HHS Public Access

Author manuscript

Curr Infect Dis Rep. Author manuscript; available in PMC 2017 September 05.

Published in final edited form as:

Curr Infect Dis Rep. 2014 October ; 16(10): 432. doi:10.1007/s11908-014-0432-y.

Hospital water and opportunities for infection prevention

Brooke K. Decker and **Tara N. Palmore**

National Institutes of Health Clinical Center, National Institutes of Health, 10 Center Drive, 12C103A, Bethesda, MD 20892, USA

Abstract

Nosocomial waterborne pathogens may reach patients through several modes of transmission. Colonization of healthcare facility waterworks can occur in the proximal infrastructure, in the distal water outlets, or both. Infections with waterborne organisms such as *Legionella*, mycobacteria, *Pseudomonas,* and others cause significant morbidity and mortality, particularly in immunocomprised patients. Hospitals should have prospective water safety plans that include preventive measures, as prevention is preferable to remediation of contaminated hospital water distribution system. Whole genome sequencing may provide more informative epidemiologic data to link patient infections with hospital water isolates.

Introduction

Hospital water safety is a major priority and constant challenge for healthcare epidemiologists, safety officers, engineers, and administrators. Waterborne infections incur significant morbidity and mortality, and some are preventable. As with other healthcare-associated infections, occurrence of nosocomial waterborne infections erodes public confidence in healthcare facilities. Pathogens such as *Legionella* and nontuberculous mycobacteria can colonize the deep infrastructure or outlets of hospital water distribution systems, while other Gram-negative bacteria and molds tend to adhere to biofilms at or near the distal points of use. In this review we discuss frequent routes of transmission, categories of waterborne organisms, considerations for prevention and management of waterborne transmission to patients, and future directions in investigation of waterborne outbreaks.

Routes of transmission of waterborne pathogens

Waterborne infections can occur from proximal (central pipes) or distal (points of use) contamination of the hospital water supply. Municipal and hospital tap water are not expected to be free of pathogens, but municipal water undergoes routine microbiological surveillance to assure safe levels of important community pathogens such as coliform bacteria. Although contaminated municipal water can cause outbreaks that affect immunocompromised patients in healthcare settings [1], contamination of hospital water

Compliance with Ethics Guidelines

Conflict of Interest: Tara Palmore and Brooke Decker have no conflicts of interest.

Human and Animal Rights and Informed Consent: This article does not contain any studies with human or animal subjects performed by the authors.

usually occurs within the infrastructure of the healthcare facility [2]. Bacteria that would not sicken most users of potable water in the community may infect hospitalized patients because of underlying conditions, immunosuppression, and the presence of invasive devices. Waterborne pathogens can be transmitted to patients in a number of ways. Following are examples of the diverse modes of transmission:

- Direct aerosol transmission from water to patients: aerosol from a shower or room humidifier [3, 4], or cooling tower [5], aspiration while drinking water;
- Indirect transmission from fomites that had contact with contaminated water: bath supplies and linens [6]; inappropriate use of nonsterile water for tasks that warrant higher measures of caution, such as oral/tracheostomy care of ventilated patents [7] [8] and rinsing of respiratory therapy or endoscopic equipment in tap water [9, 10];
- Exposure of implanted devices to water (e.g., bathing with a central venous catheter improperly covered) [11, 12];
- Transmission on the hands of healthcare personnel: failure to perform hand hygiene after contact with a contaminated environment or patients colonized with waterborne organisms [8]; hand washing with contaminated water [13]; splashback from contaminated sink drains [14].

All of the above defects or practices represent opportunities for prevention through hospital policies, education and monitoring of healthcare personnel practices, and proper cleaning and maintenance of equipment. The precise route of transmission is often unknown, even when infections can be linked to a water source [15].

Biofilms of water distribution systems and points of use have long been recognized as a rich environment for growth of *Legionella,* mycobacteria, *Pseudomonas*, and other waterborne organisms. Eliminating biofilms and their pathogenic residents is a major challenge, as organisms dwelling in these environments may be especially impervious to disinfectants [16, 17]. Biofilms occur in the pipes and the points of use of the water distribution system. Stagnation promotes the ideal conditions for biofilm formation. Guidelines for hospital construction recommend against water pipe formations (such as dead legs and long, horizontal runs [18, 19]) and practices (such as intermittent water usage leading to low flow) that contribute to stagnation. Older facilities may have predated these recommendations, and convoluted pipe architecture may render their structures more vulnerable to water system colonization with *Legionella,* mycobacteria, and others. Cooling towers have been implicated in multiple nosocomial outbreaks, including a recent case reported from a hospital in Japan [5]. Water can also stagnate at the points of use; colonization of fixtures such as electronic eye faucets [20–23], aerators, sink drainage pipes [19], ice machines, decorative fountains [18, 24], and others, has been reported, and some implicated in nosocomial transmission.

Studies of hospital water contamination and some waterborne infections have shown an increase in culture positivity during the summer and early fall. A series of 1154 cases of Legionnaire’s disease, occurring over 16 years in the United States Military Health System,

revealed a seasonal predominance favoring the fall and summer months. Over half of cases occurred between June and October, with July and September having the highest and March the lowest prevalence [25]. Others have shown similar results [26]. Other hospital-acquired Gram-negative infections have also been shown to vary seasonally and correlate with humidity [27]. This seasonality of water contamination levels and disease may confound uncontrolled studies or retrospective comparisons between outbreaks and interventions occurring at different times of the year.

Role of the host

Most waterborne pathogens that cause opportunistic infections in hospital patients do so in a small proportion of patients. Although many more may be exposed to contaminated water, nosocomial infections with *Legionella*, other Gram-negative bacteria, mycobacteria, and molds usually occur in patients who have specific host characteristics—which may differ from the host characteristics that predispose to community-acquired infection because of the concentration of vulnerable patients in healthcare facilities. For example, risk factors for community-acquired Legionnaire's disease include male sex, age over 60, history of tobacco use, chronic underlying lung disease, and diabetes. Risk factors for infection among hospitalized patients who are exposed to *Legionella* include stem cell or organ transplantation, chronic comorbid conditions, and receipt of immunosuppressive therapies [18, 28–31]. Healthy hosts (including healthcare personnel) who are exposed to *Legionella* in aqueous aerosols may develop Pontiac fever, which is a self-limited hypersensitivity reaction to the bacteria, or may seroconvert without symptoms [32, 33]. Hospitalized patients who develop infections with Gram-negative nonfermenters, such as *Sphingomonas, Achromobacter, Stenotrophomonas, Burkholderia,* and *Acinetobacter* spp., often have endotracheal tubes, central venous catheters, or other implanted devices that allow the bacteria to circumvent the body's physiological barriers.

Legionnaire's Disease

Legionnaire's disease, a potentially lethal pneumonia caused by members of the genus *Legionella,* is an important nosocomial infection. *Legionella pneumophila,* the species responsible for the vast majority of infections, is one of the most dreaded waterborne pathogens. Although most Legionnaire's disease is acquired in the community, approximately 10% of cases are thought to result from hospital water exposure. As described above, the most immunologically vulnerable patients in the hospital, as well as those with advanced age and chronic lung disease, are at the greatest risk for nosocomial Legionnaire's disease. The mortality rate of nosocomial Legionnaire's disease is approximately 32%, more than fourfold higher than that of community-acquired infection, likely because of the underlying comorbidities of hospitalized patients [34].

Legionella grow within free-living amoebae, which are hardy and tolerate a wide range of temperatures and other environmental conditions [35, 36]. *Legionella* replicate between 20°C and 50°C, with fastest growth as temperatures approach 40°C—when most bacteria are killed but *Legionella* continue to thrive within amoebae. Temperatures above 60°C are highly effective at suppressing (but not eradicating) *Legionella* and do so within minutes.

However, heat flushing that provides effective bacterial suppression may also pose safety risks to building occupants.

There are significant geographic variations in the epidemiology of *Legionella* spp. While the infection is common throughout the developed world, different *Legionella* species and serogroups may predominate among countries and continents. Whereas most Legionnaire's disease in Australia is attributed to *L. longbeachae,* most cases of Legionnaire's disease in the USA are caused by *Legionella pneumophila* serogroup 1, with a minority of infections attributed to other species of *Legionella* and other subtypes of *L. pneumophila.* In the USA, infection with other species and serogroups may be under-recognized because of the diagnostic bias of the *Legionella* urine antigen, which detects only *L. pneumophila* serogroup 1 and accounts for 97% of *Legionella* tests performed in the USA [37]. *Legionella* PCR and culturing on buffered charcoal yeast extract (BCYE) are means of detecting species other than *L. pneumophila.*

Prevention of hospital-acquired legionellosis requires a multidisciplinary approach involving healthcare epidemiologists and hospital safety officers, facilities architects and maintenance engineers, as well as the clinicians and the microbiology lab. Construction of new hospitals or hospital wings must incorporate published guidance that aims to minimize the structural features of water distribution systems that promote growth of *Legionella* [38, 39]. Healthcare epidemiologists must conduct close surveillance for possible cases of nosocomial *Legionella* infection; a single hospital-acquired case should generate an urgent investigation to identify nosocomial sources and preempt further transmission [40].

Microbiology labs should have prompt *Legionella* testing available, and clinicians should order diagnostic testing for Legionnaire's disease in patients who have compatible clinical syndromes. Testing for suspected *Legionella* need not stop with a negative urine antigen, as the test rules out only one serogroup of one species of *Legionella.* In our hospital, all bronchoalveolar lavage fluid is tested for *Legionella* by both *L. pneumophila* PCR and *Legionella* culture in order to detect rapidly the most likely species and to avoid missing less common species of *Legonella*.

Other Gram-negative bacteria

Waterborne Gram-negative bacteria (other than *Legionella)* that have been reported in recent years to infect hospitalized patients tend to be multidrug resistant. The pathogens may be introduced to the water supply via colonized patients, and then spread through the environment or to points of water use through the routes of transmission described above.

Although the role of the environment is unclear in many types of nosocomial outbreaks, sink drains have been repeatedly implicated in reports of transmissions of Gram-negative bacteria, including Pseudomonas [13, 14], *Klebsiella* spp.[19, 41, 42], and others. Sink aerators [43], drains [23, 44], and more distal drainage sites [19] may become colonized with waterborne human pathogens that enter via hand hygiene of patients or healthcare personnel, rinsing of patient care equipment, or pouring of patient material down the drains. The bacteria may then become incorporated into the existing (and expected) biofilm in these areas that are proximal to patients but distal to the water distribution system [2]. Splashback

from sink drains, documented in one clever study using dye [14], and may be responsible for transmission of sinkbound pathogens to patients either directly or indirectly on equipment rinsed in or stored near the sink, or on the recently washed hands of healthcare providers [8]. The attribution of an outbreak to sink drain contamination has usually been based on similar genotypic pattern and the termination of the outbreak following remediation of the contaminated sink drain [14, 19, 23]. This set of circumstantial evidence can be convincing but does not definitively demonstrate the drain's responsibility.

Sink biofilm contamination with multidrug-resistant bacteria has proven challenging to remediate, as evidenced by the struggles of Kotsanas et al, Vergara-Lopez et al, and others, to eradicate multidrug-resistant organisms from sink drains [19, 23]. Reflux of more distal contamination may contribute to the tenacity of the outbreak strains in the sink drain. In one prolonged ICU-based outbreak of metallo-β-lactamase-producing *K. oxytoca* in a Spanish hospital, a contaminated sink drain was eventually removed in an attempt to end the outbreak. The intervention reduced the rate of new cases but did not end the outbreak. Further investigation revealed that the outbreak organism could be cultured from a horizontal wastewater pipe in the ICU, and elimination of that drainage system was associated with termination of the outbreak [19].

Because of the difficulty in achieving enduring disinfection of hospital sinks, preventing transmission from sink drains via optimizing sink design (slow flow of water, far from the sink drain, and location of the sink away from patient and patient care equipment) may be worth the investment of resources [14].

It behooves the healthcare epidemiology team to keep an open mind regarding patients' less common direct or indirect exposures to water. Several reports have documented transmission of *Aeromonas* spp. to patients via leeches (kept in tanks that were inadequately cleaned) [45]. As might be expected, some *A. hydrophila* isolates have been resistant to the antibiotics that are used as prophylaxis against infection [46, 47]. Proper cleaning and maintenance of leech tanks would seem to provide enduring value in preventing leech-associated infections.

Nontuberculous mycobacteria

Hospital water has been linked to nosocomial outbreaks and pseudo-outbreaks of nontuberculous mycobacteria of diverse species, some more pathogenic than others. Unlike Gram-negative bacteria, which characteristically colonize the distal components of the water distribution system, mycobacteria often colonize both the proximal and distal components of a building's water infrastructure [2, 7, 48, 49]. Once they colonize a water distribution system, it is very difficult to suppress their growth using means that are effective for *Legionella* and other waterborne organisms [7].

Those belonging to *Mycobacterium avium* complex, the most frequent nontuberculous mycobacterial causes of community-acquired infection, have been isolated from potable water in the community [50] and in hospitals [51, 52]. Other mycobacterial species that are not generally pathogens in the community (e.g., *M.* mucogenicum and *M. smegmatis)* may cause opportunistic infections—particularly central venous catheter-related bacteremia--in

highly immunocompromised, hospitalized patients. A recent outbreak of rapidly growing mycobacteria reported among stem cell transplant recipients at a children's hospital was traced to ice machines and the potable water supply [53]. Pseudo-outbreaks of mycobacteria traced to water contamination of respiratory specimens have also been reported, leading to avoidable antibiotic exposure in some of the affected patients [7, 49, 54, 55].

Molds

Studies have shown contamination of hospital water outlets with molds that are potentially pathogenic to immunocompromised patients [56–60]. Species such as *Aspergillus* and *Fusarium* cause opportunistic sinopulmonary infections in neutropenic patients as well as stem cell and organ transplant recipients. They are usually thought to derive from inhalation of airborne fungal spores, but their presence in cultures of showerheads and faucets raises the possibility that they could be transmitted from these points of water use [56–60].

Prevention and Control of Water System Colonization

Proximal water management

The water system of a hospital may not inspire much consideration until a problem occurs. Published guidelines recommend that each healthcare facility develop prospectively and follow a comprehensive water management program [38, 39]. This plan should include a risk assessment that identifies all water treatment systems at play and all points of water use that pose potential hazards, and control strategies to mitigate any hazards. Krageschmidt et. al. describe in detail such a program in a complex healthcare system [61].

Actions that help prevent *Legionella* contamination of hospital water distribution systems include avoiding of water temperatures between 20°C and 50°C; avoiding areas of water stagnation and low flow that promote biofilm growth, avoiding plumbing components that provide nutrients and hospitable environments for *Legionella* (such as rubber hoses), and managing the accumulation of sediment and scale that can nourish and harbor *Legionella*.

Many disinfection systems are now available for use in hospital water distribution systems, each with optimal operational levels and limits. Several of these modalities have been reviewed by Lin et. al. [62]. A growing body of data supports the effectiveness of monochloramine over other modalities of *Legionella* control; chloramination is used widely in municipal water systems, but there are insufficient data on its supplemental use in hospitals. Marchesi et. al. monitored *Legionella* contamination of water in three hospital buildings (two occupied by patients) for three years, comparing *Legionella* counts with the use of chlorine dioxide, monochloramine, and control [63]. Both modalities reduced levels of *Legionella* below those in the control building, but monochloramine was significantly more effective [63]. Monochloramine may have a greater effect than other disinfectants on bacteria associated with biofilm [64].

Regardless of the methodology used, monitoring is critical. In addition to monitoring levels of any added disinfectant, a facility may need to test levels of relevant breakdown products [65]. Close monitoring of levels is crucial, but not sufficient, and the presence of adequate biocide parameters does not guarantee water is safe. Outbreaks have occurred when systems

were operating within recommended guidelines. Copper-silver levels were measured at goal levels in a recent outbreak of *Legionella* in a Pittsburgh area hospital [66], and a functioning ozone system and bromide filter were in place prior to an outbreak of *Legionella* associated with a decorative fountain in a hospital [18]. Studies have noted the persistence of *Legionella* tolerant to disinfectants [67–69].

The decision to switch treatment modality to ameliorate overgrowth of one pathogen may have unintended consequences on growth of other potential pathogens. Casini et al. describe the successful reduction in *Legionella* growth after switching from chlorine to monochloramine, although the switch was associated with increased detection of mycobacteria [68]. In an ICU-based outbreak of metallo-β-lactamase-producing *K. oxytoca* in Spain, each wave of the outbreak terminated after annual hyperchlorination was performed for *Legionella* prevention; the *K. oxytoca* in wastewater pipe biofilms was presumably suppressed following the intervention and recrudesced each time several months later [19]. Due to the limitations of our current water treatment strategies, it is critical for the infection prevention team to maintain a high level of suspicion, even in the setting of what appears to be optimal disinfection.

The literature and published guidelines on the prevention of nosocomial *Legionella* infection reflects varying levels of conservatism with respect to primary prevention. The Centers for Disease Control and Prevention recommend close clinical surveillance for *Legionella* infection and a low threshold for investigation when a suspected nosocomial case occurs, and suggest prospective sampling and testing of water on wards that house stem cell or organ transplant patients [40]. Guidelines in much of Europe recommend prospective, regular sampling and testing of water in a range of buildings, including hospitals.

Water disinfection systems are often implemented urgently to avoid or ameliorate a catastrophe. There is a great need for long-term, methodologically rigorous studies to move the field forward and answer clinical questions with data rather than expert opinion whenever possible.

Distal water management

Many reports implicate colonization point-of-use water sources in outbreaks of all categories of pathogens discussed above. Nosocomial infections associated with showers [70], ice machines [53, 71, 72], decorative fountains [18, 24] steam towel warmers [6], and dental water lines [73–76] have all been reported. Management of these contaminated fixtures ranges from attempted cleaning and disinfection to outright removal or replacement. Reporting bias in the literature may favor publications that describe the eventual success of measures to suppress or eradicate contamination at water outlets. There is no one method, including removal and replacement, that has proven successful in all cases. In the case of equipment that serves as a water outlet, such as ice machines, regular cleaning and maintenance are critical to avoid contamination that can overwhelm bandaid measures such as inline filtration [7].

In addition to preventing proximal colonization of water systems and obligatory removal of colonized hardware, point-of-use filtration may serve as a final barrier and safety measure in some settings. The Centers for Disease Control and Prevention suggest such filters in patient care units that serve transplant recipients. A liver transplant unit within a 1,600-bed hospital with no internal disinfection system succeeded in eliminating cold-water colony counts of *Legionella*, mycobacteria and filamentous fungi by installing faucet filters, while control sources without filters continued to grow these organisms. Interestingly, over time, the heterotrophic plate count increased, suggesting splashback from the sink drain or breakthrough contamination of the filters [26]. Williams et al. noted similar findings after they installed point-of-use filters in a select handful of faucets in a skilled nursing facility that had experienced an outbreak/pseudo-outbreak of rapidly growing mycobacteria [7]. In that setting, faucet filters prevented mycobacterial (but not heterotrophic plate count bacteria) contamination of patient water; an inline filter failed to prevent contamination of the ice machine with either mycobacteria or heterotrophic plate count bacteria in a setting of low water use and low free chlorine levels [7]. Point-of-use filters may be a useful approach in a strategic set of faucets, but are not a practical solution for an entire hospital, as they must be changed regularly. However, they can be considered as a last-resort safety measure to lower the exposure risk for the most vulnerable patients.

In some settings, avoidance of water may be an appropriate interim intervention—particularly when highly vulnerable patients are at risk and the water contamination cannot be solved promptly. In the aforementioned cluster of mycobacterial infections and colonization among pediatric stem cell transplant recipients, a switch to bottled water and a two-minute flush of all showers preceding use by this patient population ended the outbreak [53].

Future Research

Finding on a water fixture the same genus and species of organism from a site that is responsible for a healthcare-associated infection is merely suggestive of an environmental role in transmission to the patient. Genotypic confirmation of this association, and, when possible, the use of more granular technology such as whole genome sequencing, can further elucidate the direction, timing, and likelihood of intermediate steps involved in transmission.

Most outbreak reports, including those described in this review, utilized limited molecular strain typing methods to link potential sources to patient isolates. Time-honored techniques such as pulsed field gel electrophoresis and repetitive element PCR (REP-PCR) have narrow ability to detect genotypic differences among isolates. In the past 4 years, the use of whole genome microbial sequencing to document and describe outbreaks both within [41, 77] and outside [78, 79] healthcare settings has accelerated rapidly. Whole genome sequencing provides the high resolution needed to make connections among patients isolates and water or other environmental isolates and to detect the minute variations that occur with chronic colonization of a water distribution system [80, 81]. In combination with epidemiologic meta-data, whole genome microbial sequencing, and techniques based on that technology such as pan-PCR [82] promise increasingly to replace speculation about the dynamics of transmission of waterborne pathogens. Healthcare epidemiologists may be able to act on

these data to target more effectively their preventive measures and outbreak control interventions to interrupt the transmission of waterborne pathogens to patients.

CONCLUSION

Microbial contamination of a healthcare facility water supply is better prevented than remediated. Many waterborne infections are preventable with adherence to optimal healthcare hygiene practices. Hospitals must have prospective water management program that are updated regularly. Even if that program includes properly monitored, supplemental disinfection of the water distribution system, healthcare epidemiologists and clinicians must be vigilant to the possibility of breakthrough contamination and infections, which tend to occur first among the most immunocompromised or critically ill patients. Whole genome microbial sequencing has the potential to provide a higher level of actionable epidemiologic data in assessing transmission of waterborne pathogens. Facilities can consider the limited use of extra measures such as point-of-use filters as a last line of protection for the most vulnerable patients.

Acknowledgments

This work was supported by the Intramural Research Program of the NIH Clinical Center.

References

1. Conger NG, et al. Mycobacterium simae outbreak associated with a hospital water supply. Infect Control Hosp Epidemiol. 2004; 25(12):1050–5. [PubMed: 15636291]
2. Williams MM, Armbruster CR, Arduino MJ. Plumbing of hospital premises is a reservoir for opportunistically pathogenic microorganisms: a review. Biofouling. 2013; 29(2):147–62. [PubMed: 23327332]
3. Yiallouros PK, PT, Karaoli C, Papamichael E, Zeniou M, Pieridou-Bagatzouni D, Papageorgiou GT, Pissarides N, Harrison TG, Hadjidemetriou A. First outbreak of nosocomial Legionella infection in term neonates caused by a cold mist ultrasonic humidifier. Clin Infect Dis. 2013; 57(1):48–56. [PubMed: 23511302]
4. Moran-Gilad J, LT, Mentasti M, Harrison T, Weinberger M, Mordish Y, Mor Z, Stocki T, Anis E, Sadik C, Amitai Z, Grotto I. Humidifier-associated paediatric Legionnaires' disease, Israel, February 2012. Euro Surveill. 2012; 17(41):20293. [PubMed: 23078810]
5. Osawa K, et al. A case of nosocomial Legionella pneumonia associated with a contaminated hospital cooling tower. J Infect Chemother. 2014; 20(1):68–70. [PubMed: 24462430]
6. Higa F, et al. Legionella pneumophila contamination in a steam towel warmer in a hospital setting. J Hosp Infect. 2012; 80(3):259–61. [PubMed: 22266131]
7. Williams MM, et al. Point-of-use membrane filtration and hyperchlorination to prevent patient exposure to rapidly growing mycobacteria in the potable water supply of a skilled nursing facility. Infect Control Hosp Epidemiol. 2011; 32(9):837–44. [PubMed: 21828963]
8. Lucero CA, et al. Outbreak of Burkholderia cepacia complex among ventilated pediatric patients linked to hospital sinks. Am J Infect Control. 2011; 39(9):775–8. [PubMed: 21664002]
9. Peterson AE, et al. Clonally related Burkholderia contaminans among ventilated patients without cystic fibrosis. Am J Infect Control. 2013; 41(12):1298–300. [PubMed: 23973426]
10. Levy PY, et al. A nosocomial outbreak of Legionella pneumophila caused by contaminated transesophageal echocardiography probes. Infect Control Hosp Epidemiol. 2003; 24(8):619–22. [PubMed: 12940586]
11. Cooksey RC, et al. Multiphasic approach reveals genetic diversity of environmental and patient isolates of Mycobacterium mucogenicum and Mycobacterium phocaicum associated with an

outbreak of bacteremias at a Texas hospital. Appl Environ Microbiol. 2008; 74(8):2480–7. [PubMed: 18310417]

12. Kline S, et al. An outbreak of bacteremias associated with Mycobacterium mucogenicum in a hospital water supply. Infect Control Hosp Epidemiol. 2004; 25(12):1042–9. [PubMed: 15636290]
13. Ferroni A, et al. Outbreak of nosocomial urinary tract infections due to Pseudomonas aeruginosa in a paediatric surgical unit associated with tap-water contamination. J Hosp Infect. 1998; 39(4):301–7. [PubMed: 9749401]
14. Hota S, et al. Outbreak of multidrug-resistant Pseudomonas aeruginosa colonization and infection secondary to imperfect intensive care unit room design. Infect Control Hosp Epidemiol. 2009; 30(1):25–33. [PubMed: 19046054]
15. Johansson E, Welinder-Olsson C, Gilljam M. Genotyping of Pseudomonas aeruginosa isolates from lung transplant recipients and aquatic environment-detected in-hospital transmission. APMIS. 2014; 122(2):85–91. [PubMed: 24450429]
16. Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov. 2003; 2(2):114–22. [PubMed: 12563302]
17. Simoes M, Simoes LC, Vieira MJ. Species association increases biofilm resistance to chemical and mechanical treatments. Water Res. 2009; 43(1):229–37. [PubMed: 18977505]
18. Palmore TN, SF, White M, Bordner M, Michelin A, Bennett JE, Murray PR, Henderson DK. A cluster of cases of nosocomial legionnaires disease linked to a contaminated hospital decorative water fountain. Infect Control Hosp Epidemiol. 2009; 30(8):764–768. [PubMed: 19580436]
19. Vergara-Lopez S, et al. Wastewater drainage system as an occult reservoir in a protracted clonal outbreak due to metallo-beta-lactamase-producing Klebsiella oxytoca. Clin Microbiol Infect. 2013; 19(11):E490–8. [PubMed: 23829434]
20. Halabi M, et al. Non-touch fittings in hospitals: a possible source of Pseudomonas aeruginosa and Legionella spp. J Hosp Infect. 2001; 49(2):117–21. [PubMed: 11567556]
21. van der Mee-Marquet N, et al. Non-touch fittings in hospitals: a procedure to eradicate Pseudomonas aeruginosa contamination. J Hosp Infect. 2005; 60(3):235–9. [PubMed: 15949615]
22. Sydnor ER, BG, Gimburg A, Cosgrove SE, Perl TM, Maragakis LL. Electronic-eye faucets: Legionella species contamination in healthcare settings. Infect Control Hosp Epidemiol. 2012; 33(3):235–240. [PubMed: 22314059]
23. Kotsanas D, et al. Disinfection of Burkholderia cepacia complex from non-touch taps in a neonatal nursery. J Perinat Med. 2008; 36(3):235–9. [PubMed: 18576933]
24. Haupt TE, HR, Kazmierczak JJ, Nehls-Lowe H, Rheineck B, Powell C, Leonhardt KK, Chitnis AS, Davis JP. An outbreak of Legionnaires disease associated with a decorative water wall fountain in a hospital. Infect Control Hosp Epidemiol. 2012; 33(2):185–191. [PubMed: 22227989]
25. Armed Forces Health Surveillance Center (AFHSC). Legionellosis in Military Health System beneficiaries, 1998–2013. MSMR. 2014; 21(3):6–9.
26. Zhou ZY, HB, Qin L, Lin YE, Watanabe H, Zhou Q, Gao XD. Removal of waterborne pathogens from liver transplant unit water taps in prevention of healthcare-associated infections: a proposal for a cost-effective, proactive infection control strategy. Clin Microbiol Infect. 2014; 20(4):310–314. [PubMed: 23879308]
27. H R. Seasonality in Gram-negative and healthcare-associated infections. Clin Microbiol Infect. 2012; 18(10):934–940. [PubMed: 22784293]
28. Campins M, et al. Nosocomial Legionnaire's disease in a children's hospital. Pediatr Infect Dis J. 2000; 19(3):228–34. [PubMed: 10749465]
29. Aringer M, et al. Adverse events and efficacy of TNF-alpha blockade with infliximab in patients with systemic lupus erythematosus: long-term follow-up of 13 patients. Rheumatology (Oxford). 2009; 48(11):1451–4. [PubMed: 19748965]
30. Kool JL, et al. More than 10 years of unrecognized nosocomial transmission of legionnaires' disease among transplant patients. Infect Control Hosp Epidemiol. 1998; 19(12):898–904. [PubMed: 9872525]
31. Lepine LA, et al. A recurrent outbreak of nosocomial legionnaires' disease detected by urinary antigen testing: evidence for long-term colonization of a hospital plumbing system. Infect Control Hosp Epidemiol. 1998; 19(12):905–10. [PubMed: 9872526]

32. Rudbeck M, et al. Legionella antibodies in a Danish hospital staff with known occupational exposure. J Environ Public Health. 2009; 2009:812829. [PubMed: 20041020]
33. Remen T, et al. Pontiac fever among retirement home nurses associated with airborne legionella. J Hosp Infect. 2011; 78(4):269–73. [PubMed: 21752493]
34. Dominguez A, et al. Factors influencing the case-fatality rate of Legionnaires' disease. Int J Tuberc Lung Dis. 2009; 13(3):407–12. [PubMed: 19275805]
35. Greub G, Raoult D. Microorganisms resistant to free-living amoebae. Clin Microbiol Rev. 2004; 17(2):413–33. [PubMed: 15084508]
36. Cervero-Arago S, et al. Effect of thermal treatment on free-living amoeba inactivation. J Appl Microbiol. 2013
37. Centers for Disease Control. Legionellosis --- United States, 2000--2009. Morbidity and Mortality Weekly Report. 2011; 60(32):1083–6. [PubMed: 21849965]
38. Facility Guidelines Institute. Guidelines for design and construction of hospitals and outpatient facilities. Chicago, IL: American Society for Healthcare Engineering; 2014. Guidelines for design and construction of health care facilities.
39. ASHRAE Standards Committee. Prevention of Legionellosis Associated with Building Water Systems. ASHRAE. 2011 (Second Public Review Draft).
40. Tablan OC, et al. Guidelines for preventing health-care--associated pneumonia, 2003: recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee. MMWR Recomm Rep. 2004; 53(RR-3):1–36.
41. Snitkin ES, et al. Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med. 2012; 4(148):148ra116.
42. Lowe C, et al. Outbreak of extended-spectrum beta-lactamase-producing Klebsiella oxytoca infections associated with contaminated handwashing sinks(1). Emerg Infect Dis. 2012; 18(8): 1242–7. [PubMed: 22841005]
43. Cristina ML, SA, Casini B, Baggiani A, Del Giudice P, Brusaferro S, Poscia A, Moscato U, Perdelli F, Orlando P. The impact of aerators on water contamination by emerging gram-negative opportunists in at-risk hospital departments. Infect Control Hosp Epidemiol. 2014; 35(2):122–129. [PubMed: 24442072]
44. Gillespie TA, et al. Eradication of a resistant Pseudomonas aeruginosa strain after a cluster of infections in a hematology/oncology unit. Clin Microbiol Infect. 2000; 6(3):125–30. [PubMed: 11168087]
45. Sartor C, L-PF, Legré R, Casanova D, Bongrand MC, Sambuc R, Drancourt M. Nosocomial Infections with Aeromonas hydrophila from Leeches. Clin Infect Dis. 2002; 35(1):E1–E5. [PubMed: 12060892]
46. Wilmer A, SK, Yip J, Carr N, Grant J. The role of leech water sampling in choice of prophylactic antibiotics in medical leech therapy. Microsurgery. 2013; 33(4):301–304. [PubMed: 23417901]
47. Giltner CL, et al. Ciprofloxacin-resistant Aeromonas hydrophila cellulitis following leech therapy. J Clin Microbiol. 2013; 51(4):1324–6. [PubMed: 23363826]
48. Crago B, et al. Surveillance and molecular characterization of non-tuberculous mycobacteria in a hospital water distribution system over a three-year period. J Hosp Infect. 2014; 87(1):59–62. [PubMed: 24746232]
49. Gebo KA, et al. Pseudo-outbreak of Mycobacterium fortuitum on a Human Immunodeficiency Virus Ward: transient respiratory tract colonization from a contaminated ice machine. Clin Infect Dis. 2002; 35(1):32–8. [PubMed: 12060872]
50. Whiley H, et al. Mycobacterium avium complex--the role of potable water in disease transmission. J Appl Microbiol. 2012; 113(2):223–32. [PubMed: 22471411]
51. Aronson T, et al. Comparison of large restriction fragments of Mycobacterium avium isolates recovered from AIDS and non-AIDS patients with those of isolates from potable water. J Clin Microbiol. 1999; 37(4):1008–12. [PubMed: 10074518]
52. Tobin-D'Angelo MJ, et al. Hospital water as a source of Mycobacterium avium complex isolates in respiratory specimens. J Infect Dis. 2004; 189(1):98–104. [PubMed: 14702159]
53. Iroh Tam PY, et al. Rapidly Growing Mycobacteria among Pediatric Hematopoietic Cell Transplant Patients Traced to the Hospital Water Supply. Pediatr Infect Dis J. 2014

54. Brown-Elliott BA, et al. Five-year outbreak of community- and hospital-acquired Mycobacterium porcinum infections related to public water supplies. J Clin Microbiol. 2011; 49(12):4231–8. [PubMed: 21998415]
55. Wallace RJ Jr, Brown BA, Griffith DE. Nosocomial outbreaks/pseudo-outbreaks caused by nontuberculous mycobacteria. Annu Rev Microbiol. 1998; 52:453–90. [PubMed: 9891805]
56. Anaissie EJ, Costa SF. Nosocomial aspergillosis is waterborne. Clin Infect Dis. 2001; 33(9):1546–8. [PubMed: 11568850]
57. Anaissie EJ, et al. Fusariosis associated with pathogenic fusarium species colonization of a hospital water system: a new paradigm for the epidemiology of opportunistic mold infections. Clin Infect Dis. 2001; 33(11):1871–8. [PubMed: 11692299]
58. Anaissie EJ, et al. Pathogenic molds (including Aspergillus species) in hospital water distribution systems: a 3-year prospective study and clinical implications for patients with hematologic malignancies. Blood. 2003; 101(7):2542–6. [PubMed: 12468437]
59. Berger P, et al. Ameba-associated microorganisms and diagnosis of nosocomial pneumonia. Emerg Infect Dis. 2006; 12(2):248–55. [PubMed: 16494750]
60. Panagopoulou P, et al. Filamentous fungi in a tertiary care hospital: environmental surveillance and susceptibility to antifungal drugs. Infect Control Hosp Epidemiol. 2007; 28(1):60–7. [PubMed: 17230389]
61. Krageschmidt DA, KA, Browning MS, Wright AJ, Lonneman JD, Detmer MJ, McCoy WF. A Comprehensive Water Management Program for Multicampus Healthcare Facilities. Infect Control Hosp Epidemiol. 2014; 35(5):556–563. [PubMed: 24709725]
62. Lin YE, SJ, Yu VL. Controlling Legionella in hospital drinking water: an evidence-based review of disinfection methods. Infect Control Hosp Epidemiol. 2011; 32(2):166–173. [PubMed: 21460472]
63. Marchesi I, FG, Bargellini A, Marchegiano P, Predieri G, Stout JE, Borella P. Monochloramine and chlorine dioxide for controlling Legionella pneumophila contamination: biocide levels and disinfection by-product formation in hospital water networks. J Water Health. 2013; 11(4):738–747. [PubMed: 24334848]
64. Kandiah S, Yassin MH, Stout J. Monochloramine use for prevention of Legionella in hospital water systems. Infect Disord Drug Targets. 2013; 13(3):184–90. [PubMed: 23961741]
65. Comprehensive Disinfectants and Disinfection Byproducts Rules (Stage 1 and Stage 2). Environmental Protection Agency. 2006; 71(2)
66. Centers for Disease Control and Prevention. The CDC investigation of Legionnaires' disease among patients at the VA Pittsburgh Healthcare System. 2013.
67. Casini B, VP, Baggiani A, Torracca F, Frateschi S, Nelli LC, Privitera G. Molecular epidemiology of Legionella pneumophila serogroup 1 isolates following long-term chlorine dioxide treatment in a university hospital water system. J Hosp Infect. 2008; 69(2):141–147. [PubMed: 18439718]
68. Casini B, BA, Cristina ML, Spagnolo AM, Del Giudice P, Brusaferro S, Poscia A, Moscato U, Valentini P, Baggiani A, Privitera G. Long-term effects of hospital water network disinfection on Legionella and other waterborne bacteria in an Italian university hospital. Infect Control Hosp Epidemiol. 2014; 35(3):293–299. [PubMed: 24521596]
69. Jakubek D, et al. Susceptibility of Legionella strains to the chlorinated biocide, monochloramine. Microbes Environ. 2013; 28(3):336–45. [PubMed: 24005820]
70. Johansson PJ, AK, Wiebe T, Schalén C, Bernander S. Nosocomial transmission of Legionella pneumophila to a child from a hospital's cold-water supply. Scand J Infect Dis. 2006; 38(11–12): 1023–1027. [PubMed: 17148071]
71. Stout JE, YV, Muraca P. Isolation of Legionella pneumophila from the cold water of hospital ice machines: implications for origin and transmission of the organism. Infect Control. 1985; 6(4): 141–146. [PubMed: 3886578]
72. Bencini MA1YE, Koornstra RH, Nolte CC, den Boer JW, Bruin JP. A case of Legionnaires' disease caused by aspiration of ice water. Arch Environ Occup Health. 2005; 60(6):302–306. [PubMed: 17447574]
73. Borella P, BA, Marchesi I, Rovesti S, Stancanelli G, Scaltriti S, Moro M, Montagna MT, Tatò D, Napoli C, Triassi M, Montegrosso S, Pennino F, Zotti CM, Ditommaso S, Giacomuzzi M.

Prevalence of anti-legionella antibodies among Italian hospital workers. J Hosp Infect. 2008; 69(2):148–155. [PubMed: 18448198]

74. Chikte UM, KO, Gildenhuys I. A case study of a dental receptionist diagnosed with Legionnaires' disease. SADJ. 2011; 66(6):284–287. [PubMed: 23198477]
75. Lal S, SS, Bricknell M, Pearce M, Morton LH, Ahmed W, Crean SJ. Monitoring Dental-Unit-Water-Line Output Water by Current In-office Test Kits. Curr Microbiol. 2014
76. Singh T, CM. Isolation of pathogenic Legionella species and legionella-laden amoebae in dental unit waterlines. J Hosp Infect. 2005; 61(3):257–262. [PubMed: 16099073]
77. Epson EE, et al. Carbapenem-resistant Klebsiella pneumoniae producing New Delhi metallo-beta-lactamase at an acute care hospital, Colorado, 2012. Infect Control Hosp Epidemiol. 2014; 35(4): 390–7. [PubMed: 24602944]
78. Witney AA, et al. Genome sequencing and characterization of an extensively drug-resistant sequence type 111 serotype O12 hospital outbreak strain of Pseudomonas aeruginosa. Clin Microbiol Infect. 2014
79. Kundu S, et al. Next-generation whole genome sequencing identifies the direction of norovirus transmission in linked patients. Clin Infect Dis. 2013; 57(3):407–14. [PubMed: 23645848]
80. Reuter S, et al. A pilot study of rapid whole-genome sequencing for the investigation of a Legionella outbreak. BMJ Open. 2013; 3(1)
81. Graham RM, Doyle CJ, Jennison AV. Real-time investigation of a Legionella pneumophila outbreak using whole genome sequencing. Epidemiol Infect. 2014:1–5.
82. Yang JY, et al. Pan-PCR, a computational method for designing bacterium-typing assays based on whole-genome sequence data. J Clin Microbiol. 2013; 51(3):752–8. [PubMed: 23254127]