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BIOMI 290

Case Studies 1 Biol

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Part I: The Disease ✖ ✓

Legionellosis, also known as Legionnaires' disease, is caused by the bacterium of the *Legionella* species growing intracellularly within protozoa in biofilms. *L. pneumophila* is a specific species found naturally in aquatic environments. The pneumonia associated with Legionellosis is caused by the invasion of alveolar macrophages in the lung tissue of mammals by the *Legionella* bacterium. The protozoa inhaled serve as cofactors for pulmonary disease induced by inhalation of respiratory pathogens, causing pneumonia in the affected. Transmission occurs via aerosols from environmental conditions, such as those found in hot tubs, water coolers, spas, vegetable misters, and showerheads. *L. pneumophila*, often associated with pneumonic symptoms in Legionellosis, is commonly found in potable water plumbing systems, such as that on a cruise ship. The protozoa that harbor *L. pneumophila* have been found to be tolerant of temperatures above 50°C (122°F), which is why it was found in the hot tub pipes. In addition, *L. pneumophila* populations were shown to be most abundant in plastic water plumbing systems at 40°C (104°F). In one study, amoebas were also found to be hosting *L. pneumophila* in 42% of whirlpools and hot tubs. The passengers got Legionellosis from inhaling the steam coming from the hot tub water which contained the *L. pneumophila* pathogen picked up from the pipes.

Part II: The Environment ✖ ✓

Biofilms are, simply put, surfaces covered with a layer of bacteria. In reality, they are much more complex. The surfaces most commonly occur in aqueous environments, and there can be more than just bacteria living on them. Biofilms can consist of fungi, algae, protozoa, corrosion products, and other debris. They form when a planktonic bacterial cell anchors itself to the surface, sometimes using fimbriae to aid in anchoring itself. Other planktonic cells receive chemical signals in a system called "quorum" sensing, where special genes are activated and the cells all begin to secrete a polysaccharide-rich substance. This will eventually form a glycocalyx, a protective, slimy layer that helps the cells stay anchored, communicate, share nutrients, and protect themselves from the environment. They can excrete other metabolic byproducts that can be supportive to other bacteria but be harmful to humans and even the surface of the biofilm itself. In the case of Legionellosis, biofilms are important because the pathogen, a *Legionella* bacterium, grows on the hot tub pipe surfaces on the cruise ship. This is the passengers' source of exposure to the *L. pneumophila* on the cruise ship and what caused them to become ill. BIOFILMS ARE DIFFICULT TO REMOVE

Part III: Experimental Results ✖ 3

The experiment used included a model of a water system like that on the ship. There was a system of 24 removable square PVC pieces on one side; each piece could be removed for sampling. These were the surfaces on which the biofilms would grow. The flow rate of the water, its temperature, and its contents through the

PVC piping could be controlled with this setup. The water came from the cruise ship *Endemic* and was pumped through the pipe for 2 weeks. The samples all contained a small sub-sample of the *Legionella* biofilm. Different treatments were applied to the water affecting flow rate and additives. These were added on day 0. Samples of the biofilms were taken 10 and 25 days after the treatments began. Analysis of the biofilms was done using a *Legionella*- specific tagged fluorescence antibody assay, viewable under fluorescence microscopy. This was done to view the cells in their natural habitats and to assure specificity of the organism of interest. In addition, biofilms samples were viewed using differential interference contrast microscopy. This allowed live samples to be viewed with a more 3-dimensional aspect.

The treatments included high pulse flow (2 m/s as opposed to regular at 0.2 m/s), free chlorine (0.5mg/mL), monochloramine (0.5mg/mL), erythromycin antibiotic (5mg/mL), or some combination thereof. In all, there were 7 different treatments used. Temperature was held constant at 38°C (101°F) for all treatments.

The first of just high pulse flow, applied on day 9 for 2 hours, showed a significant reduction the day after treatment on day 10. Day 0 showed about 30 microns diameter of growth, while day 10 showed only 10 microns. However, after discontinuation of the treatment, day 25 showed significant growth filling the microscopy field.

Treatment of just free chlorine showed no reduction in growth. Day 0 showed 25 microns diameter of growth; day 10 showed 50 microns, and day 25 showed significant growth filling the microscopy field.

A treatment of free chlorine and high pulse flow for 2 hours on day 9 reduced growth from 25 microns on day 0 to almost no growth (less than 5 microns) on day 10. However, day 25 showed enough growth to almost fill the microscopy field.

Treatment with monochloramine reduced growth from 25 microns on day 0 to a thin, almost absent growth on day 10. Day 25 showed select growth spots of 10 and 20 microns across.

Monochloramine combined with high pulse flow on day 9 for 2 hours seemed successful. Day 0 showed 25 microns of growth, while there were only a few spots on day 10 following high pulse flow treatment. On day 25, there was very thin growth of barely 10 microns across present.

Treatment with antibiotics (erythromycin) did not affect growth. Biofilm growth was more than 50 microns across on day 10, double from 25 microns on day 0. By day 25, there was enough growth to fill completely the microscopy field.

Antibiotics combined with high pulse flow reduced growth from 25 microns on day 0 to a few small spots up to 10 microns in diameter on day 10. However, by day 25, growth was significant enough to fill the entire microscopy field.

Part IV: My Conclusion ✱ \

Based on the treatments, I recommend combining 0.5 mg/mL monochloramine with high pulse flow of 2m/s for 2 hours every 9 or 10 days. This is better than the other methods used because of the combination of physical and chemical attack. The monochloramine is a more reactive species of chlorine and therefore can better attack the tough surface of the biofilm. The high pulse flow physically disrupts the integrity of the glycocalyx and erodes some of the bacterial growth, exposing the more susceptible inner

layers. Prolonged exposure to the monochloramine helps keep bacterial growth to a minimum and eliminates the rest of the colony that was originally present. With repeated sessions of high pulse flow and exposure to monochloramine, the biofilm can help be kept clean and free of disease-causing bacterial pathogens, specifically *Legionella pneumophila*.