Prevalence of Moulds in Households Drinking Water of Some Local Government Areas of Kano, Nigeria

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ABSTRACT

Molds, as filamentous fungi, may occur almost everywhere, even in water. They grow well in water that they can affect the health of the population or may have negative effects on food production. The aim of this study was carried out to determine the prevalence of molds in households’ drinking water. A total of 212 water samples were collected from 4 different Local Government areas of Kano Metropolis, out of which 3 were from boreholes, 135 from households, 36 from taps and 38 from wells. Mould species were isolated by using membrane filtration method with subsequent cultivation on agar plates. The different genera of moulds were identified using cultural and microscopic techniques. A total of 206 fungal colonies were isolated from water samples and cultivated for identification and the most frequently occurred genera were Aspergillus (36.4%), Penicillium (23.3%), Fusarium (19.4%), and Bipolaris (5.8%). The mean value colony forming units for moulds 4.50, 3.60, 3.13 and 3.43cfu/100ml for borehole, household, tap and well respectively.

Keywords:
Prevalence, Moulds, Drinking water, Household
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). 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 treatment. Many Nigerians are engaged in packaging water, popularly called “pure water” in polythene bags of about 60 – 65cl and selling to the public. The safety 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.

Fungal infections are becoming more and more important because of increasing numbers of immune suppressed 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 (1986) demonstrated that fungi isolated from portable 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 immune suppressed individuals such as AIDS, cancer, 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). 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 moulds in household’s drinking water in Kano state, Nigeria.

MATERIALS AND METHODS

Study area

The study was conducted in four local government areas of Kano State viz: Ungogo, Dala, Kunbotso, and Gwale.
Sample size

A total of 212 water samples were collected for the study. Samples collection was done in four local government areas of Kano state.

Sample collection

Standard method described by American Public Health Association (APHA, 1999), was used for the collection of samples. During collection of samples, in each targeted house, 300ml of water was poured aseptically into 300ml sterilized bottles. For tap water and borehole, the samples were collected by allowing the water to run to waste for 2 or 3 minutes and then the water were aseptically collected in sterile bottles. Water from wells was collected by means of a sterilized bottle fitted with a
weight at the base. All samples collected were then labeled with sample number, date of collection and sample source for analysis purposes, and then sealed. After sampling, a structured questionnaire was administered to each participating household. The questionnaire included variables such as family size, devices used to collect and store water, storage duration and water treatment. Samples collected were then transported to the laboratory in an iced cooler for storage as soon as possible.

Sample filtration

Membrane filter assembly was set up by inserting the glass funnel bottom into the opening of a jar flask. At the side of the flask, there is a narrow opening; this was then connected to the vacuum pump machine through rubber tubing. During filtration, the membrane filter was placed into the funnel using sterile forceps. Sample was shaken vigorously at least 25 times up and down to mix the sample and then 100ml of sample was poured into the funnel and the vacuum pump was then turned on to drain the sample through the sterile 47mm and 0.45µm membrane filters (Whatman, Maidstone, Japan). After filtering, the funnel walls were rinsed three times with 20-30ml of sterile peptone water, then the vacuum pump was turned off and the funnel top was lifted up to remove the membrane filter using sterile forceps and the filter was placed on Sabouraud Dextrose Agar, followed by incubation at 20-25°C for 3-5 days (APHA, 1999).

Isolation and identification of mould

Colonies on SDA were sub cultured in the same medium to isolate a pure single colony for identification test. During identification, a drop of 95% ethanol was placed on a microscope slide. Using a sterile inoculating needle, a small portion of fungal growth was gently removed and placed on 95% ethanol and then gently spread it out with two dissecting needles so that it can easily be identified when viewing. When most of the ethanol has evaporated, a drop of lacto-phenol cotton blue was added and covered with a cover glass and then examined microscopically (Chei et al., 2000; Food and Agriculture Organization, 1979).

RESULTS

A total of 212 water samples were collected from 4 different Local Government areas of Kano Metropolis, out of which 3 were from boreholes, 135 from households, 36 from taps and 38 from wells. 206 fungal colonies were isolated from water samples and cultivated for identification. Of these, (75) 36.4% was **Aspergillus** (12)5.8% **Bipolaris**, (7)3.4% **Alternaria**, (6)2.9% **Cladosporium**, (40)19.4% **fusarium**, (4)1.9% **Gliocardium**, (7)3.4% **Mucor**, (4)1.9% **Rhizopus**, (48)23.3% **Penicillium**, and (3)1.5% **Pullularia**. (Table 1).

Of the 75 Aspergillus, (34)20.9% was A. niger, (16)9.8% A. terreus, (8)4.9% A. glaucus, (14)8.6% A. nidulans and (3)1.8% A. clavatus. Of the 48 Penicillium, (26)16.0% was P. rugulosum, and (22)13.5% P. rubrum. Of the 40 Fusarium, (19)11.7% was F. tricinctum, (15)9.2% F. nivale, and (6)3.7% F. oxyporium (Table 2).

The average Colony Forming Units (CFU) were 4.50±0.71, 3.60±1.61, 3.13±1.96, and 3.43±1.95cfu/100ml for borehole, household, tap and well respectively. The average colony forming units (CFU) were 3.38±1.82, 3.90±1.83, 3.28±1.54 and 3.89±1.78cfu/100ml for Kumbotso, Gwale, Dala and Ungogo respectively (Table 3).

### Table: Genera of moulds identified in contaminated drinking water

<table>
<thead>
<tr>
<th>Mould</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus</td>
<td>75</td>
<td>36.4</td>
</tr>
<tr>
<td>Bipolaris</td>
<td>12</td>
<td>5.8</td>
</tr>
<tr>
<td>Alternaria</td>
<td>7</td>
<td>3.4</td>
</tr>
<tr>
<td>Cladosporium</td>
<td>6</td>
<td>2.9</td>
</tr>
<tr>
<td>Fusarium</td>
<td>40</td>
<td>19.4</td>
</tr>
<tr>
<td>Gliocardium</td>
<td>4</td>
<td>1.9</td>
</tr>
<tr>
<td>Mucor</td>
<td>7</td>
<td>3.4</td>
</tr>
<tr>
<td>Rhizopus</td>
<td>4</td>
<td>1.9</td>
</tr>
<tr>
<td>Penicillium</td>
<td>48</td>
<td>23.3</td>
</tr>
<tr>
<td>Pullularia</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>Total</td>
<td>206</td>
<td>99.9</td>
</tr>
</tbody>
</table>
### Table 2: Species of moulds identified in drinking water

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus niger</td>
<td>34</td>
<td>20.9</td>
</tr>
<tr>
<td>A. terreus</td>
<td>16</td>
<td>9.8</td>
</tr>
<tr>
<td>A. glaucus</td>
<td>8</td>
<td>4.9</td>
</tr>
<tr>
<td>A. nidulans</td>
<td>14</td>
<td>8.6</td>
</tr>
<tr>
<td>A. clavatus</td>
<td>3</td>
<td>1.8</td>
</tr>
<tr>
<td>P. rugulosum</td>
<td>26</td>
<td>16.0</td>
</tr>
<tr>
<td>P. rubrum</td>
<td>22</td>
<td>13.4</td>
</tr>
<tr>
<td>F. tricinctum</td>
<td>19</td>
<td>11.7</td>
</tr>
<tr>
<td>F. nivale</td>
<td>15</td>
<td>9.2</td>
</tr>
<tr>
<td>F. oxysporum</td>
<td>6</td>
<td>3.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>163</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

### Table 3: Mean, standard deviation of moulds cfu/100ml with respect to sources and sites

<table>
<thead>
<tr>
<th>Source</th>
<th>Moulds</th>
<th>Site</th>
<th>Moulds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cfus/100ml</td>
<td></td>
<td>Cfus/100ml</td>
</tr>
<tr>
<td></td>
<td>x±mx</td>
<td></td>
<td>x±mx</td>
</tr>
<tr>
<td>Borehole</td>
<td>4.50±0.71</td>
<td>Kumbotso</td>
<td>3.38±1.82</td>
</tr>
<tr>
<td>Household</td>
<td>3.60±1.67</td>
<td>Gwale</td>
<td>3.90±1.83</td>
</tr>
<tr>
<td>Tap</td>
<td>3.13±1.96</td>
<td>Dala</td>
<td>3.28±1.54</td>
</tr>
<tr>
<td>Well</td>
<td>3.43±1.95</td>
<td>Ungogo</td>
<td>3.89±1.78</td>
</tr>
</tbody>
</table>

### DISCUSSION

In the study, the most frequently isolated mould was the genus *Aspergillus*. These findings are consistent with the works conducted by Arvanitidou *et al.* (1999 and 2000) and Gunhild *et al.* (2006), that *Aspergillus* was the most commonly isolated genera in water. *Aspergillus* 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 angio-invasions. *A. niger, A. terreus* and *A. nidulans* were found on several occasions during this study. The finding of *A. niger* was in agreement with the work conducted by Hageskal *et al.* (2006) in which he also reported the frequent occurrence of *A. niger* in drinking water. However, the presence of *A. terreus* and *A. nidulans* was not consistent with the work of Okpako *et al.*, (2009) in that he reported the frequent occurrence of *A. flavus* in borehole water samples. *A. niger* is a common allergen and may cause opportunistic invasive infections in hospitalized immunized patients (De Hooget al., 2000).

*Penicillium* species were especially abundantly distributed and clearly have the ability to survive water treatment and contaminate water reaching various network installations. Only heating of water seems to inhibit the recovery of viable *Penicillium* spores or hyphae. The implication of *Penicillium* species in allergy, asthma, or other respiratory problems has been a subject of several studies worldwide (Schwab and Straus, 2004). Strong associations between *Penicillium* spp. and health problems were also reported by Cooley *et al.* (1998). Hence, many of the species isolated in the present investigation may have allergic potential if susceptible individuals are exposed. Furthermore, several of the demonstrated *Penicillium* species have been reported to be active mycotoxin producers (Frisvad *et al.*, 1998, Moreau, 1979, Samson *et al.*, 2004). This fact raises the question of potential mycotoxin production in water and further investigations into this problem are merited. The genus *Penicillium* also includes common contaminants of food and beverages (Pitt and Hocking, 1999, Samson *et al.*, 2004). It is not unlikely that water can be the route of transmission for mold contamination and spoilage of foods.

*Fusarium* species were also found in large numbers 40(19.4%). Several researches have been found to show the occurrence of *Fusarium* species in water environments. Okpako *et al.*, (2009), reported the
occurrence of *Fusarium* species on several samples 16(15.7%). Similarly, Anaissie et al., (2002), recorded 14 species of *Fusarium* from water sampled in hospital. *Fusarium* species have been recognized as agents 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 immune compromised 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).

Very small percentage of *Rhizopus* was found in the research. Okpako et al., (2009), recovered a significant percentage of *Rhizopus*in sachet and borehole water. Zygomycetes are known to cause diseases in immune compromised 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.

The presence of these filamentous fungi may be mainly associated with post treatment contamination from outside sources, or post collection contamination, or from populations growing within biofilms or other materials (such as pipe joints and seals) in the distribution system, or they were able to escape the treatment procedures or the contamination is the source. (Bays et al., 1970, Grabinska et al., 2007, Ramirez-toro and Minnigh, 2002).

The detection of pathogenic microorganisms in different sources of drinking water also reveals the alarming situation for households’ water. The high prevalence of filamentous fungi in households’ drinking water is a matter of serious concern.

On comparison, there was no significant difference in the occurrence of moulds between water collected from boreholes, households, taps and wells (p>0.05). Also a significant difference was not found in the occurrence of moulds in waters collected from Kumbotso, Gwale, Dala and Ungogo (p>0.05). The lack of a difference between these sites may be associated to the fact that most of them have the same sources of water.

The mean number of cfu/100ml was 4.50, 3.60, 3.13 and 3.43 for boreholes, households, taps and wells respectively. Yamaguchi et al., (2007), also reported the mean number of cfu/ml as 10.8 and 11.6 for yeasts and moulds in bottled mineral water, respectively. In municipal tap water, he also reported 2.8cfu/ml for yeasts and 1.0cfu/ml for moulds.

It is unlikely that the occurrence of moulds in water at the concentrations observed in this study would cause disease in healthy individuals. However, if the right conditions are present and regrowth of moulds occurs in water systems, exposure of humans to large amounts of potentially harmful mold species could become a problem. Several of the molds are potential toxin producers and exposure to small amounts of toxins for several years may have negative effects on the immune system (Letsher-Bru et al., 2002, Vismer et al., 2002).

**CONCLUSION AND RECOMMENDATIONS**

Since most of the species of molds isolated are pathogenic to humans, it is likely that exposure of humans to very large amounts of potentially harmful mold species could cause infections to healthy individuals. Owing to the health hazards associated with the fungi isolated from drinking water used for this study, the following recommendations are necessary; Prevention of storm flooding into springs and wells. If source water is microbiological clean, then use of containers with a narrow mouth and lid, would render boiling unnecessary. Governments should improve dissemination of information on private water testing, personal hygiene and sanitation.

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