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Research Article

Bacteriological Quality of Bottled Water and Water from Dispensers Distributed in the School of Bioanalysis and Department of Mental Health of the University of Carabobo

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Abstract

The purpose of the study to determine the bacteriological quality of the bottled water consumed by students and workers of Bioanalysis and Department of Mental Health, and the water placed in the dispensers, is to compare the bacterial quality before and after the water is placed in the dispenser. To this end, mesophilic aerobes were determined by the plate pouring technique, total coliforms, heat-resistant coliforms, *Escherichia coli* and *Pseudomonas aeruginosa* by the membrane filtration. We randomly selected 11 sealed bottles and 11 samples from the dispensers. The mesophiles aerobic load in all the samples of drinking water showed a higher growth than that established by the Official Gazette (GO) of the Bolivarian Republic of Venezuela No. 36,395; for total and heat-resistant coliforms 27% and 36% respectively of the samples do not meet the criteria established in the Venezuelan standard COVENIN Nº 1431-82 and in the GO Nº 36,395; It also occurs with the results obtained for *Pseudomonas aeruginosa*, where it is observed that 91% of the samples do not meet these requirements. In none of the analyzed samples was the presence of *Escherichia coli* observed. The water samples from the dispenser showed a significant increase in microorganisms compared to water in the sealed bottles, which allows us to conclude that the dispensers represent a source of contamination for bottled water.

Keywords: Quality; Contamination; Indicators; Bottled Water

Introduction

As the years go by, diseases related to inadequate water, sanitation and hygiene conditions have a negative impact on the population of developing countries. It is estimated that 80% of diarrhoeal diseases are caused by the supply of unsafe water [1]. Similarly, drinking water contaminated with bacteria and other microorganisms can favor urinary tract infections, diarrhea, meningitis and gastrointinal diseases [2].

By virtue of this, the World Health Organization (WHO) set out general considerations that countries must comply with to ensure that the population's drinking water meets minimum acceptance requirements [3]. In the case of the Bolivarian Republic of Venezuela, the Ministry of People's Power for Health decreed in Official Gazette number 36,395 the Sanitary Standards of Drinking Water Quality, which establish parameters for the microbiological, physicochemical and organoleptic quality of the same. Among

the most relevant are articles 2, 3, 8, 9, 10, 11, 12 and 13, which establishes that the entities responsible for the supply of drinking water, whether public or private, are subject to compliance with these standards and that they are responsible for ensuring that it does not contain microorganisms that transmit or cause diseases, consequently no sample of 100 milliliters (mL) of drinking water should contain heat-resistant coliforms or total coliforms, nor should it contain pathogens or other aerobic heterotrophic organisms at density greater than 100 colony-forming units per mL (CFU/mL), in addition they must be considered permanent health surveillance programmes and apply appropriate specific corrective measures [4].

In conjunction with this regulation, another of the agencies responsible for coordinating and centralizing the approval of technical standards for the evaluation of the quality of drinking water is the Venezuelan Commission of Industrial Standards (COVENIN) No. 1431-82: Bottled drinking water, specific requirements [5], which is established in its Article 10 XIII-002 that requirements must be met for water to be considered drinkable, among these are: a) organoleptic: color, smell, appearance and taste; b) physicochemical: hardness, presence of metals and nonmetals; c) microbiological: in which the presence of total and fecal coliforms is evaluated, tolerable impurities are also evaluated and finally establishes packaging, marking and labeling requirements [6].

In Venezuela, the institution responsible for monitoring compliance with the standards established for drinking water is the Hidrológica de Venezuela (HIDROVEN) according to Official Gazette No. 38,763 Law on Partial Reform of the Organic Law for the Provision of Drinking Water and Sanitation Services in its article 2 [7]; having as a responsibility to develop policies and programs in the field of drinking water supply, collection and treatment of wastewater and urban drainage, as well as the establishment of guidelines for the administration, operation, maintenance and expansion of the systems served by each of its subsidiaries; it also regulates and supervises these hydrological and decentralized companies, enforces the Organic Law for the provision of the services of drinking water and sanitation; encourages citizen participation, and develops projects proposed by communities and technical water tables [8].

However, there is no complete application of the aspects indicated in the aforementioned Organic Law, both in the structural aspects and in the regulatory agents, which could condition a greater risk of contamination in the bottled water [9].

On the other hand, the water in the state of Carabobo, Aragua and part of Cojedes, arrives from its original sources to its final destination thanks to HIDROCENTRO, which is responsible for capturing, processing, driving and distributing it before and after being used. This process consists of 5 main stages: The first is the storage stage, because normally the sources of water depend on the rainy season, so it is necessary to store it, that is what reservoirs are for; the second stage is based on obtaining water in its natural sources: watersheds (rivers, lakes, groundwater, among others) this etapa is called catchment; the third stage consists of the conduction which consists of carrying the water from the sources to the purification plants; the fourth stage is purification where raw water is transformed into drinking water through physical and chemical processes, and the last stage is responsible for distributing the water to be consumed by people [10].

The services to supply drinking water to the Venezuelan inhabitants are characterized by insufficient coverage and low level of quality. Investments in this sector are changing and depend on fluctuations in oil prices. According to all the sources found, the coverage of Drinking Water is between 82%, according to the 2007 census, and 92%, according to HIDROVEN, there is talk of between 2,250,000 and 3,500,000 Venezuelans who do not have access to this service. In addition, in Venezuela the population is concentrated in the Center-North-Coastal axis and the water resources are located in the axis of Apure and Orinoco, which includes the largest rivers in the country such as the Aro, the Caura and the Caroní. This situation has repercussions on long distances of transfer and the loss of considerable amount that must be taken into account. The National Institute of Statistics, in 2001 conducted studies on the quality of water service in the 335 existing municipalities in Venezuela, finding that they were insufficient in 70% of them, that represents 231 municipalities, which causes an important demand for this product [11].

On the other hand, advertising in various media shows bottled water as a product of absolute purity, favoring the progressive consumption of it. These characteristics have boosted a considerable growth in the bottled water industry in Venezuela, which is not exempt from suffering from many of the pollution

problems that have so far occurred in various parts of the world [12]. That is why it is necessary to alert and inform about the microbiological characteristics of it.

It is important to note, in bottled water the bacteria can have their origin from the water source or can be introduced during the course of processing and packaging. Once the container is full and sealed, bottled water can remain on the shelf of the grocery store or stored in the house for weeks or sometimes months, so any bacteria present will stick to the inner surfaces of the container and multiply thanks to small portions of organic matter present in the water. Thus, water containing few organisms, once bottled, can present an exponential increase in the number of bacteria [12].

From the microbiological point of view, the examination of the sanitary quality of water aims to determine the presence of certain groups of bacteria, which reveal recent contamination by fecal matter or organic matter. The group of coliform bacteria has always been the main indicator of quality of the different types of water; the presence of coliforms in a sample is used as a criterion for contamination and, therefore, for its sanitary quality, because they provide important information about the source and type of contamination present [13].

These are bacilli with relatively simple nutritional requirements and are generally identified by their ability to ferment glucose and lactose by fermentation giving acids as a final product [14]. Within the heatoresistant coliform bacteria, the presence of *Escherichia coli* is investigated, being a classic indicator of enteric pathogens in water, due to the direct relationship between this bacterium and fecal contamination or external contamination derived from the environment [12].

On the other hand, *Pseudomonas aeruginosa* is another of the most common pollutants in water supply sources because it is able to multiply in a very wide range of substrates since it can proliferate thanks to the great variety of organic compounds that they use as sources of carbon and energy [15]. In addition, it adheres to inert surfaces by forming biofilms, these are microbial communities that grow in a self-produced exopolysaccharide matrix which allows it to adhere to plastic surfaces, representing a high risk of contamination for those drinking waters that are distributed in plastic containers [16]. In addition, it is a regular member of the microflora of the soil and water of tropical areas, presenting an optimal temperature of development around 42ºC and constituting itself as an opportunistic pathogen. The analysis of *Pseudomonas aeruginosa* in water can inform processes of degradation of the water quality distributed to the user [17].

In relation to this, various investigations in bottled water demonstrated the presence of mesophilic aerobic bacteria, *Pseudomonas aeruginosa,* total coliforms and, to a lesser extent, thermoresistant coliforms such as *Escherichia coli,* revealing a potential risk to the population. Lation that consumes them in the belief that being packaged are safer and healthier, ignoring that a bad manipulation of them can contaminate them [13,18-21,24].

It is important to note that in the study carried out by Rojas T., *et al*. (2012), where the formation of biofilms and antimicrobial susceptibility among coliforms isolated in bottled drinking water in the state of Carabobo was evaluated, the presence of other bacterial groups such as *Klebsiella pneumoniae, Enterobacter cloacae, Enterobacter aerogenes and Klebsiella was also confirmed. oxytoca,* these were listed using standard methods and filtration through membrane [22].

Taking into account the possible contamination of the dispensers, Campo and Chaidez (2003) conducted a study where the microbiological quality of tap water and dispensers located in residences and workplaces was compared. The incidence of contamination with coliforms or indicators and pathogenic bacteria was significantly lower in tap water than in the samples of water dispensers. Based on the study, the authors concluded that the bacteriological quality of tap water is superior to the water quality of water dispensers, so the latter may favor the multiplication of heterotrophic bacteria. They recommended cleaning water dispensers every two months to limit contamination [23].

In the state of Carabobo, studies have indicated an increase in the consumption of bottled water, which is in breach of the provisions of the Organic Law for the Provision of Drinking Water and Sanitation Service on the quality of the vital liquid, a situation that is generating health problems in the population, according to complaints made by specialists in environmental matters [25], and taking into account that the process of purification, filling and packaging of the bottles as well as the disinfection of the dispensers is unknown, in the present study, the bacteriological quality of samples of bottled drinking water and the water supplied

in the dispensers of the School of Bioanalysis and Department of Mental Health during the period October and November 2015 was determined.

Materials and Methods

Samples

The samples were taken for 3 consecutive weeks in the months of October and November 2015, from where 11 samples of 100 ml of packaged drinking water were obtained at random and 11 samples were taken directly from the 4 dispensers distributed in the school and department, obtaining a total of 22 samples, 11 from dispensers and 11 from water bottles. They were handled based on the Venezuelan Standard COVENIN No. 2614 [26] and finally were transferred in refrigerated cava to be analyzed in the Center for Applied Microbiological Research for processing.

Methodological procedure

Quantification of mesophilic aerobic bacteria

For the count of mesophilic aerobic bacteria, the plate pouring technique was used, in which 1 mL and 0.1 mL of sample respectively were sown in depth in 2 sterile petri dishes, then the culture medium of nutritive agar enrichment was added and incubated at 37ºC in aerobiosis for 48 hours. Subsequently, the count was performed using a colony counter and was reported as CFU/mL of sample [27].

Quantification of bacteria of the coliform group

The membrane filtration technique was used to detect and quantify total and fecal coliforms or thermissive. It was carried out based on the Standardized Methods for the analysis of drinking and waste water: 100 mL of water sample was filtered through a cellulose membrane of 47 mm and 0.2 µm of porosity in partial vacuum, this membrane was placed in a petri dish with agar MacConkey, which was done in duplicate since one plate was incubated at 37ºC to obtain the growth of total coliforms and another at 44ºC for the growth of thermoresistant coliforms, both were incubated for 24 hours and finally the count was carried out with the help of a colony counter, reporting as UFC/100 mL [28].

Identification of *Escherichia coli*

The method of sowing by plate stria was used, from the lactose fermenting colonies in the growth of the thermoresistant coliform plate. A colony with a sterilized planting handle was taken and en zigzag stretch marks were performed on nutritious agar plates, then incubated at 37ºC for 24 hours. Macroscopic, microscopic characterization, manual biochemical tests were performed: Gram staining, kliger and confirmation through API 20E 28 galleries.

Identification of *Pseudomonas aeruginosa*

For the detection and counting of *Pseudomonas aeruginosa*, the technique of filtration of 100 mL of sample was performed through a cellulose membrane of 0.2 µm, which was sown in cetrimide agar plate and incubated at 37ºC for 48 hours. The count was performed with the help of a colony counter and reported as UFC/100 mL. For its confirmation, the oxidase test was used for colonies with greenish coloration [27].

Comparison of results

Samples that met the microbiological criteria established in COVENIN 1431-82 were considered suitable, which stipulates that the maximum limit allowed for total coliforms is 10 CFUs/100 mL, and that no sample of 100 mL of bottled water should contain thermoresistant coliforms or *Pseudomonas aeruginosa* [6]; and that it also complies with the requirements set forth in the Official Gazette of the Bolivarian Republic of Venezuela No. 36,395 in which it is argued that the maximum limit allowed for mesophilic aerobes must be 100 CFU/mL and that no sample of 100 mL of bottled water should contain total coliform and thermoresistant microorganisms [4].

Results

Ensuring the quality of water for human consumption has as its main objective to protect the health of the consumer, for this reason it was considered the determination of microorganisms indicators of sanitary quality, among them, mesophilic aerobes, total coliforms, thermoresistant coliforms and *Pseudomonas aeruginosa*, because they provide information on the hygienic practices applied in the manufacture and process And indicate possible contamination, consequently an increase in their number, alerts about the possible presence of pathogenic microorganisms.

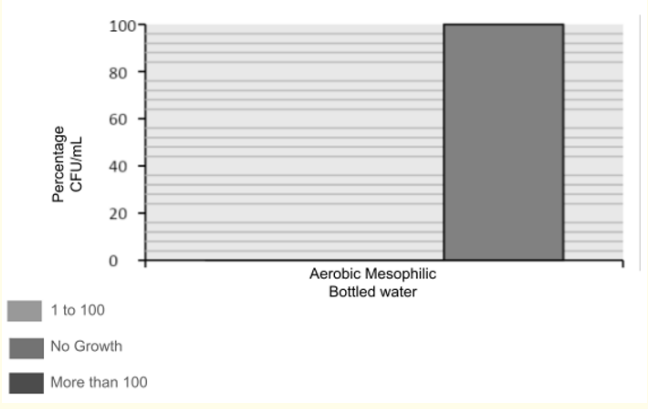

Figure 1: Graphical representation percentage of growth of mesophilic aerobes in bottled water.

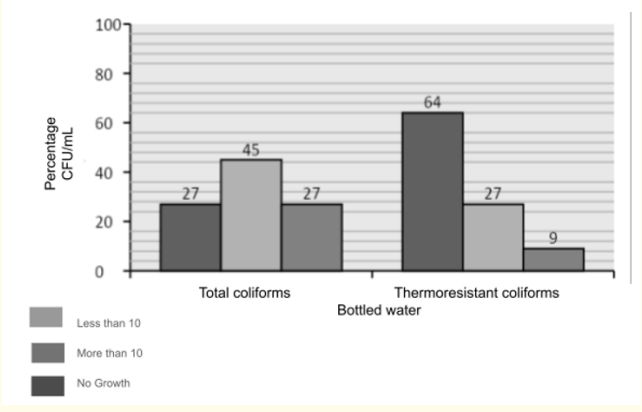

Figure 2: Representation of the percentage of growth of total coliform and thermoresistant microorganisms in bottled water.

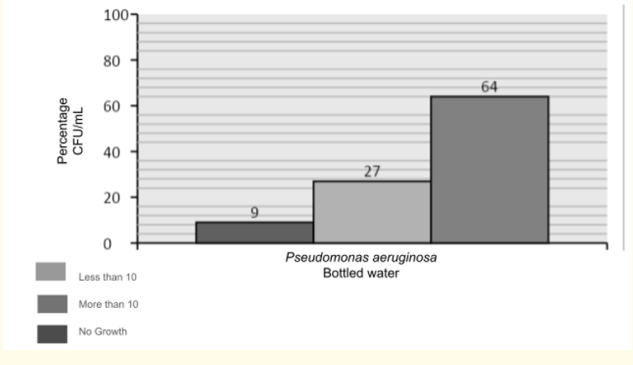

Figure 3: Graphical representation of the percentage of growth of *Pseudomonas aeruginosa* in bottled water.

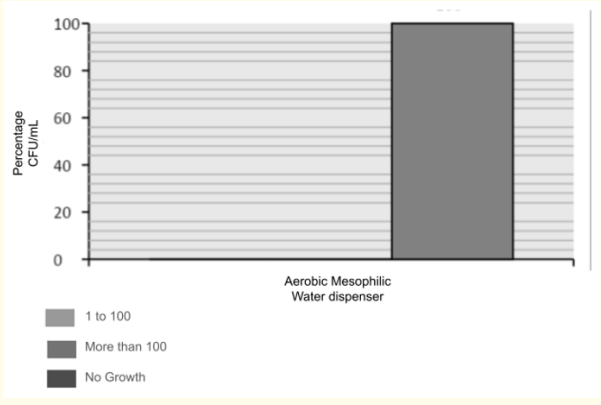

Figure 4: Graphical representation of the percentage of growth of mesophilic aerobes in dispenser water

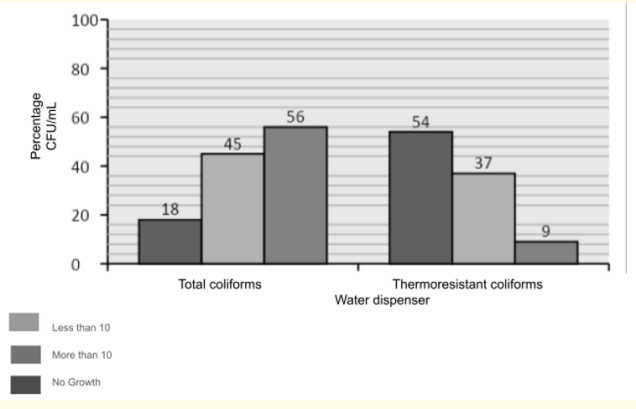

Figure 5: Representation of the percentage of growth of total coliform and thermoresistant microorganisms in dispenser water.

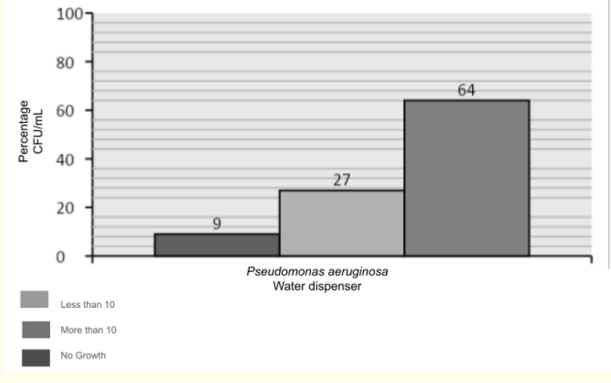

Figure 6: Graphical representation of the percentage of growth of *Pseudomonas aeruginosa* in dispenser water.

	Bottled water		Dispenser water			
Microorganisms	**My.**	**Max.**	**My.**	**Max.**	**Limit Values Venezuelan Standard**	**Limit Values G.O. of the Bolivarian Republic of**
					COVENIN No. 1431-82	**Venezuela**
						No. 36,395
Mesophilic aerobes UFC/mL	176	1232	238	1680	ON	<100
Total coliforms UFC/100 mL	0	58	0	1000	<10	0
Fecal coliforms UFC/100 mL	0	15	0	51	0	0
Pseudomonas aeruginosa UFC/100 mL	0	1000	0	1000	0	ON

Table 1: Minimum and maximum representative values of the bacterial load of mesophilic aerobiums, total coliforms, fecal coliforms and *Pseudomonas aeruginosa* in bottled water and in dispenser water.

NA = No Applies.

Discussion and Conclusion

As for mesophilic aerobes, all bottled water samples analyzed had counts greater than 100 CFUs/mL. Similar results were observed in the study conducted by Silva., *et al*. (2004), who determined in a total of 30 samples of bottled and distributed drinking water in the state of Carabobo, Venezuela, the presence of mesophilic aerobes, of which all samples (100%) presented a growth greater than 100 CFU/mL [24], evidencing the non-compliance with the Official Gazette of the Republic Bolivarian of Venezuela Nº36.395. The determination of aerobes is of the utmost importance, given that their count of less than 100 CFU/mL indicates adequate water purification, allowing to obtain information on the alteration of water quality and identify failures in the process of purification, filling and packaging of the same [30]. On the other hand, they comprise most of the microorganisms that are hosted by man and other warm-blooded animals, so the presence of these in packaged drinking water can represent a high risk for the consumer [29].

In the case of total coliform bacteria, 27% of the bottled water samples analyzed had a count greater than 10 CFU/100mL. Similar results were observed in the research of Rojas., *et al*. (2012), who determined the formation of biofilms and antimicrobial susceptibility among coliforms isolated in bottled drinking water in Carabobo, Venezuela [22], observing that at least 36% of the samples obtained levels higher than 10 CFU/100 mL for total coliforms, while 58% presented indices of at least 1 CFU/100 mL.

The presence of coliforms in water is associated with external ambiental contamination, but mainly of fecal origin. Enteric diseases caused by coliform bacteria are transmitted almost exclusively by contamination of water and food of fecal origin, since this type of microorganism is located in the intestinal tract of mammals and warm-blooded animals. Hence the importance of routine analyses for the verification of the non-existence of this type of bacteria in drinking water and public consumption [29].

Of the samples obtained from bottled water, 36% presented counts greater than 1 CFU/100 mL of heat-resistant coliforms; contrasting these results Vidal's research when evaluating the microbiological quality of the bottled water produced in Sincelejo - Colombia (2009), of a total of 30 samples, only one showed the growth of fecal coliforms or thermoresistant [20].

Due to the importance of *Escherichia coli,* not only as an indicator of sanitary quality, but as an agent that by itself represents a potential threat to public health, since it can lead to virulence factors and resistance mechanisms [29], we proceeded

to detect the presence of this microorganism in drinking water samples, where the absence of growth of this microorganism was evidenced; this result is contrary to what was observed in the research of Martínez., *et al*. who determined the bacteriological quality of the drinking water commercially packaged in Ciudad Bolívar observing the presence of this microorganism in one of the samples analyzed [30].

Of the samples obtained from bottled water, 64% presented growth greater than 10 CFU/mL for *Pseudomonas aeruginosa*; contrary results were observed in the study by Martínez., *et al*. (2010), where they did not obtain growth of this microorganism in any of the samples analyzed [30].

The presence of these microorganisms in bottled water is related to their ability to form biofilms, these are microbial communities that constitute the most successful form of colonization among microorganisms, they are considered communities of bacteria that grow embedded in a self-produced exopolysaccharide matrix and are adhered to an inert surface like plastic containers. The formation of biofilms occurs as a continuous process according to several phases of development which are: conditioning, adhesion, extracellular matrix synthesis, maturation and dispersion, leading to the formation of a uniform structure in form of homogeneous deposits and cellular viscous accumulations surrounded by a polymer matrix with open channels for the movement of water and nutrients [16].

Pseudomonas aeruginosa is an opportunistic pathogen, responsible for a wide range of infections, mainly nosocomial. Particularly patients with immunosuppression, as well as those who have suffered severe burns, chemotherapy-induced neutropenia or have underlying lung diseases are prone to develop the infection, so an exponential increase in this in bottled water poses a risk to the susceptible population [31].

A large proportion of the population in developing countries drink water of dubious microbiological quality; Even when no is contaminated and the quality of the water supplied is high, contamination usually occurs in the time it is stored in the dispensers, so the latter can favor the multiplication of heterotrophic bacteria [32]. For this reason, the level of contamination of the dispensers distributed in the School of Bioanalysis and Department of Mental Health was considered.

We proceeded to evaluate mesophilic aerobic organisms, where all dispenser water samples presented counts greater than 100 CFUs/mL; 56% of the samples analyzed had counts greater than 10 CFUs/mL of total coliforms, 46% presented thermoresistant coliforms and 64% presented *Pseudomonas aeruginosa*.

It should be noted that in none of the dispenser water samples analyzed *was Escherichia coli* detected; however, other microorganisms were identified. Thermoresistants such as *Enterobacter cloacae,* which, found in the human digestive system, is not a principal pathogen, but has been considered a major cause of nosocomial infections [33]; and *Enterobacter sakazakii,* which has been associated with sporadic cases or small outbreaks of sepsis, meningitis and necrotizing enterocolitis, its mortality has been recorded as greater than 50% but has decreased to less than 20% in recent years [34].

Letarte., *et al*. (2001), compared the microbiological quality of bottled water and water systems of 50 water chillers located in residences and workplaces in Quebec Canada, evaluating facultative aerobic and anaerobic heterotrophic bacteria, total coliforms, and two indicators of fecal contamination (fecal coliforms and fecal streptococci), as well as three types of pathogenic bacteria (*Staphylococcus aureus), Pseudomonas aeruginosa*, and *Aeromonas* spp.), who found that 36 and 28% of nursing home and workplace water dispenser samples, respectively, were contaminated by at least one coliform or indicator bacterium and/or at least one pathogenic bacteria, while samples from bottled water systems obtained much lessos counts than those observed for water chillers [32]. It is important to note that the results of *Pseudomonas aeruginosa* were similar in samples of bottles and dispenser, which shows that the formation of biofilms may be occurring in both sites in an equivalent way.

Based on the provisions of the Venezuelan COVENIN Standard No. 1431-82, it can be concluded with respect to total coliforms that 27% of the samples of bottled water and 56% of the samples from the dispensers are not complying with this standard, because they have counts greater than 10 CFU/mL, a value established as a reference. While for thermoresistant coliforms and *Pseudomonas aeruginosa* this standard stipulates that no sample of 100 mL of bottled water must contain these microorganisms, noting that only 64% of the samples of bottled water and 54% of the samples

obtained through the dispenser are free of heat-resistant coliforms, while 64% of both samples showed counts of *Pseudomonas aeruginosa*.

Regarding the Official Gazette of the Bolivarian Republic of Venezuela No. 36,395 establishes that for mesophilic aerobes the maximum permissible value for bottled water is 100 CFU/mL, which shows that the results obtained in this investigation also do not comply with the provisions of this gazette; with respect to total and fecal coliforms or thermoresistants, it states that no water sample of 100 mL must contain the presence of these microorganisms so only 27% and 64% that correspond to those samples of bottled water where no growth of total coliforms and thermoresistant respectively were observed comply with the Gazette, as for the water of the dispensers only 18% of these were free of total coliforms and 54% did not obtain growths for thermoresistant coliforms.

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