**Original Article**

**Conjunctival Fungal Flora in a Tertiary Eye Hospital in Nigeria**

**Abstract**

**Objectives:** The aim of this study is to determine the hospital incidence and pattern of conjunctival fungal flora in adult patients at the Guinness Eye Center Onitsha, Nigeria. **Materials and Methods:** New adult patients, without anterior segment disease, were randomly recruited. Using a sterile swab stick, specimen was taken from the inferior conjunctival fornix of each participant’s right eye and inoculated into Sabouraud dextrose agar slant in a test tube and incubated at 27°C. The specimens were examined for fungal growth every 48 h for 4 weeks. Specimens with fungal growth were further examined under a high power microscope for fungal identification and characterization. **Results:** A total of 225 patients (105 males, 120 females) were examined. The age range was 18–75 years; mean age was 41±17.1 years; 62 (27.6%) were culture-positive: 25 (40.3%) were males and 37 (59.7%) were females (*P* >0.05). Both moulds and yeasts were isolated with moulds constituting 44 (74.2%). *Aspergillus* [26 (41.9%)] and *Candida* [16 (25.8%)] were the commonest organisms. Participants >60 years had the greatest burden. Pensioners (61.5%), traders (44.0%), farmers (30.1%), and artisans (27.3%) were occupational groups with significantly higher proportions of culture-positive specimens (*P* < 0.05). **Conclusion:** Over a quarter of new adult patients without anterior segment disease harbour fungi, some of which are pathogenic, in their conjunctival fungal organisms. While *Aspergillus* and *Candida* were the commonest isolates, older participants, pensioners, traders, farmers, and artisans had significantly higher proportion of culture-positive specimens. These findings should be considered when formulating pre-operative guidelines for ocular surgery in our environment.

**Keywords:** *Conjunctiva, flora, fungus, Nigeria*

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**Introduction**

Fungi are non-motile, multicellular organisms that exist in the form of either branching filaments or spores.[1] They are commonly found in the soil and in wet organic materials including plants. In humans, fungi could be found in mucous membranes and epithelial tissues where they reside as commensals and only cause infection when the mucous membrane or epithelium is breached.[1] They may infect the intact human skin or epithelium, especially in the immunocompromised.[1]

The ocular surface and adnexa are exposed to the external environment. They are therefore at risk of being colonized and infected by fungi. Ocular infections due to fungi had been reported in previous studies in Nigeria.[2,3] Gugnani *et al.*[2] reported a seasonal variation in the incidence of fungal keratitis in Nigeria. A previous study of corneal ulcers at the Guinness Eye Centre Onitsha reported that 11.6%

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were due to fungal infection.[3] Yeasts such as *Candida* species and *Malassezia furfur* may inhabit the normal eye lid margin, whereas filamentous fungi could colonize the normal conjunctiva.[4]

The conjunctival surface being made wet by the tear film is favourable for fungal colonization with or without clinical infection.[4] The worry about fungal conjunctival flora, especially in the absence of clinical infection, is that during ocular surgery, fungus could be inadvertently inoculated into ocular tissues with devastating consequences.

Studies on conjunctival fungal flora had been conducted in Sierra Leone,[5] India,[6] USA,[7] Japan,[8] and UK.[9,10] The results of these studies varied from 2.9% to 27.4% of hospital incidence of fungus isolation from healthy conjunctival specimens.[5-10] Some of these studies also reported an increasing incidence of fungal conjunctival flora with increasing age of the study subjects.[6,7,10] To the best of the authors’ knowledge, there has not been any report on the study of fungal

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conjunctival flora in our environment. In an attempt to fill this knowledge gap, the present study was embarked upon. This article aims at reporting the hospital incidence and pattern of conjunctival fungal flora at the Guinness Eye Centre Onitsha, Nigeria.

**Materials and Methods**

This was a study of adult Nigerians (aged ≥ 18 years) seen at the Guinness Eye Centre Onitsha between August and October 2012. The study was approved by the Ethical Committee of the Nnamdi Azikiwe University Teaching Hospital, Nnewi. The minimum sample size based on 95% confidence interval was calculated using the modified Leslie–Kish formula[11] for population less than 10,000. The laboratory could handle only 15 specimens per day.

The study participants were consenting new adult patients who were without ocular or adnexal infection. Excluded were new patients younger than 18 years; new adult patients with ocular and/or adnexal infections; those with ocular trauma; and those who had used topical antimicrobial agents or topical traditional eye medicine (TEM) in the 3 weeks preceding recruitment.

The participants were selected by simple random sampling, viz: new adult patients presenting on each clinic day were assembled and given health talk. The objectives of the study, benefits including voluntary participation, were explained. The patients were numbered serially. A 2 cm by 2 cm piece of paper was used to write patients’ numbers. The papers were folded, dropped in a bag, and churned. From the bag, an assistant, naive to the numbering, picked out 15 folded papers. Patients with the corresponding numbers were thus selected.

Fungal identification was as follows: From each culture-positive specimen, a piece of mycelium mat was removed using a wire loop and teased in a drop of lactophenol cotton blue stain on the surface of glass slide; the slide was then covered with a glass slip cover and examined under ×10 and ×40 microscopic magnification. The final identity of the isolate was determined using the criteria of Campbell and Stewart[12] and Larone.[13] Data obtained were analysed using descriptive and inferential statistics (χ2 test) with an alpha value at 0.05.

**Results**

Of the 225 participants, 105 (46.7%) were males and 120 (53.3%) were females. Table 1 shows the age distribution of the participants. The age range of the participants was 18– 75 years and the mean age was 41±17.1 years. There was a bimodal peak in the 21–30 year and 51–60 year age brackets, both of which constituted 51.1% of the participants. Table 2 shows the occupation of the participants. Farmers and students constituted 137 (60.8%) participants; pensioners were the least [13 (5.8%)].

Sixty-two specimens were culture-positive, thus giving an incidence of 27.6% among the participants; 25 (40.3%) males vs. 37 (59.7%) females were culture-positive. However, the difference was not statistically significant (*χ*2 *=* 1.38; df *=* 1; *P* >0.05). *Aspergillus* [26 (41.9%)] and *Candida* [16 (25.8%)] were the commonest isolates [Table 3]. Apart from *Candida* which is classified as yeast, all the other isolates [44 (74.2%)] were moulds.

Table 4 shows a proportion of fungal isolates in relation to participants’ age. Compared with other age groups, the

Each participant’s sociodemographic profile was documented. The anterior segment and ocular adnexa were assessed with a pen-torch and a slit-lamp biomicroscope to exclude infections and signs of previous surgery or trauma. The posterior segment assessment was by direct ophthalmoscopy.

The conjunctival swab was obtained as follows. Under strict aseptic conditions, sterile swab (Sterrin, Lot No. 120308) was used to obtain specimen from the inferior conjunctival sac of each participant’s right eye. After specimen collection, topical anesthetic (4% proparacaine, Ashford Laboratories) was instilled into the eye to minimize discomfort. The specimen was immediately sent to the laboratory.

**Age (years)** ≤20

21–30 31–40 41–50 51–60 61–70 ≥71 Total

**Occupation** Farmers Students

**Table 1: Age distribution**

**No.** **%** 28 12.4 60 26.7 25 11.1 27 12.0 55 24.4 26 11.6

4 1.8 225 100.0

**Table 2: Occupation**

**No.** **%** 73 32.4 64 28.4

In the laboratory, the specimen was processed as follows: Each specimen was inoculated into Sabouraud dextrose agar slants of two test tubes; incubation was at room temperature (27oC). The tubes were examined for fungal growth every 2 days for 4 weeks. After 4 weeks, the specimen was discarded if there was no growth.

Civil servants 28 12.4 Traders 25 11.2 Artisans\* 22 9.8 Pensioners 13 5.8 Total 225 100.0

\*Artisans = bricklayer, mechanic, driver, tailor, and so on

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61–70 age bracket had a significantly higher proportion of fungal isolates (*χ*2 *=* 17.72; df = 6; *P* < 0.05). However, the age-specific burden of fungal conjunctival flora expressed as percentage of culture-positive cases showed that older participants aged 61 years and above had the greatest burden [Table 4]. Table 5 shows participants’ occupation vs. culture-positive specimens. Farmers had the highest number of culture-positive specimens (*χ*2 *=* 16.49; df = 5; *P* < 0.05). However, the occupation-specific burden of fungal conjunctival flora expressed as percentage of culture-positive cases showed that pensioners, traders, farmers, and artisans were at the greatest risk [Table 5].

**Discussion**

The results of this study confirm the suspicion that some healthy-looking conjunctiva of adult Nigerians harbour fungi, some of which are potentially pathogenic. The 27.6% culture-positive rate obtained in the present study is comparable to the 27.4% documented by Ainley and Smith in the UK,[9] 26.0% recorded by Capriotti *et al.* in

**Table 3: Fungal isolates**

**Isolate** **No.** **%** *Aspergillus* 26 41.9 *Candida* 16 25.8 *Mucor* 9 14.5 *Scopulariopsis* 5 8.2 *Acremonium* 3 4.8 *Rhizopus* 2 3.2 *Paecilomyces* 1 1.6 Total 62 100.0



Sierra Leone,[5] and 25.4% obtained by Fazakas[14] in Central Europe. However, lower incidence rates were obtained by Hammeke and Ellis[7] (10.2%) in the USA and Ando and Takatori[8] (6.6%) in Japan.

Several studies suggest that gender, age, occupation, domicile, and weather conditions influence the incidence and pattern of conjunctival fungal flora and fungal infections.[2,7,10,14-18] Other predisposing factors include use of cosmetics, steroids, and TEM.[3,6,10,19] Although the present study was conducted only in adults, the influence of some of these factors was nonetheless noticeable. Females, farmers, and participants >60 years had a greater burden of fungal culture-positive specimen; the occupation-specific burden of conjunctival fungal flora expressed as percentage of culture-positive cases showed that pensioners, traders, farmers, and artisans were at the greatest risk [Table 5].

Our findings are comparable with the results of the previous studies. A study by Williamson *et al.*[10] reported that the incidence of conjunctival fungal flora increased in older subjects. Similarly, Hammeke and Ellis[7] in a study involving both children and adults documented fungal culture-positive specimens in 10.2% of adults, 5% of children and 0.1% of neonates. Saxena and Goswami[6] reported that females were twice as likely to harbour microbes including fungi in the conjunctiva than males. The authors attributed this to the use, by females, of mascara and other cosmetics, which might have been contaminated by fungi. *Candida parapsilosis* had been grown from mascara.[20] Fazakas[14] in a study of rice harvesters and threshers reported that 83% of the external eye and adnexa (lids, conjunctival sac and lacrimal passages) were fungal culture-positive.

**Table 4: Age vs. culture-positive specimens**

**Age (years)** ≤20

21–30 31–40 41–50 51–60 61–70 ≥71 Total

**Total participants** 28 58 25 27 50 26 11

225

**Culture-positive (%)** 8 (12.9) 11 (17.7) 5 (8.1) 6 (9.7)

10 (16.1) 16 (25.8) 6 (9.7)

62 (100.0)

**Age-specific culture-positive (%)** 28.6 19.0 20.0 22.2 20.0 61.5 54.5

*χ*2 = 17.72; df = 6; *P* < 0.05

**Table 5: Occupation vs. culture-positive specimens**

**Occupation** Farmers Students Civil servants Traders Artisans Pensioners Total

**Total participants** 73

64 28 25 22 13 225

**Culture-positive (%)** 22 (35.5)

10 (16.1) 5 (8.1) 11 (17.7) 6 (9.7)

8 (12.9) 62 (100.0)

**Occupation-specific culture-positive (%)** 30.1 15.6 17.9 44.0 27.3 61.5

*χ*2 = 16.49; df = 5; *P* < 0.05

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The deleterious effects of inappropriate use of steroids and TEM on the eye had been documented in various studies in Nigeria.[3,19] A study of corneal ulcers at the Guinness Eye Centre Onitsha had documented that 11.6% were fungal culture-positive.[3] Up to 34% of the patients in that study had used topical steroids and TEM.[3] In Benin City, Ukponmwan and Momoh[19] reported that TEM use was responsible for 8% of corneal ulcers. The present study differs from the studies cited earlier as it was conducted among participants without clinical external ocular infection or disorder and participants who had not used topical medication in the 3 weeks preceding enrolment into the study.

In India, Rao and Rao[15] observed that fungal colonization of the conjunctiva tends to be higher in dry, cool weather. In Nigeria, Gugnani *et al.*[2] observed two peaks, namely, February–March and November. These peaks corresponded to agricultural planting and harvesting seasons, respectively. Our study was conducted during the rainy season (August– October) when significant farming activities still go on. Currently, mechanized farming is not widely practised in Nigeria. Most farmers in Nigeria are still at the subsistent level using basic implements such as hoe and machete with persistent exposure of the facial structures to dust, soil, and vegetable matter.

The present study isolated seven different types of fungi. *Aspergillus* and *Candida* made up more than two-thirds with *Aspergillus*alone constituting 41.9% [Table 3]. Separate studies by Jones *et al.*[21] in the UK, Soleymani *et al.*[22] in Iran, and Dasgupta *et al.*[23] in India also had documented the preponderance of *Aspergillus* in the healthy conjunctiva of their study subjects. However, this is different from the findings of Liesegang and Foster[24] in the USA who reported*Fusarium solani* as the commonest fungi. None of the participants in the present study was culture-positive for *Fusarium*. But this does not necessarily translate to the absence of *Fusarium* species in our environment. A review of mycotic keratitis in Enugu, Nigeria reported *F. solani* as the predominant fungus.[2] But our study differs from that of Gugnani *et al.*[2] in Enugu in two important respects, namely, ours was a cross-sectional study and specimens were taken from healthy conjunctiva, whereas the work of Gugnani *et al.* was on persons with infected conjunctivae and corneas.

Potentially pathogenic organisms, such as *Aspergillus* and *Candida*, as part of the conjunctival flora increase the risk of infection. External ocular infection is an important cause of blindness in Nigeria.[25-27] In Anambra State, Nigeria, complications of external eye infections was responsible for 9.9% of the blindness.[25] Ayanru[26] in Benin City reported that corneal opacity complicating infective keratitis was the commonest indication for penetrating keratoplasty. The Nigeria National Blindness and Visual Impairment Survey[27] reported that 12% of blindness was

due to corneal opacity, most of which were secondary to infections. It bears to emphasize that fungus is not the only cause of external ocular infection. Bacteria, virus, and parasites are also culprits. While the present study is on conjunctival fungal flora, a previous study in our hospital had also determined conjunctival bacterial commensals and some of the organisms isolated were potentially pathogenic.[28]

The strength of the present study lies in its being conducted among participants without anterior segment infection and also with careful exclusion of confounding variables such as use of agents that may encourage the proliferation of fungi in the participants’ conjunctiva. Adrawback is that this was a hospital-based study with its inherent patient selection bias. These participants had some ocular complaints that necessitated their hospital visit, even though the disorder may not be in the anterior segment. Therefore, they could be considered different from normal inhabitants of their communities. Secondly, the antimicrobial sensitivity of the isolated fungi was not tested. It was therefore not possible to determine which available antifungal agents best cleared the organisms from the conjunctival sac. A population-based study incorporating anti-microbial sensitivity tests would address these weaknesses.

In conclusion, more than a quarter of new adult patients without anterior segment disease harbour fungi in their healthy-looking conjunctiva. Some of these fungi are pathogenic. While *Aspergillus* and *Candida* were the commonest fungal isolates, pensioners, traders, farmers, and artisans had a significantly higher proportion of culture-positive specimens. These findings should be considered when formulating pre-operative guidelines for ocular surgery in our environment.

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**Conflicts of interest**

There are no conflicts of interest.

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