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Epidemiology of *Legionella* and Climatic Variables in Seoul, Korea

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Legionella species are abundant in the built environment and are increasingly recognized as a cause of Legionnaires' disease (LD). As the number of cases of Legionnaires' disease acquired by local communities in the Seoul metropolitan area in Korea has been increased, there was concern that changes in environmental factors could affect disease outbreaks. We described the association between climatic variables and occurrence of legionellosis in Korea and *Legionella* detection rate in Seoul area. A total of 418 cases of legionellosis were reported between 2014 and 2017. There was a seasonal peak in summer. LD continuously occurred from early spring to winter every year and rapidly increased in summer. In the regression analysis, the primary variables of interest- PM2.5 (μg/m^3), NO$_2$ (ppb), and a number of the date of issue O$_3$ warning were not significant except for average temperature (R^2=0.8075). The *Legionella* detection rate in Seoul, Korea showed a trend similar to precipitation (P=0.708, ANOVA). A relatively high proportion of *Legionella* detection rate was shown, especially cooling tower (17.7%) and public bath (19.3%). This finding is in line with current understanding of the ecological profile of this pathogen and supports the assertion that legionellosis occurs through contamination of water sources.

Key Words: *Legionella*, Climatic variables, Regression analysis

INTRODUCTION

Legionella is a genus of pathogenic Gram-negative bacteria responsible for a serious disease known as legionellosis, which is transmitted via inhalation of this pathogen in aerosol form. There are two forms of Legionnaires' disease (LD), a potentially fatal pneumonia and Pontiac fever, a milder self-limiting flu-like illness (1, 2).

Legionella, highly diverse (3) and pleomorphic (4) bacteria, can be found in a variety of environments and are naturally occurring in freshwater (3) and soil (5), because their life cycle typically involves endoparasitization and replication within eukaryotic host such as amoebae (6).

Incidence of LD has been rising consistently since the discovery of the disease (7), with a nearly 3.5-fold increase between 2000 and 2011 in the United States (8), 3 fold in Europe since 1995 (9, 10), 2.5-fold increase between 2015 and 2016 in Korea (11), leading to legionellosis being considered an increasingly important disease from a public health standpoint.

Numerous factors could possibly contribute to an increase of notified LD cases in Europe, USA, and Korea. Previous studies have suggested that changing the environmental conditions, such as rainfall and temperature, can affect the incidence of sporadic community-acquired LD cases in Europe (12, 13, 14). Additionally, population growth and increase in aging, which are accompanied with greater use of immunosuppressant drugs and higher incidence of comorbid conditions, are other important risk factors of incases in LD (15).

The objective of this study was to investigate the link between climatic changes and the prevalence of *Legionella* spp. and the incidence of Legionellosis in cooling towers and baths of public buildings in Seoul, Korea from 2013 to 2017.

MATERIALS AND METHODS

Samplings

Total 6,598 water samples were collected annually from the public use facilities from the 25 districts in Seoul, Korea between 2013 and 2017. Two litters of water samples were collected aseptically in sterile plastic containers and kept them at 6 - 18 °C for transportation with light-free condition. The samples were categorized by their originated buildings, such as hospitals, decorative fountains, welfare facilities, subway, shopping malls, and department store.

Legionella Culture in Water Samples

One litter of water sample was filtered through 0.2-μm nitrocellulose membrane under vacuum. Each membrane was then placed on buffered charcoal yeast extract (BCYE) agar supplemented with L-cysteine and antibiotics followed by incubating for 10 days at 37°C. Colonies growing on BCYE agar, but not on blood agar, were identified as *Legionella* spp.

Detection of *Legionella* by PCR

PCR analysis was performed to identify *Legionella pneumophila* and *Legionella* spp. by amplifying 16S rRNA and *mip* gene as described previously (Table 1) (16, 17). Amplification reactions were subjected to an initial denaturation step of 95◦C for 4 min, followed by 30 amplification cycles of 30 sec at 95◦C, 30 sec at 55◦C, and 30 sec at 72◦C, followed by a final extension at 72◦C for 10 min.

Table 1. Primers used in this study

Primer	Sequence (5' → 3')	Product size
16S rRNA	F: AGGGTTGATAGGTTAAGAGG	386 bp
	R: CAACAGCTAGTTGACATGG	
mip	F: GGTGACTGCGGCTGTTATGG	630 bp
	R: GGCCAATAGGTCCGCCAACG	

Detection of *Legionella* by MALDI-TOF

Legionella isolates were identified by matrix assisted laser desorption and ionization time of flight mass spectrometry (MALDI-ToF; Bruker, Billerica, MA, USA) according to the manufacturer's protocols.

Legionellosis Data Collection

Legionellosis data were collected from the National Notifiable Diseases Surveillance System database of Korea Centers for Disease Control and Prevention (KCDC).

Meteorological Data

Climate data in Korea including average temperature, precipitation, PM2.5 ($\mu g/m^3$), NO_2 (ppb), and the number of O_3 warning were obtained from the Korea Meteorological Administration during 2013-2016.

Statistical Analysis

Analyses were conducted using SPSS version 24.0 (IBM Corp., Armonk, NY, USA). The relationships among the incidence of LD and climatic factors were examined using linear regression analysis.

RESULTS

In total 6,598 water samples collected from 2013 to 2017 in Seoul, Korea, *Legionella* spp. were isolated by culture and identified by PCR and MALDI-TOF analysis. *Legionella* spp. was detected in 191 out of 1,092 (17.5%), 180 out of 1,015 (17.7%), 109 out of 920 (11.8%), 158 out of 1,149 (13.8%) and 306 out of 2,422 (12.6%) resulted positive to *Legionella* spp. by year indicating that an annual average isolation rate of *Legionella* spp. was 14.3% (Table 2).

Among 944 Legionella isolates, 17.7% (283 isolates out of 1,595 samples), 9.4% (190 isolate out of 2,025 samples), 19.3% (439 isolates out of 2,272 samples), 1.5% (2 isolates out of 132 samples), and 5.2% (30 isolates out of 574 samples) were isolated from cooling towers, hospitals, public bath, fountains, and others, respectively (Table 2).

Table 2. Number of *Legionella pneumophila* and *Legionella* spp. positive samples from the public facilities in Seoul, Korea from 2013 to 2017

Year	No. of + Samples[a]/No. of Total Samples[b] (Detection Rate; %)					Total
	Cooling Tower[c]	Hospital[d]	Public bath[e]	Fountain[†]	Others	
2013	66/317(20.8)	24/270(8.9)	101/396(25.5)	0/24(0.0)	0/85(0.0)	191/1,092(17.5)
2014	55/283(19.4)	45/242(18.6)	74/370(20.0)	0/25(0)	6/95(6.3)	180/1,015(17.7)
2015	38/251(15.1)	20/203(9.9)	49/351(14.0)	1/27(3.7)	1/88(1.1)	109/920(11.8)
2016	45/292(15.4)	21/332(6.3)	87/376(23.1)	1/27(3.7)	4/122(3.3)	158/1,149(13.8)
2017	79/452(17.5)	80/978(8.2)	128/779(16.4)	0/29(0.0)	19/184(10.3)	306/2422(12.6)
Subtotal	283/1,595(17.7)	190/2,025(9.4)	439/2,272(19.3)	2/132(1.5)	30/574(5.2)	944/6,598(14.3)

[a]Number of *Legionella pneumophila* and *Legionella* spp. positive water samples; [b]Number of water samples collected and tested for *Legionella* spp.; [c]Water samples of colling tower were collcted from cooling tower basins of large shopping malls, department stores, hotels, and large buildings; [d]Water samples were collected from toilets and showers in hospitals; [e]Water samples were collected from taps, showers, and hot tubes of public bath houses; [f]Water samples were obtained from the decorative fountains in the parks and squares.

The surveillance of LD is coordinated by KCDC, and Legionellosis data were collected from the National Notifiable Diseases Surveillance System database of KCDC. The latest data from 2013 to 2017 revealed 443 cases of LD in Korean population. The incidence of LD steadily increased every year (Fig. 1). However, it must be noted that the incidence of LD in 2016 was increased 184.4% compared with previous year, 2015 (Fig. 1). The monthly distribution of LD case showed that it occurred throughout the year with peaks in September of 2014, November of 2015, September of 2016, and July and November of 2017 (Fig. 1).
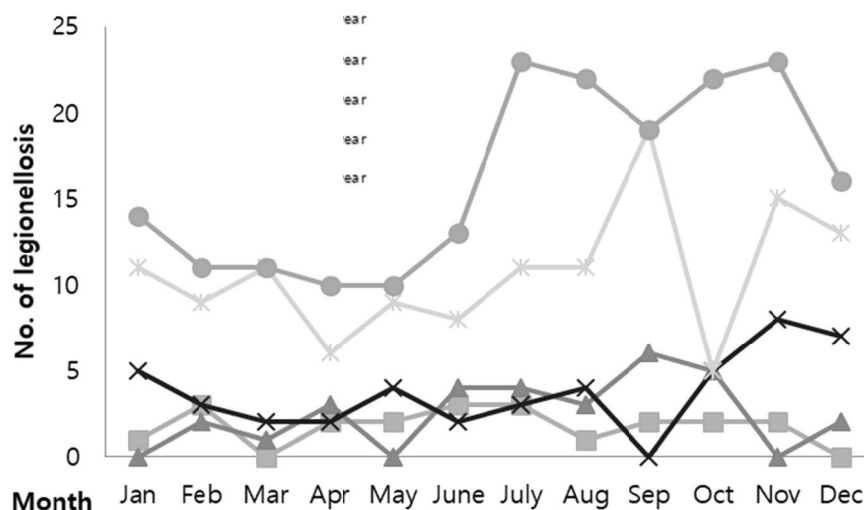

Figure 1. Monthly Distribution of Legionellosis from 2013 to 2017.

Correlation analysis between the incidence of LD and climatic factors showed no significant correlation except average temperature. The average temperature showed significantly positive linearity with the incidence of LD (R^2=0.8075) (Table 3) (Fig. 2). In addition, changes in the isolation rate of *Legionella* spp. showed a similar trend to that of precipitation (P=0.708, ANOVA) (Fig. 3.).
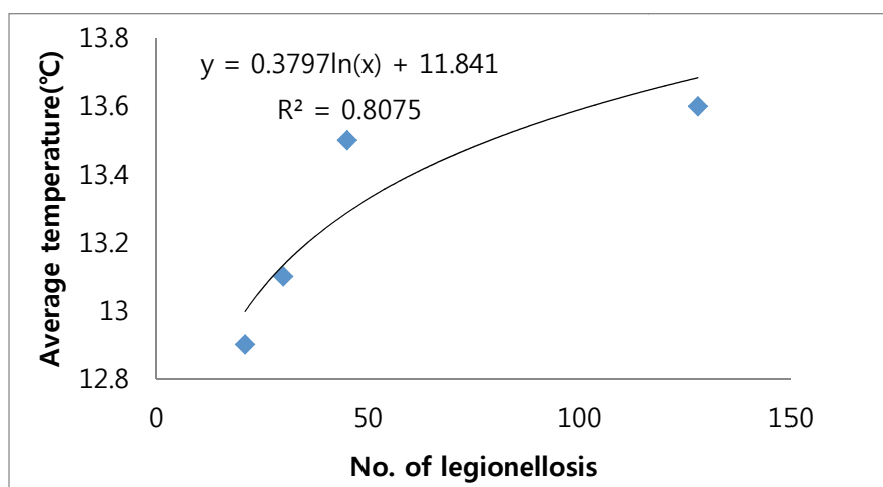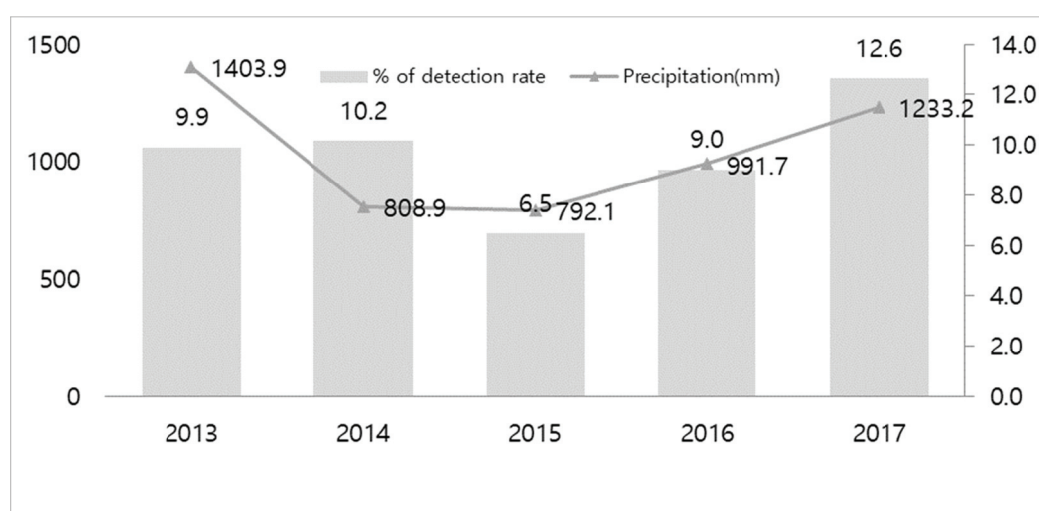

Figure 2. Simple regression analysis between average temperature and number of Legionellosis.

Table 3. Correlaton between Legionellosis and climate variables from 2013 to 2016

Year	2013	2014	2015	2016
[a] No. of Legionellosis	21	30	45	128
Average temperature (℃)	12.9	13.1	13.4	13.6
Precipitation (mm)	1162.4	1158.4	939.2	1264.1
PM2.5 (μg/㎥)	25	24	23	26
NO_2 (ppb)	33	33	32	31
Number of the date of issue O_3 warning	9	8	2	17

[a] Number of Legionellosis

Figure 3. Yearly changes in precipitation and isolation rate of *Legionella* spp.

DISCUSSION

During the summer of 2015, New York, NY, USA, had one of the largest and deadliest outbreaks of Legionnaires' disease in the history of the United States. A total of 138 cases and 16 deaths were linked to a single cooling tower in the South Bronx (18). Based on the increase in LD in Europe and Korea, we have explored the current knowledge about LD, engineering controls that might prevent future disease outbreaks and future research that is needed to investigate the seasonality of LD and its association with climatic factors to predict the epidemic in Korea (2). Reported LD incidence and patterns of disease can vary greatly based on political boundaries, potentially due to factors other than climate and geography (7).

Considering the high percentage of *Legionella* community-acquired infections, Italian and European literature data on *Legionella* contamination in air conditioning systems and hot water networks are available both for hospitals (19, 20), and small residential buildings (21). A previous report based on epidemiological data, suggests that one is more likely to contract legionellosis in public places, such as hospitals, hotels, and nursing homes in air conditioning systems, than in private ones (22, 23). Cooling towers are perhaps the most widely known source of legionellosis (24, 25). A relatively high proportion of detection rate, especially in cooling towers (17.7%) and public water bath (19.3%), is consistent with the results of other recent investigations on the *Legionella* distribution surveys (26, 27). The rapid increase in the number of cooling towers is the reason behind the recent large outbreaks, in the Bronx, NY, USA (28). Previous reports indicate that these devices can

generate large volumes of aerosols from a heated water source that is frequently contaminated by *Legionella*, and their aerosols can be produced over extended periods of time and disseminated over long distances (29, 30). Legionellosis outbreaks have also been associated with water sources in the built environment including plumbing sources (e.g., showers, faucets, and toilets), cooling towers, respiratory devices (e.g., humidifiers, vaporizers, and nebulizers), swimming pools (including spas/hot tubs and whirlpools), steam-producing appliances, and ornamental fountains (2, 31~35).

Recently, a steady increase in LD has been described, and the increase has been reported to be consistent with the improvement in surveillance and effect of demographic change with increasing numbers of at-risk older people (13). Taken together, the reasons for this increase are unknown but might be partly a result of the increase in awareness about LD and the consequently increased testing for LD (9, 23).

Usually, in the summer and fall season (July to November), an increase in the cased of LD has been noted. A monthly analysis of cases in order to determine the proportion of *Legionella* infected patients with LD in 2017, revealed a peak of LD infection in July. The reason for such a high prevalence could be explained based on 2015 MERS Outbreak in Korea that led to increased awareness, testing, and susceptibility of the population. Although, there is no direct evidence, we speculated increase in *Legionella* in the environment, or some combination of factors as the possible cause.

Several studies strongly suggested that LD occurs mainly in the summer months (23, 36~42). In the United States, 62% of LD cases occur during June–October (Centers for Disease Control and Prevention (43), a period of generally warm weather when commercial air conditioning systems, including those with cooling towers (CTs), are in operation (44). Our results suggest that the incidence of LD varies considerably based on meteorological data and local weather pattern. Previous studies have demonstrated the effects of seasonality and weather patterns on LD. And the associations among weather, particularly temperature and humidity, drive the summer spike in incidence (36, 37, 40, 41). In the regression analysis, the primary variables of interest- PM2.5 (μg/m^3), NO$_2$ (ppb), and number of the date of issue O3 warning precipitation – were not significant with LD except for average temperature (R^2=0.8075 *Legionella* detection rate in Seoul, Korea showed a trend similar to precipitation (P=0.708, ANOVA). A similar pattern of occurrence of LD in warm (60°–80°F) and very humid ($\geq$80.0%) months of was observed in a few reports (45). Increase in rainfall led to an increase in the risk of occurrence; however, regardless of rainfall, warm and humid weather were a major risk factors. (13, 23, 38, 45). In the absence of any direct evidence, we speculated that *Legionella* spp. thrives in warm, and wet environments, which support the survival of the pathogen and existence of aerosolization.

Since 2000, LD has been designated as reportable infectious disease in Korea, and the incidence was between 0.04 and 0.09 per 100,000 people from 2010 to 2015 (46). However, there has been a rise in the number of annual reported cases, with an incidence of 0.39 per 100,000 people in 2017 (11). The reason for the rapid increase in remains unclear, although increased testing for differential diagnosis of pneumonia, enhancement in reporting, and growth of the old-age population are considered as probable contributing factors (47). In recent years, large outbreaks of LD such as in the Bronx, NY, USA (28), have raised public attention and generated momentum for stronger prevention efforts. There are many factors that contribute to the increased incidence of Legionnaires' disease. Although, there is no direct evidence, we speculated that people are at increased risk because of aging of the population, greater use of immunosuppressant drugs, and higher prevalence of comorbid conditions. It is also possible that the change in environmental conditions facilitates human exposure to aerosolized water containing *Legionella* spp. (15).

To reduce the incidence of LD, the World Health Organization and the US Center for Disease Control and Prevention have recommended the development of a regular monitoring program for water systems where *Legionella* is most likely to thrive (48, 49). It is well established that the heating process can degrade the level of disinfectant (49). Although our work has several limitations, we expect that the results will be useful for the design of a preventive approach for the surveillance of *Legionella*.

In conclusion, our work is the first study to evaluate the link between climatic variables in Korea from 2013 to 2017, monthly incidence of legionellosis, and the prevalence of *Legionella* spp. in cooling towers in public building and public baths in Korea.

In summary, our study describes that the prevalence of the recent increase in legionellosis was associated with marked summertime seasonality. In the regression analysis, the primary variables of interest, PM2.5 ($\mu g/m^3$), NO_2 (ppb), and the number of days of issue O_3 warning, were not significant except for average temperature (R^2=0.8075). The *Legionella* detection rate in Seoul, Korea showed a trend similar to precipitation (P=0.708, ANOVA). Although further studies are needed, our finding is consistent with the contention that contamination of cooling towers and public water bath are important causes of legionellosis. Our data promotes a preventive approach for the surveillance of *Legionella* in Seoul, Korea.

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