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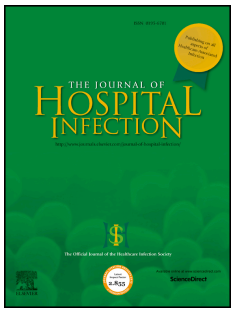

The rate of *Legionella pneumophila* colonization in hospital hot water network after time flow taps installation

M. Totaro, P. Valentini, B, A.L. Costa, S. Giorgi, B. Casini, A. Baggiani

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SHORT REPORT TITLE: The rate of *Legionella pneumophila* colonization in hospital hot water network after time flow taps installation.

Authors: M. Totaro, P. Valentini, B., A.L. Costa, S. Giorgi, B. Casini, A. Baggiani

Affiliations: **Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy.*
° *Department of Clinical and Experimental Medicine, University of Pisa.*
Corresponding author: Prof. Angelo Baggiani. *Department of Translational Research and New Technologies in Medicine and Surgery, University of Pisa, Italy.*
Tel. 050 2213583; Fax. 050 2213588; E-mail: angelo.baggiani@med.unipi.it
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Abstract

In hospital water systems legionellae may be resistant to disinfectants in pipework, which is a problem particularly in areas where there is low flo or stagnation of water. We evaluated legionella colonization of a water network of an Italian hospital after time flow taps (TFTs) installation in proximity to dead legs. The water volume flushed was 64 L/day from May 2016, and 192 L/day from December 2016. Before TFTs installation, *Legionella pneumophila* sg2-14 was detected in all points ($4x10^4 \pm 3.1x10^4$CFU/L). All sites remained positive ($2.9x10^4 \pm 1.9x10^4$CFU/L) through November 2016. From December 2016 Legionella persisted in one point only ($2x10^2$ to $6.8x10^3$CFU/L). TFTs with chemical disinfection may reduce legionella colonization associated with dead legs.

Introduction

Legionella spp. are waterborne pathogens frequently associated with nosocomial infections, particularly among immunosuppressed patients. Some of the outbreaks of Legionnaires' disease reported by the European surveillance scheme over the last years in Europe have been linked to healthcare facilities having a hot water system colonized by legionellae.[1] Italian guidelines indicate that the complexity of the water distribution systems, and the presence of corroded pipelines and dead leg branches, may induce the growth of legionellae in water networks.[2] These critical points in the water distribution system cause a low water flow with a higher likelihood of biofilm development.[3] Hot water networks may be treated with different compounds to ensure continuous disinfection; however, such treatments do not completely eradicate colonization.[4] First, legionellae demonstrate resistance to chemical agents at concentrations usually applied for water disinfection,[5] and second, they can shelter in free-living protozoa, where they are protected from the effects of even higher concentrations of disinfectants. Moreover, disinfectants will tend to disappear from stagnant water in deadlegs, creating a high risk for microbial re-growth.[6]

The risks associated with stagnant or low flow water may be reduced by implementation of scheduled automatic or manual flushing plans. We report on our experience of managing the legionella risk in the hot water distribution system of an Italian hospital, initially using a continuous disinfection treatment and then installation of time flow taps (TFTs) in the vicinity of all dead legs to increase the local water flow.

Methods

Setting, water disinfection and TFT installation

The setting is a general hospital of the North-Western Tuscany region local health unit, (Italy), a 401-beds hospital with a catchment area of 160,000 inhabitants. The hospital opened in 2002 and

has steel pipework. The hospital is a monoblock with a central area on 6 levels that hosts high-risk areas (Haematology, Oncology, Transplant Surgery, etc).

In January 2016 a Water Safety Plan (WSP) implementation programme was initiated that included a systematic monitoring program of the hot water system with sampling at the final points of use.

Municipal water supplied to the hospital is not pre-filtered, but is softened before entering the hospital hot water distribution system. As part of the WSP the hot water network was treated with continuous chlorine dioxide disinfection. A survey of the water network indentified five dead legs, ranging from 5 m to 7 m in length.

After evaluating the hot water consumption in the hospital (60 m^3/month), from May 2016 five TFTs were installed in correspondence with dead-end branch. TFTs were programmed in order to obtain a 1 minute flush every 6 hours (64 L/day) with a water flow of 16 liter per minute. From December 2016, the TFTs scheduling was changed with a 1 minute flush every 2 hours (192L/day) with the same water flow in order to obtain an increase of water daily flow.

Hot water sampling and Legionella research

From January 2016, the hot water distribution system was sampled at the recirculation of the central heating system (R) and at five points of use (located in different floors) for legionella detection. From May 2016 samplings were performed at the same points of use where TFT devices were placed.

Legionella testing and physical-chemical parameters assessment (chlorine concentration, pH and water temperature) were initially performed on a monthly basis. After achieving a reduction in legionella concentrations, from February 2017 onwards water sampling, at the same points, was undertaken four-monthly.

Methods of sampling an culture for *Legionella* spp. in hot water samples were as recommended by the Italian guidelines.[2] Approximately 10% of the colonies obtained were subjected to species and serogroup identification analysis using a multi-purpose latex agglutination test (Legionella Latex

Test, Oxoid Ltd, Basingstoke, UK; *L. pneumophila* group sera set Biogenetics, Ponte San Nicolò, Italy).

Sampling for chemical analysis was performed on a six-monthly basis (January 2016, July 2016, January 2017) at the same sampling points chosen for microbiology tests. Chemical parameters as iron ions, zinc ions and trihalomethanes (THMs) were assayed as established by Council Directive 98/83/EC. [7]

Results

Table I shows the mean concentration of *Legionella* spp., and the physical and chemical parameters at each sampling points before and after TFTs installation.

Before installation of the TFTs high *L. pneumophila* serogroups 3,10-14 concentrations (from $8x10^3$ to $1.3x10^5$ CFU/L) were detected in all the tested water outlets. These data indicated significant colonization of the hospital building water system despite the presence of total chlorine concentrations ranging from 0 to 0.23 mg/L. Moreover, a good chemical quality was observed in all the water samples, with all values within the limits recommended by Council Directive 98/83/EC.[7]

Following the installation of TFTs, between May 2016 and November 2016, all samples remained positive *L. pneumophila* serogroups 3,6,10-14, with counts ranging from $1x10^2$ to $1.05x10^5$ CFU/L. Chlorine concentrations ranged from 0 to 0.3 mg/L. Once again, the good chemical quality of water was maintained and all physical-chemical and chemical data were similar to the values found before the TFTs activity.

The initial TFT flushing regimen made little difference to the numbers of legionella isolated, and all sampling points remained positive. However, the revised more frequent flushing regimen decreased legionella counts to the point where only one sampling point was positive (Figure 1). *L. pneumophila* serogroups 3,6 were detected at only the TFT3 point, with counts of $2x10^2$ to $6.8x10^3$ CFU/L. The persistence of these serogroups is probably due to their chlorine-tolerance.

Discussion

Despite the use of continuous chlorination of a 15 year-old water plant we found *L. pneumophila* sg2-14 to be ubiquitous in water samples. Evaluating the structural complexity of pipeworks we hypothesized that the low hot water consumption in the hospital caused the persistence of Legionella strains in biofilm despite the chemical disinfection. As previously described, the presence of dead legs in water networks allows the biofilm growth in sites where the disinfectant can not be effective.[8]

By installation of TFTs in proximity to dead leg sections an increase in hot water use from 60 m^3 to 70 m^3 per month was achieved during the period between May 2016 to June 2017. In the first months of the study we did not observe a reduction in *L. pneumophila* sg2-14, despite the increase in the hot water flow. Only after a more frequent flusging was introduced did we see a significant impact on legionella counts.

Our preliminary results suggest that frequent water flushing in the vicinity of dead legs can enhance the effectiveness of chlorine dioxide in situations where the dead legs cannot be removed. Presumably, a reduction in stagnant water reduces the biofilm growth that protects microorrgansims from the effects of chemical disinfectants.[9]

Despite the short duration, we believe that our study is the first to assess the application of new TFTs devices aimed to increase the efficacy of the water disinfection. Maintaining the current disinfectants, TFTs installation may aid Legionella control where it is impossible to eliminate dead legs from the plumbing system. Although the contamination decrease is slow, these devices could represent a valid choice intended to improve water quality and disinfectants efficacy reducing the critical points of water network and the biofilm proliferation in dead-end sections.

Competing Interests

All authors have no conflict of interest to declare.

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Table I: Mean values of *Legionella* spp., physical-chemical and chemical parameters detected at each sampling points before and after time flow tap (TFT) installation. Sampling Points (R – Recirculation); (TFT1 - General Medicine; Floor 2); (TFT2 - Emergency Department; Floor 0); (TFT3 – General Surgery; Floor 1); (TFT4 - Locker Room; Floor -1); (TFT5 - Health Management; Floor 4).

BEFORE TFTs INSTALLATION (January 2016 – April 2016) – 24 samples							
SAMPLING POINT	**LEGIONELLA (mean±SD CFU/L)**	**TOTAL CHLORINE (mean±SD)**	**TEMPERATURE (mean±SD °C)**	**mean pH±SD**	**IRON IONS (µg/L)**	**ZINC IONS (µg/L)**	**THMs (µg/L)**
R	$1.8x10^4±2.2x10^3$	0.21±0.01	46.8±2.1	6.51±0.12	84	104	< 3
TFT1	$7.5x10^4±4.7x10^4$	0.21±0.01	41.2±1	6.49±0.14	88	99	< 3
TFT2	$2.5x10^4±5.7x10^3$	0.05±0.007	42.3±4.9	6.48±0.21	94	112	< 3
TFT3	$3.7x10^4±5.4x10^3$	0.14±0.11	38.2±4.4	6.51±0.20	98	96	< 3
TFT4	$6.2x10^4±4.6x10^4$	0.21±0.09	40±4.3	6.52±0.18	74	97	< 3
TFT5	$1.9x10^4±3.9x10^3$	0.12±0.08	40±4	6.49±0.11	86	78	< 3
AFTER TFTs INSTALLATION (May 2016 – October 2016) – 36 samples							
SAMPLING POINT	**LEGIONELLA (mean±SD CFU/L)**	**TOTAL CHLORINE (mean±SD)**	**TEMPERATURE (mean±SD °C)**	**mean pH±SD**	**IRON IONS (µg/L)**	**ZINC IONS (µg/L)**	**THMs (µg/L)**
R	$8.7x10^3±1.9x10^3$	0.24±0.01	44.9±1.9	6.58±0.23	91	110	< 3
TFT1	$4.2x10^5±5.5x10^4$	0.19±0.03	43.3±2.6	6.56±0.24	85	98	< 3
TFT2	$1.3x10^4±2.8x10^3$	0.1±0.9	41.9±1.8	6.52±0.19	96	96	< 3
TFT3	$4x10^4±3x10^3$	0.28±0.11	39±4.9	6.5±0.21	88	89	< 3
TFT4	$5.2x10^4±2.9x10^4$	0.8±0.11	38.7±4.2	6.48±0.17	71	87	< 3
TFT5	$2.1x10^4±2.9x10^3$	0.19±0.1	40.4±3.6	6.46±0.13	80	85	< 3
AFTER TFTs INSTALLATION (December 2016 – June 2017) – 24 samples							
SAMPLING POINT	**LEGIONELLA (mean±SD CFU/L)**	**TOTAL CHLORINE (mean±SD)**	**TEMPERATURE (mean±SD °C)**	**mean pH±SD**	**IRON IONS (µg/l)**	**ZINC IONS (µg/l)**	**THMs (µg/l)**
R	0	0.29±0.02	43.4±2.3	6.58±0.12	87	98	< 3
TFT1	0	0.21±0.04	42.5±0.5	6.51±0.23	99	101	< 3
TFT2	0	0.27±0.03	42,1±0.6	6.45±0.22	92	94	< 3
TFT3	$3x10^3±2x10^3$	0.30±0.01	39.1±1.1	6.44±0.13	94	84	< 3
TFT4	0	0.27±0.02	38.2±2.1	6.51±0.22	81	85	< 3
TFT5	0	0.25±0.01	41.6±2.7	6.52±0.08	89	88	< 3

Figure 1

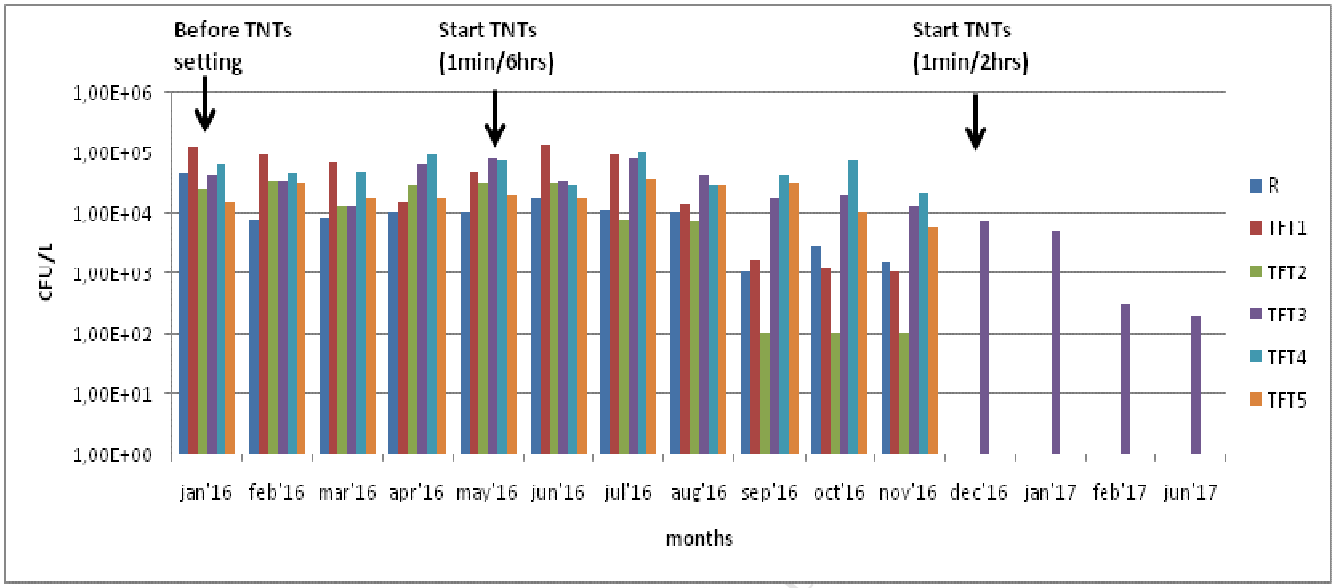

Figure 1: *Legionella* spp. counts in all the points of sampling during the period of the study.