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Microbial contamination analysis of drinking water from bulk dispensers and fast-food restaurants in the Eastern Coachella Valley, California

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ABSTRACT

Safe drinking water is a fundamental requirement for human life. The deterioration of water quality primarily involves microbiological hazards, since most evident water-related health problems are the result of microbial contamination. The aim of this study was to evaluate the microbial contamination of drinking water from three sources: water vending machines (WVMs), soda fountains (SFs), and tap water (TW) in the Eastern Coachella Valley (ECV) using physico-chemical parameters, conventional cultivable methods, including IDEXX technology and molecular methods. A total of 72 samples were analyzed and results indicated heterotrophic plate counts (HPCs) bacteria in 20% of samples from WVMs, 25% of samples from SFs, 33.3% of TW samples, and 76% of swab samples. Results also demonstrated 20% of WVM, 88% of swab samples, 41% of SF, and 50% of TW samples had total coliforms. Our qPCR indicated the presence of genetic materials of all six selected microorganisms (*Salmonella spp*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Campylobacter jejuni*, *Escherichia coli*, *Enterococcus faecalis*) used as indicators of pathogenic microorganisms in water from WVMs, SFs, and TW, and were represented at different concentrations and predominance.

Key words: disadvantage communities, microbes, restaurants, water dispensers

HIGHLIGHTS

- First study in the Eastern Coachella Valley to assess the water quality from soda fountains, water vending machines, swabs, and tap water.
- This study reinforces the health risk of biofilms in water dispensers and the importance to develop surveillance and regulation that target water dispensers.
- The study coupled physico-chemical parameters, DEXX Quanti-Trays, HPC, and qPCR.

1. INTRODUCTION

Drinking water is vital to human existence, and its bacterial contamination poses a serious public health threat worldwide. Despite the continued efforts to maintain water safety, waterborne outbreaks are still reported globally (Ramírez-Castillo *et al.* 2015). While drinking water quality can deteriorate through contaminants, such as toxic chemicals and microbes during transport, storage, and handling, distribution lines and systems may also influence the quality of drinking water (Bitton 2005). Approximately 2 billion people globally are obligated to utilize contaminated drinking water with excreta, while 1.2 billion people lack basic drinking water services, and more than 829,000 people die each year from contaminated drinking water (World Health Organization (WHO) 2022). Although the quality of tap water (TW) in most high-income countries is stringently regulated and monitored, the demand for bottled and dispensed water has been on the rise worldwide (Hu *et al.* 2011; Patel *et al.* 2016; Praveena *et al.* 2018). The consumption of bottled water has been increasing by at least 10% every year since 2008, with the fastest growth observed in Asia and South America (Gleick *et al.* 2012). In the United States, bottled water consumption has grown as much as 44% since 2010, with 9 out of 10 Americans wanting bottled water to be available whenever other drinks are sold, turning a $36.3 billion profit in 2020 (IBWA 2021). The use of different drinking water sources for human consumption instead of TW has been increasing (Girolamini *et al.* 2019).

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Examples of two new sources of drinking water are self-standing microfiltered water vending machines (WVMs) and drinking water from soda fountains (SFs). These are commonly preferred alternatives to bottled water, and appear to be environmentally friendly, overcoming bottled water disposal and pollution drawbacks. These sources are typically equipped with reverse osmosis or activated carbon filters that can remove chlorine taste, odors, and organic and inorganic contaminants (Ramírez-Castillo *et al.* 2015).

SFs are commonly used to dispense beverages in most fast-food establishments where consumers either dispense their own beverages in a purchased cup, or employees use SFs to dispense purchased beverages for customers. SF machines dispense carbonated soft drinks and drinking water. The global soda water dispenser market was valued at $1.0 billion in 2018 and is expected to grow at a compound annual growth rate of 5.4% from 2019 to 2025 (GVR 2022). The majority of reports of water contamination from SFs come from mass media sources (Cox 2010; Park 2010). Water quality studies of drinking water from SFs in fast-food restaurants are scarce (Hertin 2011; Hu *et al.* 2011; Godard *et al.* 2013) despite the rapid growth and use of these devices.

Self-standing WVMs (or simply WVMs) are generally located inside or outside grocery stores, pharmacies, and convenience stores where consumers are able to purchase drinking water while shopping or eating. WVMs are connected to TW from the local water district and make use of various filtration systems. The use of water from WVMs has considerably increased due to its affordability compared to bottled water. WVMs can dispense 5-gallon, 3-gallon, and 1-gallon units of water, depending on the funds inserted into the machine. The largest WVM provider in California is the Primo Water Company, which owns the Glacier vending machine network. In 2001, the non-profit organization, Environmental Working Group, released a report in which it verified that the Glacier Water Company reported over 60% of its sales went to Latino or Asian customers (Bitton 2005; WHO 2022). It has been demonstrated that WVMs are generally located in low-income and immigrant communities (Cardaci *et al.* 2016). Although microbial contamination of drinking water from WVMs is understudied, Hile *et al.* (2020) reported the presence of genetic material from *Salmonella spp*, *Listeria monocytogenes,* and other pathogenic microorganisms from WVM in the Coachella Valley of Southern California. Of these samples, Hile *et al.* (2000) found that 32% had coliforms and 21% had heterotrophic plate counts (HPCs). Although outbreaks related to the consumption of bottled water are rarely reported, they nevertheless do occur (CDC 2020). In Italy, a study by Liguori *et al.* (2010) found that HPCs counted at 22 °C were 71 and 86% higher than the allowable values in non-carbonated water and carbonated water, respectively, and at 37 °C, HPCs counted were 81 and 88% for non-carbonated and carbonated water, respectively. The United States has several federal-, state-, and county-level drinking water regulations, although there is only limited regular monitoring and unenforced authority for drinking water from WVMs and SFs (White *et al.* 2010).

Waterborne pathogens and their related diseases are a major public health concern worldwide (Ramírez-Castillo *et al.* 2015). The presence of pathogenic microorganisms in drinking water is a serious public health concern and cannot be overemphasized. Although waterborne outbreaks have considerably declined over the past 20 years (Jacobsen & Koopman 2004; Levy *et al.* 2018; Barrett 2019; Gharpure *et al.* 2019), waterborne microbial agents, such as *Salmonella typhimurium, Vibrio cholerae, Legionella, E. coli O157:H7, and Pseudomonas* have been implicated in acute gastrointestinal illnesses, acute respiratory illnesses, hepatitis, and several deaths (Ramírez-Castillo *et al.* 2015). In the United Kingdom, and many other developed countries, *Campylobacter jejuni* is the cause of most rapid onset of gastrointestinal infections resulting in acute morbidity and mortality, with an estimated 2 million cases per year, and mortalities estimated to be greater than 2,000 people annually. In these cases, the majority of infections are sporadic and the sources of infection are rarely determined (Cowden 1992).

It has been demonstrated that water quality deterioration may often be related to biofilm formation (Farhadkhani *et al.* 2014). In devices such as WVMs and SFs, high surface-to-volume ratios, the absence or low concentrations of residual chlorine, and stagnation periods are all factors that influence bacterial growth and proliferation (Farhadkhani *et al.* 2014). Biofilms are organized communities of organisms widely present in nature that represent serious problems in environmental, industrial, and medical settings (Szymanska 2003). They also play an important role in bacterial persistence in water lines and water systems, shielding them from disinfectants and adverse environmental conditions (Farhadkhani *et al.* 2014). Additionally, these films can also harbor pathogenic microorganisms (Flemming 2002), causing serious public health concerns.

There is a well-known disparity (Madison 2019) in drinking water monitoring where urban high-income areas have better monitoring and water quality than rural areas. This disparity exists in the rural and unincorporated communities known as Thermal, Oasis, Mecca, and North Shore located in the Eastern Coachella Valley (ECV) of Riverside County, Southern

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California, USA. In these communities, the water contamination issue is well known among community members and stigmatized due to previous reports of arsenic contamination in groundwater. This contamination is present for many of the rural residents who live in mobile homes that are supplied with untreated well water (Pierce & Jimenez 2015).

Given the importance of drinking water safety, this study was conducted to assess the quality of drinking water from WVMs and SFs in the ECV and compare them to TW collected in the community. We hypothesized that drinking water from WVMs and restaurants in the ECV is contaminated with pathogenic bacteria and that our swab samples from the machine spigots contain more bacteria than samples from the corresponding bulk water samples. We also postulated that drinking water from SFs is contaminated with elevated bacterial colony-forming units (CFUs) and pathogenic microorganisms. We hypothesized that TW samples from the ECV have fewer bacteria than water samples from WVMs and SFs.

Because our overall aim is to improve the quality of drinking water in this community, we adopted a problem-solving method that characterized the physico-chemical parameters that influence the microbial contamination. We focused on identifying the presence of DNA of select microbes with parallel assessments of pH, electrical conductivity (EC), free chlorine, and total dissolved solids (TDS). This problem-solving focus recognizes that microbial contamination could be exacerbated by other water quality issues. We used quantitative polymerase chain reaction (qPCR) for the identification of the six selected microorganisms.

2. MATERIALS AND METHODS

2.1. Study site

The present study was carried out in the ECV, a desert area situated within the Imperial Valley in Southern California (33°36′46.9″N, 116°08′47.2″W) characterized by an arid climate. It extends southeast into Riverside County for approximately 72.4 km, from the San Bernardino Mountains to the northern shore of the Salton Sea. It is limited on the West by the San Jacinto Mountains and the Santa Rosa Mountains, and on the North and East by the Little San Bernardino Mountains (Figure 1). The ECV includes the city of Coachella and the unincorporated communities of Thermal, Oasis, Mecca, and North Shore. These communities are largely Latino, made up of approximately 14% of undocumented immigrants and agricultural-worker families that contribute approximately $430 million per annum to the gross domestic product of the USA (PUCDC 2020).

2.2. Sample collection

Prior to sampling, we sterilized sampling bottles (1-L glass bottles) for WVMs, and metal water bottles (0.5 L) for SFs, by washing with soap and bleach, then rinsing with deionized water before autoclaving at 121 °C for 15 min. Bottle tops and SF sampling bottle covers (made of plastic) were immersed in a water bath before autoclaving to prevent melting. We aseptically added 1 mg of sodium thiosulfate to each 1 L bottle used for WVMs, and 0.5 mg of sodium thiosulfate to each 0.5 L bottle used to sample water from SFs, to neutralize the chlorine (Cl_2) in sampled water.

In the field, we collected a total of 2 L of water samples from each WVM, and swabbed the interior surface of spigots which were uniformly 5 cm deep and 2 cm wide in diameter. We additionally collected 50 mL of water sample for physico-chemical parameters that were measured on site. We also collected a matched tap water (TW) sample from the same building that provided water to the WVM. We used the HM digital TDS-4 meter (HM digital®, Redondo Beach, California, USA) to measure total dissolved solids (TDS) in $mg{\cdot}L^{-1}$ and EC in $\mu S{\cdot}cm^{-1}$. We measured temperature (°C) and pH using a portable Omega meter (Connecticut, USA), and free Cl_2 ($mg{\cdot}L^{-1}$ Cl_2) using the HACH DR 300 DPD colorimetric method 8021 (Hach, Loveland, Colorado, USA). Instruments used for physico-chemical parameters were all calibrated in the laboratory prior to sampling according to manufacturer instructions. Each sampling bottle was labeled with the WVM code, sampling date, time, and site. Collected water samples were immediately placed on ice in a cooler and transported to the laboratory, where samples were immediately processed within 6 h. Unless otherwise indicated, all samples were processed at room temperature in duplicates.

We collected 0.5 L of drinking water from SFs by either directly placing a sterile sampling bottled under the water spigot of a soda dispenser in a fast-food restaurant, or by requesting a water sample from a restaurant staff member. The fast-food restaurants were not aware of the study. We purchased a meal or a small drink before collecting water from their dispensers. For water sampled directly from the SFs, care was taken not to contaminate the lid during the opening and closing of the sample bottles. For water samples handed out by waiters in a cup, we carefully transferred SF water samples into sterile water bottles. All samples were labeled and within 1 h transported on ice to our laboratory for analysis.

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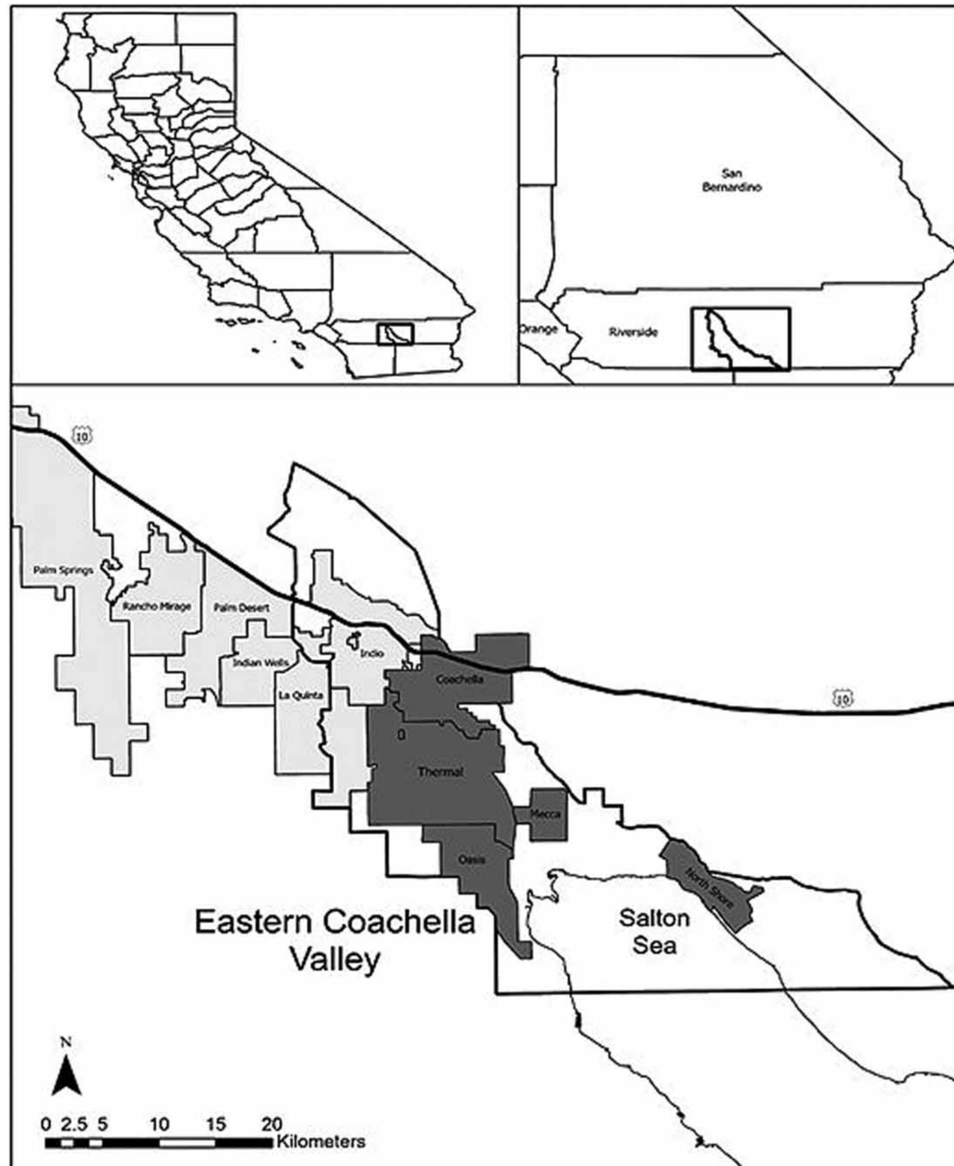

Figure 1 | Map featuring California State, San Bernardino County, and the Eastern Coachella Valley.

2.3. Heterotrophic plate counts

We prepared 100 mm plates for heterotrophic bacteria plate counts using BD™ Difco™ R2A Agar (Fisher Scientific, Ontario, California, USA) according to the manufacturer's recommendations, a day before sample collection.

To test for heterotrophic bacteria in our samples, we streaked 100 μL of water sample directly onto prelabeled R2A agar plates in duplicates without dilution (10^0) and 1 mL of water sample was diluted 1:10 with sterile phosphate buffer saline (PBS) in three microcentrifuge tubes. All streaked R2A plates were incubated at 35 °C for 48 h. Colonies were counted afterward, and results expressed as colony-forming units per milliliter ($CFU \cdot mL^{-1}$) for WVMs and tap water samples. For swab samples, the inner surface area for the spigot was swabbed (approximately 1 cm radius × 5 cm height = 34.5 cm^2) and was used to calculate HPCs from swabs in $CFU \cdot cm^{-2}$. We also set the detection limit for HPC to be below 10 $CFU \cdot mL^{-1}$.

2.4. Coliforms/*E. coli*

We used the Colilert reagent test (IDEXX, Maine, USA) to detect and count total coliform and *E. coli* cells in water samples. We mixed 100 mL of water samples with Colilert reagent in a sterile 100-mL bottle that was later sealed and shaken

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thoroughly to mix. This solution was poured into a Quanti-Tray labeled with the sample identification number and sealed with the IDEXX Quanti-Tray Sealer. Quanti-Trays were incubated at 35 °C for 24 h, and the presence of total coliform was confirmed by a yellow color in Quanti-Tray wells. We determined the presence of *E. coli* by fluorescence emitted when Quanti-Trays were exposed to long-wave (365 nm) UV light.

2.5. Filtration

After physico-chemical and Quanti-Trays measurements, we assembled a Whatman vacuum filtration system (Cleves, Ohio, USA) to filter approximately 1,800 mL of water sample from each WVM visited, and 0.5 L from each fast-food restaurant the same day through a 0.45 μm MF Millipore™ membrane (Millipore Sigma, USA). All glass funnels and filter frit were sterilized by bleaching, then rinsing with deionized water, then autoclaving. We further sterilized the glassware using a benchtop U.V. sterilizer (Millipore, Sigma, USA) for approximately 5 min. After the vacuum and filter were assembled, each water sample was poured in and filtered. Each filter was aseptically removed after filtration and placed in a labeled sterile 50-mL micro-centrifuge tube, containing 10 μL of sterile PBS. A water bath sonicator was used for 5 min at 37 °C to remove bacteria from the filters. Samples were further centrifuged at 12,000 rpm for 5 min for DNA extraction.

2.6. In-house standard curve

The quantitative analyses from qPCR were completed by creating standard curves with the reference microbes. Ten-fold serial dilutions of genomic DNA extracted from stock solutions were prepared. In this study, we used stock solutions of *Salmonella typhimurium* (ENVH Carolina 155351A), *L. monocytogenes* (ATCC® 7644), *Pseudomonas aeruginosa* (ATCC® 27853), *Enterococcus faecalis* (ATCC® 29212), *C. jejuni* (ATCC® 33291 and *Escherichia coli* (ATCC® 25922). The stock microorganisms were enriched overnight in liquid BD™ trypticase soy broth (Fisher Scientific, USA) to an approximate concentration of 1E8·mL^{-1} for *P. aeruginosa*, *S. typhimurum*, and *E. coli*. We used an approximate concentration of 1E6·mL^{-1} for *L. monocytogenes* and *C. jejuni*. To further prepare the stock for the standard curve calculation, we used selective media to validate the concentration in each stock solution. From there we used a DNA extraction kit on the stock solution in parallel with our plate count methods so that we could have a CFU·mL^{-1} estimates and a DNA extraction for the same stock. To estimate CFU·mL^{-1}, plate colonies were counted the next day and averaged to determine the original suspension. The suspensions were serially diluted 1:10 in PBS, and 100 μL of the last three dilutions were plated in duplicate on selective media, then incubated overnight at 35 °C. In addition, 1.5 mL of the suspension was used for immediate DNA extraction. A 10-fold dilution series was then created from each template and assayed in duplicate using a SYBR Green I assay (Sigma–Aldrich, St. Louis, Missouri, USA). Regression lines from the dilution curve with $R^2 > 0.95$ were used to determine the concentration of unknown samples.

2.7. DNA extraction

To extract DNA from environmental microorganisms, we used GenElute™ Bacterial Genomic DNA KIT (Sigma–Aldrich, Missouri, USA). For field samples, we used the modified gram-positive extraction procedure described by the manufacture. After filtration and sonication, we centrifuged samples for 2 min at 12,000 rpm without the overnight enrichment step recommended by the manufacturer, to preserve original concentrations. Pelleted cells were resuspended in 200 mL of lysozyme solution and incubated for 30 min at 37 °C. We then added 20 μL of Proteinase K solution to the sample, followed by 200 μL of lysis solution, then incubated at 55 °C for 10 min after thorough vortexing. Columns were prepared by adding 500 μL of the column preparation solution to each pre-assembled GenElute Miniprep Binding Column and centrifuging for 1 min at 12,000 rpm. We then added 200 μL of 95% ethanol to the lysate and mixed thoroughly. The entire lysate was transferred into the binding column and centrifuged at 12,000 rpm for 1 min, after which the column was placed in a new collection tube and 500 μL of wash solution # 1 was added to the column and centrifuged for 1 min at 12,000 rpm. After discarding the eluate, we placed 500 μL of wash solution # 2 into the binding column and centrifuged for 3 min at 12,000 rpm. For DNA elution, we poured 200 μL of elution solution onto the column and allowed it to incubate for 5 min at room temperature, then centrifuged it for 1 min at 8,000× *g*. DNA concentration was estimated using a Nanodrop™ 1000 (Thermo-Scientific, Ramsey, Minnesota, USA).

2.8. Primer design

Primers used in this study were designed using the SILVA database (Quast *et al.* 2012). We also cross-checked all primers in the literature, and provided references as listed in Table 1. We selected primers based on annealing temperature, small

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Table 1 | Primer sets of selected microorganisms with their various amplicon sizes and justification references used in qPCR

Target microorganisms		Primer sets (5′–3′)	Size	Justification	References
Salmonella spp.	F	FGGAAACGGTGGCTAATACC	103	Local	Liu *et al.* (2018)
	R	CCTCACCAACAAGCTAATCC		contaminant	
Listeria monocytogenes	F	GATGATCAGGTAGATAGGTTTGG	119	Local	Gião & Keevil (2014)
Campylobacter jejuni	R	CCTAACTGAGCCCTTTCTTC		contaminant	
	F	CCCTATCAAACTCCGAATACC	93	Local	Whiley *et al.* (2013)
Pseudomonas aeruginosa	R	GGTAGTCTGGGTTGTTTCC		contaminant	
	F	GAGCAGGTTGAAGGTTAGG	111	Similar	Liguori *et al.* (2010)
Enterococcus faecalis	R	GCTAATCAAGCTCGGAGATAG		study	
	F	TTGTGTTATGAACCCTCTAACC	124	Indicator	Girolamini *et al.* (2019)
E. coli	R	GGTTCCCTCAGAATGGTTG		bacteria	
	F	CTATGTGTTGTTGGGTAGGG	423	Indicator	Luby *et al.* (2015)
	R	GATGTTACCTGATGCTTAGAGG		bacteria	

amplicon size, and specificity to the selected microorganisms. Specificity of each primer was tested using Primer-Blast-NCBI (Boratyn *et al.* 2019).

2.9. Real-time PCR (qPCR)

Real-time PCR was performed using a C1000 Touch Thermal Cycle CFX 96 (Bio-Rad, Hercules, USA). Each 96-well plate reaction mixture (20 μL) contained 10 μL of iTaq Universal SYBR Green Supermix (Bio-Rad, California, USA), 7 μL of nanopure distilled water, 1 μL of forward primers, 1 μL of reverse primers, and 1 μL of the extracted sample's genomic deoxyribonucleic acid (gDNA). In the 96-well plate set-up, we had positive control wells with gDNA from target microorganisms, negative controls with only deionized water, and a common mix without the sample gDNA as our no-template control (NTC). This NTC allowed quality control for contamination, and any plates with compromised NTCs were not used. The thermocycling program was 40 cycles at 95 °C for 3 min for the initial cycle, 95 °C for 10 s, and 55 °C for 30 s. All amplifications and standards were run on the same CFX 96 Real-Time instrument.

3. RESULTS

3.1. Physico-chemical parameters

A total of 72 samples were collected in this study. The physico-chemical parameters considered were limited to temperature, EC, pH, free chlorine, and TDS. Table 2 presents physico-chemical parameters for WVMs. Water temperature from WVMs ranged from 18.2 °C for WVM11 to 32.8 °C for WVM13.

The physicho-chemical results for SFs are presented in Table 3. Except for SF11 that presented a water temperature of 32 °C, all SF water temperatures ranged from 14.3 to 28 °C. We also recorded pH ranging from 4.9 to 8.1 for SF7 and SF3, respectively.

The physico-chemical parameters of TW samples are presented in Table 4. We found the average Cl_2 was 0.12·mg L^{-1} with a maximum of 0.5 mg·L^{-1}. When comparing WVMs, TW and SF, TW samples had the highest average pH at 5.12. Our results also indicated that TDS ranged from 16 mg·L^{-1} for TW16 to 3,720 mg·L^{-1} for TW10. The EC recorded ranged from 0.3 μS·cm^{-1} for TW14 to 34 μS·cm^{-1} for TW10. When comparing physico-chemical parameters of WVMs, SFs, and TW, we observed that WVMs had the highest water temperature followed by TW. However, for EC, pH, and Cl_2, TW ranged higher compared to WVM and SF samples (Figure 2).

3.2. Microbiological analysis

Results of bacteriological analysis of WVMs ($n = 24$), swabs from WVMs ($n = 24$), SFs ($n = 12$), and TW ($n = 12$) are presented in Tables 5–8, respectively. Results for HPC in Table 5 demonstrated that 20% of WVMs had HPC above 500 CFU·mL^{-1}. Table 5 also indicated that WVM10, WVM12, WVM13, and WVM16 had total coliforms. No microbial growth was observed in the negative control blanks for HPCs or IDEXX methods.

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Table 2 | Physico-chemical parameters including temperature, electrical conductivity (EC), total dissolved solids (TDS), pH, and free chlorine, measured from water vending machines (WVMs) of the Eastern Coachella Valley

Sample	Temp (°C)	EC ($\mu S \cdot cm^{1}$)	TDS ($mg \cdot L^{-1}$)	pH	Free chlorine ($mg \cdot L^{-1}$)
WVM1	19.45	2.5	1.81	6.99	0.2
WVM2	30.55	0.8	0.51	6.91	0.1
WVM3	30.5	1.2	0.80	6.67	0.26
WVM4	28.4	2.1	1.49	7.41	0.1
WVM5	28.05	1.2	0.80	7.31	0.1
WVM6	32.15	7.4	6.06	7.36	0.02
WVM7	26.8	0.8	0.51	7.58	0.1
WVM8	30.9	2	1.42	7.05	0.1
WVM9	25	1.2	0.80	6.96	0.05
WVM10	22.5	0.1	0.05	7.41	0.1
WVM11	18.2	0.1	0.05	6.65	0.1
WVM12	19.4	2.7	1.98	7.38	0.05
WVM13	32.8	2.1	1.49	7.14	0.12
WVM14	32.1	0.1	0.05	7.31	0.08
WVM15	8.1	0.3	0.17	7.77	0.07
WVM16	28.5	0.1	0.05	7.92	0.2
WVM17	29.5	0.3	0.17	7.59	0.1
WVM18	20.1	5.4	4.27	7.49	0.08
WVM19	26	0.2	0.11	7.22	0.03
WVM20	29	32	30.89	6.12	0.1
WVM21	22	<1	0.05	5.9	0.01
WVM22	29	9	7.54	5.57	0.1
WVM23	27	0.3	0.17	6.4	0.1
WVM24	31	41	40.70	5.9	0.03
WVM25	32	28	26.63	6.2	0.01
WHO	/	400–600	50–250	6–8.5	<0.2

We present results from microbial analysis of swabs in Table 6, that demonstrate that 76% of swabs had HPC above 500 CFU.mL^{-1}. Results for the presence of total coliforms for swab samples are also presented in Table 6 and ranged from 3.1 to 435.2 MPN·100 mL^{-1}. We found that 60% of swabs had total coliforms. In Table 7, we show 25% of SF samples had HPC values above 500 CFU·mL$^{-1.}$ We also show in Table 7 that 42% of SFs had total coliforms. For TW, we demonstrate in Table 8 that 33% of samples collected had HPC values above 500 CFU mL$^{-1,}$ and 50% of collected samples had total coliforms. We compare the concentration of HPCs from WVMs, swabs, SFs, and TW in Figure 3 and found that TW samples had the highest concentrations of HPCs, followed by swab samples. We also compared the concentration of total coliforms between WVMs, swabs, SFs, and TW samples, shown in Figure 4 and found that SFs had the highest concentrations of total coliforms, followed by swabs. We also observed that water in SF water bottles handled by employees were all positive for coliforms. In Figure 5(a), we demonstrated that *E. faecalis* was the predominant gene targets identified in WVMs when using the qPCR quantification method described in the current study. We also show in Figure 5(b) that *Salmonella spp* was the most identified gene target in TW samples, followed by *P. aeruginosa*, when using qPCR. In swab samples, *P. aeruginosa* was the most predominant gene target identified, followed by *L. monocytogenes*, as shown in Figure 5(c). When comparing swabs and WVMs for microbial gene target concentration using our qPCR method, we found that except for *E. faecalis* dominance in WVMs, all identified selected microorganism gene targets predominated in swab samples (Figure 6).

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Table 3 | Physico-chemical parameters including temperature, electrical conductivity (EC), total dissolved solids (TDS), pH, and free chlorine, measured from fast-food soda fountains (SFs) of the Eastern Coachella Valley

Fast-food ID	Temp (°C)	EC (μS·cm^{-1})	TDS (mg·L^{-1})	pH	Free chlorine (mg·L^{-1})
SF1	19.3	0.1	0.05	6.5	0
SF2	22.5	0.2	0.11	6.12	0.16
SF3	18.2	0.1	0.05	8.1	0
SF4	24	0.1	0.05	6.45	0.02
SF5	16.3	0.27	0.15	5.43	0.17
SF6	15	0.3	0.17	7.39	0.12
SF7	18	0.37	0.22	4.95	0.14
SF8	18.2	0.21	0.12	6.19	0
SF9	14.3	0.29	0.17	6.16	0.06
SF10	25	0.1	0.05	6.82	0
SF1	32	0.1	0.05	7.05	0
SF12	28	0.2	0.11	7.9	0.02
WHO	/	400–600	50–250	6–8.5	<0.2

Table 4 | Physico-chemical parameters including temperature, electrical conductivity (EC), total dissolved solids (TDS), pH, and free chlorine, measured from tap water (TW) of the Eastern Coachella Valley

Codes	Temp (°C)	EC (μS·cm^{-1})	TDS (mg·L^{-1})	pH	Free chlorine (mg·L^{-1})
TW1	24	20.3	18.63	/	/
TW2	22	20.3	18.63	/	/
TW3	21	17	15.29	/	/
TW7	21	17	15.29	/	/
TW9	21	17	15.29	/	/
TW10	22	34	33.05	/	/
TW13	29.8	1.2	0.80	8.28	0.5
TW14	27.4	0.3	0.17	8.75	0.01
TW15	27.2	0.5	0.30	7.54	0.16
TW16	28	0.6	0.37	9.12	0.02
TW17	29.5	0.6	0.37	8.78	0.02
TW18	21.9	0.4	0.24	7.77	0.02
WHO	/	400–600	50–250	6–8.5	<0.2

/ indicates missing data.

4. DISCUSSION

Although illness outbreaks related to the consumption of bottled or dispensed water are rarely reported, they do occur (Cardaci *et al.* 2016). Waterborne pathogens and related diseases are major public health concerns worldwide. However, it has been demonstrated that risk of contamination in drinking water is higher in rural areas than urban areas (Madison 2019). Moreover, drinking water quality analyses from WVMs and SFs are scarce. To the best of our knowledge, microbial contamination of drinking water from SFs, TW, WVMs and nozzle swabs of WVMs have not been previously evaluated, quantified, or analyzed, especially in the ECV. The goal of this study was to evaluate the quality of drinking water from WVMs, using 5-gallon jugs, nozzle swabs of WVMs, SFs, and TW in the ECV, using cultivable and molecular methods for estimates of contamination.

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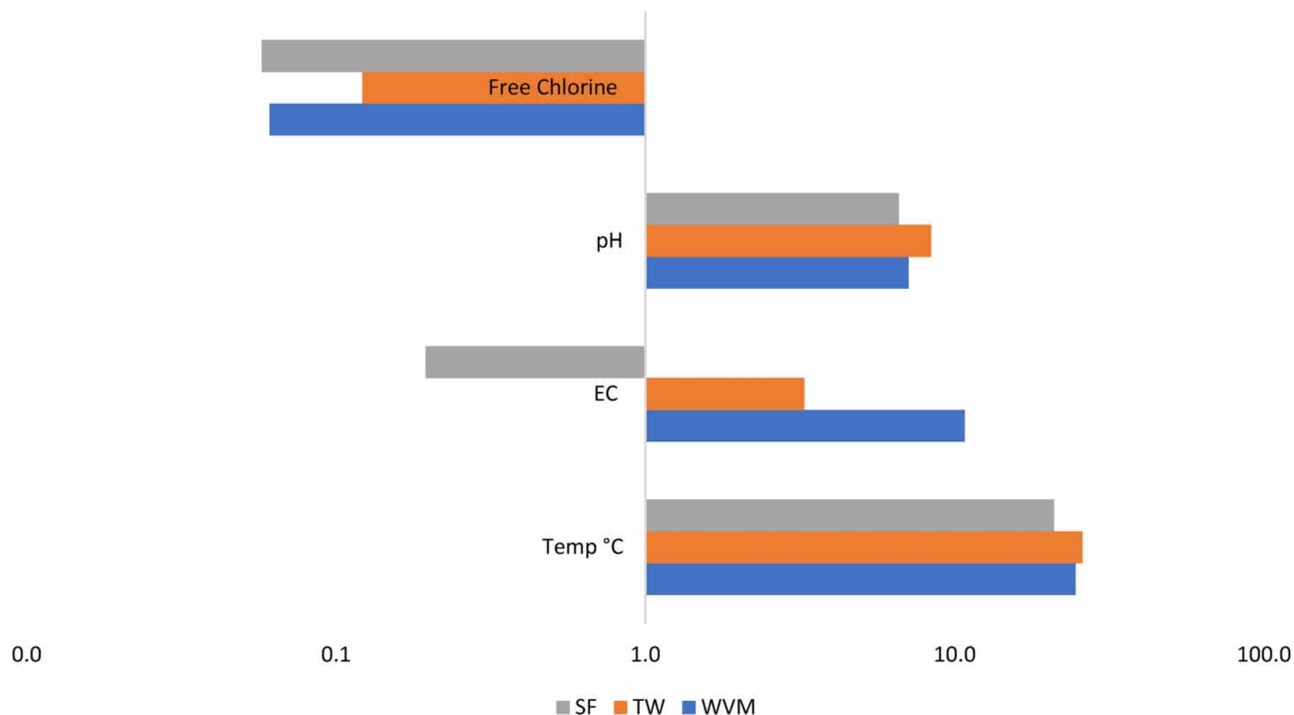

Figure 2 | Averages of physico-chemical parameters for water vending machines (WVMs), soda fountains (SFs), and tap water (TW) of the Eastern Coachella Valley.

Results for physico-chemical parameters indicated that the mean temperature of WVMs varied. These differences in mean temperature may be explained by their individual locations, and their relative exposure to ambient heat or direct sunlight. SFs are generally located indoors (in fast-food restaurants) and connected to cooling systems for customer convenience, and consequently are much cooler than other sources, such as WVMs and TW. Tap water samples were generally collected from outdoor TW spigots, where garden hoses were connected, or indoors, from sinks that are connected through ground pipes to the local water district. The majority of WVMs however, were at times exposed to ambient desert heat, resulting in elevated water temperatures. Nevertheless, drinking water temperature does not necessarily have a direct health impact, other than on aesthetic qualities. However, high temperatures between 25 and 42 °C have previously been implicated in robust biofilm formation, more so than for low temperatures (4–10 °C) (Roy *et al.* 2021). Our study showed that 72% of WVM, 25% of SF, and 41% of TW samples had adequate water temperatures for the formation and growth of biofilms, which can harbor pathogenic microorganisms and therefore pose threats to human health and public safety. These findings are supported by a study by Farhadkanhi *et al.* (2014) in Iran which found that temperature had a significant effect on the HPC population in water coolers.

The pH measures the acid–base equilibrium achieved by compounds dissolved in water (Masood *et al.* 2015). Although drinking water pH does not necessarily impact human health directly, it has been demonstrated that high pH values (above 7.5) may cause pipes to be encrusted with deposits, while low-pH water may cause corrosion of pipe metals potentially impacting water quality (USGS 2019). However, in the current study, we found no health concerns related to water pH level.

Free Cl_2 indicates the water source has been treated to kill disease-causing microorganisms. The Centers for Disease Control and Prevention (CDC) recommends a range of 0.2–2 $mg{\cdot}L^{-1}$ of Cl_2 in TW (CDC 2014), while the World Health Organization (WHO) maximum allowable value for Cl_2 concentrations in drinking water is 5 $mg{\cdot}L^{-1}$ (WHO 2017). Our results indicated that Cl_2 measurements obtained from WVMs, and SFs fall within the recommended CDC and WHO requirements. For drinking water from WVMs and SFs, we found that Cl_2 levels were mostly far below WHO recommendations, indicating filtration efficiency used by WVMs and SFs that effectively remove Cl_2.

Although no guidelines have been established for drinking water TDS limits by the EPA, WHO recommends 600 $mg{\cdot}L^{-1}$ or less for aesthetic reasons (WHO 2017). We found that 83% of TW samples had TDS above the WHO recommended value. These results may be due to human activities or water sources, such as groundwater, which came from wells with high

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Table 5 | Biological characteristics of water vending machines (WVMs) in the ECV including HPCs

Samples	HPCs ($CFU \cdot mL^{-1}$)	Total coliforms ($MPN \cdot 100\ mL^{-1}$)
WVM1	6.2×10^3	<1
WVM2	<10	<1
WVM3	<10	<1
WVM4	3.2×10^2	<1
WVM5	<10	<1
WVM6	<10	<1
WVM7	<10	<1
WVM8	9.1×10^3	<1
WVM9	<10	<1
WVM10	<10	8.5
WVM11	<10	<1
WVM12	<10	13.2
WVM13	3.6×10^4	3.1
WVM14	3.0×10^2	<1
WVM15	<10	1
WVM16	<10	435.2
WVM17	<10	<1
WVM18	<10	<1
WVM19	<10	<1
WVM20	<10	<1
WVM21	<10	<1
WVM22	<10	<1
WVM23	<10	<1
WVM24	<10	<1
WVM25	<10	<1
EPA	<500	0

concentrations of soil. Similar results were found by both Wei *et al.* (2013) in China in his study of characterizing urban runoff pollution between dissolved and particulate phases, and Roșca *et al.* (2020) in Romania in their study on the impact of anthropogenic activities on water quality parameters of glacial lakes. The TDS concentrations above the WHO recommendation indicate a problem with either the filtration system or calcification of premises plumbing that accumulated over time leading to elevated TDS at the point of use. However, WVMs and SFs had TDS far below WHO recommended value. This indicated the potential effectiveness of filtration systems used in WVMs and SFs that can remove high concentrations of TDS from tap water to which they are connected.

Maintaining the microbiological quality of drinking water between the water treatment facility and the consumer's tap constitutes a serious challenge in drinking water safety (Fengyi *et al.* 2009; Sacchetti *et al.* 2014). Consequently, the evaluation of microbial quality of drinking water from WVMs, SFs and TW, is necessary to ensure compliance with quality standards. Heterotrophs are microorganisms that require organic carbon for growth. However, only a small proportion of microorganisms present in water are able to grow and become detectable in HPC test conditions (Bartram *et al.* 2003). The EPA sets the maximum permissible level of heterotrophic bacteria in drinking water at 500 $CFU \cdot mL^{-1}$ (EPA 2021). Our results indicated that HPC bacteria were present in 20% of samples from WVMs, 25% of samples from SFs, 33.3% of TW samples, and 76% of swab samples. Similar results were found by Boonhok *et al.* (2021) in Thailand, Phiri *et al.* (2021) in New Zealand and Ang & Tham (2020) in Malaysia who all reported bacterial counts above the stated drinking water limits for HPC ($<500\ CFU \cdot mL^{-1}$) in raw water samples. The very high HPC concentrations from swab samples and low HPC concentrations in WVM bulk

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Table 6 | Biological characteristics of water vending machines (WVMs) swabs in the ECV including heterotrophic plate counts (HPCs)

Samples	HPCs (CFU·cm^{-2})	Total coliforms (MPN·1 cm^{-2})
Swab1	29	7.4
Swab2	3.3×10^3	172.3
Swab3	20	435.2
Swab4	1.1×10^3	28.2
Swab5	2.0×10^3	111.9
Swab6	1.1×10^4	4.1
Swab7	1.9×10^3	7.5
Swab8	9.5×10^2	13.4
Swab9	1.2×10^3	435.2
Swab10	2.9×10^3	410.6
Swab11	46	435.2
Swab12	2.4×10^3	7.4
Swab13	31	10.7
Swab14	1.2×10^2	435.2
Swab15	3.5×10^3	54.4
Swab16	22	23.2
Swab17	1.6×10^3	3.1
Swab18	29	<1
Swab19	7.2×10^4	435.2
Swab20	<10	435.2
Swab21	<10	<1
Swab22	<10	435.2
Swab23	<10	<1
Swab24	<10	10.7
Swab25	<10	435.2
EPA	<500	0

water samples may be explained by the presence of biofilms in spigots of WVMs, rather than the water samples themselves (Fengyi *et al.* 2009). This suggestion is supported by Cardaci *et al.* (2016) in their study in Italy about contamination in automatic vending-machines, where nozzles of WVMs were the most contaminated area. Additionally, Muhammad *et al.* (2020) in Malaysia in their study on microbiological analysis of drinking water from WVM found that all WVMs nozzles were contaminated with *Stenotrophomonas, Pseudomonas, and Bacillus.* In our study, we found 80% of WVMs had no HPC, yet 76% of swabs from the same WVMs had HPC above 500 CFU·mL^{-1}, exceeding the acceptable level for drinking water set by the EPA. The formation of biofilms in WVMs is a serious problem that exposes purified and filtered water to potentially pathogenic microorganisms forming biofilms that can leach out into drinking water bought by consumers. In Iran, Farhadkhani *et al.* (2014) found that rubber-lined hoses had high levels of plastic, which encouraged bacterial growth. Thus, waterlines made of plastic material, and used for WVMs offer the ideal environment for biofilm formation (Zanetti *et al.* 2009). Moreover, narrow bore spigots made of plastic material constitutes a suitable surface for microorganism adhesion and biofilm formation (White *et al.* 2010; Sacchetti *et al.* 2014). Therefore, the high concentration of HPCs in swab samples, rather than in the WVMs themselves, is an indicator of likely biofilm formation in the spigots. Contamination of spigots may occur over time by the accumulation of microorganisms in WVM plastic water line systems. The present study supports previous findings by Farhadkhani *et al.* (2014) in Iran, Gião & Keevil (2014) in UK, and Wingender & Flemming (2011) in Germany in their studies assessing of drinking water quality from bottled water coolers. These authors all found biofilms in tap water that enter into the viable but non-cultivable state, and all concluded that the spigots of water dispensers are

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Table 7 | Biological characteristics of drinking soda fountains (SFs) water in the ECV including heterotrophic plate counts (HPCs)

Samples	HPCs ($CFU{\cdot}mL^{-1}$)	Total coliforms ($MPN{\cdot}100\ mL^{-1}$)
SF1	<10	<1
SF2	<10	2,419.6
SF3	2.03×10^3	2,419.6
SF4	<10	22.6
SF5	<10	5.3
SF6	5.40×10^4	2,419.6
SF7	<10	<1
SF8	<10	<1
SF9	<10	<1
SF10	4.30×10^4	3.1
SF11	<10	<1
SF12	<10	<1
EPA	<500	0

Table 8 | Biological characteristics of tap water (TW) in the ECV including heterotrophic plate counts (HPCs)

Samples	HPCs ($CFU{\cdot}mL^{-1}$)	Total coliforms ($MPN{\cdot}100\ mL^{-1}$)
Tap 1	<10	<1
Tap 2	<10	11.15
Tap 3	<10	<1
Tap 7	<10	45.65
Tap 9	<10	<1
Tap10	<10	<1
Tap 13	1.1×10^6	<1
Tap 14	8.3×10^4	3.6
Tap15	<10	138.5
Tap16	9.2×10^4	6.55
Tap17	3.0×10^4	<1
Tap 18	<10	9.95
EPA	<500	0

suitable surfaces to facilitate excessive growth of bacteria and biofilm formation. Additionally, Wingender & Flemming (2011) found *Listeria monocytogens* in biofilm from tap water. Results for total coliforms demonstrated 20% of WVM, 88% of swab, 41% of SF, and 50% of TW samples had total coliforms. Also, the mean values of total coliforms in WVMs, swabs, SFs, and TW, indicated that SFs had the highest mean concentration of total coliforms, followed by swabs. A previous study by White *et al.* (2010) in the US found that many of the SFs analyzed exceeded US drinking water standards including 20% of SFs sampled, with an HPC > 500 $CFU{\cdot}mL^{-1}$, 48% with coliform bacteria, and 6.7% with *E. coli*. TW and WVMs had the lowest mean concentration of total coliforms. Our findings regarding the presence of coliforms in the present study were in accordance with studies by Liguori *et al.* (2010) in Italy, Zanetti *et al.* (2009) in Italy, Baumgartner & Grand (2006) in Switzerland and Lévesque *et al.* (1994) in Canada that also found coliforms in WVM or coolers. The presence of total coliforms in drinking water indicates that environmental contamination with biological matter into drinking water systems may

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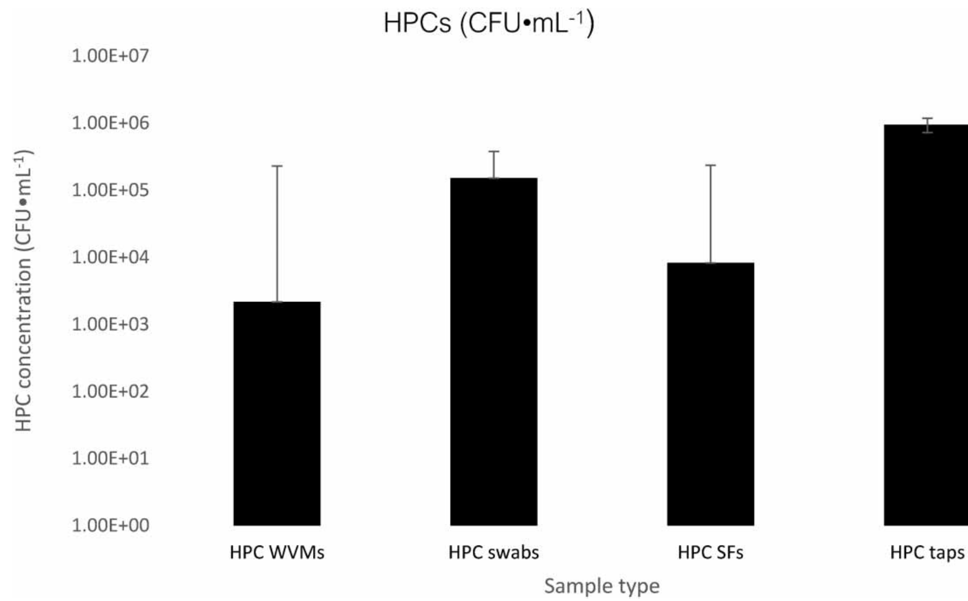

Figure 3 | Comparison of heterotrophic plate count (HPC) most probable numbers between water vending machines (WVMs) swabs, fast-food soda fountains (SFs), and tap water (TW).

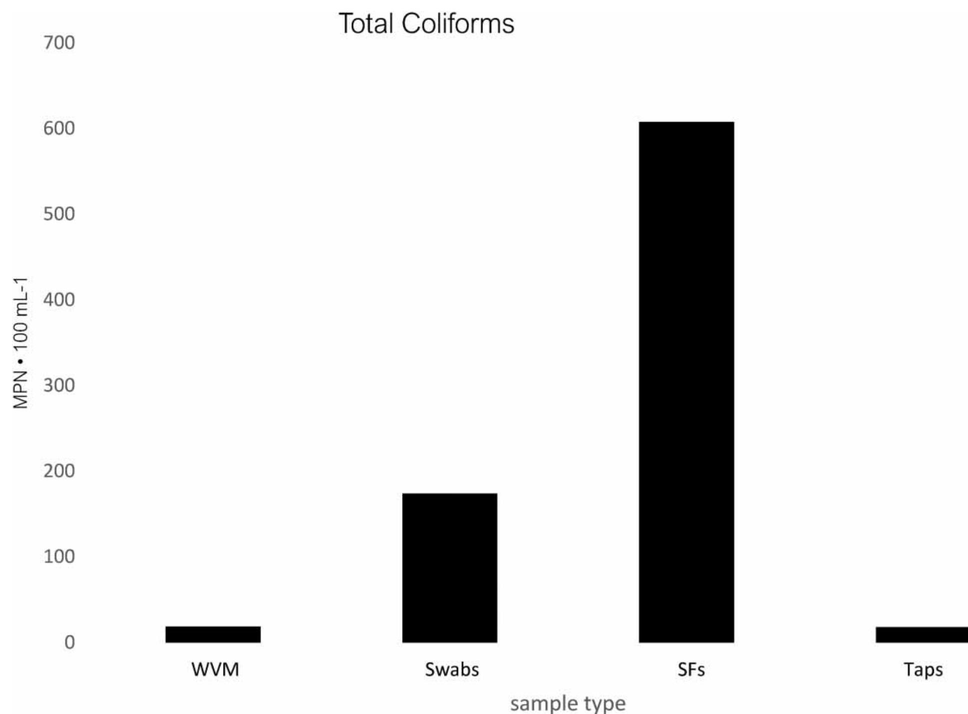

Figure 4 | Comparisons of total coliform concentrations from water vending machines (WVMs), swabs from water vending machines, soda fountains (SFs), and tap water (TW) of the Eastern Coachella Valley.

require investigation (WSDH 2016), and that there is a possible health risk to the population in relation to exposure to potential pathogenic microorganisms. Total coliforms are used as an indicator of other pathogens in drinking water, and as such, are used to determine the competence of water treatments (EPA 2021). The striking presence of coliforms in 88% of swabs,

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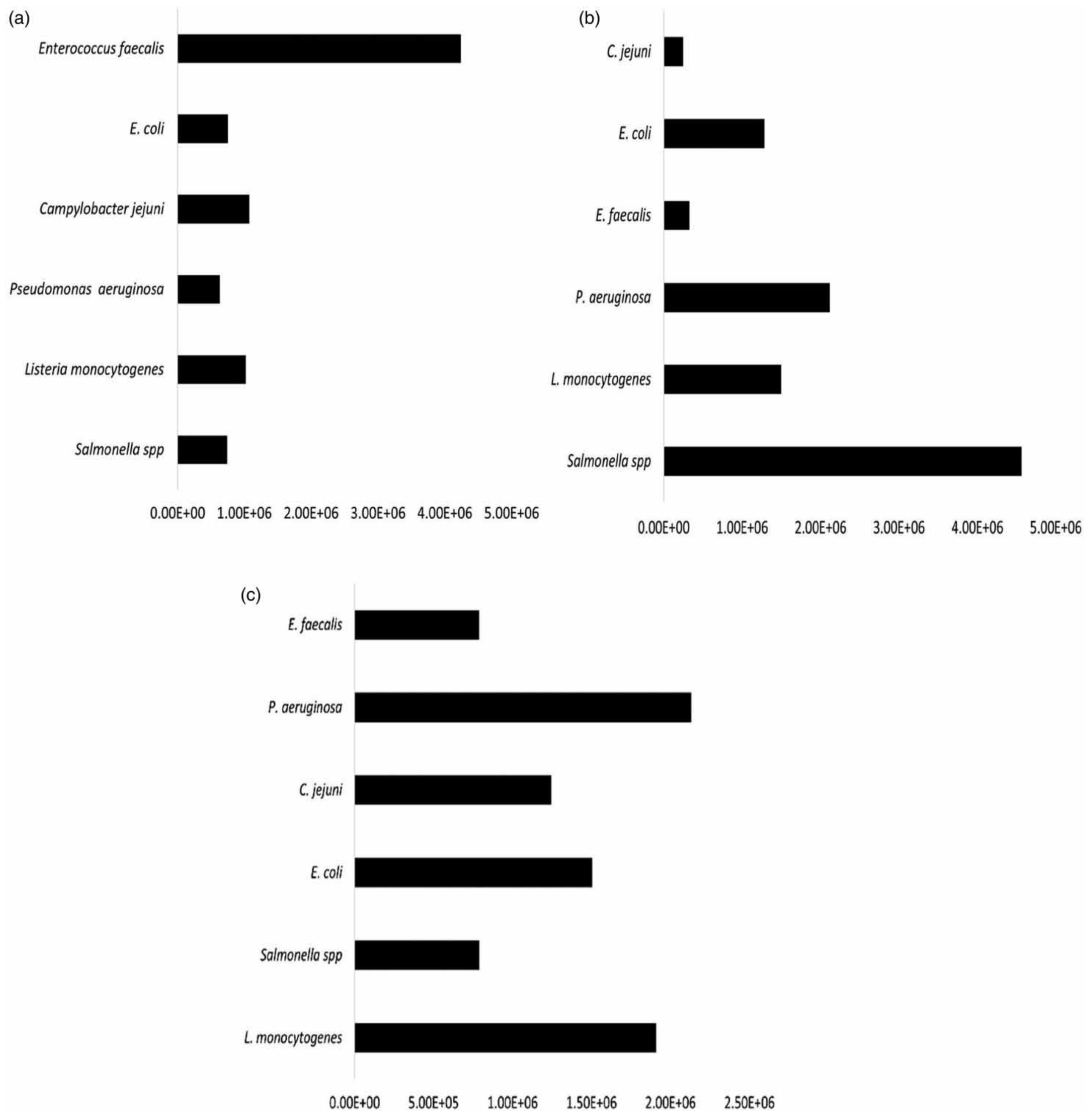

Figure 5 | Microbial concentration of selected microorganisms based on qPCR quantification in water vending machines (a), in tap water (b) and in swabs (c) of the Eastern Coachella Valley.

yet only 20% of WVMs is likely an indication of biofilm formation in spigots of WVMs that sporadically leach into water samples. The presence of HPCs exceeding the threshold set by the EPA for drinking water, and the presence of total coliforms in some WVMs and SFs is an indicator of microbial water quality deterioration, despite the effectiveness of the filtration system that was able to filter Cl_2 from TW and result in parameters, such as pH, TDS, and EC, within required limits.

Municipal water systems in the US are monitored and required to comply with drinking water regulations. The present study sampled water from taps located in the ECV along with WVMs and SFs. The elevated concentrations of HPCs and the presence of total coliforms in our TW samples was unexpected and is in contrast to other studies that found higher concentrations of HPCs in water dispensers than the TW used to supply them (Liguori *et al.* 2010; Wingender & Flemming 2011;

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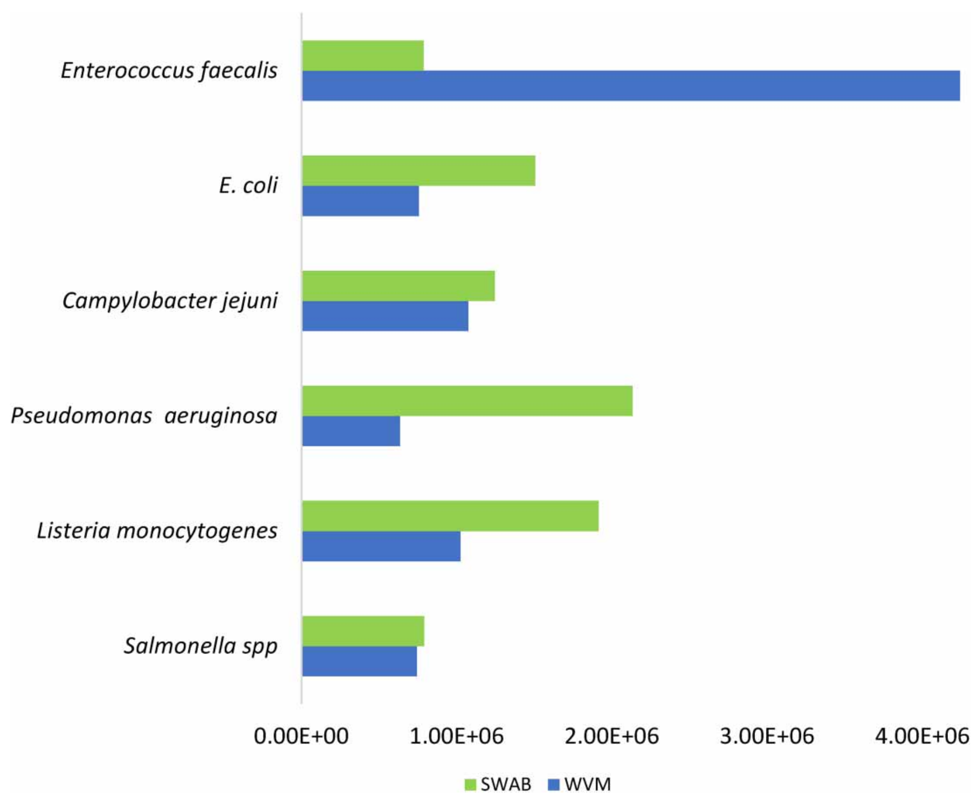

Figure 6 | Comparison of microbial quantification of selected microorganisms from water vending machines (WVMs) and swabs from water vending machines of the Eastern Coachella Valley, using the qPCR method.

Farhadkhani *et al.* 2014; Sacchetti *et al.* 2014). Heterotrophic plate count results obtained in the current study from drinking water sampled from a water tank supplied by the Coachella Valley Water District to the ECV community were all below detection limit, with no coliform or *E. coli* (data not shown). However, TW collected during this study had elevated HPCs and coliforms. This may be explained by the fact that distribution systems and service lines influence the quality of water (Bitton 2005). Although the Coachella Water District provided clean water, according to results published on their website and the analysis of the water tank we analyzed, we can infer that the TW spigots, fixtures, or other premise plumbing are likely to be contaminated with biofilms. This is supported by studies that found drinking water quality was deteriorated by microbial contamination during transport before reaching the consumer (Bitton 2005; Arnal *et al.* 2010). We assume that since water quality from the tank was sufficient for drinking, and reports from the ECV water district confirm that quality, it is likely that tap water contamination was due to premise plumbing and biofilm formation overtime. For example, Huang *et al.* (2021) in their study characterizing microbiomes in both water and biofilms in Australia showed the formation of taxonomically diverse biofilms in premise plumbing. Similar results were found by Falkinham *et al.* (2015), and Huang *et al.* (2020), in the USA.

From our results for quantified selected microorganisms using qPCR, we found all selected microorganisms were represented in WVMs at different concentrations. However, the presence of selected microorganisms varied from one WVM to another. *P. aeruginosa* is an important opportunistic pathogen, is a serious threat to drinking water safety, and its predominance in drinking water is a serious public health concern. *Pseudomonas aeruginosa* is known for its resistance to disinfectants, such as chlorine, and for its ability to form biofilms. Although the detection of the presence of selected microorganisms in WVMs using qPCR does not indicate the presence of viable microorganisms in our samples (rather, the presence of genetic materials of target microorganisms), it does not eliminate the risk of exposure to pathogenic microorganisms. We found that *P. aeruginosa* mean concentration was elevated in TW and swabs. Also, the elevated mean concentration of *P. aeruginosa* in swabs almost equal to the concentration in TW in comparison to WVM (see Figure 5(b) supplemental), indicated that microorganisms piped in WVMs form biofilms in spigots and later leach into bottled water during the purchase

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of drinking water. In this study, *E. faecalis* was predominant among selected microorganisms in WVMs. *E. faecalis* is an opportunistic pathogen frequently found in mineral water and spring water used for human consumption, and may cause human urinary tract infections, endocarditis, and neonatal sepsis (Wei *et al.* 2017). Although qPCR is unable to distinguish between live versus dead microorganisms, it is able to identify genetic material of a microorganism alive or dead in a sample to a lower concentration, and greater sensitivity in comparison to traditional bacterial counting methods. Although we were unable to detect cultivatable *E. coli*, we were nevertheless able to determine the presence of *E. coli* genetic material in our samples. The presence of *E. coli* in drinking water is generally used as an indicator of drinking water quality and the risk of contamination with pathogenic microorganisms (Brown *et al.* 2008).

We found that TW samples had the highest mean concentration of *Salmonella spp*. We also found that WVM's, and swabs presented essentially the same mean concentration of *Salmonella spp*. However, the percent positive of *Salmonella spp* showed that swab samples were more contaminated with *Salmonella spp* than WVMs. *Salmonella spp* ranks high among microorganisms causing foodborne disease outbreaks, and approximately 32.7% of *Salmonella spp* outbreaks in the US have been associated with drinking water consumption (Liu *et al.* 2018). We can infer that *Salmonella spp* and *L. monocytogenes* contamination in WVMs comes from TW and biofilm formation in spigots that will then leach into drinking water purchased by consumers. Similar to the study in the US by White *et al.* (2010), we were not able to sample the TW supply directly before entering WVMs and consequently, we are unable to infer that the municipal tap water is the source for contamination of WVMs. Moreover, TW from the ECV complied to the EPA water quality standards. The dispensing nozzles, as well as the fittings that connect the WVM and SF machines to the municipal water distribution network may be the source of contamination (White *et al.* 2010). Moreover, previous studies have found that microbial populations were greater after passing through a dispenser than before, hence we suggest the contamination of the dispensed water likely happened in the machine itself. This suggestion is supported by Zanetti *et al.* (2009) who investigated water contamination in microfiltered water dispensers in Italy. Both Kohnen *et al.* (2005) in Germany and da Silva *et al.* (2008) in Brazil found similar results from their studies of microbiological quality of carbonated drinking water and the comparison of the bacteriological quality of tap water and bottled mineral water, respectively.

Microbial contamination of drinking water from SFs is understudied. We found that SFs in the ECV had the highest mean concentration of total coliforms in comparison to TW, swabs, and WVM samples. Although White *et al.* (2010) found no significant differences in the levels of microbial contamination between self-served and employee-served SFs, our data, although insufficient from which to draw conclusions, indicated all SFs served by employees were contaminated with coliforms. We suggest that further investigations should be carried out to compare the microbial contamination between self-served and employee-served SF water.

5. CONCLUSION

International drinking water quality monitoring programs have been established to prevent waterborne disease outbreaks through a requirement of drinking water infrastructure and maintenance. Failure to monitor water can lead to infrastructure failure and may expose people to pathogenic microorganisms. Concerns have been raised about the quality of alternative drinking water sources (WVMs and SFs) to TW from municipal water districts. To ensure safety of drinking water from WVMs and water from fast-food restaurants for communities living in the ECV, we designed this study to analyze drinking water samples from WVMs, SFs, and TW for physico-chemical parameters, as well as microbiological quality. We found that WVMs and SFs had microbial contamination above limits set by the EPA. Moreover, genetic material of selected pathogenic microorganisms was represented in both WVMs and SFs. Contrary to prior studies, we found that TW samples at point of use were greatly contaminated. We suggest biofilm formation in TW distribution systems is likely the main source of contamination. Since WVMs and SFs are connected to TW systems, biofilms likely form overtime in the water distribution systems, which are mainly made of plastic piping, and are ideal for biofilm growth. The quality of filtration systems used for WVMs and SFs appears to be insufficient to prevent consumers from drinking contaminated water. Dispensing nozzle structure and location are also suitable for biofilm formation, especially during the summer season. In this study, nozzle swabs demonstrated elevated bacterial contamination, supporting the idea that their location and structure are ideal for biofilm formation and summer drinking water contamination. To improve water quality from dispensed water, we recommend regular cleaning and flushing of dispensers by owners. We recommend regular flushing and cleaning of water dispensers and the use of nanoparticle coated tubes inside water dispensers to control the growth of biofilms in water dispensers.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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