Progress in Microbes and Molecular Biology

HH PUBLISHER

Review Article

Legionella pneumophila — The causative agent of Legionnaires' disease

Loh Teng Hern Tan[1,2†], Wei Yu Tee[2†], Tahir Mehmood Khan[3,4], Long Chiau Ming[5], Vengadesh Letchumanan[2*]

Article History

Received: 3rd March 2021;

Received in Revised Form: 3rd April 2021;

Accepted: 5th April 2021;

Available Online: 8th April 2021

†These authors contributed equally to the writing.

[1]Clinical School Johor Bahru, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Johor Bahru 80100, Malaysia; loh.teng.hern@monash.edu (LT-HT)

[2]Novel Bacteria and Drug Discovery (NBDD) Research Group, Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, 47500, Selangor Darul Ehsan, Malaysia; weiyuwy1@gmail.com (W-YT)

[3]Institute of Pharmaceutical Sciences (IPS), University of Veterinary & Animal Sciences (UVAS), Pakistan; tahir.khan@uvas.edu.pk (TMK)

[4]School of Pharmacy, Monash University Malaysia, 47500 Bandar Sunway, Selangor Darul Ehsan, Malaysia

[5]PAP Rashidah Sa'adatul Bolkiah Institute of Health Sciences, Universiti Brunei Darussalam, Gadong, Brunei Darussalam; long.ming@ubd.edu.bn (LCM)

*Corresponding author: Vengadesh Letchumanan, vengadesh.letchumanan1@monash.edu

Abstract: Over the years, *Legionella pneumophila* has increasingly become a public health threat that causes sporadic and epidemic community-acquired and nosocomial-acquired pneumonia. Thus, this review aims to discuss the current knowledge of *L. pneumophila*, focusing on the global epidemiology, clinical features, diagnosis and treatment of Legionnaires' disease (LD). *Legionella* bacteria are Gram-negative rod-shaped bacteria that are ubiquitous in aquatic environments. *L. pneumophila* was first discovered in 1976 and recognized as the causative agent of LD. *L. pneumophila* is a facultative intracellular pathogen that infects and replicates within eukaryotic host cells such as macrophages and protozoan. Diagnosis of LD remains a significant challenge as the clinical manifestation of LD is hardly distinguishable from pneumonia caused by other respiratory pathogens. Therefore, early testing and appropriate treatment are keys to alleviating the rising morbidity and mortality caused by LD.

Keywords: *Legionella*; pneumonia; amoeba; Legionnaires' disease; respiratory disease

1. Introduction

Throughout history, human populations have encountered several serious epidemics caused by pathogens[1–3]. Pathogens are various organisms that cause severe illnesses to their hosts, including bacteria, viruses, fungi and protozoa[4–11]. Among the various illnesses, respiratory infections represent the largest category of human disease and the leading cause of mortality globally[12–16]. Apart from the pneumonic plague caused by *Yersinia pestis* as one of the most significant outbreaks in human history, *Legionella* is another pathogenic bacteria that were discovered from investigations of an epidemic of a mysterious pneumonia outbreak in Philadelphia in 1976[17].

At the time of writing, there are more than 60 species and serogroups under the genus *Legionella*, a great variety of them can inflict human disease or legionellosis. Legionellosis is defined as an infection caused by bacteria under the genus *Legionella*, of which there are two distinct clinical manifestations in humans — Pontiac fever (PF) and Legionnaires' disease (LD)[18]. *Legionella pneumophila* serogroup 1 is the clinically most relevant species that cause LD in human, accounting for 90% of the total reported human infections. Meanwhile, *L. bozemanii*, *L. longbeachae*, *L. dumoffii* are the several species also known to cause LD[19]. Primarily, LD is caused by inhalation of aerosols or aspiration of water containing *Legionella* bacteria. Given the continued increasing incidence of LD globally, *Legionella* bacteria present a significant threat to human health. There was a 286% increase in legionellosis cases reported in the United States from the year 2000 to 2014[20]. Furthermore, a steady increase of LD cases was also observed in European countries, whereby more than 80% of the infections contributed by *L. pneumophila* serogroup 1[21]. Therefore, this review aims to update the current knowledge of *Legionella* bacteria prevalence, clinical manifestations, pathogenesis, detection and treatment strategies (Figure 1).

2. Sources of *Legionella* bacteria

Legionella is a Gram-negative, facultative aerobic and intracellular waterborne bacterium present in natural aquatic environments, such as lakes, rivers and groundwater, but at low concentrations[22]. The widespread distribution of *Legionella* bacteria is attributed to their ability to proliferate within diverse types of niches, including live in planktonic form, infect and replicate within protozoan hosts as well as coexist within multi-organismal biofilms developed on the surfaces of water systems[23,24]. Given the ubiquitous nature of *Legionella* bacteria in aquatic habitats, *Legionella* bacteria can easily enter and proliferate in man-manufactured water systems, including cooling towers and water distribution system of large buildings[25]. A relatively high prevalence of *Legionella* bacteria has also been found in public facilities such as hospitals, showers, hotels and hot water systems[26–29]. Several factors that enhance the colonization of *Legionella* bacteria in the water systems include lukewarm water temperature, obstruction and stagnation of water flow, plumbing materials, biofilm formation and presence of amoeba, which support the growth of *Legionella* sp. These risk factors are commonly present in the water distribution system of antiquated buildings,

especially in old hospitals[30]. In fact, half of the sporadic cases of hospital-acquired pneumonia are attributed to *Legionella* bacteria, where patients are infected via inhalation of *Legionella* sp. contaminated aerosols from medical devices like respiratory equipment, misting devices, cooling towers and hot tubs[31].

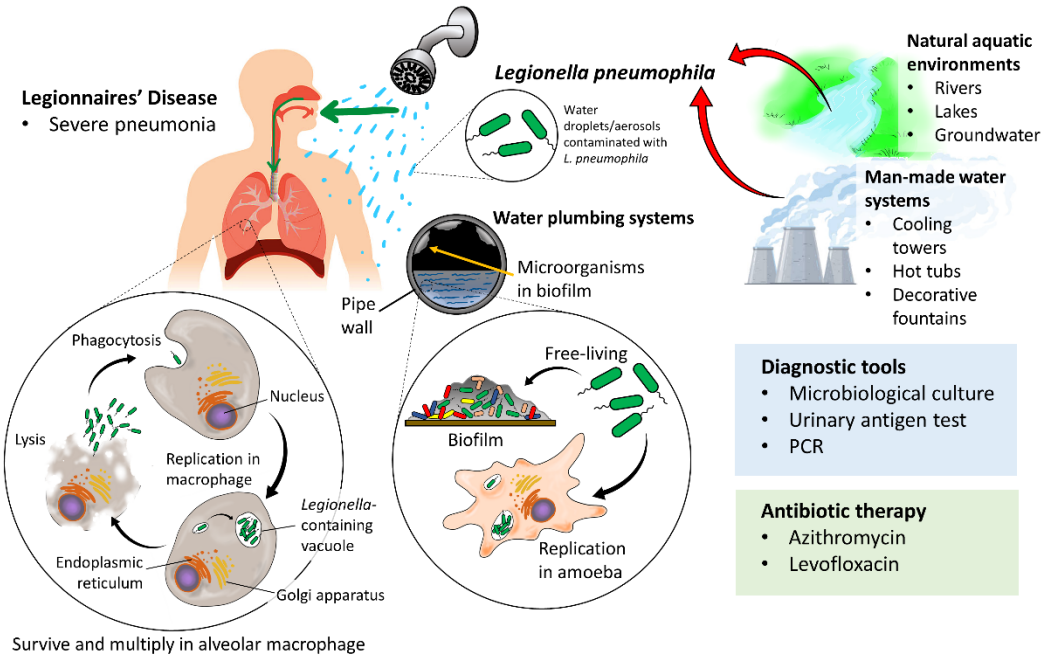

Figure 1. The transmission sources, life cycle within water systems and human macrophage, the diagnostic tools and antibiotic treatment for *Legionella pneumonia*.

Besides living in the planktonic phase, *L. pneumophila* utilizes amoebae as a host for survival and replication. Currently, there have been more than 40 species of *Legionella* sp. identified to possess the ability to parasitize amoebae, specifically *Acanthamoeba* and *Naegleria*[32]. Thus, these protozoans act as a vector of various pathogenic microorganisms[33], including *Legionella*, and facilitates pathogen dissemination. Being an intracellular pathogen, *Legionella* sp. also can infect and thrive within human macrophage [34]. A recent study demonstrated that the infection processes and the intracellular life cycle of *Legionella* within amoeba are remarkably similar to that occurred within human macrophages[35], although both are evolutionary distant hosts. Researchers believe that the *L. pneumophila* is primed for subsequent infection in the protist host and becoming more virulent and infectious after escaping from its environmental host, resulting in a more robust disease in humans[35,36]. In fact, a previous study indicated that intracellularly pre-grown *L. pneumophila* acquired higher resistance to disinfectants and antimicrobials, suggesting to confer increased infectivity to mammalian cells[37].

3. Epidemiology of Legionnaires' disease

Legionella pneumophila is a significant bacterial pathogen that causes severe sporadic and epidemic community-acquired and nosocomial acquired pneumonia[38]. An

estimate of 12 to 15% of overall mortality rate among hospitalized patients[39], the number may go up further to 27% for those who failed to receive adequate antibiotic treatment. Furthermore, LD is also commonly associated with poorer outcomes with the increasing length of hospitalization[40].

Dated back to July 1976 in Philadelphia, the first-ever reported LD outbreak due to *L. pneumophila* resulted in 29 deaths out of the total 180 persons attending the 56th annual American Legion Convention[41]. Over the years, the burden of LD is increasing each year in the United States and European countries. In the United States, there was an incidence of 1.89 cases of LD per 100,000 populations in 2015. Similarly, 1.4 cases of LD per 100,000 populations were reported by the European Centre for Disease Prevention and Control in 2016. Recently, a study in Italy reported an incidence rate of 48.9 cases per 100,000 populations for legionellosis in 2018, of which 3.4% was of nosocomially acquired cases[42]. In Hong Kong, the incidence of LD was increasing from 0.16 cases/100,000 population in 2005 to 0.91 cases/100,000 population in 2015[43]. Among all the *Legionella* sp. identified to date, *L. pneumophila* has responsible for 80–90% of the LD cases reported in the United States and Europe. Within the similar *Legionella* species, *L. pneumophila* serogroup 1 accounts for approximately 90% of cases.

Generally, the sources of outbreaks for nosocomial acquired LD are attributed to the contaminated water in the hospital plumbing system and the exposure to contaminated cooling towers. From 2006 to 2017, cooling waters, air conditions and evaporative condensers were reported to have contributed a major portion (60%) of outbreak-associated deaths due to LD or PF. Moreover, building water systems contributed to approximately 13% and 17% of the outbreak-related cases and deaths, respectively[44]. Besides that, The European Working Group for *Legionella* Infections and the United States Centers for Disease Control and Prevention (CDC) have identified numerous cases of travel-associated LD where the most common source is the contaminated water in hotels. Travel-associated LD has also been linked to cruise ships[45].

The mode of transmission of LD to human is mainly by the inhalation of contaminated aerosols droplets where the source can disseminate water droplets such as the cooling towers. Due to the small sizes of water droplets containing the *Legionella* sp., which can be deeply inhaled into the respiratory airways, it has increased the ease of transmission of LD. Besides that, there is limited evidence to indicate the person-to-person transmission of LD. Not until recently, the first strong evidence of person-to-person transmission of LD was reported in Vila Franca de Xira, Portugal, in 2014[46].

The host-related risk factors for LD infection may be associated with factors such as old age, cigarette smoking, organ transplantation, the use of immunosuppressive medications, obstructive pulmonary disease as well as patients with acquired immune deficiency syndrome. Moreover, the elevated risk of nosocomial infection may attribute to general anaesthesia and endotracheal intubation. Besides that, there are reports of infection in children and premature neonates[45]. In addition, those workers who maintain the water

cooling towers and air-conditioning system are the most at-risk group to be infected with LD where the circulated air that contaminated with water droplets containing *Legionella* sp. can be inhaled easily into the respiratory system[45,47].

4. Pathogenesis of *Legionella pneumophila*

Predominantly, human exposure occurs when *L. pneumophila* are inhaled into the lungs through inhalation of contaminated aerosols which results in pneumonic respiratory disease. A combination of bacterial virulence factors and host immunity determines the outcome of *Legionella* infection, whether leading to a self-limiting and mild respiratory disease (PF) or severe pneumonia (LD)[48]. Once *L. pneumophila* gets into the lung, the bacteria are phagocytosed by the alveolar macrophages, where they can multiply intracellularly. This is due to the ineffective killing of *L. pneumophila*, which has been demonstrated through several *in vitro* models of infection by specific antibody and neutrophils[49]. Meanwhile, the pathogenesis of PF may involve a host response to endotoxin from the lipopolysaccharide of *Legionella* without replication of the bacteria in the host[50].

The infection process of *L. pneumophila* on protozoa and mammalian phagocytic cells are shown to be similar where *Legionella* uses common gene and gene products to infect mammalian and protozoan cells[35]. The uptake of *L. pneumophila* by monocytes and macrophages is by the conventional and coiling phagocytosis which is an important feature in the pathogenesis of the bacteria[51]. After internalization of the pathogen by phagocytosis, *L. pneumophila* evades endocytic degradation, controls the innate immune response, and triggers the *Legionella*-containing vacuole (LCV) biogenesis. The LCV biogenesis involves the recruitment of the rough endoplasmic reticulum and mitochondria result in formation of a specialized vacuole for the intracellular replication of *L. pneumophila*. This mechanism is directed by the type IV secretion system encoded by the Dot/Icm genes. The Dot/Icm type IV secretion system is the main virulence system of *L. pneumophila* which consists of bacterial proteins that promote the secretion of bacterial virulence factors suggested to be responsible for inhibition of phagosome-lyzosome fusion[52]. Hence, *L. pneumophila* can multiply intracellularly in human macrophages by avoiding phagosome-lyzosome fusion[53]. As *Legionella* becomes vigorously motile in the massive intracellular vesicle, it causes the host cell to become overwhelmed by the infection resulting in lysis of the host cell. This has led to the death of the host cell macrophage and the release of the progeny of the pathogen from the cell to the environment where new host cells are found.

5. Detection and diagnosis of *Legionella* pneumonia

Today, various diagnostic techniques are available for *Legionella* pneumonia, including urinary antigen tests, respiratory specimen cultures, molecular detection tests, and serum antibody tests[54]. Despite being the diagnostic gold standard, culture of *Legionella* bacteria isolated from the clinical respiratory sample is limited by the requirement of a specific culture medium[55] and long laboratory turnaround time, which requires at least 3 to 5 days to identify culture positivity[56].

Serological test for antibodies against *Legionella* bacteria was the principal diagnostic tool for LD in the early 1980s. However, serological test has been replaced by more rapid and definitive analyses in the modern laboratory, such as the urinary antigen test (UAT) and molecular methods. The use of serology was reported to have declined from 61% to 6% from 1995 to 2010[57], due to the widespread adoption of the less technically demanding UAT. The UAT has been widely utilized globally due to its straight-forward procedure and rapid turnaround time. In Europe and the United States, UAT represents more than 80% to 90% of the diagnostic tools used for LD confirmation. The specific urinary antigens of *Legionella* can be detected in most of the *L. pneumophila* infections in the first few days of clinical symptom onset and up to several days to more than ten months. Typically, the specific urinary *Legionella* antigen is no longer detected in most cases after 1 to 2 months of antibiotic therapy. However, the current commercially available UAT for non-*L. pneumophila* serogroup 1 exhibit lower sensitivity and highly variable than those for *L. pneumophila* associated disease when tested with urine from confirmed LD patients[58,59]. Remarkably, a recent study demonstrated a novel urinary antigen test kit (LAC-116) that can detect non-*L. pneumophila* serogroup 1 pneumonia on top of showing comparable accuracy with the other existing kits for *Legionella* pneumonia[54].

With the advances of molecular biology and the development of new technologies, the field of diagnostic and detection of pathogens has been improved tremendously, with many new molecular methods being developed to generate rapid, sensitive and accurate results[60]. Currently, real-time PCR is regarded as the molecular method of choice for the detection of *Legionella* bacteria. Targeting the macrophage inhibitor protein *mip* gene, highly conserved in all *L. pneumophila* isolates, has been widely employed by numerous published studies, showing a 15% increased yield in detecting any *L. pneumophila* serogroup than that of culture method[61]. Some studies performed PCR targeting 16S rDNA gene of *Legionella* sp. which was suggested to offer greater sensitivity. However, the CAPNETZ study demonstrated a low detection rate of legionellosis (10%) with *Legionella* sp. PCR targeting 16S rDNA and only 60% of these samples were confirmed by DNA sequencing. Although *Legionella* nucleic acid-based detection methods offer higher sensitivity and rapid turnaround time, there are several notable disadvantages and limitations. For instance, non-lower respiratory tract samples, such as urine and serum, are not suitable for the PCR test, and the inherent limitation of PCR which cannot assess bacterial viability. Furthermore, nucleic acid amplification technologies also require highly trained personnel to handle sophisticated equipment to perform the diagnostic test[62] but are increasingly accessible to many laboratories on a moderate budget[61]. Nevertheless, nucleic acid-based methods are valuable additions to LD diagnostic and detection methods and complement well with the use of other testing modalities to enhance the rate of successful detection[61].

In the United States, a clinical case of *Legionella* pneumonia is considered when laboratory results show positive UAT, bacterial culture and serology test for *L. pneumophila* serogroup 1. Meanwhile, a suspected or probable case is considered when *Legionella*

antigens in respiratory specimens are detected by serology test, detection of seroconversion to non-serogroup 1 or non-*L. pneumophila*, and/or detection of *Legionella* nucleic acid[63].

6. Clinical manifestation and management of *Legionella* pneumonia

Legionellosis presents two distinct clinical manifestations in humans: milder respiratory disease PF and severe pneumonia LD[64]. PF manifests as a milder form of LD that involves respiratory illness without pneumonia, which resembles the self-limiting flu-like illness with symptoms lasting for approximately a week. Meanwhile, LD is a more severe form of pneumonia that could also affect other organs, including the liver and kidneys[65,66]. Typically, LD is a severe pneumonia that often occurs in susceptible persons, including old ages, smokers and those with comorbid conditions or immunosuppression[67]. LD does not present any specific or defining clinical features but shows a range of clinical manifestations and symptoms. LD basically begins with a headache, muscle pain and general feeling of sickness. Then, these typical symptoms are followed by severe high fever, dry cough, shaking chills, nausea, vomiting and diarrhoea. The infected person also might experience chest pain and difficulty in breathing.

In some rare incidences, a persistent or relapsing LD may develop into slow or non-resolving pneumonia which is characterized by the persistence of pulmonary infiltrates for more than 30 days since the disease onset[68]. Majority of the previously reported cases occurred in immunocompromised patients as a result of intracellular survival of *Legionella* pathogens due to ineffective antibiotic treatment[69,70] rather than de novo reinfection via exposure to contaminated sources. These slowly or non-resolving LD patients experience severe pulmonary complications, including pleural effusion, abscess formation and empyema, over the course of the infection[71].

Considering the high mortality and morbidity associated with untreated LD, early diagnosis and prompt treatment with effective antibiotics are extremely crucial, together with appropriate clinical management of complications and underlying comorbidities should be prioritized for LD patients[72]. Given that LD does not present any defining clinical features, empiric antibiotic therapy is well justifiable for the initial management of all moderate-to-severe community- and nosocomial-acquired pneumonia prior to LD confirmation made from microbiological diagnosis.

Primarily, therapy for LD is the antibiotic treatment of the infection and management of complications. In general, all *Legionella* sp. tested are found to be susceptible to commonly prescribed macrolides (erythromycin, azithromycin) and fluoroquinolones (levofloxacin)[73]. Antibiotics, such as macrolides and fluoroquinolones, are also known to achieve therapeutic intracellular concentration within tissue and alveolar macrophages where *L. pneumophila* resides. Since 1976, erythromycin had been the treatment of choice for *L. pneumonia* but only until 1990s, because of its side effects when given intravenously. To date, azithromycin and levofloxacin have become the mainstay LD treatment in both healthy and immunocompromised patients[18]. A recent meta-analysis revealed that levofloxacin

resulted in a significant reduction in the length of hospitalization, lowered mortality, shorter time to apyrexia, and reduced risks of adverse effects compared to macrolides[74].

World Health Organization (WHO) has developed a framework that can be used to assess and manage the risks posed by *Legionella*[31]. The framework includes health-based targets, water safety plans and surveillance. In addition to the framework proposed by WHO, many approaches of disinfection have been tried over the past 13 years with variable success. Superheating of water up to 70˚C and 80˚C, hyperchlorination[75] and copper-silver oxidation method were also implemented to halt the LD outbreak.

7. Conclusion

Legionella pathogen is one of the top causative agents of severe respiratory disease, which requires swift treatment and rapid diagnosis to achieve successful disease resolution with appropriate antibiotic therapy. *Legionella* pathogen replicates intracellularly in the host cells, especially amoeba and macrophages. The process of invasion, development and replication of *Legionella* sp. in the environment has been well studied. LD occurs as outbreaks or as sporadic cases in the community and hospital. The transmission of this disease is through inhalation of contaminated aerosols or aspiration of contaminated water. Furthermore, the diagnosis and treatment of LD have changed over the years, where the urinary antigen detection test and molecular methods are commonly used nowadays. Early testing and appropriate treatment are keys to mortality and morbidity caused by LD. Ultimately, greater effort is required from all facets of *Legionella* research to facilitate better disease surveillance, improve disease diagnosis and detection, and improve clinical outcomes for LD patients.

Author Contributions: LT-HT and W-YT performed literature search and manuscript writing. TMK, LCM and VL provided critical review and proofreading of this manuscript. LT-HT and VL conceptualize this review writing project.

Funding: The SEED Funding funded this work from Microbiome and Bioresource Research Strength (MBRS), Jeffrey Cheah School of Medicine and Health Sciences, (Vote Number: MBRS/JCSMHS/02/2020) awarded to VL.

Acknowledgements: The authors thank Professor Dr. Shajahan Yasin, Professor and Head of School, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia for the technical support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Letchumanan V, Loo K-Y, Law JW-F, *et al. Vibrio parahaemolyticus*: the protagonist of foodborne diseases. Prog Microbes Mol Biol 2019; 2(1): a0000029.

2. Goonewardene ST, Tang C, Tan LT-H, *et al.* Safety and efficacy of pneumococcal vaccination in pediatric nephrotic syndrome. Front Pediatr 2019; 7: 339.

3. Janik E, Ceremuga M, Niemcewicz M, *et al.* Dangerous pathogens as a potential problem for public health. Medicina (Kaunas) 2020; 56(11): 591.

4. Rayanakorn A, Katip W, Lee LH, *et al.* Endophthalmitis with bilateral deafness from disseminated *Streptococcus* suis infection. BMJ Case Rep 2019; 12(2): e228501.

5. Rayanakorn A, Katip W, Goh BH, *et al.* Clinical manifestations and risk factors of *Streptococcus* suis mortality among northern Thai population: retrospective 13-year cohort study. Infect Drug Resist 2019; 12: 3955.

6. Letchumanan V, Wong P-C, Goh B-H, *et al.* A review on the characteristics, taxanomy and prevalence of *Listeria monocytogenes.* Prog Microbes Mol Biol 2018; 1(1): a0000007.

7. Kemung HM, Tan LT-H, Khan TM, *et al. Streptomyces* as a prominent resource of future anti-MRSA drugs. Front Microbiol 2018; 9: 2221.

8. Rayanakorn A, Goh B-H, Lee L-H, *et al.* Risk factors for *Streptococcus* suis infection: a systematic review and meta-analysis. Sci Rep 2018; 8(1): 1–9.

9. Letchumanan V, Pusparajah P, Tan LT-H, *et al.* Occurrence and antibiotic resistance of *Vibrio parahaemolyticus* from shellfish in Selangor, Malaysia. Front Microbiol 2015; 6: 1417.

10. Eng S-K, Pusparajah P, Ab Mutalib N-S, *et al. Salmonella*: a review on pathogenesis, epidemiology and antibiotic resistance. Front Life Sci 2015; 8(3): 284–293.

11. Tan T, Chan K and Lee L. Application of bacteriophage in biocontrol of major foodborne bacterial pathogens. J Mol Biol Mol Imaging 2014; 1(1): id1004

12. Loo K-Y, Letchumanan V, Ser H-L, *et al.* COVID-19: Insights into potential vaccines. Microorganisms 2021; 9(3): 605.

13. Ser H-L, Tan LT-H, Law JW-F, *et al.* Genomic analysis of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) strains isolated in Malaysia. Prog Microbes Mol Biol 2020; 3(1): a0000093.

14. Tan LT-H, Letchumanan V, Ser H-L, *et al.* PMMB COVID-19 Bulletin: United Kingdom (22nd April 2020). Prog Microbes Mol Biol 2020; 3(1): a0000078.

15. Ser H-L, Letchumanan V, Law JW-F, *et al.* PMMB COVID-19 Bulletin: Spain (18th April 2020). Prog Microbes Mol Biol 2020; 3(1)**:** a0000074.

16. Letchumanan V, Ab Mutalib N-S, Goh B-H, *et al.* Novel coronavirus 2019-nCoV: could this virus become a possible global pandemic. Prog Microbes Mol Biol 2020; 3(1): a0000068.

17. McDade JE, Shepard CC, Fraser DW, *et al.* Legionnaires' disease: isolation of a bacterium and demonstration of its role in other respiratory disease. N Engl J Med 1977; 297(22): 1197–1203.

18. Carratalà J and Garcia-Vidal C. An update on *Legionella.* Curr Opin Infect Dis 2010; 23(2): 152–157.

19. Fliermans CB. Ecology of *Legionella:* From data to knowledge with a little wisdom. Microb Ecol 1996; 32(2): 203–228.

20. Garrison LE, Kunz JM, Cooley LA, *et al. Vital signs: deficiencies in environmental control identified in outbreaks of Legionnaires' disease—North America, 2000–2014*. 2016, Wiley Online Library.

21. Beauté J. Legionnaires' disease in Europe, 2011 to 2015. Eurosurveillance 2017; 22(27): 30566.

22. Quan F-S, Kong H-H, Lee H-A, *et al.* Identification of differentially expressed *Legionella* genes during its intracellular growth in *Acanthamoeba.* Heliyon 2020; 6(10): e05238.

23. Lau H and Ashbolt N. The role of biofilms and protozoa in *Legionella* pathogenesis: implications for drinking water. J Appl Microbiol 2009; 107(2): 368–378.

24. Hilbi H, Hoffmann C, and Harrison CF. *Legionella* spp. outdoors: colonization, communication and persistence. Environ Microbiol Rep 2011; 3(3): 286–296.

25. Buse HY, Schoen ME, and Ashbolt NJ. *Legionellae* in engineered systems and use of quantitative microbial risk assessment to predict exposure. Water Res 2012; 46(4): 921–933.

26. Boppe I, Bédard E, Taillandier C, *et al.* Investigative approach to improve hot water system hydraulics through temperature monitoring to reduce building environmental quality hazard associated to *Legionella.* Build Environ 2016; 108: 230–239.

27. Bargellini A, Marchesi I, Righi E, *et al.* Parameters predictive of *Legionella* contamination in hot water systems: association with trace elements and heterotrophic plate counts. Water Res 2011; 45(6): 2315–2321.

28. Lytle DA, Pfaller S, Muhlen C, *et al.* A comprehensive evaluation of monochloramine disinfection on water quality, *Legionella* and other important microorganisms in a hospital. Water Res 2021; 189: 116656.

29. Yakunin E, Kostyal E, Agmon V, *et al.* A snapshot of the prevalence and molecular diversity of *Legionella pneumophila* in the water systems of Israeli hotels. Pathogens 2020; 9(6): 414.

30. Nisar MA, Ross KE, Brown MH, *et al. Legionella pneumophila* and protozoan hosts: implications for the control of hospital and potable water systems. Pathogens 2020; 9(4): 286.

31. Bartram J, Chartier Y, Lee JV, *et al. Legionella and the prevention of legionellosis*. 2007: World Health Organization.

32. Rowbotham TJ. Preliminary report on the pathogenicity of *Legionella pneumophila* for freshwater and soil amoebae. J Clin Pathol 1980; 33(12): 1179–1183.

33. Tan LT-H, Raghunath P, Ming LC, *et al. Mycobacterium ulcerans* and *Mycobacterium marinum*: Pathogenesis, diagnosis and treatment. Prog Microbes Mol Biol 2020; 3(1): a0000114.

34. Boamah DK, Zhou G, Ensminger AW, *et al.* From many hosts, one accidental pathogen: The diverse protozoan hosts of *Legionella.* Front Cell Infect Microbiol 2017; 7: 477.

35. Best A and Kwaik YA. Evolution of the arsenal of *Legionella pneumophila* effectors to modulate protist hosts. mBio 2018; 9: e01313-18.

36. Gomez-Valero L and Buchrieser C. Intracellular parasitism, the driving force of evolution of *Legionella pneumophila* and the genus *Legionella.* Microb Infect 2019; 21(5): 230–236.

37. Barker J, Brown MR, Collier PJ, *et al.* Relationship between *Legionella pneumophila* and *Acanthamoeba polyphaga*: physiological status and susceptibility to chemical inactivation. Appl Environ Microbiol 1992; 58(8): 2420–2425.

38. Phin N, Parry-Ford F, Harrison T, *et al.* Epidemiology and clinical management of Legionnaires' disease. Lancet Infect Dis 2014; 14(10): 1011–1021.

39. Campese C, Bitar D, Jarraud S, *et al.* Progress in the surveillance and control of *Legionella* infection in France, 1998–2008. Int J Infect Dis 2011; 15(1): e30-e37.

40. Falcó V, de Sevilla TF, Alegre J, *et al. Legionella pneumophila*: a cause of severe community-acquired pneumonia. Chest 1991; 100(4): 1007–1011.

41. Fraser DW, Tsai TR, Orenstein W, *et al.* Legionnaires' disease: description of an epidemic of pneumonia. N Engl J Med 1977; 297(22): 1189–1197.

42. De Giglio O, Diella G, Lopuzzo M, *et al.* Impact of lockdown on the microbiological status of the hospital water network during COVID-19 pandemic. Environ Res 2020; 191: 110231.

43. Leung Y-H, Lam C-K, Cheung Y-Y, *et al.* Epidemiology of Legionnaires' disease, Hong Kong, China, 2005–2015. Emerg Infect Dis 2020; 26(8): 1695–1702.

44. Hamilton KA, Prussin AJ, Ahmed W, *et al.* Outbreaks of Legionnaires' disease and Pontiac fever 2006–2017. Curr Environ Health Rep 2018; 5(2): 263–271.

45. Guyard C and Low DE. *Legionella* infections and travel associated legionellosis. Travel Med Infect Dis 2011; 9(4): 176–186.

46. Correia AM, Ferreira JS, Borges V, *et al.* Probable person-to-person transmission of Legionnaires' disease. N Engl J Med 2016; 374(5): 497–498.

47. Falkinham JO. Living with *Legionella* and other waterborne pathogens. Microorganisms 2020; 8(12): 2026.

48. Liu X and Shin S. Viewing *Legionella pneumophila* Pathogenesis through an Immunological Lens. J Mol Biol 2019; 431(21): 4321–4344.

49. Horwitz MA and Silverstein SC. Interaction of the Legionnaires' disease bacterium (*Legionella pneumophila*) with human phagocytes. I. *L. pneumophila* resists killing by polymorphonuclear leukocytes, antibody, and complement. J Exp Med 1981; 153(2): 386–397.

50. Rubin LG. *Chapter 177 - Legionella Species*, in *Principles and Practice of Pediatric Infectious Disease (Third Edition)*, S.S. Long, Editor. 2008, W.B. Saunders: Edinburgh. p. 912–915.

51. Bozue JA and Johnson W. Interaction of *Legionella pneumophila* with *Acanthamoeba castellani*i: uptake by coiling phagocytosis and inhibition of phagosome-lysosome fusion. Infect Immun 1996; 64(2): 668–673.

52. Copenhaver AM, Casson CN, Nguyen HT, *et al.* Alveolar macrophages and neutrophils are the primary reservoirs for *Legionella pneumophila* and mediate cytosolic surveillance of type IV secretion. Infect Immun 2014; 82(10): 4325–4336.

53. Ge J and Shao F. Manipulation of host vesicular trafficking and innate immune defence by *Legionella* Dot/Icm effectors. Cell Microbiol 2011; 13(12): 1870–1880.

54. Ito A, Yamamoto Y, Ishii Y, *et al.* Evaluation of a novel urinary antigen test kit for diagnosing *Legionella pneumonia*. Int J Infect Dis 2021; 103: 42–47.

55. Fields BS, Benson RF, and Besser RE. *Legionella* and Legionnaires' disease: 25 years of investigation. Clin Microbiol Rev 2002; 15(3): 506–526.

56. Cunha BA, Burillo A, and Bouza E. Legionnaires' disease. Lancet 2016; 387(10016): 376–385.

57. Ricketts KD and Joseph C. Legionnaires' disease in Europe 2003–2004. Eurosurveillance 2005; 10(12): 256–259.

58. Olsen CW, Elverdal P, Jørgensen CS, *et al.* Comparison of the sensitivity of the *Legionella* urinary antigen EIA kits from Binax and Biotest with urine from patients with infections caused by less common serogroups and subgroups of *Legionella*. Eur J Clin Microbiol Infect Dis 2009; 28(7): 817–820.

59. Svarrer CW, Lück C, Elverdal PL, *et al.* Immunochromatic kits Xpect *Legionella* and BinaxNOW *Legionella* for detection of *Legionella pneumophila* urinary antigen have low sensitivities for the diagnosis of Legionnaires' disease. J Med Microbiol 2012; 61: 213–217.

60. Robinson BWS, Erle DJ, Jones DA, *et al.* Recent advances in molecular biological techniques and their relevance to pulmonary research. Thorax 2000; 55(4): 329–339.

61. Mentasti M, Fry N, Afshar B, *et al.* Application of *Legionella pneumophila*-specific quantitative real-time PCR combined with direct amplification and sequence-based typing in the diagnosis and epidemiological investigation of Legionnaires' disease. Eur J Clin Microbiol Infect Dis 2012; 31(8): 2017–2028.

62. Mercante JW and Winchell JM. Current and emerging *Legionella* diagnostics for laboratory and outbreak investigations. Clin Microbiol Rev 2015; 28(1): 95–133.

63. Control ECfD and Prevention. *European Legionnaires' Disease Surveillance Network (ELDSNet): Operating procedures*. 2012, ECDC Stockholm.

64. Soda EA, Barskey AE, Shah PP, *et al.* Vital signs: health care–associated Legionnaires' disease surveillance data from 20 states and a large metropolitan area—United States, 2015. Am J Transplant 2017; 17(8): 2215–2220.

65. Diederen B. *Legionella* spp. and Legionnaires' disease. J Infect 2008; 56(1): 1–12.

66. Daumas A, El-Mekaoui F, Bataille S, *et al.* Acute tubulointerstitial nephritis complicating Legionnaires' disease: a case report. J Med Case Reports 2012; 6(1): 1–6.

67. Dooling KL, Toews K-A, Hicks LA, *et al.* Active bacterial core surveillance for legionellosis—United States, 2011–2013. Morbidity and Mortality Weekly Report 2015; 64(42): 1190–1193.

68. Mandell LA, Wunderink RG, Anzueto A, *et al.* Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 2007; 44(Supplement 2): S27–S72.

69. Sheldon PA, Tight RR, and Renner ED. Fatal legionnaires' disease coincident with initiation of immunosuppressive therapy. Arch Intern Med 1985; 145(6): 1138–1139.

70. Gump DW, Frank RO, Winn Jr WC, *et al.* Legionnaires' disease in patients with associated serious disease. Ann Intern Med 1979; 90(4): 538–542.

71. Pouderoux C, Ginevra C, Descours G, *et al.*, Slowly or nonresolving Legionnaires' disease: Case series and literature review. Clin Infect Dis 2019; 70(9): 1933–1940.

72. Allgaier J, Lagu T, Haessler S, *et al.* Risk factors, management, and outcomes of *Legionella* pneumonia in a large, nationally representative sample. Chest 2020; 19: 1–11.

73. Chahin A and Opal SM. Severe pneumonia caused by *Legionella pneumophila*: differential diagnosis and therapeutic considerations. Infect Dis Clin North Am 2017; 31(1): 111–121.

74. Kato H, Hagihara M, Asai N, *et al.* Meta-analysis of fluoroquinolones versus macrolides for treatment of *Legionella* pneumonia. J Infect Chemother 2021; 27(3): 424–433.

75. Orsi GB, Vitali M, Marinelli L, *et al. Legionella* control in the water system of antiquated hospital buildings by shock and continuous hyperchlorination: 5 years experience. BMC Infect Dis 2014; 14(1): 394.