**Pseudomonas aeruginosa as an Indicator for Opportunistic Premise Plumbing Pathogens**

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**Abstract**

There is widespread evidence of increases in the prevalence and incidence of opportunistic premise plumbing pathogens (OPPPs) such as *Legionella pneumophila*, *Mycobacterium avium*, *Acinetobacter baumanii*, and *Stenotrophomonas maltophilia*. The prevalence of Legionnaire's disease, caused by *L. pneumophila*, is increasing at 10% per year to almost 20,000 currently and pulmonary mycobacterial disease, caused by *M. avium* complex and other nontuberculous mycobacteria (NTM) is rising at a rate of between 5 and 15% to 85,000 based on current estimates. In spite of the increasing prevalence of these infections, proof that the source of these microorganisms is drinking water, and the cost and complexity of treatment, there is almost no monitoring for their presence and number. Currently, only cases of Legionnaire’s Disease must be reported. Standard does not measure of microbiological quality of water, fecal coliform nor heterotrophic plate count (HPC) predicts numbers or presence of OPPPs. However, *Pseudomonas aeruginosa* could be used as an indicator for OPPPs. *P. aeruginosa* occupies the same habitats as OPPPs (e.g., drinking water) and shares many characteristics (e.g., disinfectant-resistance and biofilm-formation) in common with OPPPs. The literature shows that the numbers of *P. aeruginosa* correlate with *L. pneumophila*, *M. avium*, *A. baumanii*, or *S. maltophilia*. Herein, I propose that studies be performed to determine whether presence/absence or numbers of *P. aeruginosa* correlate with those of OPPPs. Fortunately, both cultural- and qPCR-based methods can be used for specific detection and enumeration of *P. aeruginosa* suggesting that *P. aeruginosa* testing of water could be implemented widely.

**ABBREVIATIONS**

OPPP: Opportunistic Premise Plumbing Pathogens

**INTRODUCTION**

In the last several decades, there has been increasing awareness of the emergence of a group of opportunistic microbial pathogens whose source of infection is water. Further, in many instances, infection has been traced to water in homes and hospitals; hence the name, opportunistic premise plumbing pathogens (OPPPs). A list of OPPPs is provided in (Table 1). The prevalence and incidence of OPPP-infection and disease is rising. Over 18,000 cases of Legionnaire’s disease, caused by *Legionella pneumophila*, were reported in the United States in 2011 and the number is increasing at an annual rate of 5% per year [1,2]. Approximately 85,000 individuals in the United States and Canada have pulmonary mycobacterial disease [3]; a debilitating and chronic lung infection caused by *Mycobacterium avium* complex (MAC) and other nontuberculous mycobacteria (NTM). The cost of treatment of infections caused by OPPPs is enormous; almost $1 billion annually in the United States for just *L. pneumophila* and *M. avium* complex [4]. *P. aeruginosa* is a well known nosocomial opportunistic pathogen causing ventilator-associated pneumonia [5] and infections in intensive care units [6]. *Acinetobacter baumanii*, a relative of *P. aeruginosa*, became well known after infections amongst U.S. troops in the Middle East and is associated with antibiotic-resistant, community-onset pneumonia of high mortality whose source of infection has been traced to drinking water [7]. *S. maltophilia* is an environmental global opportunistic pathogen associated with respiratory and tissue and soft skin infections and bacteremia in humans whose sources include drinking water and medical devices rinsed with non-sterile water [8]. Independent risk factors for infection by

**Table 1: Opportunistic Premise Plumbing Pathogens (OPPPs).**

<table>
<thead>
<tr>
<th><strong>Opportunistic Premise Plumbing Pathogens (OPPPs).</strong></th>
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<tr>
<td><em>Legionella pneumophila</em></td>
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<td><em>Mycobacterium avium complex (MAC)</em></td>
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<td><em>Pseudomonas aeruginosa</em></td>
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<td><em>Acinetobacter baumanii</em></td>
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<td><em>Stenotrophomonas maltophilia</em></td>
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<td><em>Sphingomonas paucimobilis</em></td>
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<td><em>Aeromonas hydrophila</em></td>
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<td><em>Acanthamoeba</em></td>
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<td><em>Vermamoeba</em></td>
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most OPPPs include age, lung damage, and immunodeficiency. For example, older, slimmer, and taller women are at increased risk for pulmonary nontuberculous mycobacteria infection [9]. The prevalence of NTM disease in individuals over 60 years is 100 per 100,000 population whereas it is 15 per 100,000 for persons younger than 50 [3]. Increased susceptibility of older individuals is also characteristic of *L. pneumophila* infection [2]. As the United States population ages, so will the prevalence of OPPP infections increase. It is estimated that by 2025, 25 % of United States citizens will be over 60 years [10]. Another risk factor for OPPP infection and disease is immunodeficiency, whether due to cancer, chemotherapy, or infection by agents such as HIV [3]. As it has been anticipated that more individuals will be undergoing chemotherapy for treatment of disease and those same individuals are at increased risk for OPPP disease, we expect an increasing prevalence of OPPP disease in the future. A common risk factor of most OPPP-infections is residence in a hospital. Specifically, *P. aeruginosa* [6], *Acinetobacter baumanii* [7], and *S. maltophilia* [8] infections have been traced to drinking water, aerosols, ice machines, or water in medical devices in hospitals. As the cost of hospital-acquired infections continues to increase [4], hospitals might consider routine monitoring for OPPPs. Such predictions suggest that public health programs should anticipate these increases and map out plans for dealing with their medical and economic ramifications. As OPPP infections are a result of exposure to water or water aerosols colonized by these opportunistic pathogens, it follows that a viable approach to reducing the incidence and prevalence of OPPP disease is reduction of OPPP numbers in water supplies. This would be especially effective amongst those of increased susceptibility and likely more cost-effective than relying upon expensive antibiotic therapy.

**COMMON CHARACTERISTICS OF OPPORTUNISTIC PREMISE PLUMBING PATHOGENS (OPPPS)**

OPPPs share a number of structural, morphological, physiological, and metabolic features that are determinants of their ecology (Table 2) [11,12]. All of those characteristics mean that OPPPs share common habitats. Further, many of those OPPP habitats are occupied by humans; for example, households, hospitals and more specifically, showers, therapy pools, spas and hot tubs. Premise plumbing in households, apartments, condominiums, hospitals, and office buildings share a number of common features; all advantageous for persistence and growth of OPPPs (Table 2). These include: (1) a high surface to volume ratio, (2) low disinfectant concentration, (3) low organic carbon concentration, (4) periods of stagnation, (5) dead ends or unused areas of plumbing, and (6) exposure to high temperature in water heaters. The high surface-to-volume ratio of premise plumbing provides surfaces for OPPP adherence and biofilm formation. That means the slowly growing OPPP will not be washed out. The low disinfectant concentration selects for OPPPs as all are relatively disinfectant-resistant compared to *Escherichia coli* and other fecal contaminants. For example, *P. aeruginosa* [13], *A. baumanii* [7], *S. maltophilia* [8], and *M. avium* [14] are disinfectant-resistant. Low organic carbon is not a barrier to OPPP growth as a number have been shown to grow in water; even distilled water, like *P. aeruginosa* [15]. Further, though much of the assimilable organic carbon (AOC) in drinking water consists of humic and fulvic acids and other humic substances [16] that do not serve as nutrient for most heterotrophs and fecal coliforms, OPPPs can grow. In fact, humic and fulvic acids stimulate the growth of *M. avium* complex [17] and *P. aeruginosa* [16]. Dead-ends in plumbing are regions of stagnation with low oxygen levels where the microaerobic *M. avium* complex [18] and nitrate reductase-producing *P. aeruginosa* [19] can grow. Dead-ends also serve as sources for re-inoculation of premise plumbing following disinfection. Finally, OPPPs are relatively resistant to water temperatures encountered in water heaters (e.g. < 125°F, 50°C). Not only do OPPPs survive in water heaters, but also they provide a site for growth and increase in numbers. Specifically, water heater temperatures were found conducive for *L. pneumophila* growth [20]. *P. aeruginosa* tolerates exposure to water heater temperatures [21], and *M. avium* isolates from water heaters in an area with a high proportion of taller, slimmer, older women in Montgomery County, PA [22]. Finally, even if there are bacterial-grazing amoebae, the OPPPs, including *L. pneumophila*, *M. avium*, *S. maltophilia*, and *P. aeruginosa* can grow inside the amoebae [23]; and there by collectively referred to as amoeba-resisting bacteria (ARB). Thus, the common characteristics of OPPPs mean that they can colonize and grow in drinking water distribution systems, premise plumbing, and in medical equipment. From those sources, OPPPs can be transmitted and infect a population of individuals who are more likely to be pre-disposed to OPPP infection.

**CHARACTERISTICS OF PSEUDOMONAS AERUGINOSA**

*P. aeruginosa* is an OPPP and has long been associated with water borne infections, particularly amongst burn patients and those with cystic fibrosis [24]. *P. aeruginosa* is a cause of hospital-acquired infections and community-associated life-threatening pneumonias. It is estimated that *P. aeruginosa* is responsible for approximately 1,000 cases per year in the United States [25]. *P. aeruginosa*-associated infections are quite severe and require immediate attention. In a meta-analysis of *P. aeruginosa*-associated bronchiectas is of 3,683 patients, *P. aeruginosa* led to increased hospital admissions, severity of disease symptoms, and 3-fold increase in mortality [26]. *P. aeruginosa* has been detected in a variety of habitats that it shares with humans, including: rivers and lakes [27], drinking water [28], premise plumbing [29], chlorinated swimming pools [30, 31], hospital water baths [32], hospital sink drains [33, 34], bottled waters [35-37], humidifiers [38], and even distilled water [15]. A variety of techniques has been used to fingerprint

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Table 2. Premise Plumbing Characteristics and Their Relevance for OPPPs.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Relevance for OPPPs</th>
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<tr>
<td>High Surface to Volume Ratio</td>
<td>Surfaces for Adherence and Biofilm Formation</td>
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<tr>
<td>Low Disinfectant Concentration</td>
<td>Selection against OPPP Competitors</td>
</tr>
<tr>
<td>Low Organic Carbon Concentration</td>
<td>Selection against OPPP Competitors</td>
</tr>
<tr>
<td>Periods of Stagnation</td>
<td>Selection for Microaerobic OPPPs</td>
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<tr>
<td>Dead Ends or Unused Plumbing</td>
<td>Sites for OPPP Growth under Low Oxygen</td>
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<tr>
<td>High Temperature in Water Heaters</td>
<td>Selection for Heat-tolerant OPPPs</td>
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and thereby trace *P. aeruginosa* isolates in outbreaks. Pulsed-field gel electrophoresis (PFGE) and repetitive sequence-based PCR (rep-PCR) were used to fingerprint and track metallo-β-lactamase-producing, carbapenem-resistant hospital isolates of *P. aeruginosa* [39]. Whole genome sequencing has been used to track a *P. aeruginosa* outbreak in a neonatal intensive care unit [40]. *P. aeruginosaa* adheres to a variety of surfaces, such as ethylene-propylene-diene-monomer (EDPM) rubber and silane cross-linked polyethylene (PE-X) [41]. Biofilms provide a means whereby OPPPs can persist and grow in flowing systems (e.g., pipes) and protect cells from disinfectants, due to the layers of cells within an extracellular polymeric matrix. In addition to biofilm-enabling protection from disinfectants [42], *P. aeruginosa* cells in water suspension are relatively resistant to disinfectants in chlorine, particularly due to reduced outer membrane permeability [29].

**LACK OF CORRELATION BETWEEN FECAL COLIFORM, TOTAL VIABLE COUNT, OR HETEROOTRPHIC PLATE COUNT BACTERIA**

Although there have been a limited number of relevant studies, there appears to be no association of any OPPPs with the presence of coliforms, fecal coliforms, or *Escherichia coli* or heterotrophic plate count (HPC) bacteria numbers. The available data consists of studies that looked for and enumerated OPPPs and only incidentally were coliforms or HPC bacteria enumerated. Thus, the samples were not chosen to represent the range of possible coliform or HPC bacteria densities. However, there is no evidence of correlation or association between OPPP numbers and either coliform or heterotrophic plate count bacteria numbers. In the absence of any demonstration of a positive correlation, the data suggest that neither coliform nor HPC bacterial numbers can serve as an indicator for OPPP numbers. Below is a brief review of the available data. In a study including 8 different water distribution systems across the United States, it was found that although a variety of *Mycobacterium* species, including *M. avium*, were recovered, there was no correlation between *Mycobacterium* spp. numbers and fecal coliforms [43]. Similarly, in freshly squeezed orange juice samples collected from street vendors in Mexico City, mycobacterial presence did not correlate with total coliforms, fecal coliforms, or *Escherichia coli* [44]. *Legionella* spp. numbers as measured by PCR also fail to be correlated with total viable counts (TVCs) on yeast extract agar incubated at either 22°C or 37°C using samples collected from dental unit water lines [45]. In Table 2 of that publication are reported values for *Legionella* spp. numbers by PCR and TVCs measured at both incubation temperatures. Statistical analysis showed that there was no correlation between the two groups of values over a wide range of both *Legionella* (i.e. 5,200 – 830,000 gene units/L) and TVCs at 22°C (i.e. 5-3,000 CFU/ml) and 37°C (i.e. 1-470 CFU/ml). Generally, there appears to be no correlation between heterotrophic plate count (HPC) bacterial numbers and health effects, suggesting that monitoring HPC numbers do not serve to inform public health [46, 47]. Further, heterotrophic plate count (HPC) bacteria are in drinking water, but as is the case with coliform counts, HPC counts do not correlate with the presence or absence of OPPPs. In a study of rainwater tanks in Japan, it was shown that *Legionella* spp. positive samples by PCR were associated with samples with higher than 10,000 HPC/ml, whereas in samples with 1,000 HPC/ml no *Legionella* spp. were detected by PCR [48]. However, it has been pointed out that HPC species composition were different depending upon the incubation temperature and composition of the medium used for enumeration [49]. Thus, the demonstration of either a positive-negative correlation of HPC with any of the OPPPs might not be a correct representation.

**EVIDENCE OF CORRELATIONS BETWEEN THE PRESENCE OF PSEUDOMONAS AERUGINOSA AND OPPPS**

**Correlations between *P. aeruginosa* and *Mycobacterium chimaera***

For the past 18 months, my colleagues and I have been developing methods for the disinfection of heater-coolers that have been implicated in *Mycobacterium chimaera* infections in patients undergoing cardiac surgery [50]. In sampling a wide variety of heater-coolers we found that they were colonized by both *M. chimaera* and *P. aeruginosa*. In fact, there was a positive correlation (*r = 0.6189*) between numbers of *M. chimaera* and *P. aeruginosa*. In a study of samples collected over time from a drinking water distribution system, numbers of *Legionella* spp. *Mycobacterium* spp. and *Pseudomonas aeruginosa* were measured by PCR [51]. In Figure 1 of that excellent publication [51], the data on numbers of those three bacteria (and other) is presented and allowed a statistical analysis of the correlation between the numbers. High correlations were calculated for numbers of *P. aeruginosa* and *Mycobacterium* spp. (*r = 0.6486*), *P. aeruginosa* and *Legionella* spp. (*r = 0.8197*), and *Mycobacterium* spp. and *Legionella* spp. (*r = 0.8200*).

**Correlations between *P. aeruginosa* and *A. baumanii***

In a study of seasonal and temperature-associated increases in Gram-negative blood-stream infections in hospitalized patients, it was shown that *A. baumanii* and *P. aeruginosa* increases coincided during the summers [52]. Further, the pattern of percentage changes in blood stream infections plotted against increases in monthly temperatures caused by *A. baumanii* and *P. aeruginosa* also reflected the same patterns [52]. This data suggests the two bacteria are responding to the same environmental influences.

**Correlations between *P. aeruginosa* and *S. maltophilia***

*S. maltophilia* appears to modulate the virulence of *P. aeruginosa* in mixed biofilms [53]. When grown in biofilms...
together, the presence of *S. maltophilia* led to over-expression of genes for protease and alginate synthesis [53]. As both are involved in virulence, *S. maltophilia* should be considered a regulator of *P. aeruginosa* virulence.

**PROPOSAL**

Based on the foregoing analysis, I propose that studies be initiated to determine whether *P. aeruginosa* is a suitable indicator for OPPPs. Such studies would involve collection of a water and biofilm samples from premise plumbing and distribution systems. Samples would be analyzed for the presence and numbers of *P. aeruginosa* and different OPPPs, principally *M. avium, L. pneumophila*, and one or more of the other OPPPs listed in (Table 1). Both culture- and PCR-based methods would be employed. For that goal to be achievable, it is necessary that the methods for detection and enumeration be accurate and amenable to high-throughput. In an excellent review of risk assessment of *P. aeruginosa* in drinking water, Hardalo and Edberg [24] identified many of the same features of that waterborne opportunistic pathogen as listed above. At that time it was their conclusion that “...because there is no readily available sensitive and specific means to detect and identify *P. aeruginosa* available in the field, any potential regulation governing control would not have a defined laboratory test measure of outcome.” [24]. However, that is no longer the case; both culture- and DNA-based methods are available to specifically and sensitively enumerate *P. aeruginosa*. Cultivation and enumeration can be easily performed on any water or biofilm sample using *Pseudomonas* Isolation Agar. In fact, Hardalo and Edberg report it as being selective and sensitive (Table 4) [24]. The medium is quite selective and *P. aeruginosa* colonies can be readily identified by their production of a green diffusible pigment and yellow-green fluorescence of colonies. Quantitative polymerase chain reaction (qPCR)-based methods have been employed for the detection and enumeration of *P. aeruginosa* in water [54,55], animal cagewater [54], aerosols [55], in sputum from cystic fibrosis patients [56,57]. There appears to be quite a high correlation between the results of culture- and PCR-based methods for *P. aeruginosa* detection. Results from 55 sputum cultures of cystic fibrosis patients, 52 (95 %) were both culture- and PCR-positive, only 3 were culture-negative and PCR-positive, and none were culture-positive and PCR-negative [56]. In the report of Lee et al. [55], it was shown that the PCR method worked even in samples that were chlorinated, unless the chlorine concentration was 10 ppm or greater. I trust this brief review of OPPPs, *P. aeruginosa*, and premise plumbing provides sufficient information to research groups to collaborate in a study directed towards determining whether *P. aeruginosa* presence/absence and counts correlate and serve as an adequate indicator for OPPP presence/absence and counts. Water and biofilm samples can be collected from different premises in different geographic areas to capture the widest range of *P. aeruginosa* and OPPP numbers and water quality variables. That approach would offer the most stringent test of the hypothesis proposed here.

**REFERENCES**


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