%), 4 Enterobacter (14.8%), 0 Staphylococcus (0%) 2 Pseudomonas (7.4%), 0 with mix of Staphylococcus and Pseudomonas (0.0%), 0 with a mixture of Bacillus and Enterobacter (0.0%), 2 with a mixture of Bacillus and Pseudomonas (66.7%) and 0 with a mixture of Pseudomonas, Staphylococcus, and Enterobacter (0.0%), while the students and workers who sterilized the hair cutter

before use had 0 Bacillus (0.0%), 0 Enterobacter (0.0%), 1 Staphylococcus (33.3%), 0 Pseudomonas (0.0%), 0 with a mixture of Staphylococcus and Pseudomonas (0.0%), 0 with a mixture of Bacillus and Enterobacter (0.0%), 2 with a mixture of Bacillus and Pseudomonas (66.7%) and 0 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (0.0%).

Twenty of the students and workers said they don't using shampoo during hair washing and 10 said they used. This study revealed 9 of students and workers with singes of continues hair fall, 12 students and workers with singes of all hair fall, 8 with hair fall singe accompanied with dandruff, and 1 with hair fall singe accompanied with itching.

The frequency of microorganism according to using shampoo during hair washing is shown in Table 10. The result showed that the students and workers who not using shampoo during hair washing had 12 Bacillus (60.0%), 4 Enterobacter (20.0%), 0 Staphylococcus (0.0%), 0 Pseudomonas (0.0%), 1 with a mixture of Staphylococcus and Pseudomonas (5.0%), 0 with a mixture of Bacillus and Enterobacter (0.0%), 0 with a mixture of Bacillus and Pseudomonas (0.0%) and 3 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (15.0%), while the students and workers who using shampoo during hair washing had 0 Bacillus (0.0%), 0 Enterobacter (0.0%), 1 Staphylococcus (10.0%), 2 Pseudomonas (20.0%), 0 with mix of Staphylococcus and Pseudomonas (0.0%), 5 with a mixture of Bacillus and Enterobacter (50.0%), 2 with a mixture of Bacillus and Pseudomonas (20.0%) and 0 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (0.0%).

The frequency of microorganism according to sings of hair loss is shown in Table 11. The result show 4, 8, 0, and 0 of *Bacillus* organism among students and workers who had a sings of continues hair fall (44.4%), all hair fall (66.7%), hair fall accompanied with dandruff (0.0%), and hair fall accompanied with itching (0.0%) respectively. Thefrequency of *Enterobacter* organism was 4, 0, 0, and 0 among students and workers who had a sings of continues hair fall (44.4%), all hair fall (0.0%), hair fall accompanied with dandruff (0.0%), and hair fall accompanied with itching (0.0%) respectively. The frequency of *Staphylococcus* organism was 0, 0, 0, and 1 among students and workers who had a sings of continues hair fall (0.0%), all hair fall (0.0%), hair fall accompanied with dandruff (0.0%), and hair fall accompanied with itching (100.0%) respectively. The frequency of *Pseudomonas* organism was 0, 0, 2, and 0 among students and workers who had a sings of continues hair fall (0.0%), all hair fall (0.0%), hair fall accompanied with dandruff (25.0%), and hair fall accompanied with itching (0.0%) respectively. The frequency of a mixture of Staphylococcus and Pseudomonas was 1, 0, 0, and 0 among students and workers who had a sings of continues hair fall (11.1%), all hair fall (0.0%), hair fall accompanied with dandruff (0.0%), and hair fall accompanied with itching (0.0%) respectively. The frequency of a mixture of Bacillus and Enterobacter was 0, 1, 4, and 0 among students and workers who had a sings of continues hair fall (0.0%), all hair fall (8.3%), hair fall accompanied with dandruff (50.0%), and hair fall accompanied with itching (0.0%) respectively. The frequency of a mixture of Bacillus and Pseudomonas was 0, 0, 2, and 0 among students and workers who had a sings of continues hair fall (0.0%), all hair fall (0.0%), hair fall accompanied with dandruff (25.0%), and hair fall accompanied with itching (0.0%) respectively. The frequency of a mixture of Pseudomonas, Staphylococcus and Enterobacter was 0, 3, 0, and 0 among students and workers who had a sings of continues hair fall (0.0%), all hair fall (25.0%), hair fall accompanied with dandruff (0.0%), and hair fall accompanied with itching (0.0%) respectively.

The results on Table 12 students and workers had *Bacillus* organism (40.0%), 1 had *Staphylococcus* (3.3%), 2 had *Pseudomonas* (6.7%), 4 had *Enterobacter* (13.3%), 1 with mix of Staphylococcus and Pseudomonas (3.3%), 5 with a mixture of Bacillus and Enterobacter (16.7%), 2 with mix of Bacillus and Pseudomonas (6.7%) and 3 with a mixture of Pseudomonas, Staphylococcus and Enterobacter (10.0%). The frequency of each individual type of microorganism was showed in table 12. The study revealed 21 Bacillus, 7 Pseudomonas, 12 Enterobacter, and 4 Staphylococcus, with total parentage 47.8 %, 15.9 %, 27.3 %, and 9 % respectively.

**Table 3:** The results of the questionnaire:

| Variables | Categories | Frequency | Valid Percent | Cumulative Percent |
|---|---|---|---|---|
| Sex | Male | 26 | 86.7 | 86.7 |
| | Female | 4 | 13.3 | 100.0 |
| Age | 20 – 25 | 13 | 43.3 | 43.3 |
| | 26 – 30 | 7 | 23.3 | 66.7 |
| | 31 – 35 | 4 | 13.3 | 80.0 |
| | 36 – 40 | 30 | 20.0 | 100.0 |
| Location of live | In | 16 | 53.3 | 53.3 |
| | Out | 14 | 46.7 | 100.0 |
| Location of work | In | 100.0 | 100.0 | 100.0 |
| | Out | - | - | - |
| History of hair loss | No | 19 | 63.3 | 63.3 |
| | Yes | 11 | 36.7 | 100.0 |
| Cutter sterilization | No | 27 | 90.0 | 90.0 |
| | Yes | 3 | 10.0 | 100.0 |
| Using shampoo | No | 20 | 66.7 | 66.7 |
| | Yes | 10 | 33.3 | 100.0 |
| Singes | Continuous hair fall | 9 | 30.0 | 30.0 |
| | Fall of all hair | 12 | 40.0 | 70.0 |
| | Hair fall with dandruff | 8 | 26.7 | 96.7 |
| | Hair fall with itching | 1 | 3.3 | 100.0 |
| Type of bacteria | stap+ pseudo | 1 | 3.3 | 3.3 |
| | Entero | 4 | 13.3 | 16.7 |
| | Bacillus | 12 | 40.0 | 56.7 |
| | pseudo+staph+entero | 3 | 10.0 | 66.7 |
| | bacillus+entero | 5 | 16.7 | 83.3 |
| | Pseudo | 2 | 6.7 | 90.0 |
| | bacillus+psedo | 2 | 6.7 | 96.7 |
| | Staph | 1 | 3.3 | 100.0 |

**Table 4:** The frequency of microorganism according to students and workers gender

| | Gender | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sataph+Pseudo | Entero | Bacillus | Pseudo+Staph+entero | Bacillus+Entero | Pseudo | Bacillus + Pseudo | Staph |
| Male | Frequency | ND | ND | ND | ND | ND | 1 | 2 | 1 |
| | Percentage | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 25.0 | 50.0 | 25.0 |
| Female | Frequency | 1 | 34 | 12 | 3 | 5 | 1 | ND | ND |
| | Percentage | 3.8 | 15.4 | 46.2 | 11.5 | 19.2 | 3.8 | 0.0 | 0.0 |

Staph= staphylococcus spp ,Pseudo= pseudomonas spp ,Entero=enterobacterspp , ND= not detected

**Table 5:** The frequency of microorganism according to students and workers age:

| | | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Age | Sataph + Pseudo | Entero | Bacillus | Pseudo + Staph+ entero | Bacillus+ Entero | Pseudo | Bacillus+ Pseudo | Staph |
| 20-25 | Frequency | 1 | 4 | 8 | ND | ND | ND | ND | ND |
| | Percentage | 7.7 | 30.8 | 61.5 | 0 | 0 | 0 | 0 | 0 |
| 26-30 | Frequency | ND | ND | 4 | 3 | ND | ND | ND | ND |
| | Percentage | 0.00% | 0 | 57.1 | 42.9 | 0 | 0 | 0 | 0 |
| 31-35 | Frequency | ND | ND | ND | ND | 4 | ND | ND | ND |
| | Percentage | 0.00% | 0 | 0 | 0 | 100 | 0 | 0 | 0 |
| 36-40 | Frequency | ND | ND | ND | ND | 1 | 2 | 2 | 1 |
| | Percentage | 0 | 0 | 0 | 0 | 16.7 | 33.3 | 33.3 | 16.7 |

**Table 6:** The frequency of microorganism according to students and workers living:

| | Live | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sataph + Pseudo | Entero | Bacillus | Pseudo + Staph + entero | Bacillus + Entero | Pseudo | Bacillus + Pseudo | Staph |
| in | Frequency | 1 | 4 | 11 | ND | ND | ND | ND | ND |
| | Percentage | 6.2 | 25 | 68.8 | 0 | 0 | 0 | 0 | 0 |
| out | Frequency | ND | ND | 1 | 3 | 5 | 2 | 2 | 1 |
| | Percentage | 0 | 0 | 7.10% | 21.40% | 35.70% | 14.30% | 14.30% | 7.10% |

Staph= staphylococcus spp , Pseudo= pseudomonas spp , Entero=enterobacterspp ,ND= not detected

**Table 7:** The frequency of microorganism according to students and workers working:

| | Work | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sataph + Pseudo | Entero | Bacillus | Pseudo + Staph + entero | Bacillus + Entero | Pseudo | Bacillus + Pseudo | Staph |
| in | Frequency | 1 | 4 | 12 | 3 | 5 | 2 | 2 | 1 |
| | Percentage | 3.3 | 13.3 | 40.0 | 10.0 | 16.7 | 6.7 | 6.7 | 3.3 |

**Table 8:** The frequency of microorganism according to previous history hair loss

| | Pervious history | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sataph + Pseudo | Entero | Bacillus | Pseudo + Staph + entero | Bacillus + Entero | Pseudo | Bacillus + Pseudo | Staph |
| No | Frequency | 1 | 4 | 12 | 2 | ND | ND | ND | ND |
| | Percentage | 5.3 | 21.1 | 63.2 | 10.5 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yes | Frequency | ND | ND | ND | 1 | 5 | 2 | 2 | 1 |
| | Percentage | 0 | 0.0 | 0.0 | 9.1 | 45.5 | 18.2 | 18.2 | 9.1 |

Staph= staphylococcus spp , Pseudo= pseudomonas spp , Entero=enterobacterspp ,ND= not detected

**Table 9:** The frequency of microorganism according to sterilization of the hair cutter before use

| | Cutter sterilization | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sataph + Pseudo | Entero | Bacillus | Pseudo + Staph + entero | Bacillus + Entero | Pseudo | Bacillus + Pseudo | Staph |
| No | Frequency | 1 | 4 | 12 | 3 | 5 | 2 | ND | ND |
| | Percentage | 3.7 | 14.8 | 44.4 | 11.1 | 18.5 | 7.4 | 0.0 | 0.0 |
| Yes | Frequency | ND | ND | ND | ND | ND | ND | 2 | 1 |
| | Percentage | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 66.7 | 33.3 |

**Table 10: The frequency of microorganism according to using shampoo during hair washing**

| | Using shampoo | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sataph + Pseudo | Entero | Bacillus | Pseudo + Staph + entero | Bacillus + Entero | Pseudo | Bacillus + Pseudo | Staph |
| No | Frequency | 1 | 4 | 12 | 3 | ND | ND | ND | ND |
| | Percentage | 5.0 | 20.0 | 60.0 | 15.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Yes | Frequency | ND | ND | ND | ND | 5 | 2 | 2 | 1 |
| | Percentage | 0.0 | 0.0 | 0.0 | 0.0 | 50.0 | 20.0 | 20.0 | 10.0 |

Staph= staphylococcus spp , Pseudo= pseudomonas spp , Entero=enterobacterspp ,ND= not detected

**Table 11:** The frequency of microorganism according to sings of hair loss

| | Signs | Bacteria | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sataph+Pseudo | Entero | Bacillus | Pseudo+Staph+entero | Bacillus+ Entero | Pseudo | Bacillus+Pseudo | Staph |
| contenious fall | Frequency | 1 | 4 | 4 | ND | ND | ND | ND | ND |
| | Percentage | 11.1 | 44.4 | 44.4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| all fall | Frequency | ND | ND | 8 | 3 | 1 | ND | ND | ND |
| | Percentage | 0.0 | 0.0 | 66.7 | 25.0 | 8.3 | 0.0 | 0.0 | 0.0 |
| fall+ dandruff | Frequency | ND | ND | ND | ND | 4 | 2 | 2 | ND |
| | Percentage | 0.0 | 0.0 | 0.0 | 0.0 | 50.0 | 25.0 | 25.0 | 0.0 |
| fall+itchy | Frequency | ND | ND | ND | ND | ND | ND | ND | 1 |
| | Percentage | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |

Staph= staphylococcus spp , Pseudo= pseudomonas spp , Entero=enterobacterspp ,ND= not detected

**Table 12:** The frequency of each type of microorganism.

| Bacteria type | Frequency | Percentage |
|---|---|---|
| Bacillus | 21 | 47.8 |
| Enterobacter | 12 | 27.3 |
| Staphylococcus | 4 | 9 |
| Pseudomonas | 7 | 15.9 |
| Total | 44 | 100 |

Summary of the Chi-square test showed that from a total of 7 variables were analyzed analysis using Chi-square test to determined the associated between risk factors and hair loss in Gezira State, 6 variables were significant with p-value ≤ 0.05 except Location of working which are not computed (because the Location of work is a constant among all students and workers). The significant factors were: Age ($X^2 = 13.77$, $p = 0.003$), Gender ($X^2 = 41.03$, $p = 0.000$), Location of living ($X^2 = 18.28$, $p = 0.000$), History of hair loss ($X^2 = 22.82$, $p = 0.000$), and hair cutter sterilization ($X^2 = 13.33$, $p = 0.004$), using of shampoos ($X^2 = 25.87$, $p = 0.000$) (Appendix.1).

## 4. Discussion

Hair loss is one of the most common complaints among all patients consulting a dermatologist and is usually associated with severe psychological disturbances, distress and symptoms of depression (Thiago*et al.*, 2013).

The human scalp normally contains 100,000 hairs that follow a specific growth cycle (Odom *et al.*, 2000). The first phase is the anagen, or growing phase, which lasts about 3 years. During this period, hair grows about 0.37mm daily. Hairs then enter the catagen phase, which is a controlled regression of hair growth, a transitional phase between the growing and resting phase that lasts 1 or 2 weeks. Finally hairs enter the telogen phase, a resting state that lasts between 3 and 4 months until the hair falls out. A healthy human scalp contains 90% anagen hairs, and about 10% catagen or telogen hairs.

In pattern baldness (PB), the hair loss is a result of an increase in the ratio of telogen to anagen hairs and the miniaturization of the hair follicle ( Sinclair, 1998). The anagen phase becomes progressively shorter and the telogen phase becomes longer. Since the length of the hair is a result of the duration of the anagen phase, each hair becomes progressively shorter than its precursor. Eventually, the hair becomes so short that it does not reach the skin surface. In addition, because telogen hairs are not as well attached to the follicle as are anagen hairs, the increase in telogen hair count results in an increase of hair shedding in individuals with PB. With every completion of the hair cycle the hair follicle becomes progressively smaller, and thus the hair they produce becomes smaller as well.

Pattern baldness (PB), also called androgenetic alopecia, is characterized by a progressive patterned hair loss from the scalp, which begins usually during the twenties or thirties (Odom *et al.*, 2000), a finding that goes in line with our study results in the presence of a significant association between hair loss and students and worker age ($p = 0.003$). Moreover, the study showed a significant association between hair loss and gender ($p = 0.000$), a finding that agreed with Sinclair, (1998) who found that PB affects about 30% of Caucasian men in their thirties, 40% in their forties, and so on until age 70. In Caucasian women it is less common and it was found that in one study of over 500 women , PB affected 13% of premenopausal women and 37% of postmenopausal women (Venning and Dauber, 1988 cited in Keratin.com) . The rates of PB are also different in other ethnic groups. Male African Americans are four times less likely to develop PB than are Caucasian males (Setty, 1970).

PB was initially thought to occur as a result of shaving (Blaine, 1999). Because frequent use of the razor was believed to stimulate hair growth, researchers believed that the energetic drain to supply the rapid growth of the beard necessarily caused a weakness in other parts of the body and led to decreased hair growth on the scalp. In addition, according to our study findings a significant relation was found between the sterilization of hair cutter ($p = 0.004$). This could be due to the fact that contaminated or improperly sterilized equipment can increase the risk of dandruff, which is is associated with a yeast fungus called Pityrosporumovale . Although, in case of hair fall and dandruff some hair follicle could be damaged or destroyed .But there is no evidence in the medical journals that the baldness is caused by ordinary dandruff.

The use of shampoo showed significant association with hair loss ($p = 0.000$) .Although Shampoos clean the hair ,but there are real problems with using shampoos: although their product claims are often exaggerated and unrealistic. Shampoos coat the hair with synthetic compounds that boast the appearance of the claims on their bottles. After a few washes, the effects are gone. That's why you'll never see a claim for permanent volume lifts, frizz reduction, dandruff control, or shininess **,**their ingredients are carcinogenic and hormone-disrupting**.** Depending on your frequency of exposure, this can have a compounded

negative effect on your health and shampoos can accelerate the pathway to thinning hair**.** Shampoo strips your hair of the oils your body naturally produces to protect it, which worsens the health of your scalp and creates excess sebum production, a precursor to pattern baldness (Internet,. 1)

A number of research has shown evidence that infectious causation may be partly responsible for PB. First, some types of baldness have already been shown to be a result of infectious diseases such as influenza, typhus, lyme meningitis, tick-borne encephalitis (Cimperman, 1999), HIV (Jan and Roudier-Pujol, 2000), scarlet fever, and pneumonia (Weigand, 1969). Second, there is growing evidence of the presence of inflammation in the scalp of balding individuals (Kligman, 1988; Young *et al.*, 1991; Jaworsky *et al.*, 1992; Piérard *et al.*, 1996; Sueki *et al.*, 1999; Mahé *et al.*, 2000). Finally, PB has been associated with an increased risk of heart disease (Herrera and Lynch, 1990; Trevisan*et al.*, 1993; Ford *et al.*, 1996; Sasmaz *et al.*, 1999). Since heart disease shows evidence of infectious causation (Ewald and Cochran, 2000), it is very probable that infection may be partly responsible for PB as well.

Kligman (1988) was the first to notice the difference in the degree of inflammation in balding as opposed to control subjects. Finally, Kligman suggested studying PB in connection with seborrheic dermatitis and dandruff. Since those diseases are now known to be caused by pathogens (Odom *et al.*, 2000). Subsequent studies have also reported the presence of inflammation on the scalp of individuals with PB. Young *et al.* (1991) found immunoglobulin M or C3 (or both) present on the basement membrane of 96% of individuals with PB and on 12% of controls. The presence of immunoglobulins on the scalp indicates that an antigen is present, perhaps a microbe.

With the growing evidence of the presence of inflammation in PB, one group decided to study the effects of an antimicrobial lotion on the progress of PB. Piérard and others (1996) supplied 20 men with PB with a lotion containing piroctoneolamine and triclosan. Piroctoneolamine is a powerful antifungal agent and triclosan is a common antibacterial. Patients were told to apply the lotion daily to the scalp. Long-term application of the cream resulted in decreased inflammation of the scalp as revealed by a decrease in the number of lymphosites, decreased number of skin flora, and decreased hair loss. The researchers suggested preventing PB by controlling the microorganisms that inhabit the scalp, which have been shown to be potent elicitors of the immune response (Piérard-Franchimont, 1995- cited in Piérard, 1996).

With regard to all mentioned facts our study for the microbial isolation and identification, revealed the presence of different microorganisms from all students and workers, including: staphylococcus, Bacillus, Enterobacter and Pseudomonas, a finding which was also stated by Morales-Sánchez , (2010), who concluded that a carriers of *Streptococcus pyogenes* were considered a risk factors for alopecia areata .Never the less, he also isolated *Spyogenes and* , *Pseudomonas aeruginosa*from cultures .

Moreover, the study reported a significant association between hair loss and site of living weather inside or outside Wad medani (p = 0.000). This could be explained by the fact that students and workers who live inside Wad medani exposed to high environmental contamination and pollution due to the nature of the locality which characterized with high density of population facilities and vehicles, and this could expose hair easily to bacterial and fungal contamination and infection. Prolonged exposure to the sun dries out the hair and disrupts the cells of the cuticle causing the hair, if untreated, to become brittle and prone to breakage. This is an easily avoidable cause of hair loss. A finding which was supported by Young *et al.* (1991), ) who found immunoglobulin M or C3 (or both) present on the basement membrane of 96% of individuals with PB and on 12% of controls. The presence of immunoglobulins on the scalp indicatesthat an antigen is present, perhaps a microbe. They also found porphyrins in 58% of PB subjects and in 12 % of controls. Porphyrins are water-soluble, nitrogenous biological pigments (Encyclopædia Britannica, 2001). Because *Propionibacterium acnes* has been shown to produce porphyrins (Young *et al.*, 1991), they suggested that *P. acnes* may be involved in the process of PB. The production of porphyrins by this organism may result in the observed inflammation when light-activated porphyrinsoxydizesqualene to produce a toxic inflammation. Since PB begins in areas that are most frequently exposed to the sun, Young and coworkers argued that light may excite porphyrin production by the resident flora, producing an inflammatory reaction. They concluded their paper by suggesting the use of antimicrobials to reduce the number of natural flora and a reduction in sun exposure to help control the extent of PB.

Finally, the study showed that Family history was found statistically significant with hair loss (p = 0.000). A similar findings have been documented by Hasan*et al.*, (2011) who reported the relationship between extensive alopecia areata and family history. Beside, Rhodes*et al*,(1998) whose results suggested the probability of male pattern hair loss to be dependent on family history ,i.e hair loss in a man's father appears to play an important role in increasing a man's risk of hair loss, either in conjunction with a history of hair loss in the mother or hair loss in the maternal grandfather.

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Appendices:

**Appendix 1:** Summary of the Chi-square test

| Variables | Categories | Frequency | Percent | $X^2$ | DF | P - value |
|---|---|---|---|---|---|---|
| Gender | Male<br>Female | 26<br>4 | 86.7<br>13.3 | 13.77 | 3 | 0.003 |
| Age | 20 – 25<br>26 – 30<br>31 – 35<br>36 – 40 | 13<br>7<br>4<br>6 | 43.3<br>23.3<br>13.3<br>20.0 | 41.03 | 9 | 0.000 |
| Location of live | In<br>Out | 16<br>14 | 53.3<br>46.7 | 18.28 | 3 | 0.000 |
| Location of work | In<br>Out | 100.0<br>- | 100.0<br>- | * | * | * |
| History of hair loss | No<br>Yes | 19<br>11 | 63.3<br>36.7 | 22.82 | 3 | 0.000 |
| Cutter sterilization | No<br>Yes | 27<br>3 | 90.0<br>10.0 | 13.33 | 3 | 0.004 |
| Using shampoo | No<br>Yes | 20<br>10 | 66.7<br>33.3 | 25.87 | 3 | 0.000 |

- * mean that no statistics was computed because work is a constant.

**Appendix (2):** Questionnaire

**Questionnaire form for factors associated with hair loss**

Date: ……..……..

Student or worker name:…….

1. Age:
   20 – 25 ☐ 26 – 30 ☐ 31 – 35 ☐ 36 – 40 ☐
2. Gender:
   Male ☐ Female ☐
3. Location of living:
   Inside Wad medani ☐ Outside Wadmedani ☐
4. Location of working:
   Inside Wad medani ☐ Outside Wad medani ☐
5. Sterilization of hair cutter:
   Yes ☐ No ☐
6. Using shampoo:
   Yes ☐ No ☐
7. Sings of hair loss:
   Continuous ☐ All fall ☐ Fall with dandruff ☐ Fall with itching ☐