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Bacteriological Quality Of Drinking Water That Sterilized By Reverse Osmosis system Al Nasiriyah City

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ABSTRACT

The treated water by reverse osmosis is widely used as one of the types of drinking water, and for the correlation of this water with the health of individuals, this study was aimed at detecting the bacteriological quality of this water. Samples were collected based on three drinking water supply stations using reverse osmosis system . three samples (from the same station ,from water seller that supplied by station water , from houses that supplied by station water) were collected per station per month, for the period from November 2017 to march 2018 .The bacteriological analyses were Heterotrophic plat count (HPC) ,Total coliform (TC) count and *E.coli* count . It was appeared , the high rates of numbers of HPC in all stations ,they registered 190 ,70 and 5×10^2 (CFU / 100 ml). The rates of total coliform (TC)from same stations , as it reached 0 , 67 , 2×10^2 (cell / ml) in stations respectively , houses recorded the highest rates of these bacteria for all stations 53 , 100 , 4 $\times10^2$ (CFU / 100 ml) in A , B and C respectively . The rates of *E.coli* in RO treated drinking water samples that collected from the same stations were 0, 13,1$\times10^2$ (CFU / 100 ml). The higher numbers of *E coli* were appeared in houses for all stations , it registered 50,27 and 4 $\times10^2$ (CFU / 100 ml) for stations A ,B and C respectively . Numbers of these indicators in most samples of RO treated drinking water exceeding the international standards. the number of bacteria that isolated from these water were 36 isolates that distributed to 27 (75%) Gram negative and 9 (25%) Gram positive bacteria ,*Klebsiella* spp. was the more frequency isolate among Gram negative bacteria , and *Enterococcus fecalis* was the predominant among Gram positive bacteria.

Keywords: Drinking Water ,Reverse Osmosis ,Heterotrophic plat count (HPC) ,Total coliform (TC) , *E.coli*

INTRODUCTION

Water is important element for life on earth .The structure and synthesis of cell components , cell metabolism and transfer nutrients into cells depend on water (Sharma & Bhattacharya,2016).Drinking water sources include water pipes , reservoirs , streams , rivers , springs, rain and ponds . studies it has shown that the way water is collected , and handled after collecting and storing at home causes quality degradation to the extent that water have potential risks infection to users (Ampofo& Karikari , 2006).

Since the beginning of recorded history water has been recognized as a potential carrier of disease (presscott *et al*., 2001), microbial growth in drinking water can degrade water quality

and become a major area of concern for affected utilities (Rice *et al*., 1991).in general, the greatest risk of microbes is associated with ingestion of water that is carrying human or animal faces . The discharge of waste water into rivers and coastal waters are the major source of contamination by fecal microbes that including pathogens (WHO, 2008).

Water-borne diseases caused by different microbes such as enteric bacteria ,enteric protozoa and enteric viruses (Leclerc *et al*., 2002),water borne disease outbreaks are most affect the developing countries in the world , However , these outbreaks also targets developed countries , especially when it is a strict health system standards are not performed (Karanis ,2006). typhoid fever and Cholera and are classic examples of waterborne diseases, as only a few highly pathogenic pathogens are required to cause severe diarrhea, hepatitis A, Shigellosis, amoebic dysentery, and other digestive diseases can also be transmitted through water (WHO, 2011a).The main of the major microbiological contaminants of drinking water are the pathogenic bacteria , e.g. *Escherichia coli , Salmonella , Campylobacter , Shigella* ,Viruses and parasite e.g. *Giardia lamblia , Cryptospordium parvum* (Odonker & Ampofo, 2013).

Isolation and identification of the organisms that causes these diseases can be complex , expensive and less quantitative (WHO, 1983), therefore , the development of very promising techniques for the analysis of pathogens (Castillo *et al*., 2015).

Heterotrophic plate count (HPC) test was used as indicators of the proper functioning of water treatment processes and as indirect indicator of water safety (Bartram *et al*.,2003).Coliform bacteria ,fecal coliform , and *E.coli* were used as indicators of microbiological safety of water supply (Griffin *et al*., 2008),members of two groups of bacteria ; coliform and fecal streptococcus are used as indicators of possible contamination of water and wastewater because they are usually found in human and animal wastes . Although they are not generally harmful they suggest to indicate to presence of pathogenic bacteria , viruses and protozoa that also live in human and animal digestive tracts , thus , their presence in the water indicates that pathogenic microorganisms may also be present (Burres , 2009).

Bacterial indicators are used to determine whether the sources of drinking water are microbiologically safe , if drinking water treatment is sufficient and good maintenance of drinking water distribution system (NRC, 2004). Total coliform is considered useful indicator in testing treated drinking water where soil or plant contamination is a concern . The drinking water quality based on the total coliform density are determined in the safe drinking water act , as amended in 1986 (USEPA , 1986). In 1986, *Escherichia coli* replaced total coliform and fecal coliform and enterococci as the recommended indicator bacteria , *E.coli* or enterococci are recommend for freshwater monitoring , whereas enterococci are the preferred bacteria for marine water detection because they are tolerated for salinity (USEPA, 2004).

Reverse osmosis (RO)can elimination many types of ions molecules from solutions, it is used in both industrial and the production of potable water. The result is that the solute remains on the compact side of the membrane and pure solvent, that in most cases is water, is forced across the membranes on the other side, where it is accumulated. RO is used in multiple implementations, comprising recycling, treatment of wastewater , processing of food and beverage, and power generation. Different techniques and processes include the use of RO processing plants. RO is one of the few effective ways to elimination volatile organic ,Compounds, Minerals , fluorides and other chemical pollutants from drinking water

(Wimalawansa, 2013). Because of the frequent use of drinking water which sterilized by reverse osmosis in the city of Nasiriyah , this study was aimed to estimate the bacteriological quality of reverse osmosis water .

MATERIALS AND METHODS

Samples collection

Samples were collected based on three drinking water supply stations* using reverse osmosis system .These stations supply vendors who specialize in selling this water, who transport it by containers made of plastic or small water tanks and transport it to residential areas and sell it to houses, three samples in duplicate (from the same station ,from water seller that supplied by station water , from houses that supplied by station water) were collected per station per month, for the period from November 2017 to march 2018 .

Water samples was collected in a sterile 500 ml glass bottle , and then were transported in the icebox to the laboratory of microbiology in department of pathological analysis in college of science , thi Qar university.

*Station A : is located behind the Holandy bridge

Station B : is located in the area of Sayed Dakhil

Station C : is located in the city of Nasiriyah

Microbial analysis

1 - ***Heterotrophic plat count*** **(HPC):** the method was used to detected aerobic plate count (APC) was pour plate technique by using nutrient agar , duplicate water samples of 0.1 and 1 ml from the original water sample , and 1ml , 0.1ml from the same original sample was diluted to 10^{-2} inoculated into petri dishes , and 20-25 ml of nutrient agar was poured into each petri dishes , mixed well , and then incubated at 35 C° for 48hr the colonies were calculated as CFU/ ml (APHA,AWWA&WEF;2012).

2-Total colifrom bacteria and *E.coli* were detection by membrane filtration technique (using MacConkay agar medium ,and EMB agar was used for the identification and numeration of *E.coli*) according to methodology describe in the √ (WHO, 1997).

3- Identification of Bacteria:

For identification and diagnosis of bacterial isolates, the following criteria were considered, colonial, cellular morphology, and biochemical reaction according to (Collee *et al*., 1996; MacFaddin, 2000).

RESULTS

Table (1) : Rates of numbers of HPC in RO treated water samples

The stations	The station	seller The	The houses	The total
	Rate×10^2 (CFU / 100 ml) (Range ×10^2 (CFU / 100 ml))			
A	190 (0-340)	196 (0-300)	197 (0-300)	194 (0-340)
B	70 (0-100)	197 (0-300)	423 (0-1000)	230 (0-1000)
C	5 (0-10)	21 (0-42)	36 (0-72)	21 (0-72)

Table (1) was appeared , the high rates of numbers of HPC in all stations ,they registered 190 ,70 and 5×10^2 (CFU / 100 ml), and RO treated water samples that collected from houses of all stations showed the higher number of these bacteria than stations and seller . they were registered 197 ,423,36(×10^2 (CFU / 100 ml)) in stations A,B and C respectively.

Table (2) : Rates of numbers of TC in RO treated water samples

The stations	station The	The seller	The houses	The total
	Rate×10^2 (CFU / 100 ml) (Range ×10^2 (CFU / 100 ml)			
A	0	13 (0-40)	53 (0-95)	22 (0-95)
B	67 (0-200)	100 (0-300)	100 (0-300)	89 (0-300)
C	2 (0-6)	2 (0-4)	4 (0-8)	3 (0-8)

The results revealed that the higher rates of TC were detected in station B it was registered 89 ×10^2 (CFU / 100 ml)and houses recorded the highest rates of these bacteria for all stations studied as recorded 53 , 100 , 4 ×10^2 (CFU / 100 ml) in A , B and C respectively while the lowest rates of these is bacteria were from same stations , as it reached 0 , 67 , 2 (cell / ml) in stations respectively.

Table (3) : Rates of numbers of *E.coli* in RO treated water samples

The stations	Station The	The seller	The houses	The total
	Rate×10^2 (CFU / 100 ml) (Range ×10^2 (CFU / 100 ml))			
A	0	7 (0-22)	50 (0-110)	19 (0-110)
B	13 (0-40)	23 (0-69)	27 (30-50)	21 (0-69)

C	1 (0-1)	1 (0-1)	4 (0-4)	2 (0-4)

The results revealed that the rates of *E.coli* in RO treated drinking water samples that collected from the same stations were 0, 13,1×10^2 (CFU / 100 ml) , and higher rates of numbers were in station B in rate 21 ×10^2 (CFU / 100 ml), and smallest rates in station C in rate 2 cell /ml . The higher numbers of *E coli* were appeared in houses for all stations , it registered 50,27 and 4 ×10^2 (CFU / 100 ml) for stations A ,B and C respectively .

Table (4): Types of bacteria which isolated from RO treated water samples

The Stations	Gram negative bacteria	No.&(%)	Gram positive bacteria	No.&(%)
A	*Klepsiella pneumonaie* *Enterobacter* spp. *Pseudomonas aruginosa*	3(11.1) 4 (14.8) 3 (11.1)	*Enterococcus facalis* *Bacillus* spp.	2(22.2) 2(22.2)
B	*Klepsiella pneumonaie* *Enterobacter* spp. *Pseudomonas aeruginosa* *Proteus* spp.	3(11.1) 4 (14.8) 3 (11.1) 2(7.4)	*Enterococcus facalis* *Bacillus* spp.	2(22.2) 1(11.1)
C	*Klepsiella* spp *Proteus* spp	3 (11.1) 2(7.4)	*Micrococcus* spp.	2(22.2)
Total	36	27 (75%)		9 (25%)

Table (4) Was showed the types of bacteria that isolated from RO treated water samples the results showed , the number of bacteria were 36 isolates that distributed to 27 (75%) isolates were gram negative bacteria and 9 (25%) isolates were gram positive bacteria .It was revealed ,*Klebsiella* spp. was the more frequency isolate among Gram negative bacteria , and *Enterococcus fecalis* was the predominant among Gram positive bacteria.

DISCUSSION

Bacterial growth is a big trouble in both water distribution pipes and domestic water purification units such as reverse osmosis system (Geldreich *et al*., 1972; Payment, 1989; Reasener , 1989).

The results of this study were showed that the RO treated water samples that collected from all stations were containing high rates of HPC , therefore , this water does not meet the international standards such as European Union (1998) that limited the numbers of HPC for drinking water are not exceed on 20 CFU/ml when incubated at 37C for 48 hours . The numbers of HPC agreements with US EPA (2009) acceptable HPC levels in drinking water is less than 500 CFU /ml , Although no specific numeric guidelines are recommended for HPC in drinking water , is suggested to be maintained of the lowest level possible as evidence of processing efficiency (WHO,2011b), and not meet of some these numbers with Iraqi standard specification (Central organization for standardization and control quality , 1984) that

determined the HPC levels in drinking water should be less than 50 CFU /ml ,this increase of HPC may indicate a problem with water treatment or disinfection (Bartram , *et al*., 2003)or may be result from contamination during transport processes .A significant increase in HPC levels can be an early sign of contamination (NHMRC.2004),the results of our study agreement with the results of Massoudinejad *et al*.(2016) who founded 15% of RO water samples were positive for HPC and 70% of them have more than 100 colonies based on the milliliter but less than 500 CFU /ml .

In the United States , coliform bacteria has been recognized under the EPA safe drinking water act since 1989 as an occasion microbial index of drinking water quality (USEPA , 2001a).the results of this study showed that the total coliform was different among the stations that supplied the reverse osmosis water and inconformity with the Iraqi standard specifications for drinking water (Central organization for standardization and control quality , 1984) which determined the total number of total coliform does not exceed 5 cell/ 100ml , while the international standard specifications (WHO,1996) that determined the validity of drinking water free of any presence of TC , this shows that this water does not meet international standards ,except for water taken from station A , which was free of these bacteria .

Detection of TC in drinking water may indicate failure of the treatment system, re growth of coliform or infiltration of the system , any of which may be dangerous health effects, as such TC continue to be acceptable indicators of effectiveness of disinfection and treatment process (FPTCDW,2002).

preliminary studies have shown that *Escherichia coli* a more better indicator is preferred for the risk of disease than others in the fecal coliform (Odonkor &Ampofo,2013), the results of this study showed that the OR treated drinking water samples of all stations were found to be contaminated with these bacteria except for water taken from station A , which was free of these bacteria .

In this study , the numbers of *E.coli* were above the EPA (2001b) and WHO(1997) guideline value (the standards require zero of this indicator to be presence per 100 ml of drinking water sample),these results were agreement with results of Pesewu *et al*.(2014) who found all specified home storage water poly tanks were contaminated with indicator bacteria such as fecal coliform and *E.coli* above World Health Organization recommended standards ,the study carried out by Ali *et al* . (2011) showed that all samples of drinking water contaminated with total coliform and 66% of samples were contaminated with *E.coli* , the presence of total coliform and *E.coli* in RO treated drinking water may be indicates to exposure theses water to the external modes of contamination , because these bacteria are spread in environment. The use of indicators , such as *E.coli* and total coliform are indication of assessing the potential presence of enteric pathogens that causes water borne diseases (Hijnen *et al*.,2000) .our study showed that the numbers of indicator bacteria in the houses samples for all stations increased away from the original water sources (stations) ,this may be due to the contamination of containers that used in water transportation and handling . Some studies concludes that handling and storage of drinking water after collection increases the numbers bacteria in water ,these studies have shown that plastic water containers are susceptible to contamination in

addition to fecal contamination of people's hands ,it also contributes to contamination of water (Eschol *et al*., 2009 ;Trevett *et al*., 2005 ; Nichdson *et al*., 2017).

The present study was appeared the differences in numbers and types of bacteria isolated from osmosis reverse treated drinking water , the reason may be due to formation biofimls in internal surface tubes of water treatment system and increased the numbers of bacteria in biofilm such as Gram negative (Geldreich , 1996), it was notice the almost of bacteria that isolated in this study were opportunistic pathogens such as *Klebsiella* , *Pseudomonas* , *Enterobacter* and *Proteus* , when such bacteria are present in drinking water , they cause diseases among people whose defense mechanisms are impaired such as very old and very young persons and those suffering from AIDS , if water carrying excessive numbers of these organisms , it may causes a variety of infections such as eye , skin and mucous membrane ,ear , throat and nose (WHO, 1996) . These results were agreement with Massoudinejad *et al*.(2016) who isolated five species of Gram negative bacteria from RO treated water device in desalination center in city of Kashan.

We conclude that most of samples of RO treated water especially that collected from sellers and houses that taken from all three stations were found to be contaminated with indicator bacteria (HPC. TC and *E.coli*) in numbers exceeding the international standards. Highlights the importance of testing and monitoring of distributing this water ,improve personal hygiene in handling and storing water in the domestic area is suitable measures to reduce the contamination.

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