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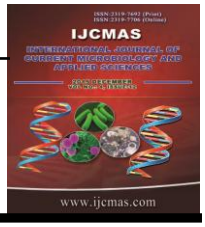

Original Research Article

Bacteriological Evaluation of Tap Water and Bottled Mineral Water in Taif, Western Saudi Arabia

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Keywords

Chemical parameters, Water quality, Tap water, Bottled water, Coliforms, Fecal indicator bacteria, *Streptococcus* pp, *P.aeruginosa*, 16S-rRNA

A B S T R A C T

In this study, chemical and bacteriological characteristics of drinking bottled and tap water were comparatively studied. Total 103 bottled water representing 17 brands and 21 tap water samples were collected from different locations in and around Taif city. Acidity degree (pH), electrical conductivity (EC) and total dissolved solids (TDS) were analyzed for different water samples. Total coliforms, fecal coliforms, *E. coli*, fecal streptococci, *P.aeruginosa* and heterotrophic plate count (HPC) were detected. The results indicated that pH, EC and TDS of bottled water were highest in (B2 and B11), (B1 and B16) and B16, respectively. However, pH, EC and TDS were variable in tap water. Total coliform and fecal coliform were equal inboth tap water 9.5% and in bottled water 2.9%, respectively. No *E. coli* was observed in any samples of tap or bottled water. No fecal streptococci were observed intap water but 0.97 % only in bottled water. HPC represent 23.8% in tap water and 1.9% only in bottled water. *Pseudomonas* spp. was higher in tap water (14.3%) than bottled water (1.9%). *Pseudomonas* strains were detected in two brands of the bottled water samples. Total aerobic microbial count (HPC) was higher in tap water 23.8% than bottled water 1.9%. According to morphological, physiological characteristics, APi Profiling and sequencing of 16S-rRNA gene, the selected isolates were identified as *Entrobacteraerogenes, S. pneumoniae, Acinetobacter calcoaceticus, S. pyogenes, K. pneumonia, P. aerogenosa*. Generally, the water quality depends on its chemical and microbiological condition. Our results suggest that microbiological quality of bottled waters sold in Taif, Saudi Arabia is highly variable. To protect public health, stringent quality control is recommended for the bottled water industry.

Introduction

Water plays a significant role in maintaining the human health and welfare. Clean drinking water is now recognized as a fundamental right of human beings. The transmission of waterborne diseases is still a matter of major concern, despite worldwide

efforts and modern technology being utilized for the production of safe drinking water (Venter, 2000). Studies showed that approximately 3.1% of deaths (1.7 million) and 3.7% of disability-adjusted-life-years (WHO 2004) (54.2 million) worldwide are attributable to unsafe water, poor sanitation and hygiene (WHO 2004).

Drinking water is important for survival, and biological and chemical contamination is a serious matter that may have serious health effects. There is a worldwide concern over the quality of tap water, due to pollution, bacterial contamination, and the associated taste and odor (Saleh*et al*, 2001, Ikem*et al*. 2002); therefore, people are turning to bottled water for safety and quality characteristics (Mahajan*et al*. 2006). As a result, the global market for bottled water is huge and is growing continuously to meet the increase in demand and the search for good quality drinking water(Mahajan*et al*. 2006, Guler, 2007).

Water is indispensible to life as it is required for all physiological processes which demand that all living organisms have ready access to water. This need is no less important to human beings who have to drink plenty of water every day (Nester *et al*. 2004). Apart from drinking, man uses water for many domestic, industrial and recreational purposes which include washing, bathing, cooking, food processing, brewing and beverage bottling as well as sporting activities. This means that there is a need for the constant supply of potable water to all human communities and in areas where such supplies are lacking, a great deal of time and effort are devoted to finding a suitable source of supply. Unfortunately, such water sources even when they are available, are seldom safe or reliable and waters obtained there from need to be treated appropriately in order to make them potable.

Local bottled water consumption increased during the summer, in particular during Umrah and Haj seasons in Saudi Arabia. The Kingdom is the largest consumer of desalinated water worldwide, with sales increasing during the summer, Umrah and Haj seasons due to the hot weather. The abundant supply of bottled water and competition in the industry benefits consumers in the end, as companies work to reduce their prices in order to win the largest number of customers. This increase in consumption of bottled water could be attributed to the growth in the population, as well as increased awareness of the importance of drinking clean and safe water (Bin-Zouma 2015). Bin-Zouma (2015) pointed to the significant increase, more than 6.5 billion liters per year, in the production capacity of local factories. The Saudi Food and Drugs Authority (SFDA) estimates 45 companies are working in the field of bottled water, with investments estimated at SR 8 billion. It is predicted that the number is much higher and likely to grow even more in the future.

Bottled water consumption has significantly increased during the last decade. The European Federation of Bottled Water (EFBW, 2006) estimates the consumption of bottled water in the European Union during 2003 as 45,000 ml and the consumption in Greece as 60 L per capita. The water-sales worldwide exceed a value of 5 billion euros (Rosenberg, 2003). Non-carbonated bottled water has become more popular than carbonated, being a substitute for tap water in many homes (Armas& Sutherland, 1999). A number of reasons has been reported: consumer awareness about increasing water pollution; deficiencies in municipal water supply in terms of odor, taste, fluoride, chlorine (Tamagnini and Gonzalez, 1997), as well as successful marketing strategies of the bottling mineral water companies (Misund *et al*. 1999).

Saudi Food and Drug Authority (SFDA) has recently collected samples of various sizes of potable drinking water, locally produced from two different factories in Qassim and Tabuk, from different production dates and batches, in order to verify their conformity with GCC Standard Specifications, setting the maximum allowed percentage of Bromates at (10 ppb). Laboratory analysis revealed that they exceeded the allowed level of Bromate (SFDA, 2015). Therefore, SFDA advises consumers not to use this water and dispose of any stock they may possess of this brand. However, SFDA has addressed the concerned authorities to take the necessary action to force the said factory to recall their products from the local market and stop production of the said water until they comply with the above GCC Standard Specifications for Bromate.

Apart from microbiological considerations, the upsurge in the demand for bottled water has prompted the interest of many manufacturers in the production of bottled water. Two decades ago, bottled water was a product of a few multinational and large scale food processing and beverage producing companies in Saudi Arabia. Presently however, there is the involvement of very many water bottling companies ranging from large scale multinational companies to medium scale business enterprises, institutional and government business investment companies as well as small scale entrepreneurs (Manufacturing Today 2011). These water bottling companies use various water purification methods which may be one of or a combination of two of filtration, ionization, ultra violet irradiation and chlorination.

Bottled mineral water has been reported to be associated with outbreaks of infections in the last few years in some countries e.g. Nigeria. In 2006, *Salmonella entericaserovar Kottbus* from bottled water was significantly associated with 41 cases in an outbreak in infants in Nigeria. The bacteia was isolated from bottled mineral water randomly selected from the markets and in the local factory where the water was bottled (Palmera-Suarez *et al*. 2007). Eckmanns *et al*. (2008) have described an outbreak of hospital-acquired *P. aeruginosa* infection caused by contaminated bottled mineral water in intensive care units in a hospital in Germany (Eckmanns *et al*. 2008). Studies on the quality of bottled mineral water in many parts of the world including Canada, South Africa, Iran, Egypt and Nigeria have shown that bottled water samples are not always of the required microbiological quality (Martins 2011, Semerjian2011, Varga 2011).

Microbial surveys carried out worldwide indicated various problems with bottled water such as: high Heterotrophic Plate Count (HPC) levels (Warburton, 2000). Therefore, the bottled water industry has to exhibit strict quality standards in terms of microbial parameters, production processing, bottling, transportation and storage (Cowman and Kelsey, 1992 and Hunter, 1993), while Hazard Analysis Critical Control Points (HACCP) systems should be implemented in the bottling process (Venieri et al., 2006).

Bottled water microbiological quality parameters are clearly defined by Saudi food and Drug authority (SFDA) according to which: "water should be free from parasites and pathogenic organisms, total coliforms, *Escherichia coli*, *Enterococcus* spp., *P. aeruginosa* should not be detectable in any 250 ml bottled water sample analyzed, while HPC, at 22 ℃, and 37 ℃, should not exceed 100/ml, and 20/ml CFU, respectively" (SFDA 2015). The 37 ℃HPC can provide an indication of fast growing bacteria,

related to pathogenic types and the 22°C HPC an indication of characteristic bacteria that develop slowly (Ramalho *et al*. 2001).

Water quality is often related to the degree of bacterial contamination. Drinking water distribution systems are colonized by saprophytic heterotrophic microorganisms that grow on biodegradable organic matter (Servais *et al*. 1992). Potentially pathogenic microorganisms (e.g., *Pseudomonas aeruginosa*) and microorganisms of fecal origin (e.g., *Escherichia coli*) may also find favorable conditions and proliferate in these systems. The quantity of bacteria in commercial mineral water is generally dependent on the disinfecting process of natural spring-water use at the factory. It is well known that natural mineral water is characterized by its bacterial flora, chemical and physical composition. The quantity of flora of spring water is usually high. Spring water contains a natural microbial flora composed mainly of species of the genera *Alcaligenes*, *Achromobacter*, *Cytophaga*, *Pseudomonas*, *Flavobacterium*, *Moraxella* and *Acinetobacter*. If these bacteria are not adequately removed during processing and bottling, bacterial multiplication may occur for 1–2 weeks after bottling, and the bacterial count can reach 10^3–10^4 bacteria mL^{-1} at 37 °C (Tamagnini and Gonzalez, 1997). In addition to natural contamination, the product can also spoilage before it reaches the consumer.

The use of PCR, over the last years, rapid purification, and automated DNA sequencing has significantly reduced the time to yield a high-quality sequence. The use of 16S- rRNA gene sequencing to study the relatedness of prokaryotic species is well established and has led to increased availability of 16S- rRNA databases. The convergence of these technical and computational advances has also enhanced the application of 16S- rRNA gene sequence analysis to bacterial identification (Rantakokko-Jalava *et al.*, 2000). Recently, it was reported that subtle sequence differences in the 16S rRNA gene could be used for bacteria identification (Sacchi *et al.*, 2002) and for subtyping and identifying bacterial clones (Nilsson *et al.*, 2003).

The aims of this study were (i) to compare quality of tap and bottled water brands produced by different classes of water bottling companies in south-western Saudi Arabia (ii) to investigate the prevalence and numbers of fecal indicators (*E. coli* and enterococci) in water samples and (iii) to characterize isolated bacteria using morphological and biochemical methods as well as 16S-rRNA gene method.

Materials and Methods

Study Area and Sample Collection

In kingdom of Saudi Arabia, different sites locatedin Taif State and its surroundings, were selected.

Collection of Samples

A total of 103 bottled mineral water samples representing 17 brands of bottled water and 21 tap water samples were collected on different occasions within a period of 14 months were tested for bacteriological quality. The bottled water samples were inspected and ascertained to be in good condition with the caps and protective seal intact before purchase. The dates of production as well as the batch numbers were documented. Tap water samples were collected in sterile 500 mL glass bottles. They were taken to the laboratory and analyzed for total bacterial load and the presence of bacterial indicators of drinking water quality. The sampling was collected

bi-weekly in the period between January 2013 and September 2014to include representative samples of each brand of bottled water available for sale in city. The bottled water brands were coded B1- B17 for the purpose of this study and also categorized into two groups based on the size or capacity as shown in Table1.Tap water samples were coded T1-T21. Samples were stored at 4°C till further investigation and analysis of water-quality parameters was carried out as per standard methods of Martel et al (2006), APHA (2012), IBWA (2012). Inoculations into selective media were conducted within 24 h after collection of the water samples. Collected samples were transferred in an ice box to Microbiology Laboratory in Scientific Research Center, Taif University, Saudi Arabia.

Sample Examination

Samples were examined individually. Subjected to Chemical, and bacteriological examination.The procedure for analysis was followed as per standard methods of analysis of water and wastewater (IBWA, 2012).

Chemical parameters

pH and Conductivity (EC)

The pH is measured by Jenway, 3505 pH meter and EC byS700 Seven Excellence™. Mettler Toledo's wide range of TDS/conductivity meters, which gives direct value of pH and EC according to the American Public Health Organization (APHA, 2012) instructions.

Total Dissolved Solids (TDS)

Total dissolved solids is determined by TDSB enchtop Meters **(**S700 Seven Excellence™ Conductivity)**.** The premium and routine level bench top instruments allow for electrical conductivity(µS/cm, mS/cm) and TDS (mg/L) measurements. Mettler Toledo's wide range of TDS/conductivity meters according to (PME, 2015), Saudi Arabia instructions.

Microbiological Parameters

Total heterotrophicplate count (HPC), most probable number (MPN) of coliform bacteria and IMViC test for differentiation in coliform bacteria were also determined in collected 21 tap water samples and 17 bottled mineral water samples.

Determination of Total Heterotrophic Bacteria

The enumeration of heterotrophic bacterial count was carried out using both the serial dilution and the pour plate technique (HPC). Serial 10 fold dilutions in sterile water were carried out and 1 ml of each dilution was aseptically placed in sterile petri-dishes in triplicates. 20 ml of molten plate count agar (Oxoid) cooled to 45°C was then added to each of the plates and mixed thoroughly. The mixture was allowed to solidify and the plates incubated at 22°C and 37°C for 24–72 hours. The number of bacterial colonies were counted and reported as colony-forming units permillilitre. The heterotrophic plate count (HPC) was determined by the pour plate technique as described by the standard methods (APHA, 2005).

Detection of Coliforms and *E. coli*

The examination of the water samples using standard coliform Multiple- tube (MPN) fermentation techniques (Clesceri *et al*., 1998). The isolation of Fecal *E. coli* was achieved in water samples by monitoring the acidification and gas production during growth in MacConky broth (Oxoid, UK) at

44±0.5°C for 24±3 h. From the fermentation tube, further recovered on eosin methylene blue (EMB) agar (Scharlau, Spain, EU) as metallic sheen.

The identification of *E. coli* was confirmed by performing API identification strips (bioMerieux-France). Bacterial glycerol stocks were prepared using two aliquots of the bacterial suspension (0.5 mL) diluted with 0.5 mL of 2X tryptic soy broth (TSB - Oxoid) containing 20% glycerol and stored at-70ºC.

Detection and Enumeration of *P.aeruginosa*

The presence of *P. aeruginosa* was determined by plating 0.1 ml of serially diluted samples onto MacConkey agar, with incubation for 24 h at 37°C. Identification of *P. aeruginosa* was done using standard methods (Api, 2009). All other growths on MacConkey agar were also subjected to identification.

Detection and Enumeration of Fecal Streptocccci

The presence of other indicator organisms which include Streptocccci was determined by filtering 100 ml volumes of each sample (in triplicate) as described above. The filter membranes were placed on nutrient agar plates and incubated at 37°C for 24–48 hours. Colonies arising after incubation were observed and streaked onto fresh agar plates.

Isolation of Bacteria

A 500 ml water sample was concentrated by filtration on 0.25µmcellulose nitrate membrane filters (Sartorius AG, G¨ottingen, Germany) (Thomson et al. 2008). The filters were macerated in 3ml sterile distilled water. From the 3 ml of supernatant, 0.1ml aliquotswere then transferred in triplicate to nutrient agar plates. The biofilm swabs were directly spread on the same medium.

Identification of Bacteria

The morphological characteristics of the isolates were identified by gram stain and biochemical reactions. The biochemical reactions include glucose fermentation, oxidase test, catalase production reaction, cell motility and reaction in tryptose soya broth were performed. According to Bergey's Manual of Determinative Bacteriology (Holt *et al.*, 1994). Particular isolates were re-identified using API 20E strips (API 2009, bioMerieux, Inc., France,) isolates were isolated, identified and named based on morphological, physiological, biochemical characteristics.Also, 16S-rRNA sequencing techniques were adopted to characterize and identify the selected isolates at molecular level.

Maintenance of Strain

All pure isolated colonies were sub-cultured onto blood agar plates (for growth of heterotrophic bacteria) and MacConkey agar plates (for coliforms) for 24 hrs at 37^{o}C for colony isolation and morphological identification. Distinct colonies of each isolate from the samples were stored in nutrient agar at 4°C and cryo-preserved in glycerol-nutrient broth in a deepfreezer.

Molecular Genetics Analysis

DNA Extraction

The genomic DNA of bacterial isolates was extracted using the CTAB-method described by Ausubel *et al.* (1992).

PCR Amplification of 16S-rRNA Gene

Primer sequences used to amplify the 16S-rRNA gene fragment were: U1 [5CCA GCA

GCC GCG GTA ATA CG3] and U2 [5ATC GG(C/T)TAC CTT GTT ACG ACT TC3] according to Kumara et al. (2006). The PCR master mix contained 10 *P*mol of each primer and 12.5 µl of 2xSuperHot PCR Master Mix (Bioron, Ludwigshafen, Germany) mixed with 50 to 100 ng of DNA template. Sterile d.H_2O was added to a final volume of 25 µl. Thermal cycler (Uno II, Biometra, Germany) program was 94 °C for 4 min., 94 °C for 1 min., 55 °C for 1 min., 72 °C for 1.5 min, the number of cycles was 35 cycle and the post PCR reaction time was 72°C for 5 min.

Analysis of the PCR Products

After the amplification, the PCR reaction products were electrophoresed with 100 bp ladder marker (Fermentas, Germany) on 10 x 14 cm 1.5% -agarose gel (Bioshop;Canada) for 30 min using Tris-borate- EDTA Buffer. The gels were stained with 0.5ug/ml of ethidium bromide (Bioshop; Canada), visualized under the UV light and documented using a GeneSnap 4.00- Gene Genius Bio Imaging System (Syngene; Frederick, Maryland, USA).

DNA Sequencing

The PCR-products of each isolate were purified from excess primers and nucleotides by the use of AxyPrep PCR Clean-up kit (AXYGEN Biosciences, Union City, California, USA) and directly sequenced using the same primers as described for the amplification process. The products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (ABI Applied Biosystems, Foster City, California, USA) on a 3130XL Genetic Analyzer (Applied Biosystems, UK). These fragments were sequenced in both directions. The bacterial 16S- rDNA sequences obtained were then aligned with known 16S-rDNA sequences in Genbank using the basic local alignment search tool (BLAST) at the National Center for Biotechnology Information, and percent homology scores were generated to identify bacteria.

Data Analysis

Data was presented as average of replicates. The data was subjected to statistical analysis by conducting analysis of variance (ANOVA), using SPSS software package (version 10.0). The significant differences of their means were compared using least significance difference (LSD) tests and p value was considered significant at 95%.

Results and Discussion

Analysis of pH, EC and TDS

Acidity Degree (pH)

pH is classed as one of the most significant water quality parameters. Measurement of pH relates to the acidity or alkalinity of the water. A sample is considered to be acidic if the pH is below 7.0. Meanwhile, it is alkaline if the pH is higher than 7.0. Acidic water can lead to corrosion of metal pipes and plumping system. Meanwhile, alkaline water shows disinfection in water. The normal drinking water pH range mentioned in WHO guidelines is between 6.5 and 8.5 (Table 2). pH is affected not only by the reaction of carbon dioxide but also by organic and inorganic solutes present in water (Table 1). All the samples were found to be clear in visibility and acceptable in taste. The pH of all samples (bottled, and tap water) was around neutral and within the recommended maximum concentration limit guidelines by WHO, HACCP and SFDA. pH in tap water samples in range 7.09-7.99 except two samples T12 and T15 (data no shown). Average of pH was found to be in

the order of tap water > bottled water. A change in water pH is accompanied by the change in other physical and chemical parameters (Shrivastava et al. 2013). pH value is one of the most important attributes of any aquatic system since all the biochemical reactions depend on pH of the water. It was concluded that the pH of water were slightly alkaline (7.5 - 8.0) and were within the maximum limit set for domestic use as per HACCP (2012). Both low and high pH is problem because water with low pH can be acidic which can damage metal pipes and water with high pH signifies high level of alkalinity (Bujaret al. 2013). Higher pH prevents the corrosion and contamination from pipes (Ahmad and Bajahlan, 2009).

High value of pH of liquids may results due to waste discharge, microbial decomposition of organic matter in the water body (Raj 2008, Patilet al. 2012). The high pH in this case may be attributed to organic and inorganic solutes present in water. For B11 bottled water sample, the measured pH is 7.44 which are almost the same as the pH stated by the manufacturer on the labeled of the container, that is, 7.34. This indicates that the manufacturer did not provide any inaccurate information on the label.

Electrical Conductivity (EC)

Conductivity is a measure of water capability to transmit electric current and also it is a tool to assess the purity of water (Table 1). Conductivity of bottled water found in the range 234-330µS/cm and in range 175-413 µS/cm for tap water. Conductivity in tap water samples were not shown. One of the reason of salinity is the high concentration of captions and anions (Raja 2008, Chauhan *et al* 2012).The presence of dissolved solids such as calcium, chloride, and magnesium in water samples carries the electric current through water. The measured conductivity values of all the bottled drinking water samples are illustrated in Table (1). According to WHO(2011) and SASO (1984)., the maximum allowable level of conductivity is 1000 µS/cm. The results show that the measured conductivity of all water samples ranges from 244 µS/cm to 330 µS/cm, and the average conductivity value of all bottled water brands is 260.12 µS/cm (Table 1). The lowest and highest conductivity values correspond to Brand code B17 and B1, B16 samples, respectively. This can be explained as the reverse osmosis treatment technique is used to remove dissolved solids, turbidity, colloidal matters, and others, and thus it gives lowest conductivity value. Similarly, it is expected to find high mineral contents in bottled water, which resulted in higher conductivity value (Table 1).

Moreover, according to Azrina*et al*. (2011), the wide differences among the values of the conductivity of tap water are not yet known. Scatena (2000) explained the differences based on various factors such as agricultural and industrial activities and land use, which affect the mineral contents and thus the conductivity of the water. Conductivity does not have direct impact on human health. High conductivity may lead to lowering the aesthetic value of the water by giving mineral taste to the water. For the industrial and agricultural activity, conductivity of water is critical to monitor. Water with high conductivity may cause corrosion of metal surface of equipment such as boiler. It is also applicable to home appliances such as water heater system and faucets. Food-plant and habitat-forming plant species are also eliminated by excessive conductivity (Jia et al. 2010, Heydari and Bidgoli , 2012, Katsoyiannis and Zouboulis, 2013, Tuzen and Soylak, 2006).

Total Dissolved Solids (TDS)

The value of TDS were observed minimum 100 mg/L of brand code B1 andmaximum173 mg/Lin brand code B16.The most remarkable observation of investigation was the alarmingly high level of total dissolved solids (Table 1). The TDS of all the bottled water samples were in range of 100-173 mg/L while the maximum permissible limiting value of TDS for potable wateris500 mg/L according to WHO and EPA. TDS for tap water samples were in range of 120-352 mg/L (data not shown). The lowest value in T2 and the highest in T16 sample. High level of TDS in water used for drinking purposes leads to many diseases which are not water-borne but due to excess salts. The US Environmental Protection Agency (EPA) presents a conceptual approach for developing a national estimate of endemic acute gastrointestinal illness(Martel et al. 2006) due to drinking water and a national estimate analysis developed through a model application(Colford 2006). Most of the analyzed parameters in different brands of bottled water were found to be in concentrations far below the recommended maximum concentration limit guideline by WHO (2011) and SASO (1984).

Microbiological Quality of Tap Water and bottled Mineral Water

The bacteriological analysis performed on samples of tap water from municipal water supplies and samples of bottles water appear in Table 3. The number of samples with HPC were 5 (23.8%) for tap water and 2 (1.9%) bottled mineral water, respectively. Total coliforms (TC) bacteria were detected in 2 out of 21 (9.5%), and 3 out of 103 (2.9%) of the tap water, and bottles of mineral water, respectively. Two (9.5%) of the tap water and 3(2.9%) of bottled water samples were positive for fecal coliform (FC). Neither tap water samples nor bottled water samples were contaminated with *Escherichia coli*. Fecal streptococci (FS) were not detected on the EIA plates while detected in 1 out of 103 (0.97%) of the bottled water, respectively. According to WHO (2011), the term ‘‘fecal streptococci’’ refers to those streptococci generally present in the feces of humans and animals. Their primary value in water quality examination is therefore as additional indicators of treatment efficiency. *P.aeruginosa* contamination was evident in 3 (14.3%) of the tap water samples and 2 (1.9%) of bottled water samples. Considering that HPC is an indicator of hygienic condition and that disinfection does not completely eliminate these bacteria, different ranges of total bacterial densities were established.

On the basis of results obtained with a sampling of initial streams of water, the bacteriological quality of municipal tap water is almost similar to the quality of bottled water. Of the 124 samples examined, 23 (18.5%) were contaminated by at least one coliform or indicator bacterium and/or at least one pathogenic bacterium, including 12 (57.1%) of the 21 tap water samples from municipal supplies, and 11 (10.7%) of the 103 bottled water. No *Clostridium* was found in any of the samples (data not shown). Results are in agreement with data of various researchers in different parts of the world (Bartram et al. 2003,Mena and Gerba 2009, Hunter 1993, Marzano 2011).

Morphological and Biochemical Characterization of isolates

Isolates from household tap water and bottled mineral water were isolated by enrichment technique and deposited in our microbial bank at Taif University, Saudi Arabia in our laboratory. The isolates were identified on the basis of their cultural and biochemical characteristics according to

Bergey's Manual of Determinative Bacteriology (9th edition) (Holt et al., 1994) and Api kit profiles (ApiBioMerieuxsa, 2009). Phenotypic examination of the recovered microorganisms revealed that they belong to the genera of *Acintobacter*, *Streptococcus*, *Klebseilla*, *Enterobacter*, and *Pseudomonas* (Table 3). A total of 33 isolates were isolated 22 from tap water and 11 from bottled mineral water (Table 5). All selected strains showed optimal growth at 30°C. Strains were local isolates isolated by enrichment technique and deposited in our microbial bank at Taif University, Saudi Arabia in our laboratory. These microorganisms were isolated from tap and bottled water by various investigators in different countries (Magda *et al*. 2008, Gholam*et al.* 2010, Onweluzo*et al*. 2010)

Table.1 Brand Codes, Capacity, Number of Samples, pH,TDS and EC Properties of Bottled Mineral Waters

Tested Brand Code	Capacity (liter)	No. Samples	pH*	TDS* (mg/L)	EC µS/cm
B1	0.66	8	7-7.5	100-156	330
B2	0.33	6	7-7.6	121-142	270
B3	0.33	5	7.2	114	253
B4	0.60	8	7.1-7.3	112	250
B5	0.33	12	7.0-7.2	111	245
B6	0.6	5	7.2	109	240
B7	0.6	7	7.2	126-133	260
B8	0.33	5	7.1	113-130	245
B9	0.33	4	7.2	110	244
B10	0.33	4	7.2	119	248
B11	0.33	5	7.4-7.6	121	271
B12	0.66	6	7.0	119	247
B13	0.33	5	7.1	112	249
B14	0.33	4	7.1	124	261
B15	0.66	6	7.2	110	245
B16	0.33	5	7.1-7.5	155-173	330
B17	0.66	8	7.0-7.1	111	234
Total		103			

*, pH= 6.5- 8; TDS= 1500mg/L; EC = nd, (PME, Saudi Arabia)

Table.2 Limits of Bottled Water and Tap Water in Saudi Arabia and its Comparison with WHO and SASO Guidelines for Drinking Water

Parameter	Water brands	Tap water*	SASO	IBWA	WHO
pH	7.0-8.0	7.3-8.4	6.5- 8.5	6.5-8.5	6.5-8.5
TDS (mg/L)	100-173	120-352	100-500	500	500
EC (µS/cm)	175-860	-	-	-	-

*, average of 21 tap water samples; SASO, Saudi Arabian Standards Organization; IBWA, International Bottled Water Association; WHO, World Health Organization

Table.3.Microbiological Quality of Tap Water and Bottled Mineral Water

Indicator bacteria or pathogen	No. of positive samples (%)	
	Tap water (21)	Bottled water (103)
HPC (CFUmL-1)	5(23.8)	2(1.9)
Total coliform	2(9.5)	3(2.9)
Fecal coliform	2(9.5)	3(2.9)
E. coli	0 (0.0)	0 (0.0)
Fecal streptococci	0 (0.0)	1(0.97)
P. aeroginosa	3(14.3)	2(1.9)

Table.4 Microbiological Limits* of Bottled Water and Tap Water in Saudi Arabia**

Characteristic	Tap water	Bottled water
Heterotrophic plate count (HPC)**	500	500
Total coliforms	0	0
Fecal coliforms	0	0
Escherichia coli	0	0
Staphylococcus spp.	–	–
Pseudomonas aeruginosa	–	0
Fecal streptococci	–	0
Sulfite-reducing clostridia	–	0

Not determined; *, Colony-forming units (CFU) per 100 mL;
**, Colony-forming units (CFU) per mL (PME, KSA)

Table.5 Prevalence of Bacterial Isolates in Household Tap Water and Bottled Mineral Water

Isolates	Tap water	Bottled water	Total
Entrobacter spp.	7	2	9
Streptococcus spp.	-	3	3
K. pneumoniae	3	1	4
Acinetobacter spp.	2	-	2
S. pneumoniae	3	2	5
P. aerogenosa	7	3	10
Total	22	11	33

Molecular Characterization and Genetic Identification of Isolates

Sequencing of 16S- rRNA gene as a PCR based technique was used to identify the selected bacterial isolates. According to the alignment at the National Center for Biotechnology Information (NCBI), the sequences of studied isolates in (Table 5) were identified as *Entrobacter aerogenes, S. pneumoniae, Acinetobacter calcoaceticus, S. pyogenes, K. pneumonia, P. aerogenosa* (DeSantis *et al*, 2006). Many researchers employed 16S-rRNA biotechnology to

identify isolated bacterial species from water and other environments (Qualls *et al.* 1983, Szewzyk *et al.* 2000, Shahaby *et al*. 2013, 2014, 2015).

In conclusion, bottled water is an important source of drinking water in the KSA, which has limited resources of fresh water. Continuous monitoring of water quality and effectiveness of the treatment processes, and obeying regulations, are required to ensure that the water quality meets the set standards and to meet the increasing demand for good quality tap or bottled water. The study revealed that most of the chemical parameters were within World Health Organization limits (WHO) regardless of the brand name. The concern however was the high TDS and conductivity in some brands and tap water samples. No *E. coli* was detected throughout the study period. There is a need for a rigorous inspection and follow-up of water bottling facilities and municipal water so that only those companies which consistently produce water of acceptable bacteriological quality are allowed to produce water for public consumption. It is recommended that Saudi Food and Drugs Authority (SFDA) should promulgate standardized method of bottled water industry in order to increase its characters and shelf life. Also, the results of these analyses indicated the need to improve Hazard Analysis Critical Control Points (HACCP) systems, in order to continuously monitor the water supply source in bottling plants, and at supermarkets.

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