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(Research Article)

Comparison of the efficiency of George fisher and metal pipes in water piping system

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Abstract

Background and Aim: The health concern caused by the respiration of drinking water aerosols containing opportunistic pathogens such as *Legionella pneumophila* is increasing. This study aimed to investigate the effect of George Fisher's piping system on controlling *Legionella pneumophila* growth.

Methods: A total of 32 samples with a volume of 1 liter per hot and cold water system of the gallery commercial-office complex and selective hospital were collected. Then, filtration, concentration, and heat treatment were performed and cultured on selected BCYE agar medium

Results: The culture results showed that 5.8% of George Fisher samples and 37.5% of metal tubes were positive for *Legionella pneumophila*.

Conclusion: *Legionella pneumophila* stopper pipes and fittings of George Fisher Company have a good ability to control the growth and density of *Legionella pneumophila* in the indoor water supply system and can be considered a suitable option for use in plumbing and indoor water supply.

Keywords: *Legionella pneumophila*; George Fisher Plumbing; Metal Pipe; Culture

1. Introduction

Legionella is bacil of gram-negative, motile, aerobic, without spores and capsules, hard growing, and has a non-fermentative respiratory metabolism based on amino acid catabolism. Water distribution networks are an important source of Legionnaire's disease [1]. With increasing population, improving living standards and consequently increasing water consumption and decrease in water resources, and increasing physical, chemical, and microbial pollution of water, the water crisis has been raised as one of the major global problems [2-5].

Legionella pneumophila is a ubiquitous aquatic bacterium. The resulting mortality rate is estimated at 5 to 40%. Group 1 is responsible for almost 70% of all infections caused by this bacterium and group 6 is in second place in this field [6-1].

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The mortality rate from Legionellosis is 5-20% and in cases of nosocomial infections, it is 30%, 40%, and above 50% [1]. Risk factors include age, sex, underlying diseases, delay in treatment, smoking, chronic bronchitis, treatment of patients with steroids, chemotherapy for cancer and patients with diabetes mellitus, transplant patients, chronic obstructive pulmonary disease, tube insertion into the trachea and the use of drugs that weaken the immune system. Reports of an epidemic of Legionnaires' disease and the waters used have been reported. Since the direct human-to-human transmission has not been reported so far, disruption of the transmission chain of this infection has often been focused on identifying epidemiological foci and destroying them.

On the other hand, identification of foci of disease requires identification of *Legionella pneumophila* in environmental specimens. Much research has been done using different methods to remove *Legionella* from water, including thermal disinfectant, hyper chlorination, ionization, ultraviolet light, and copper-silver ionization. Even the type of disinfectant, water composition (water-soluble organic matter, hardness, iron, manganese, etc.), pipe material, and water supply facilities in terms of the reaction of the disinfectant material with the pipe material, which may be metal or plastic in terms of roughness The inner surface of the pipe, biofilm condition and age of water used can be effective in influencing the type of disinfection used [7].

The results of studies show that none of these methods have been able to eliminate this bacterium. This study aimed to evaluate the efficacy of George Fisher's piping system containing Legionella pneumophila growth inhibitors and its bactericidal activity.

2. Material and methods

2.1 Sample collection and filtration

This descriptive study was conducted in 2020 in the water supply system of two commercial centers with two different types of plumbing (George Fisher and metal pipes and fittings). One center used *Legionella* Stopper pipes and fittings made by George Fisher for indoor water supply, and the second center used ordinary metal pipes and fittings. The geographical location of the chemical quality of the inflow from the urban network was not different in both centers. 16 samples (8 samples of cold water and 8 samples of hot water) from the administrative-commercial center with pipes and fittings of George Fisher and 16 samples (8 samples of cold water and 8 samples of hot water) from the ward equipped with ordinary metal pipes and fittings of the hospital Selected Tehran was collected.

Samples were collected in sterile containers made of polyethylene with a volume of 1.5 liters from different parts of the office-commercial complex of the gallery and the selected hospital in Tehran and were cultured and analyzed for the presence of *Legionella pneumophila*. The samples were immediately transferred to the laboratory in the vicinity of iceboxes and analyzed at a maximum interval of 8 hours using membrane filtration and vacuum pump (model VE115N) and polycarbonate micron carbon filter (Uflow Membrane Filters, pore size: 0.22-0.45 μm). An autoclave (temperature 121 °C, pressure 15 ppm, and time 15 minutes) was used to sterilize the components of the filtration system. After filtration, the filter was separated and filtered in 50 ml of water and crushed into sterile glass containers, and suspended. To ensure the separation of bacteria from the water filter containing the filter particles, the mixture was mixed for 24 hours using an orbital shaker (GFL-3017) at a speed of 230 rpm and stored in a refrigerator at + 4 °C [8].

2.2 Culture and biochemical tests

BCYE agar medium (manufactured by Biomark) containing L-cysteine supplements (manufactured by Merck Germany), ferric pyrophosphate (manufactured by Sigma Aldrich), and GVPC (Glycine, Vancomycin, Polymyxin B, Cycloheximide) (manufactured by Biomark) according to the instructions and was used for bacterial culture. To ensure the control of intrusive bacteria, the samples were heat-treated using a water bath (56 ° C for 11 minutes) before planting. 100 μl of the sample was inoculated with flame on the culture medium and the plates were placed in a candlestick jar containing 2-5% carbon dioxide for 7-14 days at 37 ° C in a humid incubator. Legionella colonies were identified based on size, color, and biochemical properties (catalase, oxidase, hydrolyzate, hyporate, and hot staining tests).

To be sure, the grown colonies were re-inoculated on blood agar medium (base material made by German Merck Company and Iranian sheep spring defibrillated blood) and incubated at 35 °C. After ensuring no growth, they were assigned to *Legionella c*onfirmed (8). *Legionella* growth was monitored and recorded on the third to fourteenth days. The colonies that appear on the first and second day are not *Legionella*, and the colonies that appear on the third and later should be thoroughly examined. *Legionella* colonies are white-gray or blue-green, convex, shiny with a diameter of 2-4 mm. The central part of the young colonies appears as light gray and granular, like a glass background, while the peripheral part of the colony is light pink or blue.

Legionella colonies were detected under ultraviolet light by their size, color, type, special characteristics, and fluorescence properties. In addition, the grown colonies were re-cultured on BCYE unenriched sheep blood agar medium and their appointment to *Legionella* was confirmed after ensuring no growth (6-11). Data were analyzed and reported using Excel 2018 software and descriptive statistics.

2.3 Catalase test

To perform this test, a solution of 3% oxygenated water with a volume of 0.05 cc (one drop) was added to a suspicious colony (on a slide). The formation of oxygen bubbles in this test is a sign of bacterial catalase.

2.4 Oxidase test

The enzyme cytochrome oxidase oxidizes the reduced cytochrome by molecular oxygen to produce H2O. Tetramethylene para-phenylenediamine dihydrochloride 1% reagent was used for the oxidase test.

2.5 Sodium hydrate hydrolysis test

This bacterial test is performed in liquid broth nutrient medium or BHI broth. Add to the pyrophosphate and L-cysteine medium as growth supplements and allow to incubate at 37 °C for 18 hours. Then added ferric chloride reagent to the tubes. The formation of a white clot deposit indicates that the test is positive.

3. Results and discussion

3.1 Culture and diagnostic tests

Figure 1 Cultivation of Legionella from contaminated water in the BCYE environment

Figure 2 Candle jar containing carbon dioxide and cultured plates

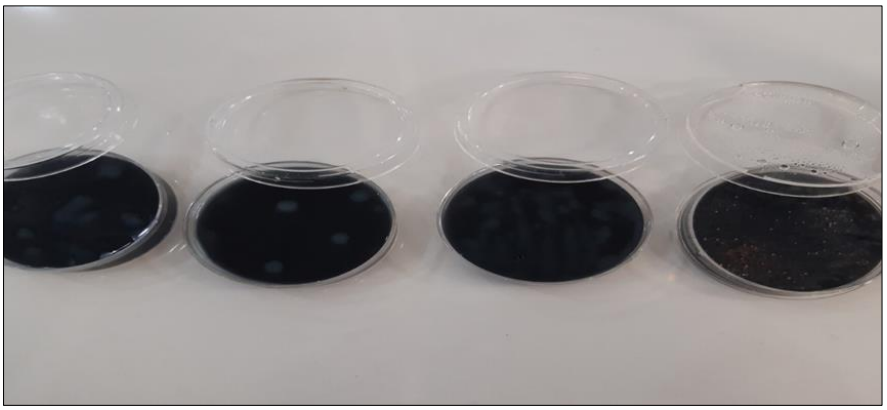

Figure 3 Culture plates containing Legionella pneumophila colonies after 7 days of incubation

3.2 Morphology of Legionella

Legionella is found in fresh culture as a bacil and in old culture as a filamentous.

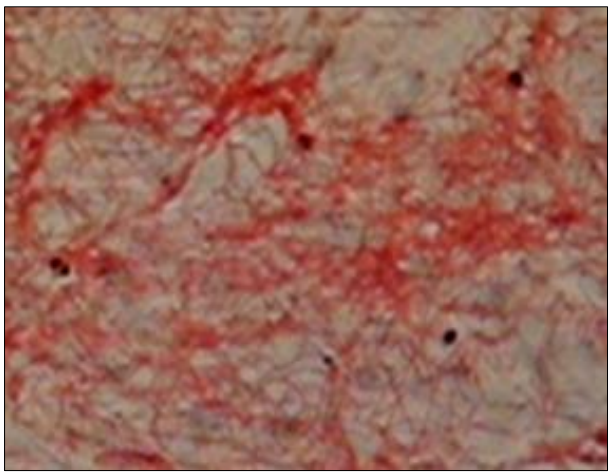

Figure 4 *Legionella pneumophila* gram staining smear from an old culture seen as filamentous

3.3 Catalase test

The bacterial colony produced a bubble in the presence of hydrogen peroxide, and the release of oxygen indicates a positive presence of catalase.

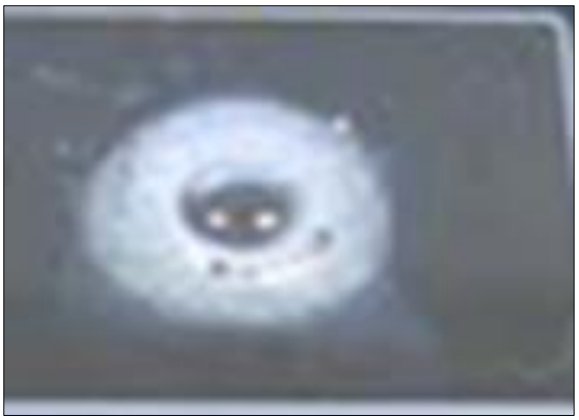

Figure 5 Catalase test result of Legionella pneumophila colony with 3% hydrogen peroxide

3.4 Oxidase test

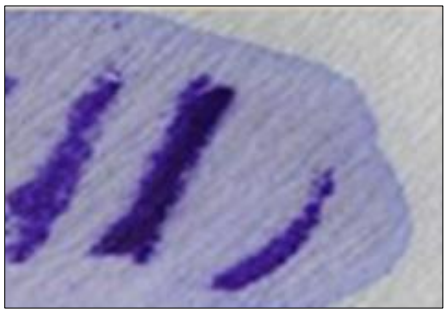

Figure 6 Oxidase test result with 1% tetramethylene paraphenyl diamine dihydrochloride reagent which appeared purple

3.5 Sodium hydrate hydrolysis test

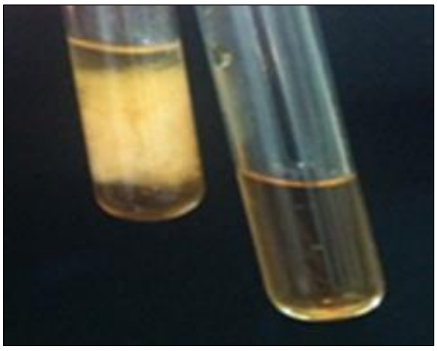

Figure 7 Hydrolysis test of sodium hypurate in tube culture medium containing *Legionella pneumophila*

Legionella pneumophila contains the enzyme hypurase, which is a culture medium containing 1% sodium hypurate decomposes into glycine and benzoic acid. Addition of ferric chloride reagent results in a white precipitate.

Table 1 Culture results of *Legionella pneumophila* aqueous samples from George Fisher and metal piping system

Pipe material	Total number of samples	Hot water	Cold water	Percentage of positive culture
George Fisher	16	1	0	%6.25
Metal	16	4	2	%37.5

4. Discussion

Water is one of the common sources of Legionella transmission in hospitalized patients. Hospital water networks are an important source of *Legionella pneumophila* disease in such centers [14-20]. Many kinds of research have been done using different methods in removing Legionella from water such as the thermal method, using chlorine compounds, ultraviolet light, copper-silver ionization, photocatalytic oxidation, using ozone gas, and catalytic ozonation, and the above methods although They may have advantages, their disadvantages and side effects are also noteworthy, including short-term bactericidal stability, carcinogenicity, high investment cost, need for preparation, use of chemicals, and more. Kurdish [1, 13, 12].

The results show that George Fisher pipes and fittings are much less polluted and only out of 16 samples related to the George Fisher pipe and fittings system, 6.25% of the samples show Legionella contamination, based on which it can be said that George Fisher pipes and fittings in 93.75% prevent the growth of Legionella pneumophila in water. Also, the use of metal pipe and fittings system has higher contamination than George Fisher pipe and fittings system, so out of 16 samples collected from Ray Ho pipe and fittings, 37.5% of the samples were Legionella pneumophila contamination. In general, the advantages of this research over other research in this field include the long-term stability of George Fisher

tube bactericide, no chemicals, no carcinogenicity, no corrosion of tubes, no side effects, no need for preparation, and low investment cost [20-a].

Examination of the results in terms of *Legionella bacterial* density in these systems also shows that George Fisher pipes and fittings are not only less contaminated, but also better in terms of contamination density than metal pipes and fittings.

5. Conclusion

The use of Legionella Stopper pipes and fittings by George Fisher Company due to its material and having antibacterial compounds has good properties in controlling the growth and increase of Legionella density in the water supply system and can be a suitable option for use in piping and water supply to kill *Legionella pneumophila* to be considered.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

No conflict of interest.

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