

## Introduction

*Pseudomonas aeruginosa* has been described as an environmental bacterium which can survive in polymicrobial societies independently of man.... [which are] nutritionally undemanding, copiously endowed with enzymes, toxins, pigments, glycocalyx, and useful biochemical capabilities, and forms coherent layers on moist surfaces inside and outside of living organisms...[with these properties culminating in] a hardy resistance to mechanical cleansing and flushing as well as to disinfectants, antibiotics, and antibodies.<sup>1</sup> This gram-negative bacillus is found in warm, moist environments, and can be frequently isolated from soil, water, and occasionally from normal human skin.<sup>2</sup> *P. aeruginosa* can inhabit the nasopharynx and lower digestive tract, but is only occasionally associated with disease, primarily as an opportunistic pathogen in immunocompromised hosts, who are prone to development of septicemia.<sup>3</sup>

In humans, *P. aeruginosa* is the second most frequent gram-negative nosocomial pathogen in hospitals<sup>4</sup> (accounting for 10-11% of all nosocomial infections), and has the highest case-fatality rate of all hospital-acquired bacteremias.<sup>5</sup> During the years 1991-1992, *Pseudomonas* spp. were the most commonly identified etiologic agents causing dermatitis, conjunctivitis, or otitis in humans following recreational water exposures (i. e., hot tubs, whirlpools, and swimming pools) where water pH or free residual chlorine levels failed to meet CDC guidelines for public spas and hot tubs.<sup>6</sup>

Within laboratory animal facilities, *P. aeruginosa* is normally an organism of low invasiveness, low virulence, and minimal significance to research, except in immunosuppressed and immunocompromised animals. Studies on the pathogenesis of *P. aeruginosa* in normal experimental laboratory animals are hindered because of the inherent resistance of most species to disease by this organism. Generally, the laboratory animal host is rendered susceptible only by the use of agents or procedures which severely depress defense mechanisms.<sup>7</sup>

Many questions remain regarding the pathogenicity, adherence mechanisms, ecological and growth characteristics, and effective therapeutic modalities for this organism. The intent of this article is to provide an overview of references and data on *P. aeruginosa*. We will attempt to evaluate its effect on research animals and research results, and to make recommendations for prevention, treatment, and control in laboratory animal facilities.

## Characteristics and Pathogenesis

*Pseudomonas aeruginosa* is a bacterium of the class *Schizomycetes*, order *Eubacteriales*, and family *Pseudomonadaceae*.<sup>8</sup> It is a gram-negative, non-spore forming, motile (with one polar flagellum), aerobic bacillus which measures 1.5-3.0  $\mu\text{m}$  in length by 0.5-0.7  $\mu\text{m}$  in diameter.<sup>9</sup>

*Pseudomonas aeruginosa* (*aeruginosa* = full of copper rust or verdigris, hence green) was first named by Schroeter in 1872. It was originally described as the causative agent of "blue pus" (thus the descriptive original name of the organism -- *Bacillus pyocyaneus*).<sup>17</sup> Gessard, in an 1882 publication entitled "On the Blue and Green Coloration of Bandages"<sup>10</sup>, showed that this bacteria formed two pigments: 1) pyocyanin, a bluish-green, non-fluorescent pigment which is soluble in chloroform and water; and, 2) fluorescein, a fluorescent greenish-yellow and insoluble in chloroform but soluble in water.

In 1886, Flugge recognized two other fluorescent pseudomonads. The first is now called *P. fluorescens* and liquefies gelatin. The second, *P. putida*, does not liquefy gelatin.<sup>12</sup> Since then, numerous species have been added to the genus. Some differ only slightly from the above-mentioned three, but others differ so much that a very broad definition is necessary to include them all.<sup>12</sup>

The virulence of *P. aeruginosa* is multifactorial. Its cellular products (lipopolysaccharide [LPS], pili, leukocidin, and alginate) and extracellular products (neutral and alkaline proteases, elastase, phospholipase C, and a rhamnolipid hemolysin) ensure its ability to infect most hosts. Two additional proteins are excreted by *P. aeruginosa* -- toxin A (ETA) and exoenzyme S (exo-S), with the former being the most toxic product secreted by the organism.<sup>13</sup> The proteases have been reported to have inhibited the chemotaxis, phagocytosis, and oxidative metabolism of human neutrophils.<sup>14</sup> The proteases may also contribute to the persistence of the organism through cleavage of immunoglobulins.<sup>15</sup>

Phagocytic cells (i.e., neutrophils and mononuclear phagocytes [circulating monocytes and tissue macrophages]) are the body's primary defense against *P. aeruginosa*. Phagocytosis is enhanced in the presence of serum factors including IgG, complement, and fibronectin.<sup>16</sup> When phagocytic dysfunction occurs in diseased or immunosuppressed animals or humans, such as with neutropenia (i.e., secondary to hematological malignancy [leukemia] or following chemotherapy), following extensive thermal injuries/burns, or in individuals with Cystic Fibrosis or other congenital phagocytic disorders, opportunistic infection with *Pseudomonas* can readily occur.

Other predisposing factors include previous antibiotic or corticosteroid therapy, premature birth, and immunosuppression associated with organ transplants. In individuals with normal phagocytic function, a number of *P. aeruginosa* products mentioned above (i.e., leukocidin, elastase, ETA), and also mucoid exopolysaccharide, slime glycolipoprotein, and pyocyanin, can adversely affect phagocytes and/or phagocytosis and predispose those individuals to the pathogenic effects of the organism.<sup>16</sup>

## Ecology/Distribution

*Pseudomonas aeruginosa* can be isolated from a wide range of inanimate, animal, and human environments, and can be found in a variety of geographic settings throughout the world. *Pseudomonas aeruginosa* is widely distributed in moist environments, including soil, water and sewage.<sup>9</sup> It can be found on and is occasionally pathogenic for plants and vegetables. Strains isolated from leaf spot tobacco, identical with or similar to *P. aeruginosa*, have been named "*P. polycolor*".<sup>17</sup>

This organism can be found in fresh water sources, usually in reservoirs polluted by human or animal wastes, but is not typically described as a member of groundwater microflora. Within hospital settings, *P. aeruginosa* has been isolated from equipment that contain or use water (i.e., sink drains, toilets, showers, bathroom fixtures, sanitary plumbing, air humidifiers), with recovery of up to 10<sup>9</sup> organisms per ml after undisturbed overnight growth.<sup>4</sup> This finding should prompt consideration of procedures for disinfection or decontamination of sinks before their first use of the day.

Macrocolonies of the mucoid-encapsulated organism have been found in dental offices covering the internal surfaces of waterlines used for patient procedures.<sup>18</sup> In an aqueous environment, the organism can colonize a variety of surfaces including silicon rubber, plastic/CPVC pipe, and stainless steel. Researchers have speculated that the mucoid capsule facilitates bacterial adhesion by bridging the electrostatic repulsion barrier between the macrocolony and the environmental surface, preventing the organism(s) from being dislodged by turbulence in water-filled pipes.<sup>4</sup> Survival times for the organism can be relatively short in aerosols and on dry surfaces, but half-life increases in environments with higher relative humidity (~80% RH) to almost an hour.<sup>4</sup>

Part of *P. aeruginosa*'s versatility includes the ability to phenotypically change from nonmucoid to mucoid variants. Mucoid variants of *P. aeruginosa* produce large amounts of a thick extracellular polysaccharide (alginate) coat called the glycocalyx. The glycocalyx surrounding the macrocolony serves a number of functions, including control of cell-to-cell adhesion and macrocolony formation, exclusion of humoral and synthetic antibacterial agents from the bacterial cell, and facilitation of entrapment/acquisition of nutrients in an aquatic environment.<sup>19,20</sup>

While nutrient-rich water can easily sustain growth of *P. aeruginosa*, the organism can still grow and multiply within distilled water. One study demonstrated the organism's ability to survive in autoclaved/sterilized drinking water for over 200 days following initial inoculation with 10<sup>6</sup> cells per ml.<sup>21</sup> In contrast to other gram-negative

bacteria tested, which remained viable but nonculturable in the sterilized water, *Pseudomonas aeruginosa* was readily culturable from the water for over 95 days.<sup>21</sup> Thus, the organism's minimal growth requirements and nutritional versatility allow it to adapt to changing ecologic circumstances, ensuring its ubiquitous distribution.<sup>22</sup>

*Pseudomonas aeruginosa* has been isolated in commercial laboratory animal barrier production facilities.<sup>23</sup> It can be a constituent of the bacterial flora of the intestines of laboratory rodents, especially mice.<sup>24</sup> In facilities with conventional housing, and minimal or no water treatment, maintenance of nasopharyngeal and intestinal colonization is associated with repeated ingestion of large numbers of the organism in the drinking water.<sup>8</sup>

## Microbial Biofilm Formation and Significance

Biofilms are matrix-enclosed bacterial populations adherent to each other and/or to surfaces or interfaces (including automatic watering pipes, recoil hoses, water bottles, or sipper tubes), forming either single-species or mixed-species microcolonies which are phenotypically distinct from their planktonic counterparts, and which provide primitive homeostasis and metabolic cooperativity within the microcolony.<sup>25</sup> Mixed-species biofilms are usually thicker and more stable than monospecies biofilms, a result of commensal interaction.<sup>26</sup> By pooling their biochemical resources, several species of bacteria, each armed with different enzyme systems, can break down food supplies that no single species could digest alone.<sup>27</sup>

Over 99% of all bacteria live in biofilm communities.<sup>27</sup> The formation of stationary, metabolically cooperative biofilms ensures protection of microcolony members from adverse environmental conditions, chemical disinfectants, and antibacterial agents. Biofilm cells have been shown to be 500 times more resistant to antibacterial agents as compared to planktonic forms.<sup>25</sup>

Biofilms containing pathogenic bacteria can form on a variety of devices used in biomedical research and clinical care, including endotracheal tubes used for chronic mechanical ventilation, indwelling catheters, vascular prostheses, cardiac pacemakers, prosthetic heart valves, biliary stents, indwelling urinary catheters, chronic peritoneal dialysis catheters, extended-wear contact lenses, and artificial joints, resulting in serious infections which are unresponsive to antimicrobial therapy.<sup>28</sup> Many of these same devices are used in biomedical research and clinical veterinary medical practices. Medical device manufacturers have spent decades and hundreds of millions of dollars to identify colonization-resistant materials, but have been frustrated by versatile bacteria with adaptive adhesion mechanisms.<sup>29</sup>

*P. aeruginosa* cells, during favorable nutrient conditions, adhere to available surfaces, and through binary fission and exopolymer (glycocalyx) production, begin the establishment of a mature biofilm. The advantages of establishment of a biofilm for *P. aeruginosa* persistence are listed below:<sup>30</sup>

- microenvironment establishment and maintenance
- protection from the environment
- protection from desiccation
- retention and concentration of nutrients
- utilization of nutrients at the surface
- utilization of metabolic products of other bacteria
- retention and binding of cations
- enhanced cell-to-cell communication
- increased plasmid stability and genetic exchange
- concentration of extracellular virulence factors
- increased resistance to antibiotics and other biocides
- decreased susceptibility to killing by host defense mechanisms

Planktonic (free-floating) cells are periodically produced and released from the biofilm to colonize new locations. Some of the cells become entrained in the boundary flow layer, a quiescent zone adjacent to the pipe wall, where flow velocity falls almost to zero. Some cells may temporarily adsorb to, then desorb from, the surface (referred to as reversible adsorption), while other cells become irreversibly adsorbed.<sup>31</sup> These cells take advantage of conditioning films on surfaces which originated from wastes and nutrients released upstream from the "parent" colony.<sup>32</sup> The conditioning film is an organic layer deposited at the solid/water interface. It neutralizes excessive surface charge and surface free energy which could prevent bacterial cell attachment.<sup>33</sup>

In the absence of a conditioning film surface, *P. aeruginosa* cells were shown to adhere to electropolished stainless steel surfaces within 30 seconds of exposure.<sup>34</sup> One study of biofilm development indicated no

significant difference in the number of cells which colonized stainless steel surfaces versus PVC plastic surfaces following 167 days exposure to flowing municipal water.<sup>35</sup>

Bacterial microcolonies, composed of "sister" cells from an original planktonic progenitor, are the basic structural and functional unit of a microbial biofilm. Microcolony proliferation can result in either mound-shaped or mushroom-shaped protrusions from the adherent biofilm base. An anastomosing network of water channels, penetrating all levels of the biofilm, allows convective flow of fluid in the same direction as bulk fluid flow, akin to a primitive circulatory system, supplying nutrients to all areas of the biofilm. During unfavorable nutrient conditions, both sessile and planktonic cells undergo a reversible reduction in size to  $\pm 0.3\mu\text{m}$ , assuming a spherical shape, in a process referred to as starvation survival. When nutrient levels increase, so do vegetative forms of the bacteria.

Biofilm cells exhibit slower growth rates than do planktonic cells, but the lower growth rate and microcolony formation enhances their ability to resist antibacterial compounds. Older biofilms are more resistant to antimicrobial action than are newly formed biofilms, consistent with the ongoing production/evolution of the glycocalyx matrix. Within a mature biofilm, the glycocalyx matrix accounts for 75-95% of its mass, while the bacterial cells comprise only about 5-25% of its mass.<sup>36</sup>

Biofilm detachment (in contrast to release of planktonic cells) can occur as: 1) erosion -- the detachment of single or small clusters of cells; 2) sloughing -- detachment of large areas of the biofilm; 3) abrasion -- solid particle collision with the biofilm; and 4) human intervention -- physical or chemical intervention leading to a decrease in biofilm mass.<sup>30</sup> Other unsuitable environmental conditions which can lead to partial detachment of biofilms include starvation and anaerobiosis, both of which are unlikely in automatic watering systems. While turbulence within the surrounding environment might be expected to detach biofilms from the interior of pipes, in most situations the shear stress is inadequate to sufficiently disrupt an established biofilm.

## Host Susceptibility and Routes of Transmission

Of the common laboratory animals, mice and rats have the greatest susceptibility to infection with *P. aeruginosa*. However, there have been a few reports of naturally occurring *Pseudomonas* in rabbits, guinea pigs, nonhuman primates<sup>37</sup>, and dogs. *Pseudomonas aeruginosa* is widely distributed in conventional stocks of rodents. In one 10-year survey in Japan, *P. aeruginosa* was found in 20-50% of the rodent colonies. In this survey, *P. aeruginosa* was isolated from nasal passages, oropharynx, large intestines, and skin in many healthy rodent colonies.<sup>38</sup>

The normal flora of the nasopharynx and the gastrointestinal tract are effective in controlling the population *in vivo*. However, prevalence determined by culture with specific media, e.g., PIA, can be as high as 100% in non-manipulated mice, as well as in lethally irradiated mice and rats.<sup>3</sup>

*P. aeruginosa* can be transmitted by fomites (such as food, bedding, and water), human carriers, and contact with infected rodents.<sup>8</sup> The likelihood of humans (i.e., researchers or animal care staff) serving as a reservoir for transmission of the bacteria is significant, since *P. aeruginosa* is occasionally recovered and isolated from healthy humans. It has been isolated occasionally from the oral cavities of overtly healthy adults, with the highest isolation rate occurring in the 54 to 63 year-old age group.<sup>39</sup> *P. aeruginosa* was found in the feces of 1.2 to 2.3% of otherwise healthy individuals in several studies.<sup>4</sup> Within the intestinal tract, numbers of the organism may be limited by gastric acidity and substances produced by anaerobic and facultative anaerobic flora (i.e., volatile fatty acids produced by *Bacteroides* spp.).<sup>40</sup> Should normal bacterial flora be eliminated following antibiotic therapy, then *P. aeruginosa* can opportunistically colonize the intestinal tract.<sup>41</sup>

The importance of maintaining normal intestinal microflora has also been demonstrated in laboratory rodents. In the early 1960's, *P. aeruginosa* spread rapidly through one cesarean-derived mouse colony, and was attributed to limited numbers of beneficial intestinal microflora.<sup>42</sup>

Disease occurs when the normal flora is altered or host defenses are impaired.<sup>43,44,45</sup> As in humans, antibiotic treatment can facilitate the colonization of *Pseudomonas* in laboratory animals, presumably by reducing the inhibitory effect of normal microflora.<sup>46</sup>

In carrier animals, the organism normally localizes in the oropharynx, upper respiratory tract, and large intestine.<sup>2</sup> *P. aeruginosa* is capable of living for long periods of time outside the body in moist environments. The organism grows well at room temperature. Contaminated and soiled bedding, when pushed out of cage openings (i.e., the cage top or automatic watering grommet opening) by mice, can fall into cages on lower shelves, leading to contamination of animals in those cages.<sup>46</sup> Twenty-five percent of the cockroaches in one study were found to harbor the organism.<sup>46</sup>

*Pseudomonas* has been shown to contaminate animal facility equipment including faucets, sinks, water bottles, and sipper tubes. *P. aeruginosa* has been isolated from the walls and floors of animal rooms, as well as equipment.<sup>24</sup> In one facility, it was observed that personnel who emptied and cleaned water bottles, and who subsequently filled and assembled clean water bottles, were a prime source for transfer and contamination with the organism.<sup>47</sup> At the same facility, where inadequate ventilation in the washroom led to condensation on the ceiling, *P. aeruginosa* was frequently cultured from the condensate, which would drip on and contaminate caging and equipment.<sup>47</sup> This underscores the necessity for adequate ventilation and exhaust systems in areas with bottle washers, rack washers, tunnel washers, and autoclaves.

Many disinfectants or washing compounds in ordinarily recommended working solutions are sometimes ineffective in killing *Pseudomonas*, which are frequently found in community water supplies despite chlorination.<sup>44</sup> Drinking water is considered important for spreading contamination within animal colonies. Macrocolonies of mucoid-encapsulated *P. aeruginosa* lining the inside of automatic water distribution pipes and rack manifolds can shed single, nonmucoid, flagellated "swarmer cells" which can contaminate inanimate objects, humans, or animals.

Human-to-animal transmission of *P. aeruginosa* has been documented, but conversely, animal-to-human transmission has not been documented.<sup>48</sup> Ungloved hands or other exposed skin are considered to be one likely source of the organism in animal facilities. Two outbreaks of *Pseudomonas* infection within a mouse colony were linked to infected animal caretakers who served as reservoirs.<sup>49</sup> Those outbreaks were curtailed by transferring the infected personnel and by implementing strict sanitation and hygienic procedures. Aerosols generated in *P. aeruginosa*-contaminated sinks have been shown to colonize the hands of personnel who wash without using soap or disinfectant solutions.<sup>50</sup>

Important predisposing factors for *Pseudomonas* disease in animals include any procedure that will compromise the normal immune response, including irradiation and treatment with steroids or other immunosuppressants. Phagocytic cells, primarily neutrophils, are the first line of defense of the host against the organism, with humoral immunity providing the second line of defense.<sup>51</sup> A number of factors may predispose animals to disease caused by *P. aeruginosa*. B-lymphocyte-deficient mice are more susceptible to *P. aeruginosa* infection than B-lymphocyte-immunocompetent mice and have been used as a model for studying the protective efficacy of monoclonal antibodies against the organism.<sup>52</sup> Mice are generally resistant to challenge with a wide variety of gram-negative bacteria such that lethal challenge doses may be required. This is due mostly to an efficient phagocytic system and to the presence of naturally occurring antibodies against O-serotypic determinants on lipopolysaccharides.<sup>52</sup>

One study done on whether neutrophil-induced lung protection and injury are dependent on the amount of *Pseudomonas aeruginosa* administered via airways in guinea pigs concluded that neutrophils protect against lung injury during low-level bacterial challenge, but enhance lung injury and contribute to mortality during high-level bacterial challenge.<sup>53</sup>

Another study conducted on whether *Pseudomonas* siderophore pyochelin enhances neutrophil-mediated endothelial cell injury, found results consistent with the possibility that the interaction of *Pseudomonas*- and phagocyte-derived secretory products could contribute to local tissue injury at sites of *P. aeruginosa* infection by causing the generation of OH.<sup>54</sup>

Models which increase the susceptibility of animals to the disease have included whole body irradiation<sup>45,46,55</sup>, administration of cyclophosphamide<sup>56,57</sup>, local burns<sup>7,58</sup>, administration of ferric ions<sup>59</sup>, administration of streptozotocin<sup>60</sup>, and co-infection with murine cytomegalovirus<sup>61</sup>. The presence of *P. aeruginosa* in the oral cavity or gastrointestinal tract of lethally or sublethally irradiated mice leads to earlier than expected death in those animals.<sup>62</sup> Lactobacilli, which constituted the largest population of intestinal microflora in the rats of one study, fell to 25% of their normal levels following whole-body irradiation, and pseudomonads increased 1,000 fold within 7 days post-irradiation.<sup>43</sup> In immunosuppressed animals, the organism invades from the normal sites of localization into deeper tissues, resulting in bacteremia and high mortality.<sup>43,45,63</sup> Surgical procedures such as the implantation of indwelling jugular catheters may result in acute to chronic *Pseudomonas* infection.<sup>64</sup>

## Clinical Signs

Under usual circumstances, the organism can be part of the normal flora in the digestive tract and clinical disease and hence clinical signs are not present. After immunosuppression, fulminating septicemia can occur, with few clinical signs of infection prior to death.<sup>46</sup> Clinical signs in mice may include dyspnea, depression, facial edema, circling or tilting of the head (torticollis), weight loss in animals surviving for more than 24 hours, conjunctivitis, and nasal discharge. Clinical signs in immunosuppressed rats include facial edema (which must

be differentiated from sialodacryoadenitis virus infection), conjunctivitis, nasal discharge, and retro-orbital abscessation in nude rats.

There have been reported cases of clinical inner ear disease in mice affecting the vestibular apparatus, attributed to natural *P. aeruginosa* infection.<sup>65,66,67</sup> These were characterized clinically by either circling<sup>65,67</sup> or rolling<sup>66</sup>. Paroxysmal rolling of mice on their long axis can also occur with *Mycoplasma neurolyticum* infection.<sup>66</sup> Similar signs of spinning/circling had also been experimentally produced by intravenous inoculation of mice with *P. aeruginosa*.<sup>68,69</sup>, with intravenous *Mycobacterium* injection<sup>70,71</sup>, and with intravenous inoculation with *Nocardia seaborans*<sup>68</sup>.

## Gross and Histopathological Lesions

*Pseudomonas* spp. may be associated with fur and skin lesions and may be characterized by a bluish-green discoloration, especially in rabbits. At necropsy, in acute cases, there may be pulmonary edema, splenomegaly, and visceral ecchymoses consistent with a systemic infection gram-negative bacterial septicemia. In rats that succumb during the subacute to chronic stages of the disease, multifocal necrosis with abscessation may be present in organs such as lung, spleen and kidney. In animals with indwelling jugular catheters, vegetative lesions may be present on the tricuspid valves. On microscopic examination, lesions in acute cases are those of an acute bacterial septicemia, with vasculitis, thrombosis, hemorrhage, and neutrophil infiltration. In affected foci, changes vary from acute coagulation necrosis to suppuration, with obliteration of the normal architecture. Lesions are usually most extensive in the lung.<sup>2</sup>

In addition to hemorrhagic and thromboembolic changes, bacterial colonies and proteinaceous fluid are frequently present in alveoli. Vegetative endocarditis with septic embolization may be present, particularly in animals with indwelling venous catheters.<sup>2</sup>

In the reported cases of inner ear disease in mice attributed to *Pseudomonas aeruginosa* infection, *P. aeruginosa* was consistently isolated from the middle and inner ears and associated lesions.<sup>66,67</sup> Affected animals had suppurative otitis media, interna and localized meningencephalitis. Inner ear lesions in the cochlea and the vestibular apparatus ranged from an acute neutrophilic infiltration to a more commonly observed chronic proliferative inflammation with partial replacement of the inner ear with fibrous connective tissue invested with plasma cells.<sup>67</sup> Dissolution of bone surrounding the inner ear permits spread of the infection to the cerebellum and cerebrum, producing focal abscesses.<sup>24,67</sup>

A similar disease has been produced experimentally by inoculating *P. aeruginosa* intravenously into mice.<sup>65,67,68</sup> It appears that circling in mice is produced by *P. aeruginosa* only when conditions are optimal for the invasion and growth of the organism, although the exact conditions required are not completely understood.<sup>65</sup> The lesions produced by *P. aeruginosa* in the ears of mice are very similar to mycoplasma infection, which occurs with greater frequency.<sup>24</sup> *Mycoplasma* spp. were never recovered from reported spontaneous or induced cases of circling in mice.<sup>65,67</sup>

In humans, *P. aeruginosa* is the most commonly isolated bacterial pathogen in adults and children with a clinical history of chronic, suppurative otitis media, and a major cause of otitis media in neonates.<sup>72</sup> This is in contrast to studies which demonstrated that *P. aeruginosa* is not a normal inhabitant of the external auditory canal, having been recovered from that site in only 1% of 1,377 healthy volunteers.<sup>73</sup> Otitis externa caused by *P. aeruginosa* was seen with some frequency in diabetic humans over the age of 55.<sup>74</sup>

## Diagnostic Procedures: Culture and Identification

Cecal contents, feces, oral cavity and drinking water are suitable specimens for isolation of *Pseudomonas aeruginosa* from animals. Importantly it should be noted that *P. aeruginosa* can be cultured from the intestine of healthy animals.<sup>9</sup> Furthermore, *P. aeruginosa* can be isolated from the blood, lungs, liver, spleen, kidneys, fur, and inner and middle ear of septicemic animals. *Pseudomonas aeruginosa* can also be isolated from clinical specimens (wound, burn and urinary tract infections).<sup>17</sup>

*Pseudomonas* disease should be included on the differential diagnosis following death with lesions of septicemia of immunocompromised animals. However, a history of procedures such as immunosuppression or certain surgical manipulations, coupled with typical gross and microscopic changes is not conclusive. Often, the disease produced by *P. aeruginosa* in a susceptible or stressed host is a septicemia, and the lesions are not specific. Thus, diagnosis should be based on isolation and identification of *Pseudomonas*. Other causes of septicemias in mice include salmonellae, although this is increasingly rare in recent decades. The septicemic

disease produced in mice by *Erysipelothrix insidiosus* is also characterized by focal necrotic areas in the liver, although this has not been reported in the last 20 years.<sup>24</sup>

Gram-negative bacilli are usually identifiable in sections stained with tissue Gram stains such as Brown and Brenn.<sup>2</sup> Use of serologic methods for identification is impractical and is not recommended.<sup>9</sup> Differential diagnoses for septicemic lesions include visceral abscessation due to *Corynebacterium kutscheri* or *Pasteurella pneumotropica* infections, salmonellosis, pulmonary abscessation due to murine respiratory mycoplasmosis and Tyzzer's disease.<sup>2</sup>

Incubation of blood agar plates at 37°C and selective media such as Koser's citrate medium are recommended to isolate the organism.<sup>17</sup> Most strains are  $\beta$ -hemolytic and produce a bluish-green pigment, pyocyanin, as well as fluorescein. The use of specialized media (*Pseudomonas* P agar or *Pseudomonas* isolator agar) enhances pigment production and increases frequency of isolation. Use of such media is strongly recommended when screening healthy animals for the presence of *Pseudomonas*.<sup>48</sup> Bacteria grown on solid media have a characteristically musty, slightly aromatic odor.

Two main colony types can be observed on common solid media. One is large and smooth, with flat edges and elevated centers ("fried egg" appearance), and the other is small, rough, and convex. Clinical materials are, in general, good sources of the large colony type, while the small is commonly obtained from natural sources. Variation of the large type to the small is easy to observe, but the reverse variation is extremely rare. A third colony type (mucous) often can be obtained from respiratory and urinary tract secretions, and was first observed by Sonnenshein.<sup>75</sup> Mucoid mutants of *P. aeruginosa* can be divided into two groups according to whether the mucus (alginate) is produced in chemically defined media.<sup>76</sup> Aside from pyoverdinin and pyocyanin, other pigments may be produced by some strains, including a dark red pigment.<sup>17</sup>

The organism derives energy from carbohydrates via oxidation rather than fermentative metabolism.<sup>48</sup> In some cases, nitrate can be used as an alternate electron acceptor allowing growth to occur anaerobically.

Advances in molecular biology have led to development of nucleic acid probes for *in situ* detection of pseudomonads.<sup>77</sup>

## Interference with Research

Exhaustive control procedures for *P. aeruginosa* are not necessary for most studies using mice and rats. Interference with research is unlikely, especially in the absence of clinical signs of illness. Animals are likely to exhibit clinical signs only if they are profoundly immunosuppressed, in which case the success of studies in which these animals are used may be affected. Animals showing clinical signs should not be used for research, whether the signs are consistent with *Pseudomonas* disease or any other condition.

Experimental procedures which induce lower host resistance or impair immunocompetence may also provoke latent infection (i.e., treatment with corticosteroids, cyclosporine, burn research, or whole-body irradiation). Administration of  $10^7$  *P. aeruginosa* cells by gavage to irradiated and nonirradiated mice caused illness and death in 60% of the irradiated mice within 4 days after inoculation, while no observable clinical signs of illness were seen in the nonirradiated mice during the 30 days that they were observed.<sup>78</sup>

Mice with streptozotocin-induced diabetes mellitus have been reported to have increased susceptibility to *P. aeruginosa* infection.<sup>79</sup> Mice previously infected with murine cytomegalovirus may also be more susceptible to experimental *P. aeruginosa* disease.<sup>80</sup> Mice given *P. aeruginosa* intraperitoneally have depressed contact sensitivity to oxazolone<sup>81,82,83</sup>, and mice given *P. aeruginosa* by gavage have reduced survival when exposed to cold stress of -20°C for 2.5 hours<sup>84</sup>. Indwelling jugular catheters in rats may become infected with *P. aeruginosa* and be causally associated with septic pulmonary emboli<sup>64</sup>.

## Prevention and Control

It may not be practical or possible to exclude *Pseudomonas* from many animal facilities. Implementation of rigorous procedures and technologies necessary to exclude *Pseudomonas* is unlikely to produce any benefit to animal health or research studies for immunocompetent animals.

Exclusion requires obtaining animals free of *Pseudomonas*, maintaining them free of it, and monitoring with selective media to confirm their *Pseudomonas*-free status. It also probably requires excluding humans from the animals' microbiological environment, such as with the use of isolators or microisolator caging. Control and

prevention of *Pseudomoniasis* requires a thorough investigation of and correction of management and sanitation practices as required. *P. aeruginosa* has been shown to be highly resistant to disinfectants such as quaternary ammonium compounds and chlorinated phenols. Furthermore, contaminated disinfectant solutions have been the source of nosocomial infections in the past.<sup>4,85</sup> Listed below are several control, treatment, and prevention measures available for use in research animal facilities.

### Water Testing

The necessity to perform bacterial assays of animal drinking water will depend on the nature of research in a particular animal facility, and the perceived risk of *P. aeruginosa* to the animals. Routine scheduled water testing is required for facilities conducting studies governed by the Good Laboratory Practices Act (GLP's). However, Subpart E, Section 58.90 (g) Animal Care of the GLP's merely states that "feed and water used for the animals shall be analyzed periodically to ensure that contaminants known to be capable of interfering with the study and reasonably expected to be present in such feed and water are not present at levels above those specified in the protocol". Thus, neither the exclusion of *P. aeruginosa*, nor specific maximum acceptable levels of animal drinking water, are stated in the GLP's, and are left to the determination/discretion of research and veterinary personnel in individual facilities.

When implementing a water assessment/treatment program, an initial assessment of actual bioburden should be made. The urgency of response and the intensity and frequency of treatment regimes will depend on the results of that preliminary assessment. When designing and implementing water testing programs, the source and method(s) of sampling should be evaluated to aid in interpreting the significance of results and in determining treatment methodologies. The following baseline determinations are of value<sup>86</sup>:

- assessing the "contamination" of the public/private water source using total counts;
- assessing whether filters lower the total number of organisms [comparing pre- and post-filter counts], or whether the filters are collecting organisms which are subsequently released to the water supplying the animals;
- assessing the contamination level of water bottles following animal use/contact for a specified period;
- assessing the contamination level of water from recoil hoses [comparing results to pre- and post-filter counts]; and,
- assessing the contamination level of water at lixit valves [comparing results to pre- and post-filter counts].

Common sampling points for automatic watering systems include the quick disconnect connections in the room distribution piping, recoil hoses attached to the quick disconnects, and flush valve outlets at the end of the room distribution piping. To assess the system at its "weakest link", samples should be taken from points farthest from the water source, especially in areas where stagnation may occur. Greater than 99% of bacteria in water systems are in biofilms attached to pipe surfaces, thus when sampling water, one samples only the "strays" and not the main "herd" of the bacteria in the system.<sup>87</sup> Thus it should be recognized that standard plate count techniques will generally underestimate the total number of bacteria within an automatic watering distribution system. Water samples may contain slimy aggregates of thousands of cells which have been detached from a mature biofilm. Each aggregate may produce only a single colony forming unit during plate culture, further contributing to the underestimate of the contamination problem. By dispersing aggregates prior to dilution and plating through mechanical shaking or ultrasonication, a more accurate determination of colony forming units [CFU's] can be made.<sup>88</sup> The number of free-floating bacteria in the fluid stream can fluctuate widely, especially if portions of the biofilm are suddenly dislodged/shed, resulting in random bacterial and particle showers which may not be detected at regularly scheduled testing intervals.<sup>89</sup>

### Water Treatment

In animal facilities, contaminated untreated drinking water is the most common source of *P. aeruginosa*, and has been shown to be a source of cross-infection in animals. Decontamination of water prior to presentation to animals can be accomplished by a number of techniques including reverse osmosis, deionization, microfiltration, reverse osmosis-deionization-ultrafiltration, autoclaving, hyperchlorination, and acidification.<sup>90</sup> Additional methods include use of UV light, iodination of water, and ozonation of water. The reverse osmosis process removes minerals, bacteria, endotoxins/pyrogens, and viruses. Deionization, while removing inorganic impurities, may still allow bacterial growth, and should be coupled with another procedure (i.e., ultraviolet light exposure, hyperchlorination, or acidification) to ensure adequate disinfection. Microfiltration can remove particles of  $\geq 0.02\mu\text{m}$ , but bacterial growth and plugging of the membrane can occur when large volume systems are used.

A combined reverse osmosis-deionization-ultrafiltration system, while effective in removing ionic and organic

materials, is not an efficient method for water treatment in large facilities and may not provide sterile water, as bacteria can grow across filter. By using reverse osmosis water, with its nutrient-poor content, less bacterial growth will occur within automatic watering system pipes, resulting in a thinner biofilm for easier penetration of biocidal agents.

Biofilm associated bacteria have been found to be 150-3,000 times more resistant to free chlorine and 2-100 times more resistant to monochloramine than free-floating bacteria.<sup>91</sup> Researchers at the Centers for Disease Control and Prevention (CDC) filled sections of PVC pipes with water containing 2 strains of *Pseudomonas* and incubated the pipes for 8 weeks. They then drained them and filled them with disinfectant solutions, including chlorine, for 7 days. Upon draining the pipes again then filling them with sterile water, viable organisms were subsequently recovered and biofilm formation was discovered in the pipes.<sup>92</sup>

Chlorination (10-13 ppm) or acidification (pH 2.5-3.0) of drinking water can greatly reduce the colonization of the nasopharynx and intestines of mice, but will not eliminate an established infection.<sup>8,9</sup> Another study found that chlorinated drinking water, containing 6-8ppm of available chlorine, cleared some animals of existing infection, although a higher level (10ppm) did not.<sup>93</sup> Chlorination of the drinking water can be accomplished prior to filling of water bottles, or using proportioners which add chlorine to automatic watering systems. The bactericidal efficacy of free and combined available chlorine in water is affected by: 1) the length of exposure/contact, with longer exposure times producing greater effect; 2) the temperature of the water at the point of contact/interaction, with lower temperatures resulting in less disinfectant activity; and, 3) the pH of the water, with less effective chlorination in more acidic water.<sup>94</sup>

McPherson observed a drop in available chlorine in drinking water in water bottles from the initial 12 ppm concentration to 6 ppm after 24 hours, to 2.5 ppm after 48 hours, and finally to 0.25ppm after 72 hours. *P. aeruginosa* and other coliform bacteria can be cultured from water bottles where the available chlorine concentration was  $\leq 1$ ppm.<sup>95,96</sup> It has been demonstrated that hyperchlorination of drinking water to 25-30ppm of available chlorine can depress macrophage function in mice (i.e., peritoneal exudate macrophages), decreasing host resistance against transplantable tumors.<sup>97</sup>

The use of HCl-treated water does not appear to adversely affect reproductive rates in mice. Despite concerns that acidified water might adversely affect water consumption, one early study observed that mice had no demonstrated preference for either tap water or HCl-treated water at a pH of 2.5.<sup>95</sup> Acidification of drinking water resulted in decreased weight gain in normal and irradiated mice, and the mean number of bacterial species isolated from the terminal ileum of acid-treated groups was less than that found in control animals. Thus, use of acidified drinking water should be considered as an environmental variable during study design.<sup>117</sup>

A suppression of latent infections of *P. aeruginosa* in one mouse colony was accomplished by a combination of acidification (to pH2.5) and chlorination (to 10 ppm) of water provided to the animals.<sup>44</sup> However, Orcutt warned that a combination of acidification and hyperchlorination of water would be ill-advised, as the low pH (below pH4) would facilitate the release of chlorine gas from the water.<sup>98</sup>

#### Watering Equipment and Facility Decontamination

To effectively exclude *Pseudomonas*, gnotobiotic conditions are recommended, supported by autoclaving of cages, water bottles, and sipper tubes and prevention of human contact. Most *P. aeruginosa* strains can be killed by exposure to 55°C for 1 hour, 80°C for 5 minutes, or 100°C for 1 minute. *P. aeruginosa* is inactivated by UV irradiation. The organisms are sensitive to acid and to silver salts. Disinfectant solutions with a pH of 3 or lower have been shown to have a bactericidal effect on *P. aeruginosa* within 60 seconds.<sup>99</sup> Solutions at pH 12 are also effective in killing the organism.<sup>100</sup> Many kinds of disinfectants, including 70% ethanol, 50% isopropanol and 3% saponated cresol are effective.<sup>9</sup>

To achieve sanitization temperatures of 82.2 C or higher, as recommended by The Guide for the Care and Use of Lab Animals, several critical factors are necessary in processing rack manifolds in auto rack washers.

Before introducing the rack manifolds into the rack washer, all residual water must be removed. Residual water within the pipes will adversely affect the auto washers' ability to achieve sanitization temperatures. The material in which the rack manifolds are constructed will also affect proper sanitization. In one study, interior and exterior temperature of stainless steel manifolds were consistently found to exceed 82.2 C during rack washer cycles, while in 3 of 6 trials using CPVC pipe, interior temperatures failed to reach 82.2 C (11). It is recommended that both types of manifolds be secondarily/routinely treated with pressurized flushing and

chemical agents which are compatible with their chemical make up.

Lastly, the pre-treatment of the rack manifolds with physical or chemical agents prior to introduction into the rack washer should also be considered. This process will reduce the bioburden (biofilm) within the rack manifold prior to sanitization.

In addition to processing water bottles through mechanical washers (using thermal and chemical methods to destroy bacteria), post-sanitization autoclaving of bottles and use of acidified water (pH 2.5) have been shown to be effective in preventing growth of *P. aeruginosa*.<sup>116</sup> Water equipment sanitization procedures in place at the National Center for Toxicological Research included autoclaving caging and sipper tubes at 250°F for 30 minutes, and processing water bottles in a mechanical bottle washer which included a final rinse at >180°F.<sup>101</sup> Those procedures, coupled with autoclaving drinking water for 30 minutes at 200°F and subsequent filtration through 0.45µ millipore filters, resulted in only 35 monitoring samples exceeding established standards (i.e., 0 CFU/swab) out of 14,849 total samples taken from water, sipper tubes, and water bottles following sanitization during 10 years of sampling. Handling of sipper tubes (i.e., reinserting them into stoppers) following sanitization could lead to their recontamination if personal hygiene practices are determined to be inadequate, or if the area in which assembly takes place is contaminated.<sup>102</sup>

Sanitization by acidification of room automatic watering system pipes, recoil hoses, or rack water manifolds, while destroying viable bacteria, does not remove established biofilms within those structures, resulting in diminished flow rates or an ideal surface for later colonization.<sup>103</sup>

### System Flushing

High water flow rates, while altering biofilm structure and growth (i.e., leading to a denser, more tenacious biofilm and limited biofilm thickness), do not prevent attachment of planktonic forms to pipe surfaces<sup>103</sup> by virtue of: 1) lower flow velocity at the laminar sublayer near the wall of the pipe as compared to its center, with insufficient shear forces necessary to dislodge bacterial cells<sup>104</sup>; and, 2) the ability of *Pseudomonas* to anchor itself to the surface with its glycocalyx.

In an automatic watering system that is regularly flushed, the biofilm thickness would be expected to be less than 200µ, with sloughing of those portions of the biofilm which extend beyond the laminar sublayer into zones of more turbulent flow, resulting in particle showers of bacteria.<sup>105</sup> At a flush velocity of 2 ft/sec (~1.25 gpm), provided by commercially available room distribution system flush units (Edstrom Industries, Waterford, WI), the laminar sublayer thickness within a 1/2" pipe, where turbulence is minimal or nonexistent, has been determined to be ~125µ.<sup>104</sup> By increasing the flush flow velocity to 5 ft/sec (~2.3 gpm), the biofilm thickness could be limited to ~50µ.

Flushing can be coupled with physical and chemical treatments to enhance reduction or destruction of biofilms. One physical method, mechanical scrubbing, is not practical or possible within water distribution system piping, but may be required in water storage tanks. A second physical method, use of heat (i.e., hot water >80°C) on a scheduled basis, will destroy planktonic forms and reduce, but not eliminate, biofilm mass.<sup>106</sup> Periodic treatment of systems at 95°C for 100 minutes has been shown to destroy bacteria within the biofilm,<sup>107</sup> but would be impractical for maintenance of an animal facility automatic watering system.

Chemical treatments include the use of nonoxidizing and oxidizing biocides. Nonoxidizing biocides include quaternary ammonium compounds, formaldehyde, and anionic and nonionic surface-active agents. The first two are impractical or pose health and safety risks to animals and personnel within the animal facility, and the latter would need to be coupled with other biocides to enhance biofilm removal. Oxidizing biocides, in order of effectiveness on an equal mg/L dosage basis, include: ozone, chlorine dioxide, chlorine, iodine, and hydrogen peroxide.

Chlorine, the least expensive and most commonly used biocidal agent in water systems, can be more effectively used by increasing the chlorine concentration at the water-biofilm interface. At low chlorine concentrations, biofilm bacteria produce their slime matrix material faster than chlorine can diffuse through it. Exposure to high chlorine concentrations for a short period of time is more effective in penetrating deeper into the biofilm with greater resultant action.<sup>108</sup>

Flushing, combined with high chlorine concentrations, can result in greater uptake of chlorine and greater detachment of the biofilm by: 1) disrupting the biofilm through shear forces, exposing new surfaces for chlorine action; 2) decreasing the thickness of the laminar sublayer and increasing chlorine exposure; and, 3) increasing the transfer of chlorine from the bulk water to the biofilm.<sup>108</sup> Higher pH favors hypochlorite ion-promoted detachment of mature biofilms, and shock chlorination at pH 8, while not rigorously tested, might be of some benefit in reducing biofilms within water lines.<sup>108</sup>

The effectiveness of flushing automatic watering rack manifolds to diminish or eliminate contamination depends upon the configuration and hydrodynamics of the manifolds -- horizontal (similar to a ladder, with two vertical pipes connected by horizontal piping at each shelf level) or serpentine. Even with daily flushing of the horizontal manifold system (in the absence of acidification or hyperchlorination), contamination was still verified.<sup>109</sup> Using chemical tracers, it was determined that waterflow was unequal in horizontal manifold systems, and areas of stagnation were evident even after five minutes of flushing.<sup>110</sup> These areas of minimal flow would be conducive to bacterial proliferation and biofilm development. An upfeed serpentine manifold was found to be more effectively flushed than was the horizontal manifold system.<sup>110</sup>

Similar areas of stagnation can occur in facility automatic watering lines, especially in unoccupied rooms or at the terminus of the lines. In an unpublished study performed in an animal research facility at a major pharmaceutical company, initial analyses of water from its automatic watering system revealed high bacteria levels ( $\approx 15,000$  CFU/ml), attributed to stagnation of water flow in the lines.<sup>105</sup> Once weekly flushing was performed for either five or fifteen minutes at 3 PSI, with the fifteen minute flush (but not the five minute flush) yielding post-flush counts below 500 CFU/ml; however, bacterial counts again exceeded 500 CFU/ml within 24 hours after flushing. Following installation of an automatic system flushing device, which operated at 12 PSI for three minutes per day, and daily flushing of the lines, standard plate count levels were virtually the same prior to and immediately after flushing, averaging about 35 CFU/ml in the four rooms which were tested.

In a survey of nine research animal facilities which use automatic watering systems, Edstrom Industries found that the facility with the highest water quality standards (plate counts  $<1$  CFU/ml) utilized the following equipment and procedures to maintain those standards: 1) use of stainless steel corridor header and room piping; 2) use of reverse osmosis water containing 1-1.5ppm chlorine; 3) room piping and rack manifolds flushed daily (rack manifolds flushed at 15 PSI for 1 minutes once daily); and, 4) rack manifolds and recoil hoses sanitized every two weeks with exposure to 5 ppm chlorine for 30 minutes.<sup>111</sup> The frequency of flushing of room piping varied from 2-10 times per day at the other facilities surveyed, and concentration of chlorine used for sanitization of manifolds and recoil hoses ranged from 10-20ppm. One facility flushed its lines with 149°F water for three minutes once a month, with plate counts  $<1$  CFU/ml.

### Personal Hygiene and Facility Practices

One study of hospital personnel indicated that their hands were virtually free of *P. aeruginosa* before entering the facility at the start of the work day, but that 46% of the same individuals yielded positive hand cultures during the course of the day. This was likely associated with contamination from sinks and inadequate hygiene practices.<sup>4</sup> In another study, *P. aeruginosa* was found and cultured on agar plates placed 10 feet from a known-contaminated sink, following activation of the faucet for three ten-second intervals and generation of aerosols.<sup>112</sup>

Thus, in animal facilities where *P. aeruginosa* has been identified, it might be advisable to require daily initial decontamination of sinks before use, and to ensure that personnel use appropriate soap and/or disinfectants when washing their hands. Additionally, in conventional facilities where no separation of "clean" and "dirty" cagewash areas exists, personnel should take great care in avoiding cross-contamination of water bottles, stoppers, and sipper tubes. Hands should also be thoroughly washed/disinfected following handling of "dirty" watering equipment to prevent possible contamination of clean equipment.

By providing physical and differential air pressure barriers between the clean and dirty cagewash areas, direct and aerosol transmission of the organism can be deterred. Adequate ventilation (exhaust) in cagewash areas will help to reduce or eliminate condensate accumulation on ceilings and walls, reducing favorable environments for *Pseudomonas* growth. Handling mice with tongs or forceps, chemically disinfected between each animal, helps to reduce contamination of animals.<sup>116</sup>

### Animal Treatments / Procedures

More emphasis should be placed on prevention rather than on medical treatment of *Pseudomonas aeruginosa* infection in experimental animals in those situations where *Pseudomonas* might prove deleterious. Detection of carriers/shedders by culturing sipper tubes, improved sanitation, and chlorination or acidification of the drinking water are recommended procedures. Incoming animals should be quarantined and cultured using special media.

Rederivation by cesarean or embryo transfer can effectively eliminate *P. aeruginosa* infection.<sup>116</sup> Since *P. aeruginosa* can opportunistically colonize the intestinal tract in the absence of favorable bacterial flora, axenic mice, with their absence of indigenous intestinal flora, are at significant risk for infection. When administering a Schaedler's cocktail for establishment of intestinal flora in gnotobiotic mice, the inclusion of *Bacteroides* spp., clostridia, and lactobacilli have been shown to provide relative colonization resistance against *P. aeruginosa*.<sup>113</sup>

The organism is not susceptible to many of the conventionally used antibiotics, and may develop several mechanisms of resistance against a variety of antibiotics. A few antibiotics are effective in treating *P. aeruginosa* infections, including  $\beta$ -lactams (carbenicillin, ticarcillin, imipenem, cefotaxime, and ceftriaxone), aminoglycosides (gentamicin, amikacin, and tobramycin), and quinolones (ciprofloxacin).<sup>114</sup> Mass antibiotic treatment is ineffective in controlling *P. aeruginosa* in animal breeding colonies. However, oral treatment with 1.0 g gentamicin per liter of drinking water has been shown to be effective in eliminating the infection in mice.<sup>9</sup>

Several experimental vaccines against *P. aeruginosa* were developed for use in rats and mice, providing some protection for those species<sup>115</sup>, but appear to have limited commercial and clinical value in the majority of research facilities.<sup>8</sup>

## Summary

*Pseudomonas aeruginosa* is a tenacious foe in the war against microbiological contamination in animal facilities. Fortunately, in most instances it is a paper tiger, posing minimal or no threat to animals or research integrity when immunocompetent animals are used. When immunosuppressed/immunocompromised animals are used, common sense procedures can be implemented (i.e., autoclaving food, water, bedding, water bottles and sipper tubes; housing in microisolator caging or isolators; changing cages in a laminar air flow hood; using aseptic handling procedures; practicing good personal hygiene) to prevent contamination with or transmission of *P. aeruginosa*.

As the prototypical biofilm bacteria, once it becomes established in supportive environments (i.e., room and rack automatic water manifolds, sinks and faucets, and other water lines) it is difficult, if not impossible, to completely eradicate all of the organisms or all of the biofilm. The use of routine flushing of lines (at higher flow rates) to reduce the thickness of the biofilm and its protrusion into the standard water flow current, coupled with periodic or continuous treatment of water through hyperchlorination or acidification, will help reduce the potential for "embolic showers" of biofilm colonies or planktonic individuals.

Periodic sampling at multiple sites of the automatic water distribution system will help to assess the efficacy of treatment/prevention methods, and allow adjustments to be made to either increase the stringency of treatment and monitoring, or to reduce the frequency of monitoring and thus reduce labor and testing costs.

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