Bacteriological and Chemical study on different types of Drinking Water

Nuha Abdulridha Saad Dosh Ph.D.
Alia Essa Bashboosh M.Sc.
Thanaa Shams Al-Deen Abbass M.Sc.
Dep.,of Microbiology / College of Medicine / Kufa Univ.

Abstract:
The aim of this study is to evaluate the effects of the quality and bacteriological content of water on the health of consumer.
Locally prepared bottled water was stored at room temperature for three months and undergone bacteriological examination monthly for three successive months. Household RO and tap waters also examined bacteriologically.
pH, EC, TDS, total hardness, minerals (Mg, Ca) and chlorides were determined in each type of water.
Household RO water has given to male rats for two months and three animals were sacrificed at two weeks intervals. Liver, kidney and heart were removed and pathologically studied.

It was found that bottled water was not sterile and contain low number of bacteria that naturally present or accidently introduced during processing. Household RO water showed pathological changes in sacrificed animals studied.

Keywords: BW (bottled water), E.C.(electrical conductivity), MPL( maximal permitted levels), RO(reverse osmosis), TDS(total dissolved solids), TH(total hardness) and THM(trihalomethan).
Tap water is municipal water that comes out of the faucets and has been treated, processed and disinfected. Municipal water supplies is purified with chlorine, which kills disease-causing bacteria that the water or its transport pipes contain (3). The municipal water systems are becoming increasingly dangerous to our health. In many of the older systems the pipes are made of clay and lead, the later cause severe neurological damage particularly in children, elevated blood pressure and chronic anemia. Also chlorine combine with organic chemicals to form byproducts trihalomethans (THMs) and haloacetic acids, which have been linked to reproductive disorder and cancer (4, 5).

The deterioration taste and quality of tap water and the fear of unknown contaminants have made bottled water a solution for many families. Bottled water may be purified or mineral water. Purified water produced by distillation, deionization and reverse osmosis, while mineral water could be natural spring water or artesian water, comes from underground source that has been treated to be suitable for human consumption (6,7).

Reverse osmosis (RO) is a filtration process whereby water is forced through semi-permeable membrane, while contaminants are held back. Its effectiveness depends on the quality of the filters and the frequency of filter cleaning or replacement (8) RO water is filtered usually acidic and has little or no minerals (9). Because of low attention on bottled water in Iraq this study is regarded as the first one in our country aimed to check the sterility of bottled water and to detect the effects of the quality and content of water on the health of consumer.

**Materials and Methods:**

Thirteen samples of bottled water were collected from Najaf markets and stored at room temperature for three months then undergone bacteriological examination monthly to check their bacterial content.

Water from household RO system and tap water also subjected to bacteriological examination, and they have been given to male rats for two successive months, in order to observe the pathological effects of water compositions on different organ tissues.

**Bacteriological examination:**
This test aimed to detect the most probable number of *Escherichia coli* and *Enterococcus faecalis* in water, thus their presence in water indicates the fecal material has contaminated the water supply.

Total count determination and *E.coli*, *E.faecalis* detection tests were achieved according to (10).

**Chemical Determinations:**
The amounts of pH, electrical conductivity (E.C.), total dissolved solid (TDS), hardness, minerals (Mg, Ca) and chlorides were determined in each type of water by (Central lab./Directorate of water in Najaf).

**Laboratory animals:**
Twenty four male rats at ten weeks of age were obtained from animal house in college of medicine/University of Kufa. Rats were divided into two groups, the first one of twelve animals have been given water from household RO system for two months, while the second group of other twelve rats have been given tap water for the same period of time as a control. Three animals from each of two groups were
sacrificed at two weeks intervals, and the livers, kidneys and hearts were removed and fixed in 10% buffered formalin for histological examination (11).

Results:

From table (1), it was observed that bottled and tap waters, the pH values ranged from (6.96-7.12) and (7-7.8) in respectively, while in water from household RO systems the pH values ranged between (6.47-7.25). From the same table it can be seen the elevation of E.C. and TDS values in tap water, while these values were decreased in each of bottled and household RO water. There were significant variation in total hardness in water from household RO system and bottled water in which the values ranged from (24-78) and (16-88) in respectively in compare to tap water which read (440).

There was obvious depression in values of caliciume and maginisme ions and chlorides in both bottled and RO waters in compare to tap water and MPL. Also the results revealed the absence of ferric ions in both tap and bottled waters, while there was trace amounts of these ions in water from household RO systems in compare MPL value.

Table (1) : Physical and Chemical analysis for different types of water

<table>
<thead>
<tr>
<th>Physica&amp; chemical parameters in mg/L</th>
<th>MPL</th>
<th>Tap water</th>
<th>Bottled waters</th>
<th>Household RO waters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample 1</td>
<td>Sample 2</td>
<td>Sample 3</td>
</tr>
<tr>
<td>pH</td>
<td>6.5 - 8.5</td>
<td>7.83</td>
<td>7.1</td>
<td>6.96</td>
</tr>
<tr>
<td>E.C.(μs/cm)</td>
<td>200 0</td>
<td>1513</td>
<td>247</td>
<td>181</td>
</tr>
<tr>
<td>TDS</td>
<td>100 0</td>
<td>774</td>
<td>122</td>
<td>89</td>
</tr>
<tr>
<td>Total hardness</td>
<td>500</td>
<td>440</td>
<td>78</td>
<td>46</td>
</tr>
<tr>
<td>Ca</td>
<td>150</td>
<td>94.4</td>
<td>16.8</td>
<td>0.8</td>
</tr>
<tr>
<td>Mg</td>
<td>50</td>
<td>49.7</td>
<td>8.7</td>
<td>10.7</td>
</tr>
<tr>
<td>Fe</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chlorides</td>
<td>250</td>
<td>218</td>
<td>51</td>
<td>47</td>
</tr>
</tbody>
</table>

In bottled water samples there was elevation in total count of aerobic bacteria and Ps.aeruginosa during storage for three months. There were no growth of E.coli and E.faecalis in these samples. (table 2)

The results demonstrated that the total count of aerobic bacteria and the number of Ps.aeruginosa in tap water are larger than in household RO water. Also E.coli and E.faecalis absent from them. (table 3)
Table (2): Bacterial composition of thirteen samples of bottled water that were stored for three months

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Bottled Water</th>
<th>Storage for 1 month</th>
<th>Storage for 2 months</th>
<th>Storage for 3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total counts</td>
<td>0 – 10^{-2}</td>
<td>10^{-2} - 10^{-3}</td>
<td>0 – 10^{-3}</td>
<td>10^{-3} - 10^{-5}</td>
</tr>
<tr>
<td>E. coli</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>0 – 10^{-1}</td>
<td>10^{-1} - 10^{-2}</td>
<td>0 -10^{-2}</td>
<td>10^{-3} - 10^{-4}</td>
</tr>
</tbody>
</table>

Table (3): Bacterial composition of water from household RO system in compare with tap water

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Tap water</th>
<th>RO water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total counts</td>
<td>0 – 10^{-2}</td>
<td>10^{-1} - 10^{-3}</td>
</tr>
<tr>
<td>E. coli</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>E. faecalis</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Ps. aeruginosa</td>
<td>10^{-1} - 10^{-3}</td>
<td>0 – 10^{-2}</td>
</tr>
</tbody>
</table>

Regarding histological examination, it was observed that liver, kidney and heart affected by drinking RO water for long period and its effectiveness ranged from mild to severe chronic disease. The changes represented by cytoplasmic vacuole and cellular swelling in liver and congestion with tubular cell degeneration in kidney after second and forth week of drinking water from household RO system. (fig.1)

Fig.(1): cytoplasmic vacuole and cellular swelling in liver and congestion with tubular cell degeneration in kidney after the 2nd and 4th week of drinking water from household RO system. A- cytoplasmic vacuole and cellular swelling in liver B- congestion with tubular cell degeneration in kidney: 100x(Hematoxylin and eosin stain)
Marked tubular necrosis and hemorrhage in kidney and perivascular lymphatic infiltration and degenerative changes in liver were observed after the sixth and eighth week of drinking water from household RO system. (fig.2).

Fig. (2): tubular necrosis and hemorrhage in kidney and perivascular lymphatic infiltration and degenerative changes in liver after the 6th and 8th week of drinking water from household RO system. A- perivascular lymphatic infiltration and degeneration in liver B- tubular necrosis and hemorrhage in kidney : 100x(Hematoxylin and eosin stain)

After the last week (eighth week) of drinking the same type of sample, the heart appeared large, white and hard, that means the animal suffer from chronic vascular disease represented by deposition of adipose tissue in the heart. (fig.3)

There were no changes occur in tissue of control animals that drank tap water.

Fig. (3): chronic vascular disease represented by deposition of adipose tissue in heart after the 8th week of drinking water from household RO system : 100x(Hematoxylin and eosin stain)
Discussion:
From table (1) it was observed that tap and bottled waters were with neutral pH while water from household RO systems was slightly acidic, this may be due to bottled water in some cases made from city water system processed and packaged in plastic bottles that can block carbon dioxide (CO2) penetration, while water from household RO systems sits exposed to open air, it absorbs CO2, which dissolve in water forming carbonic acid, making it acidic (12,13).

\[
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^- 
\]

The same table reveals depression in the values of each of (E.C., TDS, TH, Ca, Mg and chlorides) in both commercially bottled and household RO waters, because these types of water were processed by reverse osmosis process in which water is forced through a semi permeable membrane removing a large amount of minerals, TDS and other impurities (8). The variation in the values of these parameters within each type of these waters, belong to that water composition depends on their sources and how it was processed. Also RO systems vary in their effectiveness depends on the quality of the filters and the frequency of filter cleaning or replacement (14). The appearance of trace amounts of iron ions in samples from household RO systems related to decrease in pH values in these samples that made it acidic waters which cause oxidation to water pipes (15). The elevation of these values in tap water may be due to no reverse osmosis process in the treatment of tap water in compare to bottled and reverse osmosis waters which are considered as purified waters (16). From table (2) it was observed that bottled waters contain few numbers of bacteria either naturally present or produced during processing, because filters were not changed or cleaned in frequently and the storage for months at room temperature promote bacterial growth which form biofilm inside the bottle and multiply there. This may be due to the absent of chlorine or to the ozone level that its half-life affected by time and temperature (17,18). So bottled water should not be stored for weeks or months at or above room temperature, which promotes the bacterial growth and the leaching of components from plastic bottles into the water, some of them may be carcinogenic (19).

In comparison between household RO water with its source tap water, the results demonstrated that tap water was not free from bacteria which collect inside water pipes during rotation forming sticky layer through which water flows (20). While the former type of water becomes contaminated as a result of overtime clogs of membrane pores, salts and bacteria accumulate on membrane surface causing contamination which vary in its adversity depending on quality and maintenance of filters in RO system (21). Versus if water disinfected with high chlorine, it will kill useful bacteria and react with the organic materials in intestines and form THMs in the body (22).

All types of water that have undergone the study were free from *E.coli* and *E.faecalis* and the presence of *Ps.aeruginosa* in fresh water not considered to be contaminants (19).

The pathogenic effects of drinking water with acidic pH and deficient in minerals were demonstrated in (fig.1,2,3). These results may be due to when more and longer purified water an individual drinks lead to development of minerals deficiency and acid state (23). The acidity like rust corrode tissue, veins and arteries and cause degenerative disease. Also over acidity leading to heart attack reduce oxygen transport and blood supply to the heart tissue and body create cholesterol (fatty
deposit) (13). In addition to, if there were no minerals in drinking water, the body will rob minerals from somewhere in it to satisfy its need (23). Mg deficiency accelerates the induction of thrombocyte aggregation, therefore it is described as risk factor for acute myocardial infarction (24,25).

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