

MINI REVIEW

Microbial drinking water monitoring now and in the future

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Abstract

Over time, humanity has addressed microbial water contamination in various ways. Historically, individuals resorted to producing beer to combat the issue. Fast forward to the 19th century, and we witnessed a scientific approach by Robert Koch. His groundbreaking gelatine plating method aimed to identify and quantify bacteria, with a proposed limit of 100 colony-forming units per millilitre (CFU/mL) to avoid Cholera outbreaks. Despite considerable advancements in plating techniques through experimentation with media compositions and growth temperatures, the reliance on a century-old method for water safety remains the state-of-the-art. Even though most countries succeed in producing qualitative water at the end of the production centres, it is difficult to control, and guarantee, the same quality during distribution. Rather than focusing solely on specific sampling points, we propose a holistic examination of the entire water network to ensure comprehensive safety. Current practices leave room for uncertainties, especially given the low concentrations of pathogens. Innovative methods like flow cytometry and flow cytometric fingerprinting offer the ability to detect changes in the microbiome of drinking water. Additionally, molecular techniques and emerging sequencing technologies, such as third-generation sequencing (MinION), mark a significant leap forward, enhancing detection limits and emphasizing the identification of unwanted genes rather than the unwanted bacteria/microorganisms itself. Over the last decades, there has been the realization that the drinking water distribution networks are complex ecosystems that, beside bacteria, comprise of viruses, protozoans and even isopods. Sequencing techniques to find eukaryotic DNA are necessary to monitor the entire microbiome of the drinking water distribution network. Or will artificial intelligence, big data and machine learning prove to be the way to go for (microbial) drinking water monitoring? In essence, it is time to transcend century-old practices and embrace modern technologies to ensure the safety of our drinking water from production to consumption.

MICROBIOLOGICAL DRINKING WATER SAFETY STILL RELIES ON CENTURY-OLD METHODS

Over time, humanity has addressed microbial water contamination in various ways. Historically, individuals resorted to products such as beer to overcome the

health risks of drinking contaminated water (Antman & Flynn, 2022). Both the boiling and fermentation processes, which result in a small amount of alcohol, ensure that during the production and storage of beer, the water is purified and unwanted microorganisms are killed (Bamforth & Wiley InterScience, 2004; Homan, 2004).

Thomas Pluym and Fien Waegenaar contributed equally to this work and share the first authorship

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In the 19th century, people began to grasp the importance of clean and microbiologically safe water. In 1854, there was a cholera outbreak in London. An anaesthetologist, John Snow, was able to trace the source of the outbreak and was the first to describe cholera as a waterborne disease (Snow, 1854; Tulchinsky, 2018). Three decades later, Robert Koch invented a technique to isolate and cultivate 'pure' cultures of bacteria by growing them in plates with gelatine based, solid growing media, allowing for their identification and quantification (Blevins & Bronze, 2010; Koch, 1881). Richard Petri later modified Koch's technique to make handling and pouring easier (Petri, 1887). By means of this new method, Koch was able to identify the bacteria, describe the mode of action and locate the source of a *Vibrio cholera* outbreak in Egypt (Blevins & Bronze, 2010). This method with 'Petri' dishes into which media could be poured, cooled and solidified to grow and isolate pure cultures to evaluate the drinking water microbiology is still bound to a legal standard (Commission European, 2020; WHO, 2022). This standard, was first introduced by Koch, as he stated that lowering the amount of pathogenic cholera bacteria below 100 CFU/mL could prevent a cholera outbreak (Exner et al., 2003; Koch, 1893). Both Snow's and Koch's discoveries lead to the realization that access to clean water was necessary to prevent the spread of cholera and disease outbreaks in general. The access to clean and safe drinking water was later even declared a basic human right by the World Health Organization (WHO) and now one of their Sustainable Development Goals (SDG 6: Clean Water and Sanitation), as it is essential for preserving the health, dignity and prosperity of every individual (WHO, 2017, 2022).

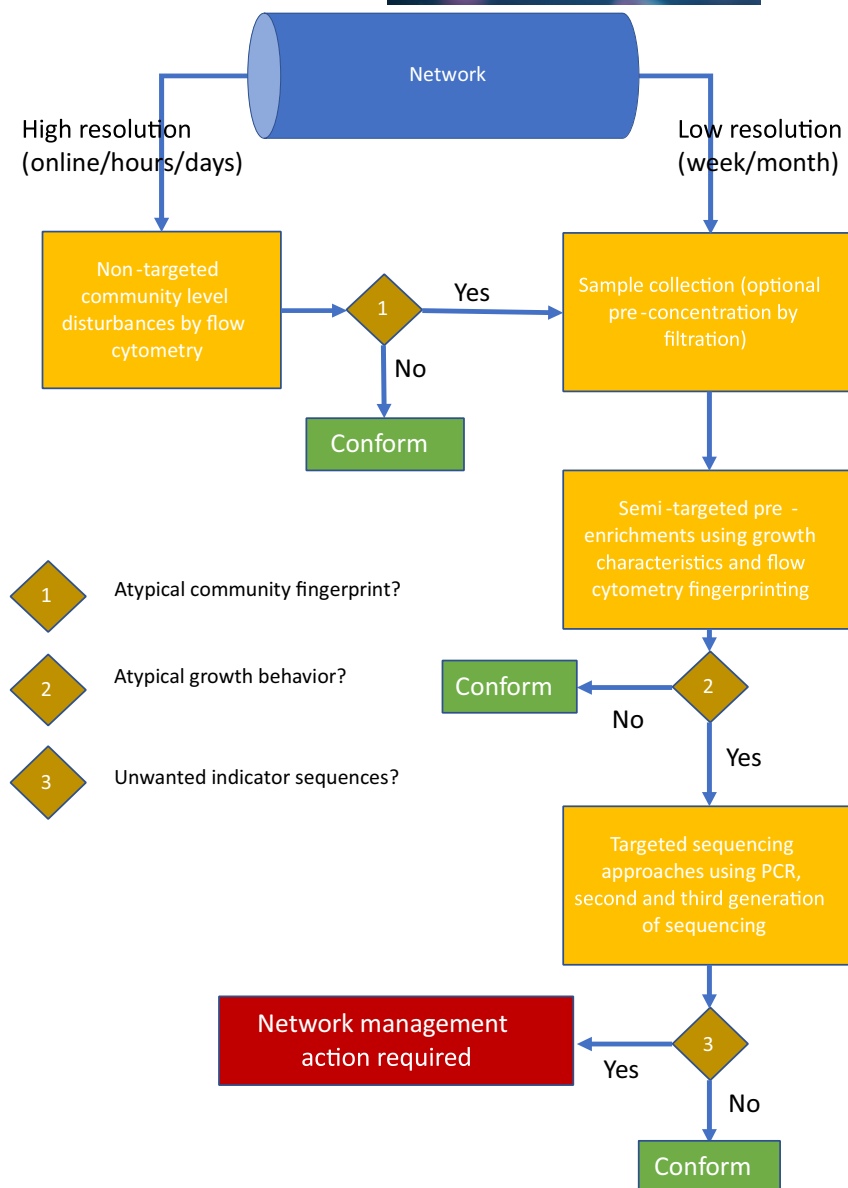
Their inventions led to the heterotrophic plate counting (HPC) technique and its corresponding upper limits to prevent waterborne diseases. These were the foundations for the (legal) standards today (Figure 1). Heterotrophic bacteria are broadly defined as bacteria that require organic compounds as their carbon and nutrient source for metabolic synthesis and thus their growth. In general, HPC includes a variety of simple culture-based tests that are intended to recover a wide range of heterotrophic bacteria from drinking water. The abundance of microorganisms and the grown genera depend on media composition, time, temperature of incubation and means of medium inoculation (Allen et al., 2004; Gensberger et al., 2015). Today, bacteria are inoculated on semi-solid nutrient-rich media and incubated under defined incubation conditions. For drinking water analysis the most commonly used media are Reasoner's 2A (R2A) and yeast extract agar (YEA) (Frilabo, 2016; Gibbs & Hayes, 1988; Sartory et al., 2008). R2A medium has been specifically developed for drinking water analysis, as it is a low nutrient medium that in combination with lower growing temperatures stimulates the growth

of stressed and chlorine-tolerant bacteria (Reasoner & Geldreich, 1985). The European and Flemish (Farys, Pidpa, Water-Link, De Watergroep) drinking water providers use these methods at an incubation temperature of 22°C/36°C for the detection of mostly aquatic coliforms, as described by the European directive (Commission European, 2020). Now, depending on the country and sampling location the 100 CFU/mL threshold has been altered, even to the point that within Europe (Allen et al., 2004; Van Nevel et al., 2017). Instead, there are guidelines that state that 'no abnormal change' (NAC), should be detected (Sartory, 2004). This 'degree of acceptable change' fits within the idea of creating a biostable environment drinking water distribution with a stable bacterial abundance and community composition (Favere, Barbosa, et al., 2021). As the concept of biostability, defined by Favere, Barbosa et al., reaches further than microbial abundance, HPC is not an ideal method to monitor biostability. Although HPC only detects a small portion (<1%) of cells in the drinking water and thus fails to detect the 'viable but non-culturable' (VBNC) bacteria, they are simple (Park et al., 2023). Despite remaining the primary parameter for microbiological drinking water quality, these plating techniques are labour intensive (Craun et al., 2002; Hammes et al., 2008). Moreover, the outcomes of HPC only provide information about a specific region of the entire distribution network (at an accessible point, the source water, at the tap, ...) at a specific point in time (no continuous monitoring).

Over the years, Koch's plating technique underwent several adaptations and in 1885 it was first used as a routine drinking water analysis in London (Hutchinson & Ridgway, 1977). Since the direct detection of all pathogenic bacteria is not feasible, specific growing media were designed to identify and enumerate indicator bacteria (Dufour, 2013; Means et al., 1981; Méndez et al., 2004). These indicator bacteria are considered to signal the presence of faecal material and waterborne diseases (Ashbolt et al., 2001). Over time, *Escherichia coli* had become one of the key indicators, as it was found that 'faecal' coliforms not always had a faecal origin and a lot of methods were specifically improved for the detection of *E. coli* (Tallon et al., 2005). The detection and enumeration of coliforms is a vital parameter to monitor disinfection and the drinking water distribution systems (DWDS) integrity (WHO, 2022).

Early 20th century, based on these first cultivation techniques, other plating/growing methods were derived. For example, the most probable number (MPN) technique, in which it was specified that not more than one of five 10 mL portions of drinking water should contain *E. coli* (Ashbolt et al., 2001). A full MPN screening took between 24 and 96 h, since the media lacked specificity and a subculture and confirmation step was necessary (George et al., 2000; Watkins & Xiangrong, 1997). With the MPN technique, the USA

FIGURE 1 An overview of the evolution of microbiological monitoring of drinking water.



Public Health Service Drinking Water Standard invented a bacteriological standard that was applicable to any drinking water system (Ashbolt et al., 2001).

From the 1970's on, membrane filtration methods had gained wide acceptance, as these were faster (14 h) and typical colonies could be identified (Watkins & Xiangrong, 1997). Later, specific enzymes incorporated in the media were used to detect the hydrolysis products of fluorogenic or chromogenic substrates (George et al., 2000). This method allowed for a better identification and recovery of the target bacteria. These substrate based methods have then evolved into different enzymatic assays based on the MPN technique for the detection of several indicator bacteria, such as Enterolert for *Enterococci*, Legiolert for *Legionella*, Colilert for total coliforms and *E. coli* (Boubetra et al., 2011; Eckner, 1998; Rech et al., 2018). The Colilert technique specifically proved to be as sensitive for *E. coli* and even more sensitive for coliform

detection compared to the standard MPN and membrane filtration techniques (Eckner, 1998; Edberg et al., 1988, 1990). The Colilert technique for drinking water testing had improved sensitivity, specificity, cost, labour and speed compared to the standard cultivation based methods. Up until now (2024), Colilert is still the standard method to control the integrity of the drinking water distribution networks for the drinking water providers (Farys, De Watergroep, Pidpa, Waterlink, AGSO Knokke-Heist). However, the previously used cultivation based methods lacked the ability to detect the 'viable but non-culturable' bacteria (Colwell et al., 1985).

All the above mentioned methods (MPN, Colilert, HPC and membrane filtration) remain the legal standards (established in the European Drinking Water Directive (EU 2020/2184), and are in Flanders still implemented in the Flemish drinking water legislation (Belgian Official Gazette, 20/01/2023)). They are also found in the water analysis compendium (WAC) (De

Watergroep, 2024; Farys, 2024; Pidpa, 2024). Despite advancements in media composition and reducing the analysis time and labour, we thus still rely on century-old methods and upper limits for microbiological monitoring and drinking water safety. In such a fast moving society where everything is connected, it seems odd that for something as precious as our drinking water and associated human health, we seem to fall behind concerning technology in microbial monitoring. However, with the use of modern day techniques, such as MALDI-TOF, these plating techniques have been further modified to better identify the detected bacteria (Farys, 2024; Pidpa, 2024; Pinar-Méndez et al., 2021; Sala-Comorera et al., 2016; Schumann & Maier, 2014). It is important to note that a multitude of these plating, Colilert and Enterolert techniques are also registered as legally obliged methods to monitor the drinking water (ISO 17025). Undoubtedly, these methods are still very valuable for drinking water monitoring today. The fact that they are still the current state-of-the-art show how revolutionary the inventions of Koch and Petri and the adaptations over the last centuries were for microbiology.

THE RISE OF NEW HOLISTIC METHODS FOR MICROBIOLOGICAL MONITORING

The previously discussed plating and enzymatic techniques often fail to provide the drinking water providers with a holistic overview of the entire distribution network. They are not sufficient for the detection of short-term water quality changes, as the time-to-result takes too long (24 h). This, in combination with the low abundance of pathogenic bacteria in the drinking water distribution networks leaves room for uncertainties (Buysschaert et al., 2019; European Communities, 1998). Accurate and sensitive monitoring of the bacteria (and other organisms) in the drinking water distribution networks is not only important to assure safe and qualitative drinking water at the customer's tap, but also to monitor and control the treatment processes (Hammes et al., 2012). Innovative technologies are thus necessary to shift from slow and inaccurate to fast, accurate and sensitive microbiological monitoring methods.

Recently, biological online monitoring devices based on cultivation-independent techniques have been developed, enabling fast and direct monitoring of the microbial quality of drinking water. For example, measurement of specific and/or total enzymatic activity by adding a substrate fluorescent compound such as p-nitrophenyl phosphate or 4-methylubelliferyl- β -D-galactopyranoside that is converted by the respective enzymes such as alkaline phosphatase activity and β -D-galactosidase (e.g. ColiMinder (VWM, Austria), BACTControl (MicroLan, The Netherlands)). As a

result, a fluorescent signal is generated and measured by laser detection. Coliminder has recently been applied to assess quality of the intake water for drinking water production, as the β -D-glucuronidase shows a good correlation to the *E. coli* and can thus be used as a real-time faecal indicator (Frank et al., 2022; Hachad et al., 2024). Because these methods are often not sensitive enough to detect the low concentration of unwanted bacteria in the drinking water that they have been applied to the source water. The above mentioned studies show that safeguarding water quality can actually already start by monitoring the source water that is used for the production itself. It could be argued that it might be easier to monitor the source itself (only one location). These monitoring methods leave the drinking water providers with the question what part of the drinking water production, treatment and distribution pipeline is the correct place to sample/monitor and is definitely food for thought. As an alternative to these culture-based and enzymatic assays for the detection of *E. coli* and Enterococci, reverse-transcriptase-polymerase chain reaction (RT-PCR) has been applied (Molaei et al., 2015). This offline technique was already used for the detection of *E. coli*, but has recently been optimized and validated (according to: ISO16140-2: 2016) for Enterococci as well (Heijnen, 2018; Heijnen et al., 2024). Results showed that four strains of Enterococci can be identified. By means of RT-PCR, a contamination can be detected within a matter of hours instead of days, when compared to conventional methods.

Over the years, several alternative on- and offline monitoring techniques have been applied to monitor drinking water microbiology. The measurement of adenosine triphosphate (ATP) is an indirect measurement that determines the biological activity of the cells that are present in the drinking water by detecting the fluorescent conversion of luciferin by the enzyme luciferase (Stoddart et al., n.d.; de Vera & Wert, 2019). This offline measurement can be done in less than an hour. Although ATP measurements are fast and relatively easy in practice, the conventional methods, such as HPC and Colilert exceed the ATP measurement for the detection of surface and wastewater intrusion (Vang et al., 2014). Despite its short time-to-result and thus, potential use as an early warning system for high microbial loads, ATP measurements are not often used in practice because interpreting the results is difficult, and there is often no clear indication if a problem occurs and what that problem is (Favere, Waegenaar, et al., 2021; Vang et al., 2014).

Flow cytometry, a cultivation-independent method known for its high throughput, is used to sensitively measure bacterial cell densities, both online and offline. In recent decades, it has been increasingly employed for the analysis of drinking water (Berney et al., 2008; Hoefel et al., 2003). With the use of different staining

techniques, flow cytometry offers the opportunity to gain insight into bacterial physiology, activity and viability (Hammes & Egli, 2010; Hatzepichler et al., 2020; Nescerecka et al., 2016). Based on the flow cytometric measurements, a phenotypic fingerprint of the microbial community can be generated. Flow cytometric fingerprinting relies on physiological data acquired through the rapid optical characterization of thousands of cells. With the flow cytometric data, a phenotypic fingerprint that represents the multivariate optical data of more than 10,000 cells can be generated. This high-throughput flow cytometry-based technique was designed to track the biodiversity of microbial communities at fine temporal resolution (Props et al., 2016). The outcome of this phenotypic fingerprinting enables the identification of key transitions in the community structure of drinking water samples. These microbial dynamics as a response to changing water quality allow us to use the microbial community as an (indirect) indicator for water quality changes as well. As such, flow cytometry has been successfully applied to detect the community response to operational events in the drinking water production and transport systems, regrowth in the DWDS, the intrusion of rain- and groundwater, and/or seasonal changes in the drinking water (sources) (Buysschaert et al., 2019; Favere et al., 2020; Pluym et al., 2023). These studies demonstrate that flow cytometry and flow cytometric fingerprinting can be used as a sensitive monitoring and event detection for drinking water microbiology and ultimately as an early warning method for drinking water quality. In a study by Favere et al. flow cytometry even proved to be more sensitive to rain- and groundwater contaminations than plate counting (Favere, Waegenaar, et al., 2021). Although flow cytometry has the potential to do high-throughput and accurate measurements of the bacteria present in the DWDS, it can currently only indicate or predict a calamity as an unexpected change in the phenotypic fingerprint. Additionally, flow cytometers are expensive machines, that not only require certain environmental conditions (e.g. temperature variations, humidity, etc.), but also trained personnel to maintain and interpret the data. In practice, this means that implementing flow cytometry in the routine analysis of the drinking water laboratories, would increase operational costs. Despite their fast and accurate nature, interpretation and translation of these online tools (ATP, flow cytometry) to drinking water norms are still difficult. It is currently suggested that these online technologies can serve as a first line of screening placed at crucial points in the distribution network. If abnormal or unwanted changes are detected, additional samples for HPC, qPCR, RT-PCR and/or MALDI-TOF MS are taken to determine both the exact problem and precise consequence for the water quality.

Over recent years there has been a focus on computational and statistical advancements as well, which has

led to higher resolution flow cytometric fingerprints and thus more tools to analyse the microbiology (Claveau, Hudson, Jeffrey, & Hassard, 2024). The latter has proven to be useful in operational event detection and disinfection monitoring (Claveau, Hudson, Jarvis, et al., 2024). The collection of this big data (high-resolution flow cytometry and other online data) in combination with machine learning will lead to a more precise monitoring and possibly even event prediction based on several online parameters (Sadler et al., 2020). Although the application of machine learning and artificial intelligence (AI) for drinking water monitoring is still under construction or constrained by legislation, it could have the potential to provide both the supplier and costumer with useful insights regarding water quality and safety (Maroju et al., 2023) (Figure 2). It has already been successfully applied for surface water monitoring, but, since we are only at the start of grasping AI's capabilities, full-scale drinking water applications will probably follow soon (Pérez-Beltrán et al., 2024; Rana et al., 2023).

BACTERIA ARE ONLY THE 'TIP OF THE ICEBERG'

Over the last centuries, there has primarily been a focus on (the detection) bacteria. It is important to understand that these bacteria are only a small part of the complex ecosystem that a drinking water distribution network is (Bichai et al., 2008). This means that the presence of viruses, bacteriophages, protozoa and higher organisms are a largely unexplored and, more importantly, unmonitored part of the drinking water distribution network ecosystem. Recent advancements and technical improvements in flow cytometry have contributed to the development of a full flow virometry pipeline, enabling the quantification of viruses (Safford et al., 2023). As these can infect humans (and bacteriophages bacteria), these organisms are potential vital parts of the drinking water ecosystem. Viruses on their part may enter the distribution network through leaks or when the system is opened for maintenance works and could then be infectious for human when consumed (Lambertini et al., 2011; Teunis et al., 2010).

Protozoa in particular have even been called the 'Trojan horses' of the microbial world, because of the fact that they can harbour (e.g. protection from disinfection, grow inside protozoa) specific (unwanted) bacteria, which can be released again later (Barker & Brown, 1994; King et al., 1988). There are some traditional microscopic counting techniques for higher organisms, but these require pipe flushing at limited study locations.

Furthermore, a recent study revealed the presence of flies in water towers, which can lead to increased coliform detection and alterations in the general microbial community of drinking water when the basin in a

The evolution of microbiological monitoring in drinking water

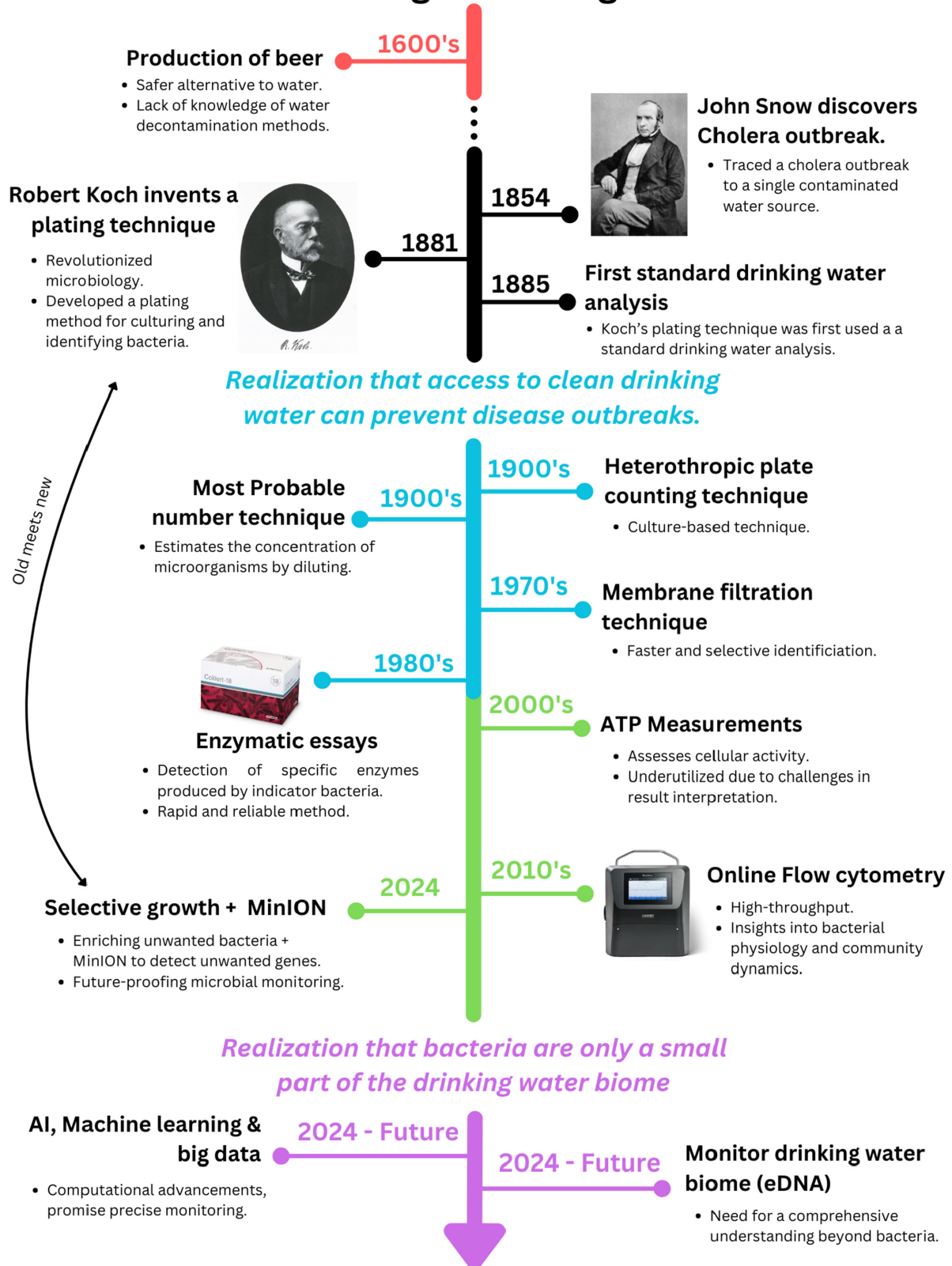


FIGURE 2 Overview of a multi-analysis approach to detect microbiological calamities in the drinking water distribution network. By combining flow cytometry, enrichments and sequencing microbiological calamities can be detected.

water tower is left uncovered (Baele et al., 2023). Other macroinvertebrates, such as *Asellus aquaticus* and Oligochaeta have also been observed in the DWDS (Gunkel et al., 2021; Ketelaars et al., 2023). The presence of these organisms also has an impact on the bacteria and overall water quality, as it has been seen that *Asellus aquaticus* is associated with the presence of *Aeromonas* and coliforms (Christensen et al., 2013; Hijnen et al., 2024; Ketelaars et al., 2023).

We believe that a comprehensive understanding of the DWDS-biome, including protists, invertebrates, bacteria, fungi and archaea is currently missing. New techniques using environmental/eukaryotic DNA (eDNA) to detect macroorganisms are necessary to unravel the complex interplays within the drinking water biome (Figure 2). Moreover, a well-established eDNA analysis will broaden the potential sampling locations and improve dataset robustness (Xie et al., 2021).

'OLD' MEETS 'NEW': THE FUTURE OF DRINKING WATER MONITORING

With the rise of new sequencing techniques, such as third-generation metagenomic sequencing, there are new possibilities for drinking water monitoring (Werner et al., 2022). Oxford Nanopore's MinION, for example, one of these third-generation sequencing techniques, is faster than second-generation sequencing and can even be performed in the field. These rising metagenomic sequencing techniques do not only allow for taxonomic identification, but unravel all gene sequences found in a sample. The latter could be an important aspect for (drinking) water monitoring. The presence of a certain opportunistic pathogen does not always immediately imply an effect on drinking water quality (Siponen et al., 2024). As unwanted traits are correlated with the transcription of specific genes, we hypothesize that the detection of these specific odour and taste, pathogenic, virulence and antibiotic resistance genes alone could be sufficient to evaluate drinking water quality, rather than screening for indicator and/or specific bacteria. By means of the different growth dynamics of r/K-strategists, there is the possibility to selectively enrich the low-abundant, unwanted bacteria in the drinking water distribution system, as proposed by Favere, Barbosa, et al. (2021). It is hypothesized that these unwanted bacteria are r-strategists (ideal conditions means fast growth) and the resident community are K-strategists (no matter the conditions, slow but steady growth) (Andrews & Harris, 1986). This combination of specifically enriching the unwanted bacteria in non-specific liquid growing media with the MinION (third-generation sequencing) sequencing analysis, could revolutionize the drinking water microbiology monitoring. By means of this new analysis pipeline the drinking water providers now have the ability to get

high-resolution data on the drinking water microbiome, and additionally, on the presence of genes that affect water quality in around 48 h. Although this analysis will take as long as the standard plating techniques, a lot more high-resolution data and information about the network will be generated. This will be a step in the right direction for future-proofing the microbial monitoring of drinking water. Especially since it combines the plating/growing methods, which have been the state-of-the-art for centuries, with some of the latest advancements in sequencing and bacteriology. An integrated approach in which multiple analysis are combined (flow cytometry, enrichments and third-generation sequencing) will allow for an accurate detection of calamities and will help the drinking water providers decide if action needs to be taken (Figure 2). This multi-analysis approach, will give better insights into the microbiology of the network than any analysis alone, and, if a problem occurs, allow for a more targeted solution. Furthermore, the upcoming treats of climate change (e.g. floods and extreme weather...) will enhance the spread of pathogens and put a strain on surface water availability (Jia et al., 2024; Leveque et al., 2021). As these consequences of climate change might limit water availability, accurate and fast water quality monitoring techniques to monitor all types of water (drinking water, surface water, ...) will be detrimental.

CONCLUSION

As of today, the drinking water sector primarily relies on HPC plating (22°C/36°C, for aquatic coliform detection), the MPN technique (Colilert) and membrane filtration methods for their drinking water monitoring (De Watergroep, 2024; Farys, 2024; Pidpa, 2024; Waterlink, 2024). These techniques, which were based on Koch's original plating method, have undergone several adaptations to meet today's microbiological monitoring demands, and are implemented in the (European) legislation as a result. Nowadays, these are combined with more advanced techniques such as MALDI-TOF to rapidly identify the bacterial cultures grown on the plates (Pinar-Méndez et al., 2021; Pidpa, 2024). Their robustness and the existence of century old data ensure that they remain the current state-of-the-art and legal standard in Flanders, based on the European Drinking Water Directive. Over the last decades, there has been a lot of experimentation with different alternative off- and online techniques, such as flow cytometry, RT PCR, qPCR and enzymatic assays. Despite some successful applications of for example (online) flow cytometry and enzymatic assays, these alternative methods can, and probably will never completely replace the 'century-old' plating methods. They need to be regarded as supporting methods to the plating methods currently established in the legislation,

because these alternative methods can serve as an early warning system because of the short time-to-result. When an unexpected or unwanted event is detected, additional tests are still required. Simply, because flow cytometry and enzymatic assays still lack a certain specificity. However, flow cytometry (off- and online) could replace HPC, especially since current standards require 'no abnormal change' (NAC), which can perfectly be monitored by FCM. However, we hypothesize that a combination of plating/growing methods with (third generation) sequencing might be a leap forward for drinking water monitoring. This combination of an 'old' and 'new' technique can provide the drinking water suppliers with information about odour and taste, virulence and antibiotic resistance genes and can thus indicate the possible cause of a certain problem. This pipeline allows for a more adequate and fast solution. Nevertheless, the drinking water distribution networks still remain a largely unexplored ecosystem, containing protozoa, fungi, macroorganisms, viruses, etc., in which bacteria are possibly only a small part. Other techniques, such as the development of an eDNA sequencing pipeline that can provide a holistic overview of the drinking water ecosystem will be necessary in the near future. This combination of new and old microbial monitoring techniques will contribute to clean and safe drinking water and thus prevent the spread of diseases now and in the future (SDG 3: Ensure healthy lives and promote well-being for all at all ages).

Although advancements are being made, there has not been a major leap forward in terms of microbial drinking water monitoring since the discoveries of Koch. In a smart and connected society, we still rely on century-old methods that are relatively slow, while not always revealing the origin of the problem. This is striking for something as precious as our drinking water. After all, access to safe and qualitative drinking is a basic human right, as declared by the WHO. As of today, new techniques are being applied to drinking water, but we are still waiting for the next revolutionary discovery to improve microbiological monitoring in drinking water. Is this the era in which AI, big data and machine learning will drastically change microbial drinking water monitoring as well?

AUTHOR CONTRIBUTIONS

Thomas Pluym: Writing – original draft; writing – review and editing; visualization. **Fien Waegenaar:** Writing – original draft; writing – review and editing; visualization. **Bart De Gussemme:** Conceptualization; writing – review and editing; supervision. **Nico Boon:** Conceptualization; writing – review and editing; supervision.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflict of interest to declare.

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