



INVESTIGATION OF THE TYPE OF BACTERIA *L.PNEUMOPHILA* IN WATER SYSTEMS OF FIVE-STAR HOTELS IN ISTANBUL

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ABSTRACT

This study was performed to understand the terms of the bacterium *Legionella* spp of the major hotels' water in Istanbul and to prevent new cases and epidemics in the same hotels. In our study, there is a marked increase in March in the samples obtaining from air conditioning return water of A five-star hotel. B is a five-star hotel in a room in the months of January and February were reproduction in the shower water, C, D and E in the samples taken from five-star hotel compared to the month in general, a reproduction was found. Despite of the fact that samples were taken on different dates from some of our hotels, the continuous *Legionella* bacteria are found. According to obtained by examining statistics, it is been in the opinion of protection methods from *Legionella* are not implemented adequately in our luxury hotel. In this study, it is aimed to introduce the importance of valuing to the human health and as a result of our study on the water systems of the hotels it is stated expressly that European and World-class hotels having high quality in Istanbul do not show the necessary sensitivity to human health.

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INTRODUCTION

Legionella bacteria have been known to cause Legionnaires' diseases (pneumonic legionellosis) and Pontiac fever (severe influenza-like illness) (Kim, et al., 2002). Since then, the bacterium has been isolated from numerous sources in the environment (WHO, 1990), and revealed to be a ubiquitous freshwater inhabitant. It is believed that bacterial transmission to humans occurs through droplets generated from environmental sources such as cooling towers, showerheads, whirlpools and other human-made devices that generate aerosols (Aurel, et al., 2004, Ishimatsu, et al., 2001, Liu, et al., 1995, Lin, et al., 1998, Leoni, et al, 2001, Ohno, et al.,2003, Sabria and Yu, 2003, Stout and Yu, 2003, Moens, et al.,2002). Since legionellae widely inhabit many water environments, the significance of isolating the bacteria from water reservoirs of aerosol-producing devices is often uncertain (Redd and Cohen, 1987).

The first epidemic on *Legionella* was diagnosed in a hotel and attracted attention all over the world. 50 species and 70 serogroups were identified of *Legionella* genus bacteria. (Edelstein, et al., 2005). Depending on the temperature of aquatic environment, bacteria decreases at lower than 20°C, starts reproduction and reaches the maximum level at 37°C, remains at 46°C, stays alive for a few hours and for a few minutes at 50°C and 60°C, respectively.

Breathing of water grains containing bacteria and amoeba causes infection. Presence of lower than 10 cfu/mL bacteria in cooling towers has no meaning. Nevertheless thousands of bacteria can be found in an amoeba. This bacteria has been studied for more than 30 years in spite of the fact that it has a low potential to cause an infection.

The aim of this study is to prevent new occurrences and epidemics due to the colonisation of *Legionella* bacteria in various five-star hotels of Istanbul.

MATERIAL AND METHOD

109 samples which are the total of 8 samples of A hotel, 62 samples of B hotel, 20 samples of C hotel, 12 samples of D hotel, 7 samples of E hotel were collected from showerheads, whirlpools of each room, besides restaurant kitchen, boiler exit and cooling tower systems of five different five star hotels between January- October 2014 using 1000 ml brown amber glass and they were transported to the laboratory keeping in cold chain. Samples were filtered and concentrated through a 0, 2 µm membrane filter (Millipore HAWG047S1, lot H6PM10572). Filters were taken into the Falcon tubes including 5 ml Tryptone(Tryptlc)Soya Broth and provided the transition of bacteria into the Tryptone(Tryptlc)Soya Broth by shaking at vortex. Then they were cultured into the BCYE-a, MWY ve BMPA mediums (Eaton, et al., 1992, Baron and Finegold, 1990)

Application of acid to the samples: 0,1 ml sample liquids were added into the sterilized tubes having acid solutions (5.3 ml 0.2N HCl + 25 ml 0.2N KCl ve 100 ml distile su, pH 2.2) and shaken. After 4 minutes they were shaken again and cultered into the mediums as 150 µl. (Rodgers, *et al.*, 1991).

The plates were wrapped with parafine and incubated for 14 days at 35°C (at the medium having 5% CO₂). They were controlled once at 48 hours if there are Legionella colonies and incubated at 35°C in a medium having CO₂, then investigated once at 48 hours during 14 days. Gram and Giemsa preparates were prepared from the S type of colonies reproducing at BCYE-a mediums (Rodgers, *et al.*, 1991).

RESULTS

It can be seen from the tables whether there is any reproduction of Legionella bacteria. (Table1, 2, 3, 4, 5). 7 samples from air condition return water and 1 sample from room shower were taken in A hotel. Whereas there are reproduction in 4 samples from air condition return water taken at the end of March, June and July, there are no reproduction in 3 samples from air condition return water and 1 sample from a room shower water taken at the beginning of March, June and July.

Table 1 "A" Five-Star Hotel

Sampling Date	Sampling Part	Number of Samples	Reproduction Amount/Place
04.01.2014	Air conditioning Return Water	1	No reproduction
27.01.2014	Air conditioning Return Water	1	No reproduction
16.02.2014	Air conditioning Return Water	1	No reproduction
17.03.2014	Air conditioning Return Water	1	220 cfu/ml- Sg-1
17.06.2014	Air conditioning Return Water	1	14 cfu/ml- Sg-1
08.07.2014	Air conditioning Return Water	1	60 cfu/ml- Sg-1
29.07.2014	Air conditioning Return Water	1	No reproduction
21.10.2014	Room	1	100 cfu/ml- Sg-1

Cfu/ml: colonies per ml

Table 2 "B" Five-Star Hotel

Sampling Date	Sampling Part	Number of Samples	Reproduction Amount/Place
04.01.2014	Room	12	1 room - 4 cfu/ml- Sg-1
18.02.2014	Room	12	1 room - 12 cfu/ml- Sg-1
04.04.2014	Room	12	No reproduction
14.06.2014	Room	14	No reproduction
30.09.2014	Room	6	No reproduction
30.09.2014	Cooling Tower	6	1 Cooling tower - 4 cfu/ml- Sg-1

Cfu/ml: colonies per ml

Table 3 "C" Five-Star Hotel

Sampling Date	Sampling Part	Number of Samples	Reproduction Amount/Place
25.02.2014	Room	9	No reproduction
25.02.2014	Boiler Exit	1	No reproduction
28.10.2014	Room	9	3 room - 2 cfu/ml, 2 cfu/ml, 4 cfu/ml - Sg-1
28.10.2014	Boiler Exit	1	No reproduction

Shower water samples were taken from various rooms of hotel B at different dates. There are reproduction in two samples taken in January and February, yet there are no reproductions from samples taken in April and June. In September only one reproduction was observed among six different rooms and

cooling towers of the same hotel. One sample from boiler exit in February and October and 9 samples from room shower were collected from hotel C.

Table 4 "D" Five-Star Hotel

Sampling Date	Sampling Part	Number of Samples	Reproduction Amount/Place
08.03.2014	Room	12	1 room- 18 cfu/ml – Sg-1 Cfu/ml: colonies per ml

Table 5 "E" Five-Star Hotel

Sampling Date	Sampling Part	Number of Samples	Reproduction Amount/Place
28.03.2014	Room	4	3 room- 40 cfu/ml, 36 cfu/ml, 32 cfu/ml – 2-14
28.03.2014	Restaurant Kitchen	3	1 kitchen 60 cfu/ml 2-14 Cfu/ml: colonies per ml

There is no reproduction in the sample of February but reproductions were found in samples taken from three rooms in October. 12 samples were taken from room shower of hotel D and reproduction was seen at only one sample. 4 different room shower samples and 3 restaurant kitchen samples were taken in March at hotel E and reproductions were found at three rooms and one kitchen samples. However these results were given as A,B,C,D,E due to the privacy of hotel names.

DISCUSSION

Legionella bacteria have widely been found at nature and some species cause a type of pneumonia called Legionnaires Disease. Particularly water systems of hotels and hospital could be reservoir for bacteria. In such circumstances, epidemic diseases can be occurred. Therefore water systems of hotels should be maintained regularly to prevent the settling of the bacteria.

Equipments (cooling towers, showerheads, whirlpool baths) form water to aerosols are widely used in touristic facilities and cause contamination of bacteria to human. (Köksal *et al.*, 2002, Arnow, *et al.*, 1985, Borella, *et al.*, 2005)

It is reported that between 8.000-18.000 Legionary events are seen in the USA every year. The presence of the disease is known in Turkey but the number of patient per year is not known due to deficiency of statistical knowledge. Mortality rate of the people suffering this disease is 5-15% among the world. Whereas in 1997, 360 Legionary events were seen, this proportion exceed 2000 in 1999 (Kantaroglu, 2006, Stout *et al.*, 1992, Aalry and Joly, 1991, Tiefenbrunner, *et al.*, 1993).

Legionella bacteria was obtained from tap waters, hospital hot water systems at various countries (Musellim, *et al.*, 1994., Baskin, *et al.*, 1998, Baulanger and Edelstain, 1995, Liu, *et al.*, 1993, Perente, *et al.*, 1999, Rogers, *et al.*, 1994). In our country different researchers isolated legionella from environmental water samples (Baskin, *et al.*, 1998) and *L.pneumophila* from hospital humidifiers (Musellim, *et al.*, 1994, Perente, *et al.*, 1999, Yildirim, 1996, Nakiboglu and Gurler, 2000).

In our study, while there is no reproduction in samples of A five-star hotel's air conditioning return water in January and February, a sharp increase was observed in March. There was a reproduction of only one room among 12 rooms of B five-

star hotel in January- February and there was no reproduction in April and June. Generally reproductions were observed in samples from C, D and E. This case is related to hotels do not show enough sensitivity for public health. Consequently, these results are not pleasant for our country being very attractive for tourists. Water samples should be monitored periodically even though this process has high cost to protect public health.

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