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Research Article

Profile of Fungi Isolated from Domestic Water Used by Street Vendors: The Public Health Implication

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Abstract:

This study aimed at establishing the profile of fungi isolated from domestic water used by street vendors in Owerri metropolis, Imo State from August 2020 to January 2021. Fiftynine water samples collected from 25 street vendors with reference to usage. Data obtained was statistically analyzed with p-value set at 0.05 significance level. The following fungi species were identified in the study; *Coccidiodes* spp. (5.08%), *Penicillium* spp. (23.73%), *Drechsler* spp. (8.47%), *Candida* spp (5.08%), and *Paecilomyces* spp. (5.08%). Three *Aspergillus* species were identified, namely *A. flavus* (16.95%), *A. niger* (15.25%) and *A. fumigatus* (11.84). The prevalence relative to fungi species was statistically significant. The result showed that 28(93.33%) of samples from water used for food preparation was contaminated with fungi, while 26 (89.66%) from drinking water were contaminated, however, the difference was not statistically significant. The result recorded (2.01±1.11 TCFU/100 ml) colony-forming units. The overall number of colonies formed by fungi species was significantly different ($p<0.05$). *Drechsler* spp had the highest count of 4.63±4.41TCFU/100 ml, while the least was observed in *Aspergillus niger*, 1.07±0.10TCFU/100 ml. The fungi total colony-forming units count was not dependent on the type of water usage, however, samples from water used for food preparation (2.56±2.19TCFU/100 ml) had higher counts than drinking water samples (1.43±0.20 TCFU/100 ml). The result has revealed the presence of some fungi of hazardous inclinations in water used by food vendors in Owerri. There is a need for concerted public enlightenment on the risk of fungi contaminated water and observance of sound safety and hygienic practices.

Keywords: fungus, Domestic water, food vendor, Owerri.

Introduction

Nigeria has a huge number of local eateries where many people eat daily (Oyeneho and Hedberg, 2013). In Owerri, the administrative capital of Imo State, there has also been an upsurge in the acceptance of ready-to-eat food sellers (Oyeneho and Hedberg, 2013). Their inadequately regulated businesses, however, create major concerns about food safety and sanitary standards. Owerri is a rapidly increasing metropolitan hub that is defined by "people on the go," which generates a booming atmosphere for the street food trade, which unfortunately thrives in unsanitary conditions (Barro *et al*.,2006). These circumstances necessitate a timely and inescapable examination of the safety and hygiene of street foods being sold in Owerri town to identify emerging food safety issues and prevent them from becoming health risks.

Fungi are ubiquitous, heterotrophic organisms existing as single-celled yeasts or multi-cellular filamentous forms. Fungi are natural inhabitants of composting plants, water and soil (Calvo-Polanco *et al*., 2016). They may be leached or released from farmland, soil or air, to contaminate surface water. Many fungal species that appear in surface and piped water often reside in the water system pipes as biofilms. The fungi in such biofilms periodically re-contaminate water sources that end up as tap water.

Fungal diseases in humans can arise from breathing-in aerosolized mold spores and hyphal fragments in indoor air when polluted water passes through shower-heads, taps, or toilet cisterns (Green *et al*., 2003) or dietary exposure to mycotoxin-contaminated food and feed products (Kanzler *et al*., 2007). Fungal species like *Fusarium* and *Aspergillus* species have been found to multiply in water reservoirs where they have been incriminated in water-borne infections (Kanzler *et al*., 2007).

Fungi are considered harmful to humans and they present a health risk, when consumed in contaminated water, food or inhaled. Some contagious species are known to be emphatically allergenic (e.g., causing skin disturbance), or may cause contaminations in the immunocompromised (e.g., those experiencing AIDS, malignant growth, asthma or other respiratory illnesses, or recuperating from organ transfers) (Green *et al*., 2003). The prominent genera of fungi occurring in various water sources tested (e.g., reservoir) are of these genera: *Cladosporium*, *Penicillium*, *Aspergillus*, *Fusarium*, *Trichoderma*, *Pithomyces*, *Alternaria*, *Phialophora*, *Paecilomyces*, *Acremonium*, *Epicoccum* and *Curvularia* (Sammon *et al*., 2010).

Mycotoxins found in water may be much diluted and may not be of main concern. However, when water is kept in reservoirs, cisterns or even in bottles, for a long time their concentrations may rise (Siqueira, 2011). Immense amounts of water are taken daily, and daily intake over several years of even microscopic

amounts of mycotoxins may be risky to human health. Mycotoxins have serious and chronic impact on humans as several are believed to be carcinogenic, cytotoxic, mutagenic and can lead to immunosuppressive complexes (Arroyo-Manzanares *et al*., 2015). Furthermore, fungal infections are a challenge to manage as fungal cells are eukaryotic, just like human cells (Yamaguchi *et al*. 2007). Although there are reports regarding advances in antifungal therapy, it is worth noting that cases of infection and antifungal resistance are also alarmingly high, hence, fungal disease control does not indicate any promise of being attained soon (Pellon *et al*., 2018).

Consequently, this research was undertaken to assess the profile of fungi isolated from domestic water used by street food vendors in Owerri. It is intended to highlight the public health implications of fungi contaminated water used for street foods.

Methodology

Study Area

Owerri town, the capital of Imo State, located in the South-East geopolitical zone of Nigeria, lies within longitude 5˚29'06"N and latitude 7˚02'06"E occupying an area between the lower River Niger and the upper and middle Imo River (Government of Imo State, 2006). The Nigeria census in 2006 recorded 3.93 million (2.03 million males and 1.9 million females) as the population of Imo State and the populace is made up of the mainly Igbo ethnic group. The State has a population density of about 707.9 per square kilometer and occupies an area of 5289.49 square kilometers (Government of Imo State, 2006). The major sources of water in Owerri are sub-surface water (Nworie and Otamiri Rivers), and numerous private / commercial bore holes that litter the town.

Methodology

The study was conducted among restaurants food vendors in Owerri town for eight months (August 2020 - January 2021). Food vendors operating from makeshift and temporary roadside structures, food kiosks, bukaterias and roadside food sellers were profiled as Street food vendors.

A total of 59 water samples was collected from 25 street food vendors. Samples were taken from boreholes and public water systems, storage tanks and containers, packaged water and bottled water according to standard procedures (Simon-Oke *et al*., 2020). Water used to prepare food and for drinking were studied. The samples were labelled noting the dates of collection and source of water. Samples were transported to the laboratory of the Department of Animal and Environmental Biology, Imo State University, Owerri, within three hours of collection in a light-proof insulated cold box containing ice packs for further analysis.

Morphological characteristics of the isolates were used for primary identification. Fungi isolates were identified morphologically using cultural and morphological traits with the aid of suitable references (McGinnis, 1980; Navi et al., 1999). On macroscopic examination, the colonies of the isolates were observed for peculiar characteristic morphology and this was done utilizing the colour of colonies, texture of colonies, colonial appearance, reverse side or colour of the underside and vegetative pattern. Microscopic identification was done according to the method of Auwal and Taura, 2013.

Fungi isolates observed were identified by comparing the macroscopic and microscopic characteristics using appropriate taxonomic guides (Siqueira et al., 2011).

Result

Table 1 reveals the morphology of fungi found on the different SDA Plates, while Table 2 illustrates the occurrence rate of the fungi species isolated from water samples in the study. The following fungi species were identified in the study; *Coccidiodes* spp. (5.08%), *Penicillium* spp.(23.73%), *Drechsler* spp.(8.47%), *Candida* spp (5.08%), and *Paecilomyces* spp. (5.08%). Three *Aspergillus* species were identified, namely *A. flavus* (16.95%), *A. niger* (15.25%) and *A. fumigatus* (11.84). The prevalence relative to fungi species was not statistically significant ($p>0.05$). The result also showed that 28(93.33%) of samples from water used for food preparation was contaminated with fungi, while 26 (89.66%) from drinking water were contaminated, however, the difference was not statistically significant.

The distribution of the total colony-forming units of fungi species isolated is illustrated in Table 3. The result showed the mean total colony-forming units recorded to be 2.01±1.11 TCFU/100 ml. *Drechsler* spp had the highest count of 4.63±4.41TCFU/100 ml followed by *Paecilomyces* spp. (2.16±0.22TCFU/100 ml), *Candida* spp (1.92±1.39 TCFU/100 ml) and *Aspergillus fumigatus* (1.74±0.09 TCFU/100 ml). *Aspergillus flavus* had 1.61±0.40TCFU/100 ml, while *Penicillium* spp. recorded 1.65±0.12TCFU/100 ml and *Coccidiodes* spp., 1.30±0.42 TCFU/100 ml). The result showed that 1.07±0.10TCFU/100 ml was observed in *Aspergillus niger*. The overall number of colonies formed by fungi species was significantly different ($p<0.05$). The fungi total colony-forming units count was not dependent on the type of water usage, however, samples from water used for food preparation (2.56±2.19TCFU/100 ml) had higher counts than drinking water samples (1.43±0.20 TCFU/100 ml).

Table 1: Morphology of Fungi Found on The Different SDA Plates

S/N	PROBABLE FUNGI	CULTURAL MORPHOLOGY	MICROSCOPIC MORPHOLOGY
1	Penicillium spp.	White fluffy colonies that turn to blue-green pigment with time.	Brush-like spore bearing structure; round to ovoid conidia in chains. Hyphae septate, hyaline.
2	Candida spp.	Creamy mucoid colony.	Chlamydospores are numerous, borne in clusters.

3	Paecilomyces spp	Colonies are granular which develop to green-blue spores.	Hyphae are hyaline and septate,conidiophores branch freely into brush-like structure.
4	Aspergillus niger	Colonies appear purple to brown at early stage and turn mix white and purple-black at maturity.	Hyphae are hyaline and distinctly septate.
5	Aspergillus flavus	White soft velvety colonies that turn yellowish-green and may have a white border.	Septate hyphae with long rough or spiny conidiophores.
6	Aspergillus fumigatus	Colonies has some shade of blue-green or green-brown colour with a powdery appearance from profuse production of pigmented spores.	Hyphae are hyaline and distinctly septate.Conidiophores are long, terminating in a large club-shaped vesicle.
7	Drechslera spp.	Suede-like dark brown colonies with a black reverse side.	Conidiophores are sympodial with pale round conidia.
8	Coccidiodes spp.	Gray white, moist and membranous colonies initially. Colonies become tan to brown in colour with age.	Hyphae are hyaline,septate and thin. Arthroconidia are thick-walled and barrel-shaped.

Table 2: Occurrence of fungi species isolated from the study samples

Vending Type	Water usage	Coccidiodesspp	Penicillum Spp	Drechsler spp	Aspergillus flavus	Aspergillus niger	Aspergillus fumigatus	Candida spp	Paecilomyces spp	All Species
Street	Food preparation N=30	2	9	3	4	4	2	2	2	28 (93.33)
	Drinking N=29	1	5	2	6	5	5	1	1	26 (89.66)
Street Total N=59(%)		3(5.08)	14(23.73)	5(8.47)	10(16.95)	9(15.25)	7(11.84)	3(5.08)	3(5.08)	54 (91.52)

□□□-value 0.2

Table 3: Distribution of the total colony-forming units of fungi species isolated in the study

Vending Type	Water usage	Coccidiodes spp	Penicillium spp.	Drechsler spp.	Aspergillus flavus	Aspergillus niger	Aspergillus fumigatus	Candida spp	Paecilomyces spp.	Total
		Mean TCFU/ 100 ml	Mean TCFU/ 100 ml	Mean TCFU/ 100 ml	Mean TCFU/ 100 ml	Mean TCFU/ 100 ml	Mean TCFU/ 100 ml	Mean TCFU/ 100 ml	Mean TCFU/ 100 ml	Mean TCFU/ 100 ml
Street (S)	Food preparation (26)	1±0.0	1.73±0.52	7.75±4.66	1.89±0.73	1±0.0	1.81±0.81	2.96±0.48	2.31±0.43	2.56±2.19
	Drinking (25)	1.59±0.0	1.56±0.79	1.51±0.91	1.33±0.51	1.15±0.44	1.67±0.82	1±0.0	2±0.0	1.43±0.20
Street Total (51)		1.30±0.42	1.65±0.12	4.63±4.41	1.61±0.40	1.07±0.10	1.74±0.09	1.92±1.39	2.16±0.22	2.01±1.11

Discussion

The result of this research identified isolates from six (6) genera (*Penicillium*, *Aspergillus*, *Paecilomyces*, *Candida*, *Drechslera* and *Coccidiodes*). The most frequently isolated genus is *Penicillium* (23.73%), followed by *Aspergillus* with three species identified, namely *A. flavus*, *A. niger* and *A. fumigatus* while *Paecilomyces* and *Coccidiodes* were the least with occurrence rate of 5.08% each (Table 2). This agrees with findings by Okpako *et al*. (2009) on sachet and borehole drinking water in Calabar, Nigeria which showed *Aspergillus* (29.4%) as the major genus isolated in their study. Anaissie *et al*. (2003) noted that species of *Aspergillus* and *Penicillium* have been the fungi most often found.

The World Health Organization (WHO) and Food and Agricultural Organization (FAO) allowable limit of bacteria count for potable water and Standards Organization of Nigeria (SON) permissible level of coliform did not address fungi,

however, wholesome water for human consumption should be free from any micro-organisms, parasites or substances which in numbers or concentrations constitute a potential danger to human health.

The fungal counts recorded in this study may be attributed to the fact that most street food sellers operate in open structures and side streets under very gross conditions with little attention paid to hygiene practices.Hence, the ease of contamination via exposure to dust, insects, the hands of the food handlers and customers is high. In some instances, water fetchers and cups were seen dropped carelessly on the floor with no safety precautions, while some vendors displayed bottled and sachet water on bare ground, under the sun and even on dirty slabs while some water storage vessels were noted to be uncovered or poorly covered with periodic cleaning of these water containers not guaranteed.

Further analysis showed that higher fungi counts were observed in water samples used for food preparation than those for drinking by customers. This may be as a result of the belief that the cooking process will eliminate possible contaminants in water for food preparation hence priority is not placed on the water source and its handling. Another possible contributory factor is the fact that most of the vendors served packaged water (bottled or satchet water) for drinking which to an extent had undergone some level of treatment during its processing and packaging. The use of packaged water especially bottled water by food vendors is merely based on the assumption of purity and this can be misleading. Over the years, the use of packaged water has grown astronomically in Nigeria with several hundreds of million litres of these water products consumed every year by Nigerians (Ogundipe, 2008). This high demand for packaged water by consumers has led to springing up of many water producing industries where water is packaged without proper regard for hygiene. Therefore concerns about the safety, microbial quality and public health implications of these products have been raised (Olaoye and Onilude, 2009; Onoja *et al*., 2015).

Research suggests that some of the sachet water products sold in Nigeria are non-compliant with the NAFDAC (National Agency for Food and Drug Administration and Control) and/or NIS (Nigerian Industrial standard) standards for drinking quality (Akunyili, 2003; Onoja *et al*.,2015). The presence of different species of fungi in supposedly "fungi-free" bottled water is of great concern. Whether the species of fungi present in the water samples are pathogenic or not, the fact that these are present, the hazards of contamination, and health risks to consumers should not be taken for granted (Abed and Alwakeel, 2007).

Fungal contaminants are known to cause allergies, opportunistic infections and intoxications. *Aspergillus* spp., the most prevalent fungi isolated in this study are opportunistic and can cause health problems in immune-compromised patients. *Aspergillus fumigatus* is the most common cause of all forms of invasive and non-invasive aspergillosis (Morgan *et al*., 2005). *A. flavus* produces mycotoxins called aflatoxins, which are toxic to humans and animals (Mesquita-Rocha *et al*.,2013). Aflatoxins are potent carcinogens which are responsible for many thousands of human deaths mostly in non-industrialized tropical countries (Shephard, 2006). *Penicillium* species are opportunistic and capable of causing infections such as allergies, asthma and other respiratory problems in immune-compromised patients (Schwab and Strauss, 2004). *Coccidiodes* species is associated with systemic mycosis which can run a fulminant course. The fungal species isolated in this study may have these potentials if susceptible individuals are exposed to it.

The result has revealed the presence of some fungi of hazardous inclinations in water used by street food vendors in Owerri. There is a need for concerted public enlightenment on the risk posed by consumption of fungi contaminated water and observance of sound food safety and hygienic practices.

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