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MICROBES ASSOCIATED WITH BEARD HAIR AND THEIR SPORE FORMATION CAPACITY

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Abstract: The study aimed at evaluating microbes associated with beard hair and their spore formation features, following the significance of spore dispersal and the trend observed with beard keeping. Fifty (50) beard hair samples were collected from adult male volunteers, who subjected them-self for swab collection exercise. Thereafter, the study employed a spread plate and spore stain microbiological procedures for isolation of isolates and determination of spore features respectively. The result showed a mean count of 8 x 10^3, 2.1 x 10^3, and 1.5 x 10^3 cfu/ml for Heterotrophic bacteria, Staphylococcal and Heterotrophic fungal respectively. Statistically, the counts showed a significant difference exist at 0.05. The isolates were characterized and identified as *Staphylococcus aureus*, *Aspergillus* spp., *Cladosporium* spp., *Bacillus* spp., *Conidiophores* spp. and *Aspergillus* spp. On spore formation capacity, *Bacillus* spp. and *Aspergillus niger* produced or formed spores. The recoveries of these microbes may be due to deficient sterilization and the persistence of spore. The study concludes that microbes and spores they form persist in beard hair even after possible treatment, thus the study recommends regular beard hair hygiene and the use of therapeutic agents on the beard hair to prevent the evasion of *Bacillus* spp. and *Aspergillus* spp. spores.
Keywords: Microbes; Beard hair; Spore formation; Spore dispersal

1 INTRODUCTION

The keeping of beard hair by some male is becoming a trend in the southern part of Nigeria as it remains popular amongst adolescent and adult male [1]. Beard hair grows as a result of changing levels of androgen, a hormone mostly present in human male and some female. The beard hair can be trimmed, shaved-off completely or styled in different ways like the goatee, van dyke, neck beard, the chinstrap etc [1]. The trend at which adult male keep beard hair continue to receives more applause from the public, as seen in fashion gallery [2]. Basically, some individuals do not treat or care for their bearded face, they allow it to grow and cut to their choice style [2]. The negligence for beard hair care may result to the harbor of microbes. Hence, the beard hair may be a potential source of microbe and therefore, harbors pathogenic microbes [3]. The harbor of microbes by the beard hair could cause diseases such as Staph infection, skin irritation, dandruff, ingrown hairs, ance etc [4]. Similarly, uncared or un treated beard could become a threat to the carrier and the public at large due to the harbor microbial spores which are transmissible [5]. Human exposure to spore has been linked to allergic respiratory symptoms such as asthma. [5] The microbes in the beard hair are capable of forming spore considering the long time it has inhabited the facial hair [3]. The habit at which some individuals routinely touch their beard hair, can one way transmitted the spore to other surfaces, thereby exposing the public [3]. Also, given the proximity of the beard hair to the nose, reinfection can occur and thus respiratory allergies may develop. Respiratory allergies may arise unnoticed as the microbial spore have the capacity to penetrate alveoli of the lungs to cause illness [5]. In all these challenges, the study is significant as it aims at sensitizing and enlightening adult beard hair keepers, who routinely, touch their hairs, on the negative effect or the implication of transfer of microbial spore in and out of their facial hair surface. Following this the study aimed at evaluating microbes associated with beard hair and their spore formation features.

2 MATERIALS AND METHODS

2.1 Study Area

The study was carried out at the campus of the Ignatius Ajuru University of Education, Rumuolumeni, Port Harcourt with a geographical coordinate of $4^0$48’16.273’’N and $6^0$56’0.191’’E. The school campus vicinity is known for high proportion of beard hair adult men of diverse beard style such as chin-strap, goatee, neck beard, van dyke etc. Following this, ethical consent was requested from individual who indicated interest to participate in the screening exercise.

2.2 Collection of Samples

Fifty (50) beard hair samples were collected from adult male volunteers, who subjected them self for swab collection exercise as documented by Locascio et al.[6]. Sample collection involved the use of a sterile swab sticks immersed in a sterile/freshly prepared peptone broth for few seconds and thereafter streaked on the jawed beard for 10 seconds before introduction into the broth again for serial dilution. The samples were then labelled accordingly and transported to the Biology Laboratory, Ignatius Ajuru University of Education, Rumuolumeni, for microbiological analysis.

2.3 Preparation of Media

The media adopted for the analysis were the: nutrient agar, mannitol salt agar and sabouraud dextrose agar for the growth of heterotrophic bacteria, *Staphylococcus aureus* and fungi respectively. All media employed for the study were prepared according to the manufacturers instruction, as the media were weighed, autoclaved/sterilized, allowed to cool before been dispensed into sterile petri dishes for sample inoculation[7].

2.4 Enumeration of Microbial Population

One (1) ml of the broth sample was serially diluted in peptone water as carried out by Ho et al.[8], from which 0.1ml aliquot (10^1 dilution) was inoculated onto the sterile prepared media[9]. The Nutrient agar medium was used to determine counts of heterotrophic bacteria while the Mannitol salt agar medium and sabouraud dextrose were used to determine counts of Staphylococcal [10] and heterotrophic fungi [10] respectively. After inoculation of the samples into the various media plates, the nutrient and mannitol plates were incubated for twenty (20) hours at thirty-five degrees centigrade (350C) while the sabouraud media plates were incubated at room temperature for four (4) days at the end of the various incubation periods, colonies that developed were counted and recorded as colony forming unit per mill (CFU/ml)[10].

2.5 Identification and Characterization of Bacteria Isolates

The isolates were characterized and identified based on their colonial morphological features such as; texture, colour, shape, size and elevation. Biochemical tests were also carried out for proper identification of the isolates. The test carried out are as follows; catalase, indole, methyl red, Voges -Proskauer, citrate utilization, urease and sugar fermentation (glucose, lactose, sucrose and mannose) tests [10].

2.5.1 Sugar fermentation test

The sugar tested employed the use of glucose, sucrose, lactose and mannose in the right proportion accordingly. One gram (1g) either of the above stated sugar was added into eighty mill (80ml) of peptone water and stirred thoroughly to solubilize the sugar, thereafter twenty mill (20ml) of 0.2% (w/v) phenol red indicator was added to the sugar-peptone water solution. Following the addition, ten mill (10ml) of the sugar-peptone water solution was dispensed into test tubes contained in it inverted Durham's tubes and autoclaved at one hundred and twenty-one degrees centigrade (121°C) for fifteen (15) minutes. The test tubes were allowed to cool, and inoculated with the test organism. The tubes were then incubated at thirty-seven degrees centigrade (37°C) for twenty (24) hours. An orange colour change of the medium signified a positive result for both fermentation and oxidation while the presence of bubbles in the Durham's tubes signified gas production [11].

2.5.2 Catalase test

In carrying out this test, a drop of hydrogen peroxide was placed on a clean microscope slide and with the aid of a sterile wire loop the test colony was introduced into the glass slide and the component smeared. Few seconds after the procedure, the slide was observed for bubbles. Catalase-positive bacteria are signified by the production of bubbles of oxygen [11].

2.5.3 Indole test

The indole medium, a composition of nutrient broth, peptone water and tryptone broth was freshly, prepared with the use of autoclave at one hundred degrees Celsius (121°C) for fifteen (15) minutes and used for the test. Following the sterilization and subsequent dispense of the medium into test tubes. The test bacteria were then inoculated into the medium with the aid of a sterile wire loop and the medium incubate at thirty-seven degrees Celsius (37°C) for forty-eight (48) hours. After incubation, a red/pink layer formed on top of the broth indicated indole positive while a reverse of yellow indicated indole negative [11].

2.5.4 Methyl red

The test involved the use of MR/VP broth. The MR/VP broth was then sterilized, allowed to cool before the isolate was inoculated. Following the inoculation, the broth was incubated for four days at thirty-seven degrees Celsius (37°C). Thereafter 5 to 6 drops of methyl red reagent were added to the broth, stirred thoroughly, and allowed to stand for 5 minutes. The broth culture was then examined for color change [11].

2.5.5 Voges proskauer

The test involved the use of glucose phosphate broth. The glucose phosphate broth was sterilized, allowed to cool before the isolate was inoculated. Following this, the broth was incubated for four days at thirty-seven degrees Celsius (37°C). Then, 1.5 ml of 5% alcoholic alpha napthtol and 0.5 ml of forty percent (40%) aqueous Potassium Hydroxide (KOH) was added and the test tubes, stirred thoroughly, and allowed to stand for 5 minutes. The test tubes were then examined for the

development of pink colour which denoted which denoted positive Voges Proskauer test, while the formation of a yellow colour indicated a negative result [11].

2.5.6 Citrate utilization test

This test was carried out to ascertain the ability of the bacteria in the presence of bromothymol blue as an indicator to utilize citrate as its source of carbon and ammonium as its source of nitrogen. With the use of a sterile wire-loop the isolate was inoculated on the citrate medium and the medium incubated at thirty-seven degrees Celsius (37°C) for twenty-four (24) hours. A citrate positive result was indicated by a change in colour of the indicator from green to blue [11].

2.5.7 Urease test

In carrying out the urease test, the isolate was streaked on the surface of a freshly prepared urease agar media slant and the component incubated for forty-eight (48) hours at thirty-seven degrees centigrade (37°C). Following the incubation, the media was carefully examined for the development of a pink colour on the surface of the media. Thus, the development of bright pink colour indicated urease positive bacteria while the reverse, negative [11].

2.6 Identification and Characterization of Fungi Isolates

The fungi isolates recovered from the sabauraud dextroxe media plates were characterized and identified based on their colonial/ morphological features such as; texture, color, shape, size and elevation. Similarly, the fungal morphological features were determined with the use a light microscope. The investigation employed the use of Lactophenol Blue Staining technique as adopted by Leck et al.[12] The procedure for the fungal characterization involved introducing a drop of lactophenol blue on a glass slide using a pasture pipette, thereafter a sterile wire loop was used to pick the isolate onto the glass slide. The isolate on the glass slide was emulsified and a cover slip, placed on the slide. Following this the glass slide was then viewed under a light microscope.

2.7 Spore Formation Test

Spore formation test was done to see the capacity of the microbe in producing spores. In carrying out this test, Le-Veque et al. [13] was adopted, were a thin smear was prepared on a clean glass slide, allowed to air dry and thereafter flooded with methylene blue reagent for five (5) minutes. The smear was counter stained with carbon fuschin for thirty (30) seconds, rinsed with tap water and blotted with filter paper. The stained cells were then examined under the oil immersion objective lens. Green and red colored pigmented/vegetative cell view denoted spore formation.

3 RESULTS

3.1 Mean Load of Bacterial and Fungal Obtained from Beard Hair Volunteers

Table 1 showed a mean total heterotrophic bacteria count of 8 x 10^3 cfu/ml recovered from the beard samples, while 2.1 x 10^3 cfu/ml, and 1.5 x 10^3 cfu/ml counts were mean counts obtained for staphylococcal and total heterotrophic fungal respectively. Hence, counts obtained showed a significant difference across the microbes exist.

Table 1 Mean Load of Bacterial and Fungal Obtained from Beard Hair Volunteers

	THB (CFU/ml)	S(CFU/ ml)	THF(CFU/ ml)	T-test
Volunteers Beard	8 x 10^3	2.1 x 10^3	1.5 x 10^3	P<0.05

Note: THB= Total Heterotrophic Bacteria, S= Staphylococcal, THF= Total Heterotrophic Fungi

3.2 Colonial and Morphological Characteristics of the Bacteria Isolates from Beard Hair Samples

Table 2 showed colonial characterization of the bacteria isolated. On the Mannitol salt media plates, the isolates recovered were small sized, yellow coloured while off-whitish coloured colonies of small sizes were observed on nutrient media plate. Thus, on both mannitol salt media and nutrient media plates, *Staphylococcus aureus* and *Bacillus* spp. were alleged respectively.

Table 2 Colonial and Morphological Characteristics of the Bacteria Isolates from Beard Hair Samples

Size	Colour	Shape	Texture	Bacteria
Small	Yellow	Round	Smooth	*Bacillus* spp.
Small	Off-white	Round	Smooth	*Staphylococcus aureus*

3.3 Biochemical Characteristics of Bacteria Isolates

Table 3 showed the biochemical characterization of the bacteria isolates from beard hair samples. From the Table *Bacillus* spp. and *Staphylococcus aureus* reacted positively to glucose, lactose, sucrose, catalase and citrate. They were however negative to indole test while they differed with reactions to methyl red, urease and Voges- Proskaurer tests.

Table 3 Biochemical Characteristics of Bacteria Isolates

Cat	Cit	Ind	MR	Ure	VP	Glu	Lac	Suc	Bacteria
+	+	-	-	-	-	+	+	+	*Bacillus* spp.
+	+	-	+	+	+	+	+	+	*Staphylococcus aureus*

Note: Cat= Catalase, Cit= Citrate, Ind= Indole, MR=Methyl Red, Ure=Urease, VP=Voges Proscaurer, Glu= Glucose, Lac=Lactose, Suc= Sucrose

3.4 Colonial and Morphological Features of Fungi Isolates from Beard Hair Samples

Table 4 showed the colonial and morphological characteristics of the fungal isolates in the beard hair samples. From the result, *Aspergillus* spp. had globose conidia that are rough walled while *Cladosporium* spp. had *Conidiophores* spp. that are more or less distinct from the vegetative hyphae. *Fusarium* spp. had clusters of thicken cell, walled round. Similarly, budding yeast-like cells, while *Candida* had unicellular cocci or ovoid shape, larger than bacterial cells.

Table 4 Colonial and Morphological Features of Fungi Isolates from Beard Hair Samples

Colonial Features	Morphological Features	Fungi
Dark brown colonies with white edges on the surface, with a touch of yellow.	Conidia are globose and rough walked, conidia heads are dark brown	*Aspergillus* spp.
Colonies are rather slow growing, mostly brown and powdery	Conidiophores are more or less distinct from the vegetative hyphae, erect, straight or flexous, unbranched or branched only in the apical region.	*Cladosporium* spp.
Slightly red, with clustered colonies	Clusters of thick-walled round, budding yeast-like cells and short angular hyphal forms	*Fusarium* spp.
Flat, smooth, milky large coloured colonies	Unicellular cocci or ovoid shape, larger than bacterial cells	*Candida* spp.

3.5 Microbial Spore Formation Features

Table 5, showed that the microbes namely; *Bacillus* spp. and *Aspergillus* spp. produces or formed spores unlike, *Cladosporium* spp., *Fusarium* spp., *Staphylococcus aureus* and *Candida* spp. that failed to form spores.

Table 5 Microbial Spore Formation

Microbes	Spore Sensitivity
Aspergillus spp.	+
Cladosporium spp.	-
Fusarium spp.	-
Candida spp.	-
Staphylococcus aureus	-
Bacillus spp.	+

4 DISCUSSION

The study showed the bacteria counts obtained from the beard hair samples differed from counts obtained in a study conducted by Edelbi et al. [3], were bacteria load in beard hair ranged between 30 x 10^4 and 200 x 10^6 cfu/ml. However, in study carried out by Edelbi et al. [3], the bacterial nomenclature was not pointed out in counts as presented in this study. Similarly, fungal and Staphylococcal counts in beard hair have not been adequately, reported as no literature exist. Basically, this study considered loads of Staphylococcal and Fungal counts which have not be reported, therefore the presence of Staphylococcal microbe may result to Staph infection as noted by Woodhouse [14] were it has been implicated in Folliculitis condition. The Folliculitis is a condition which leads to the development of small red or white bumps that resemble pimples. *Staphylococcus aureus* may have gotten into the beard through person to person contact or through the touch of contaminated surface. In recoveries of bacteria, the recovery of *Bacillus anthracis* in the beard hair may be attributed to the use of contaminated unsterilized razor brushes that may have been used for shaving as reported by Vazquez [15]. According to Vazquez [15] unsterilized razor brushes were identified route of *Bacillus anthracis* spore out-break. The *Bacillus anthracis* outbreak were due to deficient sterilization of brushes. Sterilization of razor brushes according to Prada [16] entailed immersion of the razor into 70% alcohol. This aimed at removing the microbes present on the blades and therefor presents the zazor fit for subsequent use. In fungi recoveries, *Aspergillus* spp., *Cladosporium* spp., *Conidiophores* spp. and *Aspergillus niger* spp., which were identified morphologically, have been pointed by Oliverira et al. [17] as fungi that manifest on human as a result of human exposure. Direct or indirect contact of the beard individual through touching of an infected lesions can transmit these fungi which later cause fungal infection likely in an opportunistic host. [17] The study did not identify the fungus *Malassezia* spp., a type of fungi responsible for dandruff diseases, hence the dandruff disease is likely not to emerge in this situation. *Bacillus* spp. and *Aspergillus* spp. which were noted to produce spore may be infective to the individual accidentally, as the spores are small and travel long distance as reported by Odebode et al. [5] Inhalation of a single spore by the beard individual or the public can lead to allergen responses. Basically, this study did not consider the infective levels of *Bacillus* spp. and *Aspergillus* spp. as reported by Eduard [18]. Eduard [18] showed fungal spore level of 4 x 10^3 CFU/ml for *Trichoderma harzianum* and noted the fungi insignificant, when compared to the fungi load obtained in this study. Similarly, fungi spores from asthmatic patients could be infective at 1 x 10^4 for patients allergic to *Penicillum* spp or *Alternaria alternate*. However, this study did not identify *Penicillum* spp or *Alternaria alternate* whose infectivity does not correspond with *Aspergillus* spp., *Cladosporium* spp., *Conidiophores* spp. and *Aspergillus* spp. The capacity of *Bacillus* spp. and *Aspergillus* spp. to express spore features can be linked to resistance of the microbe. According to Odebode et al. [5], *Aspergillus* spp. and *Bacillus* spp. have the ability to withstand harsh or severe environmental condition such as the application of chemicals and other stressor that may not be favorable. [5]

5 CONCLUSION

The study pointed out heavy loads of microbes on the beard hair of individuals who subjected their beard hair for analysis. Heterotrophic bacteria, heterotrophic fungi and staphylococcal counts obtained were insignificant across the recoveries. The microbes, *Staphylococcus aureus*, *Aspergillus* spp., *Cladosporium* spp. *Bacillus* spp., *Conidiophores* spp. and *Aspergillus* spp. were morphologically and biochemical identified. Following their identification, *Bacillus* spp. and *Aspergillus* spp. produced spore for which they persist in the beard individual, hence the emergence of diseases associated with the microbes.

6 RECOMMENDATION

The study recommends regular beard hair hygiene and the use of therapeutic agents on the beard hair to prevent *Bacillus* spp. and *Aspergillus* spp. spores. This definitely will go a long way in controlling spore dispersal.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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