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Diversity and seasonal variation of fungi flora from different human skin type: A research study of people in Anambra senatorial zones

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Abstract
Background: The human skin are home to different microrganisms generally known as microbiome. The microbiome has been a research interest but there is limited scientific information on the skin fungi flora and none on its distribution according to seasonal variation, this study aimed to cover the research gap by providing information on fungal isolates from the skin among individuals from the three Anambra senatorial zones in two (Raining and dry) seasons.
Methods: Questionnaire was given to the participants to get the primary data needed for this research, skin sample was collected from five different body sites using tape stripping method, culturing was done for fungal isolation, identification of the fungal isolates were done using macroscopic, microscopic morphology and molecular characterization of the isolates. The results of this research were analyzed statistically using descriptive analysis (frequency and crosstab).
Results: The study findings include the isolation of seven moulds which include *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus tamari*, *Aspergillus tamarii*, *Lentinus squarrosulus* and *Curvularia lunata*, with *Aspergillus niger* (34.1%) having the highest distribution followed by *Aspergillus nidulans* (15.0%). Among the different body sites used the fingers showed to have the highest distribution of the isolates than the other sites, the fungal isolates distributed more during dry season, *Aspergillus niger* and *Aspergillus fumigatus* distributed more among study participants from Anambra North senatorial zone.
Conclusion: *Aspergillus* is among the commonly distributed fungi genera found in the skin, also the skin mycoflora distributes differently among the body sites with the finger showing highest distribution of the isolates, seasons and individual environment has a major role to play in distribution of this fungi isolates. This study recommends the need for us to take care of our skin differently across the season to suit the natural changes in the season.

Keywords: Skin microbiome, skin mycoflora, isolate collection, seasonal variation, and senatorial zones

1. Introduction
The biggest organ in the human body, the skin serves as the organism's outermost contact with the outside world (Boxberger *et al*., 2021) [2]. A healthy skin microbiota is one aspect that affects how well the skin functions (Skowron, 2021) [12]. Although the skin's microenvironment differs from place to location on the body, all of them are home to microorganisms that might influence the preservation or disturbance of skin health (Prescott *et al*., 2017) [10]. Micro-eukaryotes (mites), viruses, bacteria, fungi, archaea, and phages make up this diverse group of microorganisms (Skowron, 2021) [12]. The age, sex, and health of the host are only a few factors that affect the skin structure, which controls the makeup of the skin microbiome. The skin serves as a barrier against dangerous microorganisms, but it is also thought to provide roughly 30 m^2 of varied microbial habitat (Gallo, 2017) [6]. Different microbial communities made up of bacteria, fungus, viruses, and microeukaryotes may be found in the wet, sebaceous, or dry microenvironments of the skin (Swaney and Kalan, 2021) [13].
With a decline in the host's health, ageing over time, or simply a change in home or employment, the microbiome makeup may vary (Skowron, 2021) [12]. The dominance of certain bacteria, their proportions, and their interactions are influenced by the physical and chemical characteristics of the skin (Skowron, 2021) [12]. Studies based on culture have located *Malassezia* spp. according to Byrd *et al*. (2018) [4], as the dominant genus of

commensal skin fungus. Contrary to the bacterial percentage, the fungal community makeup was previously believed to be consistent across the whole body (Byrd *et al*. 2018) [4]. Recent research, however, shown that *Malassezia* spp. predominated near the body's centre and on the arms, while a more varied assortment of fungus colonised the foot locations (Byrd *et al.* 2018) [4]. The human skin microbiome is shaped by the ecosystems in which people live, which are impacted by the state of biodiversity, climate, and urbanisation, among other variables (Moskovicz *et al*., 2020 review on environmental factor and skin micobiota) (Prescott and Logan, 2016) [11].

Most studies of skin microbiome have concentrated on characterising the community structure of microbes with health and diseases (Liseung and Martin, 2012) [8], topographical diversity of skin microbiota (Boxberger *et al.*, 2021) [2], looking at the association of the skin microbiota with environment type (Xi LI *et al*., 2017) [17], also researcher have characterised the diversity of this microbial representatives from the human skin (Timm *et al*., 2020) [14]. In contrast, this present study focused on the diverse distribution of Skin Mycoflora from different human skin type living in Anambra senatorial zones, and this research was done in two (raining and dry) seasons.

2. Materials and Methods

2.1 Study Participants

The individuals for this study were selected from the three Anambra senatorial zone consisting of Anambra South, Anambra North, and Anambra Central using random sampling method. The participants were given consent form to read and sign after that they were issued questionnaire for the collection of their primary data.

2.2 Sample Size Determination

The minimum sample size was determined using the below formula (Bujang, 2021) [3].

$$n = \frac{Z^2 P (1-P)}{d^2}$$

Where, n = minimum sample size

Z = confidence interval of 95% equivalent to confidence coefficient of 1.96

P = prevalence rate in % which is 4.9% (Umeanaeto *et al.*, 2016) [15].

d = the desired level of significance (0.05)

Hence, 392 participants were sampled by random sampling technique.

2.3 Sample Collection

Collection of skin sample was done at two different season of the year (rainy and dry season) from individuals living in the three Anambra senatorial zones (Anambra North, Anambra south and Anambra Central) using tape – stripping method collection of skin fungi. The tape stripping method was used. Firstly, medical air permeable tape with acrylic glue was sterilized by ultraviolet radiation. Three sterilized tapes were applied to each designated region of the participant skin for 1 minute. Two tapes were peeled off from the skin with sterile forceps and applied to individual SDA agar plates then incubated. The samples were collected from 5 different sites which include face, back, chest, and finger and toe web (Ogai *et al*., 2018) [9].

2.4 Culturing, Isolation and Identification

In order to isolate and identify the aetiology agents, the samples were grown in a Petri dish with Sabouraud dextrose agar. Sabouraud dextrose plate were prepared following the manufacturers instruction, 0.05 mg/ml Chloramphenicol was added to the prepared SDA plate to prevent bacterial development (Uthansingh, 2019) [16]. The tapes peeled off from the skin were placed on the plates and they were incubated at 37 ^{0}C in the incubator for 3–5 days; they showed rapid growth those without growth were kept in for up to 2 weeks (Uthansingh, 2019) [16]. The fungi were isolated and identified based on their morphology; representatives of the various colonies were chosen based on their morphological traits, purified by iterative sub-culturing on Sabouraud dextrose agar for 5 days at 37°C, and identified phenotypically. The colonies from the subculture plate were injected into the Bijou bottle, which had been slanted with SDA. The Bijou bottle was then incubated at 37 °C for 3-5 days, during which time the growth on the bottle was recognised based on its colour and morphological traits (Al-Jaradi *et al*., 2018) [1].

2.5 Molecular Characterization

DNA Extraction

Following the manufacturer's instructions, the fungal DNA was extracted and purified from the samples using a DNeasy blood & tissue DNA extraction kit. (Grimshaw *et al*., 2019) [7].

PCR Amplification and Sequencing

The fungal rRNA gene's ITS2 region was amplified. The PCR process was done in triplicate. The duplicate PCR reactions from each sample were combined after the samples had been amplified. It was purified using the DNA extraction kit (Axyprep DNA gel extraction kit, Axygen) in accordance with the manufacturer's instructions after being visualised by gel electrophoresis (1.0% biology grade agarose, invitrogen, USA). After that, magnetic beads (Agencourt ampura XP, Beckiman Coulter) would be used to further purify the DNA. A composite pool for pyrosequencing was created by pooling PCR amplicons with equimolar concentrations from each sample using the Quant-IT wide range dsDNA kit from Invitrogen (Grimshaw *et al.*, 2019) [7].

2.6 Statistical Analysis

For the purpose of this research's outcome statistical analysis using descriptive analysis, SPSS version 27.0 was used.

3. Results

1. Fig 1: showed Illustrates the distribution of fungi across the different skin sites showing finger to have the highest distribution of the fungi isolate.
2. Table 1: showed the different frequency of occurrence of the fungi isolates, with *Aspergillus niger* having the highest number of occurrence followed by *Aspergillus nidulans*.
3. Table 2: represented the Colony morphology, microscopic morphology and molecular characterization of the fungi isolate.
4. Table 3 represented frequency of distribution of fungi

isolate in raining and dry season, the result showed that majority of the isolates distribution higher during dry season.

5. Table 4: Distribution of fungi isolates among study participants from Anambra senatorial zone was represented which showed that the fungi isolates distributed more among participants from Anambra North senatorial zone.

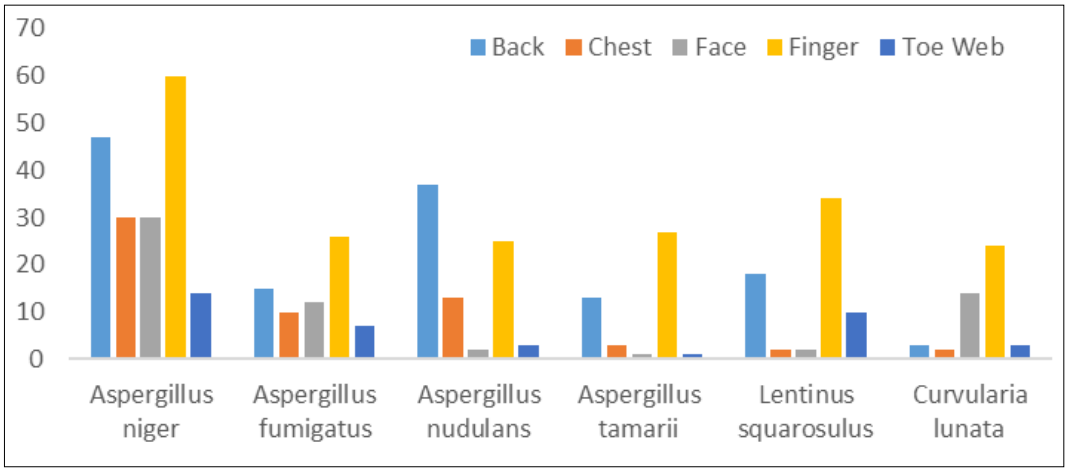

Fig 1: Distribution of fungi isolates among skin sites

Table 1: frequency of occurrence of the fungi isolates

Isolates	Frequency RS	Occurrence (%) RS	Frequency DS	Occurrence (%) DS
Aspergillus niger	181	34.1%	161	31.1%
Aspergillus fumigatus	70	13.1%	71	13.7%
Aspergillus nidulans	80	15.0%	85	16.4%
*Aspergillus tamarii*1	45	8.4%	31	6.0%
*Aspergillus tamarii*2	45	8.4%	48	9.2%
Lentinus squarrosulus	66	12.4%	74	14.3%
Curvularia lunata	46	8.6%	48	9.3%

Keys; RS = Raining season DS= Dry season

Table 2: Colony morphology, microscopic morphology and molecular characterization

S/N	Isolate	Colony Morphology	Microscopic Morphology	ITS report
1	*Aspergillus niger*	black to deep brown on the surface; pale yellow on the reverse; downy to powdery	smooth walled conidio-phores with rough walled round brown conidia	MT550026.1
2	*Aspergillus fumigatus*	blue green to grey green on the surface; pale on the reverse downy to powdery	smooth walled conidio-phores, tinted greenish; round conidia with finely roughed walls in chains	MK623263.1
3	*Aspergillus nidulans*	white colony on the Surface; dark brown with yellow surroundings on the reverse; downy and non powdry colony	short conidiophores smooth walled rough conidia	MG459155.1
4	*Aspergillus tamarri*1	white fluffy colony on the surface; white on the reverse; wooly rough	roughed walled spherical and smooth shaped conidia	MN128231.1
5	*Aspergillus tamari*2 on the surface; light	dark orange to brown with white surrounding yellow with white patches on the reverse; powdery	smooth shaped condia	OP526911.1
6	*Lentinus sqarrosulus* tend to collapse when	densely white cottony/wooly mycelia which ovoid macroconidia touched	smooth walled hyaline hyphae	KT956127.1
7	*Curvularia lunata*	light to dark grey on the surface; black with orange lining on the reverse; downy	straight conidiophores, smooth conidial wall.	KY806118.1

Table 3: Frequency of distribution of fungi isolates in rainy and dry season

Isolate	Occurrence in RS N (percentage)	Occurrence in DSN (percentage)	Total	PV≤0.05
Aspergillus niger	181(46.2)	161(41.1)	342(87.3)	0.01*
Aspergillus fumigatus	70(17.9)	71(18.1)	141(36.0)	0.01*
Aspergillus nidulans	80(20.4)	85(21.7)	165(42.1)	0.01*
Aspergillus tamarii	45(11.5)	31(7.9)	76(19.4)	0.01*
Aspergillus tamarii	45(13.3)	48(14.0)	93(27.3)	0.01*
Lentinus squarrosulus	66(16.8)	74(18.9)	140(35.7)	0.01*
Curvularia lunata	46(11.7)	48(12.2)	94(23.9)	0.01*

Significant P-value (calculated <0.05). Percentage value in parentheses
Keys; RS = Raining season, DS = Dry season, N = Number

Table 4: Distribution of fungi isolates among study participants from Anambra senatorial zone

S/N	Fungi Isolates	Anambra North (n=138)		Anambra South (n=157)		Anambra Central (n=97)		Total		P-value	
		RS	DS	RS	DS	RS	DS	RS	DS	RS	DS
1.	*Aspergillus niger*	82	75	63	56	35	30	181	161	0.001*	0.001*
2.	*Aspergillus fumigatus*	39	39	17	21	14	11	70	71	0.001*	0.001*
3.	*Aspergillus nidulans*	16	22	40	36	24	27	80	85	0.006	0.082
4.	*Aspergillus tamarii*	18	6	14	13	13	12	45	31	0.439	0.079

4. Discussion

This study isolated seven moulds with five belonging to *Aspergillus* Genera and two having same specie name but different gene, which include; *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus nidulans*, *Aspergillus tamarii*, and *Aspergillus tamarii*, the remaining two are *Lentinus squarrosulus* and *Curvularia lunata.* This finding is in contrast with Xi Li *et al.,* 2017 [17] who evaluated cutaneous microbial distribution and microbial co-occurrence at different body site and skin of Chinese women and reported *Malesezzia furfur* to be the dominating fungi isolates this could be as a result of the difference in study country, it agrees with Callewaert *et.al,* 2014 [5], who recorded *Aspergillus* as one of the genus isolated from urban skin that agrees with this study fungi isolate which is dominated by the genus *Aspergillus*, Skowron *et al*., 2021 [12] also recorded *Aspergillus* sp among the most frequently isolated skin fungi genera. *Aspergillus niger* (34.1%) showed the highest distribution followed by *Aspergillus nidulans* (15.0%). The fungi isolates distributed more among fingers of the study participants which could be as a result of the fingers being the most exposed part of the body, as it is used on daily bases for different activities ranging from opening of doors.

This study has demonstrated for the first time the relationship of the skin fungi isolates and season (dry season and rainy season) variation, and reported the distribution of the fungi isolates from the two seasons (rainy and dry season). All the result of the fungi isolates in this study showed a statistically significant difference (Pv $\leq$ 0.05) in the occurrence of the isolates in each season. *Lentinus squarosulu* which distributed 66 times with 16.8% in rainy season came to dry season and distributed 74 times which is 18.9% as well as most of the organism distributed more in dry season, this could be as a result of the natural changes which the skin experiences during dry season which include skin dryness, roughness, itchy or even scaly. Dry season also comes with high temperature and low humidity, which can also be a contributing factor in the high distribution of *Lentinus squarrosulus* and others microorganism during dry season.

The distribution of the fungi isolates among the study participants from the three senatorial zones recorded a significant result on higher occurrence of *Aspergillus niger* and *Aspergillus fumigatus* among study participants in Anambra North followed by the study participants in Anambra South this could be because of the environment difference in the study participants location. Skowron *et al*., 2021 [12] in his work on impact of intrinsic and extrinsic factors on skin microbiota revealed that the environment of a given individual is also extremely relevant in the microbial distribution.

5. Conclusion

This study isolated and identified seven moulds which majority of them belongs to the *Aspergillus* genera showing that *Aspergillus* are among the commonly distributed fungal genera on the skin. The isolates distributes differently among skin sites, with fingers showing the highest distribution. The different season has effect on the distribution of the fungi across the body, also individual's environment plays a major role in the distribution of skin microbiome.

This study recommends that there is need for us to take care of our skin differently across the season to suit the natural changes that comes with each season.

6. Declaration

Ethical Approval and Consent to participate

This study protocol got approval from Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) ethical clearance committee before proceeding. Ref: COOUTH/CMAC/ETH.C/Vol.1/FN:04/209.

7. Competing interests

The authors declare that there is no conflict of interest.

8. Author's contribution

All the authors contributed to the success of this research work, CU initiated the concept for this work and supervised the whole work, IS developed the design for the work carried out the analysis and interpretation of data and prepared the manuscript, CM did the collection of samples and also interpretation of data, LC contributed in the buildup of the work design, SN contributed in the practical analysis and design of the manuscript.

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