



Are Fungi Isolated from Water and Fish Samples in Lapai-Agaie Dam, Nigeria Pathogenic to Human Health?

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Abstract

The need to identify pathogenic fungi isolates from the water and its' fishes, in Lapai-Agaie Dam that provides for both domestic and agricultural (irrigation and fisheries) activities in order to provide an understanding of the health implication of the isolates was the panacea for this study. Water samples were collected from four characterized sites while fish samples were obtained from the landing sites at the early hours (between 7000 and 8000hours). Standardized procedures for sample collections and fungi isolation and identification were adopted. Eight (8) and seven (7) species of freshwater fungi were isolated from the water and fish samples respectively. Seventeen (17) species of freshwater fishes were identified from the landing site. The most frequent isolates in water were *Penicillium chrysogenum* 11(26%) and *Mucor varians* 09(21%) while *Aspergillus nidulans* 1(2%) was the least in occurrence. *P. chrysogenum*, *A. niger* and *A. fumigatus* were the isolates common to both water and fish samples. The presences of these fungi in both media (water and fish) may pose health risk to the populace as the water is used for domestic activities; as some populace are in the habit of eating partially cooked fish called 'half done' as delicacy meal.

Keywords: Tropical-Freshwater, Fishes, Mycoflora, Public Health

Introduction

Fungi are diverse group of organisms belonging to the Kingdom Eumycota (Schubler, *et al.*, 2001), where some are either pathogenic or parasitic that affects fishes as a result of stress or immune-compromise system due to unfavourable environmental condition, or secondary to bacterial or viral infections. They are cosmopolitan in nature due to their broad enzymatic capabilities; as they can actively degrade most complex natural substances thus exist in all strata of the aquatic ecosystem (APHA, 1989). They are known to cause zoosporic diseases (Lafevre *et al.*, 2012) that affects eggs, fingerlings and adult fishes (Eli and Abowei, 2011; Abolude *et al.*, 2013).

Fungal species diversity in aquatic environment varies by location and depth as Shearer *et al.* (2007) reported greater species diversity in the tropics, as most fungi isolated in the tropics and subtropics are anamorphic Basidiomycetes and Ascomycetes (Sridhar, 2005). Harms *et al.* (2011) reported that they provide information on the biological and functional diversity of the environment. Studies of fungi diversity in aquatic environment has been conducted in water and sediment (Parveen *et al.*, 2011, Doi, *et al.*, 2018); on plants (Motlagh, 2010; Adamu *et al.*, 2017) and on fishes (Al-Niaem *et al.*, 2015; Ali, 2015; Angahar, 2016; Atef *et al.*, 2016) as they have reported to cause serious diseases in freshwater fishes. Some fungi are disease-causing pathogens

responsible for mycoses and allergies. Walsh *et al.* (2004) reported that they are associated with disease affecting humans, plants, and animals. Studies of freshwater fishes in standing water has been conducted (Al-Niaeem, *et al.*, 2015; Angahar, 2016; Oso *et al.*, 2017).

Dams are mainly used to conserve available water for use during need periods, or for irrigation, domestic uses and municipal water supply. The Lapai-Agaie dam was constructed by the Niger State Government in partnership with the Federal Government with the sole objective of providing portable water for the Lapai and Agaie populace. Therefore, this study isolated fungi in water column and fish samples from Lapai-Agaie Dam with the objectives of identifying species of fungi isolates on landing fishes, and in water column and identify the pathogenic isolates with potential human health implications.

Materials and Methods

Sampling Site

The sampling was conducted in Lapai-Agaie Dam, located close to Bakajeba village at latitude $9^{\circ}13'N$ and Longitude $6^{\circ}35'E$ (Plate 1) in Lapai Local Government Area of Niger State, Nigeria where sampling was conducted biweekly during the months of June and July, 2017.

Sample Collection

Surface water sample was aseptically collected from one meters from the bank for fungi isolation during the 0700 and 0800 hours. The water pH and temperature were monitored using Hanna, pH meter and mercury glass thermometer respectively before sampling.

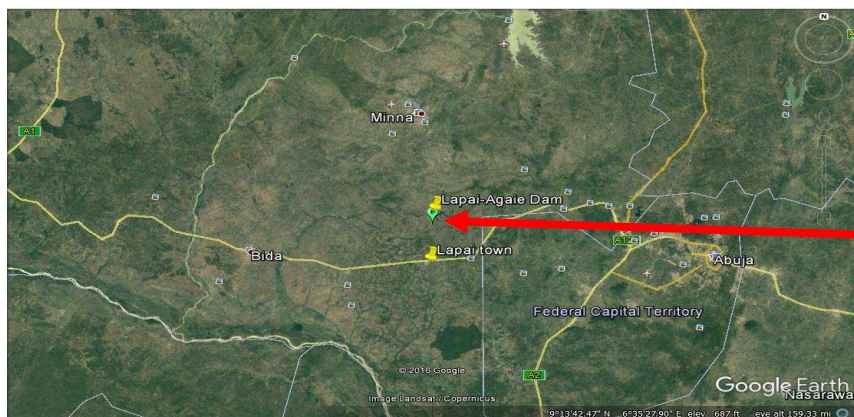


Plate 1: Pictorial representation of Lapai-Agaie Dam

source: Google Earth

The samples were collected from four identified site labeled A-D into sterilized sample bottles (Okuda *et al.*, 2000). The following distinctions were noted for the sites; Site A (rocky with aquatic plants); Site B (domestic activities such as washing and bathing), Site C (landing site/fishing activities) and Site D (clayey and calm water without aquatic plants). The identified fish samples were obtained from the landing site, swabbed with a sterile swab stick and placed in a normal saline for analyses.

Isolation and Identification of Fungi

The laboratory activities were conducted within 2-4 hours of the swab were collected. Potato Dextrose Agar (PDA) was used and prepared in accordance to the Manufacturer's instruction (Accumix®- Tulip Diagnostics (P) Ltd). The samples were serially diluted and dilution factors 10^{-2} and 10^{-3} were used as stock solution. One ml of each dilution was aseptically taken from the suspension and transferred into sterile Petri dishes. Then Potatoes Dextrose Agar (PDA) was

poured into the Petri dishes containing the suspension and 1ml of chloramphenicol. The plates were swirled gently to allow even distribution of the sample in the medium and were incubated at room temperatures ($28 \pm 2^\circ\text{C}$) for 24 hours. From the culture obtained, sub-culturing was made to get the pure culture of each fungus isolated. Fungi isolated were identified using Fungi Families of the world mycological monographs by Samson *et al.* (2004) and Amadi and Adebola (2008). Isolates were studied macroscopically by colony shape, size, colour and growth pattern. Slides were prepared from each colony and stained with 0.05% Trypan blue in lacto phenol. The slides were observed under microscope. The existing septate wall, sexual organ structure, size and arrangement of spores were also examined and recorded. The fungi species were identified with the help of available fungi identification keys and literature (Willoughby, 1994). The data obtained were presented in tables and charts, the mean fungal load were compared using one-way analysis of variance (ANOVA), followed by the student's t-test for comparison test. Analyses were aided with Microsoft Excel, 2007 version where level of significance was placed at 5% probability.

Results and Discussion

Water Samples

The water temperature and pH are presented in Table 1. Water at Site B recorded the highest water temperature and pH of $27.00 \pm 0.32^\circ\text{C}$ and 6.90 ± 0.01 respectively. There was significant difference ($p < 0.05$) in the monitored pH values.

Table 1: Mean and Standard Error of Temperature and pH of sampled water sites of Lapai-Agaie Dam

Parameters	Sampling Sites			
	A	B	C	D
Temperature ($^\circ\text{C}$)	25.00 ± 0.04	27.00 ± 0.32	26.20 ± 0.45	26.00 ± 0.02
pH	$5.30 \pm 0.41^*$	6.90 ± 0.01	6.40 ± 0.37	6.40 ± 0.23

* $p < 0.05$

The macroscopic and microscopic characterizations of fungi isolated from the four sampling sites are presented in Table 2. The different fungi isolated from the sampling sites are presented in Table 3. Eight (8) fungi isolates were identified from the water sample. *A. nidulans* was only recorded in Site C. Site B recorded the least (7) frequency of isolates while the highest (14) was recorded in Site A. The highest cumulative average fungi load on the identified fishes was recorded in *Petrocephalus soudanensis* as 13.50×10^5 cfu/ml. The least values of 6.00×10^5 cfu/ml was recorded in *Melapterururs electricus* as presented in Table 4. The total numbers of seven (7) fungal isolates were identified on the fishes as presented in Table 5 based on their morphological examination. In Table 6 revealing the different isolate per fish showed that *Aspergillus niger* was the most frequent isolate whilst *Pelvicachromis taeniatus* recorded the highest number of fungal isolates on the skin. Four (4) isolates: *Rhizopus* sp, *Geotrichum candidum*, *Candida* sp and *Trichoderma viridae* were not isolated from water sample but on the fish.

Table 2: Morphological Characterizations of Fungi Isolates in Water samples from Lapai-Agaie Dam

Morphological Characterization		Probable Fungi
Macroscopic	Microscopic	
Light yellow greenish colony. Ovoid in shape colonies.	Conidial head are radicate. Conidiophores was thick walled, hyaline and slightly roughened, erect, long, aseptate with a vesicle at the top with phialides and short conidial chains.	<i>Aspergillus flavus</i>
Widely spread black colonies with smooth white edges and spongy surface that is densely packed with the formation of hyphae	Hyphae are septate, hyaline and conidiophores are long and globase at the tip, blackish conidial head.	<i>A. niger</i>
Has a dark cress green surface, its reverse side were pale to bright yellow to deep brown.	It had branched septate, dome shaped and blue to green conidial head. The conidial surface is smooth slightly rough.	<i>A. nidulans</i>
Widely spread colony, dark green with smooth white edges and spongy surface.	Long conidiophores with narrow base and broad near the vesicle, smooth walled hyaline. Grayish conidial head.	<i>A. fumigatus</i>
Fast growing colonies, resembles white-to-grey cotton candy that darkened with time. While the reverse is light-coloured to white.	Wide hyphae, branched septate with long sporangiophores, septate and round sporangia.	<i>Mucor varians</i>
The colony was grayish-green to dark green, while reverse was creamy-yellow.	It has subglobose conidia shape that is smooth finely roughed. Septate hyphae.	<i>Penicillium chrysogenum</i>
The colony was pink with white patch on surface. Round shaped colony.	Light and dark violet, salmon-coloured, purplish brown, all with cottony mycelium, without exudates	<i>Fusarium oxysporum</i>
The colony was pink with white patch on surface. Round shaped colony.	The mycelium is orange brown with light brown exudates. In appearance it is light yellow and moist.	<i>F. solani</i>

Table 3: Frequency of Fungi Isolates in Water samples from Lapai-Agaie Dam

Fungi Isolates	Sampling Site/Sampling Period (Biweekly)												Total
	A			B			C			D			
	1	2	3	1	2	3	1	2	3	1	2	3	
<i>Aspergillus niger</i>	+	+	-	-	-	-	+	+	-	+	+	-	06
<i>A. nidulans</i>	-	-	-	-	-	-	+	-	-	-	-	-	01
<i>A. fumigates</i>	+	-	+	+	-	-	+	-	+	-	-	-	05
<i>A. flavus</i>	-	-	+	-	-	+	+	-	-	-	-	+	04
<i>Fusarium oxysporum</i>	-	+	-	-	-	-	-	+	-	-	+	-	03
<i>F. solani</i>	-	+	+	-	-	-	-	-	-	-	+	+	04
<i>Mucor varians</i>	+	+	+	+	+	+	+	+	+	-	-	-	09
<i>Penicillium chrysogenum</i>	+	+	+	-	+	+	+	+	+	+	+	+	11
Sub-Total	04	05	05	02	02	03	06	04	03	02	04	03	
Total	14			07			13			09			43

+ = Present, - = Absent

Table 4: Fungal load on the skin of sampled fishes from Lapai-Agaie Dam, Lapai

Fish sample	Sampling Periods (Biweekly)/Fungi population in Cfu/ml x10 ⁵			Cumulative Average Cfu/ml x 10 ⁵
	1	2	3	
<i>Barboides gracilis</i>	7.00	-	9.00	8.00
<i>Tylochromis sudanencis</i>	12.00	5.00	7.00	8.00
<i>Ctenopoma petherici</i>	9.00	-	-	9.00
<i>Brycinus nurse</i>	-	11.00	-	11.00
<i>Labeo senegalensis</i>	-	8.00	5.00	6.50
<i>Pelvicachromis taeniatus</i>	7.00	10.00	8.00	8.33
<i>Tilapia dageti</i>	13.00	-	10.00	11.50
<i>T. zilli</i>	-	9.00	9.00	9.00
<i>Petrocephalus soudanensis</i>	-	13.00	-	13.00
<i>Badysynodontis batensoda</i>	-	7.00	-	7.00
<i>Marcusenius mento</i>	-	8.00	11.00	9.50
<i>Marcusenius abadii</i>	8.00	12.00	-	10.00
<i>Bryconaethiops quinquesquamae</i>	11.00	-	-	11.00
<i>Clarias gariepinus</i>	10.00	-	6.00	8.00
<i>C. anguillaris</i>	15.00	6.00	12.00	11.00
<i>Petrocephalus bovei</i>	-	-	8.00	8.00
<i>Melapterurus electricus</i>	6.00	-	-	6.00

-=not sampled

Table 5: Morphological Characterizations of Fungi Isolates on the skin of sampled fishes from Lapai-Agaie Dam

Morphological Characterization		Probable Fungi
Macroscopic	Microscopic	
Surface- texture deeply cottony white becoming gray brown on surface. Reverse; pale white	Hyphae broad, not or scarcely septate, rhizoids and stolon present. Sporangiohores brown.	<i>Rhizopus</i> sp
Widely spread black colonies with smooth white edges and spongy surface that is densely packed with the formation of hyphae	Hyphae are septate, hyaline and conidiophores are long and globose at the tip, blackish conidial head.	<i>Aspergillus niger</i>
Surface- off-white to cream colored colonies with butyrous texture with a velvety, suede-like or ground glass/matt appearance.	Clear hyaline, septate hyphae. Produce chains of arthroconidia.	<i>Geotrichum candidum</i>
Surface- fluffy white tufts, green tufts may develop within the colony. Reverse; typical light tan to yellow or pale orange.	Septate hyaline hyphae, conidiophores are short and branching irregularly. Phialides are flask shaped. Conidia are globose.	<i>Trichoderma viridae</i>
Greenish to black, white mycelia at the margin, white droplet, yellow golden in the media	It has subglobose conidia shape that is smooth finely roughed. Septate hyphae.	<i>Penicillium</i> sp
Widely spread colony, dark green with smooth white edges and spongy surface.	Long conidiophores with narrow base and broad near the vesicle, smooth walled hyaline. Grayish conidial head.	<i>Aspergillus fumigates</i>
Creamy to white colonies on surface.	Septate hyphae, conidiophores are short and inflated with black and thick wall conidia.	<i>Candida</i> sp

Table 6: The different fungal isolates, identified from the skin of freshwater fishes sampled from Lapai-Agaie Dam.

Fish sample	<i>Pennicillium</i> sp	<i>Rhizopus</i> sp	<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Geotrichum candidum</i>	<i>Trichoderma viridae</i>	<i>Candida</i> sp
<i>Barboides gracilis</i>	+	+	+		+		
<i>Tylochromis sudanencis</i>	+	+	+				+
<i>Ctenopoma petherici</i>	+		+		+		
<i>Pelvicachromis taeniatus</i>	+	++	+	+	+		+
<i>Tilapia dageti</i>	+	+	+		+		
<i>Marcusenius abadii</i>		++	+		+	+	+
<i>Bryconaethiops quinquesquamae</i>	+	+	+				+
<i>Clarias gariepinus</i>	+	+			+		
<i>C. anguillaris</i>	+	+		+	+		
<i>Melapterurus electricus</i>			+		+		+
<i>Brycinus nurse</i>				+	+	+	
<i>Labeo senegalensis</i>			+			+	+
<i>Tilapia zilli</i>		++	+	+			
<i>Petrocephalus soudanensis</i>		+	+	+			
<i>Marcusenius mento</i>			+		+	+	
<i>Petrocephalus bovei</i>			+				
<i>Brachysynodontis batensoda</i>		+				+	+

++= high presence, +=present, -=absent

The highest frequency of fungi was recorded in Site A. This may be attributed to the substrata nature of the site (rocky) which could enable fungi to firmly attach to the bottom and the acidic nature of the water which may be due to the metabolites released by the isolates. However, the least frequency of isolates in Site B may be attributed to the high rate of domestic activities in the area. The fishing activities in Site C, may have been responsible for another higher fungi frequency as the presence of biotic components such as faecal matter, scales and death remains of fishes may have been the factor that is responsible for the high frequency of fungal isolates recorded in the area; in spite of having less hard substratum. The less frequency of isolates in Site D, may be attributed to the substratum (sandy clayey) and least biotic components observed thus reduced

availability of nutrient. The low rate of water flow and temperature variation in standing waters like the Lapai-Agaie Dam have been attributed to increase pathogenic fungal isolates (Bodnarska *et al.*, 2009 and Bichi and Bawaki, 2010). The absence of *A. niger* and *F. oxysporum* in site B, may be attributed to the Human activities in the area. However, the presence of *A. nidulans* in only site C, may be related to the presence of decomposing matter (bacteria) (Adamu *et al.*, 2017). Thereof, they may be opportunistic parasites that are able to take advantage of damaged or stressed fishes. The high quantum of fungal pathogen recorded in this study may be an indication of high bacteria or other pathogens present on the surface of the identified sampled fishes (Fayioye *et al.*, 2008; Junaid *et al.*, 2010; Shahbazain *et al.*, 2010). The source of these fungal pathogens may be associated with anthropogenic activities that occur around the water as well as the presence of bacterial isolates not overlooking the ubiquitous nature of these microscopic prolific giants. Contamination of fish by pathogens may occur prior to harvest (Venugopal, 2002) as pathogen in water and other aquatic biota are likely to infest the fish. According to Kirby *et al.* (2003) water could be a vehicle for the transmission of many micro-organisms.

Pathogenic Fungi isolates such as *Aspergillus spp* (Refai *et al.*, 2010) was isolated in this study which are higher than that reported by Ali, (2015). Reports have revealed that the fungi are mostly frequent in fishes (Ali, 2015, Doi *et al.*, 2018). De Hong and Horré (2002) had described *Aspergillus spp* in water as causative agents of kidney, liver disorder, allergy, burns, otitis media and increase risk of invasive infections. It causes a disease known as Aspergillomycosis in fishes (Fayioye *et al.*, 2008). Studies have reported the isolation of this fungus in fishes (Momeni Shahraki, *et al.*, 2014, Al-Niaeem *et al.*, 2015, Atef, *et al.*, 2016). *A. niger* is a common allergen and may cause opportunistic invasive respiratory infection in hospitalized immunized patients (De Hoog *et al.*, 2000). *A. flavus*, *A. fumigates* and *A. niger* are known to produce aflatoxins, and ochratoxins which are carcinogenic and are capable of causing kidney and liver disorders, invasive and non-invasive aspergillosis, allergic and sinusitis (Samson *et al.*, 2004; Thliza *et al.*, 2015). The effects of aflatoxins on animal health vary from species to species (Gourama and Bullerman, 1995).

Similarly, *Penicillium chrysogenum* was referred to as pathogenic isolate (Iqbal and Saleemi, 2013). Studies have revealed that the isolate are disease causing agents (Nazim *et al.*, 2008; Gunhild *et al.*, 2009) such as allergy, asthma and other respiratory problems (Houbraken *et al.*, 2010; Memon, 2012). The fungal is most associated with fish as supported by the study conducted by Moneni Shahraki *et al.*, (2014) that it caused 22% mortality of fish eggs and fishes (Refai *et al.*, 2010 and Shahbazain *et al.*, 2010); thereby may be affecting the population of fish in the Dam. Therefore, the presence of this terrestrial mould in aquatic habitat is an indication of contamination that may be attributed to sewage disposal in the study area. According to Hassan *et al.*, (2011), *Candida spp* (a yeast species) are one of the fungal species infesting *Tilapia spp* (Atef, *et al.*, 2016). *Candida sp* is peculiarly associated with spoilage of food (Obayamiji *et al.*, 2008). *Candida sp* may cause be any opportunistic fungus that may affect the health of human as Madhavan *et al.* (2011) reported that *Candida albicans* causes mycosis where oral candidiasis is common amongst AIDS patients, poorly nourished patients and immune-suppressed patients. Mbata *et al.* (2008) reported the presences of these fungi in Jordan River, Israel to be significant ($p < 0.05$).

Studies have revealed that *Mucor sp* and *Rhizopus sp* causes invasive diseases (Jimoh *et al.*, 2014; Al-Niaeem *et al.*, 2015; Atef *et al.*, 2016). *Trichoderma viridae* is very useful in industry as biocontrol agent (Samuel, 2006). The fungus is reported to produce trichothecenes, alamethicins, emodin, trichotoxin and suzukacillin and have been reported to cause mycosis and allergy in humans (Samson *et al.*, 2004). The presence of *T. viridae* in this study medium (PDA) had proved

its efficacy in fungi isolation (Iqbal *et al.*, 2017). The fungal community described herein represents the diversity found in this standing water during the study period. Many of the fungus species found are pathogenic and may be useful due to their ability to produce specific enzymes applicable in the biotechnological and pharmaceutical industries.

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