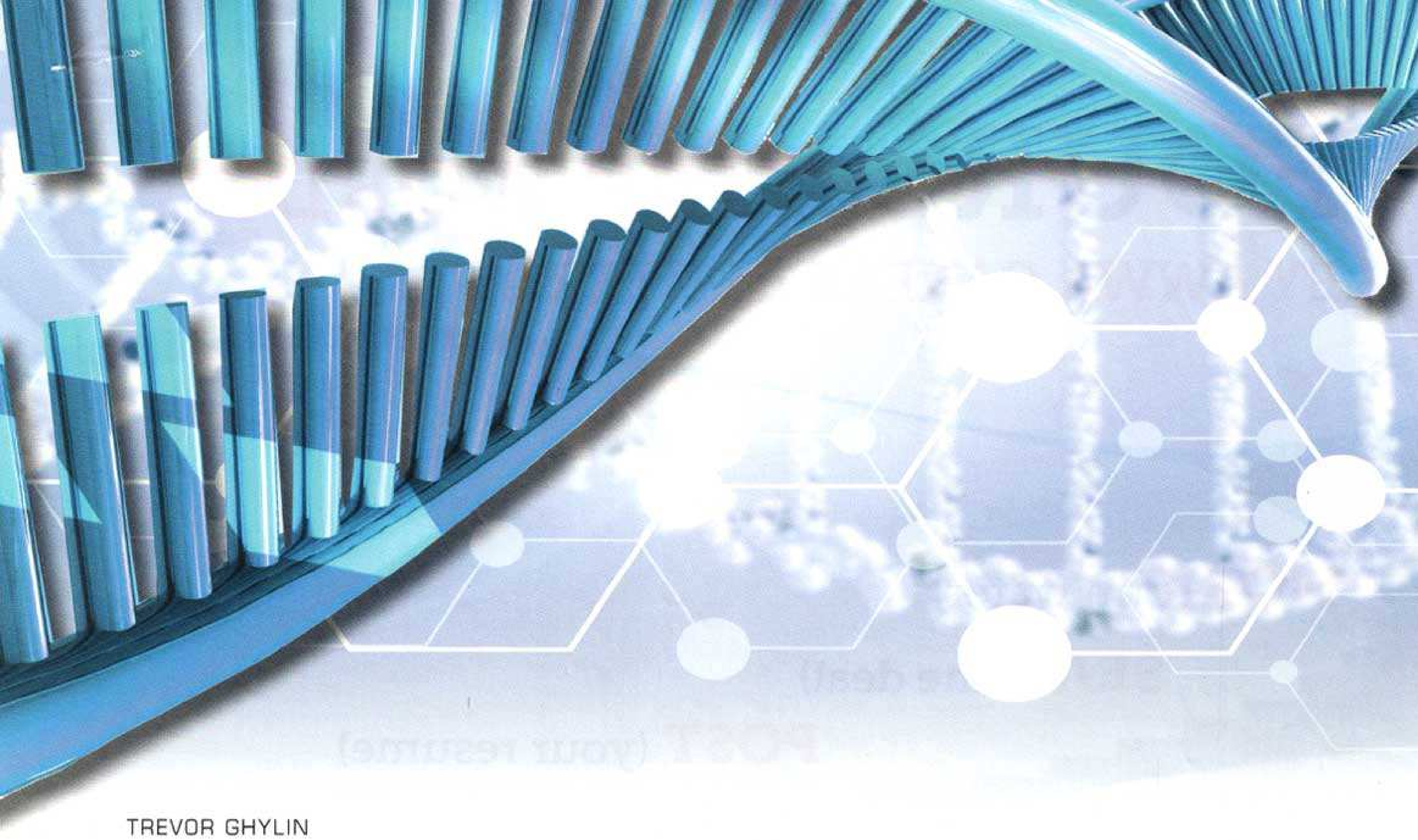


TREVOR GHYLIN

DNA-based microbial analysis detects and locates potential contamination in distribution systems

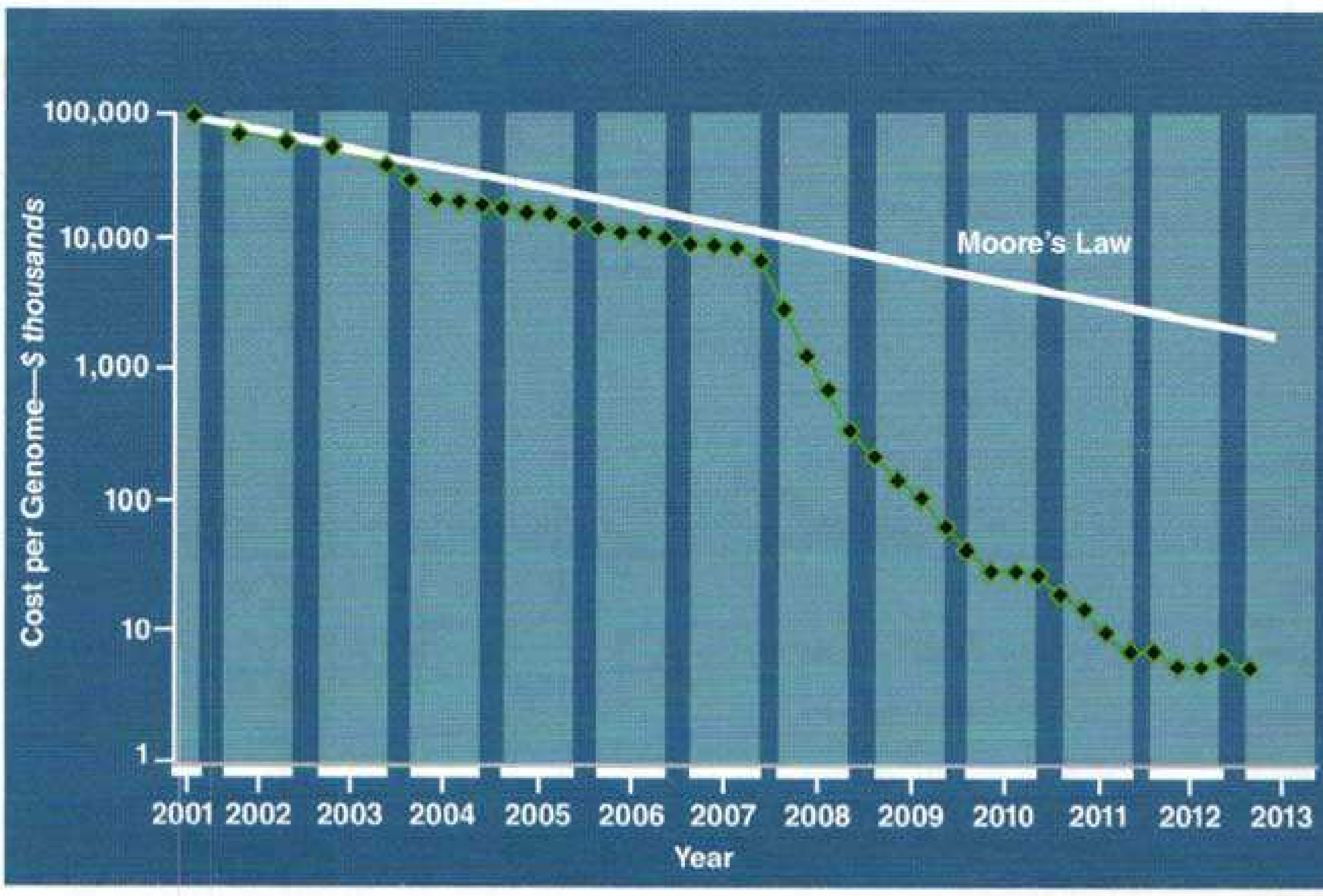
DNA TECHNOLOGY CAN FILL IN THE GAPS LEFT BY OTHER MICROBIAL TESTING METHODS.

No water utility wants to issue a boil-water notice, but contamination of drinking water still happens. One of the primary modes of such contamination occurs in distribution systems (NRC, 2005). Operators have little information on the integrity of distribution systems other than standard coliform test results, which have been documented as failing to provide warning of waterborne threats. In fact, Nwachuka et al (2002) investigated 12 water systems with documented waterborne disease outbreaks and found that only two exceeded the maximum contaminant level for total coliforms. The National Research Council (2005) states that contamination of the distribution system will typically be detected only by other means (e.g., taste and odor complaints, such as recently occurred in Charleston, W.Va.) and suggests that coliform testing may be limited in its ability to ensure public health protection from microbial contamination of distribution systems. Now, however, DNA technology provides a method for detecting and locating problems in distribution systems. Recent advances in DNA technology now allow nearly all microbes (i.e., archaea, bacteria, and protozoans) in a sample to be identified and quantified based on their DNA.

DNA TECHNOLOGY IS BECOMING MORE AFFORDABLE

DNA technologies are revolutionizing the way water samples are analyzed. We are no longer limited to microscopy and culturing methods. DNA technologies have been exponentially decreasing in cost since the first human

FIGURE 1 Historical cost of DNA sequencing



Source: National Institutes of Health, National Human Genome Research Institute (www.genome.gov/sequencingcosts)

genome was published in 2001 at a cost of $3 billion (Figure 1). The cost of sequencing a human genome has fallen to just a couple thousand dollars and within just a few years will likely be around $100. The same technology that is used to sequence human genomes can be used to analyze water samples. The costs of these technologies are at the point that it has become economically feasible to identify and quantify nearly every microbe in water samples using DNA. This technology can be used to investigate a variety of issues in drinking water systems including positive coliform tests, aesthetic complaints (taste, color, odor), biofilms, nitrification, and corrosion.

MANY MICROBES PASS THROUGH TREATMENT BARRIERS

Field studies using direct microscopic counting techniques have shown that the total bacterial populations of source waters range from 10^4 cells/mL (groundwater) to more than 10^7 cells/mL (surface water) (Brazos & O'Connor, 1984). Comprehensive pretreatment can reduce the bacterial count; however, rapid sand–filtered surface waters may still contain total bacterial cell counts of 10^6/mL. Bacterial cells are more abundant than the combined total of all other particles larger than 0.2 µm in filtered waters by three to four orders of magnitude (O'Connor & O'Connor, 2001).

Studies have shown that many microbes are able to pass through treatment barriers and survive to colonize distribution systems (O'Connor & O'Connor, 2001). Additionally, Pinto et al (2012) found that treatment filters actually harbored microbes that acted as a seed for the distribution system. The microbial community found in the distribution system was more similar to the microbial community found in the filters than to the microbial community found in the source water.

UNDERSTANDING THE MICROBIAL COMMUNITY IS CRITICAL

Microbes that survive the treatment process can attach to pipe walls and begin forming robust biofilms. Pioneer bacteria attach and begin secreting extracellular polysaccharides (i.e., slime) that protect them from residual disinfectants and provide a more hospitable environment in which successor microbes can readily attach and begin to grow a more complex biofilm community. Biofilms in drinking water systems can provide protection for coliforms or contaminant microbes that may enter the system from various sources. Biofilms may also cause pipe corrosion, taste and odor issues, and residual disinfectant consumption. Similar problems are found in many groundwater wells where iron-oxidizing and sulfur-reducing bacteria can cause poor taste and fouling or plugging of wells. Because of these significant effects, it is important to gain a better understanding of the microbial community in order to improve water quality and safety and to extend the life of the potable water infrastructure.

DISTRIBUTION SYSTEM DNA INVESTIGATION

A medium-sized Midwestern city contracted with a private firm[1] to help investigate the causes for aesthetic (taste/odor/color) complaints from customers. The city has consistently met all regulations set by the Safe Drinking Water Act, but it decided to

TABLE 1 Bacterial data as a percentage of total bacteria (sorted by abundance for location 1)

Bacteria	Origin	Sample Location Number							
		1—%	2—%	3—%	4—%	5—%	6—%	7—%	8—%
Fusobacterium	Human mouth bacteria	15	0	0	0	0	0	0	0
Burkholderia	Common distribution system bacteria	10	37	50	35	21	28	20	29
Xanthomonas	Unknown	7	3	0	0	3	0	1	1
Streptococcus	Human associated bacteria	7	0	1	0	0	3	0	0
Prevotella	Human mouth bacteria	6	0	0	0	0	0	0	0
Porphyromonas	Human mouth bacteria	6	1	0	0	0	0	0	0
Alternaria	Unknown	5	1	9	37	30	7	1	12
Actinomyces	Environmental bacteria	4	0	0	0	0	2	2	0
Stenotrophomonas	Environmental bacteria	4	0	0	0	0	8	0	5
Aggregatibacter	Human mouth bacteria	4	0	0	0	0	0	0	0
Rothia	Human mouth bacteria	3	0	0	0	0	0	0	0
Pseudomonas	Unknown	3	0	0	3	0	0	0	1
Ralstonia	Unknown	3	8	4	4	1	4	2	4
Leptotrichia	Human mouth bacteria	3	0	0	0	0	0	0	0
Acinetobacter	Environmental bacteria	2	1	9	4	4	7	2	2
Bifidobacterium	Mammal mouth and gut bacteria	2	0	0	0	0	0	0	0
Capnocytophaga	Human mouth bacteria	2	0	0	0	0	0	0	0
Gemella	Human mouth bacteria	1	0	0	0	0	0	0	0

Shaded rows indicate bacteria found only at location 1.

go beyond those requirements to ensure the safety and quality of the water. Eight samples were collected from various locations throughout the distribution system, including those from which complaints had originated. Distribution system samples were collected over a two-day period—May 29 and May 30, 2013.

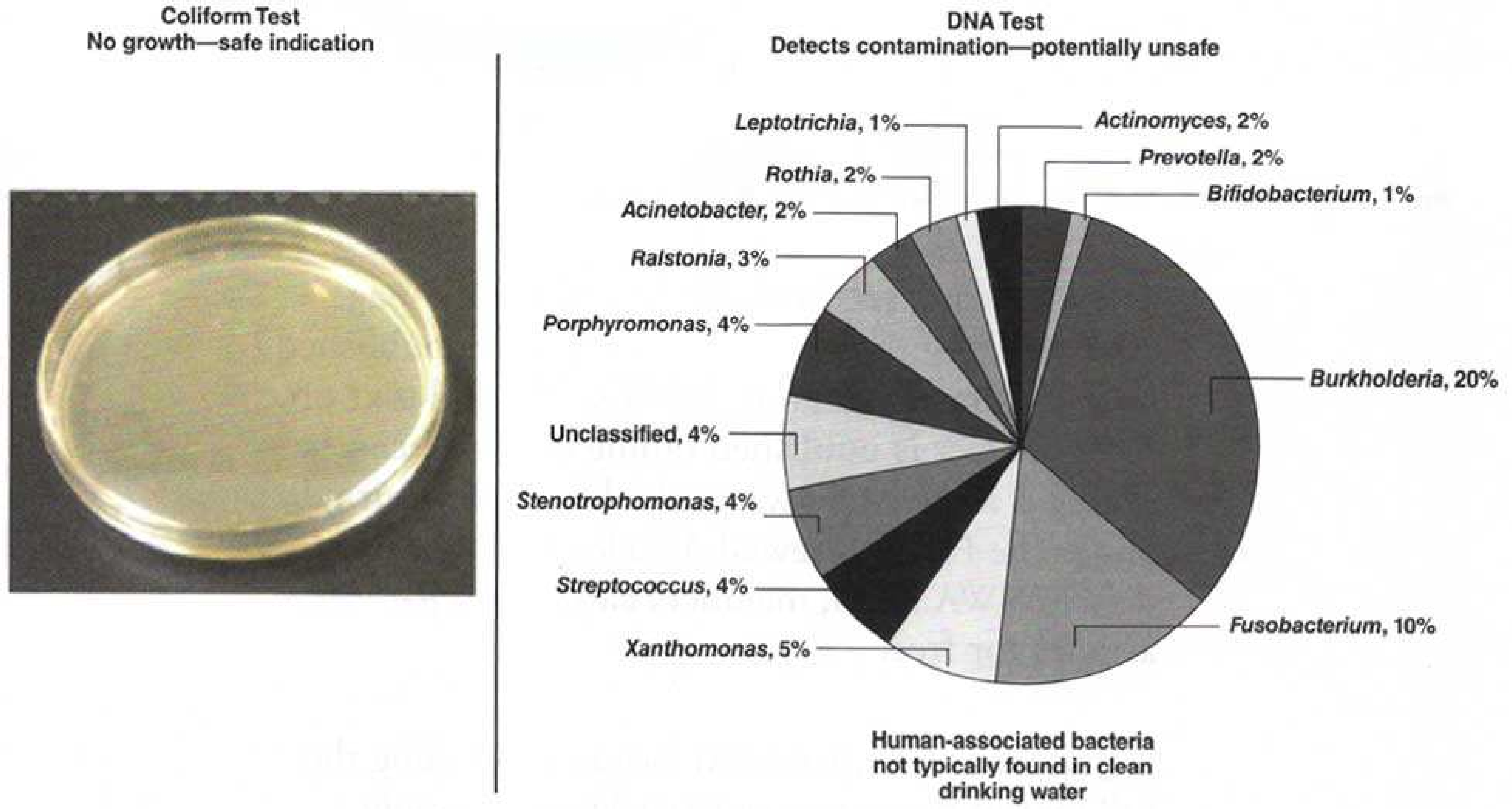
DNA technology was used to identify and quantify nearly all bacteria in the distribution system water samples. Investigators anticipated finding iron-oxidizing bacteria and/or sulfate-reducing bacteria, which are known to cause water discoloration and poor taste and odor. Iron-oxidizing bacteria and sulfate-reducing bacteria were found in a couple of locations, but the data also included some concerning results. One sample from location 1 contained significant numbers of human-associated bacteria (Table 1, Figure 2), including *Fusobacterium*, *Prevotella*, *Aggregatibacter*, *Rothia*, *Leptotrichia*, *Bifidobacterium*, and many others. These bacteria were some of the most abundant bacteria found at location 1 but were not found in any other sample. Other bacteria such as *Burkholderia* are common distribution-system bacteria and were found at all locations in high abundance.

Once these results were reported to the city, all eight locations were immediately tested for total coliform bacteria to ensure the health of the drinking water. All tests came back negative. The city inspected several facilities near location 1. Failed backflow preventers were discovered in a factory that produces animal-derived biochemicals. The city also inspected a school and found that no backflow preventers were installed. The city immediately began working to correct these deficiencies.

This project was not intended to be a thorough scientific study, so it cannot be concluded with certainty that the drinking water was contaminated without conducting further sampling and analysis. The apparent contamination cannot conclusively be linked with the lack of backflow preventers in the buildings because the city did not do any flow tracing or further investigation to prove that water was indeed flowing into the distribution system.

This study did demonstrate, however, the power of DNA technology to detect problems in distribution systems that could not be detected by routine coliform monitoring. Regardless of the viability of these organisms, the presence of apparent contamination in the distribution system is cause for concern and could have resulted in public health issues. The DNA-based testing was able to uncover and pinpoint serious problems in the distribution system that otherwise may have remained unnoticed until a health problem manifested. Fortunately the city was proactive in investigating and addressing residents'

FIGURE 2 Coliform and DNA results from water at location 1



complaints, and it ultimately solved problems that may have never shown up in coliform testing.

CONCLUSIONS

DNA technology is a powerful tool to provide data to help utilities resolve issues in distribution systems. DNA testing can be used for investigating and solving taste and odor complaints, positive coliform results, and routine monitoring for added insurance that a city's drinking water has no contamination issues. This technology can also be used to analyze well water quality. Routine water quality monitoring using DNA technology can greatly reduce water quality risks and provide confidence and peace of mind to both residents and water utility personnel that the water is truly safe to drink.

ABOUT THE AUTHOR

***Trevor Ghylin** is chief executive officer of Microbe Detectives, Global Water Center, Ste. 320, 247 Freshwater Way, Milwaukee, WI 53204 USA; trevor.ghylin@microbe-detectives.com. Ghylin is also a PhD candidate at the University of Wisconsin-Madison. He has an MS degree in civil and environmental engineering from the University of Wisconsin-Madison and a BS degree in civil engineering from the University of North Dakota. He worked as a process engineer for CH2M HILL for five years prior to returning to graduate school and launching Microbe Detectives to help bring DNA technology to the water industry.*

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FOOTNOTE

[1]Microbe Detectives, Milwaukee, Wis.

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ADDITIONAL RESOURCES

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