The Prevalence of Fungal Infections in Six Communities in Akwa Ibom State Nigeria

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Abstract

BACKGROUND

Invasive fungal infections have emerged worldwide as the cause of opportunistic infections. Hospital-acquired fungal infection has also risen over the past decade since untreated fungal infections render patients to life-threatening conditions. This study aimed at determining the prevalence of fungi infection in six communities in Akwa Ibom state, Nigeria.

MATERIALS AND METHODS

An experimental-based cross-sectional study design was used to collect random samples of 2991 from children aged 5-16years and 2240 samples from adults (20-80years and above) between May 2018 and October 2018. The samples were collected from the skin scales, crust, nail pieces and hair of individuals living in six communities namely: Essien udim, Abak, Ikot ekpene, Uyo, Nsit Ubium, Eket and Oron. The sample size of 5234 was determined using a single-stage cluster sampling technique. The samples collected were subjected to direct microscopy examination and cultured on Sabouraud dextrose agar and incubated at room temperature (25 – 37ºC) for 3-5 days. Fungal isolated were preliminarily identified by cultural methods, purified and further confirmed by genotypic methods.

RESULTS

Results obtained showed that out of the 5234 individuals examined (children and adults), 3010 (57.5%) were found to be mycologically positive lesions while 2224 (42.5%) were mycologically suggestive lesions. The present study reveals that the prevalent rate of fungal infection was 934 (31.3%) out of the total number of individuals sampled. This corresponds to individuals between the age of 10 -13 years in all three Local Government Areas (LGAs). Data obtained from the study were tested using the Chi-Square test of independence to determine if mycologically positive lesions in all three LGAs were independent of the age group and it gave a p-value of 0.47 at a 5% level of significance. This showed that the prevalence rates across various age groups were independent of the study locations. The study also reveals that fungal infections were predominant in individuals between the age ranges of 5 – 13 years and those beyond 80 years. Individuals between the ages of 40-49 years had the lowest prevalence rate of 63 (2.1%) across all three LGA. Since all three LGAs were thickly dense areas of the three senatorial districts of Akwa Ibom State, they showed a high level of prevalence of fungi infection for individuals between ages 5 – 13 years. Nine fungal species which include *Rhizopus oryzae, Aspergillus tamaril, Tricholoma matsutake, Kodamaea ohmeri, Aspergillus awamori, Aspergillus fumigatus, Aspergillus nomius, Aspergillus awamori* and *Aspergillus nomius* were identified by genotypic methods.

CONCLUSION

The study revealed that fungal infections are predominant in highly populated areas and vary from one location to the other. The infections were more among age groups of children between the ages of 5-13. *Aspergillus spp.* was the most predominant fungi identified in the present study. There is a need for further studies on the prevalence of fungal infection that would include more geographical regions compared to the present study.

Keywords: *Fungi, Incidence, Microscopy, Prevalence, Suggestive Lesion*

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Introduction

Fungal diseases are life-threatening and are responsible for the silent epidemic, often hidden killers causing morbidity and mortality in susceptible individuals [1]. Invasive fungal infections have emerged worldwide as an increasingly frequent cause of opportunistic and co-infection [2, 3, 4]. Fungi diseases are responsible for more than 1.5 million deaths worldwide even though these deaths from fungi diseases are preventable. With these increasing deaths, fungi diseases are still neglected especially in developing countries [5]. Nigeria is a populous country with a high incidence of fungal infections, especially in rural communities. It was estimated by Oladele and Denning [6] that over 11.8% of the Nigerian population is estimated to suffer from a serious fungal infection each year. Fungal infections are a major public health problem especially in school-age children in rural communities due to overcrowding, poor hygiene and poverty [7]. Some fungi can cause severe diseases in humans, which include *Aspergillosis*, *Candidiasis*, *Coccidioidomycosis*, *Cryptococcus*, *Histoplasmosis*, *Mycetomas*, and *Paracoccidioidomycosis*. People living with underlying illnesses such as immune deficiencies, asthma, cancer and organ transplant are susceptible to disease by fungi such as *Aspergillus*, *Candida*, *Cryptococcus Histoplasma*, and *Pircumocystis* [8, 9, 10]. Other fungi can attack eyes, nails, hair, and especially the skin, dermatophytic and keretinophilic fungi can cause local infections such as ringworm and athletic foot. Fungal spores are also a cause of allergies, while others can evoke allergic reactions [11]. Early and accurate detection can aid the detection of fungal infections which allows prompt therapy thus reducing the mortality rate and preventing chronic fungal infections [5]. The prevalence of superficial mycosis was reported in Uyo, Akwa Ibom state Nigeria in a clinical survey on Dermatophytosis among school children in the region, and the infection rate was higher for male children and relatively low for female children [12]. Sang-Ha [13] working on the epidemiological characterization of skin fungal infections between the years 2006 and 2010 in Korea showed that ages 60-70 years had the highest prevalence rate of fungi infection, compared to ages 20-30 years; stating that the ages increased with age. A clinical survey on the prevalence pattern of superficial fungal infections among school children in Ile-Ife, South-West Nigeria reported the high prevalence rate of fungi infection in children and linked it to the sharing of barbing tools, frequent exposure to outdoor activities, poor hygiene and overcrowded living [14]. The incidence of fungal infection varies from one community to the other within Nigeria. These variations observed in fungal infection may be due to differences in climatic and environmental conditions of the areas being studied [15, 16]. A review carried out in Zimbabwe revealed that 14.9% of Zimbabweans suffer from fungal infections annually, with 80% having *tinea capitis*, which was higher than in other African countries [17]. The study is therefore aimed at determining

the prevalence of fungi infections in six communities in Akwa Ibom state, Nigeria.

Materials and Methods

Study design

An experimental-based cross-sectional study design was used to collect random samples of 5234 individuals consisting of 2991 children aged 5-16 years and 2240 samples from adults (20-80 years and above) between May 2018 and October 2018. The samples were collected in six thickly populated urban and rural communities in Akwa Ibom State. The communities investigated were located in Uyo, Nsit Ubium, Eket and Oron, Ikot Ekpene and Essien Udim Local Government Areas which cover the three senatorial districts in the state. The choice of the communities was based on previous reports and recorded history of fungi infections in Akwa Ibom State.

Sample size

Single-stage cluster sampling technique was employed to determine the sample size. The population of interest were local government areas in three senatorial districts of Akwa Ibom State. The population was divided into sample units or clusters based on age groups in these LGAs. Finally, three sample clusters were randomly selected using simple random sampling (SRS) for use in the study. The initial sample size was determined by considering the precision level, confidence or risk level, and the degree of variability in the attributes being measured. The formula for calculating the initial sample size is based on Cochran's formula given: $\text{Sample size} = \frac{Z_{1-\propto}{}^2 \times P(1-P)}{d^2}$ [28].

Where $Z_{1-\propto}$ = Standard normal variate. $Z_{1-\propto}$ was taken in this study to be 1.96 at a 5% level of significance. The d = margin of error or confidence level which was taken as 0.05 for this study.

P = Estimated prevalence or Proportion in the population estimated based on previous or pilot studies. P was calculated as:

$$P = \frac{\text{number of mycological positive}}{\text{total number of samples}} = \frac{3010}{5234} = 0.5751 \cong 57.5\%$$

Therefore, initial sample size = $\frac{1.96 \times 0.575 \times (1-0.575)}{(0.05)^2} = 374.3255 \cong 374.$

Since the cluster sampling technique was used, the effect of intra-cluster correlation (ICC) on the sample size was considered; this is called the design effect designated as Deff [29]. The design effect is a factor that is used to adjust the sample size in cluster sampling and is usually multiplied by the initial sample size obtained from simple random sampling (SRS) to get the required sample size [27,30,31]. A correction on the sample size was made based on seven strata (5-9, 10 -13, 4-16, 20-29, 30-39, 40-49, 80 & above by age), and a design effect of 2.0 as follows:

Corrected sample size = Initial sample size $\times$ Deff $\times 7 = 374 \times 2 \times 7 = 5236$ which is approximately equal to the 5234 sample size used for the work [27].

Physical examination of mycological sample sites

Each child was stripped and examined physically with the help of a hand lens from the head to foot under light to separate infected children from non-infected ones. All those with fungi infections were identified for sampling.

Data collection technique

Mycological sample data was collected from four different sites infected which are the skin scales, crust, nail pieces and hair. A total of 5234 samples were collected of which 2991 were from children and 2243 from adults. These samples were collected from Prisons, primary schools, rehabilitation centres and hospitals within the selected communities. Before collection, all lesions were swabbed with sterile cotton wool soaked in 40% (w/v) ethanol or methylated spirit. Fluid samples were collected in a sterile container and transported to the laboratory.

All the specimens were collected with a high level of precision and aseptic measures were ensured to minimize contamination and misinterpretation of results. Samples from hair were collected by removing the dull broken hairs from the margin of the lesion using sterile tweezers or scraping the scalp with a blunt scalpel. Clinical samples were collected from patients with evidence of different fungi infections.

Mycological Studies: Direct Examination

A portion of each clinical specimen was transferred to a clean slide. A drop of 10% potassium hydroxide (KOH) was added to the specimen and covered with a clean cover slip for 10 – 30 minutes to be softened and cleared for easy identification. The preparation was gently warmed over the flame. Nail clippings and hair scrapings were similarly mounted in 40% (KOH). Microscopic examination of these slides was done using the low power (X40) objectives hens to detect the presence of hyphae and arthrospores. In the case of materials from the scalp and lesions, the pattern of hair infection (that is ectothrix or endothrix) was also noted. In the case of allergic and invasive aspergillosis sputum is usually gelatinous and thick and contains blood. It requires a mucolytic agent before the examination can be made. A portion of the sputum was transferred to a clean slide, and a drop of 40% potassium hydroxide (KOH) was added to the specimen mixed and covered with a cover slip for 10-30 minutes to be softened and cleared then warmed gently over a flame.

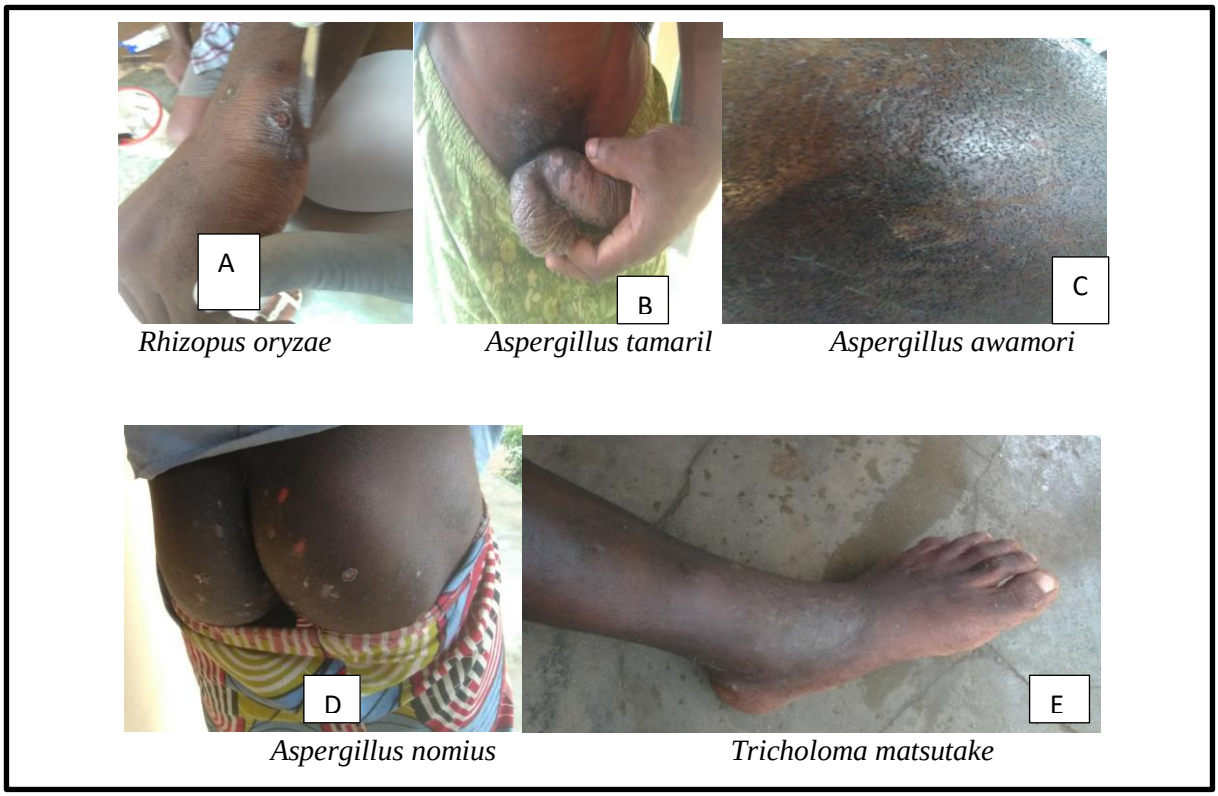

Figure 1:
Image of some samples collected

Microscopic examination of these slides was done using (x40) objective lens to detect the presence of dichotomously branching septate hyphae of uniform width conidiophore and radiating chains of conidia V-shape branching (45% angle) for mucormycosis sample were gotten from nasal discharge, and transferred into a sterile slide, a drop of 10% potassium hydrogen peroxide (KOH) was added to the specimen and covered with a clean cover slip for 5-15 minute, the preparation was view microscopically using (x40) objective lens to detect the presence of brad retractile hyphae (10-15µm) diameter with uneven thickness irregular branching and spare septations [18].

Isolation of Fungal Infections

Fungi were isolated by culturing on Sabauraud's dextrose agar (SDA). The culture medium (SDA) was prepared according to the manufacturer's instructions and supplemented with chloramphenicol 0.05mg/ml and cycloheximide 0.5mg/ml to inhibit bacterial and saprophytic fungal contamination. A portion of each specimen was inoculated on two sets of 3 plates of sterile SDA plates, one set supplemented with chloramphenicol (0.05mg/ml) and the other set containing chloramphenicol and cycloheximide (0.5mg/ml). All plates were incubated at room temperature (25 – 37ºC) for 3-5 days and examined periodically for growth development. Fungal isolates were purified according to the methods recommended [18]. The culture plates were incubated at 25 – 37ºC for 96 hours after which discrete colonies were subcultured into freshly prepared SDA slant. Pure cultures were inoculated onto a MacCartney bottle containing freshly prepared corn meal agar positioned in a slant position and incubated for 96 hours at room temperature (25 – 37ºC). After profuse growth, it was preserved by refrigerating

Characteristics and identification of fungal isolates

Fungi isolates were identified using cultural characteristics and colonial morphology of the isolates. The characteristics studied were colour, odour, surface and reverse appearance, texture, topography and the shape and sizes of the reproductive spores, as well as the different hyphae structures. A portion of each colony was placed on a clean slide with the aid of a sterile pin, and a drop of lactophenol cotton blue was added. Clean coverslips have applied a flame before examining them under the microscope using both low and high-power magnifications. The characteristic features of the hyphae, conidia, chlamydospore, conidiophore, sporangiospores, microaleunospores and macroaleurispores were detected. Cultural characteristics of most pathogenic Aspergilla have an optimum growth at 37ºC which inhibits the same fungal contaminants. *Aspergillus fumigates* grow at 45ºC at 37ºC conidiophores characteristics of the organism are present within 41hours. *Rhizopus oryzae* grows at a temperature as low as 25ºC to a maximum of 37ºC. Growth is rapid and usually noticeable 12 to 18 hours after incubation.

Genetic identification of fungal isolates: DNA extraction

Extraction was done using a ZR fungal/bacterial DNA mini prep extraction kit supplied by InqabaSouth Africa. Heavy growth of the pure culture of the suspected isolates was suspended in 200 microliters of isotonic buffer in a ZR Bashing Bead Lysis tube, and 750 microliters of lysis solution were added to the tube. The tubes were secured in a bead beater fitted with a 2ml tube holder assembly and processed at maximum speed for 5 minutes. The ZR bashing bead lysis tubes were centrifuged at 10,000xg for 1 minute. Four hundred (400) microlitres of supernatant were transferred to a Zymo-Spin filter (orange top) in a collection tube and centrifuged at 7000xg for

1 minute. One thousand two hundred (1200) microlitres of fungal/bacterial DNA binding buffer was added to the filtrate in the collection tubes bringing the final volume to 1600 microliters, 800 microliters were then transferred to a Zymo-Spin IIC column in a collection tube and centrifuged at 10,000xg for 1 minute, the flow through was discarded from the collection tube. The remaining volume was transferred to the same Zymo-Spin and spun. Two hundred (200) microliter of the DNA pre-was buffer was added to the Zymo Spin IIC in a new collection tube and spun at 10,000xg for 1 minute followed by the addition of 500 microliters of fungal/bacterial DNA wash buffer and centrifuged at 10,000xg for 1 minute. The Zymo-spin IIC column was transferred to a clean 1.5 microliter centrifuge tube, and 100 microliters of DNA elution buffer was added t the column matrix and centrifuged at 10,000xg microliter for 30 seconds to elute the DNA. The ultra-pure DNA was then stored at 20 degrees for other downstream reactions.

DNA quantification

The extracted genomic DNA was quantified using the nanodrop 1000 spectrophotometer. The equipment was initialized with 2ul of sterile distilled water and blanked using normal saline. Two microliters of the extracted DNA were loaded onto the lower pedestal; the upper pedestal was brought down to contact with the extracted DNA on the lower pedestal. The DNA concentration was measured [19].

Internal transcribed spacer (ITS) amplification

The ITS region of the isolates was amplified using the ITSIF: primers on an ABI 9700 applied Biosystems thermal cycler at a final volume of 30 microliters for 35 cycles. The PCR mix included: the x2 Dream Taq Master mix supplied by Inqaba. South Africa (Taq polymerase, dNTPs, $MgCl_2$), the primers at a concentration of 0.4M and the extracted DNA as a template. The PCR conditions were as follows: Initial denaturation, 95^0C for 5 minutes: denaturation, 95^0C for 30 seconds; annealing, 53^0C for 30 seconds; extension, 72^0C for 30 seconds for 35 cycles and final extension, 72^0C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 15 minutes and visualized on a blue light transilluminator.

Sequencing

Sequencing was done using the big Dye Terminator kit on a 3510 ABI sequencer by Inqab Biotechnological, Pretoria South Africa. The sequencing was done at a final volume of 10ul, the components included 0.25ul BigDye terminator vl.1/v3.1, 2.22ul of 5 x BigDye sequencing buffer, 10uM Primer PCR primer, and 2-1ong PCR template per 100bp. The sequencing conditions were as follows 32 cycles of 96^0C for 10s, 55^0C for 5s and 60^0C for 4min [20].

Phylogenetic analysis

Obtained sequences were edited using the bioinformatics algorithm trace edit, similar sequences were downloaded from the National Center for biotechnology information (NCBI) database using BLASTN. These sequences were aligned using MAFFT. The evolutionary history was inferred using the Neighbor-joining method in Mega 6.0.[21] The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method [20, 21].

Statistical analysis

The mycological sample data collected were analyzed using the Chi-square test of independence on the Microsoft Excel platform to test if the number (and hence the prevalence rate) of mycologically positive lesions in all three LGAs was independent of the age group considered for the study.

Ethical considerations

An ethical approval letter with the number MH/PRS/99/VOI.V/515 was obtained from the Ministry of Health Idongesit Nkanga Secretariat Uyo on the 5th of July 2019. A consent Letter with the number UPH/MCB/POL/061 for this study was obtained and sent from the University of Port Harcourt to the University of Uyo.

Results

Out of a total number of individuals examined (children and adults), some were found to be mycologically positive lesions while others were mycologically suggestive lesions. The prevalence rate of individuals with mycological positive lesions in Nsit Ubium and Uyo LGAs was highest in individuals of the age range 10-13(30%), followed by 5-9 (23%), 80 and above (20%), 20-29(15%), 14-16(7%) and 30-39(3%). The least prevalence in Nsit Ubium and Uyo LGAs was observed in the age range 40-49(2%). For Eket and Oron LGAs, the prevalence rate was highest in individuals of the age range 10-13(30%), followed by 80 and above (22%), 5-9(21%), 20-29(16%), 14-16(7%) and 30-39(3%).

Table 1:
Incidence of Fungal Disease across Different Age Groups in the Chosen Locations

Age	No. Examined	Nsit Ubium and Uyo		Eket and Oron		Ikot Ekpene and Essien Udim		Total
		Mycological suggestive	Mycological positive	Mycological suggestive	Mycological Positive	Mycological suggestive	Mycological positive	
5-9	1132	159(19.9%)	219(23.1%)	152(30%)	225(21.4%)	133(18.1%)	244(24.1%)	1132
10-13	1510	222(27.9)	282(29.7%)	192(27.8%)	312(29.7%)	162(22%)	340(33.6%)	1510
14-16	349	54(6.8%)	62(6.5%)	46(6.7%)	70(6.7%)	49(6.7%)	68(6.7%)	349
20-29	789	116(14.6%)	146(5.4%)	97(14%)	165(15.7%)	111(15.1%)	154(15.2%)	789
30-39	155	24(3%)	26(2.7%)	23(3.3%)	27(2.6%)	25(3.4%)	30(3%)	155
40-49	142	28(4%)	19(2%)	26(3.8%)	22(2%)	25(3.4%)	22(2.2%)	142
80 & above	1157	194(24.3%)	194(20.5%)	155(22.4%)	230(2.9%)	231(31.4%)	153(15.1%)	1157
Total	5234	797(100%)	948(100%)	691(100%)	1051(100%)	736(100%)	1011(100%)	5234

Table 2:
A contingency table (reproduced from Table 1)

Age	Number Examined	Nsit Ubium and Uyo	Eket & Oron	Ikot Ekpene & Essien Udim	Total
		Mycological Positive	Mycological Positive	Mycological Positive	
5-9	1132	219	225	244	688
10-13	1510	282	312	340	934
14-16	349	62	70	68	200
20-29	789	146	165	154	465
30-39	155	26	27	30	83
40-49	142	19	22	22	63
80 & above	1157	194	230	153	577
Total	5234	948	1051	1011	3010

The least prevalence in Nsit Ubium and Uyo LGAs was observed in the age range 40-49 (2%). For Ikot Ekpene and Essien Udim LGAs, the prevalence rate was highest in individuals of the age range 10-13(34%), followed by the following age ranges: 5-9(24%), 80 & above and 20-29(15%), 14-16(7%) and 30-39(3%). The least prevalence rate across all three LGAs was observed in the age range of 40-49 (2%). The summary of the prevalence and percentage distribution of mycological positive cases and clinically suggestive lesions for both the children and adult age groups across the three LGAs are presented in Table 1 and Figure 2. The Chi-square test of independence was used to test if the number (and hence the prevalence rate) of mycologically positive lesions in all three LGAs was independent of the age group considered for the study. Table 1 shows the incidence of fungal disease in the studied locations. Table 2 shows the contingency table used to determine the expected frequencies of each data cell. Appendix 1 and 2 show the calculation of frequencies and the Chi-square Test table used to find the p-value, test statistic and critical value. The result of the analysis gave a p-value of 0.47 which was greater than the significant level of 0.05, hence it was concluded that the number (and hence the prevalence rate) of mycologically positive lesions in all the three LGAs was independent of the age group at 0.05 significant level.

Discussion

Microorganisms are ubiquitous in nature which makes them found everywhere. Therefore, fungal species are not exempted [6] working on the "Burden of serious fungal infection in Nigeria' estimated that over 11.8% of the Nigerian population suffers from a serious fungal infection each year. Overcrowded living, cold humid climates, and poor sanitary conditions promote the spread of fungi. Fungal infections can cause great discomfort to humans presenting symptoms of mild infections such as irritation, scaly skin, Redness, swelling, itching and blisters. Severe symptoms may also occur which include chronic dilation of air passages, the collapse of segments of the lungs, coughing of blood, infarction and haemorrhage, endocrine disorder and neutropenia and if left untreated can be fatal [22].

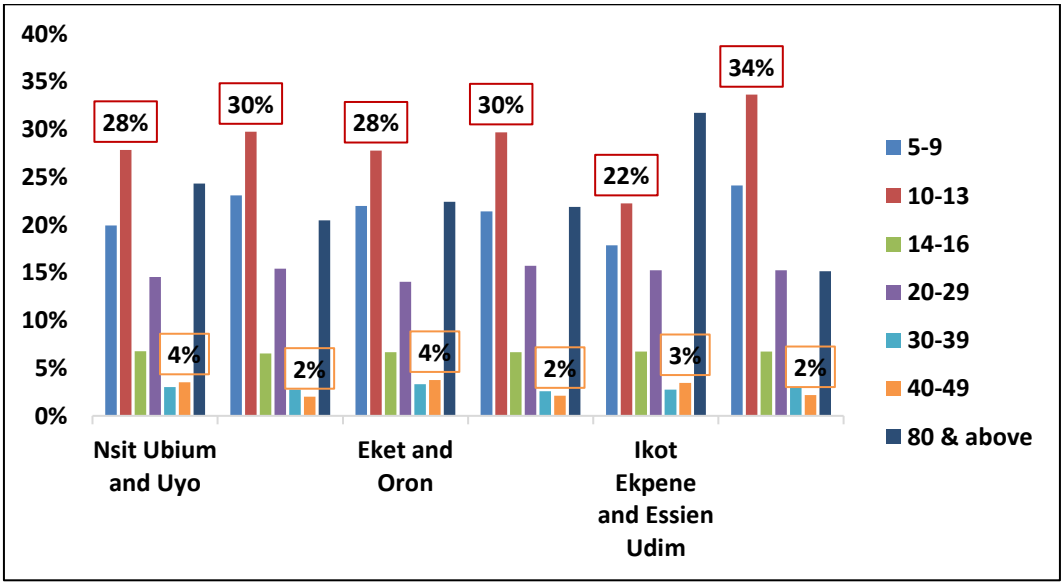

Figure 2:
Percentage distribution of fungal diseases by age among different individuals in the study locations

The present study reveals the prevalence of fungal infection with mycological positive cases to be 3010 (57.5%) and clinical suggestive lesions to be 2224(42.5%) of the total number of 5234 samples obtained. The present study reveals the highest average prevalence rate of fungal infection is found to be 934(31.3%) out of the total number sampled and this corresponds to individuals between the age of 10-13 years in all the three LGAs. This was followed by a prevalence rate of 688(22.9%) for individuals between 5 – 9 years, 577(19.2%) for individuals from 80years and above, 465(15.4%) for individuals between 20 – 29 years, 200 (6.6%) for individuals between 14 – 16 years and 83(2.8%) for individuals between 30 – 39 years. The least prevalent rate of fungi infection was found to be in adults in the age range of 40-49 years and this corresponds to 63(2.1%). The trend of the prevalence rate of fungal infections from the study shows that children between 5 – 13 and adults 80 years and above had the highest risk of being affected with fungal infections in all three LGAs. Low levels of fungistatic fatty acids, infected barbing instruments and exposure to fungi infection during play may be reasons for the high prevalence rate in children between the age of 5 - 13 years [14, 25]. The study also showed that individuals between the ages of 40–49 had the lowest risk of being infected with fungal infections. In addition, there was a high risk of being infected with fungal infections in all three LGAs since the areas considered for the study were thickly dense. The fungal isolates identified in the present correlate with other findings where they can also occur as coinfection [32]. *Aspergillus* spp was the most prevalent fungi identified in the study, it is responsible for aspergillosis. Aspergillosis is one of the most common opportunistic fungal co-infections caused by some *Aspergillus* spp., which particularly affects immunocompromised persons.

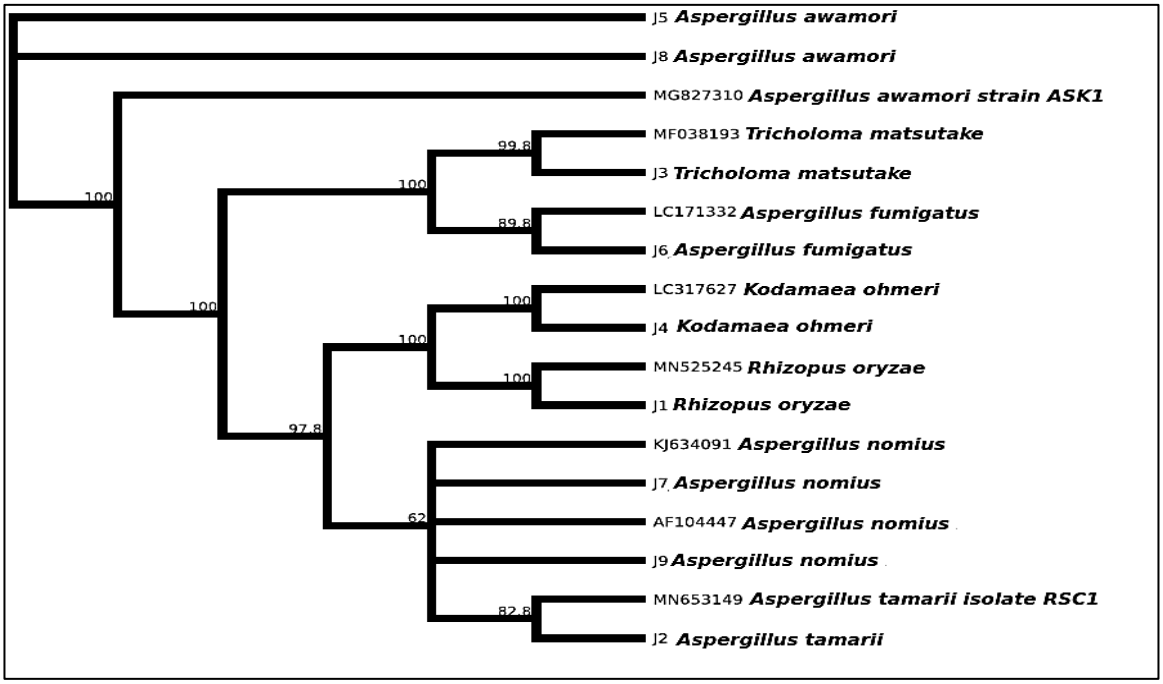

Figure 3:
Phylogenetic Tree of the Fungal Isolates

The prevalence observed in this study could be attributed to the source of the sample and the fact that Nigeria is a tropical and humid country. The prevalence could also be associated with the reality that more work is done on bacteria than fungi. Thus, fungal diseases do not receive serious attention.

Study Limitations

Due to a lack of funds, molecular analysis for the genotypic identification of fungal isolates was limited to some fungal species and the scope of the study. Lack of recent studies on fungal which posed a challenge in the present work

Conclusion and Recommendations

The study revealed that fungal infections are predominant in highly populated areas, and vary from one location to the other. These infections were more among age groups of children between the ages of 5-13. *Aspergillus spp.* was the most predominant fungi identified in the present study. There is a need for a study on the prevalence of fungal infection that would include more stratified geographical regions and capture more age groups compared to the present study.

Source of funding

Authors sponsored the study

Competing Interests

The authors declare that this manuscript was approved by all authors in its form and that no competing interest exists.

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Contribution of Authors

JOV: Sourced for the literature, did the laboratory analysis and wrote the first draft.

FN and AOE: Designed and supervised the work; AOE: Corrected the work and designed the manuscript

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Appendix 1.

Calculation of the Expected frequencies ($f_e = \frac{Row\ Total \times Column\ Total}{Total\ cases}$)

Age	Number Examined	Nsit Ubium and Uyo	Eket & Oron	Ikot Ekpene & Essien Udim
		Mycological Positive	Mycological Positive	Mycological Positive
5-9	1132	216.6857143	240.2285714	231.0857143
10-13	1510	294.1634551	326.1242525	313.7122924
14-16	349	62.99003322	69.83388704	67.17607973
20-29	789	146.4518272	162.3637874	156.1843854
30-39	155	26.14086379	28.98106312	27.87807309
40-49	142	19.84186047	21.99767442	21.16046512
80 & above	1157	181.7262458	201.4707641	193.80299

Appendix 2:

Chi-square test table

		Nsit Ubium and Uyo	Eket & Oron	Ikot Ekpene & Essien Udim
Age	Number Examined	Mycological Positive	Mycological Positive	Mycological Positive
5-9	1132	0.02471745	0.965369716	0.721718242
10-13	1510	0.502950447	0.611713195	2.202794056
14-16	349	0.015560649	0.000395131	0.010105451
20-29	789	0.001393959	0.042802752	0.030550682
30-39	155	0.000759065	0.135419846	0.161509506
40-49	142	0.03571888	2.45859E-07	0.033308286
80 & above	1157	0.828966891	4.039877962	8.590600152
The critical value of Chi^2 = 21.02606982			Test Statistic (Chi^2) = 11.75058638	
degree of Freedom = 12			P-value of Test statistic = 0.465912657	