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Prevalence and Antifungal Profile of Fungi Isolated From Drinking Water Sources in Gbam in Tai Ogoni

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ABSTRACT

Fungal contamination of water sources is a significant concern due to the potential health risks associated with fungal infections. This study investigated the prevalence and antifungal sensitivity of fungal species in various drinking water sources (surface water, borehole, well and sachet water) in Gbam community in Ogoni in Tai local government area. The samples were analyzed using standard microbiological techniques to isolate and identify fungal species. Fungal colonies were identified based on morphological and microscopic characteristics. The results revealed a high prevalence of fungal species in water sources, surface water recorded higher percentage followed by well and borehole water respectively. Various fungal species belonging to the genera *Candida* (60%), *Mucor* (21%), *Penicillium* (12%), and *Aspergillus* (7%), were identified. The results of antifungal sensitivity profile revealed that all the fungal isolates except *Candida* and *Yeast* species were resistant to Nystatin and Clostrinazole while all the isolate revealed high sensitivity level to Ketoconazole and Fluconazole except *Mucor* species that was resistant to ketoconazole. These fungal species are known to causes infections. The presence of these fungi in the water sources suggests a potential risk of fungal infections to individuals consuming contaminated water. Proper water treatment and disinfection are necessary to prevent fungal contamination and ensure the safety of drinking water. This study highlights the prevalence and diversity of fungal species in drinking water sources in Gbam in Ogoni. The findings emphasize the need for regular monitoring and protection of water sources to prevent fungal contamination and protect public health.

Keywords: Gbam-Ogoni community, drinking water sources, stream, well, borehole, fungi, prevalence.

Introduction

Water is vital to our existence in life and its importance in our daily life makes it imperative that it should have the quality characteristics of its intended use. The public health significance of water quality cannot be over emphasized. Water supplies used for human consumption (potable water) must be free from organisms and from concentration of chemical substances that may be hazardous to health (Obire and Alali, 2015). Also supplies of drinking water should be pleasant to drink as circumstances permit, it should be free of colour, taste and odor which are important for public water sources used for drinking (WHO, 2011).

According to Obire (2009), there are various sources of water for drinking and household use. These include rainwater, surface water (streams, rivers, springs, lakes etc.) and underground water (shallow wells and deep wells and springs).

Surface water is easily polluted, either by direct contamination by man and animals, or indirectly when rain washes faeces and other pollutants from the banks into the water body. Shallow wells are liable to pollution by seepage from surface water (Fish *et al.,* 2017). Sources of water pollution include effluents of untreated sewage that are dumped directly into water bodies, runoffs containing faecal materials, leaking pipes run in gutters or drainages, domestic effluents containing large microbial populations that are involved in degradative processes, and hospital effluents, etc.

A suitable water source should provide a supply that is adequate and safe. It should be free from chemical and biological hazards contaminants, and acceptable in terms of its taste, colour and softness (Obire *et al.,* 2009). Potable drinking water is a transparent liquid without colour, taste or odour.

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However, when infected with organisms like fungi, these qualities are lost and instead such water becomes harmful to both human and animal populations .Bad tastes in water have been attributed to micro-fungi for decades (Kelley *et al*., 2003). They have often been associated with pipe wall growth of microorganisms that is biofilms. Fungal infections are becoming more and more important because of increasing numbers of immunosuppressed patients. Nonetheless, waterborne fungi are associated with taste and odour problems, contamination of food and beverage preparation, and in a variety of health related effects (Doggett, 2000; Joseph and Michelle, 2003).

A wide variety of fungi species have been isolated from water in various investigations. The lists of taxa reported in these investigations vary from study to study. Some of these species isolated from water samples are known to be strong allergenic skin irritants or may cause infections in immuno-suppressed individuals such as AIDS, cancer, and organ transplant patients and persons with asthma or various respiratory problems (Gunhild *et al.,* 2006). An increase in the number of invasive diseases due to fungi has occurred recently (Kelley *et al*., 2003). In Nigeria water borne diseases are one of the main problems in rural and urban communities. These diseases are as a result of bacterial, fungal or other microbial infection of water. Unfortunately, most water screening methods in Nigeria are focused on the occurrence and significance of bacteria with little attention to other microorganisms such as fungi. It is on this note that we decided to investigate the prevalence of fungal species in different potable water sources in Gbam, Tai Local Government Area, Rivers State, Nigeria.

Materials and Method

Description of Site

This study area was Gbam community in Tai Local Government Area, Rivers State which is situated in the Niger Delta region of Nigeria, on the coast of gulf of Guinea and east of the city of Port Harcourt with a population of close to 832,000 (National Census, 2006). The major sources of drinking water supply in Gbam community are; Surface water (Stream), underground water (well, and borehole), sachet water popularly known as ('pure water').

Boreholes which are privately owned sell water to the public without any adequate form of treatment, and the recently refurbished public water supply treated in an ultramodern treatment plant before being distributed to consumers by the Rivers State Water Board (designated as borehole 2 for this study).

The GPS coordinates of the seven (7) sampling locations are as follows; Borehole samples were collected from Menedee compound (4°44'22.1724"N 7°2'51.57492") and Ntogo compound 4°45'37.962" N and 7°2'11.574"E. The streams 1 were collection location with coordinate (4°45'24.282" N 7°1'56.136"E), and Stream 2 from location with coordinate 4°45'13.098"N 7°0'46.734"E. Well water samples were collected from Barinee and from Baritordum compounds with a coordinate 4°47'12.942"N and 6°59'21.462"E, 4°45'27.963"N and 7°2'31.564"E respectively. While Sachet water sample was purchase from shop 1 at location with coordinate 4°45'14.284"N and 7°1'56.146"E

Collection of Samples

Two water samples were collected from each of the 7 water sources, monthly for a period of four months (June, July, August and September, 2023). A total of fifty-six (56) water samples of stream, well, borehole and sachet water were aseptically collected from Gbam community using standard methods. Water samples of approximately 600ml were aseptically collected during each sampling poured aseptically into sterile bottles and properly labeled and transferred into ice packed cool box. After collection of water samples from all the water sources, they were transported immediately in the cool box containing ice packs to Rivers State University Microbiology laboratory for analysis (APHA, 2002).

Sterilization

Materials (sample bottles, medium containing agar) used in the research were sterilized by autoclaving at 121°C for 15 min. For sachet water samples, Sodium thiosulphate solution 100 gl -l was added to the sample bottles before autoclaving. The use of sodium thiosulphate was to stop the fungal effect of residual chlorine from acting on any fungi that may be present in the water sample (APHA, 1992; Oni, 2004). Borehole samples were not treated with sodium thiosulphate solution.

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However, during collection taps were washed and flushed several times and allowed to run for 5 mins. Sample bottles were then opened and water quickly collected making sure that the bottles did not touch the taps before, during and after collection.

Enumeration of the Fungal Colonies

This was determined using the Sabround Dextrose Agar (SDA) amended with Tetracycline to suppress bacterial growth (Okerentugba and Ezereonye 2003). The spread plate technique as described by Prescott *et al* (2005) was adopted. An aliquot 0.1ml from 10^{-2} dilution of the serially diluted samples was inoculated onto pre-dried SDA agar plates in duplicates. The inocula were then spread evenly on the surface of the media using a flamed bent spreader. The plates were then incubated at room temperature (25 ^{o}C) for 5 days after which the colonies that developed were counted and the mean total Fungi counts were recorded accordingly.

Identification of Fungal Isolates

The fungal isolates were identified based on morphological and microscopic characteristics such as colony growth pattern, conidial morphology, and pigmentation. The technique described by (Oni mi *et al*., 2001) was also adopted for the identification of the isolated fungi using cotton blue in lactophenol stain. This was done by placing a drop of the stain on clean slide with the aid of a mounting needle, where a small portion of the aerial mycelia from the representative fungi cultures was removed and placed in a drop of lacto phenol. The mycelium was well spread on the slide with the needle. A cover slip was gently placed with little pressure to eliminate air bubbles. The slide was then mounted and viewed under the light microscope with ×10 and ×40 objective lenses. The morphological characteristics and appearance of the fungal isolates seen were identified in accordance with standard scheme for identification of fungi as adopted by Okerentugba and Ezereonye (2003).

Antifungal Sensitivity Testing

Disk diffusion method was employed. The disks contained 10µg/ml of ketoconazole, 25µg/ml of fluconazole, 100 units of nystatin, and 10µg/ml of clotrimazole were prepared in the laboratory using sterile filter paper.

Five different colonies, each measuring about 1mm, were selected from a 24-hour-old culture grown on potato dextrose agar and incubated at 35°C to create the inoculums. Five milliliters of sterile 0.85% normal saline was used to suspend the colonies. To obtain 1x 10^{5} cells/ml (0.5McFarland standards), the suspension is vortexed and its turbidity is adjusted. Applying the inoculum suspension-moistened sterile cotton swab to a plate of potato dextrose agar supplemented with 250mg of Tetracycline.

Antibiotics disc were positioned in the middle of the agar after the plates had been left to dry for five to fifteen minutes. The plates were incubated at 37°C for 18-24hours, and after 48hours, the slowly growing isolates were once more measured. Millimeters were used to measure zone sizes. The data was interpreted using the zone interpretative criteria for fluconazole (National Committee for Clinical Laboratory Standards, 2000) for susceptible (diameter≥19mm), resistant (diameter≤14mm), or dose dependent (diameter between 15 and 18 mm) (CLSI, 2012). For the plating method, 500 µl of samples were plated on agar plates.

Each colony from the primary plates was sub-cultured onto fresh Potato dextrose agar, tap water agar and malt extract agar each supplemented with 300 mgl^{-1} cefotaxime and 100 mgl^{-1} Kanamycin to inhibit bacterial growth. These were replicated three times.

Maintenance of Pure Culture

The sub-culture was carried out to purify the fungi isolates. During the sub-culture an inoculating loop flamed in a bursen-burner was used to pick the colony and smeared on the agar plate. This was further incubated at room temperature for 3 days. Fungal colonies were isolated upon formation, stained with lactophenol and observed under the microscope. Fungi so observed were identified using appropriate taxonomic guides (Doggett, 2000).

Statistical Analysis

Statistical analysis using one-way analysis of variance method to compare the results obtained in all the containers was performed. A p-value of less than or equal to (<0.05) was considered to be statistically significant.

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Results

The result of the fungi counts obtained from the water sources in the study area is presented in Table 1. The fungal counts from the surface water ranged from 3.1 ±0.14×10^3 to 4.2 ±0.12×10^{3}CFU/ml, Well water counts ranged from 2.0 ±1.21×10^3 to 2.1 ±0.70×10^{3}CFU/ml, Borehole 1.6 ±0.14×10^3 to 1.8 ±0.07×10^{3}CFU/ml and Sachet water 1.2 ±0.12×10^3 to 1.4 ±0.70×10^{2}CFU/ml .

In this study surface water had high microbial counts followed by well water, Borehole water and sachet water recorded the lowest count across the study period.

The results of the T test analysis showed that there was a significant difference between the fungal counts obtained from surface, well, borehole and sachet water samples at P≤0.05. The high counts recorded in the surface water samples could be attributed to the anthropogenic activities that takes place more frequently around the study area such as open defecation into the surface water, animal activities around the water, dropping of dry leaves, and disposal of wastes.

The occurrence (%) of fungi species isolated in the study is presented in Figure 1. *Aspergillus* (10%), *Rhizopus* (13%), *Penicillium* (15%), *Mucor* (18%), *Candida* (24%), *Yeast* (20%) were the most isolated.

Table 1: Mean Fungal Population of the Water Samples

Water Sample	Fungal Population (CFU/ml)			
	June	July	August	September
Borehole water 1	3.3±0.58 x10^{3a}	1.0±0.20 x10^{3a}	2.3±0.42 x10^{3ab}	3.0±0.21 x10^{3f}
Borehole water 2	4.2±0.10 x10^{3d}	1.2±0.20 x10^{3b}	2.9±0.31 x10^{3a}	1.2±0.15 x10^{3e}
Surface water 1	1.6±1.16 x10^{3a}	2.6±0.20 x10^{3bc}	3.2±0.30 x10^{3a}	3.8±0.10 x10^{3d}
Surface water 2	2.0±0.58 x10^{3b}	2.4±0.20 x10^{3b}	3.5±0.15 x10^{3bc}	4.9±1.00 x10^{3bc}
Sachet Water	0.3±0.58 x10^{3a}	0.9±0.21 x10^{3cd}	1.0±0.26 x10^{3cd}	1.0±1.00 x10^{3a}
Well water 1	1.0±0.00 x10^{3a}	2.1±0.21 x10^{3d}	2.1±1.00 x10^{3d}	3.3±2.52^{a} x10^{3b}
Well Water 2	2.5±0.44 x10^{3c}	1.8±0.20 x10^{3cd}	2.2±0.21 x10^{3e}	3.3±0.49 x10^{3a}
P-value	0.000	0.000	0.000	0.000

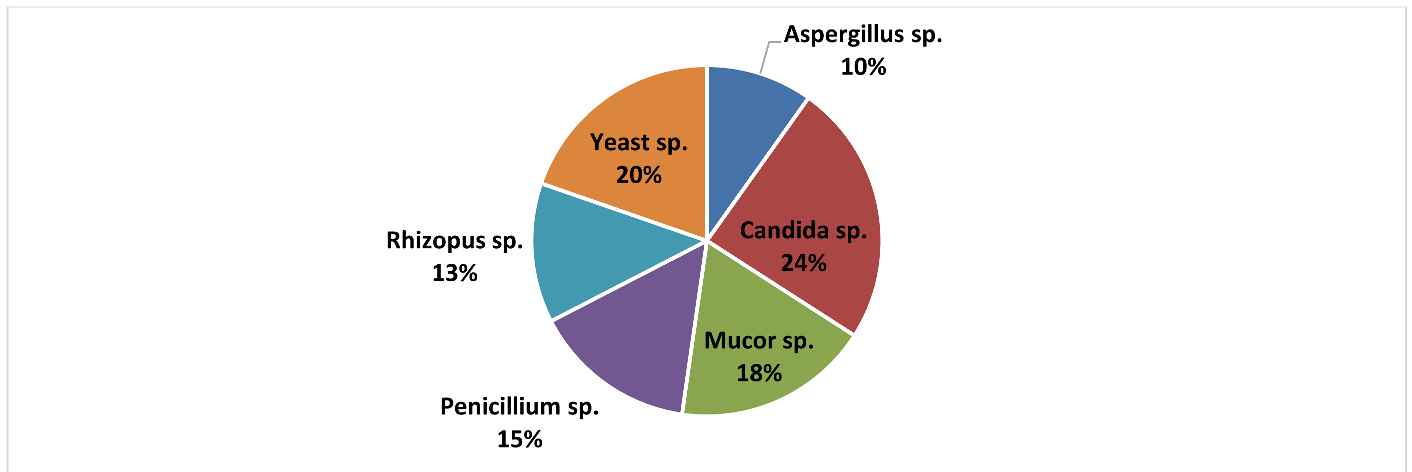

Figure 1: Percentage Fungi Species from the Different Water Samples

The result of the frequency occurrence of fungi species isolated from the different water samples is presented in Table 2. While the results of antifungal profile of the fungi species isolated from the different water in this study is presented in Table 3.

Results revealed that Fluconazole was sensitive to all the fungi isolated followed by Ketoconazole that was sensitive to all except *Mucor* species. Nystatin, Clostrinazole shows varied degree of response to the different species isolate.

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Table 2: Frequency Occurrence of Fungi Species Isolated from the Drinking Water Samples

Fungal isolate	Drinking water sample source				
	Surface water	Well	Sachet	Borehole	Total Frequency
Aspergillus sp.	8	5	0	0	13
Candida sp.	12	10	3	7	32
Mucor sp.	11	9	0	4	24
Penicillium sp.	8	4	0	0	12
Rhizopus sp.	7	10	0	0	17
Yeast sp.	11	11	0	4	26
Total	57	49	3	15	124

Table 3: Antifungal Sensitivity Profile of Fungi Isolated from the Drinking Water Samples

Fungal Isolate	Antifungal agent			
	Nystatin	Clostrinazole	Fluconazole	Ketoconazole
Aspergillus sp.	I	R	S	S
Candida sp.	R	S	S	S
Mucor sp.	R	R	S	R
Penicillium sp.	R	R	S	S
Rhizopus sp.	R	R	S	S
Yeast sp.	R	S	S	S

Key: R- Resistance, **S**- Sensitivity, **I**-Intermediate.

Discussion

The present study revealed the fungal counts obtained from the drinking water sources in Gbam community in Ogoni across the sampling period of four months (June to September 2023). The decreasing order of fungal counts of the drinking water samples was; surface water (Stream water) > Borehole water > Well water > Sachet water. This indicates that the sachet water is the least contaminated with fungi. This is attributed to some form of treatment and the packing in the presence of UV light which has the potential to inhibit some of the fungi while stream water is heavily contaminated because of its exposure to environmental factors. This observation agreed with findings of Nwankwo (2020), who reported high fungal count in groundwater in their study. The location of the streams prone to contamination considering the prevailing environmental practices and other anthropogenic activities such as sand mining around the stream could be blamed for the high fungal population. Furthermore, open defecation is still rampant in these areas and has been implicated for contamination of surface and groundwater (Kumpel, *et al.,* 2017). Poor agricultural practices may also have contributed to the fungal contamination of the stream waters in this community. This is especially true because the indigenes are mostly farmers (Asuquo *et al.,* 2016).

The results of this research show that fungi occur widely in drinking water sources in Gbam community in Ogoni. The fungal genera of *Aspergillus, Rhizopus, Penicillium*, and *Candida* were often detected in this study from the various drinking water samples namely; Borehole water, surface water, sachet water and well water, except that *Aspergillus, Rhizopus,* and *Penicillium* were not isolated from borehole and sachet water respectively while *Mucor* species was present in borehole but not detected in sachet water while *Candida* species was present in all the water sources (Table 2). Future research should address the efficiency of the different treatment processes currently used for the inactivation of fungi and whether adverse health effects may be associated with the presence of these species in drinking water sources. Furthermore, future studies should also be conducted to investigate if mycotoxins can be produced in these matrices.

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If so, removal of secondary metabolites by drinking water treatment processes should also be evaluated. The positive role of the described species in the degradation of persistent drinking water pollutants should also be considered. The sachet drinking water, which is affordable and consumed by almost everyone in Gbam community is generally regarded or termed "pure water". However, according to Akunyili (2005), the question that still remains is: how pure is the "pure water"? Four (4) genera of fungi viz: *Aspergillus, Mucor, Rhizopus* and *Penicillium* were isolated from sachet drinking water samples. This is an indication that these waters are not well treated before packaging. It could also be that the use of chlorination as a chief purification disinfectant procedure, has remained uncertain in the treatment of water by our sachet water producers or their treatment processes is probably not suitable to eliminate fungi. De Maria (2006) and Oni (2001) independently reported that purification procedures such as chlorination do not eliminate fungal spores, which implies that perhaps the treatment given to our sachet water is usually not effective enough to eliminate these microorganisms. Also, Gunhild *et al.* (2006) suggested that several mold species survive disinfection and water treatment and could thus contaminate the water that reaches the consumer.

All the borehole samples examined showed evidences of contamination (Table 2). The presence of fungi in the borehole samples probably indicates poor treatment techniques. The presence of fungi in the borehole samples may also be as a result of intrusion from compromised water mains during distribution (Hageskal *et al*., 2019). Furthermore, since these boreholes are sited within residential areas; it is probably that poorly designed septic tanks, poor drainage, human waste water disposal and poor sanitation (APHA 2002) can add endangering fungi to the water, Also, differences in raw water sources, treatment protocols and system maintenance could certainly account for the unique fungal assemblage (Doggett, 2000). These fungi cause a wide range of diseases in humans, ranging from hypersensitivity reactions to invasive infections associated with angio invasions.

Antifungal sensitivity testing revealed that, all the isolates showed resistance to Nystatin. All the isolates except *Candida* and *Yeast* species showed resistance to Clostrinazole while all the isolates except *Mucor* species that was resistant to ketoconazole, exhibited a high sensitivity level to Ketoconazole and Fluconazole. The high level of resistance of fungi in this study to Nystatin and Clostrinazole is a major concern. The resistance shown in this study could be attributed to the protective mechanisms developed by the fungi. It has also been noted that susceptibility to antimicrobial agents is not static and could be altered by environmental impact/factors and human activities (Adeyinka *et al.,* 2019).

In conclusion, the findings of this study have revealed that, waterborne fungi in this study are of clinical concern capable of causing disease. Since most fungi species/spores survive disinfection and water treatment, it is thus suggested that good water treatment techniques that would eliminate not only fungi but all forms of microorganisms that could cause water related diseases should be used to treat stream or surface water, sachet and borehole water and well water before consumption. Also, proper sanitation practices should be implemented within the vicinity of these various drinking water sources and during production of sachet water. The siting of latrines/toilets close to borehole water systems should be avoided. Furthermore, improved monitoring of water and frequent application of chlorine and other water treatment agents should be adopted.

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