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# Occurrence of Biofilm-forming *Aeromonas* spp. in Stainless Steel Water Dispensers in an Education Institute

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## Abstract

Monitoring bacterial contamination in water is essential for human health, as certain waterborne bacteria can pose serious illnesses. This pilot-scale study aimed to identify biofilm-forming *Aeromonas* spp. within stainless steel water dispensers (SWD) in an educational institute, while also elucidating the underlying contributing factors. *Aeromonas* spp. were isolated from nineteen SWDs. Distinct biochemical characteristic aeromonads were selected for biofilm-formation testing by glass tube experiment. Water samples were collected and assessed for pH, turbidity, color, and free chlorine residue. The study revealed that sixteen of nineteen SWDs had 48 aeromonads (84.21%). The most identified species were *A. bestiarum, A. hydrophila,* and *A. veronii*. Interestingly, this study documented the occurrence of globally distributed pathogens such as *A. hydrophila, A. veronii,* and *A. caviae.* All twenty-nine tested isolates were able to form biofilms, and *A. hydrophila* exhibited the most potent biofilm-producing properties. There was no association between the biofilm formation and the measured physicochemical parameters. The occurrence of biofilm-forming *Aeromonas* spp. within SWDs underscores the urgency for heightened awareness among public health professionals about the effects of biofilm-forming microorganisms and offers guidance on mitigating their presence. In addition, it is imperative to conduct regular monitoring in educational institutions and other public buildings.

***Keywords:*** *Aeromonas* spp., Biofilm, Lecture building, Stainless steel water dispenser, Drinking water

## 1. Introduction

*Aeromonas* spp. are waterborne bacteria known for their resistance to disinfectants and their ability to form biofilms in water processing systems (Fernandez-Bravo and Figueras, 2020; Barger *et al.,* 2021). These bacteria have been detected in drinking water across various countries, and their concentrations are linked to their ability to regrow within the system. This ability is influenced by their ability to produce and persist within biofilms (Kasozi *et al.,* 2021; Mori and Smith, 2019; Schmautz *et al.,* 2017). Many studies have been directed toward their role as opportunistic pathogens for humans, causing intra and extra-intestinal infections through the consumption of contaminated food and water (Pessoa *et al.,* 2022). Notable among the recognized *Aeromonas* spp. responsible for human infections are *A. dhakensis, A. hydrophila, A. caviae,* *A. veronii,* and *A. salmonicida* (Talagrand-Reboul *et al.,* 2017). *Aeromonas* spp. utilize various mechanisms, including adhesin, S/A layer, motility, lipopolysaccharide, biofilm formation, and toxins such as enterotoxin, cytotoxin, hemolysin, lipase, and proteases, to cause harm. Furthermore, the production of Shiga-like toxins by these bacteria can result in hemorrhagic septicemia, particularly in individuals with compromised immune systems and water-associated wound infections. Therefore, the presence of these bacteria in drinking water can be a public health concern due to their ability to form biofilm and produce toxins.

Stainless steel water dispensers (SWD) are commonly used in various public buildings, including educational institutes in Thailand, owing to their convenience, durability, lack of harmful chemical emissions, and promotion of hygiene. However, inadequate cleaning and maintenance can result in microbial contamination and the formation of biofilms within SWDs (Talagrand-Reboul *et al.,* 2017). Biofilms refer to complex communities of microorganisms attached to solid-water interfaces on either biotic or abiotic surfaces, living within self-produced or acquired extracellular polymeric substances (Kilic and Bali, 2023). They are ubiquitous and commonly found in water distribution systems, pipes, and dispensers (Park *et al.,* 2017). These biofilms enhance stability and shield microorganisms against environmental conditions like salinity, antimicrobial substances, or oxidative stress (Talagrand-Reboul *et al.,* 2017) thereby worsening human and animal infections. They play a pivotal role as a virulence factor, endowing bacteria with resistance to antimicrobial agents and the ability to adhere to host tissues (Dias *et al.,* 2018).

Previous studies have reported several instances of biofilm-forming *Aeromonas* spp. in drinking water, food, and clinical samples, underscoring the influence of biofilm-forming capacity on the pathogenicity of aeromonads. Furthermore, biofilm-forming strains necessitate particular attention regarding their potential sources and reservoirs for several microbes, including pathogens (Talagrand-Reboul *et al.,* 2017). Therefore, ascertaining the presence of these bacterial strains within water dispensers is critical for maintaining drinking water quality and safeguarding public health. Various physiochemical factors, such as nutrient levels, temperature, pH, and organic matter, can influence biofilm formation in aeromonad (Salvat and Ashbolt, 2019). A recent study conducted on *A. hydrophila,* which was isolated from drinking water, showed that low temperatures significantly increase the occurrence of the species (Mizan *et al.,* 2018). Furthermore, it has been found that *Aeromonas* spp. are present in water distribution sites with low chlorine residuals, highlighting the importance of water treatment system (Botero *et al.,* 2023). Nonetheless, there is still a need to investigate the physiochemical parameters that may affect the occurrence and biofilm formation of *Aeromonas* spp. in water systems.

The emergence of biofilm-forming *Aeromonas* spp. within the drinking water sources raises a significant public health concern, necessitating a meticulous evaluation of drinking water quality. To achieve this, the authors initiated an inquiry to identify biofilm-forming *Aeromonas* spp. within SWDs in the lecture buildings of a specific university. Additionally, we investigated the potential factors contributing to the prevalence of these strains. The findings of this study could provide useful information for monitoring, assessing, and managing drinking water quality in educational institutions and other public buildings.

## 2. Methodology

### *2.1 Study area and description of studied stainless steel water dispensers*

A pilot-scale study was conducted from November to December 2022 within six lecture buildings situated at a university in Pathum Thani Province, Thailand. The study focused on the examination of nineteen stainless steel water dispensers (SWDs) situated in commonly frequented areas by students and staffs. Dispensers that were not in operational condition during the examination were excluded from the study.

All the SWDs under scrutiny were of an identical model type and featured uniform filtration systems comprising five distinct stages of filters: sediment, carbon block, cation resin, reverse osmosis, and post-carbon filters. As part of the maintenance process, the sediment, carbon block, and cation resin filters underwent replacement every three months. Similarly, the reverse osmosis and post-carbon filters were replaced every six months to uphold the optimal functioning of the dispensers.

*2.2 Sample collection*

Surface swab testing was employed to examine the presence of *Aeromonas* spp. contamination within SWDs. To conduct this analysis, sterile cotton swabs were submerged in buffered peptone water (BPW) (Oxoid Ltd., Basingstoke, United Kingdom) and then swept along the inner walls of the faucets. After the swabbing process, the collected samples were placed into tubes containing sterile 5 ml of BPW. At the same time, drinking water samples were collected from each SWD to assess physicochemical parameters following the Standard Methods for the Examination of Water and Wastewater (Baird and Bridgewater, 2017). All samples were chilled in an ice box and immediately transported to the laboratory.

*2.3 Isolation and identification of Aeromonas spp.*

Tests were conducted to isolate possible *Aeromonas* spp. contaminations following the enrichment of swab samples in BPW at 37 $^{o}$C for a duration of 18 hours. Subsequently, the enriched BPW samples underwent serial dilution before being plated onto the *Aeromonas* selective agar (Himedia Ltd., Maharashtra, India). The plated agar was then incubated at 37 $^{o}$C for a period of 18 hours.

From each sample, three distinct translucent colonies, smooth, convex colonies 3 - 5 mm in diameter colonies, were chosen at random to undergo identification through a series of biochemical tests as outlined by Abbott *et al.* (2003). These tests included Triple Sugar Iron, Lysine Indole Motile, Methyl red, Voges-Proskauer, Citrate, Nitrate, Oxidase, and DNase test. The results of biochemical tests which are utilized for the identification of *Aeromonas* spp. are listed in Table 1.

**Table 1.** Biochemical characteristics of identified *Aeromonas* spp.

| Biochemical characteristics[a] | *A. hydrophila* | *A. bestiarum* | *A. salmonicida* | *A. caviae* | *A. media* | *A. eucrenophila* | *A. veronii* | *A. sobria* |
|---|---|---|---|---|---|---|---|---|
| TSI: Slant/Butt | K/A | K/A | K/A | K/A | K/A | K/A | K/A | K/A |
| $H_2S$ production | + | - | - | - | - | - | - | - |
| Lysine decarboxylase | d | d | d | - | - | - | + | + |
| Indole production | + | + | + | + | d | + | + | + |
| Motility | + | + | - | + | - | + | + | + |
| Methyl red | + | + | + | + | + | + | + | - |
| Voges-Proskauer | + | d | - | - | - | - | + | d |
| Citrate, Simmons | d | - | - | d | d | d | + | - |
| Nitrate reduction | + | + | + | + | + | + | + | + |
| Oxidase | + | + | + | + | + | + | + | + |
| DNase | + | d | + | + | + | + | + | + |

[a]Test results were read at 24 h, and incubation temperatures of 37$^{o}$C. Abbreviations: K, alkaline; A, acid; +, 90% or more of strains are positive; -, 90% or more of strains are negative; d, 11-89 of strains are positive. (Holt, 1997)

*2.4 Assessment of biofilm formation ability of Aeromonas spp.*

The glass tube method, initially described by Christensen *et al.* (1985), was employed with a slight modification. From the identified representatives of *Aeromonas* spp. in each SWD, specific strains were selected to assess their biofilm-formation abilities. A loopful of the aeromonad colony was inoculated into 5 ml of tryptone soya broth (obtained from Oxoid Ltd., Basingstoke, United Kingdom) within a borosilicate glass tube. Subsequently, the tubes were incubated at 37 °C for a duration of 48 hours. After the incubation period, the bacterial solution was drained and subjected to two successive washes using phosphate buffer saline with a pH of 7.2. To fix the biofilm, 5 ml of 2% sodium acetate solution was added and allowed to react for 10 minutes. Following this, the solution was discarded, leaving the tubes to dry at room temperature. In the subsequent steps, the tubes were stained with 0.1% crystal violet solution, with rotation of the tubes to ensure uniform staining. Post-staining, the crystal violet was removed, and the tubes were rinsed using distilled water. The tubes were then inverted and left to dry for a period of 24 hours. The extent of biofilm formation was evaluated based on the visible film present on both the walls and the bottom of the tube, thereby enabling a comparison between the control (without bacteria inoculum) and the test samples. The amount of the film was graded as absent (-), weak (+), moderate (++), and strong biofilm formation (+++), as shown in Figure 1.

*2.5 Determination of physicochemical parameters of drinking water*

The model and operational conditions of the stainless-steel water dispensers were observed. Subsequently, all drinking water samples were thoroughly analyzed for physicochemical parameters including temperature, pH, turbidity, and color. These analyses were performed in accordance with the standard methods for water and wastewater examination (Baird and Bridgewater, 2017). Furthermore, the concentration of free chlorine residual was determined utilizing a commercially available test kit (Hanna Instrument, Smithfield, RI).

*2.6 Statistical analysis*

Descriptive analysis was used to define the tendency and distribution of *Aeromonas* spp. in SWDs. Meanwhile, the correlation between the physicochemical parameters and the biofilm-formation ability was evaluated using the Chi-square test and One-way ANOVA with a significant set at $p < 0.05$.

*2.7 Ethical approval*

The project protocol was approved by the Biosafety Committee of Thammasat University, Thailand (no. 066/2565). The usage of microbiological agents was in rigorous accordance with the guidance in the Biosafety Guidelines for Work Related to Modern Biotechnology or Genetic Engineering of Thailand's Technical Biosafety Committee.

| **Score of biofilm formation** | | | |
|---|---|---|---|
| **Absent (-)** | **Weak (+)** | **Moderate (++)** | **Strong (+++)** |

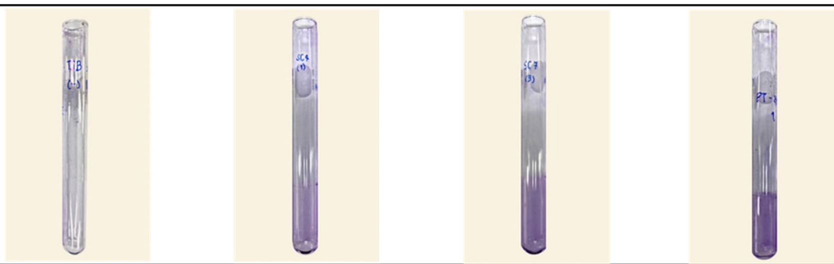

**Figure 1.** The estimation of biofilm formation ability of *Aeromonas* spp.

## 3. Results and discussion

*3.1 Isolation and identification of Aeromonas spp.*

Table 2 presents the results of the bacteriological analysis conducted on the swab samples collected from the SWDs. Among the total of nineteen SWDs assessed, a majority of sixteen units (84.2%; 16/19) exhibited the presence of *Aeromonas* spp. Subsequent to biochemical assessments, eight distinct species of aeromonads were identified. These included *A. bestiarum, A. hydrophila, A. veronii, A. sobria, A. media, A. caviae, A. salmonicida,* and *A. eucrenophila.* Of particular significance, *A. bestiarum* (constituting 41.7% of the total, or 20 out of 48) and *A. hydrophila* (comprising 20.8% or 10 out of 48) emerged as the most prevalent aeromonad species across the majority of the studied buildings. The diversity of aeromonad species observed was particularly identified in the SWDs of building B1 (with 7 species) and B2 (with 5 species).

This study found the presence of globally distributed pathogens such as *A. hydrophila* (building B1, B2, B4, and B6), *A. veronii* (building B1 and B2), and *A. caviae* (building B4) within the examined SWDs.

*3.2 Determination of the factors associated with biofilm-formation ability of Aeromonas spp.*

In order to assess the potential for biofilm formation, a selection of 29 *Aeromonas* spp. isolates derived from SWDs underwent biofilm-formation testing using the glass tube method. A total of 22 isolates were randomly selected from *A. bestiarum* (11 isolates), *A. hydrophila* (6 isolates), and *A. veronii* (5 isolates). Furthermore, all isolates of *A. sobria* (2 isolates), *A. media* (2 isolates), *A. caviae* (1 isolate), *A. salmonicida* (1 isolate), and *A. eucrenophila* (1 isolate) were subjected to testing. The results of this study are presented in Table 3. It is imperative to highlight that all examined isolates exhibited varying degrees of proficiency in forming biofilms.

Among the tested aeromonads, a significant proportion, accounting for 37.9% (11 out of 29) showcased substantial biofilm formers, with a rating of (+++). Additionally, 34.5% (10 out of 29) of the isolates displayed comparatively weaker biofilm formation with a rating of (+). Notably, specific strains such as *A. hydrophila* (5 isolates), *A. veronii* (3 isolates), and *A. caviae* (1 isolate) demonstrated particularly vigorous biofilm formation.

*3.3 Investigation of physicochemical factors contributed to the occurrence of biofilm-forming Aeromonas spp.*

Drinking water samples were collected from SWDs and subjected to a thorough analysis targeting physicochemical factors that might impact on both biofilm formation and water quality. Additionally, the obtained test results were compared against the recommended guidelines set forth by the World Health Organization (WHO, 2011) outlined in Table 4.

The temperature range of the examined water samples was between 2 to 6 $^{o}$C, and tested all the parameters, including pH, color, turbidity, and residual free chlorine. These parameters were found to adhere to the WHO-established guidelines. This study has also determined that no correlation exists between the tested physicochemical parameters and the capacity of aeromonads to produce biofilm.

*3.4 Discussion*

In this study, we have made a significant discovery that stainless steel water dispensers (SWDs) can serve as reservoirs for various types of aeromonads. This finding consistently corroborates a study conducted in Brazil, which indicated that *A. hydrophila* (26%), *A. media* (23%), *A. bestiarum* (12%), *A. eucrenophila* (11%), and *A. veronii* (6%) were commonly present in drinking water (Razzolini *et al.,* 2008). It is interesting to note that a diverse range of aeromonads was identified in buildings B1 and B2, which were found to be the most frequently contaminated areas. This result resonates with the findings of Park *et al.* (2017), who suggested that frequently used water dispensers might facilitate the transfer of bacteria from external sources, resulting to the proliferation of diverse bacterial species around the faucet.

The presence of *A. hydrophila, A. veronii,* and *A. caviae* in SWDs may indicates potential health risks for individuals consuming the water (Environmental Protection Agency, 1998; Lamy *et al.,* 2010; USEPA, 2005; Boonhok *et al.,* 2021). Pathogenic *Aeromonas* spp. can lead to infections upon ingestion, inhalation, or skin contact with contaminated drinking water. Such infections can manifest intestinal and extra-intestinal ailments (Abbott *et al.,* 2003; Pessoa *et al.,* 2022). *A. hydrophila* is known for its virulence factors, like extracellular products and enzymes, including proteases, hemolysins, cytotoxins, and enterotoxins (Casabianca *et al.,* 2015). One of the most critical factors contributing to *Aeromonas* infection is the cytotoxin produced by *A. hydrophila*, which triggers cell signaling that leads to inflammation in human epithelial cells (Galindo *et al.,* 2006). On the other hand, *A. veronii* has many enzymes, such as caseinase, lipase, gelatinase, and hemolysin, that help it invade host cells. Various toxins also play an essential role in *A. veronii,* including enterotoxin, hemolytic toxin, and cytotoxin (Zhu *et al.,* 2022). According to Lamy *et al.* (2009), *A. veronii* and *A. caviae* have the exact pathogenic mechanism, and they can cause bacteremia and gastroenteritis in humans. To our knowledge, this is the first study to explore the presence of aeromonad contamination in SWDs in Thailand. Typically, assessments of microbial quality in drinking water primarily focus on detecting fecal indicator bacteria. However, waterborne pathogens like *Aeromonas* spp. can contaminate and persist in water distribution systems without being directly linked to feces (Scoaris *et al.,* 2008). This emphasizes the necessity of regularly pathogen monitoring in drinking water, water distribution systems, and water storage containers. Furthermore, investigating the levels of waterborne pathogen contaminants and their potential health effects is imperative.

The findings of previous studies align with the current understanding that *Aeromonas* spp. has an affinity for attaching to various surfaces, including pipe materials such as polyvinyl, polyethylene, and stainless steel (Kirov *et al.,* 2004; Eboigbodin *et al.,* 2008; Kregiel, 2013). In line with these observations, our research has identified multiple *Aeromonas* spp. within the inner walls of SWD faucets, highlighting their capacity to colonize and potentially compromise the dispensers. Microorganisms in drinking water distribution systems are often associated with biofilms or particle attachment (Wingender and Flemming, 2004). The attachment abilities of various bacteria to surfaces and their biofilm-forming potential are influenced by factors such as surface characteristics, environment conditions (temperature, pH, nutrients, and ironic strength), phenotype, and genotype (Thomas *et al.,* 2022).

Biofilms, which are structures formed by bacteria, contribute to enhanced virulence and pathogenicity by facilitating the spread of antimicrobial resistance and virulence genes (Van Houdt and Michiels, 2010). Furthermore, biofilm-forming bacteria tend to display significantly higher resistance to antimicrobials and disinfectants compared to their planktonic counterparts, exhibiting a resistance range of 500 to 5,000 times higher (El-Shekh *et al.,* 2010). Notably, up to 30% of Aeromonad's gastroenteric infections are associated with biofilms (Gracey *et al.,* 1982; Rautelin *et al.,* 1995; Costerton *et al.,* 1999). *Aeromonas* spp. has been identified as a contaminant by the Environmental Protection Agency (EPA) due to the high risk of water-borne diseases associated with it, as has been documented by Figueras and Borrego (2010). Therefore, the identification of strong biofilm-forming strains as *A. hydrophila, A. veronii,* and *A. caviae,* in our study may underscores potential health risks due to their pathogenicity. Additionally, the presence of biofilm-forming strains requires particular attention, as they may serve as reservoirs for other waterborne pathogens like *Pseudomonas aeruginosa, Enterococcus* spp., *Escherichia coli,* and *Clostridium perfringens* (Farkas *et al.,* 2012).

Although our investigation did not establish a direct correlation between the tested physicochemical parameters and biofilm-forming *Aeromonas* spp., it did unveil the ability of these bacteria to persist and thrive within SWDs during regular operation. *Aeromonas* spp. are ubiquitous in aquatic environments and can endure a broad range of temperatures (0 – 45 °C) and pH levels (4.5 – 9), with their optimal pH range being 5.5 to 9 (Isonhood and Drake, 2002; Igbinosa *et al.,* 2012). Consequently, our study demonstrates that SWDs provide favorable conditions for the growth of aeromonads. Several factors contribute to the increased aeromonads population in water, including stagnant piped water with prolonged retention times, high turbidity and organic matter levels, biofilms, and low disinfectant residual levels (Salvat and Ashbolt, 2019).

The presence of low levels of residual chlorine in public water systems can potentially result in harmful bacteria in drinking water. Specifically, *Aeromonas* spp. can endure free chlorine levels to 0.3 ppm (Burke *et al.,* 1984; Egorov *et al.,* 2011). Our study found that all drinking water samples investigated maintained a free chlorine level of 0.2 mg/L, a concentration deemed safe for consumption according to WHO guidelines. Nevertheless, considering the chlorine resistance of aeromonads, it might be necessary to explore the possibility of elevating chlorine levels as a preventive measure. Notably, a study from Korea indicated that using filter systems in drinking water dispensers to remove chlorine could lead to an increased concentration of bacteria within the dispenser (Park *et al.,* 2017). Our study involves a direct-piping type of SWD equipped with five filters designed to eliminate particles and residual chlorine. This may affect the growth of aeromonads. Additional assessment of both tap water and filtrate water quality is recommended to evaluate the effectiveness of the filter systems.

It is important to acknowledge that our study utilized a culture-based approach coupled with simple biochemical tests for bacterial species identification, which may have limited the detection of certain *Aeromonas* spp. Future studies should consider a combination of conventional and molecular identification techniques for more conclusive results. The accuracy of quantifying biofilm formation was solely reliant on visual observation, which could benefit from incorporating more precise and automated methods of measurement. Additionally, it is important to recognize that the ability to form biofilms can vary based on variables, such as incubation time, initial bacterial concentration, growth media, and the testing methodology employed (Ormanci and Yucel, 2017; Dias *et al.,* 2018).

**Table 2.** Distribution of *Aeromonas* spp. isolated from stainless steel water dispensers located in the university buildings

| Building | No. of SWD[a] | No. (%) of *Aeromonas* positive | No. of tested colony | No. (%) of identified *Aeromonas* spp. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *A. bestiarum* | *A. hydrophila* | *A. veronii* | *A. sobria* | *A. media* | *A. caviae* | *A. salmonicida* | *A. eucrenophila* | *Aeromonas* spp.[b] |
| B1 | 8 | 7 (87.5) | 21 | 8 (38.0) | 3 (14.3) | 5 (23.8) | 1 (4.8) | 2 (9.5) | 0 | 1 (4.8) | 1 (4.8) | 0 |
| B2 | 4 | 3 (75) | 9 | 1 (11.1) | 2 (22.2) | 4 (44.4) | 1 (11.1) | 0 | 0 | 0 | 0 | 1 (11.1) |
| B3 | 3 | 3 (100) | 9 | 9 (100) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B4 | 2 | 2 (100) | 6 | 1 (16.7) | 3 (50) | 0 | 0 | 0 | 2 (33.3) | 0 | 0 | 0 |
| B5 | 1 | 0 (0) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| B6 | 1 | 1 (100) | 3 | 1 (33.3) | 2 (66.7) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total | 19 | 16 (84.2) | 48 | 20 (41.7) | 10 (20.8) | 9 (18.8) | 2 (4.2) | 2 (4.2) | 2 (4.2) | 1 (2.1) | 1 (2.1) | 1 (2.1) |

[a] SWD, Stainless steel water dispenser; [b]Species unknown

**Table 3.** Biofilm formation ability of *Aeromonas* spp. isolated from stainless steel water dispensers

| *Aeromonas* spp. | No. of tested isolates | No. (%) of biofilm-forming aeromonad (isolate) | | |
|---|---|---|---|---|
| | | Weak (+) | Moderate (++) | Strong (+++) |
| *A. bestiarum* | 11 | 6 (54.5) | 3 (27.3) | 2 (18.2) |
| *A. hydrophila* | 6 | 1 (16.7) | 0 | 5 (83.3) |
| *A. veronii* | 5 | 1 (20) | 1 (20) | 3 (60) |
| *A. sobria* | 2 | 1 (50) | 1 (50) | 0 |
| *A. media* | 2 | 1 (50) | 1 (50) | 0 |
| *A. caviae* | 1 | 0 | 0 | 1 (100) |
| *A. salmonicida* | 1 | 0 | 1 (100) | 0 |
| *A. eucrenophila* | 1 | 0 | 1 (100) | 0 |
| Total | 29 | 10 (34.5) | 8 (27.6) | 11 (37.9) |

**Table 4.** Analytical results of the physicochemical parameters for drinking water from stainless steel water dispensers

| Characteristic | Unit | Reference value[a] | Location | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | B1 | B2 | B3 | B4 | B5 | B6 |
| pH | - | 6.50-8.50 | 6.87-7.32 | 7.05-7.14 | 7.16-7.27 | 7.14-7.19 | 7.28 | 7.33 |
| Color | Pt-Co | 15 or less | 5 | 5 | 5 | 5 | 5 | 5 |
| Turbidity | NTU | 5 or less | 0.09-0.15 | 0.13-0.20 | 0.11-0.18 | 0.12-0.13 | 0.15 | 0.11 |
| Residual free chlorine | mg/L | 0.2-0.5 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| Temperature | °C | - | 2-6 | 2-5 | 2-5 | 4-5 | 5 | 3 |

[a]WHO Guidelines for Drinking-Water Quality (2011)

## 4. Conclusion

The findings from this study establish the presence of diverse biofilm-forming *Aeromonas* spp. within SWDs. These outcomes shed light on their potential to colonize and subsequently compromise the SWDs, thereby undermining their intended functionality. Globally recognized pathogens such as *A. hydrophila, A. veronii,* and *A. caviae,* demonstrated a strong positive tendency for biofilm formation. The emergence of these pathogens within the drinking water sources raises a significant public health concern, necessitating a meticulous evaluation of drinking water quality. Therefore, it is imperative for public health professionals to recognize the existence of biofilm-forming microorganisms within SWD and other sources of drinking water. This recognition establishes the urgency of devising efficient strategies to mitigate their presence. Furthermore, this study serves as an initial inquiry, prompting a call for more comprehensive analyses to comprehensively evaluate the risks posed by biofilm-forming *Aeromonas* spp. It is essential to undertake regular pathogen monitoring within educational institutions and other public buildings, encompassing drinking water sources, water distribution systems, and water containers. Such proactive measures are indispensable for upholding and safeguarding public health.

## Conflict of interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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