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Yeasts Contamination in Drinking Water in Kano, Nigeria

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ABSTRACT

Water used for domestic purposes is ideally required to be free from contaminants. Various contaminants have frequently affect the quality of such water. This study was carried out to determine the frequency of occurrence of yeasts in 2 different sources of household's drinking water. A total of 173 water samples were collected from four different Local Government Areas of Kano State, Nigeria. Samples were processed by using membrane filter, followed by subsequent cultivation on agar plates. A total of 108 isolates of yeasts were isolated and identified using microscopic and biochemical methods. *Candida* spp were most frequent with total 77 (71.3%) occurrence from both sources of water. The least was *Geotrichum* spp with total frequency of 6 (5.6%) from both sources. Among the *Candida*, *C. krusei* shows the highest frequency of occurrence in both sources. *C. kefei* was the least in terms of occurrence with only 1 occurrence in stored water and none in well water. Dala and Gwale Local Governments reported the highest level of contamination. Poor hygienic and sanitation practices of sources of drinking water were thought to be associated with the occurrence of these organisms.

Keywords: *Candida*, yeast, drinking water, poor hygiene, contamination.

INTRODUCTION

Water is a universal liquid which is rightly considered a major component of life. It is used by everyone, and has many and different functions. Water used for domestic purposes is ideally required to be free from contaminants; however, this has not been the case since such water has been found to contain a variety of chemical and biological contaminants [1-5]. Absence of odor, color and taste are vital aspects of water quality which must always be considered, as these serve as indicators of water contamination. One of the greatest concerns for the water consumers with respect to the quality of drinking water is contamination with pathogenic microorganisms [6]. In the past, fungi were not frequently considered when discussing pathogenic microorganisms in water. A possible reason for this is that, the occurrence of other microorganisms like bacteria and viruses in drinking water often leads to relatively acute symptoms of diseases in humans. However, the consumption of fungal contaminated drinking water has not lead to acute disease. Fungi in water have mostly been overlooked, but may be regarded as a chronic problem in drinking water distribution systems, and are possibly an underestimated problem. But recently it has been observed that, several fungal diseases have been caused by drinking water contaminated by volatile products or toxins of fungi [7, 4]. Others, such as wound contamination, skin infections, allergic diseases and ear infections, were equally observed [3, 2, 8-11].

Several studies have resulted in increased knowledge on the occurrence of fungi in drinking water; some of them include those conducted by [12-25]. Some general results for all the studies were, for example, that the recovery of

fungi varied between 7.5–89% positive samples, and that the levels of fungi in the samples varied considerably in the various investigations. Fungi have been reported from all types of water, from raw water to treated water, and from heavily polluted water to distilled or ultra-pure water. Fungi have also been reported from bottled drinking water [26, 27, 28].

Many species of fungi have been found in water used for different purposes, such as drinking, cooking, bathing, washing, etc., obtained from different sources such as wells, taps, streams, swimming pools, ponds, boreholes and storage tanks [1, 2, 3]. Fungi from air and soil enter water distribution networks from various sources, such as sediments, plants debris, biofilms and animals. Fungi which accumulate in stored water are capable of multiplying and producing toxins in high concentrations, and this may affect the user in several ways. For example, many of them are known to cause infections in immunosuppressed individuals such as AIDS, cancer, organ transplant patients and persons with asthma or various respiratory problems [29]. Some species have been recognized as agents of superficial infections (keratitis and cutaneous infections, onychomycosis and infections of wounds and burns) [30].

Fungi are capable of attached and unattached growth in water and hence colonize biofilms on pipe materials after a significant period of exposure [10, 4]. Isolation of fungi from water for recreational purposes, bathing, washing and other purposes has been reported [9, 3, 4]. Some of these uses serve as avenues along which these organisms spread among users. Water contamination may increase through poor sanitation and the unhygienic practices and other human activities in the vicinity of the water facility, especially surface and ground water sources. On the basis of dangers that can be posed to humans by these microorganisms through consumption of contaminated drinking water, this research was aimed at determining the frequency of occurrence of yeasts in 2 different sources of household's drinking water.

MATERIALS AND METHODS

Study Area

The study was conducted in four Local Government Areas of Kano State viz: Ungogo, Dala, Kumbotso, and Gwale within Kano metropolis (Figure 1).

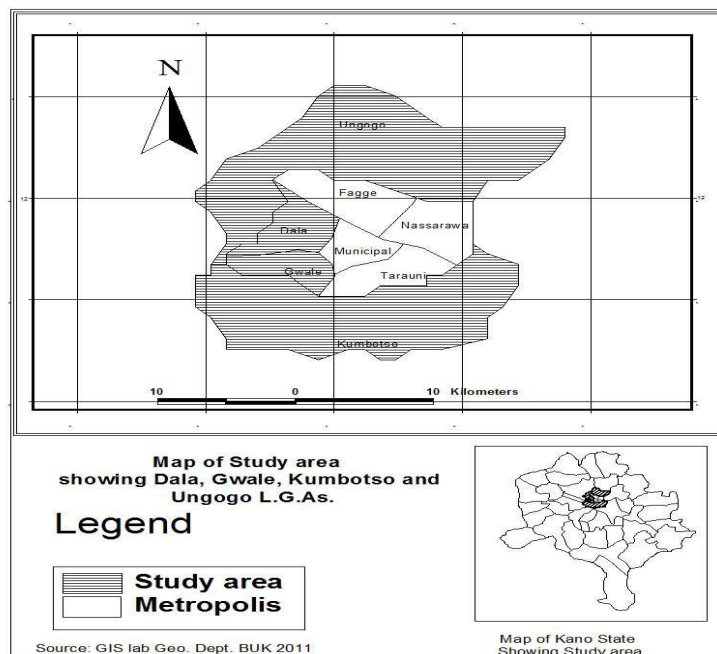


Figure 1: A map of Kano metropolis showing study areas and sample site shaded in black.
Source: GIS Lab Geo. Dept. BUK 2011.

Sample Size

A total of 173 water samples were collected for the study. Samples collection was done in four Local Government Areas of Kano State which include: Kumbotso (Kumbotso Town), Dala (Dala Kan Tudu, Gwammaja), Gwale (Gwale Yamma, Galadanci) and Ungogo (Ungogo Town, Fanisau). Selection of Local Governments was done randomly within the Metropolitan Local Governments.

Sample Collection

Standard method described by [31], was used for the collection of samples. In each targeted house, both stored and well water samples were collected separately. During collection of samples, 300ml of water sample was aseptically poured in to 300ml sterilized bottles from water stored for domestic use. 34 stored water samples were collected from three different Local Governments each, while 33 samples were also collected from one Local Government which is Kumbotso based on the availability of stored water because not all Households store water. Also 11 well water samples were collected from three Local Governments each and 5 samples were collected from Kumbotso Town because most households do not have well because of availability of Tap water. Water samples from wells were 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 in laboratory. 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. During filtration, samples were shaken vigorously at least 25 times up and down and then 100ml of sample was filtered through the sterile 47mm and 0.45 μm membrane filters (Whatman, Maidstone, Japan). After filtration, the filter was placed on sabouraud dextrose agar, followed by incubation at 25°C for 5 days [31].

Isolation and Identification of Yeasts

Based on their colonial appearances e.g. color, texture, shape, yeast colonies were differentiated from filamentous fungi. The identification was carried out by mixing a small portion of the yeast colony with a small drop of distilled water on a clean slide. A clean cover slip was placed over it and then observed immediately under the microscope at X10 Magnification. Colonies that appeared as small, round or spherical and some are budding were recorded as yeasts [32].

Dalamau Plate Technique for Identification of Yeast Species

Seventeen (17g) grams of Cornmeal agar medium was dissolved in one litre of distilled water, this was then heated with frequent agitation until it boiled, and sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to cool to about 45°C and then poured in to sterile petridishes. When the medium gelled, a small portion of the yeast colony was picked up using sterilized wireloop, and three parallel cuts were made in to the agar plates. Sterile cover slips were placed to cover the portion of the inoculating streaks on the agar. This was incubated at 25°C for 48 hours. After incubation, the plates were examined microscopically under X10 objectives through the cover slips [32].

RESULTS

Total samples of 173 were collected from 4 different Local Government Areas of Kano State. Out of these, 135 (78%) were stored water and 38 (22%) were collected from well (well water) (Table 1). A total of 108 isolates of yeasts were isolated and out of these, 67 (62%) were isolated from stored water and 41 (38%) from well water because, in some samples, more than one yeast species were found. Of those from stored water, 47 (70.1%) were *Candida* spp, 15 (22.4%) *Rhodotorula* spp and 5 (7.5%) *Geotrichum*. For those isolated from well water, 30 (73.2%) were *Candida* spp, 10 (24.4%) *Rhodotorula* spp and 1 (2.4%) *Geotrichum* spp (Table 2). Yeast distribution in stored water in respect to locations, a total of 47 *Candida* spp were identified from 4 Local Governments, out of these, 9 (13.4%) were from Kumbotso, 19 (28.4%) from Dala, 13 (19.4%) from Gwale and 6 (9.0%) from Ungogo. The frequency of occurrence of *Rhodotorula* spp was 15, out of these, 2 (3.0%) were from Kumbotso, 1 (1.5%) from Dala, 7 (10.4%) from Gwale and 5 (7.5%) from Ungogo. In case of *Geotrichum*, the frequency was 5 with 1 (1.5%) from Kumbotso and Dala each, 3 (4.5%) from Gwale and none from Ungogo (Table 3).

In case of yeasts from well water in relation to locations, a total of 30 *Candida* spp were isolated. Of these, 6 (14.6%) were from Kumbotso, 8 (19.5%) from Dala, 10 (24.4%) from Gwale and 6 (14.6%) from Ungogo.

Occurrence of *Rhodotorula* were 10, and out of these, none was from Kumbotso and Ungogo, 6 (14.6%) from Dala and 4 (9.8%) from Gwale. *Geotrichum* occurred only once which was from Kumbotso (Table 4). Four spp of *Candida* were identified from both stored and well water. These includes: *Candida albicans*, *C. glabrata*, *C. krusei* and *C. kefei*. In case of stored water, the occurrence of these spp is as follows; *C. albicans* 2 (4.3%) from Kumbotso, 5 (10.6%) from Dala, 1 (2.1%) from Gwale and 2 (4.3%) from Ungogo. *C. glabrata* 1 (2.1%) from Kumbotso and Gwale each, 3 (6.4%) from Dala and none from Ungogo. *C. krusei*, 6 (12.8%) from Kumbotso, 11 (23.4%) from Dala, 10 (21.3%) from Gwale and 4 (8.5%) were from Ungogo. *C. kefei* occurred only once which was from Gwale (Table 5). Also in well water, no *C. albicans* was recorded from Kumbotso, 5 (16.7%) from Dala, 2 (6.7%) from Gwale and 1 (3.3%) from Ungogo. *C. krusei* 6 (20.0%) were from Kumbotso, 3 (10.0%) from Dala, 8 (26.4%) from Gwale and 5 (16.7%) were from Ungogo. *C. glabrata* and *C. kefei* do not occur in well water at all (Table 6). *Rhodotorula* and *Geotrichum* were not identified to species level.

Table 1: Sources of Drinking Water Used for the Research

Sources	Number	Percentage (%)
Stored water	135	78
Well water	38	22
Total	173	100

Table 2: Genera of Yeasts Identified in Drinking Water

Yeasts	Sources of water (%)		
	Stored Water	Well Water	Total
<i>Candida</i> spps	47 (61)	30 (39)	77 (100)
<i>Rhodotorula</i> spps	15 (60)	10 (40)	25 (100)
<i>Geotrichum</i> spps	5 (83.3)	1 (16.7)	6 (100)
Total	67	41	108

Table 3: Distribution of Yeasts in Stored Water in Relation to LGA

Yeasts	Total Number of Yeast (N=67)			
	Location (%)			
	Kumbotso	Dala	Gwale	Ungogo
<i>Candida</i> spps	9 (13.4)	19 (28.4)	13 (19.4)	6 (9.0)
<i>Rhodotorula</i> spps	2 (3.0)	1 (1.5)	7 (10.4)	5 (7.5)
<i>Geotrichum</i> spps	1 (1.5)	1 (1.5)	3 (4.5)	0 (0)
Total	12	21	23	11

Key: LGA = Local Government Area.

Table 4: Distribution of Yeasts in Well Water in Relation to LGA

Yeasts	Total Number of Yeast (N=41)			
	Location (%)			
	Kumbotso	Dala	Gwale	Ungogo
<i>Candida</i> spps	6 (14.6)	8 (19.5)	10 (24.4)	6 (14.6)
<i>Rhodotorula</i> spps	0 (0)	6 (14.6)	4 (9.8)	0 (0)
<i>Geotrichum</i> spps	1 (2.4)	0 (0)	0 (0)	0 (0)
Total	9	19	14	6

Table 5: *Candida* Species in Stored Water in Relation to LGA

Yeasts	Total Number of <i>Candida</i> (N=47)			
	Location (%)			
	Kumbotso	Dala	Gwale	Ungogo
<i>C. albicans</i>	2 (4.3)	5 (10.6)	1 (2.1)	2 (4.3)
<i>C. glabrata</i>	1 (2.1)	3 (6.4)	1 (2.1)	0 (0)
<i>C. krusei</i>	6 (12.8)	11 (23.4)	10 (21.3)	4 (8.5)
<i>C. kefei</i>	0 (0)	0 (0)	1 (2.1)	0 (0)
Total	9	19	13	6

Table 6: *Candida* Species in Well Water in Relation to LGA

Yeasts	Total Number of <i>Candida</i> (N=30)			
	Location (%)			
	Kumbotso	Dala	Gwale	Ungogo
<i>C. albicans</i>	0(0)	5(16.7)	2(6.7)	1(3.3)
<i>C. glabrata</i>	0(0)	0(0)	0(0)	0(0)
<i>C. krusei</i>	6(20.0)	3(10.0)	8(26.4)	5(16.7)
<i>C. kefeyi</i>	0(0)	0(0)	0(0)	0(0)
Total	6	8	10	6

DISCUSSION

In this research, well water revealed the highest level of contamination even though the number of samples collected from wells (38) was less than those collected from stored water (135), but the occurrences of yeasts in well water (41) were more than half of that of stored water (67). This might be due to the fact that most of the wells were not well protected and were sited close to the sources of contamination (pit latrine and septic tanks). But in the case of stored water, it might not be necessarily that the stored water was from the well, it might be from borehole that has been kept in clean covered container even though the sources of stored waters were not enquired. Genus *Candida* was found to be the highest in terms of occurrence in both stored and well water but the occurrence was higher in stored water. However, there was no significant difference in the occurrence between the 2 sources ($P>0.05$). This occurrence was in agreement with the work of [33] who also reported higher occurrences of *Candida* 51 (77.3%) among the yeasts recovered in their research. This result was also in conformity with that of [34] for the fact that, out of the total yeasts isolated (27) in their research, *Candida* spp were the highest with the frequency of occurrence of 26 (96.1%). *Geotrichum* happens to be the least to occur, with lowest occurrence in well water. The occurrence of *Candida* species particularly in higher number is a matter of serious concern. *Candida* strains are known to cause nosocomial infections. The incidence of these nosocomial infections caused by *Candida* strains has risen with change of the implicated species and risk factors predisposing to Candidaemia, including colonization with *Candida* of sites other than blood [35].

On comparison on the occurrence of yeasts in stored water in relation to location, there was statistically significant difference in the occurrence of *Candida*, *Rhodotorula* and *Geotrichum* between the Local Governments ($P<0.05$), with the highest occurrence of *Candida* in Dala 19 (28.4%). Also in well water, the occurrence of *Candida* was higher 10 (24.4%) than that of other species of yeasts and this occurrence was in Gwale. These high occurrences in Dala and Gwale might be attributed to the fact that both of these Local Governments are densely populated which means the possibility of contamination is very high. However, there were no significant differences in the occurrence of these yeasts spp within a particular Local Government ($P>0.05$).

With regards to individual *Candida* spp, *C. krusei* and *C. albicans* had the highest number 31 and 10 respectively in stored water and 22 and 8 respectively in well water. On the other hand, *C. kefeyi* had the lowest number in both stored and well water, in that, it occurred only once in stored water and none in well water. The high numbers of *C. krusei* and *C. albicans* did not correlate with the results of [33]. In their research, they reported high number of *C. tropicalis*, 19 (32.8%). Also [34] recorded high occurrence of other *Candida* species that's *C. parapsilosis* 14 (53.8%). *C. albicans* is pathogenic to people in temperate climates primarily as a vaginal infection in women. It can be a health hazard in recreational waters and it has also been found in shower rooms. Even though, there are no definitive epidemiological studies linking infections with water levels of *C. albicans*, but women swimming in contaminated marine beaches have higher instance of vaginal infections due to *C. albicans*. There has been a recent increase in infections due to non-*albicans Candida*, such as *C. krusei*. The occurrence of both *C. krusei* and *C. albicans* in stored water was statistically higher in Dala than in all other local governments ($P<0.05$), also occurrence of *C. albicans* in well water was higher in Dala, however, *C. krusei* in this case was statistically higher in Gwale. So in both stored and well water, the high occurrence in both Dala and Gwale suggests improper hygienic and sanitation practices in these places.

Rhodotorula spp was also found in large number in this research 25 (23.1%). This figure was higher than those obtained by [33] 8 (9.8%). *Rhodotorula* spp have emerged as opportunistic pathogen with the ability to colonize and infect susceptible patients [36, 37]. Localized infections without fungemia including endophthalmitis, onychomycosis, meningitis, prosthetic joint infections and peritonitis have been reported in immunocompromised and immunocompetent patients.

Ungogo had the lowest number of yeasts from both sources of water. The low level in well water is clearly an indication of how protected their wells are, this is because, most of their wells are very deep as compared to those of others like Dala and Gwale in which in just few meters deep, water can be collected. It has been reported that contamination is more often if the source of water is close to sources of contamination (pit latrine and septic tanks), and this contamination might be due to infiltration of contaminated water (sewage) through cross connection, leakage points and back siphonage [38].

The presence of fungal organisms in drinking water has been attributed to the fact that fungi have the ability to grow attached to a substrate, forming part of a microbial biofilms on pipe surfaces, debris or sediments. They are likely to become established where there are cracks, pitting or dead ends [6]. Also several of these organisms are known to resist water treatment and can colonize filters in treatment plants [17].

CONCLUSION

From the results of this work, it was observed that all the sources of water reported the presence of species of yeast and by considering the high frequency of these organisms and the health threats they posed to humans, it can be concluded that the drinking water sources used in this research are significant routes for transmission of pathogenic microorganisms to human systems. Owing to the health hazards associated with yeast, the following recommendations should be given much consideration; since many of the households employ the use of well water, the wells should be protected from runoff in to the water, they should always be covered properly in order to prevent contamination. They should also not be sited close to sources of contamination (e.g. pit latrine and septic tank). Always collection and storage devices should be clean and covered in order not to allow post collection and storage contamination, there should be effective personal hygiene and sanitation as water can always become contaminated with fecal organisms even during usage.

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