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Research Article

Microbial Quality Assessment of Packaged Natural Mineral Water in Lorestan Province, Southwest of Iran

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ABSTRACT
Natural mineral water which todays attract more trend rather that tap water in urban societies and has been appreciated for high level hygiene. This study considered microbiological quality of packaged natural mineral water marketed in Lorestan province where 35 samples were purchased randomly, were tested for presence of different indicator microorganisms. 31.42% out of 35 samples didn't fulfilled standard condition in terms of unacceptable total Coliforms count while E. coli, *Staphylococcal*, *Pseudomonas aeruginosa*, *Streptococcal*, Sulfite-reducing *Clostridia* counts weren't observed. Those results revealed the necessity of more control on treatment and filling process which might be the most probable steps for sanitation.

Keywords: Mineral water, Total Coliforms, E. coli, *Staphylococcal*, *Pseudomonas aeruginosa*, *Streptococcal*, Lorestan province, Iran.

INTRODUCTION

Todays, mineral water consumption instead tap water is more preferred in homes[1,2]. The growth of urbanization and industrialization[3], undesirable taste and odor caused by over use of chlorine and fluoride in municipal water supply[4] besides marketing strategies might be the most important reasons for emerging public trend for bottled water[5].

Although in public perception, bottled water consider as a hygiene drink, it can contain biological and chemical contamination[6]. Recently worldwide reports have indicated high load of microbial contamination of bottled water in terms of, total count of Heterotrophic bacteria, Coliforms, Pseudomonas sp., Vibrio cholera, *Staphylococcus aureus*, *Aeromonas sp.*, *Salmonella sp.* and fungi. The obtained results really challenge water industry to meet certain standard level in production process, storage and transportation[2]. The main sources of natural mineral water in Iran are provided by spring and ground water[7]. Based on European Community (EC) Directive of 1980, natural mineral water has also been defined as "microbiologically wholesome water, originating in an underground water table or deposit and emerging from a spring tapped at one or more natural or bore hole exits"[8] and a standard was legislated which emphasized that the supplied water should be free from parasites and pathogenic organisms, *Escherichia coli*, *Coliforms* and fecal *Streptococci* in any 250 ml sample at all steps from source to marketing[9]. Spring water naturally contain some bacterial flora and they can reach to irritant quantity because of inadequate removal process[10]. The initial quality of source water, type of manufacturing and hygienic location is the most important factor affecting overall quality of bottled water[11]. Some researches claimed that the number microbial load can even increase during 1-3 weeks of storage[5] while that might be intensified due to water agitation, more available surface area of bottle also providing trace element and favorable temperature of storage condition[12]. Considering that natural mineral water is not pasteurized and sterilized it couldn't be recommended for infant and vulnerable patient like immuno-compromised, kidney and urinary disorders as well[12].

Battled water can also causes the outbreaks of "traveller's disease", cholera and typhoid as it has been reported several times[13]. This study is going to evaluate the compliance of mineral water products with standard criteria in domestic market of Lorestan province. So, several water samples from different outlets in province were tested for microbial load counting based on International Standards Organization (INSO 5869) techniques.

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Table 1: The results of collected samples evolution based on microbial parameters.

Indicator microorganisms	Number of positive sample	Number of negative sample	Percentage of positive sample	Percentage of negative sample
Total Coliforms	11	24	31.42	68.58
Escherichia coli	0	35	0	100
Staphylococcus sp.	0	35	0	100
Pseudomonas aeruginosa	0	35	0	100
Streptococcus sp.	0	35	0	100
Sulfite-reducing clostridia	0	35	0	100

MATERIALS AND METHODS

Sampling

35 samples were collected from supermarkets and bottling factories during March to April 2014 while all samples were sealed in polyvinyl chloride (PVC) bottles and they may were stored for 1, 2, and 4 months. Samples were analyzed according to the Iran standard protocol that is authorized for microbial quality assessment of mineral water, ISO number 5869. Indeed, samples were chosen from different brands name and random places**.**

Indicator microorganisms enumeration

At first samples were shacked and blended. For Clostridium sp. isolation, sample was heated at 75˚C for 15 minutes. The presence of total *Coliforms, E. coli, Enterococcus sp*., *Clostridum sp.* and *Pseudomonas aeruginosa* was evaluated by filtering of 250 ml sample through nitrocellulose membranes (0.22 or 0.45 µm pore size) under aseptic condition then filter papers were located on the selective media. For Pseudomonas aeruginosa CN agar (or Cetrimide agar and MPC agar) were prepared and plates were incubated at 37 or 35˚C for 48 hours. Lactose TTC agar contain heptadecyl sulfate were used for total *Coliforms* and *E.coli* isolation followed by incubation at 36˚C for 21 hours. Slanetz and Bartley medium was used for *Enterococcus faecalis* by incubating at 36˚C for 44 hours. *Clostridium sp.* which are anaerobic microorganisms should be located at the bottom of plate and liquid cooled sulfite iron agar medium was added and incubated at 37˚C for 44 hours. Some cultures were maintained for 2 to 4 hours around 25˚C revitalizing for further main incubation at optimum condition **.**

Pseudomonas sp. colonies were counted under ultra violet emission. Yellow colonies which revealed lactose positive exhibition were counted and supposed as Coliforms. Colonies which exhibit red outstanding appearance were considered as intestinal *Enterococcus species*. Each group of microorganisms was cultured in enriched agar medium and colonies were used for confirmation test of oxidase, acetamide, TSA, Indol and sculin-azid. Enterococcus sp. isolates were confirmed by transferring the membranes onto Bile- Aesculin-Azide agar preheated at 44 -C. Enterococcus sp. hydrolyse aesculin on this medium in 2 hours**.**

RESULTS

Filtrations technique is a flexible qualification and quantification method for evaluation of water supplies[14]. The results which were observed in filtration technique are shown in Table 1. 11 samples out of 35 *Coliforms* contamination were detected while they were free from other indicator microorganisms. In other word 31.42% of natural mineral water products which are supplied in market were contaminated at least by one total *Coliforms* indicator bacterium. The comparison among presence of Coliforms and the absence of other indicator give a general perception of sanitary condition of products. It should be considered those total Coliforms which are associated with soil and human or animal waste contamination are different from specific definition of fecal coliforms **.**

DISCUSSION

Bacterial populations such as Coliforms are natural component of mineral water supplies and it has never been reported any public outbreaks of disease caused by natural mineral water consumption but the high level of Coliforms could be inferred as more risk of protozoan and viral contamination. These dangerous agents survive in water and can't be easily captured in sanitizing process[15]. Microbial contamination of bottled sample directly depends on natural water source, treatment process and sanitation equipment which is adapted by factories[16]. A large proportion of waterborne diseases are caused by groundwater contamination[13]. The rainy season and periodic flooding are the most probable time for rising of surface water pollutions and the zoonotic pathogen population usually is affected by wild life in the vicinity of water sources like animals and birds. The appearance of Coliforms mostly is attributed to inadequate and ineffective treatment process though, a group of researches admit that this method functionally remove pathogenic microorganism and don’t sterilize the products[17]. Therefore safe packaging and filling system seems the most vulnerable step of production needing more attention and control[18]. Heterotrophic plate counts (HPC) which is another main method for bacterial contamination evaluation has been adapted by many researches. In a similar study 75 samples of bottled mineral water were analyzed and 28% to 68% didn't comply HPC standards. Because of lacking the correlation between HPC and total coliform count results can’t be compaired. *Coliforms* excluding *E.coli* and *Pseudomonas aeruginosa* are indicator of surface water contamination although transportation of water from source to bottling plant , temperature, static condition of water and type of filtration method[19] can also affect microbial contamination of water. Defective water treatment can be deteriorated during long storage feeding on organic or bacterial metabolite from

raw attended microbial population. There are some important reasons that show why microbiological quality necessarily can't be underestimated: 1) water might be taken between food and meals when acidity of stomach decreases, 2) bottled water generally is supposed to be intended for patients and vulnerable population such as children, the elders, pregnant women and immune deficient people[13]. Lacking of appropriate equipment and treatment process of bottled water in developing countries should receive more attention to avoid water born outbreaks**.** In some researches it has been demonstrated that many pathogens can persist in water during storage and then decrease after 1 or 2 month[15].

CONCLUSION

As it has been investigated 31.42% of sample didn't meet the standard of total *Coliforms* count for bottled water which indicates the potential of more hazardous microorganism like *Vibrio cholera*, *Salmonella sp.*, parasites and viruses. Additionally, the difference between Coliforms contamination in this study and similar researches and can't be neglected demanding for improvement of quality assessment in factories and related administer organizations.

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