International Journal of Current Science Research
www.drbgrpublications.in

Identification of different types of microorganisms that remain in potable water filters

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Abstract

Water is very crucial for life and it is easy to be contaminated. Drinking water is also one of the key vectors for the spread of several diseases. So, water supplies must be free from pathogens especially pathogenic bacteria and algae. The aim of this study is isolation, identification of bacteria and algae in filtrated drinking water in El Fayoum governorate, Egypt. *Aeromonas sobria* and *Chromobacterium violaceum* bacterial strains were identified using 16s rDNA gene sequence technique. In addition, different strains of algae were morphologically identified and were *Chlorella vulgaris, Scenedesmus quadricuda, Padorina morum, Surirella ovalis* and *Chlamydomons variabills*. In this investigation, the bacterial and algal count in filtered water samples exceeded the bacterial and algal in tap water as a result of using filters through one month only.

Keywords: *Water filter, bacteria, algae and drinking water*.

Introduction

Water is requiring for humans; it makes up roughly 60% of the body's weight. Water that we drink on a regular basis must be free of any pollutants, such as organisms or chemical substances, that could harm our health and quality of life (Hussain *et al*., 2020). Pure water and safe water are the two forms of drinking water available. Pure water is described as water that is free of all foreign substances, whether they are harmful or not. While safe water may contain impurities, these contaminants will not harm humans and must be kept under acceptable limits (Hussain *et al*., 2020). Thousands of bacteria live in water, some of which cause sickness. Various diarrhea-related disorders, such as cholera, are caused by pathogens (bacteria, viruses, protozoa, and helminthes) in water (Das & Bose, 2017; Pal *et al*., 2018).

Every day, each individual requires approximately 2 litres of clean drinking water (Yassi *et al*., 2001). Management of microbiological hazards in drinking water is crucial to ensure the safety of drinking water for public health protection (Villanueva *et al*., 2014) .

Water is a vital component of all living forms on Earth, and when contaminated, it becomes a major cause of morbidity and mortality among humans, as it is thought to be an effective disease transporter. As a result, safe drinking water (free of infections and other health hazards) is critical for human life and survival (Gadgil, 1998).The purpose of this study was isolation and identification of the different types of microorganisms that remain in potable water.

Material and Methods

Isolation and identification of bacteria and algae

Water samples were collected from EL Fayuom Governorate, Egypt in February, 2018 to November 2019.

Media used for bacterial isolation

Nutrient agar was purchased from Riedel-de Haën (Sigma-Aldrich, Seelze, Germany). Nutrient agar (NA) Ronald (2010) "Handbook of microbiological Media"

Bacterial isolation

100 ml filtered water samples obtained from each water filters after one month of using filters. Water was divided and placed on to the plates containing isolation medium (Nutrient agar that was previously sterilized at 121˚C for 20 min. 100µl from the water was spread on the surface of nutrient agar plates. incubation at 28°C for 3 days (Janarthine & Eganathan, 2012).

Bacterial count

The total bacterial count was carried out using agar plate count according to Chouhan (2015).

The16S rDNA identification

The method was used to identify bacterial strains. Genomic DNA from bacterial isolates was extracted using normal bacterial methods (Sambrook *et al*., 1989). Forward primers were employed in the amplification of the 16S rDNA gene (F1; AGA GTT TGA TCC TGG CTC AG) and reverse primer (R1; GGT TAC CTT GTT ACG ACT T). The PCR mixture was prepared as the following; 10 µL (10x) PCR buffer, 3 µL (50 mM) MgCl2, 1 µL (20 pmole/µL) of each primer, 1 µL (10, and thevolume is completed to 100 sµL by SDH2O. PCR were carried mM) dNTPs mixture, 0.5 µL (2.5U) Taq DNA polymerase, 2 µL total DNA extract out for 35 cycles under the following conditions: denaturation step at 94°C for 40 sec, annealing step at 55°C for 1 min, extension step at 72°C for 2 min and final extension at 72°C for 10 min. An aliquot of the

PCR products (10 µL) was mixed with 2 µL of DNA loading buffer and analyzed by electrophoresis (15 V/cm, 60 min) on 0.7% horizontal agarose gel in TBE buffer having 0.5 µg/mL ethidium bromide, then visualized on an UV transilluminator. GATC Biotech in Constance, Germany, did the sequencing of the amplified fragments.

Isolation of algae

The algae in this investigation were isolated from different filtered water samples obtained from filters after one month of using filters collected from El-Fayoum Governorate, Egypt using Woods Hole MBL media pH 7.2(Nichols, 1973).

Identification of isolated algae

The pure isolated algal were identified according to the keys described in the following literatures: A manual of fresh water algae by Whitford and Schumacher (1973) and

-How to know the fresh water algae by Prescott (1978).

-A Key to the more frequently occurring fresh water algae phytoplankton

Counting procedure

Cell count was carried out using a standard haemocytometer (Moheimani *et al*., 2013) under an Olympus BH-2 light microscope.

Statistical

The data given here are the mean values of three replicates. Standard errors were calculated forall the values using MS Excel 2010.

Results

Two pathogenic bacteria isolated from different water filters in EL Fayoum Governorate, Egypt using enrichment technique. Algal species that isolated and identified by light microscope where *Chlorella vulgaris, Scenedesmus quadricuda, padorina morum, surirella ovalis* and *chlamydomons variabills.*

Identification of bacteria isolated AQ1.

The AQ1 isolate was identified as *Aeromonas Sobria* using 16S rDNA gene sequencing technique.

Identification of bacteria isolated AQ2.

The AQ2 isolate was identified as *Chromobacterium violaceum* using 16S rDNA gene sequencing technique.

Isolation of algae

From the algal isolation, the most predominant algae were *Chlorella vulgaris, Scenedesmus*

quadricuda, padorina morum, surirella ovalis and chlamydomons variabills.

Algae species was shown as follow:

Chlorella vulgaris: Is a green eukaryotic microalga, a spherical microscopic cell with 0.5 - 7.5 µm diameter, unicellular algae and non-motile

Scenedesmus quadricuda: Is green eukaryotic microalgae, ellonged ellipsoidal, 11 - 18 µm Non motile and usually consist of 2,4, or 8, arranged lineally or zigzag cell body. Cells are uni nucleated and have a laminate chloroplast with 1 pyrenoid.

Chlamydomons Variabills: Unicellular green algae in the shape of a spheroid measuring about 10 µmin diameters, motile. The cell is surrounded by a smooth, thin and firm cell wall. The cell wall at the anterior end is extended to make apical papilla.

Padorina morum: Sub spherical to ellipsoidal solid colony, with 4,8,16 or 32 biflagellate, flattened by mutual compression, pyriform or spherical in shape, cells embedded in a common gelatinous envelope, motility by flagellum, multicellular organism.

Surirell aovalis: Unicellular cells, spherical or slightly cylindrical. Their length ranges from 16 to 61.5µm and their width from 11 to 35µm. It is a large and common freshwater to marine. Cells found in the benthos of hard waters.

Table (1) Total bacterial viable count in different types of filtrated water samples:

Filter type	Log number of colony forming unit (cfu)/ml
Tap water	2.4 ± 0.2
One stage filter (fresh)	2.8 ± 0.4
Three stage filter (tank)	3.04 ± 0.4
Five stage filter (reverse Osmosis system)	3.26 ± 0.3

Values are means ± standard error

Table (2) Total algal viable (No of cell $\times 10^{4}$) count in different types of filtrated water samples.

Type of filter	*C. vulgaris*	*S. opoliensis*	*S. quadricuda*	*S. ovalis*	*Chlamydomonas variabills*	*P. morum*
Tab water	1.2 ± 9	0.2 ± 5	0.3 ± 8	0.1 ± 13	0.2 ± 9	0.5 ± 8
One stage filter (fresh)	3.4 ± 7	1.1 ± 6	1.2 ± 7	1.1 ± 11	1.5 ± 11	2.2 ± 7
Three stage filter (tank)	17.2 ± 8	2.1 ± 11	1.5 ± 9	1.5 ± 13	4.3 ± 12	6.1 ± 6
five stage filter (R.O system)	24.6 ± 12	2.4 ± 10	2.7 ± 11	2.7 ± 9	6.1 ± 14	15 ± 9

Bacterial and algal count: bacterial count and algal in tap water and in different filtered water samples shown in figure (1) and (2).

Volume: 7; Issue: 12; December 2021; pp 2301-2308; ISSN: 2454-5422

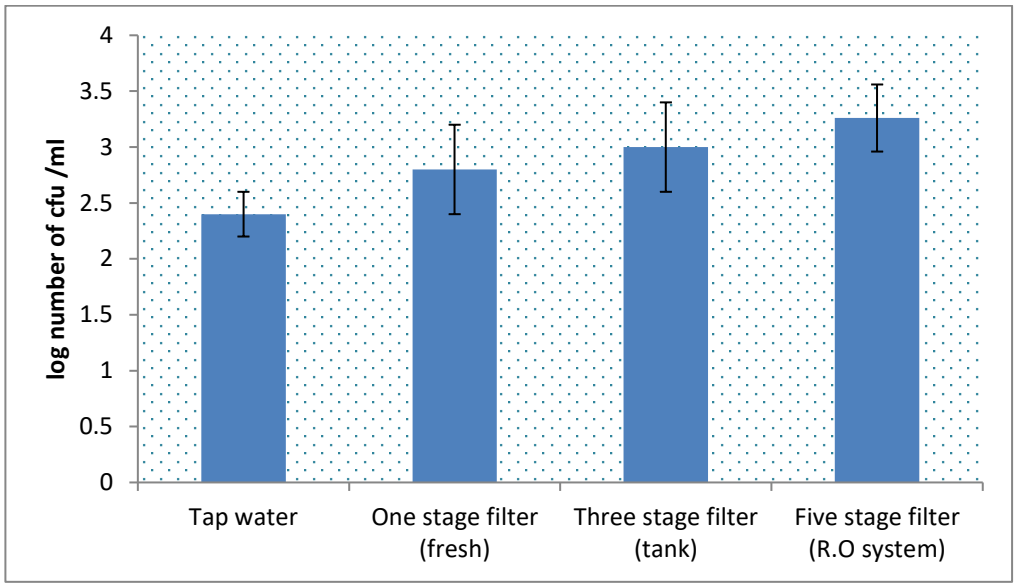

Fig1. Bacterial Count in tap water and in filtered water

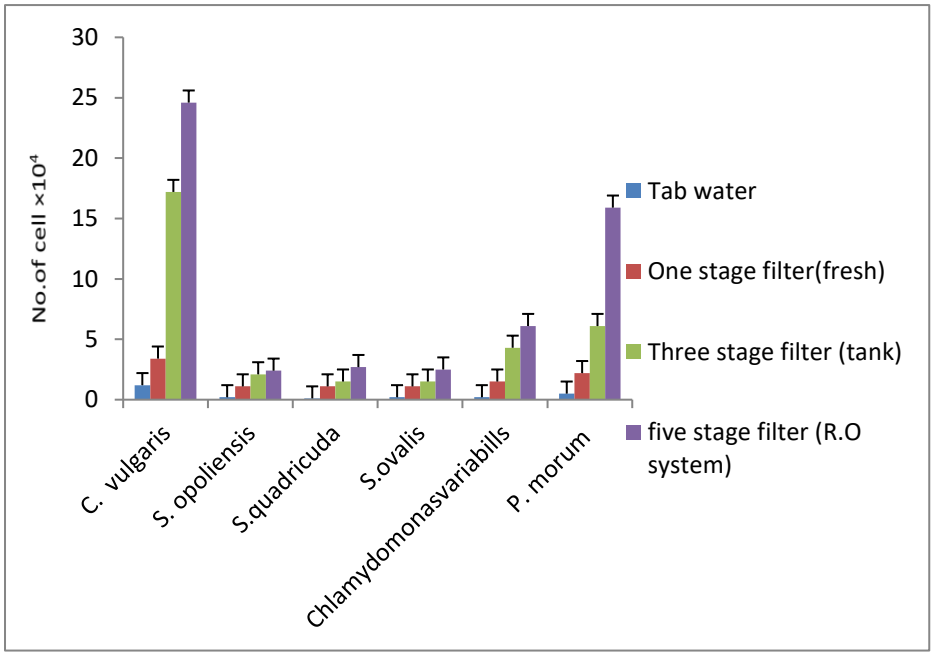

Fig2. Total algal viable count in different types of filtrated water samples

Discussion

The contamination of drinking water by bacteria that cause diarrheal diseases is the most important aspect of drinking water quality (Fawell& Nieuwenhuijsen, 2003). In both water distribution systems and activated charcoal-based residential water filtration systems, bacterial regrowth is a serious problem (Daschner *et al*., 1996; Hansen *et al*., 2013).

The obtained results in table (1 & 2) indicated that filtered water samples revealed positive results for bacterial growth as Gram-negative bacteria represented by: *Aeromonas sobria* and *Chromobacterium violaceum*. This is in agreement with previous studies of Ahmed *et al*., (2004). The most common bacteria found in drinking water are Gram-negative bacteria that are naturally prevalent in the environment.

Nriagu *et al*., (2018) found that domestic water distribution system, the filters increase microbial growth and biofilm formation, Most bacterial species were isolated from the filtered water in our study were *Aeromonas sobria* and *Chromobacterium violaceum*. In extremely immunocompromised people, this bacteria can cause life-threatening infections, especially after colonising the gastrointestinal tract. Because *Chromobacterium violaceum* is widely dispersed in natural aquatic environments and is temperature sensitive, it prefers tropical and subtropical climates (Justo & Durán, 2017; Ponte & Jenkins, 1992).

The present study showed positive results for the presence of *Aeromonas sobria* and *chromobacterium violaceum.* Suggests that the water was contaminated, which is one of the leading causes of diarrhoea. In agree with Fadhil (2016) who indicated that the water was polluted with faeces *as Streptococcus faecalis*, one of the most common signs of faecal contamination in drinking water.

In this study, isolation of the most common algae in drinking water filters *Chlorella vulgaris, Scenedesmus quadricuda padorina morum , surirella ovalis and chlamydomons variabills*.

In the United States, the Environmental Protection Agency recommends that bacterial levels in drinking water not exceed 500 cfu/ml; conversely, in Germany, the maximum bacterial count is 100 cfu/ml. Although the United States and Canada created a 500 bacteria/ml standard, the 100 cfu/ml level became the norm in many European countries where it was deemed a fair aim. Although pathogen contamination is independent to the amount of heterotrophic bacteria in drinking water. Nonetheless, it is still used as an indicator of overall water quality in most national drinking water laws. (Medema *et al* 2003).

Conclusion

In this investigation two strains of bacteria were isolated and identified by 16S rDNA techniques also five algal strains were isolated and morphologically identified. The data showed that the bacterial and algal count in filtered water samples exceeded the bacterial and algal in tap water as a result of using filters through one month only.

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