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PREVALENCE OF FUNGAL CONTAMINATION IN BOTTLED WATER AND PLASTIC CONTAINERS IN IRAQI COMPANIES

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ABSTRACT: *This study was designed to examine 100 samples of bottled drinking water from 20 different markets across Iraq. Samples were examined using the membrane method. Among of 32 samples, the fungi ranged between 29 - 388 colonies/100 ml, with 19 samples having a colony percentage below the permissible limit and 13 samples having a colony percentage higher than the maximum level specified in the International Standard for Water (no more than 100 colonies/100 ml). So 12 different fungal species were found in bottled water product (Aspergillus niger, Aspergillus terreus, Aspergillus parasiticus, Penicillium italicum, Penicillium expansum, Penicillium glabrum, Alternaria alternate, Bipolaris spp., Trichoderma spp., Rhizopus spp., Chladosporium cladosporioides, and yeast) The results showed that the same fungi were isolated from both water and plastic packaging. Antibiotic susceptibility tests showed that (ketoconazole, griseofulvin, and itraconazole)* more *effective against the isolated fungi.*

KEYWORDS: Fungi, bottled water , plastic containers , Iraq.

INTRODUCTION

Fungal contamination of bottled water will soon head the list of water-related concerns due to its compromising health risks to humans and because of humidity the grow well in the water container (1).

Fungi are widely distributed environments , where it is also present in sources of drinking water systems. According to studies, contamination with fungi is one of the most vital issues especially, if this is about bottled the fungus are widely distributed in environments so the sources of drinking water systems and bottled water may be a good habitat to these organisms (1).

Some fungus like *Aspergillus , Penicillium , Penicillium spp and Cladosporium spp* isolated from tap water, also filamentous fungus was obtained from mineral water samples ,in other hand some of these fungus may produce mycotoxins that may cause health problems in consumers so the contaminated water or plastic containers with fungus leads to human health problems(2).

The pollution of the fungal microorganisms in bottled water has become a serious issue, and most of the regulatory agencies and consumers must Take attention to this problem. Strict monitoring steps and purification processes are fundamental elements to verify the safety of water for drinking. Moreover, more research has to be done around the area to better understand this problem along with the entirety of the public health effects caused by fungal contamination (3).

The fungus found in deferent place containing water like oceans, fresh water and drinking water so these organisms able to adapt to deferent environment and these organisms are important ecologically by breaking down the complex organic materials through saprophytic life also causes problem to organisms (animals, plants and human) through parasitic life also it has benefit through symbiosis (4) .

In recent years, a wide range of fungi have been discovered in hydric feeder sources intended for human consumption. *Acremonium spp , Alternaria spp , Aspergillus spp, Cladosporium spp, Fusarium spp, Penicillium spp,* and *Trichoderma spp* all exhibit asexual reproduction through hyphal branching and conidia production on a daily basis. On the contrary, yeast cells were only seen significantly in surface water, ground water, and tap water due to biases that may have been introduced during the process of cultivating these samples (4)(5).

The diversity of the fungi was higher on the surface water than the ground or tap water observed due to variations in environmental factors such as the presence or content of nutrients, temperature, pH levels, and flow pattern of water. Despite tap water undergoing cleaning processes like particle removal and chlorine addition during production to decrease fungal presence, certain species can persist and create biofilms within distribution systems (6)

The discovery of potentially pathogenic filamentous fungus, such as *Aspergillus spp* and *Rhizopus spp*., in natural bathing sites poses a health risk to persons. However, the existing microbiological criteria employed to evaluate drinking or recreational waters do not provide adequate indicators for detecting fungal contamination (7) .

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Recognizing the prevalence of fungi in bottled water sources is crucial for ensuring public health safety. Enhancing monitoring and regulation for fungal contaminants could aid in reducing health risks associated with consuming contaminated water sources(8).

The ecology of fungi in biofilms has been studied only to a small degree, and further research should aim to investigate the features of fungi grown in biofilms (9).

The research is intended to determine to what extent have the fungi contamination of bottled water taken place, and if this contamination is related to the frequency of the bottle use in a plastic container or simply to the plasticity material itself.

MATERIAL AND METHOD

Sampling collection

During the period from October 2023 until March 2024, a study was conducted in Kirkuk City \ Iraq to involved the acquisition of 100 samples of bottled drinking water from 20 distinct brands all around in Iraq , specifically focusing on 32 local products with the code (B1 - B32) and three bottled water products single-use plastic containers with the codes (B30 , B31 ,B32).

Detection of fungi in water

Membrane filtration procedure. (10) used for detecting fungus in bottled water, which involved passing 100 millilitres of water through a Sartorius Stedim-Cellulose Nitrate Filterpore (Size 0.45 µm) for each sample. The filter membrane was then applied on the surface of the Sabouraud (Dextrose) growth medium and Sabouraud glucose agar (SGA), or malt extract agar (MEA), which promoted the growth of filamentous fungi after the tainted products had been diluted 1:100. antibiotic chloramphenicol was added to the SDA (Himedia, India) at a concentration of 100 mg/L . The plates were incubated at 25°C for a week and checked every day. They were replanting the fungal colonies on SDA media produced on solitary, pure colonies to facilitate diagnosis

CFU/100 mL = (# of colonies counted ÷ sample volume filtered in mL) x 100

(CFU = colony forming units)

Examining fungus on plastic container interior surfaces

After draining the plastic containers from the water, sterile swabs were used to remove the contents. (11) As stated in his instructions, the swabs were placed upon the surface of a solid culture media, the plates were incubated at 25°C, and they were given a daily check-up in the following week.

Preparation of Antifungal Drugs

three kinds of antifungal drugs have been used this work, (ketoconazole, griseofulvin, and itraconazole). The antifungal tablets were ground into small powdery form by mortar and pestle. It was then made 0.02g/ml, 0.04g/ml, and 0.06g/ml by adding the respective drugs in

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proper amounts and then dissolving in 95% ethanol. Distilled water was added to make the concentration different. A rotary shaker was used to make the mixture.

Determination of Antibiotic Sensitivity Pattern

The antibiotic sensitivity patterns of the antifungal drugs against the isolated fungi were determined using agar well diffusion method. To date there is currently no standardized agar based susceptibility testing method for moulds. We develop an agar based method using agar well method to check susceptibility of moulds isolates to 3 antifungals A homogenous concentration of inoculums (10^{-4}) serial with a fixed volume (1ml) was poured uniformly over the surface of gelled agar medium of potato-dextrose agar medium. A fixed volume of different concentrations of the antifungal drugs was then pipetted into the bored agar well. The plates were incubated at 25oC for 72 hours and the zones of inhibitions were measured in mm using a measuring ruler(12).

RESULTS AND DISCUSSION

The purpose of specimen selection for the research study was to collect samples from various sources in order to comprehend the dynamics of fungal proliferation in bottled waters. The purpose of the meeting was to enable the binding of 100 drinking water samples from 20 different national brands.

Maintaining the consistency and accuracy of the sampling technique is attributed to the well-trained individuals who injected the samples from private, commercial bottling facilities. To prevent any potential contamination, data was conveyed to the lab in insulated containers within 24 hours of being held at a proper temperature of 4°C.

Each batch of bottles was carefully mixed upon arrival at the laboratory to ensure that their contents were almost identical. Once the uniform batches of samples were ready, equal parts of each were merged to create the composite samples that would be the subject of the inquiry. The objective of the sample selection process was to collect representative samples with a general amount of fungal contamination from bottled water from various brands and manufacturing areas (13).

Test of water in bottles with local production, including twenty-liter bottles, showed that 19 out of 32 samples Contaminated with fungi. Fungi in 19 samples were within safe levels (less than 100 colonies per 100 milliliters), but 13 samples had fungal contamination exceeding safe levels. The specified limit (more than 100 colonies per 100 milliliters) is within internationally recommended drinking water guidelines as outlined in Table 1.

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Table (1): Results of fungi tests for bottled water and swabs for plastic containers.

	Product code	Preparation of fungi in water Colony/100 ml	Types of fungi in bottled water	Types of fungi on the inner surfaces of containers
1	B1	30	*Aspergillus niger* *Bipolaris spp*	*Aspergillus niger*
2	B2	79	*Penicillium italicum* *Aspergillus niger*	*Penicillium italicum*
3	B3	287	*Penicillium glabrum* *Yeast*	*Yeast*
4	B4	220	*Aspergillus niger* *Penicillium expansum* *Rhizopus spp*	*Aspergillus niger*
5	B5	86	*Aspergillus terreus* *Rhizopus spp*	*Aspergillus terreus*
6	B6	380	*Cladosporium cladosporioides* *Aspergillus parasiticus* *Aspergillus niger*	*Cladosporium cladosporioides* *Aspergillus niger*
7	B7	350	*Aspergillus parasiticus* *Alternaria alternata* *Aspergillus niger*	*Alternaria alternata*
8	B8	39	*Penicillium italicum* *Cladosporium cladosporioides* *Yeast*	*Penicillum italicum* *Yeast*
9	B9	320	*Penicillium expansum* *Alternaria alternata* *Aspergillus niger*	*Alternaria alternata* *Aspergillus niger*
10	B10	44	*Aspergillus terreus* *Penicillium expansum*	*Penicillium expansum*
11	B11	388	*Penicillium glabrum* *Bipolaris spp* *Aspergillus niger*	*Bipolaris spp* *Aspergillus niger*
12	B12	350	*Yeast* *Aspergillus niger*	*Aspergillus niger*

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13	B13	270	*Rhizopus spp* *Penicillium glabrum* *Bipolaris spp*	*Penicillium glabrum* *Bipolaris spp*
14	B14	45	*Penicillium italicum* *Aspergillus niger* *Penicillium glabrum* *Yeast*	*Penicillium italicum* *Aspergillus niger*
15	B15	320	*Aspergillus terreus* *Aspergillus niger*	*Aspergillus niger*
16	B16	27	*Aspergillus terreus* *Penicillium expansum*	*Aspergillus niger*
17	B17	71	*Alternaria alternate* *Aspergillus parasiticus* *Penicillium italicum*	*Aspergillus parasiticus* *Penicillium italicum*
18	B18	59	*Aspergillus terreus* *Bipolaris spp* *Aspergillus niger*	*Aspergillus terreus* *Aspergillus niger*
19	B19	280	*Yeast* *Aspergillus terreus* *Penicillium glabrum*	*Aspergillus terreus* *Penicillium glabrum*
20	B20	58	*Penicillium expansum* *Rhizopus spp* *Chladosporium cladosporioides*	*Penicillium expansum*
21	B21	320	*Alternaria alternate* *Penicillium italicum* *Bipolaris spp*	*Penicillium italicum*
22	B22	300	*Aspergillus parasiticus* *Aspergillus terreus* *Aspergillus niger*	*Aspergillus parasiticus* *Aspergillus niger*
23	B23	340	*Trichoderma spp* *Aspergillus niger* *Aspergillus terreus*	*Trichoderma spp* *Aspergillus niger*
24	B24	29	*Bipolaris spp* *Yeast* *Penicillium glabrum*	*Yeast*
25	B25	380	*Aspergillus niger* *Trichoderma spp*	*Trichoderma spp*
26	B26	69	*Yeast* *Penicillium glabrum* *Rhizopus spp*	*Penicillium glabrum*
27	B27	330	*Aspergillus terreus* *Penicillium expansum* *Chladosporium cladosporioides*	*Penicillium expansum*
28	B28	376	*Bipolaris spp* *Penicillium italicum*	*Penicillium italicum*

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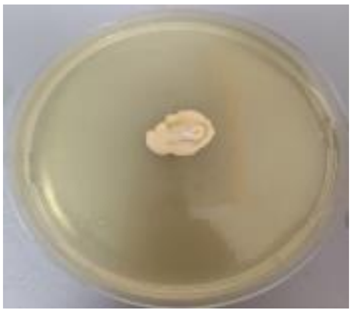
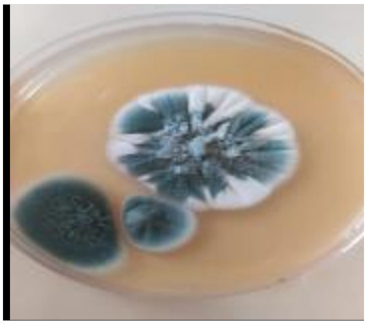
29	B29	230	*Trichoderma spp* *Yeast* *Aspergillus niger*	*Aspergillus niger*
30	B30	370	*Yeast*	Negative
31	B31	44	*Yeast*	Negative
32	B32	46	*Yeast*	Negative

Twelve different types of fungi were found in the goods made with bottled water. *Aspergillus niger, Aspergillus terreus, Aspergillus parasiticus, Penicillium italicum, Penicillium expansum, Penicillium glabrum, Alternaria alternate, Bipolaris spp., Trichoderma spp., Rhizopus spp., Chladosporium cladosporioides*, and *yeast* are included in Figure 1.

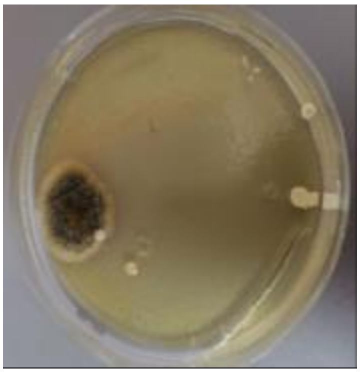


Alternaria alternate

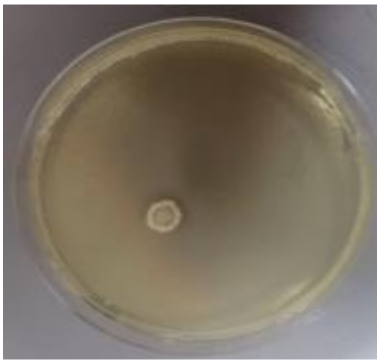
Aspergillus parasiticus



Penicillium italicum



Trichoderma spp



Bipolaris spp

Aspergillus niger

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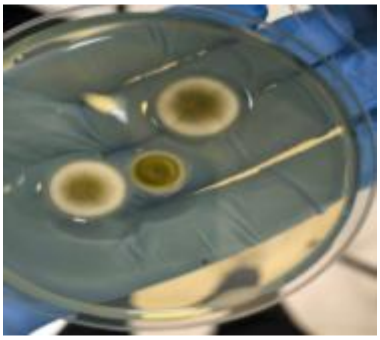
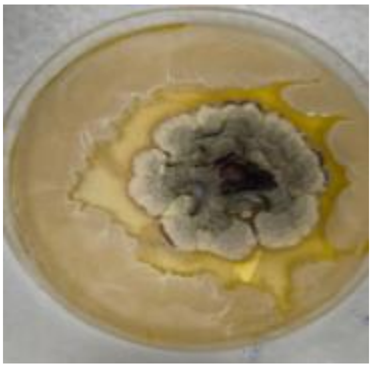
Yeast



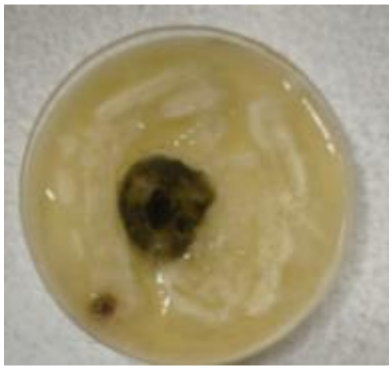
Aspergillus terreus

Penicillium glabrum

Penicillium expansum



Rhizopus spp



Chladosporium cladosporioides

Figar (1) Fungi isolated from bottled water and plastic surfaces of containers

Upon analyzing the swabs from the plastic containers, it was evident that each container harbored at least one strain of fungi that matched those found in the water. This conclusive finding established that all samples of bottled water contain multiple varieties of fungi. Furthermore, various types of fungi were also discovered on the inner surfaces of all plastic containers, providing further evidence of a correlation between the fungi present in the bottled water and the container itself, as well as the samples of fungi isolated from the internal surfaces of plastic containers.

As for water bottled in single-use plastic containers (B30, B31, and B32), Some samples are free of any type of fungus except yeast, and there is no presence of any type of fungus in plastic containers.

Most of the different concentrations of antifungal tablets have effects on the isolates. However, as the concentration of the antifungal drugs increased, the diameter of the zone of inhibition also increased (Table 2). Ketoconazole exhibited greater inhibitory potential than griseofulvin and itraconazole. This finding is in consonance with the work of (14) The results of ketoconazole and griseofulvin have also been reported in the work by (15)where not all the isolates of a particular strain were sensitive to these drugs.

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Table 2: Antibiotic sensitivity pattern of ketoconazole and griseofulvin and itraconazole on the Fungal isolates

Fungal isolates	Concentrations (g/ml)	Diameter of zone of inhibition (mm)			
		Ketoconazole	Griseofulvin	itraconazole	Control (water)
Aspergillus niger	0.02	**19.00±0.56e**	**15.00±0.97c**	**14.00±0.77a**	0.00±0.00a
	0.04	**22.00±0.77e**	**18.00±0.97c**	**10.00±0.77a**	0.00±0.00a
	0.06	**23.00±0.79e**	**19.00±0.97c**	**8.00±0.75a**	0.00±0.00a
Aspergillus terreus	0.02	**21.00±0.77e**	**19.00±0.49c**	**16.00±0.31a**	0.00±0.00a
	0.04	**23.00±0.79e**	**20.00±0.51c**	**18.00±0.33a**	0.00±0.00a
	0.06	**25.00±0.79e**	**22.00±0.52c**	**20.00±0.34a**	0.00±0.00a
Aspergillus parasiticus	0.02	**25.00±0.58e**	**20.00±0.50c**	**14.00±0.55a**	0.00±0.00a
	0.04	**27.00±0.62e**	**21.00±0.53c**	**15.00±0.58a**	0.00±0.00a
	0.06	**29.00±0.68e**	**22.00±0.57c**	**17.00±0.58a**	0.00±0.00a
Penicillium italicum	0.02	**34.00±0.79e**	**31.00±0.78c**	**19.00±0.66a**	0.00±0.00a
	0.04	**37.00±0.79e**	**33.00±0.79c**	**21.00±0.68a**	0.00±0.00a
	0.06	**39.00±0.79e**	**34.00±0.79c**	**23.00±0.68a**	0.00±0.00a
Penicillium expansum	0.02	**32.00±0.88e**	**23.00±0.77c**	**18.00±0.88a**	0.00±0.00a
	0.04	**35.00±0.88e**	**26.00±0.78c**	**19.00±088a**	0.00±0.00a
	0.06	**39.00±0.88e**	**27.00±0.78c**	**22.00±0.88a**	0.00±0.00a
Penicillium glabrum	0.02	**31.00±0.66e**	**25.00±0.61c**	**14.00±0.77a**	0.00±0.00a
	0.04	**33.00±0.68e**	**28.00±0.61c**	**17.00±0.78a**	0.00±0.00a
	0.06	**35.00±0.69e**	**30.00±0.62c**	**20.00±0.78a**	0.00±0.00a
Alternaria alternate	0.02	**26.00±0.89e**	**17.00±0.58c**	**9.00±0.19a**	0.00±0.00a
	0.04	**28.00±0.91e**	**19.00±0.56c**	**13.00±0.19a**	0.00±0.00a
	0.06	**30.00±0.94e**	**22.00±0.58c**	**15.00±0.19a**	0.00±0.00a
Bipolaris spp	0.02	**34.00±0.66e**	**25.00±0.59c**	**13.00±0.22a**	0.00±0.00a
	0.04	**37.00±0.67e**	**29.00±0.60c**	**17.00±0.23a**	0.00±0.00a
	0.06	**41.00±0.67e**	**33.00±0.63c**	**21.00±0.23a**	0.00±0.00a
Trichoderma spp	0.02	**33.00±0.67e**	**21.00±0.58c**	**9.00±0.24a**	0.00±0.00a
	0.04	**34.00±0.68e**	**24.00±0.58c**	**13.00±0.24a**	0.00±0.00a
	0.06	**37.00±0.68e**	**30.00±0.58c**	**14.00±0.25a**	0.00±0.00a
Rhizopus spp	0.02	**22.00±0.69e**	**17.00±0.58c**	**7.00±0.25a**	0.00±0.00a
	0.04	**23.00±0.69e**	**19.00±0.58c**	**9.00±0.25a**	0.00±0.00a
	0.06	**26.00±0.69e**	**21.00±0.58c**	**12.00±0.25a**	0.00±0.00a
Chladosporium cladosporioides	0.02	**23.00±0.56e**	**13.00±0.58c**	**7.00±0.54a**	0.00±0.00a
	0.04	**26.00±0.58e**	**15.00±0.58c**	**10.00±0.55a**	0.00±0.00a
	0.06	**29.00±0.58e**	**19.00±0.58c**	**11.00±0.55a**	0.00±0.00a
Yeast	0.02	**31.00±0.54e**	**25.00±0.58c**	**12.00±0.57a**	0.00±0.00a
	0.04	**35.00±0.54e**	**27.00±0.58c**	**15.00±0.58a**	0.00±0.00a
	0.06	**37.00±0.57e**	**28.00±0.58c**	**18.00±0.58a**	0.00±0.00a

The problem of fungi in bottled water is an issue of terrific concern that could have tremendous repercussions on public health. Research has indicated that numerous varieties of

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fungi, together with opportunistic and rising pathogens, can infect water assets and withstand conventional disinfection methods. This resilience allows them to increase biofilms in water distribution systems, improving their capacity to live and thrive in indoor settings (16)(17).

Regarding bottled water, fungal contamination poses a risk to prone populations, including the elderly, babies, hospitalized people, and those with weakened immune systems. The expanded stages of heterotrophic plate dependence (HPC) and the identification of spoilage fungi, as well as signs of fecal infection like *Staphylococcus aureus, Shigella spp* and *Salmonella spp* , underscore the fitness risks linked to eating tainted water(16) .

In addition to the above studies, some have found correlations between the occurrence of filamentous fungi and yeast in the samples of bottled water, showing an intricate fungal ecosystem where different species interact constantly (18). Different incidence patterns of fungi are demonstrated when comparing faucet water with tank water. This may be attributed to biofilm formation within hydraulic systems, which could enhance fungal levels in particular conditions (19).

In the literature, there is relative scarcity of information or report on the presence of yeasts and fungi in treated and bottled mineral waters (20)(21). From filamentous fungi, *Penicillium citrinum, Penicillium glabrum, Cladosporium cladosporioides, Alternaria alternate, Cladosporium, Rhizopus, Aspergillus,* and *Phoma* were isolated most common. Some of the aforementioned microorganisms are also likely to be pathogenic, allergenic, and toxigenic species. (22)(23)

In addition, examinations focusing on yeasts and filamentous fungi in mineral water and faucet water have revealed the need to include fungal controls in addition to the traditional microbial testing method (24)(25). The fact that the fungi were spotted in the water we were analyzing serves as a clear indication of the significance of an integrated quality assurance strategy for ensuring the uninterrupted distribution of quality water to customers. Scientists can correlate various strains and indicator bacteria, determine demographic structure and microbial infection layers successfully, and enforce sanitation measures to improve water intensity (3).

In a broader sense, these early studies provided fundamental information about fungal pollution in various water reservoirs, helping to build a precious knowledge base on the presence of microorganisms and their consequences for public health. By placing completed research side by side with recent investigations of fungi invading bottled waters, we have the right data to understand the problems and provide the most effective ways of dealing with microbial contaminants (26).

The impact of the growth of fungi in the bottled water open to everyone is undervalued. The genus *Aspergillus* and other opportunistic pathogens (whose resistance to employment of common disinfectant methods is proven by research) belong to a group of resilient microorganisms. This permits them to penetrate the water distribution systems and to participate in the programming of microbial communities, coexisting in biofilms with other microorganism populations, making their survival in this environment much easier, and we can suppose that this very fact will lead to increased contamination in indoor environments, where water is frequently used (27).

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A significant concern for public health is among individuals with immune system disorders or diabetic patients, i.e., patients with weakened immune systems due to the presence of fungi in drinking water. The greatest effect is the growth of fungus cells inside bottles and dispensers, which means that we have to strictly monitor and check these sites and that ongoing practices have to be maintained. Proper maintenance practices, standard garage protocols, and making sure we have disposable items are a must to manage waste and mitigate health issues from fungi and contaminants (4).

Finally, the proof postulates that fungal invasion in bottled water may lead to severe consequences in the area of health, particularly in the vulnerable groups of citizens. The prevention of fungi in ingesting water sources remains significant for the safety of the customers notably in high risk zones like healthcare centers (5).

CONCLUSION

In summary, it may be ascertained that the presence of fungi in bottled water can have serious implications on consumers' health. Since fungal colonies have been confirmed in the water samples collected from the reservoirs, it points to the ongoing need for regular surveillance and intervention. Another potential problem that should not be dismissed is the belief that frequent consumption of food which is preserved inside plastic containers leads to fungal infection. Some water fungi that cannot be disinfected can settle on the surface of pipes and form a biofilm that is hazardous to the health of the end users. This only emphasizes the need for surveillance of fungal colonization in drinking water in a bid to protect the vulnerable population, including the immunocompromised.

Considering the risks accompanied with fungal penetration into bottled water, policy makers and relevant authorities should include packaged water products into relevant monitoring systems. Some of the measures that can be taken to reduce the risks include proper drying of the bottles as well as avoiding storage of bottles for long time for processing and using mycotoxin-binding agent during processing of the bottled water.

RECOMMENDATIONS

The problem of fungal contamination in bottled water can be reduced only by putting stringent measures about the monitoring norms at each phase of production. At different production phases, samples of water should be regularly subjected to tests with a view of identifying areas of potential contamination and spread of fungi. It is equally important to ensure that bottles used in packaging the water are well cleaned and other equipment used in the process are well maintained to minimize chances of fungal growth.

Furthermore, there is the need to inform the consumers on the various dangers associated with fungi found in bottled water. To minimize the consumers' exposure to the otherwise detrimental effects of fungi, clear, unambiguous and detailed instructions on the label regarding storage conditions and shelf life should be provided. Besides, educating people on the advantages of using reusable covered containers from safe materials may reduce the utilization of disposable plastic bottles that may cause fungal contamination in the long run.

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