

Full Length Research Paper

Prevalence and significance of fungi in sachet and borehole drinking water in Calabar, Nigeria

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Borehole and sachet (“pure water”) water are the major sources of drinking water in Calabar. Such waters are required to comply with minimum standards in order to protect public health and accepted as safe drinking water. In this paper, a study was conducted to investigate the presence and effects of fungi in sachet and borehole drinking water in Calabar using 4 sachet water samples and 10 borehole samples. Sachet water aged 2 h and 2 weeks since manufactured and each borehole sample (collected in the dry and wet seasons) was subjected to membrane filtration technique and plating method to determine the fungal content of the water. Only one sample, Usua water (sachet water) tested negative for the presence of fungi at CFU (colony forming unit) /100 ml. Laura water (sachet water), Abua water, Edgerly water and Mayne-Avenue water (borehole water) were the most infected. Percentage of fungi in borehole water was higher in the rainy than in the dry season. *Aspergillus* (29.4%), *Rhizopus* (21.6%), *Fusarium* (15.7%) and *Penicillium* (13.7%) were the most frequently isolated. These fungi have the potential to cause allergic reactions or diseases in humans.

Key words: Borehole water, fungi, membrane filtration, plating method, sachet water.

INTRODUCTION

Water is one of the most abundant and essential commodities of man occupying about 70% of the earth's surface, yet a greater percentage of the world's population, most especially in developing countries live without access to safe water (Hazen and Toranzos, 1990; Adriano and Joana, 2007). The growing population of most developing countries occurs disproportionately in urban areas. These places due to considerable pressure on already overburdened budgets make it difficult to increase the water supply infrastructure. Moreover, little or no resources are left to supply, let alone, improve water supplies (Gadgil, 1998; deVilliers, 2002 and Helweg, 2002). Nigeria for example is located in coastal West Africa where water is abundant, yet most of the population lacks adequate and safe drinking water. This thus prompted the sinking of boreholes by rich individuals and selling the water to the ever growing population without any major form of treatment. Also many Nigerians are engaged in package-ing water, popularly called “pure water” in polythene bags of about 60 – 65 cl and selling to the public. The safety of

of this “pure water” is still questionable because many who are engaged in its production do not follow strictly the standards set by FEPA (1999) and WHO (2006) for safe drinking water.

Portable drinking water is a transparent liquid without colour, taste or odour. But when infected with organisms like fungi, these qualities are lost and instead such water becomes harmful to both human and animal populations (Gerba and Bitton, 1984; Mader, 1996; Eja, 1999; Leoni et al., 1999; ATSDR, 2004). Bad tastes in water have been attributed to microfungi 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 (Nagy and Olson, 1982, 1985; Hinzelin and Block, 1986; Geldrich, 1996; 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. West

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(1986) demonstrated that fungi isolated from potable water were dematiaceous (63%) and more especially *Cladosporium* (27%), *Phoma* (9%), *Alternaria* and *Exophiala* (each 7%). Arvanitidou et al. (1999) reported *Penicillium*, *Aspergillus* and *Candida* as the major genera isolated in their study while Ana et al. (2006) indicated *Acremonium* (38.2%) and *Penicillium* (40.59%) as the major isolates amongst others in tap water in Braga, Portugal. Gunhild et al. (2006) found *Penicillium* spp, *Absidia* spp, *Acremonium* spp, *Aspergillus* spp and *Mucor* spp to be the major fungi genera inhabiting Norwegian drinking water. 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 (Arvanitidou et al., 1999; Anaissie et al., 2003). For example, the genotype of *A. fumigatus* from water was related to that of isolates from two patients (Warris, 2003) indicating that water was the source of infection.

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 and significance of fungi in sachet and borehole drinking water in Calabar, Nigeria.

MATERIALS AND METHODS

Description of site

Calabar, is located within the south eastern ecological/forest region of Nigeria and lies between latitude 4.5°N to 5.2°N and longitude 8.0°E, with an annual rainfall ranging from 3,500 to 5,000 mm and an average monthly temperature of 22 - 27.5°C. The city stretches southwards and borders with the Calabar River, while to the east, it is bordered by the Great Qua River.

The major sources of water supply are; the recently refurbished public water supply from Cross River State Water Board, which is a limited liability company owned by the Cross River State Government, and the boreholes which are privately owned and are sold to the public without any adequate form of treatment. On the other hand, the public water from Water Board is treated in an ultra-modern treatment plant before being distributed to consumers. The public water is only limited to a few individuals.

Collection of samples

Samples were collected from privately owned boreholes and sachet water packers in sterile sample bottles (400 ml). A total of 10 boreholes and 4 sachet water samples were subjected to both membrane filtration and plating (APHA, 1992; Gleeson and Gray, 1997). For boreholes, samples were collected from the public distribution tap in December 2006, January and February (2007) (dry season)

and in June, July and August (wet season of 2007) while sachet water samples were obtained from 2 hr and 2 weeks old packed sachet water.

The borehole samples were collected from Dunca, Federal Housing Estate, Etta-Agbor, Fenton, Ibom Layout, Abua, Marian, Edgerly, Mayne-Avenue and State Housing Estate. Sachet water samples were collected from Dukon water, Usua water, Laura water, and So-good water.

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 g^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 (Cabelli, 1987; APHA, 1992; Oni, 2001; Rocus, 2004). Borehole samples were not treated with sodium thiosulphate solution. However, during collection taps were washed and flushed several times and allowed to run for 5 min. Sample bottles were then opened and water quickly collected making sure that the bottles did not touch the taps before, during and after collection.

Fungal isolation/characterization

Fungi were isolated using two different methods: membrane filtration and plating methods (Nagy and Olson, 1982, 1985; Cabelli, 1987; Eja, 1999; Pond et al., 2000; Leoni et al., 2001; Ana et al., 2006; Adriano and Joana, 2007; Kanzler et al., 2007). For the membrane filtration method, 100 ml of water sample was filtered through membrane filters with a diameter of 47 mm and a pore size of 0.45 µm. The filtrates were placed in the center of agar plates after filtration and incubated at 25°C. For the plating method, 500 µl of samples were plated on agar plates. Each colony from the primary plates was sub-cultured onto fresh sabouraud dextrose agar, tap water agar and malt extract agar each supplemented with 300 mg^l cefotaxime and 100 mg^l Kanamycin to inhibit bacterial growth. These were replicated three times. 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 7 days. Fungal colonies were isolated upon formation, stained with lactophenol and observed under the microscope. Fungi so observed were identified using appropriate taxonomic guides (Watanabe, 1994; Larone, 1995; Doggett, 2000).

Statistical analysis

Analysis of variance (ANOVA) test was used to analyze the data for fungal count. Means were separated using LSD (least significant difference) test. Where necessary t-test was used (Pond et al., 2000).

RESULTS

A summary of fungi species isolated in the study is presented in Table 1. From the Table, *Aspergillus* (29.4%), *Rhizopus* (21.6%), *Fusarium* (15.7%) and *Penicillium* (13.7%) were the most frequently isolated. *Trichoderma* (1.96%) and *Geotriculum* (1.96%) were present in low frequencies. Many of the species were isolated from borehole water. The genus *Aspergillus* was represented

Table 1. Fungi species identified in sachet and borehole water samples.

Fungi	Number	%
<i>Aspergillus</i>	30	29.4
<i>Rhizopus</i>	22	21.6
<i>Fusarium</i>	16	15.7
<i>Penicillium</i>	14	13.7
<i>Mucor</i>	10	9.8
<i>Oospora</i>	6	5.88
<i>Trichoderma</i>	2	1.96
<i>Geotriculum</i>	2	1.96
Total	102	100

Table 2. Fungi species isolated in different sachet water samples investigated.

Water sample	Fungi
Usau	ND
Laura	<i>Aspergillus flavus</i> <i>Rhizopus stolonifer</i> <i>Penicillium sp</i>
Dukon	<i>Fusarium solani</i> <i>Rhizopus stolonifer</i>
So-good	<i>Aspergillus niger</i> <i>Rhizopus stolonifer</i>

The species of fungi presented here were consistent in 2 h and 2 weeks old sachet water. ND = no fungus identified.

by only two species, *A. flavus* and *A. niger* (Tables 2 and 3). The genus *Penicillium* and *Oospora* were not identified to the species level.

Sachet water

The species of fungi isolated from sachet water 2 hr and 2 weeks after production are summarized in Table 2. The results show that the species of fungi were consistent in 2 hr and 2 week old sachet water.

From the Table, only Usua water tested negative for the presence of fungi as no fungal colony was identified during the period of culture. Three species of fungi (*Aspergillus flavus*, *Rhizopus stolonifer* and *Penicillium spp*) were isolated from Laura water whereas Dukon and So-good water had 2 species each. Fungal counts at CFU/100 ml were significantly ($p < 0.05$) higher in Laura water at 2 hr and 2 weeks after production (Figure 1). Comparison in the mean fungal count (t-test) between 2 hr and 2 weeks old sachet water showed higher values ($p < 0.05$) in 2 weeks old sachet water than in 2 h old water.

Borehole water

Table 3 shows the species of fungi identified from the

Table 3. Fungi species isolated in borehole samples in the different seasons examined.

Borehole sample	Fungi species	
	Dry season	Rainy season
Edgerly	<i>M. plumbeus</i> <i>Penicillium spp</i>	<i>F. oxysporum</i> <i>A. flavus</i> <i>R. stolonifer</i> <i>Penicillium spp</i>
Abua	<i>R. stolonifer</i> <i>A. niger</i> <i>Oospora spp</i>	<i>F. solani</i> <i>A. flavus</i> <i>R. stolonifer</i> <i>Geotriculum spp</i>
State Housing Estate	ND	<i>A. flavus</i> <i>Penicillium spp</i> <i>R. stolonifer</i> <i>F. oxysporum</i>
Dunca	<i>F. solani</i>	<i>A. flavus</i> <i>R. stolonifer</i>
Mayne-Avenue	<i>M. plumbeus</i> <i>Oospora sp</i>	<i>Oospora spp</i> <i>M. plumbeus</i> <i>R. stolonifer</i>
Marian	<i>A. niger</i>	<i>F. stolonifer</i> <i>A. flavus</i> <i>Penicillium spp</i>
Fenton	<i>R. stolonifer</i>	<i>F. oxysporum</i> <i>A. niger</i> <i>R. stolonifer</i>
Federal Housing	<i>A. niger</i>	<i>F. solani</i> <i>Penicillium spp</i>
Etta-Agbor	<i>A. niger</i>	<i>A. flavus</i> <i>Trichoderma spp</i>
Ibom layout	<i>A. flavus</i> <i>M. plumbeus</i>	<i>M. plumbeus</i> <i>A. flavus</i> <i>Penicillium spp</i>

borehole samples examined. The mycobiota was dominated by species of *Aspergillus* and *Rhizopus*. *Penicillium*, *Fusarium* and *Mucor* were also frequently isolated. 5 different species were each recovered from Abua and Edgerly water, and only two were found in State Housing Estate water. A number of species in both dry and rainy seasons was observed in the water samples studied. However, the species of fungi in the different water samples varied with seasons. Rainy season recorded the highest percentage (68.42%) of fungi as against (31.58%) isolated in the dry season (Table 4). The genera *Aspergillus*, *Fusarium* and *Penicillium* were found to be particularly wide spread in the rainy than in the dry season. *A. niger* was dominant in dry season whereas *A. flavus* was frequently isolated in the rainy season (Table 4). *Trichoderma spp* and *Geotriculum spp* were not found in the dry season but appeared in the rainy season at low percentages. No fungal species was isolated from State Housing Water in dry season (Table 3). Fungal count at

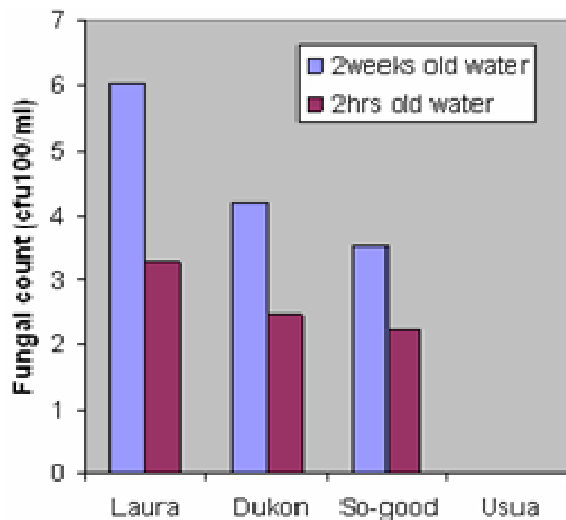


Figure 1. Fungal count in sachet water aged 2 h and 2 weeks since manufactured. No fungal colony was observed in Usua water.

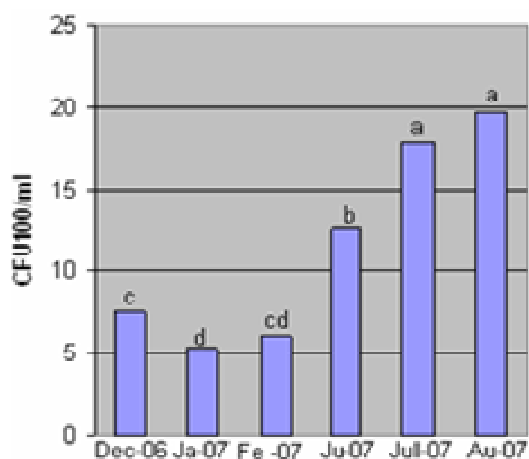


Figure 2. Fungal count in borehole water in the months studied. Counts are means of three replicates. Means with same case letter do not differ significantly at $P > 0.05$.

CFU/100 ml was significantly ($p < 0.05$) higher in the months of July and August (Figure 2 and 3).

DISCUSSION

The sachet drinking water, which is affordable and consumed by almost every Calabarian and/or Nigerian is generally regarded or termed “pure water”. However, according to Akunyili (2005), the question that still remains is: how pure is our “pure water”? 4 genera of fungi viz: *Aspergillus*, *Fusarium*, *Rhizopus* and *penicillium* were isolated from sachet drinking water (Table 2). This is an indication that these waters are not well treated. It could

Table 4. Distribution of fungi species in borehole water in dry and rainy seasons.

Fungi	No. of samples	
	Dry season	Rainy season
<i>Aspergillus</i>	10	18
<i>Fusarium</i>	5	10
<i>Penicillium</i>	3	10
<i>Rhizopus</i>	6	13
<i>Mucor</i>	4	6
<i>Trichoderma</i>	ND	2
<i>Oospora</i>	2	4
<i>Geotriculum</i>	ND	2
Total	30 (31.58%)	65 (68.42%)

also be that the use of chlorination as a chief purification procedure, which has remained dogmatic in the treatment of water by our sachet water producers is probably not suitable to eliminate fungi. De Maria (1996) 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.

Among the sachet water samples, Laura water recorded 3 species of fungi and higher colony counts than the others indicating that it is the most polluted. Although the species of fungi isolated were consistent between 2 h and 2 weeks old manufactured water, fungal counts were significantly higher in 2 weeks old water suggesting that the longer the period of storage the higher the degree of contamination supporting reports made by FEPA (1999) and Rocus (2004).

No fungal population was identified in Usua water (Table 2) indicating that the treatment technique applied by Usua water is probably effective enough to eliminate the fungi. We could therefore suggest that Usua water may be a safe and portable drinking water provided that other standards for safe drinking water as set up by FEPA (1999) and WHO (2006), which are not considered in our research are met.

All the borehole samples examined showed evidences of contamination with 2, 3 or 5 species of fungi (Table 3). The presence of fungi in the borehole samples probably indicates poor treatment techniques. The presence of fungi in the borehole samples may be as a result of intrusion from compromised water mains during distribution (Gerba and Bitton, 1984; Geldrich, 1996; Leoni et al., 1999; Adriano and Joana, 2007).

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 (WHO, 2006; Adriano and Joana, 2007)

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). The influence of physical and chemical factors unique to a given distribution system should be recognized as an important determinant of fungal disposition and growth. Water temperature could appear particularly relevant in consideration of physiological constraints for spore dormancy and viability (Cabelli, 1987; Mader, 1996; Helwey, 2002).

The distribution of fungal species isolated from borehole water varied with seasons. The total number of species isolated in the rainy season was higher than the number of species isolated in the dry season. This may be related to the fact that in the rainy season, the water table increases as a result of infiltration. During this process microorganisms from human wastes may be added to water. Our findings are in line with those of Ana et al. (2006).

The genus *Apergillus* was the most frequently isolated in our investigation. Our results are consistent with the findings of Arvanitidou et al. (1999) and Gunhild et al. (2006) that *Apergillus* is the most common isolated genera in water. *Apergillus* spp are known to produce aflatoxins (B1, B2, G1 and G2), the most toxic and potent hepatocarcinogenic natural compounds ever characterized (Bennett and Klich, 2003). These fungi cause a wide range of diseases in humans, ranging from hypersensitivity reactions to invasive infections associated with angioinvasions. *A. flavus* was frequently isolated in our investigation, particularly in borehole samples during the rainy season. This species is known to be the second leading cause of invasive and non-invasive aspergillosis (Morgan et al., 2005). *A. niger* is a common allergen and may cause opportunistic invasive infections in hospitalized immunized patients (de Hoog et al., 2000).

Fusarium spp were found in several samples in our study. *Fusarium* spp has been recognized as an agent of superficial infections (keratitis and cutaneous infections, onychomycosis and infections of wounds and burns) (Guarro and Gene, 1995). In recent years, deep-seated and disseminated infections have been increasingly described in immunocompromised patients, especially in neutropenic patients (Guarro and Gene, 1995). The prognosis is very poor and death occurs in up to 70% of cases despite antifungal therapy (Musa et al., 2000).

Penicillium species were mostly abundant in the borehole samples studied. *Penicillium* is known to cause allergy, asthma and some respiratory problems (Cooley et al., 1998; Frisvad et al., 1998 and Gunhild et al., 2006). Therefore the species isolated in this study may have allergic potentials if susceptible individuals are exposed.

A significant percentage of *Rhizopus* (Table 1) was recovered in this study. Zygomycetes are known to cause diseases in immunocompromised patients (Sheppard et al., 2004 and Ana et al., 2006). The genus *Mucor* is known to be a major cause of thrombosis, infarction, nasal or paranasal sinus infection and GI disorders.

Trichoderma species are soil borne and are characterized by rapidly growing colonies that have a great potential for spore production (Gunhild et al., 2006). The genus includes species reported to cause mycosis and allergy in humans (Jaakkola et al., 2002 and Tang et al., 2003). In our study, *Trichoderma* spp was isolated in just one borehole sample (Etta-Agbor water). This may suggest that *Trichoderma* spp is not a common inhabitant of our borehole water system.

Although it is unlikely that concentrations as low as those reported in our study can cause fungal infection in healthy people, immunosuppressed persons are at risk of infection. Kanzler et al. (2007) suggested that routine microbiological investigations should be made in hospitals or institutions where immunosuppressed individuals are treated.

Conclusion

The results presented in the present paper are, of course, not evidence that waterborne fungi are involved in disease. However, it is important to be aware that several of the same species which are of clinical concern are also present in water. Since most fungi species survive disinfection and water treatment, it is thus suggested that good treatment techniques that would eliminate not only fungi but all forms of microorganisms that could cause water related diseases should be used to treat both sachet and borehole water before distributing to the consuming population. Also, proper sanitation practices should be implemented within the vicinity of borehole water, reservoirs and during production of sachet water. Furthermore, improved monitoring of water and frequent application of chlorine and other water treatment agent should be adopted. Finally, sitting of latrines/toilets close to borehole systems should be avoided.

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