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Heterotrophic plate counts (HPC) in drinking water distribution systems: A comprehensive review and meta-analysis

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ABSTRACT

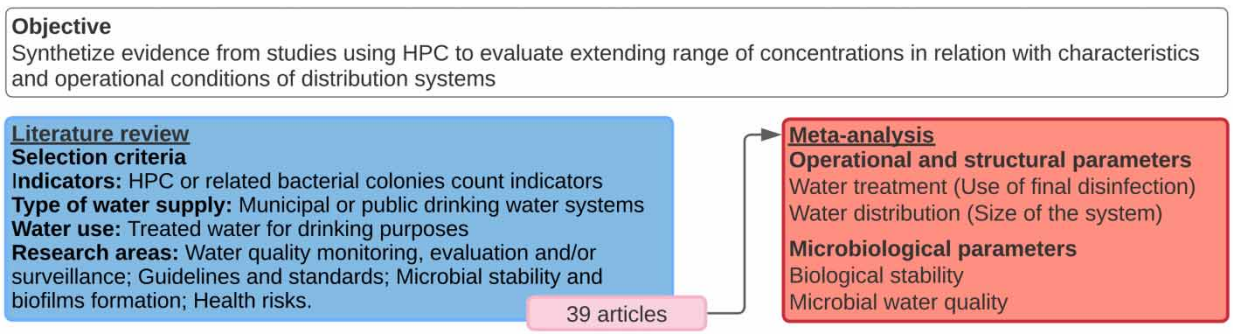
Heterotrophic plate count (HPC) is widely assessed in drinking water distribution systems. However, methodological standards and guidelines on the use of HPC are not clearly defined. This comprehensive review and meta-analysis aim to evaluate HPC concentration and how they relate to the characteristics and operational conditions of systems. The size of the distribution system, use of chlorine or chloramine as secondary disinfection and the carbon content of the water were considered. Among 839 MEDLINE® records, 39 met our criteria and were included in the meta-analysis. Overall, wide ranges of HPC levels were observed in drinking water distribution systems. Results from the meta-analysis show a significant difference in concentrations between systems using chlorine or chloramine as secondary disinfectant and those that are not using any form of secondary disinfection. Similarly, results demonstrate a positive correlation between HPC levels and assimilable organic carbon. Assessing the spatial and temporal variations of HPC can provide useful information about the biological stability of the water and allow for routine analyses within individual drinking water systems. Due to its limitations as a global and unique indicator of water quality, HPC should be applied as part of a multi-parameter approach for microbial growth analysis in distribution networks.

Key words: biological stability, heterotrophic plate count, literature review, meta-analysis, microbial water quality, water distribution system

HIGHLIGHTS

- A wide range of HPC concentrations can be found in drinking water distribution systems.
- Significant difference in HPC levels is observed in systems using secondary disinfectant.
- There is a lack of methodological standards and guidelines on the use of HPC.
- Log-linear correlation between AOC and HPC was tested using pooled data.

GRAPHICAL ABSTRACT



1. INTRODUCTION

Drinking water systems (DWSs) are dynamic environments that can harbour a large diversity of microbial communities (Eichler *et al.* 2006). Characteristics that relate to the production and distribution of drinking water, including structural and operational parameters, seasonal and temporal variations and availability of organic and inorganic nutrients can influence microbial community composition (Scheili *et al.* 2015; Prest *et al.* 2016b). Through physical, chemical and microbiological interactions, drinking water supply systems are favourable habitats for bacterial regrowth from the entry point of the distribution system to the consumer tap (LeChevallier 1990). Microorganisms can develop or enter in the distribution systems through the supply chain. Intermittent water supply systems (IWS) (i.e., water available less than 24 h/day) and/or open drinking water distribution systems (e.g., truck-to-cistern systems) are particularly vulnerable to microbiological recontamination (Daley *et al.* 2018). Despite drinking water treatment processes such as primary and secondary disinfection as well as operational and structural maintenance, microbial regrowth and biofilms may demand increased residual disinfectant or cause recontamination, which can affect overall drinking water quality (Liu *et al.* 2016).

Drinking water quality monitoring includes a broad range of parameters, which can vary at different levels according to regulations and standards for drinking water (WHO 2021). Multiple parameters and proxy indicators can be used to assess the presence of microorganisms in raw and potable treated water (Cabral 2010; Saxena *et al.* 2014). Total, faecal coliforms and *Escherichia coli* are generally recognized as the most common and/or faecal indicators (Fewtrell & Bartram 2001; Levy *et al.* 2012). However, other general microbial indicators including heterotrophic bacteria, which refer to bacteria that use organic nutrients for growth, are also widely used (Allen *et al.* 2004), using heterotrophic plate counts (HPCs).

HPC is an analytic method used to measure general heterotrophic bacteria in water. The literature shows that heterotrophic bacteria have been measured according to standard plate count, total viable count, plate count, total bacterial count, water plate count, colony count, aerobic mesophilic viable count and autochthonous flora (Allen *et al.* 2004). Various factors led to the use of heterotrophic bacteria as indicators for general drinking water quality starting in the 1800s, such as the lack of a specific method for measuring diverse pathogens and technical feasibility (Robertson & Brooks 2003). In a paper published in 1883, Robert Koch was the first to develop a methodology for the enumeration of HPCs in drinking water (Bartram *et al.* 2003). Although HPC can indicate a multitude of heterotrophic aerobic and facultatively anaerobic species in the water, some of which may include opportunistic pathogens, its relevance as an indicator of water quality and faecal contamination is widely debated. Previous studies have questioned the sensitivity and specificity of HPC measurements, highlighting that different recovery methods could result in a wide range of concentrations, from <0.01 to 10^4 CFU/mL (Allen *et al.* 2004).

There is currently no consensus on the relevance of HPC in water management programs. The WHO does not recommend the use of HPC as an indicator of water safety as HPC does not directly identify the potential adverse effects on human health (Bartram *et al.* 2003a). However, previous studies found that high levels of HPC reflect the health of the distribution network, the availability of nutrients for opportunistic pathogens (e.g., assimilable organic carbon (AOC), biodegradable dissolved organic carbon (BDOC)) and their potential to promote certain infectious waterborne diseases (Chowdhury 2012). For example, the steady-state density of HPC and opportunistic pathogens in biofilm were found to be strongly correlated (Pozos *et al.* 2004). In addition, low HPC levels in a system suggest that other pathogens such as total *E. coli* and *Pseudomonas* spp. are likely absent or present at undetectable levels, the latter being less resistant to disinfection (Robertson & Brooks 2003). There is also no consensus regarding the efficacy of HPC as a regrowth indicator due to the fact that some authors have demonstrated that HPC only enumerates a very small fraction of bacteria (both alive and deceased) compared with other methods to evaluate bacterial biomass, such as flowcytometry or ATP analysis (Hammes *et al.* 2008; Prest *et al.* 2016b).

Regulations for the monitoring of heterotrophic bacteria in drinking water are inconsistent across North America. For example, the United States Environmental Protection Agency (US EPA) refers to the National Primary Drinking Water Regulations (Health Canada 2020) which indicates an HPC objective of 500 CFU/mL through a treatment technique approach ('TT') (United States Environmental Protection Agency 2022). Meanwhile, Health Canada offers the Guidelines for Canadian Drinking Water Quality which recommends the use of HPC as a parameter for monitoring biological stability but does not report any specific HPC levels. Several public water supply systems continue to conduct HPC analyses to monitor overall bacteriological quality in their networks. Previous studies support the use of HPC as a tool to assess

changes in the supply system (Allen *et al.* 2004; Bartram *et al.* 2004; Ikonen *et al.* 2013), as well as the use of HPC to monitor drinking water. However, none has evaluated the wide range of colony count units and variations of HPC and how these relate to the characteristics and operational conditions of drinking water supply systems, including water treatment and distribution processes.

The overall objective of our comprehensive review is to evaluate the validity of using HPC as an indicator for biological stability and characterize how HPC might contribute to an integrated system for water quality management in distribution systems. Our specific objectives were to (1) provide an overview of HPC levels in different distribution systems, (2) assess the relationship between main physicochemical properties of the water and the biological stability according to HPC and (3) determine/define the role of HPC as a control indicator for microbiological activity and overall water quality.

2. METHODS

Our comprehensive review was conducted using the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) as a guide (Tricco *et al.* 2018). The established initial search strategy was revised by all the authors and by institutional librarians. Two reviewers (ACarabin, ACassivi) conducted the review independently. They were tasked with selection and extraction, while a third reviewer (CHuot) was consulted if there were any disagreements.

2.1. Eligibility criteria

Articles that met specific criteria were included in the review. The extracted articles were peer-reviewed, published in English or French and were published between 1970 and 2024. These papers also focused on HPC for water quality monitoring, evaluation and surveillance in public drinking water supply systems. Although literature reviews and grey literature were not considered eligible, they were searched using the same keywords as the peer-reviewed articles and some of these materials were cited in the discussion section when relevant.

2.2. Data sources and search strategy

The Ovid MEDLINE® database was searched for relevant articles using the full search strategy (Table 1). The list of terms that were used met a number of criteria. The terms were related to (1) HPC or related bacterial colony count indicators, (2) microbial water quality/stability, (3) drinking water systems and (4) monitoring, evaluation or surveillance. The Boolean operators 'AND' and 'OR' were used. Searches were initially conducted between April and August 2022 before data evaluation and extraction. In order to ensure the comprehensiveness of the literature review, a second search was conducted to incorporate newly published studies.

Table 1 | Search strategy used to retrieve articles in Ovid MEDLINE

#	Requests
1	('total viable count*' or 'total count*' or 'plate count*' or 'total bacterial count*' or 'colony count*' or 'aerobic mesophilic viable count*' or 'autochthonous flora' or 'HPC flora' OR 'AMVC' OR (heterotrophic and count*)).ti,ab. or *colony count, microbial/
2	(((bacteri* or biologic* or microb*) adj5 (activit* or communit* or ecolog* or growth* or regrowth* or stabilit* or quality or control)) or biofilm* or biostabilit*).ti,ab. or water microbiology/ or water quality/
3	(((drink* or domestic or ground or municipal or suppl* or tap or stored or distribut* or truck*) adj2 water) OR potable OR groundwater* OR watershed* OR 'cooling tower*').ti,ab. or exp water supply/ or drinking water/
4	(surveillance or monitor* or variability or evaluat* or assess* or track*).ti,ab.
5	1 and 2 and 3 and 4
6	limit 5 to yr = '1970-Current'
7	*Remove duplicates*

2.3. Selection process

All articles that were identified using the search strategy were downloaded into EndNote 20 reference management software. The identified articles were then subjected to a preliminary review to remove duplicates before proceeding with the screening process, which included a title and abstract review and then a full-text review. The selection of the articles was based on the inclusion and exclusion criteria list (Table 2). Additionally, studies using culture-based methods (e.g., pour plate, spread plate and membrane filter methods) for HPC were specifically selected, as culture-based method standard analytic techniques are recommended by Health Canada and the WHO to monitor HPC in drinking water (Bartram *et al.* 2003b; Health Canada 2022).

2.4. Data collection and analysis

Once the studies were thoroughly reviewed for eligibility, relevant data pertaining to characteristics of supply networks, physical and chemical parameters of water quality, types of disinfection processes, carbon content, the presence of other microorganisms and levels of HPC were extracted using a structured Excel extraction file.

Variability in HPC levels at the various drinking water supply systems from the identified studies were evaluated and classified according to three characteristics: the use of chlorine and chloramine disinfection, total population served and the use of organic matter indicators. First, all systems were classified based on whether chlorine or chloramine disinfection was used as a primary and/or secondary treatment before distribution. The terminology 'secondary disinfection' was consistently employed throughout the manuscript to refer to the process of disinfecting water with chlorine or chloramine prior to its distribution. Second, because information on network length was limited in the extracted studies, the total served population was used as a proxy for system size. The population sizes were grouped based on Statistics Canada classifications for population centres: small population centres have between 1,000 and 29,999 inhabitants; medium population centres have between 30,000 and 99,999 inhabitants and large urban population centres have 100,000 inhabitants or more. Finally, systems were classified according to whether they monitored AOC colony count units. Although other nutrients play a role in controlling microbial growth, AOC, a fraction of dissolved organic carbon, was selected specifically because it is recognized as a useful indicator and proxy of bacterial regrowth in drinking water (Health Canada 2022). Finally, the relationship between HPC, other microorganisms and possible health effects were also analysed.

To perform the meta-analysis and assess the relationships between type of disinfection, system size and HPC levels, the minimum, median, mean and maximum HPC values were pooled from every article. All those values were used as they all indicate different levels of HPC in distribution networks as well as the spatial and temporal variability of HPC levels. It should be noted that several papers did not explicitly identified HPC levels in the results section and/or used unscaled histogram, which prevented the extraction of all data. Furthermore, it is noteworthy that some papers did not provide

Table 2 | Inclusion and exclusion criteria list for article selection

	Inclusion	Exclusion
Indicators	HPC/'Total bacteria counts' and related indicator organisms	Viruses or protozoa, *Legionella*, other opportunistic bacteria, other non-HPC related indicators of water quality
Type of water supply	Municipal or public drinking water supply systems (open, such as water trucks or closed)	Private water systems, bottled water, raw water such as lakes and rainwater, premise plumbing, non-drinking water or direct potable reuse such as greywater, other systems not meant for drinking water
Water use	Treated water for drinking	Dental unit water lines and equipment; medical units; cooling towers; hot water systems and water heaters; irrigation and agriculture
Research areas	Water quality monitoring, evaluation and/or surveillance; guidelines and standards; microbial stability and biofilm formation; health risks	Alternative water treatment technologies such as 'MgO filter', bacterial removal through laboratory experiments, point-of-use water treatments
Type of study	Original research, empirical studies	Literature review, small-scale test or pilot studies

information regarding the size of the system, or we could not retrieve the information, making it more difficult to incorporate such information in the meta-analysis. To explore the relationship between levels of HPC and carbon content, a log-linear regression model was developed based on studies that used AOC as an input parameter and for which median or mean levels were reported. Only HPC means and medians were used to assess the correlation with AOC as a way to compare between studies. Median or mean HPC levels were used as output data. The linear and log-linear correlation techniques were used since previous studies indicated that HPC is correlated with AOC in linear and log-linear relationships in full-scale distribution systems (Escobar *et al.* 2001; Zhang *et al.* 2016). Non-parametric statistical techniques, Wilcoxon-Mann-Whitney and Kruskal–Wallis tests were used to analyse differences between systems ($\alpha = 0.05$). All statistical analyses were performed using XLSTAT 2022.1.2. HPC concentrations were classified using colony-forming units per millilitre (CFU/mL).

3. RESULTS

3.1. Search results

The initial search database search yielded 839 results, of which 444 were screened for relevant titles and abstracts after duplicates were removed (Figure 1). The full text of 95 eligible articles were assessed for eligibility according to the inclusion

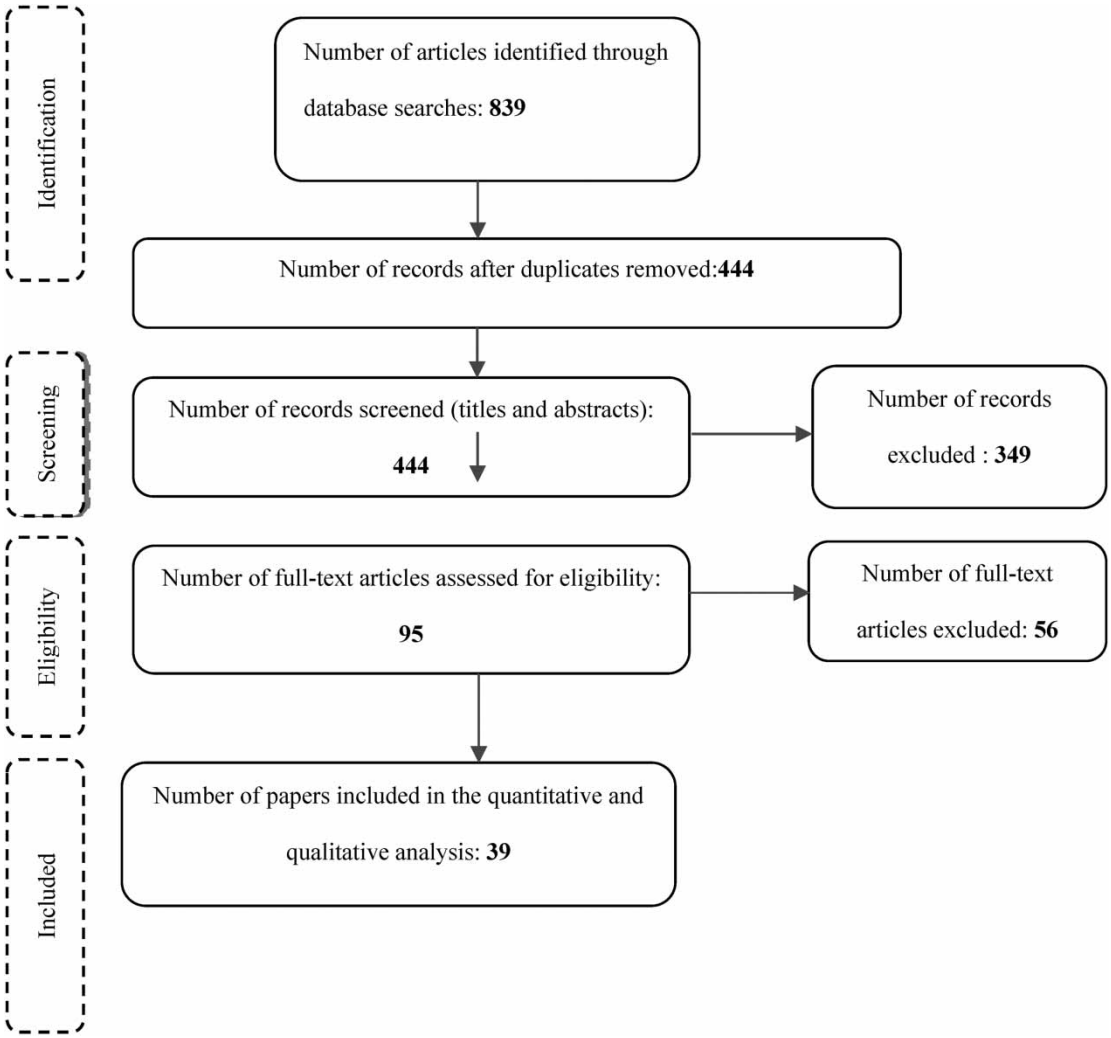


Figure 1 | PRISMA diagram.

criteria and 56 were excluded. In the end, 39 articles were included in the qualitative and quantitative analysis. Results are presented in Tables 3 and 4.

3.2. General characteristics of the studies and the included systems

Studies were conducted on municipal or public drinking water supply systems from all regions of the world, as classified by the World Bank. Most studies were conducted in Europe and Central Asia (15 studies), North America (13) and East Asia and the Pacific (five). Other studies were conducted in South Asia (two), the Middle East and North Africa (two) and Latin America (one). The location for one study was not specified. Overall, 82% of the studies (32) were conducted in high-income economies. The remaining studies were conducted in lower and upper middle-income economies, such as Brazil, China, Egypt, India and Pakistan, where intermittent water supply systems are more likely to be found (Kumpel & Nelson 2016). Studies were conducted in various system sizes based on size of population centres, with some studies including multiple systems. More than half of the studies included large population centres (22 studies) while other studies were conducted in medium and/or small population centres (equal or fewer than 99,999 inhabitants).

Surface water was the most common source of water in the selected studies compared with groundwater, groundwater recharge or spring water. While a few papers only examined the quality of tap water (Volker *et al.* 2010; Ohkouchi *et al.* 2014; Donohue 2021), most studies investigated the quality of water from source to tap. When specified, water treatment varied between studies. However, coagulation/flocculation/settling and multimedia and/or activated carbon filtration were the most common treatments (16 studies). Only a few studies used advanced techniques such as ultrafiltration or nanofiltration (Escobar & Randall 2001; Ohkouchi *et al.* 2014). Ozonation was also common among studies (eight). UV disinfection was used in combination with ozone or alone in a few studies. Fifteen percent of all the studies did not use secondary disinfection (Table 3), while 85% used disinfection techniques such as chloramine or chlorine as a primary and/or secondary treatment (Table 4). Study details and general characteristics for systems that do not use chlorine or chloramine disinfection are provided in Table 3 and the information for systems using chlorine or chloramine disinfection is presented in Table 4.

3.3. HPC methods

All 39 studies used standard culture-based methods to estimate the number of heterotrophic bacteria in the water. All studies when indicated, except three that used a standard membrane filtration method with HPC media, used pour plate or spread plate methods with standard agar or R2A media. Compared with the other high-nutrient media (high-nutrient yeast extract agar), the R2A medium is a nutrient-poor medium developed to quantify the maximum number of heterotrophic colonies in water, hence not giving the same exact picture of the present bacterial community (Gensberger *et al.* 2015). Among studies that detailed their methods, the incubation temperature and time varied. Temperatures oscillated/ranged from 20 to 35 °C and the time period ranged from 24 h to 14 days. Results showed that the most common procedure was to incubate media at 20–24 °C for 7 days maximum and at 35–37 °C for 72 h (five studies). Other studies used procedures such as incubating at 30 °C for 14 days (Richards *et al.* 2018) or at 28 °C for 5 days (Scott *et al.* 2015). Furthermore, Reasoner *et al.* (1989) enumerated HPCs using three types of media (SPC, m-SPC and R2A) and three incubation times (2, 3 and 7 days) at 35 °C. It is interesting to note that the results were significantly different when compared between media and incubation periods, i.e., ranging from 156 to 59,000 CFU/mL.

3.4. Physical and chemical parameters that can impact HPC

The impact of water systems, including physical and chemical characteristics of piped water systems on HPC colony count units, was commonly reported in studies.

3.4.1. Temperature and seasonality

Multiple studies found that levels of HPC were positively correlated with water temperature (Inkinen *et al.* 2014; Lu *et al.* 2014; Prest *et al.* 2016b; Zhang *et al.* 2016). Higher levels of HPC were consistently reported in warmer water temperatures. Results from Zhang & DiGiano (2002) also show a strong correlation between temperature and HPC for two different systems. Significant increases in levels of HPC and AOC were observed in summer, both in chlorinated and non-chlorinated systems and in public buildings (Volker *et al.* 2010; Inkinen *et al.* 2014; Lu *et al.* 2014; Prest *et al.* 2016b). In a longitudinal study conducted in Canada, HPC was not detected in more than 75% of the samples collected in cold-water winter conditions (below 4 °C) (Francisque *et al.* 2009). Similarly, lower levels of HPC were reported in colder seasons in five small public systems (Tung & Xie 2009).

Table 3 | Drinking water distribution systems without secondary disinfection and residual[a] ($n = 6$)

		Water distribution system			HPC		Organic and microbial content	
Authors and year	**Location**	**Population size**	**Number of systems**	**Water treatment processes**	**Methods**	**Results (CFU/mL)**	**Methods**	**Results**
Lautenschlager *et al.* (2013)	Switzerland	Medium/large (402,762)	1	Pre-ozonation, rapid sand filtration, intermediate ozonation, granular active carbon filtration, slow sand filtration (information extracted from Lautenschlager *et al.* (2010)) Water is distributed without the addition of any residual disinfectant	R2A plate agar counts	<10	Simplified version of AOC assay using flow cytometry	AOC below the quantification limit (<10 mg/L)
Gunnarsdottir *et al.* (2012)	Iceland	Large (87,942)	5	Groundwater is typically not treated prior to distribution, surface water is generally treated by filtration followed by UV disinfection and residual disinfection is not practiced in Iceland	22 °C, Icelandic Drinking Water Regulation, media not specified. Pour plate method (9215B)	All samples in distribution network: Before water safety plans (mean): 54 After water safety plan (mean): 15.9	–	–
Volker *et al.* (2010)	Germany	Small, medium and large	Not specified	No further detail provided	Plate agar counts 20/22 °C and 36 °C	Approximately 3.5% of all samples for HPC 36 °C exceeded the indicated limit of 100 CFU/mL. HPC 20/22 °C exceeds the acceptable limit in 1.2%	–	–
Lautenschlager *et al.* (2010)	Switzerland	Medium (619,294)	1	Ozonation and sequential biofiltration Water is distributed without the addition of any residual disinfectant	Plate agar counts, 30 °C for 72 h	Stagnated water: $0.87 \pm 1.0 \times 10^3$ After flushing: 9.1 ± 9.8	Simplified version of AOC assay using flow cytometry (indirect measure using net-regrowth)	TOC: 0.8 ± 0.0 mg/L (mean, flushed and stagnant averages) AOC concentration of about 2 μg/L from flushed and first litre samples from 10 households for 4 days
Liu *et al.* (2013)	Netherlands	Medium (private company)	2	Powdered activated carbon, rapid sand filtration (RSF) and slow sand filtration (SSF); water is supplied without disinfectant	R2A plate agar counts, 25 °C for 10 days	Level below 600 (values not specified)	Flow cytometry and ATP	AOC (μg C/L): 5 (±2) (plant 1) and 11 (±8) (plant 2)
Hijnen *et al.* (2018)	Netherlands	Large	3	Coagulation/separation, dual media filtration, primary disinfection with UV or ozone, followed by	22 °C; ISO 6222:1999 Nutrient agar	Plant 1 (P50–P90[b]): 11–88, Plant 2 (P50–P90): 3–18, Plant 3 (P50–P90): 9–63 during	AOC-P17/NOX; AOC-A3 and biomass	Average quality of drinking water sampled after the clean water reservoir:

(*Continued.*)

Table 3 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				biologically activated carbon filtration (BACF) and post-disinfection with ClO_2 at low doses (0.03–0.08 mg/L) Water is distributed without a disinfectant residual	culture medium	distribution at Plant #1, Plant #2 and Plant #3. Average quality of drinking water sampled after the clean water reservoir: Plant 1, P50: <1 and P90: 21 Plant 2, P50: <1 and P90: 2 Plant 3, P50: <1 and P90: 22	production potential	AOC (P17/NOX[c]): Plant 1: 17.5 ± 5.9 $\mu g\,C\,L^{-1}$ Plant 2: 26.6 ± 9.8 $\mu g\,C\,L^{-1}$ Plant 3: 27.9 ± 12.0 $\mu g\,C\,L^{-1}$

[a]Secondary disinfection refers to the chlorination or chloramination of finished water prior to its entry into the distribution system.

[b]P50 refers to the 50th percentile, while P90 is the 90th percentile.

[c]AOC-P17/NOX was used in a few studies and combines *Pseudomonas fluorescens* strain P17 and *Spirillum* sp. strain NOX in mixed inoculum. The AOC concentrations were calculated using the maximum colony counts and the yield value of the organisms on acetate.

Table 4 | Drinking water distribution systems using secondary disinfection (chlorine or chloramine[a]) prior to distribution ($n = 33$)

		Water distribution system			HPC		Organic and microbial content	
Authors and year	Location	Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
LeChevallier *et al.* (1987)	United States	Medium	2	Pretreatment, flocculation, clarification, filtration and disinfection (chlorine)	R2A agar, 20–24 °C for 7 days	~1.5 to 4.7 log HPC (extracted from figure in article) Site PE: ~1.5 log Site 1: ~1.5 log Site 2: ~2 log Site 3: ~2.8 log Site 4: ~4.8 log Site 5: ~2.2 log Site 6: ~3.1 log Site 7: ~4.7 log	AOC measurements were based on growth of *Pseudomonas fluorescens* (P17)	Extracted from figure in article: Plant effluent: ~135 µg AOC-C/L Site 1: ~80 Site 4: ~50
Gavriel *et al.* (1998)	United Kingdom	Not specified	1	Storage reservoir settlement, coagulation (aluminium sulphate), rapid gravity sand filtration and chlorine disinfection using chlorine gas. 31 reservoirs (3 zones: A, B, C) + secondary disinfection (sodium hypochlorite)	Yeast extract agar, 22 °C for 72 h and 37 °C for 24 h	For all 31 reservoirs and for the two cultivation methods (average): <1 to ~110	–	–
Reasoner *et al.* (1989)	United States	Large	1	Settling, coagulation/ flocculation, chlorination rapid sand filtration	(i) The pour plate procedure (SPC) with plate count agar; (ii) the m-SPC medium membrane filter procedure (m-HPC) and (iii) R2A medium. 35 °C for 7 days	Sample site, mean (SPC, m-SPC, R2A, 2 days): #1: 156.5, 2,905.3, 38,241.7 #2: 8.8, 542.8, 6,088.3 #3: 7.1, 580.4, 3,742.5 #4: 0, 0.44, 45.1 #5: 0, 0.33, 2 #6: 0.04, 4.3, 358.6 #7: 0, 83.0, 1,770.0	–	–

(*Continued.*)

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
						#8: –, –, 48.0 #9: 0, –, 1,600.0, #10: 5.1, –, 23,466.7 #11: 16.7, –, – Sample site, mean (SPC, m-SPC, R2A, 3 days): #1: 13,447.1, 18,000, 28,742.6 #2: 2 231.7, 2 819.7, 9,176.9 #3: 624.9, 2,676.8, 4,114.3 #4: 9.6, 8.9, 11.8 #5: 1.0, 1.5, 2.0 #6: 23.3, 79.6, 1,262.8 #7: 380.3, 130.0, 4,206.0 #8: –, – . 90.0 #9: 0.30, –, 7,900.0 #10: 950.2, –, 37,650.0 #11: –, –, – Sample site, mean (SPC, m-SPC, R2A, 7 days): #1: 15,121.1, 24,173.7, 59,731.3 #2: 3,487.9, 4,104.4, 12,000.0 #3: 1,120.9, 3,576.2, 18,046.0 #4: 20.4, 16.0, 22.3 #5: 2.4, 3.1, 10.2 #6: 70.4, 398.1,		

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
						3,035.9 #7: 2,991.7, 130.0, 46,036.7 #8: –, –, 250.0 #9: 0.3, –, 45,820.0 #10: 7,078.0, –, 45,820.0 #11: 64.0, –, –		
Edberg & Smith (1989)	United States	Not specified	Not specified	Not specified	R2A agar by the spread plate technique	Only detection percentages were presented	–	–
Payment *et al.* (1988)	Canada	Small	2	Not specified	R2A at 20 °C for 7–8 days or 35 °C for 48 h	Sample sites (20 °C, 35 °C), mean: #1: 2,685,385, 142,455 #2: 4,861,800, 63,667 #3: 11,669,333, 51,777 #4: 11,281,875, 350,231 #5: 13,961,333, 432,692 #6: 14,480,833, 253,846	–	–
Wolfe *et al.* (1990)	United States	Medium	1	Coagulation, flocculation, sedimentation, filtration + chloramination and disinfection (chlorine). Additional chlorination at the reservoirs	(1) Pour plate procedure with tryptone glucose extract agar for 48 h at 35 °C (2) Membrane filtration technique, with R2A medium incubated for 7 days at 28 °C	Weymouth plant effluent: 18.5 (mean), 12.0–27 (min–max) Garvey Reservoir water column: 19.0 (mean), 12.5–26.0 (min–max) Gary reservoir effluent: 19.6 (mean), 12.5–27.5 (min–max) Orange County	–	–

(*Continued.*)

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
						reservoir influent: 22.2 (mean), 14.0–25.5 (min–max) Orange County reservoir water column: 20.8 (mean), 13.0–25.0 (min–max) Orange County reservoir effluent: 20.6 (mean), 12.5–25.0 (min–max)		
Zhang & DiGiano (2002)	United States	Large	3	Coagulation (alum or ferric sulphate), sedimentation, anthracite–sand filtration, fluoridation (three water plants). Free chlorine is added just above the filters. Corrosion inhibitor is used in all three water plants. Chlorination is performed in Durham and chloramination is performed in Raleigh	Membrane-filter technique with R2A agar, 28 °C for 7 days	Williams WTP (Durham): <1 (mean) EM Johnson WTP (Raleigh): <1 (mean)	AOC analyses were conducted with the technique SM 9217 B (NOX and P-17)	Williams WTP (Durham): TOC: 2.8 ± 0.3 mg/L (mean) AOC: 110 ± 60 μg/L EM Johnson WTP (Raleigh): TOC: 2.4 ± 0.4 mg/L AOC: 120 ± 50 μg/L
Lipponen *et al.* (2002)	Finland	Not specified	15	1A: Coagulation + rapid sand filtration, slow sand filtration, chloramination 1B: Coagulation + rapid sand filtration, slow sand filtration, chloramination 2A: Coagulation + rapid sand filtration,	R2A agar by a spread plate method (7 days, 20 °C)	160 (mean), <10–7.7 E03 (min–max) (all systems)	AOC was measured using *Pseudomonas fluorescens* and *Aquaspirillium* NOX	AOC: 150 μg AOC-C/L (mean), 38–350 μg AOC-C/L (all systems)

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				Granular activated carbon (GAC), chlorination (surface water), rapid sand filtration, pH adjustment, chlorination (groundwater) 2C: Coagulation + rapid sand filtration, GAC, chlorination 3A: Liming, KMnO4 oxidation + coagulation, limestone filtration 3B: KMnO4 oxidation, rapid sand filtration 3C: Coagulation + rapid sand filtration, GAC, chlorination 4A: Coagulation, rapid sand filtration, O_3, GAC, UV, chlorination 4B: Coagulation, rapid sand filtration, O_3, GAC, chloramination 5A: Coagulation + rapid sand filtration, O_3, chloramination 5B: Coagulation + rapid sand filtration, O_3, chloramination 6: Coagulation + rapid sand filtration, O_3, chloramination 7: Coagulation + rapid sand filtration, O_3, chloramination			

(*Continued.*)

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				8A: No treatment 8B: slow sand filtration				
Zacheus *et al.* (2001)	Finland	Not specified	16	1/A: Conventional, chloramine 1/B: Conventional, chloramine 2/A: Conventional, GAC, hypochlorite 2/B: GAC, hypochlorite 2/C: Conventional, GAC, hypochlorite 3/A: No treatments 3/B: No treatments 3/C: Conventional, GAC, hypochlorite 4/A: Conventional, O_3, GAC, hypochlorite 4/B: Conventional, O_3, GAC, chloramine 5/A: Conventional, O_3, chloramine 5/B: Conventional, O_3, chloramine 6/A: Surface water Conventional, O_3, chloramine 7/A: Conventional, O_3, chloramine 8/A: No treatment 8/B: No treatment	Spread plate method. R2A agar and on tryptone yeast extract agar, at 20 °C (3 and 7 days)	R2A and tryptone yeast (TY) media, 3 days: 7.0×10^3 and 1×10^3 (finished water) and 9.0×10^4 and 1.2×10^4 (distribution networks) (all systems)	–	–
Abdul *et al.* (2011)	India	Medium (34,672)	1	Not specified	SPC method, plate agar count, 37 °C for 24 h	1.0×10^5–18×10^7	–	–
Nagymate *et al.* (2018)	Hungary	Medium (417,651)	3	Only chlorination	R2A plate media counts, 22 °C for 7 days	E1–DW (distributed water): $4.0 \times 10^3 \pm 2.8 \times 10^3$	–	–

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
						E2–DW (distributed water): $8.8 \times 10^5 \pm 7 \times 10^4$ E3–DW (distributed water): $9 \times 10^5 \pm 1.9 \times 10^5$		
Tung & Xie (2009)	–	Small (1,900–4,300)	5	Conventional treatment processes + chlorination GP: NaClO NO: Cl_2 (g) JT: Cl_2 (g) LY: Cl_2 (g) LK: Cl_2 (g)	Standard membrane filtration method with HPC media	$< 10^1$–10^5 Data shown as histograms and presented as levels in warm and cold water	TOC analysed using persulphate oxidation method with TOC analyzer	TOC: System GP: 2.4 ± 0.9 mg/L System NO: 1.3 ± 0.2 mg/L System JT: 1.3 ± 0.3 mg/L System LY: 1.4 ± 0.2 mg/L System LK: 1.2 ± 0.3 mg/L
Escobar *et al.* (2001)	United States	Medium (284,817)	2	First system: ozonation + chlorination (1.5 mg/L of chlorine) Second system: nanofiltration in parallel with lime softening + chlorination (4.0 mg/L)	Spread plating on R2A agar, 22 °C for 7 days	Treated water: nanofiltration in parallel with lime softening: 0 and 20 Treated water, system with ozonation: 8–15	AOC was measured using a mixture of *Pseudomonas fluorescens* and *Aquaspirillium* NOX	AOC: 20–440 μg/L as acetate-C BDOC: 0.00–1.39 mg/L DOC: 1.83–5.16 mg/L
Nescerecka *et al.* (2014)	Latvia	Large (632,614)	3	No further details provided except: Water treatment plant (WTP) 1: Cl_2 (0.5–3 mg/L) WTP2: Cl_2 (ca. 1.5 mg/L) WTP3: N/A	Nutrient yeast agar plates using the spread plate technique	HPC 22 °C: WTP1: 23 ± 24 WTP2: 4 ± 2 WTP3: 4 HPC 36 °C: WTP1: 16 ± 16 WTP2: 4 ± 2 WTP3: 1	TOC and AOC data from previous studies and/or provided by water utility. ATP measured using BacTiter-Glo reagent and luminometer	TOC: WTP1: 6 ± 1 mg/L WTP2: 9 ± 3 mg/L WTP3: 3 mg/L AOC: WTP1: 213 ± 37 μg/L WTP2: 209 ± 59 μg/L WTP3: N.A.

(*Continued.*)

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
								Total ATP: WTP1: 0.007 ± 0.003 nM WTP2:0.000 ± 0.004 nM WTP3: 0.001 ± 0.002 nM
Lu *et al.* (2014)	China	N/A	1	Pre-oxidation + pre-chlorination, coagulation, sand filter, ozone, biological activated carbon (BAC) filter, chloramine (sub-district D: 1.16 ± 0.26/sub-district Z: 1.41 ± 0.17)	Pour plating method with R2A media, 22 °C for 7 days	Sub-district D: 300 ± 314 (6–1,130) Sub-district Z: 220 ± 248 (8–955)	AOC measured using a mixture of *Pseudomonas fluorescens* and *Aquaspirillium* NOX	AOC: Sub-district D: 90 ± 28 (33–149) μg/L; Sub-district Z: 81 ± 21 (19–122) μg/L
Ohkouchi *et al.* (2014)	Japan	Large (2,691 million)	1	Coagulation, mid-ozonation, sedimentation, rapid sand filtration, post-ozonation, gradual activated carbon + nanofiltration and chlorination (free residual chlorine: <0.14 mg/L)	R2A media, 7 days	2–32 (nanofiltration output)	AOC measured according to Japanese Standard methods	AOC: The average levels: 62 ± 20 μg C/L (winter) and 27 ± 15 μg C/L (summer)
Rashid *et al.* (2021)	Pakistan	Large (16 million)	3	Coagulation, sedimentation, filtration + chlorination	Dilution in sterile phosphate buffered saline (PBS). One mL of each dilution was mixed with liquefied plate count agar and poured onto Petri dishes (media not specified)	1.40×10^2–2.80×10^6 5.89×10^5 (mean)	–	–
Richards *et al.* (2018)	United States	Small (~13,000)	1	N/A + chlorination (free residual chlorine: not detected to 0.34 mg/L	R2A agar, 30 °C for 2 weeks	Treated municipal: 3.57×10^2 – 5.15×10^5; 9.02×10^4	–	–

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				and not detected to 0.27 mg/L)		(arithmetic mean) Groundwater well: 2.0×10^0 -9.23×10^5 5.7×10^4 (arithmetic mean)		
Zhang *et al.* (2016)	China	N/A	2	WTP1 'conventional water treatment plant': pre-ozonation, coagulation, flocculation, filtration, chlorination WTP2 'advanced water treatment plant': pre-ozonation, coagulation, flocculation, filtration, ozonation and bacterial activated carbon filtration, chlorination as secondary disinfection before distribution (chlorine residuals: 0.7–0.8 mg/L)	R2A agar, 22 °C for 7 days	Bacterial regrowth potential: 2.01×10^4– 4.51×10^5 Bacterial regrowth potential: 2.25×10^5 (mean)	BDOC using bioassay procedure for optimal growth. AOC determined with AOC assay using P17/NOX TOC using ligni TOC trace.	BDOC: 0.152–0.508 mg/L, 0.39 mg/L (winter), 0.38 mg/L (summer) AOC: 106.6 μg C/L (winter), 124.6 μg C/L (summer) TOC: 40.5–307.9 μg C/L
Scott *et al.* (2015)	Canada	Large (2.93 million + 113,520)	2	WTP1, Toronto (one of the four plants in Toronto): pre-chlorination, coagulation, flocculation,	Filtration, R2A agar, 28 °C for 5–7 days	Not available (histograms: maximum < 1.0×10^3 for both plants)	The potential for regrowth of both AOA and AOB were measured using quantitative	No data available, only figures

(*Continued.*)

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				sedimentation, (anthracite–sand) filtration and chlorine disinfection, followed by chloramination for secondary disinfection WTP2, Waterloo: coagulation, flocculation, sedimentation, ozonation, multimedia filtration, UV disinfection and chlorine disinfection, followed by chloramination for secondary disinfection			PCR assays that target the amoA gene	
Prest *et al.* (2016b)	Netherlands	Large (623,652)	1	Coagulation, flocculation and sedimentation followed by ozonation, dual medium filtration and granular active carbon filtration. Secondary disinfection (0.1 mg/L Cl_2(g))	Pour plating, yeast extract agar, 22 °C for 3 days	82% of the HPC values were below 5	AOC data provided by water utility	Lowest AOC: 3.5 μg Ac-C eq/L Maximum ~40–42 μg C/L
Francisque *et al.* (2009)	Canada	Large (240,000)	1	Pre-chlorination followed by coagulation–flocculation–sedimentation, slow sand filtration, ozonation and post-chlorination	R2A media, 35 °C for 48 h	13.8 ± 81.2 (95th percentile: 40)		–

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				(average post-chlorination dose: 1.8 mg/L)				
Donohue (2021)	United States (46 states)	Not specified	Not specified	Different systems using chlorination and chloramination (tap water was collected from 46 states in the United States)	R2A media, 25 °C for 7 days (standard methods 9215B)	Different ranges depending on systems 0.1 ± 1,000		–
Miyagi *et al.* (2017)	Japan	Large (1,457 million)	Not specified	Chlorine (further details on treatment not provided)	Plate count agar	Below <1 for all samples		–
Rodriguez-Martinez *et al.* (2015)	Israel	Small (18,121)	1	Chlorine (details on treatment not provided)	Yeast extract agar (YEA) (ISO 6222)	Cold water: At 22 °C: 4.0 and 7.6×10^3 min and max At 37 °C: 2.0×10^1 and 1.1×10^6 Warm water: At 22 °C: 80 and 2.8×10^2 (<0.1) min and max At 37 °C: 50 and 3.31×10^5		–
Pepper *et al.* (2004)	United States	Large (542,629)	1	Sourced from groundwater. Only chlorination	Tryptic Soy Agar and R2A agar, 35–37 °C for 24–48 h	Tucson groundwater distribution sites (average): 22 (10–158) Blended distribution sites (average): 47 (10–158) The average number of bacteria in household taps: 3,072 CFU/mL	TOC analysed with standard method of Dumas	Total organic carbon (kitchen for all houses): <1 ppm
Pieri *et al.* (2014)	Cyprus	Large (276,410)	1		Yeast extract agar, 37 °C for 44 ±	Low-risk area: TBC 22 °C: 5		–

(*Continued.*)

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				Conventional water treatment + chlorination	4 h and 22 °C for 68 ± 4 h	(median) TBC 37 °C: 8 (median) High-risk area: TBC 22 °C: 4 (median) TBC 37 °C: 9 (median)		
Scott & Pepper (2010)	United States	Large (six cities) A: Portland B: Milwaukee, C: Jacksonville, D: Tampa, E: Dallas, F: Chicago	6	Conventional water treatment + chlorination	R2A media, 27 °C for 7 days	City A: 5.0×10^0 City B: 3.4×10^2 City C: 3.0×10^3 City D: 2.0×10^3 City E: 2.4×10^5 City F: None (average)		–
Sekar *et al.* (2012)	United Kingdom	Small (not specified)	1	Blend of waters: upland with ferric sulphate, upland with alum and river with alum floc-based treatment trains. Chlorination performed	2 days at 37 °C 3 days at 22 °C (media not specified)	Viable bacterial count (referred to as HPC also), 2 days at 37 °C: 0–34 Viable bacterial count (referred to as HPC also), 3 days at 22 °C: 0–42		–
Inkinen *et al.* (2014)	Finland	Medium (39,000) (building)	1	Coagulation (ferric sulphate) sedimentation, flotation and disinfection (sodium hypochlorite), manganese oxidation, active carbon filtration and ammonium chloride (to guarantee chlorine stability)	R2A media, 22 °C for 7 days	Cold water: $2.9 \times 10^4 \pm 3.9 \times 10^4$ (copper) and $1.1 \times 10^4 \pm 1.3 \times 10^4$ (PEX) Hot water: $2.0 \times 10^4 \pm 3.2 \times 10^4$ (copper) and $2.7 \times 10^1 \pm 1.5 \times 10^1$ (PEX)	AOC measured with P17/NOX. Nutrient solution added to maintain nutrient balance	AOC: 194 ± 94 mg C/L (PEX); 129 ± 82 mg C/L (copper); 125 ± 45 mg C/L (incoming water)
El-Taweel & Shaban (2001)	Egypt	Medium to large (no further details)	8	Rapid sand filtration, pre-chlorination, coagulation, clarification, sedimentation	22 °C, 37 °C No further information provided	Outlet = <1 log Treated water (average): El-Tebbien: 15 (22 °C)/13 (37 °C)		–

Table 4 | Continued

Authors and year	Location	Water distribution system			HPC		Organic and microbial content	
		Population size	Number of systems	Water treatment processes	Methods	Results (CFU/mL)	Methods	Results
				filtration and chlorination		Kafer El-Elow: 18 (22 °C)/16 (37 °C) El-Maadi: 13 (22 °C)/12 (37 °C) El-Fustat: 11 (22 °C)/9 (37 °C) El-Giza 2 (22 °C)/ 2 (37 °C) Rod El-Farag: 13 (22 °C)/11 (37 °C) Port Said: 16 (22 °C)/19 (37 °C) Ismailia: 27 (22 °C)/24 (37 °C)		
Hoefel *et al.* (2005)	Australia	Medium (28,524)	1	Coagulation (alum), flocculation, sedimentation, pre-chlorination, rapid gravity filtration (dual sand and anthracite media), chlorination	Spread plate technique (R2A and tryptic soy agar (TSA)) 35 °C for 48 h and 20 °C for 72 h	Finished water: <1 Bulk water from within the distribution system: <1		–
Zamberlan da Silva *et al.* (2008)	Brazil	Large (403,063)	1	Not specified	Plate count agar, HPC at 35 °C for 72 h	Tap water: 0–2,350 and 95 (median)		–

[a]Secondary disinfection refers to chlorination or chloramination of water prior to its entry into the distribution system.

3.4.2. Residual chlorine

Results from studies conducted in full-scale chlorinated distribution systems suggest an inverse relationship between HPC and free chlorine residuals (Lu *et al.* 2014; Nescerecka *et al.* 2014; Ohkouchi *et al.* 2014). Lu *et al.* (2014) found that maintaining a high chloramine residual concentration was the most practical way to achieve low HPC colony count units. Escobar *et al.* (2001) found that using ozonation significantly increased AOC concentrations, results show that levels of HPC remained low as chlorine residual levels were maintained. Similarly, Ohkouchi *et al.* (2014) found that maintaining consistent levels of chlorine residual was efficient for inactivating HPC and ensuring biological stability during water distribution. The effect of chlorine residuals on preventing biofilm accumulation was, however, considered small.

In some cases, chlorine residual was not sufficient to prevent microbial growth (Nescerecka *et al.* 2014; Ohkouchi *et al.* 2014; Zhang *et al.* 2016). Interrelated parameters such as water temperature and residence time can influence chlorine residual in the water distribution system and the effects on HPC levels. Chlorine depletion will generally lead to an increase in HPC levels when water temperatures are elevated (Scott *et al.* 2015; Miyagi *et al.* 2017). This is consistent with Francisque *et al.* (2009), who found that free residual chlorine was not a critical parameter for HPC colony count units in cold-water conditions.

3.4.3. Residence time/stagnation

In a non-chlorinated system in Switzerland, Lautenschlager *et al.* (2010) found that significant changes in microbial levels occurred during stagnation, meaning higher temperatures, longer residence times and nutrient contamination from the household environment and/or the pipe material. The study reported increased colony count units of HPC in stagnated water samples, ranging from 4 to 580 times higher compared with flushed water samples (Lautenschlager *et al.* 2010). In chlorinated systems, Tung & Xie (2009) found the highest HPC concentration and lowest chlorine residual to have occurred in samples with the maximum water residence time. In comparison, intact cells and ATPc concentrations only increased by 1.6–2.3 in stagnated water compared with after flushing (Tung & Xie 2009). The authors explained this difference by citing that HPC only comprises a fraction of total cell counts, as highlighted in other studies including Liu *et al.* (2013). Lack of precise data on water residence times and pipeline characteristics at different points in the distribution systems constitutes a significant limitation when modelling HPC colony count units (Francisque *et al.* 2009). Prest *et al.* (2016a) reported that water quality assessments did not include residence times in the distribution system because it could not be accurately estimated. Nevertheless, using a tracer technique, Zhang & DiGiano (2002) found a positive correlation between water residence time and log (HPC) in North Carolina. Correlation between distance from the plant and HPC levels were also investigated by Power & Nagy (1999), where a positive correlation was reported.

3.4.4. Carbon content

Most studies found a correlation between HPC and AOC concentrations. In two chlorinated systems in China and Latvia, Zhang *et al.* (2016) and Nescerecka *et al.* (2014) found a significant correlation between HPC colony count units and AOC and intact cell concentrations, respectively. Bacteria, organic carbon and chlorine concentrations were identified as the most important factors impacting the biological stability in the drinking water distribution system (Zhang *et al.* 2016).

Francisque *et al.* (2009) found that higher levels of organic matter in water were associated with higher HPC levels in a full-scale chlorinated distribution system in Canada. Similarly, in a distribution system supplied by ozonated water, Escobar *et al.* (2001) found a correlation between HPCs and AOC at different sampling points in the distribution system, both before and after ozonation. A study conducted in a non-chlorinated water distribution system in the Netherlands found consistent results (Hijnen *et al.* 2018). Those authors demonstrated a positive correlation between the particulate and/or high molecular organic carbon and regrowth indicators (HPC and Aeromonas). However, Lu *et al.* (2014) found no correlation between HPC and AOC in a large, full-scale chlorinated distribution system, despite also finding a significant correlation between HPC and temperature, dissolved oxygen and chloramine residual. Contrarily, Zhang & DiGiano (2002) found a negative correlation between HPC and organic content, both for TOC and AOC concentrations.

3.5. HPC and other proposed indicators for regrowth

Previous studies also suggest that it is possible to maintain biological stability as well as low and constant colony count units of HPC in a continuous non-chlorinated distribution system. In a medium full-scale system in Switzerland, Lautenschlager *et al.* (2013) found that HPC, TOC and AOC remained constant at all locations where HPC levels were below 10 CFU/mL.

Liu *et al.* (2013) similarly found that HPC values remained below 100 CFU/mL in a full-scale distribution system in the Netherlands, no direct relationship between HPC, TCC and ATP could be established although increased or exceeded colony count units of HPC were noted in a few cases. Results from a large study focusing on in-building installation systems in Germany showed that 3.5% of the samples, out of more than 10,000 samples, exceeded the limit of 100 CFU/mL. Among these samples, Volker *et al.* (2010) reported that higher colony count units of HPC were more likely to be found in warm water compared with cold water. This is similar to findings from Prest *et al.* (2016b), who reported that the influence of water temperature on HPC was stronger at higher temperatures. Regardless of the use of chlorination, lower water temperatures led to less biofilm formation, which may also contribute to maintaining acceptable levels of AOC (Ohkouchi *et al.* 2014).

3.6. HPC as an indicator for water safety

In a large study conducted across the United States, Donohue (2021) found that most samples that were positive for *Legionella pneumophila* had an HPC count that ranged from 101 to 1,000 CFU/mL. However, as the HPC count exceeded 10,001 CFU/mL, the authors observed a decrease in the median levels of *L. pneumophila*, showing that no consistent relationship between HPC and *Legionella pneumophila* count could be identified. Meanwhile, results from studies conducted in small chlorinated systems showed a positive correlation between HPC and *Legionella pneumophila* counts in the distribution system (Rodriguez-Martinez *et al.* 2015; Richards *et al.* 2018). Richards *et al.* (2018) used DNA extracted from biofilms and was able to also establish a relationship among detected *Mycobacterium* and quantified HPC bacteria. Similarly, in a Japanese study by Miyagi *et al.* (2017) investigating the detection of *Aeromonas*, enteric and other related bacteria in domestic water supply facilities, the authors could not establish a relationship between HPC counts and the presence of *Aeromonas* and *Enterobacteriaceae*. The HPC colony count units were below 0.1 CFU/mL for all samples in that study. In a study conducted in the UK, Gavriel *et al.* (1998) showed no relationship between incidence of *Aeromonas* and HPCs. Results from this study examining the detection percentage of pigmented bacteria (e.g., Flavobacterium and Mycobacterium species) in HPC levels, demonstrated that pigmented bacteria represent a considerable portion of HPC levels and are found to be well adapted to the distribution system environment. An association between these opportunistic pathogens and nosocomial infections was also reported (Gavriel *et al.* 1998).

3.7. Meta-analysis

3.7.1. HPC colony count units

Various levels of HPC concentration in water distribution systems were found in the literature. Overall, HPC levels ranged from <1 to 1.80×10^8.

3.7.2. Treatment and disinfection

Results from our meta-analysis revealed variations in HPC levels between systems using chlorine or chloramine disinfection before water distribution and those without secondary disinfection and disinfectant residuals (Figure 2; Table 5). Within systems using chlorination or chloramination, the observed levels ranged up to 1.80×10^8, whereas in systems without secondary disinfection, the reported range was up 8.70×10^2. The maximum level of 1.80×10^8 was observed for an intermittent supply system where infiltration of sewage water increased the likelihood of microbial contamination (Abdul *et al.* 2011). The highest level for the systems without secondary disinfection was found in overnight stagnant water (Lautenschlager *et al.* 2010). Stagnant water in pipes can affect microbial levels and composition (Lautenschlager *et al.* 2010). Regardless, lower levels of HPC were found in various types of systems and sampling locations, and a clear pattern could not be established.

Interestingly, in certain systems where secondary disinfection was not used, HPC levels were slightly lower (Figure 2). Such systems performed biofiltration and rapid and slow filtration, either in combination with coagulation as an upstream treatment or with ozone as a downstream treatment to limit growth-supporting nutrients. Lautenschlager *et al.* (2013) observed very low AOC concentrations below the detection limit of 10 μg/L in all samples and conventional cultivation-dependent HPC data consistently fell below 10 CFU/mL. These values only slightly increased with increasing water retention times (Lautenschlager *et al.* 2013). Hijnen *et al.* (2018) also reported low levels of HPC at three water treatment plants that did not add residual disinfectant after the reservoir. These levels were all below 21 CFU/mL for the 2012–2015 sampling period. The highest mean concentrations of AOC-P17/NOX was 27.9 ± 12.0 μg/L.

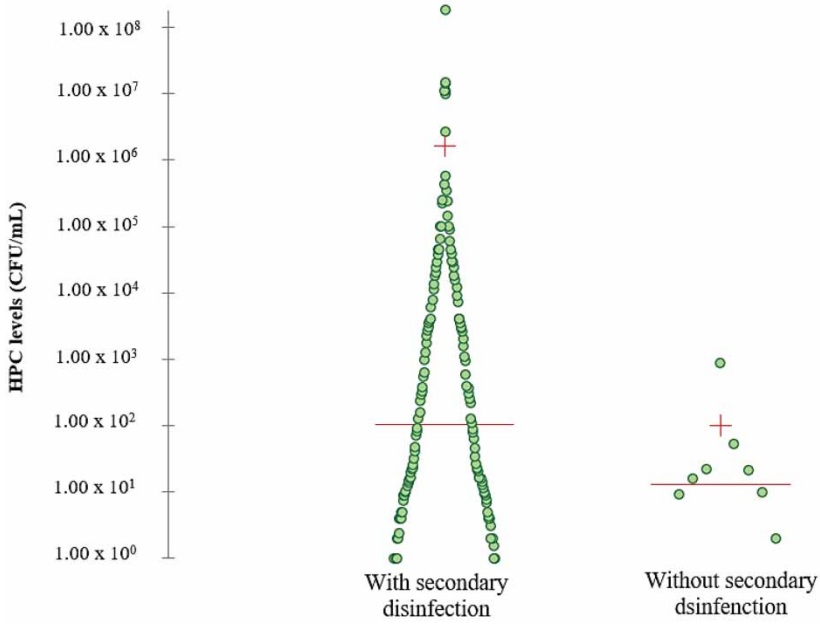


Figure 2 | Comparison of systems with and without secondary disinfection (152 and 10 observations, respectively). Red lines indicate the medians while crosses represent the means.

Table 5 | Descriptive statistics for HPC, comparing systems with and without secondary disinfection

Variable	Observations	Minimum (CFU/mL)	Maximum (CFU/mL)	Mean (CFU/mL)	Standard deviation (CFU/mL)
Systems with secondary disinfection	152	<1	1.80×10^8	1.62×10^6	1.47×10^7
Systems without secondary disinfection	10	<1	8.70×10^2	1.00×10^2	2.71×10^2

Results from a Mann–Whitney U test indicate the presence of a significant difference in HPC levels between systems using secondary disinfection and those that are not using secondary chlorination (p-value of 0.031 with a confidence interval of [0.027; 0.036], $\alpha = 0.05$). HPC levels for systems using secondary disinfection varied widely from system to system, with a standard deviation of 1.47×10^7. This suggests that HPC levels can differ despite chlorination or chloramination as a primary and/or secondary step in the water treatment process. This may also indicate that factors other than the use of chlorine or chloramine disinfectant may contribute to HPC levels.

Other factors that may have an impact on HPC variability include the water source (groundwater or surface water), type of treatment and performance (Abdul *et al.* 2011; Ohkouchi *et al.* 2014), hydraulic residence time (Sekar *et al.* 2012), flushing (Lautenschlager *et al.* 2010), water temperature (Inkinen *et al.* 2014) and pipe condition. As these effects can be combined or independent, it is difficult to determine which of these factors have the greatest impact on HPC levels. For example, a study by Francisque *et al.* (2009) shows that temperature and free residual chlorine act together to affect HPC levels. According to that study, in cold-water conditions, free residual chlorine is not an essential parameter for ensuring very low levels of HPC. However, in warm water, 0.1 mg/L of free residual chlorine must be maintained to prevent high levels of HPC (Francisque *et al.* 2009). This may be because warmer temperatures accelerate chlorine decay and promote the growth of microorganisms in drinking water.

3.7.3. Size of the system

As with hydraulic conditions, system size was also examined to determine whether this parameter could influence HPC levels (Figure 3). It was hypothesized that some smaller water distribution networks may have fewer resources to maintain pipes in good conditions over years compared with larger networks. In contrast, larger systems are more likely to have

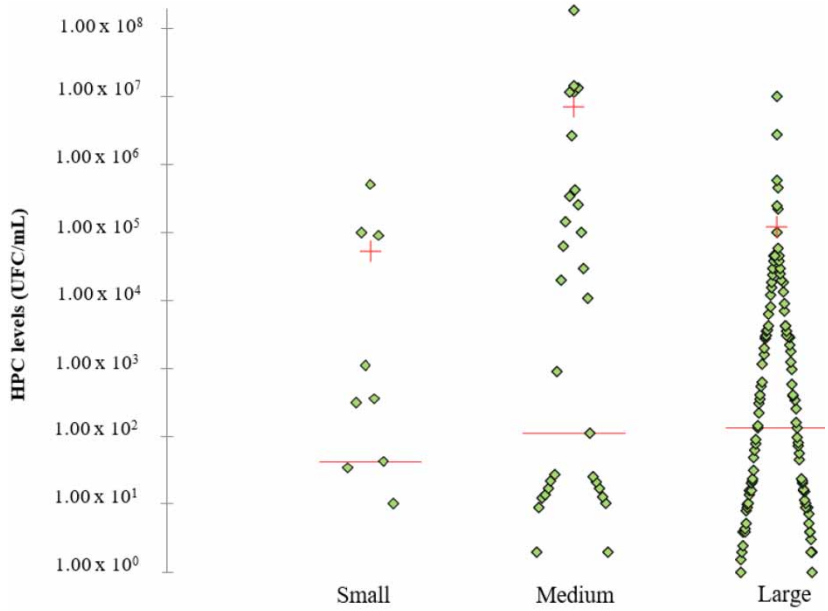


Figure 3 | Comparison between different-sized water treatment systems (Kruskal–Wallis test, $\alpha = 0.05$). Forty-one large systems, 24 medium systems and 13 small systems are shown. Red lines indicate the medians while crosses represent the means.

more resources to maintain more efficient treatment processes (McFarlane & Harris 2018). However, maintaining consistent pipe conditions remains challenging due to the greater distances, leading to more variation in hydraulic conditions and longer retention times.

The size of the population that is served by the system was used as a proxy parameter to assess system size. The level of HPC colony count units ranged from <1 to 5.15×10^5 in small systems, <1 to 1.80×10^8 in medium systems and <1 to 1.00×10^7 in large systems (Table 6). The maximum levels of 1.80×10^8 were found in a medium system. A wide range of HPC values can be observed for small, medium and large systems, with respective standard deviations of 1.43×10^5, 3.13×10^7 and 9.47×10^5. This indicates a high variability within each group of systems. For each system size, HPC colony count units below detection limits were observed (<1 CFU/mL). A Kruskal–Wallis test demonstrated that HPC colony count units did not significantly differ between small, medium and large systems. Therefore according to our data, the size of the system did not impact HPC levels (p-value = 0.999, $\alpha = 0.05$).

3.7.4. Carbon content

We developed a log-linear regression model with data extracted from relevant studies, using medians and/or means, and confirmed a positive correlation between HPC levels and AOC concentrations (Figure 4). Statistical analyses were conducted based on 15 observations (mean/median HPC levels) from both systems using and not using secondary disinfection. A few studies conducted in water distribution systems using chlorine or chloramine observed low levels of HPC and AOC concentrations (<1 CFU/mL; <1 μg C/L). Analysis shows that ~70% of the variance in HPC levels between distribution systems was explained by the AOC concentration ($R^2 = 0.70$) (Table 7). According to our data, this correlation persists between different types of systems with different characteristics, meaning that AOC is a good predictor of HPC levels regardless of system

Table 6 | Descriptive statistics comparing HPC levels in small, medium and large systems

Variable	N	Minimum (CFU/mL)	Maximum (CFU/mL)	Mean (CFU/mL)	Standard deviation (CFU/mL)
Small	13	<1	5.15×10^5	5.44×10^4	1.43×10^5
Medium	33	<1	1.80×10^8	7.12×10^6	3.13×10^7
Large	120	<1	1.00×10^7	1.25×10^5	9.47×10^5

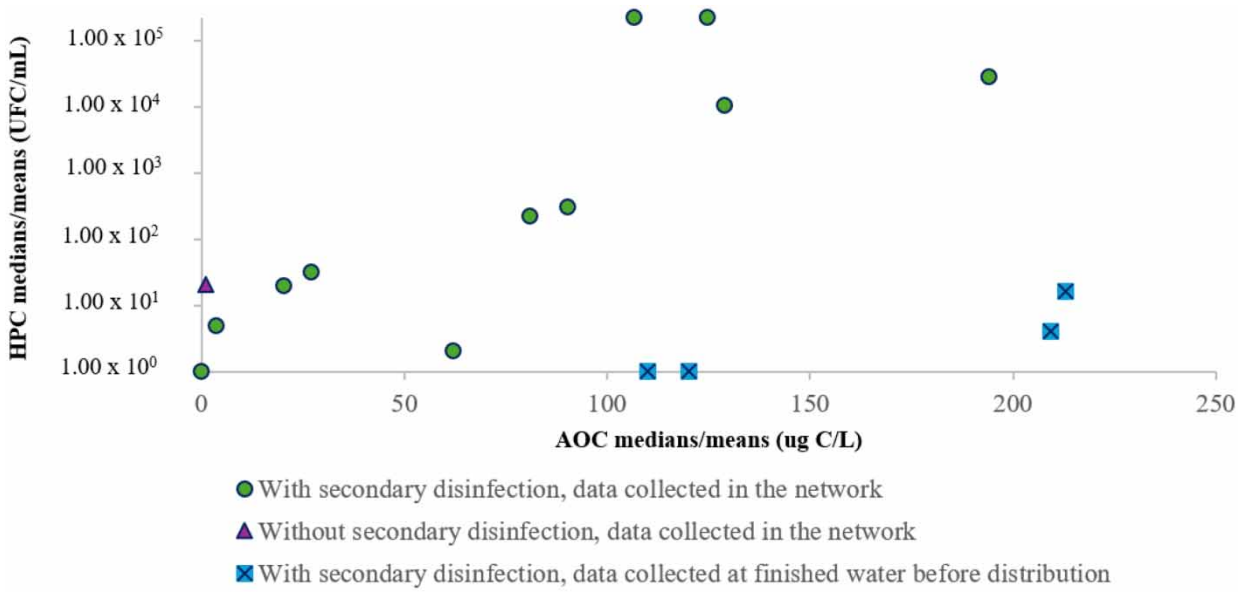


Figure 4 | Log HPC medians/means as a function of AOC medians/means ($N = 15$). Orange points indicate HPC and AOC levels at the plant before distribution for systems with secondary disinfection. The blue point indicates the HPC level measured within the network for a system without secondary disinfection. The green point indicates sampling points in the network.

Table 7 | Data used to develop the log-linear regression model

Variable	N	Min	Max	Mean	SD	Pr > Chi² (−2 log(Likelihood))	R^2 (Cox and Snell)
HPC (CFU/mL)	15	<1.00	2.25×10^5	3.27×10^4	7.85×10^4	0.005	0.70
AOC (μg C/L)	15	<1	2.13×10^2	9.93×10^1	6.99×10^1		

characteristics (e.g., treatment type, water source). Similar correlations were reported by Escobar & Randall (2001) and Zhang *et al.* (2016) using linear and log-linear models to assess the relationship between AOCs and HPC for different water treatments. To our knowledge, this is the first time that this correlation has been reported between two different types of systems (i.e., with or without secondary disinfection).

Some studies did not establish the water sampling points along the distribution system. The orange points in Figure 4 indicate the finished water sampling points in two plants prior to the water entering the water distribution network from two studies (Zhang & DiGiano 2002; Nescerecka *et al.* 2014). Based on these data, HPC levels are low compared with AOC levels, and the levels do not follow the log-linear correlation as in other papers (Figure 4). In contrast to the finished water samples used in Nescerecka *et al.* (2014) and Zhang & DiGiano (2002) (orange points, Figure 4), other studies collected water samples from within the network. This difference may have influenced the reported concentrations, as HPC levels tend to be higher within the network compared with the outlet (for the same system) (Lautenschlager *et al.* 2013; Nescerecka *et al.* 2014; Prest *et al.* 2016b). Ohkouchi *et al.* (2014) reported a low HPC level of 62 μg C/L at a water treatment plant that uses a nanofiltration pilot system that typically rejects bacterial cells in the nanofiltration permeate. As such, nanofiltration may be a more effective method for limiting HPC levels compared with conventional treatments that use coagulation and filtration (Ohkouchi *et al.* 2014). Ohkouchi *et al.* (2014) also reported that the membrane treatment reduced AOC levels from 107 ± 35 μg C/L (input) to 62 ± 20 μg C/L (output). AOC levels also tend to decrease following coagulation, sedimentation and filtration, whereas levels tend to increase following ozonation and chlorination (Zhang *et al.* 2016).

4. DISCUSSION

This paper provides a comprehensive review and meta-analysis of studies on heterotrophic bacteria occurrence in drinking water distribution systems. Overall, our review revealed varying levels of HPC in systems using secondary disinfection prior to distribution and those that do not (Bartram *et al.* 2003; Uhl & Schaule 2004; Xi *et al.* 2009; Zlatanović *et al.* 2017). Although variability within supply systems is generally common, results from the literature suggest that abnormal increases in HPC levels for one system can point to a possible deterioration of water quality and/or infrastructure within the distribution system.

Results from the meta-analysis highlight the importance of using a multi-parameter approach, as no single parameter could be considered the controlling factor for microbial growth in the distribution networks. No significant difference in HPC colony count unit was found between chlorinated and non-chlorinated systems. Findings suggest that sufficient concentrations of chlorine could help deactivate HPC under specific conditions, such as those related to water temperature and residence time (Ohkouchi *et al.* 2014). This is because HPC generally increases in the distribution network, from the point of entry to the consumer tap (Pepper *et al.* 2004; Inkinen *et al.* 2014). Although an appropriate level of chlorine is essential to maintain post-treatment protection, evidence from the literature suggests that it is not the only means of maintaining HPC levels and biological stability in water distribution networks. Other parameters such as temperature, residence time, hydraulic conditions, pipe material and sizing, limiting nutrients and the presence of protozoa/invertebrates can impact the biological stability of the water. Changes in HPC colony count units can also help identify vulnerable sectors where higher residence times, higher temperatures and/or low-pressure flow might occur. This is particularly relevant in the context of low- and middle-income countries, where systems are more likely to be intermittent (i.e., discontinuous flow and negative pressure) (Tokajian & Hashwa 2003). The bacterial growth within household distribution systems is often overlooked, in both chlorinated and non-chlorinated systems (Pepper *et al.* 2004; Lautenschlager *et al.* 2010; Inkinen *et al.* 2014). Water temperature, residence times, household pipe material and pipe diameter should also be considered at individual households (Prest *et al.* 2016b).

In systems where water is distributed without disinfectant, controlling AOC levels helps to also control and prevent the regrowth of microorganisms (van der Kooij *et al.* 2003; Liu *et al.* 2015). Biologically stable water can be achieved in part by maintaining AOC concentrations at less than 10 $\mu g\ L^{-1}$, as opposed to the 50–100 $\mu g\ L^{-1}$ seen in some chlorinated systems (van der Kooij 1992; LeChevallier *et al.* 1996). Organic matter removal can be achieved using a multifaceted approach, such as by applying advance modelling to design water systems, ensuring high flow velocities and flushing regularly under specific conditions (Smeets *et al.* 2009). Results from other studies also point to the importance of reducing organic matter to minimize disinfection by-product (THM and HAA) formation in chlorinated distribution systems (Barrett *et al.* 2000; Farré *et al.* 2013). Indeed, previous studies and results from our meta-analysis revealed a correlation between HPC and AOC, reinforcing the importance of controlling both parameters (Van Dyke *et al.* 2015). Nevertheless, it is important to note that no conclusions or inferences can be made regarding the impact of a single parameter on HPC levels, since several factors are simultaneously present and interconnected, and some factors are highly interdependent. Our meta-analysis yielded a novel finding, revealing that this correlation between HPC and AOC was found across various studies characterized by different treatment systems, water sources and operational conditions. In contrast to our findings, another study reported that there is no correlation between AOC levels and HPC in the network they investigated (Carter *et al.* 2000) The authors of that article suggested that this was due to the very low concentrations of AOC, with mean AOC concentrations of 0.09 ± 0.01 and 0.10 ± 0.02 mg/L in two sampling points in the network (detection limit was of 0.005 mg/L). However, in that study, only seven samples were collected at each site and AOC and HPC levels are subject to spatial and temporal variability within distribution networks (Sekar *et al.* 2012; Pieri *et al.* 2014).

Data from this review suggest a relationship between HPC colony count units and presence of opportunistic pathogens in DWSs, including *Legionella* spp. and *Mycobacterium* spp. (Rodriguez-Martinez *et al.* 2015; Richards *et al.* 2018). However, other research showed that there was no consistency between HPC levels and the presence of *L. pneumophila*, with HPC only being considered a 'fair' predictor (Donohue 2021). Higher levels of HPC would generally help to predict the presence of *Legionella* spp., but as HPC colony count units increase, HPC is more likely to suppress *L. pneumophila* regrowth (Donohue 2021). Although no correlation could be established, other studies found that high colony count units of HPC can be useful for determining the presence of *L. pneumophila* in cooling towers (Duda *et al.* 2015; Pierre *et al.* 2019; Sanchis *et al.* 2023). Such findings suggest that HPC and *L. pneumophila* may have similar responses to environmental conditions and

parameters in drinking water distribution systems such as water temperature (*L. pneumophila* growth occurs between 20 and 50 °C) and level of chlorine residual. Other important parameters including stagnation and residence time should also be considered (Centers for Disease Control and Prevention 2018).

Additionally, studies on the microbiological quality of drinking water found no association between HPC and other faecal and non-faecal indicators, including *E. coli* and total coliforms. Of note, previous studies have found that heterotrophic bacteria at levels greater than 500 CFU/mL can interfere with some total coliform and *E. coli* recovery methods that are lactose-based (Allen *et al.* 2004). There is no significant evidence in the existing literature associating HPC with increased health risks. Previously, evidence-based laboratory findings indicated that no significant amounts of virulence factors were retrieved from HPC bacteria (Edberg & Allen 2004). Those results are consistent with some of the earliest epidemiological studies that failed to show any association between HPC and gastroenteritis (Calderon 1988).

This review only included studies that use standard culture-based methods. Different media (plate count agar using tryptone glucose yeast agar, m-HPC agar using SPC agar, R2A agar and enzyme substrate agar using the SimPlate® method) and techniques (pour plate, spread plate, membrane filtration) (Allen *et al.* 2004; Lipps *et al.* 2023) are used to quantify HPC populations. In the literature, HPC is determined both on high- and low-nutrient media. The use of high-nutrient media is recommended for the identification of bacteria from animals and humans. For the enumeration of autochthonous bacteria from drinking water, low-nutrient media such as R2A agar are preferred (Reasoner 1990; Allen *et al.* 2004) and have been proven to be the most sensitive (Health Canada 2022). However, there is currently no established standardized universal procedure to measure HPC in drinking water distribution networks. The literature presents a wide range of procedures for determining HPC, including those with varying incubation temperatures and times. Incubation at a temperature of 35–37 °C and a brief incubation period of 34–48 h promote the growth of bacteria from animals and humans, while microorganisms that live in water benefit from a low-temperature incubation (20–28 °C) and a prolonged incubation period (5–7 days) (Reasoner 1990; Allen *et al.* 2004). Previous research that investigated HPCs results on R2A media demonstrated that counts greatly vary depending on incubation time (i.e., 2, 3 and 7 days) and sampling site (Reasoner *et al.* 1989). Different media have also been used in the literature to document HPC levels, which prevent from thoroughly interpreting and comparing water quality measurements from different distribution systems. The diversity of methods to measure HPC prevent the use of HPC as a meaningful indicator when comparing multiple systems. Further research is necessary to assess the diverse methods identified in the present literature review for enumerating HPC. Efforts should be made to standardize the enumeration process for greater consistency and comparability.

Traditional plating procedures do not recover many of the bacterial species that are present in the water. Although evidence suggests a correlation between AOC and HPC colony count units, it is apparent that HPC is not a proxy indicator for bacterial abundance (Health Canada 2022). HPC values on solid media typically only represent a fraction of the total bacterial population when measured using microscopic methods, as most drinking water bacteria cannot be readily grown on conventional media (Bartram *et al.* 2003; Lautenschlager *et al.* 2013; Health Canada 2022). An interesting alternative to explore for determining bacterial abundance and total cell concentrations is flow cytometry, a cultivation-independent microbial community analysis (Lautenschlager *et al.* 2013). HPC alone does not detect the specific composition of bacteria, including pathogenic strains (e.g., *Aeromonas* spp., *E. coli*, *Pseudomonas* spp.). Less than 1% of HPC bacteria possess possible virulence factors, of which waterborne pathogens represent approximately 0.01% of the group of culturable heterotrophic bacteria (Bartram *et al.* 2004). Screening the HPC and isolating strains of bacteria might allow for the identification of potentially pathogenic features (Pavlov *et al.* 2004; Stelma *et al.* 2004).

It is still worthwhile to determine the culturable HPC microorganism levels during the treatment, storage and distribution of drinking water, as this can provide useful information about the microbiological quality of the water (Carter *et al.* 2000). As HPC levels differ between systems and HPC enumeration methods (type of media, temperature, days of incubation), it is impractical to define clear thresholds for 'safe' global levels of HPC, as is done for other broad bacterial indicators such as total coliforms. Nonetheless, our results show that HPC can be a viable indicator for estimating bacterial population density under specific conditions and for assessing the spatial and temporal variability of these bacteria within the same distribution system. HPC can be a useful analytical method to evaluate changes in microbial populations and to determine 'hot spots' or abnormal bacterial changes in the water system. It is also possible to assess biological stability in drinking water distribution systems by combining other assessment methods with HPC monitoring. These methods include cellular adenosine triphosphate (ATPc) and AOC measurements, which provide useful information on the number of viable, active microbial cells in a multi-parametric approach (Lautenschlager *et al.* 2013; Prest *et al.* 2013; Prest *et al.* 2014; Prest *et al.*

2016b; Chorley *et al.* 2018; Health Canada 2022). Monitoring microbial growth is an important part of managing drinking water quality from the water source to the consumer tap (Pick *et al.* 2019), and having a water safety plan in place can lead to fewer incidents of high levels of HPC (Gunnarsdottir *et al.* 2012).

4.1. Limitations of the study

This meta-analysis was based on relevant data extracted from selected studies. Missing data as well as selection or incomplete reporting in the studies can influence the results and our conclusions. Heterogeneity among the included studies limited the exploration of differences among subgroups. For example, studies included in the review were predominantly conducted in large and chlorinated water distribution supplies as opposed to very small systems. Furthermore, comparisons are likely influenced by the large number of studies conducted on systems using secondary disinfection ($n = 24$) compared with those without secondary disinfection ($n = 6$). Multiple studies lacked detailed information on water treatment, which prevented thorough analysis and comparison between systems. Although the heterogeneity among the studies can pose interpretive challenges, the comparison of different studies allowed us to consider the numerous types of monitoring and analysis of HPC in drinking water distribution systems.

While HPC colony count unit data were available in most of the selected studies, other important parameters such as sampling location, water residence time, dose/levels of residual disinfectant, specifications on treatment conditions and operations, temperature and environmental conditions were scarce. In our meta-analysis, no multivariate analyses could be conducted for those parameters, which prevented us from being able to identify the relationships between them.

The present literature review focuses on factors that differentiate drinking water distribution systems, such as the use of chlorine or the size of the network. It is expected that results within these categories would likely vary if HPC levels were compared based on enumeration methods. Further research is needed to analyse the various methodological procedures for heterotrophic plate count. Implementing a standardized method for monitoring HPC would allow for data that are more representative and applicable for general use. The wide range of methods that is currently used to measure HPC makes it difficult to make comparisons between studies, control for input parameters and evaluate associations. For instance, some papers used R2A with a temperature of 22 or 37 °C that may influence the growth of HPC on this low nutrient-poor medium.

The different methods must be more thoroughly assessed to categorize the ranges of and variations in HPC colony count units to further explore the link between *Legionella*, HPC and other bacteria in the water distribution system. Finally, integrated decision support tools should be developed to guide municipal drinking water systems in microbial water quality management and risk assessment, using HPC in parallel with other physicochemical and microbial indicators to monitor biological stability.

5. CONCLUSIONS

This comprehensive review and meta-analysis presents a synthesis of the recent literature on HPC and assesses its use for monitoring biological stability and factors that influence HPC levels.

Our review demonstrates the link between HPC colony count units and the different physical and chemical parameters in water distribution systems. The results suggest that disinfectant alone cannot prevent the formation of biofilms in water distribution systems (Prest *et al.* 2016a). Other parameters such as water treatment, temperature and residence time also influence HPC levels. With so many variables at play, an integrated way to manage distribution systems is needed.

The cumulative evidence presented here corroborates previous findings that point to the lack of standardization when using HPC as a tool to monitor drinking water. While there is no evidence suggesting that HPC can be used as a water quality indicator from a health perspective, under certain circumstances, HPC seems to be a good indicator for the presence of different microorganisms. However, seasonal and temporal variations in water distribution systems influence the presence of heterotrophic bacteria and other microorganisms, including *L. pneumophila* (Francisque *et al.* 2009; Rodriguez-Martinez *et al.* 2015; Prest *et al.* 2016b). Overall, HPC is demonstrated to be a relevant parameter that can be used as a complementary tool to monitor biological water stability and water quality changes in water distribution systems.

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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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