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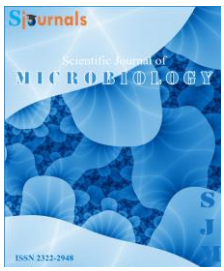

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Original article

Fungi associated with water from gallons of truck pushers sold within Sokoto metropolis and their susceptibility profile to different antifungal drugs

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ABSTRACT

This research was conducted to isolate fungi from water in gallons of truck pushers and determine their susceptibility to different antifungal drugs. The water samples from different areas in Sokoto were collected by the use of sterile plastic container and analyzed. During this research, serial dilution, isolation and identification were done. From the result obtained, different colonies were observed after incubation period for 7-14 days. The highest mean colony count was $2.4x10^3$cfu/ml and the least mean colony count was $1.6x10^3$cfu/ml. The fungi isolated include *Aspergillus niger*, *Apergillus flavus, Aspergillus fumigatus, Aspergillus oryzae* and *Rhizopus stolonifer* with *Apergillus niger* having the highest percentage frequency of occurrence (34%) while *Rhizopus stolonifer* had the least percentage frequency of occurrence (4%). Each of the fungal isolates was tested against the following antifungals: Itraconazole, ketoconazole, Griseofulvin and Fluconazole each at a concentration of l0mg/ml, 40mg/ml and 60mg/ml. In griseofulvin, a 100% zone of inhibition was recorded against *Aspergillus flavus* at the concentration of 60mg/ml while in *Aspergillus niger* it was 75%, and in *Aspergillus fumigatus* it was 87.5%, *Aspergillus oryzae* 81.25% and *Rhizopus stolonifer* 90% at concentration of 60mg/ml. In

Itraconazole, the fungal isolates were more susceptible at concentration of 60mg/ml in which *Aspergillus flavus* showed 100% zone of inhibition while *Aspergillus niger* showed 81.75%, *Aspergillus fumigatus* was 93.75%, *Aspergillus oryzae* was 81.25% and *Rhizopus stolonifer* was 91.25% at concentration of 60mg/ml. Also in ketoconazole, the isolate *Rhizopus stolonifer* and *Aspergillus fumigatus* showed 100% zone of inhibition at different concentrations of 10mg/ml, 40mg/ml and 60mg/ml. while *Aspergillus niger* was recorded at 87.50%, *Aspergillus flavus* was 93.75% and *Aspergillus oryzae* was 91.25% at concentration of 60mg/ml. fluconazole was the most effective against the fungal isolates as 100% zone of inhibition was recorded at concentration of 60mg/ml for all of the fungal isolates as well as *Aspergillus fumigatus* at concentration of 40mg/ml was recorded as 100% zone of inhibition among the tested antifungals for susceptibility testing. However, the zone of inhibition measured showed that the antifungals at all given concentrations were active against the fungal isolates. Conclusively, fungi are present in water sample and they include both pathogenic and saprophytic species. Thus attention should be given to water treatment quality and gallons used by truck pushers should always be clean to improve in hygienic practices.

1. Introduction

Water is one of the most abundant and essential commodities of man occupying about 71% 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 (Adviano and Joana, 2007). The growing population of most developing countries occurs disproportionably in urban areas. These places due to considerable pressure on already overburdened budgets make it difficult to increase the water supply infrastructure (Devilliers and Helway, 2002).

Although water can contain unwanted chemicals (from natural sources and agricultural activities), the greatest risk to human health is from faecal contamination of water supplies (*Water-borne diseases*). Serious ill health can be caused by water becoming contaminated from faeces being passed or washed into rivers, streams or pools or being allowed to seep into wells or boreholes (Cheesbrough, 2010). In piped water distribution systems, a sanitary inspection will often not detect problems occurring during distribution, e.g. pipes buried underground might be damaged, allowing pollution.

Fungi are eukaryotic, heterotrophic organisms, including both single cell i.e yeasts and multicellular filamentous fungi. They primarily function as recyclers of organic materials. Many fungal species can survive in oligotrophic environment (Paterson, 2005) through scarvenging nutrients from the substrate which they colonize, or the air or water in which they live to maximize nutrient uptake. filamentous fungi form mats of fine hyphae. Also, their dispersion is through spores. Fungi also produce secondary metabolites, some of which are toxins. Some of the fungal species and the metabolites they produce serves as human pathogens or allegens (Paterson and Lima, 2005). Due to their tolerance of oligotrophic environments, some species of fungi are able to colonize drinking water distribution system, which are typically low in nutrients. However, The significance of drinking water as an exposure pathway to their pathogenic, allergenic or toxigenic fungal specie or their metabolites is not well known (Lina, 2005).

Antimicrobial agents are drugs or chemicals that are used to kill or hinder the growth of microorganisms. They specifically target the microbes for destruction leaving other cells of the body unharmed (Rogers, 2006).

Antifungal agents have proven to be powerful antimicrobial agents against fungi,but some still shows resistance to some of the antifungal drugs (Lass et al., 2008).

Nigeria 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 fetching of water in gallons from available sources and selling it to the ever growing population without any major form of treatment. With this, the safety of this gallons water is still questionable because many who are engaged in selling it do not follow strictly the standards set by FEPA (1999) and WHO (2006) for safe drinking water.

Fungal infections are becoming of increasing concern due to the increasing numbers of immunocompromised patients and those with other risk factors (Annaise et al., 2002). Therefore, there is a need to ascertain what the exposure pathways are and whether treated drinking water has a role as a source of exposure to pathogenic fungi. The objectives of this study includes the determination of the fungal load and the determination of the susceptibility of the identified fungal isolates in the water from the gallons of the truck pushers.

2. Materials and methods

2.1. Sample collection

A total of twenty five water Samples were collected randomly from five(5) different areas in Sokoto metropolis. The areas were: Emir Yahaya, Rijiyar dorowa, Unguwan Rogo, Rijiyan Shehu and Ali Akilu. They are labeled using letters A, B, C, D and E respectively. The samples were collected in a sterile sample containers from the truck pushers that fetched the water from various places such as water board, reservoirs and wells. The samples were then transported to the Microbiology Laboratory in Usmanu Danfodiyo University Sokoto for further processing and analysis.

2.2. Sample processing and analysis

One milliliter (1ml) of each sample was taken in a sterile syringe and added to 9ml of sterile distilled water, 1ml of added sample was taken again and transferred into next testube containing 9ml of sterile distilled water, this was done serially into three testubes (10^{-1} to 10^{-3}) to reduces the fungal load for each sample. Then, 1ml of each dilute sample was inoculated into Potato Dextrose Agar (PDA) prepared and incubated at room temperature and observed for 7-14 days (Cheesebrough, 2006).

The fungal colonies observed were sub-cultured on another fresh potato dextrose Agar (PDA), this subcultured was based on the number of colonies that appeared from the first inoculation. Each colony was inoculated into three petridishes containing media in order to obtain pure isolates (Brown et al., 2004).

2.3. Identification of the fungal isolates

The macroscopic identification of fungi was carried out by checking for the colour and its appearances while the microscopic examination was carried out by taking a portion of the culture and fixing it on a clean glass slide and a drop of lacto phenol cotton blue was added using a sterile inoculating needle and covered with a clean cover slip. It was viewed under the microscope using x10 and x40 objectives. Identification was based on colonial and cellular morphology of the fungi as they appear in the mycology atlas for identification (Cheesebrough, 2000).

2.4. Fungal sensitivity testing using agar incorporation method

Fungal sensitivity test was conducted using the antifungal drugs: Griseofulvin, Itraconazole, Ketoconazole and Fluconazole at reduced concentrations. This was done by dissolving 10g, 40g, and 60g of each of the antifungal drug in 1000ml of sterilized distilled water to make the concentrations(10mg/ml, 40mg/ml and 60mg/ml). 5 ml was lastly taken from each concentration and poured into the 20ml of sterile potato Dextrose Media. The media was poured in the petridish until it solidifies, then the fungal isolates were inoculated in them. The inoculation was done with the use of sterile needle and the inoculums were incubated at 25^0C for observation (Cheesebrough, 2000). During the period of observation, the diameter for zones of inhibition were noted and recorded.

3. Results and discussion

The mean fungal colony count of the average of each designated areas are presented in Table 1. The fungi identified from the different gallon water and their percentage frequency of occurrences are presented in Table 2.

Table 3, 4, 5 and 6 shows the antifungal sensitivity test of Griseofulvin, Itraconazole, Ketoconazole and fluconazole against the fungal isolates, at their different reduced concentrations. The percentage zones of inhibition as measured shows how susceptible the fungal isolates were.

Table 1
Mean fungal colony count of the designated area.

Sample Codes	Fungal load ($x10^3$) cfu/ml
$A_1 – A_5$	1.6
$B_1 – B_5$	2.4
$C_1 – C_5$	1.8
$D_1 – D_5$	1.8
$E_1 – E_5$	2.4

Keys: A- Emir Yahaya. B- Rijiyar Dorowa. C- Unguwar Rogo. D- Rijiyar Shehu. E- Ali Akilu Area.

Table 2
Percentage of occurrence of the fungal isolates.

Fungi	Total No. of occurrence	Percentage frequency of occurrence (%)
Aspergillus niger	17	34
Aspergillus flavus	12	24
Aspergillus fumigatus	15	30
Aspergillus oryzae	4	8
Rhizopus Stolonifer	2	4
Total	50	100

Table 3
Percentage zone of inhibition of griseofulvin at different concentration on the fungal isolates.

	% Zone of inhibition of the griseofulvin at different concentration of the fungal isolates		
Fungi	**10mg/ml**	**40mg/ml**	**60mg/ml**
Aspergillus niger	50.0	56.2	75.0
Aspergillus flavus	68.7	87.5	100
Aspergillusfumigatus	58.7	77.5	87.5
Aspergillusoryzae	52.5	68.7	81.2
Rhizopus Stolonifer	50.0	71.2	90.0
Control	100	100	100

Table 4
Percentage zone of inhibition of itraconazole at different concentration on the fungal isolates.

	% Zone of inhibition of the itraconazole at different concentration of the fungal isolates		
Fungi	**10mg/ml**	**40mg/ml**	**60mg/ml**
Aspergillus niger	65.0	71.2	81.2
Aspergillus flavus	43.7	91.2	100
Aspergillus fumigatus	62.5	91.2	93.7
Aspergillus oryzae	37.5	56.2	81.2
Rhizopus stolonifer	62.5	78.7	91.2
Control	100	100	100

Table 5
Percentage zone of inhibition of ketoconazole at different concentration on the fungal isolates.

	% Zone of inhibition of the ketoconazole at different concentration of the isolates		
Fungi	**10mg/ml**	**40mg/ml**	**60mg/ml**
Aspergillus niger	56.2	66.2	87.5
Aspergillus flavus	62.5	87.5	93.7
Aspergillus Fumigatus	100	100	100
Aspergillus oryzae	58.7	77.5	91.2
Rhizopus stolonifer	100	100	100
Control	100	100	100

Table 6
Percentage zone of inhibition of fluconazole at different concentration on the fungal isolates.

	% Zone of inhibition of the fluconazole at different concentrations on the isolates.		
Fungi	**10mg/ml**	**40mg/ml**	**60mg/ml**
Aspergillus niger	85.0	95.0	100
Aspergillus flavus	62.5	87.5	100
Aspergillus fumigatus	87.5	100	100
Aspergillus oryzae	62.5	81.2	100
Rhizopus stolonifer	75.0	93.7	100
Control	100	100	100

It can be deduced from this research that portable water selling by truck pushers in gallons within Sokoto metropolis used for social amenities are colonized with fungi since the mean fungal load obtained from the designated sampled code ranges from $1.6x10^3$cfu/ml to $2.4x10^3$cfu/ml. The sample code E and B (Ali Akilu Road and Rijiyar Dorowa Roa) had the highest mean fungal load of $2.4x10^3$cfu/ml while the sample code A (Emir Yahaya Road) had the least fungal load $1.6x10^3$cfu/ml. The presence of this fungal load makes the water from the gallons of the truck pushers unsafe for drinking as it is not within the tolerable limit of microbial load as proposed by WHO (2008).

Also, from the result obtained, it was observed that, the genus *Aspergillus* is the most frequently isolated in this study, therefore the findings of this study are in general agreement with the study carried out by Gunhild et al. (2009) and Okpako (2009) who revealed that fungi are relatively common in water distribution systems but they are not found evident for any human diseases. Okpako (2009) agrees that its important to be aware of the fact that several of the species which are of clinical concern are present in the potable drinking water since most fungi survive disinfection and the water treatment is not confirmed (Okpako, 2009). Aspergillus species are known to produce aflatoxins (B_1, B_2, G_1 and G_2), the most toxic and potent hepatocarcinogenic natural compounds ever characterized (Benneth and Klich, 2003). These fungi cause a wide range of disease in humans, ranging from hypersensitivity reactions to invasive infections associated with angio invasions. *Aspergillus flavus* was frequently isolated in my investigation, mostly in “Area B” and “Area E” i.e. Rijiyar dorowa and Ali Akilu. This species is known to be the second most leading cause of invasive and non-invasive aspergillosis (Morgan et al., 2005; De Hoog et al., 2000).

Rhizopus stolonifer (4%) having the least percentage frequency of occurrences in this study is able to cause disease in immunocompromised patients (Sheppard et al., 2004; Ana et al., 2006). Although it is unlikely that it causes infection in healthy people, immunosuppressed persons are at risk of infection (Kanzler et al., 2007) thus it is important that routine microbiological investigations should be made in hospitals where immunosuppressed individuals are treated.

The sensitivity profile of these fungi to the different antifungal drugs were presented in tables (3, 4, 5 and 6) and their zones of inhibition were recorded in percentages. The fungal isolate showed greater susceptibility on the antifungals used as larger zones of inhibitions were recorded. However, minimum zones of inhibition of the fungi were recorded at the least concentration of 10mg/ml of both the itraconazole and ketoconazole while lesser growth was observed as the concentration increased to 40mg/ml and 60mg/ml.

However, the fungal isolates showed highest susceptibility to the antifungal fluconazole mostly at 60mg/ml, this however makes the percentage of inhibition recorded in fluconazole at concentration of 60mg/ml to be 100% on all the fungal isolates which in ketoconazole it also recorded 100% of inhibition at all the concentrations on *Aspergillus fumigatus*. Moreover, *Aspergillus fumigatus* is very sensitive to ketozonazole and there was high percentage zone of inhibition in fluzonazole as well. The complete action of fluconazole at highest concentration of 60mg/ml on the fungal isolates might be attributed to the fact it is a synthetic broad spectrum antifungal agents (Galgiani et al., 1997).

4. Conclusion

It is concluded that this study confirms the presence of different taxonomy of filamentous fungi in water selling truck pushers within Sokoto metropolis having the frequency of occurrence of *Aspergillus niger* (34%), *Aspergillus flavus* (24%), *Aspergillus fumigatus* (30%), *Aspergillus oryzae* (8%) and *Rhizopus stolonifer* with the lowest occurrence recorded (4%). However they are all susceptible to the antifungals griseofulvin, itraconazole, ketoconazole and fluconazole but the highest antifungal activity was recorded in fluconazole.

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