Practical Recommendations for Meeting the Objectives of the CMS Memorandum

Shivi Selvaratnam Ph.D., Michael Coughlin Ph.D., Elizabeth Sotkiewicz, and Carmel Holliday

Abstract

The Centers for Medicare and Medicaid Services issued a policy memorandum on June 2, 2017 that requires all healthcare facilities that file for Medicare or Medicaid reimbursements to implement a Water Management Plan (WMP) so as to prevent outbreaks of Legionnaires ' disease and other diseases caused by water borne pathogens. WMPs focused on Legionellosis disease prevention have become commonplace since ASHRAE approved standard 188-2015, "Legionellosis: Risk Management for Building Water Systems". However, the Centers for Medicare and Medicaid Services has expanded the scope of WMPs to require the inclusion of *Legionella* and other opportunistic water borne pathogens such as *Pseudomonas*, non-tuberculous mycobacteria, *Burkholderia*, *Stenotrophomonas*, fungi, and *Acinetobacter*. Implementing a WMP that includes a risk assessment, control measures and testing protocols specific to all water borne pathogens could be prohibitively expensive and time consuming for any infection control program. In this paper, we present realistic strategies for developing a comprehensive WMP that will not stress the human and financial resources of a healthcare facility yet provides reasonable assurances that the health of its patients, staff and visitors are protected.

Introduction

The revised memorandum, S&C 17-30, issued on June 6, 2017 by the Centers for Medicare and Medicaid Services (CMS) added clarity and detail to previously issued CFR regulations that addressed the requirement to prevent disease transmission. The federal statutes 42 CFR §482.42, 42 CFR §483.80 and 42 CFR §485.635(a)(3)(vi) stipulate that hospitals, critical access hospitals, and long-term care have infection control programs to prevent the growth and transmission of pathogens in their respective environments. S&C 17-30 makes it clear that pathogen control include all water borne pathogens that could come in contact with patients and facilities that fail to comply with this CMS regulation "are at risk of citation for non-compliance with the CMS Conditions of Participation". S&C 17-30 also states that authorities having jurisdiction will be surveying healthcare facilities to determine if compliance is being achieved. Although organizations such as OSHA, ASHRAE, AIHA and the CDC have all made it clear that Water Management Plans (WMP) are a necessary component of a pathogen control program, this is the first time that an authority having jurisdiction has been given policing and penalty enforcement power.

Although S&C 17-30 focuses on one particular waterborne pathogen, i.e., *Legionella pneumophila*, it is clear in its mandate that it includes **all** waterborne pathogens. In view of such an expansive scope of pathogen control and the financial burden to implement such programs, S&C 17-30 presents an immediate challenge to everyone in healthcare management. A necessary component of any WMP is way to validate its effectiveness. This can be done proactively by surveilling the presence of waterborne pathogens or monitoring for the presence of disease caused by waterborne pathogens. From the standpoint of disease prevention, a proactive program is of course the preferred approach. In this paper we have investigated the presence of the three most common waterborne pathogens (*Legionella spp*, *Mycobacterium spp*, and *Pseudomonas aeruginosa*) and aerobic heterotrophs in the potable water of healthcare and non-healthcare facilities. The intention of this investigation was to determine if the presence of one of these organisms might be correlated with the presence of another so as to simplify the validation requirement of a WMP. Furthermore, we wanted to determine the prevalence and proportions of these three waterborne pathogens. The overarching purpose of this investigation was to prioritize and simplify the implementation of a WMP.

Materials and Methods

Potable Water Sample Collection

One liter (1 L) potable first draw water sample from faucets were collected in sterile wide-mouth screw cap polypropylene plastic bottle containing 150-200mg sodium thiosulfate preservative.

Preparation of Samples for Bacteriological Examination

Filtration of Potable Water Samples for *Legionella* Isolation

Five hundred ml (500 ml) of each potable water sample was filter-concentrated using a 47-mm filter funnel assembly disinfected with 80% isopropyl alcohol between uses and containing a sterile 0.22 μm polycarbonate filter. After filtration, the filter was removed aseptically from the holder with sterile filter forceps, folded to the outside, and placed into a sterile, 50-ml centrifuge tube containing 5ml of sterile Butterfield's buffer. The centrifuge tube was then vortexed for one minute at maximum speed to elute bacteria from the filter.

Acid Treatment of Samples for *Legionella* Isolation

Because water samples may contain high concentrations of non-*Legionella* bacteria, it was necessary to use a selective procedure to reduce their numbers before culture (CDC, 2005). Two hundred (200) μl of the vortexed suspension was placed into a sterile 1.5 ml centrifuge tube containing 200 μl of acid buffer. The suspension was then incubated for 5 minutes at room temperature. For swab samples, 100 μl of the suspension was placed into a sterile 1.5 ml centrifuge tube containing 100 μl of acid buffer and treated as described above.

Media for *Legionella* Growth and Isolation

Buffered charcoal yeast extract (BCYE) agar containing 0.1% alpha-ketoglutarate was used as the base medium used for the recovery of *Legionella* (1). Two types of selective BCYE agar were used in the processing of the samples. The first was designated BCYE complete with antibiotics (purchased from Hardy Diagnostics); the second, BCYE complete without antibiotics.

Plating of Samples

Plates (described above) were inoculated with 0.2 ml of either acid-treated or non-acid treated suspension and distributed over the agar surface with a plastic spreader. They were then incubated at 36 ^{o}C in a humidified incubator for 14 days at a minimum humidity of 95%.

Examination of Cultures for *Legionella*

Plates were examined after 72 to 96 hours of incubation for *Legionella*. Suspect *Legionella* colonies were streaked onto BCYE agar plate without L-cysteine and antibiotics, and a positive control BCYE agar plate without antibiotics. The plates were incubated for 24-48 hours. Colonies that grew on BCYE agar, but not BCYE agar without L- cysteine, were considered to be presumptive *Legionella* species and later serotyped using the Dry Spot ™agglutination test (Oxoid, Dardilly, France) or direct fluorescent antibody (MTech).

Isolation of Heterotrophic Plate Count Bacteria from Potable Water

Culturing of HPC bacteria was carried out according to the Standard Methods for the Examination of Water and Wastewater membrane filtration method (9215D) using R2A agar (Remel, Lenexa, KS). One hundred ml of the water sample was filter-concentrated using a 47-mm filter funnel assembly disinfected with 80% isopropyl alcohol between uses and containing a sterile 0.22 μm polycarbonate filter. After filtration, the filter was removed aseptically from the holder with sterile filter forceps, and placed onto a R2A agar plate ensuring no air was trapped beneath the membrane. Plates were incubated for 48-72 hours at 36°C. If growth was present, colonies were counted and reported as CFU/ml.

Isolation of *Pseudomonas aeruginosa* from Potable Water

Culturing of *Pseudomonas aeruginosa* was carried out according to ISO16266:2006 using Cetrimide agar (BBL). One ml of the water sample was filter-concentrated using a 47-mm filter funnel assembly disinfected with 80% isopropyl alcohol between uses and containing a sterile 0.22 μm polycarbonate filter. After filtration, the filter was removed aseptically from the holder with sterile filter forceps, and placed onto a Cetrimide agar plate ensuring no air was trapped beneath the membrane. Plates were incubated for 24-48 hours at 36°C.

Examination of Cultures for *Pseudomonas aeruginosa*

If growth was present, plates were examined for pigment production. If growth and pigment production were visible, colonies were counted and CFU/ml was reported as *Pseudomonas aeruginosa.* If growth was present in the absence of pigment production, the presence of oxidase was determined by streaking a colony on an Oxistrip™. A blue color developing in 30 seconds or less was indicative of an oxidase positive reaction. If the colonies were oxidase positive, the presence of fluorescent pigment was determined using a UV light at 360 'A. If colonies were fluorescent, they were checked for the ability to deaminate by streaking an Acetamide Agar Slant with a representative colony. Development of a red color over the course of 7 days was indicative of deamination and a positive reaction.

Isolation of Nontuberculous Mycobacterium (NTM) from Potable Water

Culturing of NTM was carried out according to Standard Methods for the Examination of Water and Wastewater, Method 9260M using Middlebrook 7H10 agar (Hardy Diagnostics). Twenty ml 0.04% (w/v) cetylpridinium chloride (CPC) was added to 500 ml of water sample and left at room temperature for 24 h. The water sample was then filter-concentrated using a 47-mm filter funnel assembly disinfected with 80% isopropyl alcohol between uses and containing a sterile 0.22 μm polycarbonate filter. After filtration, the filter was removed aseptically from the holder with sterile filter forceps, and placed onto a Middlebrook 7H10 agar plate ensuring no air was trapped beneath the membrane. Plates were incubated for 7-14 days at 36°C.

Examination of Cultures of NTM

Plates were examined after 7-14 days of incubation for NTM. Colonies were examined for morphological characteristics typical of NTM. Presumptive NTM colonies were counted and select colonies were stained with acid-fast stain using Ziehl-Neelsen Method.

Results and Discussion

Potable water samples were analyzed to determine if there were correlations between (1) HPC bacteria isolated on BCYE and R2A, (2) HPC bacteria and the waterborne pathogens tested in this study, and (3) *Legionella*, *Pseudomonas aeruginosa*, and Nontuberculous Mycobacterium (NTM).

Growth of Heterotrophic Plate Count (HPC) Bacteria on BCYE and R2A

The World Health Organization (WHO) defines "heterotrophs" as micro-organisms that require organic carbon for growth and Heterotrophic Plate Count (HPC) as colony counts using a simple, culture test on an organic carbon medium (WHO 2017). Though HPC tests are not considered useful in validation of WMPs, they can be of some use in measuring the effectiveness of water treatment processes. The Environmental Protection Agency's (EPA) surface water treatment rules require systems using surface water or ground water to control HPC bacteria to no more than 500 CFU/ml.

The first goal of the study was to determine if growth of HPC bacteria on two non-selective media, namely, BCYE agar and R2A agar were comparable. Water samples were processed and HPC bacteria were isolated as noted above in Materials and Methods.

Results indicate that HPC bacteria growth on BCYE media is very similar to that on R2A (n=1288). However, at lower concentration ranges (0-1000 CFU/ml), BCYE appears to be a more sensitive growth medium compared to R2A (Table 1 and Figure 1). A two tailed paired sample t-test also indicates that the means from the two media are not statistically significant at the 0.05 level. Therefore, if potable water samples are required to meet the EPA drinking water criteria of <500 CFU/mL, BCYE is better at supporting the growth of HPC bacteria (Table 2). Interestingly, 47.5% and 65.8% of samples incubated on R2A and BCYE respectively, exceeded the EPA threshold of <500 CFU/ml of HPC. The concept that HPC bacteria are indicative of pathogen loading in a system is questionable. The presence of high levels of HPC could indicate the presence of stagnant zones, often referred to as dead legs that need to be eliminated.

Perhaps the only advantage of using R2A as opposed to BCYE is that R2A plates are typically incubated for 48 hours before reading as opposed to BCYE plates that are counted between 10-14 days. It is possible that that the 48 hour count on BCYE would yield HPC data similar to a 10-14 count but this has not been yet been verified.

Table 1: Decile Population Ranges of HPC Bacteria on BCYE and R2A

Concentration Range (CFU/ml)	R2A (n)	BCYE (n)
0	39	70
1-9	233	306
10-99	204	243
100-999	136	228
1000-9999	245	144
10000-99999	379	268
100000-999999	52	29
Total	1288	1288

Figure 1: Comparison of Growth of Heterotrophic Bacteria on BCYE and R2A

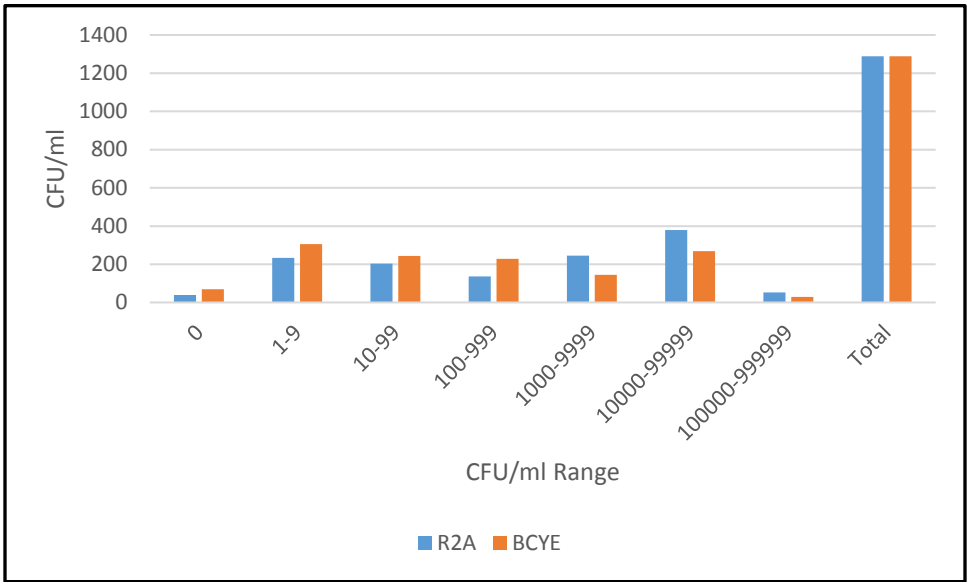


Table 2: Statistical Metrics of HPC Bacteria on BCYE and R2A

	R2A (n=1288)	BCYE (n=1288)
≤ 500 CFU/ml	612	847
Mean	25678	11212
Mode	0	0
P probability	0.065	

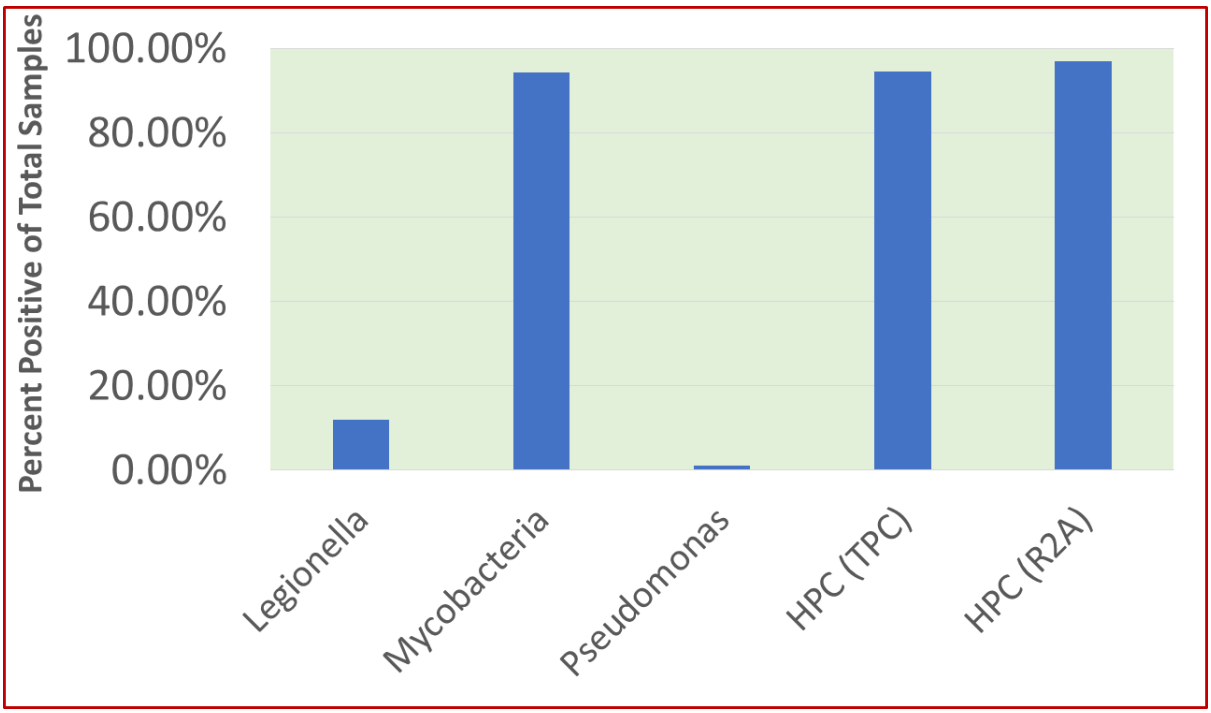
Prevalence of *Legionella*, *Pseudomonas aeruginosa*, and Non Tuberculous Mycobacterium (NTM) in Potable Water

A second objective of the study was to determine the prevalence of *Legionella*, *Pseudomonas aeruginosa*, and NTM in potable water. Water samples (n=1288) were processed and the three waterborne pathogens were isolated as noted above in Materials and Methods.

Results in Figure 2 indicate that *Legionella* was present in 12% of all samples tested while NTM was present in 94% of the samples. *Pseudomonas aeruginosa* was present in 1% of the samples. Nearly every sample contained HPC bacteria (95% when isolated on BCYE and 97% on R2A). In view of the low incidence of *Pseudomonas aeruginosa* contaminating potable water, routine environmental surveillance of this pathogen as part of a WMP is not recommended. Only when disease has been linked to this organism should water systems be investigated as a potential source of this pathogen. Conversely, the

virtual ubiquity of NTM indicates that routine testing for its presence in potable water systems would be a useful addition to a WMP.

Figure 2: Percent Positive of Total Samples



Proportion of *Legionella*, *Pseudomonas aeruginosa*, and NTM in Positive Samples

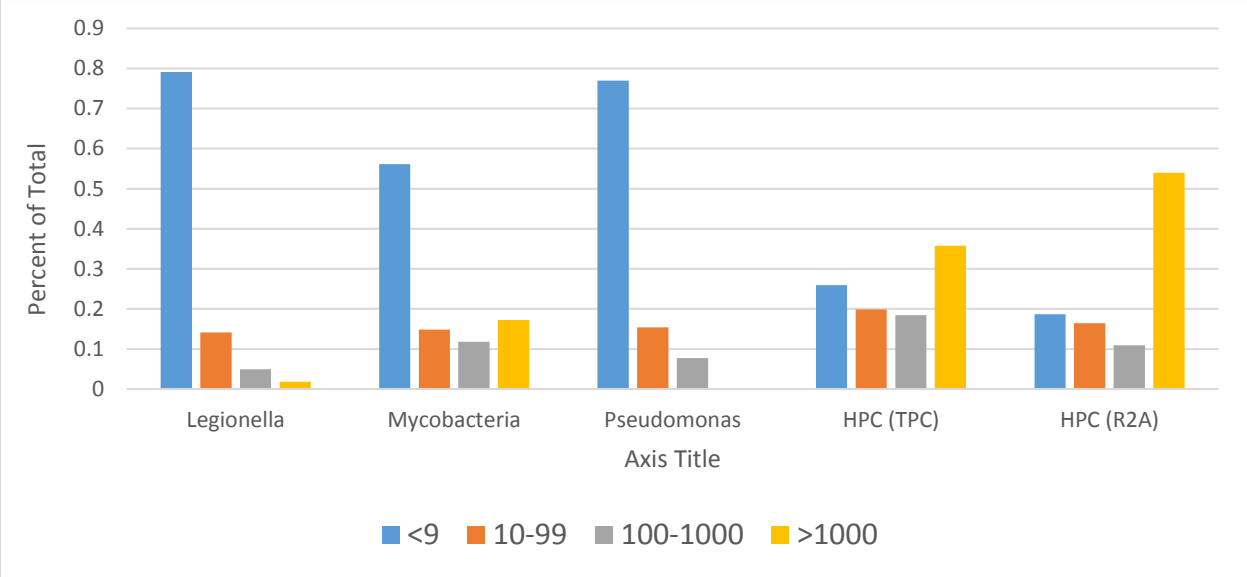
A third objective was to determine the proportion of each of the three water borne pathogens in positive samples (Figure 3). Seventy nine percent of samples that were positive for *Legionella* had a colony count of < 9 CFU/ml, 14% of the samples had colony count ranging from 10-100 CFU/ml, 5% of the samples had a colony ranging from 100-1000 CFU/ml, and 1.8% had a colony count of > 1000 CFU/ml.

Fifty six percent of samples that were positive for NTM had a colony count of < 9 CFU/ml, 14% of the samples had colony count ranging from 10-100 CFU/ml, 12% of the samples had a colony ranging from 100-1000 CFU/ml, and 17% had a colony count of > 1000 CFU/ml.

Seventy seven percent of samples that were positive for *Pseudomonas aeruginosa* had a colony count of < 9 CFU/ml, 15% of the samples had colony count ranging from 10-100 CFU/ml, 8% of the samples had a colony ranging from 100-1000 CFU/ml. No positive sample had a colony count > 1000 CFU/ml.

Additionally, more than 50% of the positive samples for HPC bacteria had >1000 CFU/ml which is double the drinking water limit set by EPA.

Figure 3: Percent Positive of Total Samples



Is there Correlation between HPC Bacteria, *Legionella*, *Pseudomonas aeruginosa*, and NTM in Potable Water?

Potable water samples were examined for the presence of HPC bacteria on BCYE and R2A, *Legionella* on BCYE, NTM on Middlebrook 7H10, and *Pseudomonas aeruginosa* on cetrimide agar.

Results (Table 3) indicate that there was no correlation between HPC and the waterborne pathogens investigated in this study. However, there was a weak correlation between HPC bacteria isolated on BCYE and HPC bacteria isolated on R2A (correlation coefficient of 0.6). The correlations between *Legionella* and HPC on BCYE (0.004), *Legionella* and HPC on R2A (0.016) and *Legionella* and NTM (0.0010) were weakly positive. The correlation between *Legionella* and *Pseudomonas aeruginosa* (-0.004) was negative. Similarly, there was a negative correlation between *Pseudomonas aeruginosa* and NTM (-0.0067). However, the negative correlations are so small as to be meaningless.

Table 3: Correlation between HPC Bacteria, *Legionella*, *Pseudomonas aeruginosa*, and NTM

	HPC (R2A)				
HPC (R2A)	1	**HPC (BCYE)**			
HPC (BCYE)	0.5834 (1337)	1	***Legionella* (BCYE)**		
***Legionella* spp**	0.0155 (1337)	0.0037 (1337)	1	***Pseudomonas aeruginosa***	
Pseudomonas aeruginosa	-0.0029 (1337)	0.0010 (1337)	-0.0041 (678)	1	**NTM**
NTM	0.0077 (620)	0.0025 (620)	0.0010 (678)	-0.0067 (678)	1

Summary Points

1. In addition to *Legionella spp*., routine environmental surveillance of NTM is recommended as a necessary component of a hospital's WMP. However, further research is required to determine which species of NTM is prevalent in potable water.
2. *Pseudomonas aeruginosa* surveillance is only recommended if this organism has been shown to be the causative agent of a hospital acquired infection. Though this organism was infrequently present in water samples, it is known to be more prevalent in biofilm.
3. From an infection prevention stand point, incorporating HPC testing into a WMP is not warranted. However, HPC may be a good indicator of water quality.
4. There is no evidence of one waterborne pathogen or heterotroph acting as a surrogate for another waterborne pathogen.
5. If an HPC analysis is required, either R2A or BCYE (with supplements) media will suffice.

References

(1) Centers for Disease Control (CDC). 2005. Procedures for the Recovery of *Legionella* from the Environment. U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia.
(2) ISO16266:2006 Water quality — Detection and enumeration of *Pseudomonas aeruginosa* — Method by membrane filtration.
(3) Standard Methods for the Examination of Water and Wastewater, Method 9215D (22nd Edition).
(4) Standard Methods for the Examination of Water and Wastewater, Method 9260M (22nd Edition).

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