THE PRESENCE OF *DOT/ICM*, *LVH*, AND *RTXA* GENES IN *LEGIONELLA* SPECIES FROM PUBLIC FACILITIES IN BUSAN, KOREA 2020-2022

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Abstract. *Legionella* spp are prevalent worldwide in both constructed and natural water systems and the rates of their detection have increased in environments such as buildings, public baths, and hospitals. The study focused on detecting *Legionella* spp in public water systems in Busan from 2020 to 2022 and on investigating the presence of the virulence genes *dot/icm*, *lvh*, and *rtxA* in *Legionella*-positive water samples. *Legionella* isolates (n = 660) were collected, with *L. pneumophila* and non-*L. pneumophila* species accounting for 80.8 and 19.2% respectively. Among *L. pneumophila* isolates (n = 533), 196 belonged to serogroup 1 and 337 to serogroups 2-14, both known to cause legionellosis. Among the non-*L. pneumphila* isolates (n = 127), *L. anisa* was the most common, followed by *L. nautarum*, then *L. busanensis* and *L. londiniensis*. At least one virulence gene was detected in 80.6% of all *Legionella*-positive isolates, with the majority of *L. pneumophila* carrying virulence genes compared to 9% of non-*L. pneumophila* spp. The presence of virulence genes in *L. pneunophila* and non-*L. pneumophila* spp collected from public water sources in Busan constitutes a potential risk of infection outbreaks within the city.

Keywords: *Legionella* spp, environmental surveillance, public facility, virulence gene

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INTRODUCTION

Legionellosis typically manifests as two distinct diseases clinically: i) Legionnaires' disease, a severe multisystem illness with pneumonia, and ii) Pontiac fever, a mild, self-

limiting flu-like illness (Fields *et al*, 2002; Bartram *et al*, 2007). There are more than 73 *Legionella* spp, divided into 94 serogroups, with approximately 50% associated with clinical cases of legionellosis (Leibniz Institute DSMZ, n.d.). Among those causing legionellosis, *L. pneumophila* serogroup 1 (sg1) is the main causative pathogen, accounting for 70-90% of cases (Fields *et al*, 2002).

Legionella spp are widely distributed at low concentrations in the environment. However, due to industrialization, *Legionella* can now thrive in hot- and cold-water pipes of apartments, hospitals, hotels, and bathrooms. Production of biofilms lining the inner walls of pipes allows *Legionella* to exist at high concentrations (Bartram *et al*, 2007). Human infection occurs after inhaling aerosol from an aquatic environment or aspirating water contaminated by *Legionella* (Fields *et al*, 2022). Many types of *Legionella* spp can multiply intracellularly, without being digested or destroyed, in various protists in the environment, such as amoeba (genera *Acanthamoeba, Dactylopodida, Naegleria*, and *Vermamoeba*), ciliated protozoon (genera *Cyclidium, Paramecium* and *Tetrahymena*) and slime mold (genus *Dictyostelium*) (Boamah *et al*, 2017; Saoud *et al*, 2021). This ability to parasitize protozoa allows *Legionella* to survive and thrive in harsh external environments (Bartram *et al*, 2007; Gattuso *et al*, 2022).

Humans are incidental hosts for *Legionella* and their ability to infect humans is believed to stem from a long co-evolution with unicellular organisms. The virulence factors that allow *Legionella* to infect these unicellular hosts also play a critical role in infecting human alveolar macrophages (Chamber *et al*, 2021). However, there are differences in the ability of different *Legionella* spp to withstand the external environment and cause infection in humans (Sreenath *et al*, 2020; Chauhan and Shames, 2021). Not only is it necessary for the *Legionella* to carry pathogenic genes but also to possess a secretion apparatus to deliver proteins or other factors for its survival into the host cell (Gattuso *et al*, 2022).

Legionella contains two secretion systems, one that exports proteins to the extracellular environment, the Type 1 secretion system (T1SS and T2SS), and another that injects

proteins into the host cell, the Type IV secretion system (T4ASS and T4BSS). Of the latter system, T4ASS *lvh* (*Legionella* vir homolog) and T4BSS *icm*/*dot* (intracellular multiplication/defective for organelle trafficking) systems are present in several *Legionella* spp (Fuche *et al*, 2015). *Lvh* is involved in conjugation, virulence and survival in the environment (Sreenath *et al*, 2020) while *icm*/*dot* is necessary for *Legionella* intracellular replication and inhibition of host cell ribosome function, which leads to cell death, allowing the bacteria to exit from the host cell (Chauhan and Shames, 2021). Additionally, TISSs are responsible for secreting repeats-in-toxin (*rtxA*) protein, which facilitates bacterial attachment and entry into host cells. *rtxA* gene also plays a crucial role in *Legionella* intracellular survival and trafficking, helping bacteria evade the host immune response and promoting persistent infection, and disrupting host cell function, thereby contributing to bacterial pathogenesis (Sreenath *et al*, 2020).

Research on *Legionella* spp has primarily focused on *L. pneumophila*, with lesser attention paid to the distribution and pathogenicity of non-*L. pneumophila* species. Thus, the study investigated not only *L. pneumophila* isolated from man-made water systems in Busan, Korea from 2020 to 2022, but also non-*L. pneumophila* spp. Strain identification of non-*L. pneumophila* spp was conducted through serotyping or DNA sequencing, and pathogenic characteristics were assessed by identifying the carriage of *icm*/*dot*, *lvh*, and *rtx*. The distribution and pathogenicity of *Legionella* spp in the water system of Busan will provide baseline data for the development of a prevention and management policy for *legionellosis* in the city.

MATERIALS AND METHODS

Samples collection

Water samples (n = 5,055) were collected from the public water systems of facilities such as buildings, public baths, hospitals, and hotels in Busan, Korea between 2020 and 2022. Water samples (one l aliquot) were aseptically collected in sterile plastic containers, stored at 4-8 ^{o}C during transportation to the laboratory, and kept in the dark until analyzed (NIER, 2013; KDCA, 2023).

Legionella culture

Water samples were concentrated by filtration through a mixed cellulose esters membrane (0.45 μm pore size, 90 mm diameter; MF-Millipore™, Merck, Darmstadt, Germany) using a vacuum system under sterile conditions. The concentrated samples (20 ml) were divided into three portions and each portion was subjected to sonication following heating at 50°C for 30 minutes, plated onto glycine-vancomycin-polymyxin B-cycloheximide (GVPC) agar plates (bioMérieux, Marcy l'Etoile, France). The agar plates were incubated at 37±1°C for 7 days in a humidified chamber. Unconcentrated water samples were also similarly processed. To confirm the identities of *Legionella* spp, all collected isolates were subcultured onto Buffered charcoal yeast extract (BCYE) agar (Difco, Detroit, MI) without L-cysteine (Merck, Darmstadt, Germany). Colonies that required L-cysteine for growth were defined as *Legionella* species. The detection limit of this procedure was 200 CFU/l (KDCA, 2023).

Identification of *Legionella* spp and serogroups

A *Legionella* Latex test (Oxoid, Basingstoke, Hampshire, UK) was employed to identify *L. pneumophila* (sg 1 and sg 2-14) and non-*L. pneumophila* spp, namely, *L. anisa, L. bozemanii* (sg 1 and 2), *L. domoffi, L. gormanii, L. jordanis, L. longbeachae* (sg 1 and 2), and *L. micdadei*.

Detection of *Legionella* spp and virulence genes by PCR

Legionella spp were also identified using PCR, employing primers for the *Legionella*-specific 16S rRNA gene, *rpoB* and *L. pneumophila*-specific *mip* (Table 1). A *Legionella* isolate was suspended in 100 μl of sterile distilled water, incubated at 100 °C for 15 minutes, centrifuged at 20,000 g for 5 minutes at 4 °C, and the supernatant was used as the source of template DNA. PCR was carried out in a 20 μl reaction mixture containing 20 pmol of each primer (Table 1), 5 μl of DNA solution and Maxime PCR PreMix (INtRON Biotechnology, Seongnam, Gyeonggi, Korea). Amplification was performed using SimpliAmp Thermal Cycler (Applied biosystems, Waltham, MA)

Table 1

List of primers used in the study

Target gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
16S rRNA	Pl.2	AGGGTTGATAGGTTAAGAGC	386	Hwang *et al* (2023)
	Cp3.2	CCAACAGCTAGTTGACATCG		
mip	Lpm-1	AGGGTTGATAGGTTAAGAGC	630	Hwang *et al* (2023)
	Lpm-2	CCAACAGCTAGTTGACATCG		
rpoB	RL1	GATGATATCGATCAYCTDGG	369	Kim *et al* (2014)
	RL2	TTCVGGCGTTTCAATNGGAC		
dot	dotDCB-F	CGATTGGTCTGGTCCGATTGA	1,900	Alli *et al* (2003)
	dotDCB-R	TCTCGAATAATGGAAGCTAACAATGTC		
icm	icmJB-F	TGCCATGTTCTTTTTTGTGCTATTAC	2,200	Alli *et al* (2003)
	icmJB-R	GAGCGTAAACCAGATCAATCCAAGTAG		
	icmLK-F	CGGAAGGCTGGGACCAATT	1,200	
	icmLK-R	CCACTCGATAATCCACGGCTTC		
	icmTSRQ-F	CACAGTTAAAACTTCAAGCTGAACC	2,500	
	icmTSRQ-R	CTGCTCAGAGCTATTTTT		
	icmWX-F	TGGGTTGGTTCCTGAGGTATGA	1,200	
	icmWX-R	TGGGGCGCTGAAATTTTGATAT		

Table 1 (cont)

Target gene	Primer	Sequence (5'-3')	Amplicon size (bp)	Reference
lvh	prpA-lvh1	GTTTTAATCCCCCAGCAAGC	260	Samrakandi *et al* (2002)
	prpA-lvh2	AATATCCCTACTCATCCTCG		
	lvhB3-lvh3	GGCTAGGTTCTTGTG	1,000	
	lvhB4-lvh4	ATTGGCCGAGATGTCCTT		
	lvhB8-lvh5	CCTCTACGCATTACAACGCC	280	
	lvhB9-lvh6	GTGGTGGTAAAGGGAATGCC		
	lvrE-lvr1	GGTCCAATGGGTCCAGCAGG	420	
	lvrE-lvr2	AGTGGCTGATTCTGGAGTGG		
rtxA	rtxA1-rtx1	GATCCGCAAGTAGCGCTCAC	630	Samrakandi *et al* (2002)
	rtxA1-rtx2	TGTAATGCTGGCATTAGGCG		
	rtxA2-rtx3	CTGATGCTGCTACGGAACAC	540	
	rtxA2-rtx4	CCGCAGTCATTACACCTGCG		

Note: In addition to known bases A (Adenine), G (Guanine), C (Cytosine), and T (Thymine), D in some of the sequences above refers to A or G or T; N refers to any base; V refers to A or C or G; and Y refers to C or T.

bp: base pair

under the following conditions: 94 °C for 3 minutes; 30 cycles of 94 °C for 20 seconds, 60 °C for 10 seconds and 72 °C for 60 seconds; followed by a final step of 75 °C for 3 minutes. For the *dot/icm* amplification, the conditions were 95 °C for 5 minutes; 30 cycles of 94 °C for 60 seconds, 60 °C for 60 seconds, and 72 °C for 60 seconds; followed by a final step of 72 °C for 5 minutes. Amplicons were analyzed using an automated capillary electrophoresis system (QIAxcel, Qiagen, Hilden, Germany). Non-*L. pneumophila* spp were identified by amplification of *rpoB*, followed by direct DNA sequencing (Macrogen, Seoul, Korea) and comparison with sequences deposited at the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

RESULTS

Legionella spp detection

Legionella spp (n = 660 isolates) were collected from water samples of multi-use facilities in Busan over three years: n = 205, 237, and 218 isolates in 2020, 2021, and 2022, respectively. During the *Legionella* and non-*Legionella* spp identification process, samples that could not be specifically identified were excluded from further analysis.

Among the 660 isolates, 533 (80.8%) were identified as *L. pneumophila* and 127 as non-*L. pneumophila* spp (Table 2). The *L. pneumophila* isolates belonged to serogroup 1 (n = 196), the major cause of Legionnaires' disease, and to serogroups 2-14 (n = 337) (Table 2). The non-*L. pneumophila* isolates consisted of *L. anisa* (n = 63), *L. erytha* (n = 4), *L. bozemanii* (n = 4), *L. busanensis* (n = 9), *L. domoffi* (n = 4), *L. londiniensis* (n = 9), *L. nautarum* (n = 17), *L. rubrilucens* (n = 6), and other *Legionella* spp (n = 12) detected in 1 or 2 isolates (Table 2).

Among the non-*L. pneumophila* spp collected from 2020 to 2022, *L. anisa* was predominant (>50%) for the first two years, and then in 2022 its relative proportion dropped to <25% and that of *L. busanensis* rose to 21% (Fig 1).

Virulence genes detection

The *Legionella* isolates were assayed for the carriage of

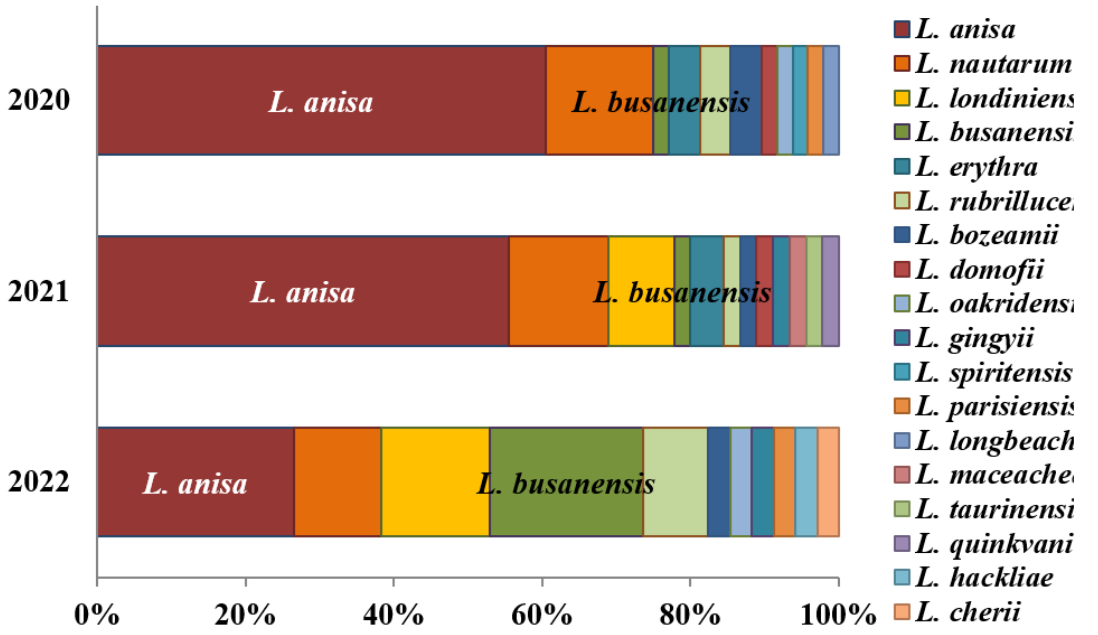

Fig 1 - Distribution of non-*Legionella pneumophila* spp isolated from public facilities, Busan, Korea 2020-2022

Note: Non- *Legionella pneumophila* spp were identified by PCR amplification of *rpoB*, followed by direct DNA sequencing (Macrogen, Seoul, Korea) and comparison with sequences deposited at the National Center for Biotechnology Information (NCBI) database (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

DNA: Deoxyribo Nucleic Acid; PCR: Polymerase Chain Reaction

pathogenic genes *dot/icm*, *lvh*, and *rtxA*; the results of which revealed the presence of all three genes in 278 isolates, *dot/icm* + *rtxA* in 90 isolates, *dot/icm* + *lvh* in 58 isolates, *lvh* + *rtxA* in 22 isolates, a single pathogenic gene in 85 isolates, with no pathogenic gene and 127 isolates (Table 2). Among *L. pneumophila* isolates, 2.4% (13/533) lacked all three pathogenic genes, while non-*L. pneumophila* isolates showed a significantly higher fraction, 90% (114/127), indicating that non-*L. pneumophila* spp were more likely to be non-pathogenic (Table 2).

Table 2

Legionella spp. and associated virulence genes isolated from public facilities in Busan, Korea 2020-2022

Legionella species	Number of samples with virulence gene(s)								
	dot/icm-lvh-rtxA	*dot/icm-lvh*	*dot/icm-rtxA*	*lvh-rtxA*	*dot/icm*	*lvh*	*rtxA*	ND	Total
L. pneumophila									
sg1	129	10	23	8	16	4	1	5	196
sg2-14	149	48	67	13	45	6	1	8	337
non-*L. pneumophila*									
L. anisa	0	0	0	1	1	4	0	57	63
L. bozemanii	0	0	0	0	0	0	0	4	4
L. busanensis	0	0	0	0	1	0	0	8	9
L. cherii	0	0	0	0	0	0	0	1	1
L. domoffi	0	0	0	0	0	1	0	1	2
L. erythra	0	0	0	0	0	0	0	4	4
L. gingyii	0	0	0	0	0	0	0	1	1
L. hackeliae	0	0	0	0	0	0	0	1	1
L. londiniensis	0	0	0	0	1	0	0	8	9
L. longbeachae	0	0	0	0	0	0	0	1	1
L. maceachemii	0	0	0	0	0	0	0	1	1

Table 2 (cont)

Legionella species	Number of samples with virulence gene(s)								
	dot/icm-lvh-rtxA	*dot/icm-lvh*	*dot/icm-rtxA*	*lvh-rtxA*	*dot/icm*	*lvh*	*rtxA*	ND	Total
L. nautarum	0	0	0	0	0	0	0	17	17
L. oakridgensis	0	0	0	0	0	1	0	1	2
L. parisiensis	0	0	0	0	1	0	0	1	2
L. qingyii	0	0	0	0	0	0	0	1	1
L. quinlivanii	0	0	0	0	0	1	0	0	1
L. rubrilucens	0	0	0	0	0	0	0	6	6
L. spiritensis	0	0	0	0	0	0	0	1	1
L. taurinensis	0	0	0	0	0	0	0	1	1
Total number (%)	278 (42.1)	58 (8.8)	90 (13.6)	22 (3.3)	65 (9.9)	17 (2.6)	2 (0.3)	128 (19.4)	660 (100.0)

ND: not detected

No non-*L. pneumophila* isolates carried all three pathogenic genes, one isolate had two genes (*lvh* + *rtx*), four isolates with *dot*/*icm*, and seven isolates with *lvh* (Table 2). Of the six *L. anisa* isolates, *lvh* was present in four isolates, and *lvh* + *rtx* and *dot*/*icm* in one isolate each. One isolate each of *L. busanensis*, *L. londiniensis* and *L. parisiensis* carried *dot*/*icm*; one isolate each of *L. domoffi*, *L. oakridgensis* and *L. quinlivanii* contained *lvh*. Of note, the presence of a pathogenic gene in one isolate each of *L. busanensis* and *L. londiniensis* is of interest as these two species as well as *L. nautarum*, *L. quinlivanii*, *L. spiritensis*, and *L. taurinenesis* are considered non-pathogenic, as they have not previously been associated with human cases.

DISCUSSION

According to an analysis conducted by the Korea Disease Control and Prevention Agency (KDCA) in Korea during 2018 – 2019 on the distribution patterns of environmental *Legionella* spp originating from domestic multi-use facilities, *L. pneumophila* accounted for 85.4% (comprising sg1, 31.7%; sg 2-14, 44.8%; undefined serogroup, 8.9%) and non-*L. pneumophila* for 14.6% (consisting of *L. anisa* (predominant), *L. feeleii*, *L. dumoffii*, *L. micdadei*, and *L. rubrilucense*) of the total isolates (Lee *et al*, 2020). These findings are consistent with the current study.

Doleans *et al* (2004) reported that in France *L. anisa* has the highest environmental isolation rate (13.8%), although its clinical isolation rate is only 0.8%. Despite this finding, *L. anisa* is known to cause pneumonia, Pontiac fever, osteomyelitis due to pneumonia, chronic endocarditis, aortic aneurysm, and arthritis (Roussotte and Massy, 2022). It is also a cause of hospital water system contamination and nosocomial infection. Furthermore, there are concerns that *L. anisa* could mask water contamination by *L. pneumophila* (van der Mee-Marquet *et al*, 2006).

L. busanensis was first discovered in a cooling tower in Busan in 1991 (Park *et al*, 2003). Its distribution is increasing in Busan, warranting continued attention despite uncertainty regarding its

infectivity. *L. rubrilucens* has been isolated from pneumonia patients (Matsui *et al*, 2010). *L. bozemanii*, *L. longbeachae* and *L. micdadei* collectively account for 2-7% of *Legionella* infections worldwide (Chauhan and Shames, 2021). Notably, *L. longbeachae* is a major cause of Legionnaires' disease in Australia and New Zealand, being commonly associated with compost and potting soil (Muder and Yu, 2002; Chamber *et al*, 2021). However, *L. busanensis*, *L. londiniensis*, *L. nautarum*, *L. quinlivanii*, *L. spiritensis*, and *L. taurinenesis* are still considered non-pathogenic to humans (Leibniz Institute DSMZ, n.d.).

Legionella spp can enter protozoa or host cells and form *Legionella*-containing vesicles (LCVs) derived from the endoplasmic reticulum. Within a few minutes after entry, the Type IV secretion system (T4SS) is expressed, transferring proteins or protein-DNA complexes into the host cell cytoplasm. This system involves the expression of *dot/icm* that plays a crucial role in cellular proliferation and pathogenicity (Cazalet *et al*, 2004).

Legionella secretion pathway (Lsp) or Type II secretion system (T2SS) is required for survival in various environmental conditions (Ridenour *et al*, 2003). Lsp-associated *lvh* influences host cell infection in a temperature-dependent manner. It is critical for intracellular survival and replication at 30 °C and at 37 °C acts as a growth factor (Ridenour *et al*, 2003).

rtxA encodes a toxin involved in *L. pneumophila* entry into host cells (Sreenath *et al*, 2020). The N- and C-terminal domains of *rtxA* are responsible for adhesion and pore formation respectively (Huang *et al*, 2006). Thus, *dot/icm*, *lvh*, and *rtxA* genes are essential for the infection and survival of *Legionella* spp in humans. The detection of these genes in *L. pneumophila* isolates should assist in predicting the virulence of *Legionella* spp distributed in Busan.

The current study demonstrated *L. pneumophila* isolates harbored at least one of the three test virulence genes, as reported in Australia, Greece, and PR China (Huang *et al*, 2004; Katsiaflaka *et al*, 2016; Zeng *et al*, 2019). On the other hand, in the current study non-*L. pneumophila*

spp had lower distribution frequencies of virulence genes than *L. pneumphila*. However, given that the non-*L. pneumophila* spp have been associated with respiratory diseases, skin and soft tissue infections, septic arthritis, and bacterial endocarditis, diagnosing *Legionella* infections caused by these pathogenic bacteria can be challenging (Lowry *et al*, 1993) and raises the possibility of underdiagnosis and underrecognition of cases caused by these *Legionella* spp. In addition, the interaction of *Legionella* with the natural environment suggests that if conditions necessary for proliferation within host cells are met, even certain non-*L. pneumophila* spp could have a high likelihood of causing disease.

While the pathogenicity of *L. pneumophila* is firmly established, the detection of pathogenicity among non-*L. pneumophila* spp, often in isolated cases or outbreaks, is increasing (Lowry *et al*, 1993; Vaccaro *et al*, 2016; Head *et al*, 2019; KDCA, 2023). Therefore, there is a need to focus on *L. busanensis* that has shown an increase in prevalence in Busan during the study period, and possibly in other regions of Korea.

In conclusion, the study of *Legionella* spp isolated from public water facilities in Busan, Korea from 2020 to 2022 revealed the prevalence of pathogenic *L. pneumophila* as well as a rise in prevalence of *L. busanensis* and a decrease in that of *L. anisa*. However, the presence of *Legionella* spp in public water supplies cannot be extrapolated to the actual impact of *Legionella* carrying pathogenic genes on humans. Further research on *Legionella* spp isolated from both the environment and humans, along with parallel studies on *Legionella* carriage of pathogenic genes and their association with human infections, are needed to deepen our understanding of *Legionella* spp and their relationship with outbreaks of Legionnaires' disease. The identification of *Legionella* sources and their associations with disease outbreaks provides important information for the development of effective response measures to infections caused by these bacteria.

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CONFLICT OF INTEREST DISCLOSURE

The authors declare no conflict of interest.

REFERENCES

Alli OAT, Zink S, von Lackum NK, Abu-Kwaik Y. Comparative assessment of virulence traits in *Legionella* spp. *Microbiol (Reading)* 2003; 149(Pt 3): 631-41.

Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S, editors. *Legionella* and the prevention of Legionellosis. Geneva, Switzerland: World Health Organization; 2007.

Boamah DK, Zhou G, Ensminger AW, O'Connor TJ. From many hosts, one accidental pathogen: the diverse protozoan hosts of *Legionella*. *Front Cell Infect Microbiol* 2017; 7: 477.

Cazalet C, Rusniok C, Brüggemann H, *et al*. Evidence in the *Legionella pneumophila* genome for exploitation of host cell functions and high genome plasticity. *Nat Genet* 2004; 36(11): 1165-73.

Chambers ST, Slow S, Scott-Thomas A, Murdoch DR. Legionellosis caused by non-*Legionella pneumophila* species, with a focus on *Legionella longbeachae*. *Microorganisms* 2021; 9(2): 291.

Chauhan D, Shames SR. Pathogenicity and virulence of *Legionella*: intracellular replication and host response. *Virulence* 2021; 12(1): 1122-44.

Doleans A, Aurell H, Reyrolle M, *et al*. Clinical and environmental distributions of *Legionella* strains in France are different. *J Clin Microbiol* 2004; 42(1): 458-60.

Fields BS, Benson RF, Besser RE. *Legionella* and Legionnaires' disease: 25 years of investigation. *Clin Microbiol Rev* 2002; 15(3): 506-26.

Fuche F, Vianney A, Andrea C, Doublet P, Gilbert C. Functional type 1 secretion system involved in *Legionella pneumophila* virulence. *J Bacteriol* 2015; 197 (3): 3563-71.

Gattuso G, Rizzo R, Lavoro A, *et al*. Overview of the clinical and molecular features *Legionella pneumophila*: focus on novel surveillance and diagnostic strategies. *Antibiotics (Basel)* 2022; 11(3): 370.

Head BM, Trajtman A, Bernard K, *et al*. *Legionella* co-infection in HIV-associated pneumonia. *Diagn Microbiol Infect Dis* 2019; 95(1): 71-6.

Huang B, Heron BA, Gray BR, Eglezos S, Bates JR, Savill J. A predominant and virulent *Legionella pneumophila* serogroup 1 strain detected in

isolates from patients and water in Queensland, Australia, by an amplified fragment length polymorphism protocol and virulence gene-based PCR assays. *J Clin Microbiol* 2004; 42(9): 4164-8.

Huang B, Yuan Z, Heron BA, *et al*. Distribution of 19 major virulence genes in *Legionella pneumophila* serogroup 1 isolates from patients and water in Queensland, Australia. *J Med Microbiol* 2006; 55(Pt 8): 993-7.

Hwang SJ, Shin SJ, Kang JH, Na YR, Park EH. Characteristics of *Legionella* species isolated from the public facilities in Busan, 2013-2022. *J Bacteriol Virol* 2023; 53(2): 100-7.

Katsiaflaka A, Pournaras S, Kristo I, *et al*. Epidemiological investigation of *Legionella pneumophila* serogroup 2 to 14 isolates from water samples by amplified fragment length polymorphism and sequence-based typing and detection of virulence traits. *Appl Environ Microbiol* 2016; 82(20): 6102-8.

Kim DU, Yeo SG, Baek KA, *et al*. *Legionella* contamination and molecular identification in water systems of the public facilities in Korea. *J Pure Appl Microbiol* 2014; 8(5): 3397-409.

Korea Disease Control and Prevention Agency (KDCA). Management guidelines for legionellosis, 2023 [cited 2023 Dec 15]. Available from: URL: https://www.kdca.go.kr/filepath/boardSyview.es?bid=0019&list_no=722227&seq=1 [in Korean]

Lee J, Jung SO, Yoo JE, Hwang KY. Distribution of *Legionella* species from environmental water sources of public facilities in South Korea, 2018-2019, 2020 [cited 2022 Aug 08]. Available from: URL: https://www.kdca.go.kr/filepath/boardSyview.es?bid=0034&list_no=367545&seq=1 [in Korean]

Lowry PW, Tompkins LS. Nosocomial legionellosis: a review of pulmonary and extrapulmonary syndromes. *Am J Infect Control* 1993; 21(1): 21-7.

Leibniz Institute DSMZ. List of prokaryotic names with standing in nomenclature (LPSN), n.d. [cited 2024 Aug 04]. Available from: URL: https://lpsn.dsmz.de/search?word=Legionella

Matsui M, Fujii SI, Shiroiwa R, *et al*. Isolation of *Legionella rubrilucens* from a pneumonia patient co-infected with *Legionella pneumophila*. *J Med Microbiol* 2010; 59(Pt 10): 1242-6.

Muder RR, Yu VL. Infection due to *Legionella* species other than

L. pneumophila. Clin Infect Dis 2002; 35(8): 990-8.

National Institute of Environmental Research (NIER). Analysis method for *Legionella* in environment, 2013 [cited 2023 Apr 06]. Available from: URL: https://www.nier.go.kr/NIER/cop/bbs/selectNoLoginBoardArticle.do?menuNo=13001&bbsId=BBSMSTR_000000000031&nttId=14892&Command=READ [in Korean]

Park MY, Ko KS, Lee HK, Park MS, Kook YH. *Legionella busanensis* sp. nov., isolated from cooling tower water in Korea. *Int J Syst Evol Microbiol* 2003; 53(Pt 1): 77-80.

Ridenour DA, Cirillo SL, Feng S, Samrakandi MM, Cirillo JD. Identification of a gene that affects the efficiency of host cell infection by *Legionella pneumophila* in a temperature-dependent fashion. *Infect Immun* 2003; 71(11): 6256-63.

Roussotte M, Massy E. Case report of arthritis caused by *Legionella anisa* and review of the literature. *BMC Infect Dis* 2022; 22(1): 633.

Samrakandi MM, Cirillo SL, Ridenour DA, Bermudez LE, Cirillo JD. Genetic and phenotypic differences between *Legionella pneumophila* strains. *J Clin Microbiol* 2002; 40(4): 1352-62.

Saoud J, Mani T, Faucher SP. The tail-specific protease is important for *Legionella pneumophila* to survive thermal stress in water and inside amoebae. *Appl Environ Microbiol* 2021; 87(9): e02975-20.

Sreenath K, Chaudhry R, Vinayaraj EV, *et al*. Distribution of virulence genes and sequence-based types among *Legionella pneumophila* isolated from the water systems of a tertiary care hospital in India. *Front Public Health* 2020; 8: 596463.

Vaccaro L, Izquierdo F, Magnet A, *et al*. First case of Legionnaire's disease caused by *Legionella anisa* in Spain and the limitations on the diagnosis of *Legionella* non-*pneumophila* infections. *PLoS One* 2016; 11(7): e0159726.

van der Mee-Marquet N, Domelier AS, Arnault L, *et al*. *Legionella anisa*, a possible indicator of water contamination by *Legionella pneumophila*. *J Clin Microbiol* 2006; 44(1): 56-9.

Zeng LZ, Liao HY, Luo LZ, *et al*. An investigation on the molecular characteristics and intracellular growth ability among environmental and clinical isolates of *Legionella pneumophila* in Sichuan Province, China. *Biomed Environ Sci* 2019; 32(7): 520-30.