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DETECTION AND INCIDENCE OF PSEUDOMONAS IN DRINKING WATER SIMPLES

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INTRODUCTION

According to data from CONAGUA [1], in Mexico, the average water consumption per person is 380 liters per day, using 4% of it for personal hygiene or for consumption. To use the water for these purposes, it must be purified, which may require a complete treatment of filtration, coagulation, sedimentation and disinfection, of which the most microbiologically relevant is disinfection.

Disinfection is intended to inactivate pathogenic microorganisms in water, including relatively sensitive forms, such as non-sporulating bacteria. The most widely used germicide in water disinfection is chlorine, in the form of hypochlorite or molecular chlorine. In water, both generate hypochlorous acid and hypochlorite ion in relative concentrations that depend on pH [2]. Another chemical agent also used to purify water is ozone, since it has the ability to inactivate bacteria and viruses more quickly than chlorine and, apparently, it has some effect against parasites [2].

Regarding physical treatments, it is known that ultraviolet light is used due to the lethal action it has on microorganisms, since their nucleic acid molecule bases absorb UV light and lose their functionality [2].

One of the microbial groups of greatest interest in water is that of Coliform Organisms, since their finding in it may indicate a lack or inefficiency in the purification process and/or recontamination during distribution. In addition, the specific finding of Escherichia coli or thermotolerant coliforms in the water constitutes evidence of recent fecal contamination and requires investigation of potential sources of contamination and eventual loss of the integrity of the conveyance and storage systems. In this sense, NOM 201-SSA1-

2015 [3] establishes in section 5.1.5.1.2 that, for water intended for human consumption, the limits for Coliform Organisms with values less than

1.1 MPN/100 mL, zero CFU/100 mL or the absence of these when the analysis technique allows it. However, a specific indicator does not show characteristics of safe application to satisfy or cover the diversity of pathogens potentially present in the water, such is the case of Pseudomonas, an opportunistic pathogen of which, the aforementioned regulations indicate its analysis only when it is From mineral water.

Pseudomonas aeruginosa is a Gram-negative, aerobic rod with a polar flagellum. When grown on suitable media it produces pyocyanin, a non-fluorescent bluish-green pigment. Many strains also produce the green fluorescent pigment pyoverdin. It is a common microorganism in the environment and can be found in feces, soil, water, and wastewater [4]. This opportunistic pathogen can cause various types of infections, predominantly colonizing damaged parts of the body, such as burns and surgical wounds, the respiratory tract of people with underlying diseases, or physical lesions in the eyes, where it can invade and cause destructive lesions or septicemia and meningitis. Cystic fibrosis or immunocompromised patients are prone to colonization by P. aeruginosa, which can lead to severe progressive pulmonary infections [4].

Its presence is significant in some settings such as health centers and, although there is no evidence that normal uses of water for human consumption are a source of infection for the general population, its ability to form biofilms represents a danger to people with compromised immune systems.

In this sense, the objective of this work was to analyze the incidence of Pseudomonas in drinking water samples from various environments by monitoring them in the presumptive phase of coliform detection by the Most Probable Number (MPN) technique and to relate the results. with the presence or absence of coliform organisms.

METHODOLOGY

66 water samples from different environments were analyzed: 14 from drinking fountains, 16 from the network water, 29 from bulk water and 7 from filtration systems. The samples were collected in sterile vials with 10% sodium thiosulfate and were transported in coolers with refrigerants without directly touching the vials. All the samples were analyzed in a time no greater than 6 hours from their sampling.

For the determination of Coliform Organisms, the analysis technique indicated in appendix H of NOM-210 SSA1-2014 [5] was followed. For the presumptive test, in the sterile area, 5 portions of 20 mL each of the sample were sown in tubes with 10 mL of Lactosed Broth (BIOXON, Becton Dickinson de México, México) triple concentration with sterile Durham bell, said tubes were They were incubated at 35°C for 48 hours. Once this time had elapsed, three rounds were taken from each tube that showed gas formation and inoculated in an equal number of tubes of 2% Brilliant Green Bile Broth (DIFCO, Becton Dickinson Company, USA) for the confirmatory test. of Total Coliform Organisms and were incubated for 48 hours at 35°C. After this, the number of tubes that presented gas formation was recorded and, with the data obtained, the MPN for Total Coliforms was calculated, taking table H.8.4.3 of NOM 210-SSA1-2014 as a reference [5].

In parallel, the tubes of the presumptive samples were kept incubated at 35°C even after taking the roast for the determination of Coliform Organisms until they presented a greenish coloration (Figure 1). Of the tubes that had this coloration, they were sown by cross streak Agar Pseudomonas (DIFCO, Becton Dickinson Company, USA) and incubated at 35°C for 24 hours. Those boxes that presented the production of green and/or blue pigments were considered as Pseudomonas (Figure 1), the data was recorded and reported in accordance with that established in table H.8.4.3 of NOM 210-SSA1-2014. [5].

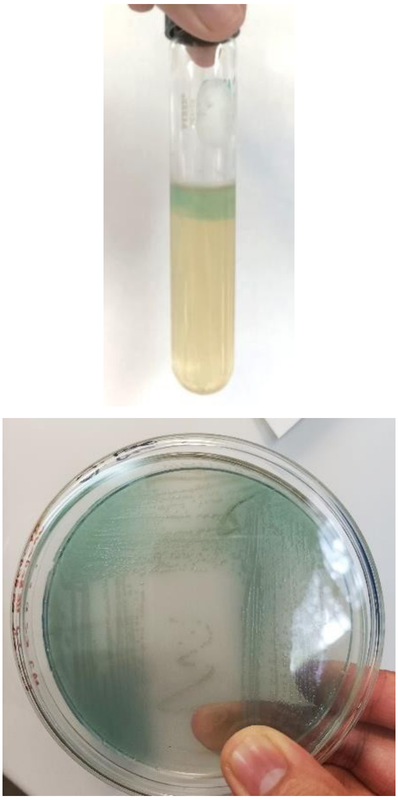


Figure 1. Presumptive test tube of the NMP technique (lactose broth) with greenish coloration and without gas and confirmatory plate in agar *Pseudomonas*

RESULTS AND DISCUSIÓN

Of the total of the 66 water samples analyzed, 37.87% (25/66) exceed the limit of coliform organisms (OCT) <1.1 NMP/100 mL, declared as indicators of sanitary quality in NOM-201-SSA1-2015 [3] that applies to ice and bottled and bulk water. This standard establishes the search for Pseudomonas only for mineral water, however, Pseudomonas spp. in 18.18% (12/66) of the water samples analyzed.

Graph 1 shows the results of the percentage of samples positive for OCT and/or Pseudomonas spp. in each type of water.

In the first instance, it was found that Pseudomonas spp. It occurred in water obtained from drinking fountains, domestic filtration systems, and bulk water. This could be due to the fact that in the three types of water, they involve the passage of it through narrow areas, such as filters or cartridges, therefore, when the system is not used, the water can remain stagnant in the areas. narrow and, if there is presence of Pseudomonas spp. In water, it can remain in these areas, creating biofilms, which would cause the microorganism to persist for a long period of time. On the other hand, the presence of OCT in the four types of water reflects deficiencies in the purification processes, for example, in bulk water, it could indicate problems during the purification treatment or even deficiencies in the washing of demijohns.

Secondly, the results obtained revealed that, for each type of water, when the incidence of Pseudomonas was high, the incidence of OCT was low, and when the incidence of OCT was high, the incidence of Pseudomonas was low. Few samples presented both OCT and Pseudomonas spp. at the same time (7.14% for water from drinking fountains; 14.28% for water from filtration systems; 6.89% for bulk water and 0% for water from the municipal network). These results agree with what was reported by Coelho et al, 2010 [6], where it is reported that the genus Pseudomonas produces bacteriostatic substances that affect some microorganisms belonging to the coliform group.

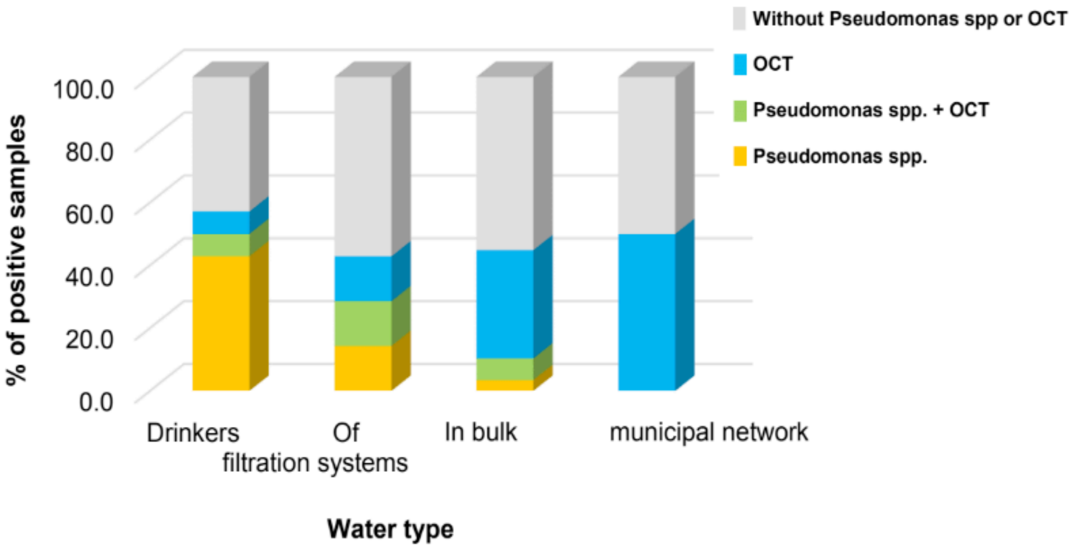
These results acquire special relevance when contrasted with the microbiological specifications of NOM-201-SSA1-2015, which indicates the absence of OCT in all types of water for human consumption, and that the determination of Pseudomonas spp must be carried out exclusively in the case of mineral water, however, given what was observed in this work, the absence of OCT does not guarantee that there is no Pseudomonas spp. So, it is recommended to reconsider the currently stipulated microbiological parameters and add the determination of Pseudomonas spp to other types of water, with the aim of guaranteeing its quality, mainly for people with a compromised immune system.

Finally, it is highlighted that the methodology used for the detection of Pseudomonas spp. it can be performed in parallel to the presumptive test in the detection of OCT, prolonging said test. This methodology could be used to identify Pseudomonas spp. without the need to acquire other types of culture broths.

CONCLUSIONS

The results obtained in this work show that the Most Probable Number detection technique for Coliform Organisms worked as an indicator of the presence of Pseudomonas in the presumptive test through the generation of green tones in the broth and this, in turn, could be confirmed by plating on agar.

Also, it must be taken into consideration that, although there is a tendency for the presence of Pseudomonas to be unrelated to the presence of OCT, not all types of water present the same behavior. Although the standards establish the search for Pseudomonas only in mineral water, this work shows the need for its determination in other types of water.



Graph 1. Porcentaje de incidencia de *Pseudomonas spp* y de OCT en muestrasde agua

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