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Microbial pathogens in source and treated waters from drinking water treatment plants in the United States and implications for human health

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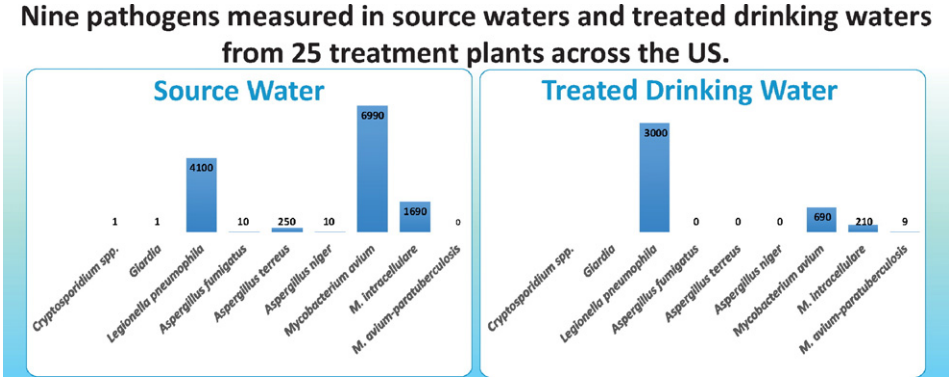
HIGHLIGHTS

- Drinking water was obtained at 25 treatment plants and screened for nine pathogens.
- Plants included a range of production volumes and drinking water treatments.
- Treatment was generally effective in reducing most pathogens below detection.
- Five pathogenic species of mycobacteria were cultured from three plants.

GRAPHICAL ABSTRACT



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ABSTRACT

An occurrence survey was conducted on selected pathogens in source and treated drinking water collected from 25 drinking water treatment plants (DWTPs) in the United States. Water samples were analyzed for the protozoa *Giardia* and *Cryptosporidium* (EPA Method 1623); the fungi *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus* (quantitative PCR [qPCR]); and the bacteria *Legionella pneumophila* (qPCR), *Mycobacterium avium*, *M. avium* subspecies *paratuberculosis*, and *Mycobacterium intracellulare* (qPCR and culture). *Cryptosporidium* and *Giardia* were detected in 25% and in 46% of the source water samples, respectively (treated waters were not tested). *Aspergillus fumigatus* was the most commonly detected fungus in source waters (48%) but none of the three fungi were detected in treated water. *Legionella pneumophila* was detected in 25% of the source water samples but in only 4% of treated water samples. *M. avium* and *M. intracellulare* were both detected in 25% of source water, while all three mycobacteria were detected in 36% of treated water samples. Five species of mycobacteria, *Mycobacterium mucogenicum*, *Mycobacterium phocaicum*, *Mycobacterium triplex*, *Mycobacterium fortuitum*, and *Mycobacterium lentiflavum* were cultured from treated water samples. Although these DWTPs represent a fraction of those in the U.S., the results suggest that many of these pathogens are widespread in source waters but that treatment is generally effective in reducing them to below detection limits. The one exception is the mycobacteria, which were commonly detected in treated water, even when not detected in source waters.
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1. Introduction

The advent of disinfection and centralized treatment has had a major impact on reducing outbreaks of disease caused by contaminated drinking water, though illnesses continue to occur, even in developed countries (Craun et al., 2010). Drinking water remains a potential source of microbial pathogens in the United States (Yoder et al., 2008) and other developed countries (Ngwenya et al., 2013) despite >40 years of improvements in treatment processes. Illnesses due to contaminated drinking water result in nearly one billion dollars annually in hospitalization costs alone in the U.S. (Collier et al., 2012).

The majority of drinking water-associated outbreaks in the U.S. in 2009–2010 were due to deficiencies in federally regulated portions of drinking water treatment plants (DWTPs) (Hilborn et al., 2013). Given this fact, few studies have evaluated drinking water treatment efficacy for microbial pathogens at DWTPs operating under real-world conditions. The Safe Drinking Water Act sets maximum contaminant levels (MCLs) or enforceable standards for some pathogens, which are published in the Code of Federal Regulations (CFR) under Title 40 CFR §141 subpart B (U.S. EPA, 2012). However many waterborne pathogens are unregulated. Monitoring directly for pathogens in treated drinking water is considered expensive and impractical, though it is recognized that monitoring for indicator bacteria (e.g. total and fecal coliforms, *Escherichia coli*) is not useful for predicting the presence of non-fecally-related environmental pathogens. Systems using surface water or groundwater under the influence of surface water that serve >10.000 people are required to monitor for the human pathogen *Cryptosporidium* in source water used for drinking water (40 CFR §141.700; U.S. EPA, 2002a), as are systems serving <10.000 people when *E. coli* levels exceed prescribed thresholds. To meet federal regulations, DWTPs use a variety of treatments to control levels of microorganisms, including filtration, chlorine (Cl_2), chloramine (NH_2Cl), chlorine dioxide (ClO_2), ultraviolet light (UV), and ozone (O_3).

A major gap exists in our understanding of the ecology of waterborne pathogens, including their occurrence and potential inactivation or removal during drinking water treatment. To begin to address this issue, this study was undertaken with the goal of obtaining occurrence information on microbial pathogens of known and emerging concern in source and treated drinking water. A secondary goal was to estimate removal, if any, of microbial pathogens from source waters by currently used drinking water treatment processes under typical plant operating conditions, and thus identify possible candidate organisms that may be amenable to enhanced reduction or removal. The occurrence of nine pathogens was estimated: two regulated protozoa, *Cryptosporidium* and *Giardia*, two bacteria listed on the U.S. Environmental Protection Agency's (USEPA) Contaminants Candidate List (U.S.EPA, 2015), *Legionella pneumophila*, and *Mycobacterium avium*, and other pathogens of concern including *Aspergillus fumigatus*, *Aspergillus niger* and *Aspergillus terreus*, *Mycobacterium avium* subspecies *paratuberculosis* and *Mycobacterium intracellulare*. Their occurrence was measured in paired source and treated water from 25 DWTPs located in 24 states across the U.S., representing small (501–3300 people) to very large (100,000 + people) people) systems using surface and/or groundwater and a variety of disinfection regimes. This paper is one of a series of related papers that comprise a national study that describes the presence, persistence, and concentration of emerging contaminants in drinking water. This paper focused specifically on microbial contaminants; the other papers in the series focus on the occurrence of chemical contaminants in source and treated drinking water. A comprehensive description of the national study can be found in this issue (Glassmeyer et al., in this issue).

2. Materials and methods

2.1. Drinking water treatment plant selection

DWTPs were nominated for inclusion in this study by field personnel from the U.S. Geological Survey (USGS). The final selection of DWTPs maximized geographical spread and represented a range of utility sizes and drinking water treatments. Table 1 describes basic information for each DWTP regarding population served, production volume, and simplified treatment train. A paired sampling scheme was designed to evaluate the treatment process with minimal potential for regrowth of microorganisms or contamination from biofilms in the distribution system.

2.2. Sample collection

One source (prior to entering the DWTP) and one paired treated water sample were collected from each of 25 DWTPs in sterile 1 L sample bottles for all bacteria and fungi. Most of the DWTPs were plumbed with sampling taps at different locations in the plant. These taps allowed collection either directly, or from a tap in the facility's laboratory. The DWTPs were instructed to collect the source water sample prior to any treatment or settling basin. The treated water sample was collected at a sampling point after final disinfection but prior to the clear well. Approximately 10 L of source water were collected for protozoa analysis. One liter of source and 1 L of treated water were collected for *A. fumigatus*, *A. niger*, and *A. terreus* analyses. Four liter of source and 4 L of treated water in total were collected for *Legionella pneumophila*, *M. avium*, *M. avium* subspecies *paratuberculosis*, and *M. intracellulare* analyses. Samples were shipped to laboratories in coolers containing ice and kept at 4 °C until analysis. Shipping temperatures were monitored using an i-button (www.maximintegrated.com); shipping temperatures were on average <15 °C.

2.3. Quantification of Cryptosporidium oocysts and Giardia cysts

Only source water was sampled from 24 of 25 DWTPs (excluding DWTP 5) for *Cryptosporidium* and *Giardia* analyses. Samples were processed using a modified USEPA Method 1623 (US EPA, 2005). Briefly, 9–11 L were filtered in the field using the EnvirochekHV capsule (Pall-Gelman, Port Washington, NY) using a flow meter. The last 250 mL of sample was not drained from the capsule. One sample used 2 capsules and only sampled 3.5 L because of clogging (DWTP 10). The outlet and then the inlet were capped and sent overnight on ice to the USEPA. Capsules were eluted as described in Method 1623 (US EPA, 2005). Immunomagnetic separation (IMS) was as described except heat dissociation was used (Ware et al., 2003). One IMS was performed per sample. Samples were stained using EasyStain (BTF Pty Ltd., North Ryde, NSW, Australia) as described by the manufacturer except all wash steps were omitted. Slides were then analyzed for *Cryptosporidium* oocysts and *Giardia* cysts and were enumerated as described in Method 1623 (US EPA, 2005).

2.4. Detection of Aspergillus species using qPCR

Methods and qPCR assays have been reported previously for detection of *Aspergillus* (Haugland et al., 2004). An aliquot of the DNA extracts, prepared according to Haugland et al. (2004), were used in this analysis. Five microliters of DNA extract, which was equivalent to analyzing 25 mL of the original source or treated water sample, was analyzed per qPCR reaction. All primer and probe sequences used have been previously published (US EPA, 2002b).

Reactions were performed with thermal cycling conditions consisting of 2 min at 50 °C, 10 min at 95 °C, followed by 40 cycles of 15 s at 95 °C for template denaturation and 1 min at 60 °C for probe and primer annealing and primer extension. Quantification was based on standard curves generated for each microorganism. The detection limit for each assay was approximately one cell per qPCR reaction.