

Fungi Contamination of Water Sources in Health Institutions and its Antifungal Susceptibility

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ABSTRACT

The presence of fungi in hospital water could cause allergic reactions or other fungal related diseases to immune-compromised people who come in contact with the water. The fungi contamination in water sources in health institutions and its antifungal susceptibility was investigated. A total of 48 water samples from water dispensers, outdoor taps, washing hand faucets in the rest-room and theatre wards were collected from the four health institutions (Immanuel clinic, Estaville hospital, Meridian hospital, La-Rosa hospital). The fungi were enumerated using the spread plate method. The American Public Health Association method was used in determining the physicochemical parameters. The disk diffusion method was used for the antifungal susceptibility testing. Sterile disks containing Nystatin (3.3 µg/ml), fluconazole (3.3 µg/ml), and Itraconazole (3.3 µg/ml) were placed on the surface of the inoculated plates and incubated for 72 hours. Results showed that the water samples had high fungal counts, with the highest fungal counts (1.5×10^6 SFU/ml) in La Rosa dispenser water, while the least was from washing hand water in La Rosa hospital. Significant differences ($P < 0.05$) were observed in all samples. *Paecilomyces* sp and *Penicillium* sp were

the most prevalent fungal isolates, while *Gliocladium* sp was the least common. Physicochemical parameters showed variations in temperature (27.4-27.6°C), pH (5.79-6.57), total dissolved solids (10-94 mg/L) and hardness (0.68-6.42 mg/L). The antifungal susceptibility tests showed that *Candida* sp was highly susceptible to fluconazole (30mm), nystatin (33mm) and itraconazole (30mm), while other isolates such as *Paecilomyces* sp and *Penicillium* sp showed no susceptibility to the antifungals. The study therefore recommends an improved water quality management in health institutions to reduce contamination.

Keywords: Fungal Contamination, Antifungal susceptibility, Hospital Water Sources.

INTRODUCTION

Fungi are known as saprophytes that degrade dead organic matter and some of the species acts as parasites or symbionts [1]. Their varied life cycle, capacity to establish extensive hyphal networks, ability to create spores, and growth as yeast cells enable them to optimise nutrient absorption and endure various environmental conditions, including oligotrophic aquatic systems [2]. Fungi in aquatic environments have mostly been neglected; however, they may be considered a persistent issue in drinking water distribution systems and could represent an underestimated concern. More so, fungi have gained more attention as contaminants in drinking water in the past ten years [3]. For water to be safe for drinking (potable), it must be void of any microbiological contamination [4].

Fungi could alter the taste and odour of drinking water, thereby making the water unpleasant. Additionally, most of the fungi are considered as pathogens that could cause allergies, respiratory illness, life-threatening meningitis, mycoses, invasive and contagious infections on the health of consumers [5]. *Aspergillus fumigatus* and other potentially harmful species have been found in drinking water, raising concerns about whether hospital water systems could act as a conduit for the spread of fungal illnesses [6].

A few others can produce hazardous secondary metabolites, such as mycotoxins, which can cause respiratory issues and cancer. They can also lower immunity, especially in immunocompromised persons, and pose a threat to human health [5]. The secondary metabolites are known to contribute to microbiological corrosion of water pipes, thus, altering the concentration of chlorine residual in the water distribution system [7]. Although mycotoxins produced in water could be extremely diluted and might not be a major concern, nevertheless, since the water is usually stored in tanks, rubbers or bottles for longer periods, the mycotoxin concentration could increase to concentrations that could impact health. Furthermore, an outbreak of skin irritations caused by bathing water contaminated with fungi on humans has been reported [6].

Antifungal susceptibility testing (AFST) is an important aspect in clinical microbiology which provide the treating clinician with useful information about the resistant, intermediate (or dose-dependent) susceptibility, or susceptibility phenotype for a combination of antifungal agents and organisms [8]. The emergence of resistant organisms such as *Candida auris*, triazole-resistant *Aspergillus fumigatus*, or fluconazole-resistant *Candida* species highlights the need for antifungal

susceptibility testing. Antifungal susceptibility testing (AFST) can provide in vitro measurements of the concentration of an antifungal agent that inhibits growth of a given fungal organism, the minimum inhibitory concentration (MIC) or minimum effective concentration (MEC) [9]. Due to the dearth of information concerning the occurrence of fungi and their antifungal resistance associated with hospital water sources, the present study was undertaken to bridge the gap.

MATERIALS AND METHODS

Study Area

The study was conducted in four different hospitals namely; Immanuel clinic (IC) (4°50'33.19188" N and 6°05'55.7040E), Estaville hospital (ELH) (4°05'58".14264N and 7°00'3.32172E) Meridian hospital (MH) (4°47'45.45023N and 6°59'13.37E) and La-Rosa hospital (LH) (4°47'44.5862N and 6°59'13.23924E) in Port Harcourt City and Obio/Akpor Local Government of Rivers State, Nigeria.

Collection of Samples

A total of forty-eight (48) water samples were collected for this study with 4 samples obtained from each of four locations (water dispensers, outdoor taps, handwashing faucets in restrooms, and theatre wards) in each hospital. This was repeated three times over three months resulting in 12 samples per location. The water supplied to these point sources other than the dispenser, originated from the borehole storage tank in each hospital.

Prior to sample collection, the tap outlet was sterilized using cotton wool soaked in 70% ethanol, and the tap was allowed to run briefly to ensure cleanliness. Each sample consisted of 10 millilitres (10 mL) of water collected in sterile biological specimen bottles. The bottles were sealed and transported in an ice-packed container to the Microbiology Laboratory, Department of Microbiology, Rivers State University for analysis.

Enumeration and Isolation of Fungi

The fungi in water samples were enumerated using the standard plate counts [10] on Sabouraud Dextrose agar (SDA). Aliquot (0.1mL) of 10^{-2} from a ten-fold serially diluted water sample was inoculated on the surface of freshly prepared SDA plates in duplicates. The medium was evenly spread with the aid of a sterile bent glass rod and incubated at 25°C for 72 hours. This was done for all the water samples. After incubation, fungal colonies were counted and distinct colonies were subcultured on freshly prepared SDA plates and incubated. The pure cultures of these spores were used for characterization and further tests. The fungal load was expressed as spore forming units per millilitre (SFU/mL). The prevalence of the fungal isolates was determined by calculating the total number of plates positive for the fungal isolates.

Characterization of Fungi

The fungal isolates, after incubation were characterized according to established methods based on macroscopic and microscopic characteristics [5]. The resulting characteristics were compared with those having similar characteristics as contained in the fungi book [11]

Determination of pH

The pH of water samples was determined based on the American Public Health Association (APHA) Standard method [12].

The pH meter was switched on and allowed for some time. It was then calibrated with buffer solutions of a high pH range between 8 and 9 as well as a lower pH range between 1 and 6 by dipping the electrodes into the buffer solutions. Fifty (50) ml of water was transferred into a 250 mL beaker and the electrode was immersed into the sample. The pH value for each sample was recorded accordingly.

Determination of Temperature

Temperature for each sample was determined on site of collection using a mercury-in-glass thermometer [12]. This was done by immersing the pH meter into a 250mL conical flask containing 50ml of the sample. The pH readings were taken after it was allowed to stabilize.

Determination Total Dissolved Solids

The total dissolved solids of the sample was determined [12]. The water sample was filtered through Whatman filter paper, and the filtrate was collected in a 250 mL beaker. A 250 mL dry beaker was weighed before the filtrate was transferred into it. This was later dried in the oven at 103°C for 24 hours. The hot beaker was removed and kept in a desiccator to cool in a dry environment for 3 to 4 hours. Once cooled, the dish was reweighed, and the dried residue weight was calculated by subtracting the initial dish weight from the final weight containing the dried residue (equation 1)

$$\text{Total Dissolved Solids } \left(\frac{\text{mg}}{\text{L}} \right) = (W2 - W1) \times 1000 \text{ mL of the filtrate used... equation 1}$$

Where W1 = initial weight of evaporating dish, W2 = Final weight of the dish (evaporating dish + residue).

Determination of Hardness

The hardness of the sample was determined [12]. The water samples were dispensed in an Erlenmeyer flask and 1mL of buffer solution was added into the water sample, 2 drops of indicator solution were added, EDTA (Ethylene Diamine tetra acetic acid) titrant was added slowly with continuous stirring until the reddish disappears from the solution, the result was taken.

Antifungal Susceptibility Testing

The antifungal susceptibility testing was carried out based on the disk diffusion method as described by the Clinical Laboratory Standard Institute [13]. In this method, the fungal inoculum was prepared by suspending isolates (*Candida* sp and moulds, respectively) in sterile 4mL normal saline. This was vortexed and adjusted using a UV spectrophotometer to a transmittance equivalent to 0.5 McFarland standard at a wavelength of 530 nm. This was further diluted by preparing a 1:100 dilution followed by a 1:20 dilution. The final inoculum concentration for the yeasts (*Candida* sp) was 2.5×10^3 while for the moulds it was 5.0×10^4 cells/ mL. Swab sticks were dipped into the standardized inoculum and spread evenly on Mueller-Hinton agar plates supplemented with 2% glucose. The plates were allowed to dry for 3-5 minutes. Sterile disks containing the antifungal drugs used: Nystatin (3.3 µg/ml), fluconazole (3.3 µg/ml), and Itraconazole (3.3 µg/ml) were placed on the surface of the inoculated plates. Plates were incubated for 24-72 hours. After incubation, plates were read and diameters of plates with zone of inhibition were measured using a graduated rule and recorded for each isolate. Azoles are known for their broad-spectrum activity, oral bioavailability and relatively low toxicity compared to polyenes.

Thus, since both azoles and polyenes cover different fungal species and resistance profile [14; 15], they were selected for this study.

Statistical Analysis

The mean and standard deviation of the fungi load in the respective samples were determined using the statistical package for social sciences (SPSS version 27). Two-way Analysis of variance (ANOVA) was carried out to check for significant differences and mean values were separated using the Duncan multiple range test (DMRT) at $P \leq 0.05$.

Results

Fungal Count of the Water Samples

The fungal count of the water samples is presented in Table 1. The result showed that the highest fungal count was observed in LH (1.5×10^6 SFU/mL), followed by MH (2.7×10^5 SFU/mL) and EH (2.0×10^5 SFU/mL). The least fungal count was recorded in LH WHW (1.0×10^4 SFU/mL). Results also showed significant differences ($P < 0.05$) across the various water samples

Table 1: Fungal Count (SFU/ml) of Water Sample

Samples	Fungi ($\times 10^4$)
EH OT	20.0 \pm 2.2 ^d
EH THW	9.5 \pm 9.2 ^c
EH TW	3.0 \pm 1.4 ^b
EH WHW	1.5 \pm 7.1 ^a
IC OT	4.0 \pm 4.2 ^b
IC WD	2.0 \pm 1.4 ^{ab}
IC TW	2.0 \pm 1.4 ^{ab}
IC WHW	8.5 \pm 2.1 ^c
LH OT	8.0 \pm 2.8 ^c
LH TW	3.5 \pm 2.1 ^b
LH WD	150.5 \pm 2.1 ^f
LH WHW	1.0 \pm 1.4 ^a
MH OT	9.0 \pm 1.4 ^c
MH TW	4.0 \pm 2.8 ^b
MH WD	2.0 \pm 1.4 ^{ab}
MH WHW	27.5 \pm 14.8 ^e

Means with similar superscript (^{abc}) down the group showed no significant differences ($P > 0.05$)

Keys: WD- Water Dispenser, OT-Outdoors tap, WHW- Washing hand water, TW- Toilet Water, THW- Theatre Water, LH, MH and EH are hospitals while IC is a clinic

Distribution of Fungal Isolates Across the Water Samples

The result showing the distribution of fungal isolates across the water samples is presented in Table 2. Based on the result, it can be seen that *Gliocladium* sp., *Aspergillus* sp., and *Candida* sp. was not isolated in LH water.

Table 2: Distribution of Fungal Isolates Across the Water Stations

The result revealed that *Paecilomyces* sp., *Penicillium* sp., *Trichoderma* sp and *Candida* sp. were isolated in IC water, while *Gliocladium* sp., *Aspergillus* sp., *Alternaria* sp., and *Geotrichum* sp were not isolated. The result also showed that *Gliocladium* sp., *Aspergillus* sp., *Paecilomyces* sp., *Alternaria* sp., *Geotrichum* sp, and *Penicillium* sp. were isolated in ES hospital water, while *Trichoderma* sp., and *Candida* sp., were not isolated. The percentage occurrence of the fungal isolates showed that *Paecilomyces* sp was the most dominant followed by *Penicillium* sp while *Gliocladium* sp was the least occurring fungi (Fig. 1).

Table 2: Distribution of Fungal Isolates Across the Water Stations

S/N	ISOLATE	LH	IC	MH	EH
1.	<i>Gliocladium</i> sp	-	-	+	+
2.	<i>Aspergillus</i> sp	-	-	+	+
3.	<i>Paecilomyces</i> sp	+	+	+	+
4.	<i>Alternaria</i> sp	+	-	+	+
5.	<i>Geotrichum</i> sp	+	-	+	+
6.	<i>Penicillium</i> sp	+	+	+	+
7.	<i>Trichoderma</i> sp	+	+	+	-
8.	<i>Candida</i> sp	-	+	-	-

Key: LH, MH and EH are hospitals while IC is a clinic

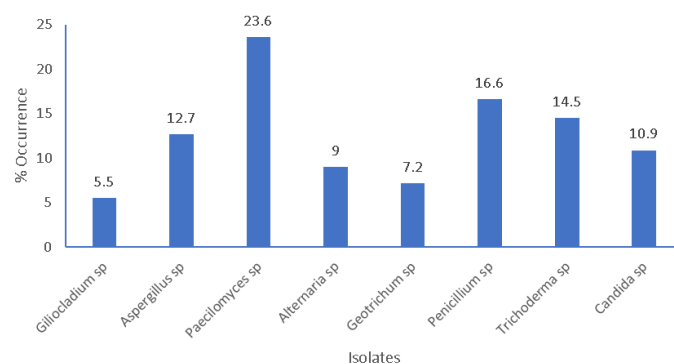


Fig. 1: Percentage occurrence of fungal Isolates

The result showing the physicochemical parameters of the water sample is presented in Table 3. The result showed that MH water dispenser and EH tap water has the highest temperature (27.6°C), while LH tap water had the least (27.4°C). Based on the result, it could be seen that the highest pH value was recorded in MH water dispenser (6.57), while the least was recorded in EH Tap water (5.79). The highest total dissolved solids was observed in MH water dispenser (94 mg/L), while the lowest was recorded in IC water dispenser (10 mg/L). The highest water Hardness was observed in MH water dispenser (6.42 mg/L), while the least was recorded in IC water dispenser (0.68 mg/L)

Table 3: Physicochemical Parameters of Water Sample

S/N	Parameters	Unit	MH water Dispenser	LH Tap Water	EH Tap Water	IC Water Dispenser
1.	Temperature	$^\circ\text{C}$	27.6	27.4	27.6	27.5
2.	pH	-	6.57	5.89	5.79	5.98
3.	TDS	mg/L	94	15	54	10
4.	Hardness	mg/L	6.42	1.02	3.69	0.68

Antifungal Susceptibility Testing

Results of the antifungal susceptibility testing is presented in Table 4. Based on the result, it was observed that Fluconazole demonstrated highest antifungal activity against *Candida* sp (30mm), and showed no antifungal activity against *Gliocladium* sp., *Paecilomyces* sp., *Penicillium* sp., *Geotrichum* sp, and *Alternaria* sp. (0mm).

From the result it was also seen that Nystatin showed a high antifungal activity on *Candida* (33mm), and showed no antifungal activity on *Paecilomyces* sp and *Penicillium* sp. (0mm). The result also showed that Itraconazole showed a high antifungal activity against *Candida* sp. (30mm), and showed no antifungal activity against *Gliocladium* sp., *Aspergillus* sp., *Paecilomyces* sp., *Penicillium* sp.

Table 4: Zone diameter (mm) of the Isolates against the Antifungal Drugs

S/N	ISOLATE	FLUCONAZOLE	NYSTATIN	ITRACONAZOLE
1.	<i>Gliocladium</i> sp	0mm	26mm	0mm
2.	<i>Aspergillus</i> sp	10mm	20mm	0mm
3.	<i>Paecilomyces</i> sp.	0mm	0mm	0mm
4.	<i>Candida</i> sp	30mm	33mm	30mm
5.	<i>Penicillium</i> sp	0mm	0mm	0mm
6.	<i>Geotrichum</i> sp	0mm	26mm	0mm
7.	<i>Alternaria</i> sp	0mm	10mm	0mm

Key: where 0 mm represent no antifungal activity on the isolate

Discussion

Yeasts and filamentous fungus are common organisms that coexist with organic substances, they are primarily opportunistic infections that can infect immune-compromised individuals and cause diseases [16]. In this study, the fungal counts were very high. Although, there is no conventional acceptable limit of fungal counts in water but their presence has been regarded as nuisance in water supply [4]. The fungal counts reported in the present study are higher than the range ($1.2 \pm 0.12 \times 10^3$ and $4.2 \pm 0.12 \times 10^3$ CFU/ml) reported by Nwogwugwu *et al.* [17]. The disparity in these counts could be attributed to the water sources investigated. While our study was on water dispensers, tap water and faucets in different wards in the health institutions, the study of Nwogwugwu *et al.* [17] investigated various open water sources including surface water, rainwater, borehole, and sachet water. More so, the highest fungal count in the present study was observed in the La Rosa hospital water dispenser (1.5×10^6 SFU/mL), followed by the water from the rest room faucet of the Meridian hospital (2.7×10^5 SFU/mL) and the outside tap water of the Estaville hospital water (2.0×10^5 SFU/mL), while the lowest fungal count was observed in the water from the faucet of the rest room in the La Rosa hospital (1.0×10^4 SFU/mL).

The high fungal counts observed in the water dispenser could imply that water dispensers are potential hotspots for fungal contamination thereby posing risks of nosocomial infections especially in immune-compromised patients or individuals who consume it. Previous studies have reported that hospital water systems could harbour fungi. Anaissie *et al.* [18] reported that water distribution systems in health care facilities could be reservoirs for opportunistic fungi such as *Aspergillus* sp and *Candida* sp. Furthermore, Babic *et al.* [2] reported that biofilm in water systems facilitate fungal persistence which contributes to high contamination levels as observed in the present study.

Most of the fungal isolates in the present study have been reported in previous study. Ghodsi *et al.* [19] reported that *Aspergillus* sp. was the most common fungus, while Nwogwugwu *et al.* [17] reported numerous fungal species of the genera *Aspergillus* (7%), *Penicillium* (12%), *Mucor* (21%), and *Candida* (60%). In the present study, *Paecilomyces* sp and *Penicillium* sp are the most prevalent fungi across the samples with *Gliocladium* sp being the least common. Additionally, *Candida* sp was only isolated in the water samples from the Immanuel clinic and *Aspergillus* sp was not isolated from La Rosa and Immanuel clinic water samples. The pattern of distribution of the isolates implied that local environmental factors and the water treatment practices adopted by the respective health institutions influenced the fungal genera. In a previous study, *Penicillium* sp and *Aspergillus* sp have been reported to thrive in hospital water systems due to their ability to form biofilms and resist standard disinfection [20]. The absence of *Candida* sp in water samples from La Rosa, Meridian and Estaville water samples could imply that effective filtration or chlorination measures in the water systems were applied.

This agreed with Warris *et al.* [21], who reported that water treatment reduces certain fungal species in health care settings. It is well known that these fungi can cause infections, and their existence in hospital water sources raises the possibility that patients who use this water either drinking or bathing could be disposed to skin irritations or fungal infections. Also, both adults and children can contract respiratory illnesses and cutaneous or systemic infections from these species, which can be harmful to their health [16; 22]. *Candida* is one of the major yeasts that cause invasive or cutaneous infections, which are linked to high mortality, extended hospital admissions, severe morbidity, and higher medical expenses [23]. Additionally, certain molds, including *Aspergillus*, *Fusarium*, and *Penicillium* species, can create hazardous secondary metabolites (aflatoxins, ochratoxins) that degrade water quality and endanger patients [7], and a few of these compounds can damage the immune system and cause cancer [24]. As opined by Mhlango *et al.* [7], even though mycotoxin levels in water are extremely low, they can increase to dangerous levels when water is kept in reservoirs for extended periods of time, and they can also remain in treated drinking water long after the fungi have perished.

The result of water sample analysis showed ranges of 27.4 to 27.6 °C for temperature, 5.79 to 6.57 for pH value, 10 to 94 mg/L for total dissolved solid, and 0.68 to 6.42 mg/L for water Hardness. The observed variations in the physicochemical parameters might have influenced the fungal counts observed. More so, the pH and temperatures of the water samples are conducive for fungal growth. Hageskal *et al.* [6] reported that neutral pH and temperatures (25-30°C) influences the growth of fungi in the water system. More so, high TDS levels in some of the water samples could be attributed to the accumulation of organic matter which, which supports the growth of fungi [2]. The high pH value recorded in this study could also be linked to alkaline filters in water dispensers which are designed to raise the water's pH for perceived health benefits [25]. More so, dispenser components, such as storage tanks or pipes, may leach alkaline substances like calcium or magnesium, increasing the pH of water [26]. Contrary to findings in this study, Sanaei *et al.* [25], reported a low alkaline content in portable water sources in their study.

The existence of biofilms is a significant problem associated with the presence of fungi in water [16]. Fungi and other microorganisms, including bacteria and protists, grow together to form the complex structures of biofilms, which serve as reservoirs for these germs [27]. Although a type of resistance is evident in the fungal cells found in a biofilm, it is unknown what processes control its growth [27]. Biofilm growth also degrades water quality and increases the expense of maintaining networks that distribute potable water by decreasing the effectiveness of residual chlorine [7]. The causes of this increased resistance to chlorine disinfection are probably due to the biofilm's consumption of disinfectants, decreased disinfectant penetration into the film, and the makeup of the microbial population within the film itself [27].

The antifungal susceptibility tests showed that *Candida* sp was highly susceptible to fluconazole (30mm), nystatin (33mm) and itraconazole (30mm), while *Paecilomyces* sp and *Penicillium* sp showed no susceptibility. Arendrup *et al.* [28] reported that *Candida* species are usually susceptible to azoles and polyenes which agrees with the present study. However, *Paecilomyces* sp and *Penicillium* sp often exhibit intrinsic resistance to azoles due to genetic adaptations in ergosterol biosynthesis pathways [29].

The observed antifungal-resistance in non-*Candida* sp is alarming since it could limit treatment options for infections caused by these fungi. Ghodsi *et al.* [19] reported similar findings with most of the fungal isolates of water dispenser showing resistance to the antifungal agents. Contrarily, all of the isolates in the study by Nwogwugwu *et al.* [17] showed high sensitivity levels to ketoconazole and fluconazole, with the exception of *Mucor* species, which was resistant to ketoconazole. According to Adeyinka *et al.* [30], susceptibility to antimicrobial agents is not constant and may be influenced by human activity and environmental factors. The resistance observed in this study may be ascribed to the protective mechanisms that the fungi have developed. The high effectiveness of fluconazole against *Candida* sp. in this study could be attributed to the inhibition of ergosterol synthesis. Fluconazole targets lanosterol 14 α -demethylase, an essential enzyme in ergosterol biosynthesis, and this disrupts the fungal cell membrane's integrity, leading to growth inhibition [31].

Conclusion

The various water sources in the hospital are contaminated with fungi, with very high fungal counts. Although there is no standard international limit for the measurement of fungi in water, but the presence of these high counts could imply the possible presence of increased mycotoxin production. Additionally, most of the molds isolated from these water sources could pose significant health challenges as they have been reported to produce mycotoxins. The antifungal susceptibility showed the presence of emerging itraconazole-resistant isolates despite the high rate of susceptibility recorded in fluconazole and Nystatin antifungals. More so, regular monitoring of water sources, disinfection of storage tanks and water flow lines, setting acceptable limits for fungi in water and installing of advanced filtration systems is highly recommended.

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