REVIEW

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Challenges for *Legionella pneumophila* detection in Indonesia

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ABSTRACT

Legionella pneumophila (*L. pneumophila*) is widely known to cause respiratory illness outbreaks and remains underdiagnosed, including in Indonesia. Several diagnostic methods are available, yet none have been implemented as a routine diagnostic panel in most clinical microbiology laboratories in Indonesia. The urine antigen test is the cheapest and easy to perform. However, it only detects serogroup 1 of *L. pneumophila*, creating a blind spot for non-serogroup 1. Culture is the gold standard, but its sensitivity and turnaround time makes culture less feasible in a clinical setting. The direct fluorescent antibody is rapid, nonetheless, expertise and experience are needed to increase the sensitivity. Molecular methods, while very sensitive, cannot rule out contamination since the bacteria are mostly found in water. In order to validate which best method to be performed in Indonesia, a nationwide surveillance and validation study should be performed.

Keywords: *Legionella pneumophila, Legionella spp., urine antigen test, culture, polymerase chain reaction, direct fluorescent antibody*.
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INTRODUCTION

In early September 2022, a cluster of severe pneumonia case was reported in San Miguel de Tucumán city, Argentina.[1] Four-deaths, three among healthcare workers, have been reported, and *Legionella spp.* was isolated from four clinical specimens from deceased patients.[1] *Legionella spp.*, especially *Legionella pneumophila* (*L. pneumophila*) serogroup 1 is widely known to cause outbreaks of respiratory illness and yet remain underdiagnosed.[2-6] In Indonesia, there are only three *Legionella spp.* reports that have been documented in several areas such as Bali, Tangerang, and Jakarta.[7,8] The low number of reports are believed to be due to the lack of capacity to diagnose or identify *Legionella spp.*, including *L. pneumophila serogrouping characterization* from clinical specimens.

Legionella spp. is consists of 66 species and more than 70 serotypes which are fastidious, aerobic, Gram-negative bacteria stained poorly by routine staining, such as Gram stain.[2,9] To cultivate *Legionella spp.* a complex and not routinely available media namely buffered charcoal yeast extract agar (BCYE) is needed with special supplementation.[2] In addition, antigen and antibody-based detection for *L. pneumophila* have variable sensitivity while molecular assay has issues related to water contamination.[2] Thus, this short review is aimed to elucidate current available diagnosis methods for *Legionella spp.*, especially *L. pneumophila* and describe the challenges that needs to overcome in order to implement the best methods for diagnosing *L. pneumophila* infection from clinical specimen in Indonesia.

LEGIONELLA URINE ANTIGEN TEST

Commercially available urine antigen tests in many form (immunochromatographic, enzyme-linked immunosorbent assay (ELISA), and radio immunoassay) for *L. pneumophila* are available and reported to have high sensitivity and specificity. The urine antigen test was designed only to target serogroup-1, which mostly responsible causing 50-80% Legionnaire's disease.[6] However, one systematic review stated that the pooled sensitivity for the urine antigen test in all methods was 74% with 99.1% specificity, despite only two studies that do not use serogroup-1 as the main population target.[10] Unfortunately, the data regarding the performance of urine antigen test for Legionella infection in Indonesia is still limited.

In 2021, study group from Japan created novel Legionella urinary antigen test kit that has agreement 96.8% with the two pre-existing kits.[11] However, when compared to culture and nucleic acid detection, the sensitivity of the new kit was only 79% despite it has enhanced capabilities to detect other serogroup than serogroup-1.[11] The main reason to use the urinary antigen test is because of its ease of performance, rapid, and cheap compare to other methods.[10] However, clinicians, especially clinical microbiologist should be mindful that no serogroup data of *Legionella spp.* circulating in Indonesia is available but also only 80% patient will secrete Legionella antigen in their urine.[12] These consideration should be addressed before implement Legionella urine antigen test in Indonesia clinical settings.

LEGIONELLA CULTURE

Culture from clinical specimens such as lower respiratory tract specimen, blood, pleural fluid, pericardial fluid, and other sites remains as the gold standard to

diagnose Legionnaires' disease.[10,13,14] The major advantages of Legionella culture is that this approach can detect all of the *Legionella spp.* and Legionella serogroups.[14,15] Buffered charcoal yeast extract medium supplemented with 0.1% α-ketoglutaric acid (BCYEα) and its modification with addition of selective antibiotics (BMPA / PAV) have been used to isolate *Legionella spp.* from clinical and environmental specimens.[13,15,16] One non-selective media (BCYEα) and two selective media (BMPA and PAV) should be used in order to obtain optimal recovery of *Legionella spp.* from clinical specimens.[13] However, the first barrier of this approach is that these media is not routinely available in clinical microbiology laboratory in Indonesia.

A systematic review pointing out that *Legionella spp.* culture has sensitivity approximately 60% (ranging from 10-80%).[17-19] The lower sensitivity is thought to be the nature of the bacteria which considered as fastidious bacteria and additional acid pretreatment or immunomagnetic separation is required in order to enhanced recovery.[20-22] Despite it can enhance recovery, the acid pretreatment can prolong Legionella recovery up to 2 weeks which is not beneficial for patient management.[14,21] In addition, suitable specimens for *Legionella culture* are hardly obtained since most of the patients only produce non-purulent sputum with less white blood cells that usually rejected by microbiology laboratory and sometimes even the patient produced no sputum.[19,23]

LEGIONELLA DIRECT FLUORESCENCE ANTIBODY (DFA)

In 1990 direct fluorescence antibody (DFA) reached its peak of popularity due to rapid diagnostic procedure compared to cultures and its ability to detect *Legionella spp.* from direct clinical specimen.[24,25] However, during these times it is also known that DFA cannot rule out *Legionella spp.* infection, since low predictive values is observed.[24] These low predictive value is contributed by several factors such as environmental contamination, airborne contamination, cross-reaction, and even reagent contamination.[25]

It is known that DFA has a wide range of sensitivity ranging from 9.5-100% compared to other methods.[26-29] Expertise is the major cause of this wide range of sensitivity since a trained and experienced microscopist plays the major role when DFA is used as diagnostic method. Furthermore, the availability of microscopic fluorescence assay has become a consideration since not all clinical microbiology laboratories are equipped with this microscope.

LEGIONELLA MOLECULAR DETECTION

Polymerase chain reaction (PCR) and in-situ hybridization (ISH), and loop-mediated isothermal amplification (LAMP) are several molecular approaches that have been evaluated for *Legionella spp.* detection in clinical specimens.[15,29-32] Despite one study mentioned that the ISH method is more sensitive in detecting *L. pneumophila* compared to PCR, PCR gained much more attention than ISH.[29] The reason PCR is more favorable than ISH is because PCR has a more rapid turnaround time (2-3 hours) compared to ISH (> 24 hours).[29] Furthermore, the LAMP method is mostly validated using environmental specimens, thus more evidences are needed to use LAMP for clinical specimens.[30]

When compared to culture and urine antigen test, PCR has a sensitivity of 92%, while culture and urine antigen test has a sensitivity of 50% and 96%, respectively in detecting serogroup-1 *L. pneumophila*.[15] However, one systematic review comparing PCR and urine antigen test showed that when using respiratory samples, PCR has an advantage in terms of sensitivity and increases diagnostic yield from 18% to 30%.[33] This increased sensitivity is due to the use of PCR that targeting the *mip* gene can also detect other serogroup of *L. pneumophila*.[28,34]

PCR could not discriminate between viable and non-viable *Legionella spp.* and a method has been developed to overcome the limitation.[35] DNA intercalating agents such as ethidium monoazide (EMA) or propidium monoazide (PMA) could infiltrate damaged membranes and bind with DNA, this interaction is used to identify viable and non-viable *Legionella* and *Legionella pneumophila*.[36] EMA combined with qPCR has been investigated compared with Bacterial Viability kit (BacLight-EM) showed accurate quantification of viable legionellae cells.[37]

Cost and equipment are the major problem, especially in a middle-income country such as Indonesia. However, because of the pandemic, most clinical microbiology laboratories have been equipped with PCR, and the cost of reagents used to perform PCR has decreased. Apart from that, we have to take into account that *Legionella spp.* are primarily found in water. Thus, contamination can lead to a false positive result when using PCR to detect *Legionella spp.*

CONCLUSION

Legionella infection and detection remains a challenge not only in Indonesia but also worldwide. To date, culture remains the gold standard for diagnosis, despite the problem with turnaround time and the need for an experienced microbiologist. However, we cannot disregard emerging methods with fast turnaround time with specific advantages and limitations. In conclusion, to implement the best and suitable methods in Indonesia, a nationwide research, education, and surveillance are urgently needed to deduct evidence-based recommendation on which methods that can be implemented to diagnose Legionella infection in clinical setting.

DISCLOSURES

Conflict of Interest

The authors declare that they have no competing interests.

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Author Contribution

All authors prepare the manuscript and agree for this final version of manuscript to be submitted to this journal.

Ethical Statement

Not applicable.

REFERENCES

1. WHO. Disease Outbreak News;Legionellosis in Argentina 2022 [Available from: https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON407
2. Morse SA, Mietzner TA, Miller S, Riedel S. Jawetz Melnick & Adelbergs Medical Microbiology 28 E: McGraw-Hill Education; 2019.
3. Spiegelman J, Pedutem T, Francisco MJ. Legionnaires' Disease Cases at a Large Community Hospital-Common and Underdiagnosed. Int J Environ Res Public Health. 2020;17(1).
4. Doleans A, Aurell H, Reyrolle M, Lina G, Freney J, Vandenesch F, et al. Clinical and environmental distributions of Legionella strains in France are different. J Clin Microbiol. 2004;42(1):458-60.
5. Helbig JH, Bernander S, Castellani Pastoris M, Etienne J, Gaia V, Lauwers S, et al. Pan-European study on culture-proven Legionnaires' disease: distribution of Legionella pneumophila serogroups and monoclonal subgroups. Eur J Clin Microbiol Infect Dis. 2002;21(10):710-6.
6. Yu VL, Plouffe JF, Pastoris MC, Stout JE, Schousboe M, Widmer A, et al. Distribution of Legionella species and serogroups isolated by culture in patients with sporadic community-acquired legionellosis: an international collaborative survey. J Infect Dis. 2002;186(1):127-8.
7. KEMENKES. Standar Pengelolaan Spesimen Legionella. Jakarta, Indonesia2003.
8. Soepandi P, Mangunnegoro H, Yunus F, Gunawan J. The pattern of micro-organisms and the efficacy of new macrolide in acute lower respiratory tract infections. Respirology. 1998;3(2):113-7.
9. Gattuso G, Rizzo R, Lavoro A, Spoto V, Porciello G, Montagnese C, et al. Overview of the Clinical and Molecular Features of Legionella Pneumophila: Focus on Novel Surveillance and Diagnostic Strategies. Antibiotics. 2022;11(3):370.
10. Shimada T, Noguchi Y, Jackson JL, Miyashita J, Hayashino Y, Kamiya T, et al. Systematic review and metaanalysis: urinary antigen tests for Legionellosis. Chest. 2009;136(6):1576-85.
11. Ito A, Yamamoto Y, Ishii Y, Okazaki A, Ishiura Y, Kawagishi Y, et al. Evaluation of a novel urinary antigen test kit for diagnosing *Legionella pneumonia*. Int J Infect Dis. 2021;103:42-7.
12. Kashuba ADM, Ballow CH. Legionella urinary antigen testing: Potential impact on diagnosis and antibiotic therapy. Diagn Microbiol Infect Dis. 1996;24(3):129-39.
13. Edelstein PH, Lück C. Legionella. Manual of Clinical Microbiology2015. p. 887-904.
14. Pierre DM, Baron J, Yu VL, Stout JE. Diagnostic testing for Legionnaires' disease. Ann. Clin. Microbiol. Antimicrob. 2017;16(1):59.
15. Chen DJ, Procop GW, Vogel S, Yen-Lieberman B, Richter SS. Utility of PCR, Culture, and Antigen Detection Methods for Diagnosis of Legionellosis. J Clin Microbiol. 2015;53(11):3474-7.
16. Raphael BH, Huynh T, Brown E, Smith JC, Ruberto I, Getsinger L, et al. Culture of Clinical Specimens Reveals Extensive Diversity of Legionella pneumophila Strains in Arizona. mSphere. 2019;4(1):e00649-18.
17. Cristovam E, Almeida D, Caldeira D, Ferreira JJ, Marques T. Accuracy of diagnostic tests for Legionnaires & apos; disease: a systematic review. J Med Microbiol. 2017;66(4):485-9.
18. Descours G, Cassier P, Forey F, Ginevra C, Etienne J, Lina G, et al. Evaluation of BMPA, MWY, GVPC and BCYE media for the isolation of Legionella species from respiratory samples. J Microbiol Methods. 2014;98:119-21.
19. Lindsay DSJ, Abraham WH, Findlay W, Christie P, Johnston F, Edwards GFS. Laboratory diagnosis of legionnaires' disease due to Legionella pneumophila serogroup 1: comparison of phenotypic and genotypic methods. J Med Microbiol. 2004;53(Pt 3):183-7.
20. Inoue H, Iwasawa T, Saruwatari Y, Agata K. Improved Acid Pretreatment for the Detection of *Legionella* species from Environmental Water Samples Using the Plate Culture Method. Biocontrol Sci. 2004;9(3):43-50.
21. Buesching WJ, Brust RA, Ayers LW. Enhanced primary isolation of *Legionella pneumophila* from clinical specimens by low-pH treatment. J Clin Microbiol. 1983;17(6):1153-5.
22. Mohammadi A, Chambers ST, Scott-Thomas A, Lewis JG, Anderson T, Podmore R, et al. Enhancement of Culture of Legionella longbeachae from Respiratory Samples by Use of Immunomagnetic Separation and Antimicrobial Decontamination. J Clin Microbiol. 2020;58(11).
23. Reller LB, Weinstein MP, Murdoch DR. Diagnosis of Legionella Infection. Clin Infect Dis. 2003;36(1):64-9.
24. Haldane DJ, Peppard R, Sumarah RK. Direct immunofluorescence for the diagnosis of legionellosis. Can J Infect Dis. 1993;4(2):101-4.
25. Puleo JA, Matar FA, McKeown PP, Conant P, Basta LL. *Legionella* pericarditis diagnosed by direct fluorescent antibody staining. Ann Thorac Surg. 1995;60(2):444-6.
26. Sethi S, Gore MT, Sethi KK. Increased sensitivity of a direct fluorescent antibody test for Legionella pneumophila in bronchoalveolar lavage samples by immunomagnetic separation based on BioMags. J Microbiol Methods. 2007;70(2):328-35.
27. She RC, Billetdeaux E, Phansalkar AR, Petti CA. Limited applicability of direct fluorescent-antibody testing for Bordetella sp. and Legionella sp. specimens for the clinical microbiology laboratory. J Clin Microbiol. 2007;45(7):2212-4.
28. Wilson DA, Yen-Lieberman B, Reischl U, Gordon SM, Procop GW. Detection of Legionella pneumophila by real-time PCR for the mip gene. J Clin Microbiol. 2003;41(7):3327-30.
29. Hayden RT, Uhl JR, Qian X, Hopkins MK, Aubry MC, Limper AH, et al. Direct detection of Legionella species from bronchoalveolar lavage and open lung biopsy specimens: comparison of LightCycler PCR, in situ hybridization, direct fluorescence antigen detection, and culture. J Clin Microbiol. 2001;39(7):2618-26.
30. Moosavian M, Seyed-Mohammadi S, Saki M, Shahi F, Khoshkholgh Sima M, Afshar D, et al. Loop-mediated isothermal amplification for detection of Legionella pneumophila in respiratory specimens of hospitalized patients in Ahvaz, southwest Iran. Infect Drug Resist. 2019;12:529-34.
31. Fain JS, Bryan RN, Cheng L, Lewin KJ, Porter DD, Grody WW. Rapid diagnosis of Legionella infection by a nonisotopic in situ hybridization method. Am J Clin Pathol. 1991;95(5):719-24.
32. Reischl U, Linde H-J, Lehn N, Landt O, Barratt K, Wellinghausen N. Direct Detection and Differentiation of *Legionella spp.* and *Legionella pneumophila* in Clinical Specimens by Dual-Color Real-Time PCR and Melting Curve Analysis. J Clin Microbiol. 2002;40(10):3814-7.
33. Avni T, Bieber A, Green H, Steinmetz T, Leibovici L, Paul M. Diagnostic Accuracy of PCR Alone and Compared to Urinary Antigen Testing for Detection of Legionella spp.: a Systematic Review. J Clin Microbiol. 2016;54(2):401-11.
34. Ballard AL, Fry NK, Chan L, Surman SB, Lee JV, Harrison TG, et al. Detection of Legionella pneumophila using a real-time PCR hybridization assay. J Clin Microbiol. 2000;38(11):4215-8.
35. Josephson KL, Gerba CP, Pepper IL. Polymerase chain reaction detection of nonviable bacterial pathogens. Appl Environ Microbiol. 1993;59(10):3513-5.
36. Inoue D, Tsutsui H, Yamazaki Y, Sei K, Soda S, Fujita M, et al. Application of real-time polymerase chain reaction (PCR) coupled with ethidium monoazide treatment for selective quantification of viable bacteria in aquatic environment. Water Sci Technol. 2008;58(5):1107-12.
37. Chen N-T, Chang C-W. Rapid quantification of viable legionellae in water and biofilm using ethidium monoazide coupled with real-time quantitative PCR. J Appl Microbiol. 2010;109(2):623-34.