MJMR, Vol. 34, No. 1, 2023, pages (1-7)

Open Access
ISSN:2682-4558

Research Article

Prevalence of Legionella Among Pneumonia Patients & Environmental Water Sample at Sohag University Hospital

Eva Reffat Nageeb[1]; Ahmed Nour El-Din Mohamed[2]; Sherif Aziz Said[2] and Laila Mahmoud Youseif [2]

[1] Department of Clinical and Chemical Pathology, Faculty of Medicine, Sohag University, Egypt.
[2] Department of Microbiology Clinical and Chemical Pathology, Faculty of Medicine, Sohag Universi

DOI: 10.21608/mjmr.2023.166120.1200

Abstract

Background: Legionellae are aerobic, gram-negative, non-spore-forming bacteria. Over 56 distinct Legionella species are encompassing at least 70 serogroups, approximately half of which have been isolated from, or detected in clinical specimens, but all species are regarded as potential human pathogens. **Aim of the study:** To detect the prevalence of legionella among pneumonia patients in environmental water samples in Sohag University Hospital. **Patients and Methods:** This study was conducted on 100 newly diagnosed patients with pneumonia on environmental water samples taken from 10 water outlets over the period from December 2016 to December 2017 at Sohag University Hospital. The following were done to all subjects: full history taking, full clinical examination, urine culture on Buffered Charcoal Yeast Extract (BCYE) Legionella Urine Ag test (UAG) and water culture on BCYE. **Results:** They were 44 males (44%), 3 of them were positive for legionella culture and 56 females (56%), 2 of them were positive for legionella culture. Their age ranged from 26-85 years with mean value 58.04±11.51 years. Cases with Legionella showed a significantly higher prevalence of neurological manifestations; need more ICU admission and ventilatory support, more likely to be males and smokers, cardiac, having more chest pain or GIT manifestations, higher degrees of dyspnea. **Conclusion:** This study found that smokers, males, having CAP with an underlying COPD and non-productive cough, GIT and neurological manifestation should be considered for Legionella infection. Patients hospitalized for more than 10 days, who acquired HAP should be suspected for Legionella until prove otherwise.

Keywords: Legionellae are aerobic, gram-negative, Pneumonia Patients

Introduction

In 1976, an outbreak of severe pneumonia among the participants of the American Legion Convention in Philadelphia led to the description of Legionnaires' disease. The disease was found to be caused by a bacterial infection[1-3]. The causative agent of Legionnaires' disease (LD) was named *Legionella*. This bacteria was found also to cause Pontiac fever[4]. Legionella has been thus retrospectively recognized as the cause of Legionnaires' disease outbreaks since 1947[1, 5].

Although *L. pneumophila* serogroup 1 is the commonest human pathogen of the genus Legionella; it is possible that, under favorable situations, the majority of other species and serotypes are also incriminated in Legionnaires' disease due to their competency for cellular invasion and intracellular proliferation[6, 7].

Legionellae are aerobic, gram-negative, non-spore-forming bacteria. Legionella spp. are ubiquitous in freshwater habitats, including rivers, lakes, streams, ponds, hot springs, and subsurface waters, and are naturally part of microbial ecosystems. Around 56 distinct Legionella species are encompassing more than 70 serogroups, with nearly half of them have been isolated from clinical specimens, but all species are regarded as possible human pathogens[8-11].

Legionella bacteria are intracellular pathogens; so antimicrobial agents that achieve intracellular concentrations higher than the minimal inhibitory concentration (MIC) are effective for their treatment, especially macrolides and quinolones[12]. The activities of levofloxacin and azithromycin are similar and both are considered the first-line therapy[13]. Patients with legionnaire disease who do not receive appropriate antibiotics can have bad prognosis and high mortality rate[14].

Legionellosis can be diagnosed with many modalities[15,16]. Detection of *Legionella* in water sources could be used as a predicting risk factor for LD [17]. However, it is important to bear several factors in mind. First, we need specific serological tests to diagnose Legionella infection. Second, it is important to escalate the discrepancy between the performances of a test in a research laboratory with what actually can be achieved in a clinical diagnostic laboratory. The substantial inter-laboratory difference has been documented for the ability to culture legionellae. Third, the good interpretation of the diagnostic tests needs a suitable "gold standard", which is lacked in our situation, where sensitivity and specificity data vary with different comparison standards. Fourth, the utility of diagnostic tests is affected by local Legionella epidemiology. L. pneumophila serogroup 1 is the chief and the most common cause of legionellosis in many areas of the world. However, other species and serogroups are more important in some regions. For example, Legionella longbeachae is a major cause of legionellosis in Australia and New Zealand, where it is often associated with exposure to the potting mix. Infection with this species will not be detected by current urinary antigen tests and will be missed by laboratories that perform serological assays only for L. pneumophila [10, 18, 19].

Advancements in Legionella in vitro diagnostics are often derived from the application of novel approaches to existing assays or through de novo development of innovative technologies. (mass spectrometry and realtime PCR-based TaqMan array cards) and emerging (immunomagnetic separation [IMS], isothermal nucleic acid amplification, high-resolution melt analysis, and wholegenome sequencing [WGS]) techniques that may enhance Legionella detection and characterization in various clinical settings[20, 21]. However, data from the developing world are still scarce[22]. In Egypt, the magnitude of legionellosis is not well recognized. Additionally, the few previous studies carried out addressed only *L. Pneumophila* [23].

Aim of the study

To detect the prevalence of legionella among pneumonia patients in environmental water samples in Sohag University Hospital.

Patients and Methods

Subjects:

This study was conducted on the available number of newly diagnosed patients with pneumonia on environmental water samples taken from 10 water outlets over the period from December 2016 to December 2017 at Sohag University Hospital.

Methods:

The following were done to all subjects:

- Full history taking.
- Full clinical examination.
- Laboratory investigation:
  - Urine culture on Buffered Charcoal Yeast Extract (BCYE) Legionella Urine Ag test (UAG) Water culture on Buffered Charcoal Yeast Extract (BCYE)

Ethical consideration:

This research was revised by the Scientific Ethical Committee of Sohag University Hospital. Informed written consent will be taken from all patients

Results

The present study was conducted on 100 subjects to determine the prevalence of Legionella among newly diagnosed pneumonia patients by full clinical examination & radiological & laboratory investigations who were attendants of outpatient clinic and inpatient departments of Sohag university hospital. They were 44 males (44%), 3 of them were positive for legionella culture and 56 females (56%), 2 of them were positive for legionella culture. Their age ranged from 26-85 years with mean value 58.04±11.51 years. Table 1 summarizes the demographic and clinical data of the study population.

In our study, we found that Cases with Legionella showed a significantly higher prevalence of neurological manifestations (100% compared to only 21% among

Legionella negative cases); need more ICU admission and ventilatory support (100% for both among Legionella positive cases compared to only 49.5% and 51.6% among Legionella negative cases; respectively).

Also, we found that Legionella positive cases are more likely to be males and smokers, being cardiac, having more chest pain or GIT manifestations, higher degrees of dyspnea. However, all these factors showed non-significant differences between the two groups, mostly due to the limited number of legionella positive cases (only 5 cases).

Table (1): Demographic and clinical data of the study population

Item		Value
Sex	Male	44%
	Female	56%
Age	Mean±SD	58.04±11.51
	Range	26-85
	≤55 years	41%
	> 55 years	59%
Chronic diseases	DM	24%
	HTN	33%
	Cardiac	40%
	Liver disease	9%
	Kidney disease	16%
	Malignancy	3%
Smoking	Positive	47%
	Heavy	18/47
	Passive	4%
	Ex-smoker	1%
X-ray	Right	55%
	Left	26%
	Bilateral	19%
Urine culture on BCYE		5%
Positive (type I) L. pneumonia		5%
Clinical	Fever	56%
	Cough	99%
	Chest pain	24%
	GIT manifestations	61%
	Neurological manifestations	24%
Sputum	Negative	6%
	Positive	94%
	Green	18(19.1%)
	Whitish	36(38.3%)
	Yellowish	21(22.3%)
	Colored	12(12.8%)
	White to green	1(1.1%)
	Scanty white	2(3.2%)
	Yellowish white	1(1.1%)
	Linged with blood	1(1.1%)
Dyspnea	Negative	4%
	Grade II	35%
	Grade III	58%
	Grade IV	3%
ICU stay		52%
Ventilator support		54%

Table (2): Comparison between Legionella positive and negative cases

Item		Urine culture on BCYT		P-value
		Negative (n=95)	Positive (n=5)	
Sex	Male	41(43.2%)	3(60%)	0.460
	Female	54(56.8%)	2(40%)	
Age	≤55 years	39(41.1%)	2(40%)	0.963
	>55 years	56(58.9%)	3(60%)	
	Mean±SD	57.6±13.1	56.2±8.3	0.818
Smoking		44(46.3%)	3(60%)	0.550
Chronic diseases	DM	23(24.2%)	1(20%)	0.830
	HTN	32(33.7%)	1(20%)	0.526
	Cardiac	37(38.9%)	3(60%)	0.349
	Liver	8(8.4%)	1(20%)	0.378
	Renal	15(15.8%)	1(20%)	0.802
	Malignancy	3(3.2%)	0	0.687
Clinical	Fever>38º	53(55.8%)	3(60%)	0.853
	Cough	94(98.9%)	5(100%)	0.818
	Chest pain	22(23.2%)	2(40%)	0.390
	GIT manifestations	56(58.9%)	5(100%)	0.067
	Neurological	20(21.1%)	5(100%)	**<0.001**
Dyspnea	Negative	4(4.2%)	0	0.938
	Grade II	33 (34.7%)	2(40%)	
	Grade III	55 (57.9%)	3(60%)	
	Grade IV	3 (3.2%)	0	
X ray	Right side	52(54.7%)	3(60%)	0.951
	Left side	25(26.3%)	1(20%)	
	Bilateral	18(18.9%)	1(20%)	
Sputum	Negative	5(5.3%)	1(20%)	0.176
	Positive	90(94.7%)	4(80%)	
	Green	17(18.9%)	1(20%)	
	Whitish	33(36.7%)	3(60%)	
	Yellowish	21(23.3%)	0	
	Colored	12(13.3%)	0	
	White to green	1(1.1%)	0	
	Scanty white	3(3.3%)	0	
	Yellowish white	1(1.1%)	0	
	Linged with blood	1(1.1%)	0	
ICU stay		47(49.5%)	5(100%)	**0.028**
Ventilator support		49(51.6%)	5(100%)	**0.034**

Discussion

Studies about LD are rare in the developing world, and the problem of Legionella is undoubtedly underestimated. Few countries of the world consider LD a notifiable disease, and incidence of L. pneumophila and other species can be obtained, whereas, most other countries have rare data because of lack of diagnostics and surveillance systems. It is worth mentioning that the worldwide occurrence of Legionnaires' disease is challenging to measure and attention should be taken in explanation of the surveillance figures [24, 25].

The diagnosis of Legionella infection can be done by many investigation arrays. Bacterial culture is considered the most specific means, but it is associated with a long time, low sensitivity and technical difficulty. Direct Fluorescent-Antibody (DFA) test is much more

rapid than culture, but it has poor sensitivity. Radioimmunoassay, enzyme immunoassay, and latex agglutination can be used for detection of only L.pneumophila (serogroup 1) in urine with moderate sensitivity. Serologic methods are extremely sensitive, but these require a long time to detect seroconversion in patients[26, 27].

So in our study, we use urine culture on Buffered Charcoal Yeast Extract Agar (BCYE) & confirm its results using urine antigen latex agglutination test as a screening test to detect the prevalence of legionella among our pneumonia patients.

In the current study, the mean age of the study population showed no difference between LD positive cases and LD negative ones, which is not agreed on by many previous studies. The fact that we had only 5 LD positive cases may explain these different results.
Similar to many previous reports, our study showed that legionellosis was more common in smokers and male patients. Smoking as a risk factor for legionellosis was found in previous studies[28]. Smoking raises the risk of legionellosis by 121% for each pack of cigarettes used up daily. This increased risk is due to the difficulty in eliminating the microorganism from the bronchial tree, due to the worsening of the respiratory mucosa and weakened cilia caused by tobacco smoking that facilitates entry into and subsequent invasion of the alveolar macrophages, depending on the individual's immune rank [29, 30].

In the current study, diabetes did not seem to be a risk factor for legionellosis, it was in about 20% of cases with legionella only, whereas former studies showed that diabetes was detected in 23-41% of patients [31].
This study found that COPD was a potential risk factor for Legionnaires' disease, found in 3 out of the 5 positive Legionnaires' cases; which agrees with the study of Maniwa et al. who reported that 21% of patients with LD suffered from COPD [32].

In the current study, neurologic symptoms showed significantly higher prevalence and GIT manifestations showed higher but non-significant prevalence among LD cases; seen in 100% of the cases. These manifestations were previously reported in LD, with a prevalence of 50% and 20%; respectively [33].

Regarding ICU and ventilatory support need, our finding showed that 100 % of patients with legionellosis were admitted to ICU and needed ventilatory support, and both were significantly higher among LD positive cases compared to negative ones. This is comparable with other studies [28]. This result can be attributed to older age (not seen in our study) and underlying co-morbidity of patients with legionella pneumonia which causes severe disease [34].

Conclusion

This study found that smokers, males, having CAP with an underlying COPD and presenting with non-productive cough, and GIT and neurological manifestation should be considered for Legionella infection. Whereas, patients hospitalized for more than 10 days, who acquired HAP should be suspected for Legionella until prove otherwise.

References

1. McDade JE, Brenner DJ, Bozeman FM. Legionnaires' disease bacterium isolated in 1947. Annals of internal medicine. 1979; 90(4):659-61.
2. Brenner DJ, Steigerwalt AG, McDade JE. Classification of the Legionnaires' disease bacterium: Legionella pneumophila, genus novum, species nova, of the family Legionellaceae, familia nova. Annals of internal medicine. 1979;90(4):656-8.
3. National Academies of Sciences E, Medicine. Management of Legionella in water systems: National Academies Press; 2020.
4. Diederen BMW. Legionella spp. and Legionnaires' disease. Journal of infection. 2008;56(1):1-12.
5. Myerowitz RL. An update on Legionnaires' disease and pneumonias caused by "new" Legionella-like bacteria. Monogr Pathol. 1982(23):123-37.
6. Fields BS, Benson RF, Besser RE. Legionella and Legionnaires' disease: 25 years of investigation. Clinical microbiology reviews. 2002;15(3):506-26.
7. Bartram J, Chartier Y, Lee JV, Pond K, Surman-Lee S. Legionella and the prevention of legionellosis: World Health Organization; 2007.
8. Molofsky AB, Swanson MS. Differentiate to thrive: lessons from the Legionella

pneumophila life cycle. Molecular microbiology. 2004;53(1):29-40.
9. Yan GB, Wang HX, Qin T, Zhou HJ, Li MC, Xu Y, et al., [Three quantitative methods to continuously monitor Legionella in spring water]. Zhonghua Yu Fang Yi Xue Za Zhi. 2013;47(7):637-40.
10. Qin T, Yan G, Ren H, Zhou H, Wang H, Xu Y, et al., High prevalence, genetic diversity and intracellular growth ability of Legionella in hot spring environments. PLoS One. 2013;8(3):e59018.
11. Pearce MM, Theodoropoulos N, Mandel MJ, Brown E, Reed KD, Cianciotto NP. Legionella cardiaca sp. nov., isolated from a case of native valve endocarditis in a human heart. Int J Syst Evol Microbiol. 2012;62(Pt 12):2946-54.
12. Roig J, Rello J. Legionnaires' disease: a rational approach to therapy. Journal of Antimicrobial Chemotherapy. 2003;51 (5): 1119-29.
13. Cunha BA, Burillo A, Bouza E. Legionnaires' disease. The Lancet. 2016; 387 (10016):376-85.
14. Abo-alella DAM. Prevalence of Legionella among pneumonia patients and environmental water samples in an Egyptian University Hospital. The International Arabic Journal of Antimicrobial Agents. 2016.
15. Borton D. Managing and preventing Legionella infections. Nursing 2020; 50(3): 54-7.
16. Carratala J, Garcia-Vidal C. An update on Legionella. Current opinion in infectious diseases. 2010;23(2):152-7.
17. Beer KD, Gargano JW, Roberts VA, Hill VR, Garrison LE, Kutty PK, et al., Surveillance for Waterborne Disease Outbreaks Associated with Drinking Water - United States, 2011-2012. MMWR Morb Mortal Wkly Rep. 2015;64(31):842-8.
18. Muder RR, Yu VL. Infection due to Legionella species other than L. pneumophila. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2002; 35(8): 990-8.
19. Yu VL. Legionella surveillance: political and social implications--a little knowledge is a dangerous thing. The Journal of infectious diseases. 2002;185(2):259-61.
20. Chang CW, Li SY, Huang SH, Huang CK, Chen YY, Chen CC. Effects of ultraviolet germicidal irradiation and swirling motion on airborne Staphylococcus aureus, Pseudomonas aeruginosa and Legionella pneumophila under various relative humidities. Indoor Air. 2013;23(1):74-84.
21. Cattan S, Thizy G, Michon A, Arlet JB, Lanternier F, Lebeaux D, et al., [Legionella spp: An update]. Rev Med Interne. 2019;40(12):791-8.
22. Phin N, Parry-Ford F, Harrison T, Stagg HR, Zhang N, Kumar K, et al., Epidemiology and clinical management of Legionnaires' disease. The Lancet infectious diseases. 2014;14(10):1011-21.
23. Mokhless NA-S, El-Mofty MF, Hanafi NF, Muhammad A, Asser SL. Atypical bacteria in ventilator-associated pneumonia; an Egyptian University Hospital experience. J Am Sci. 2010;6(12):1074-79.
24. Dabrera G, Brandsema P, Lofdahl M, Naik F, Cameron R, McMenamin J, et al., Increase in Legionnaires' disease cases associated with travel to Dubai among travellers from the United Kingdom, Sweden and the Netherlands, October 2016 to end August 2017. Euro Surveill. 2017;22(38).
25. Phin N, Parry-Ford F, Harrison T, Stagg HR, Zhang N, Kumar K, et al., Epidemiology and clinical management of Legionnaires' disease. The Lancet Infectious Diseases. 2014;14(10):1011-21.
26. Maini R, Naik F, Harrison T, Mentasti M, Spala G, Velonakis E, et al., Travel-associated Legionnaires disease in residents from England and Wales travelling to Corfu, Greece, August to October 2011. Euro Surveill. 2012;17(32).
27. Ricci ML, Grottola A, Fregni Serpini G, Bella A, Rota MC, Frascaro F, et al., Improvement of Legionnaires' disease diagnosis using real-time PCR assay: a retrospective analysis, Italy, 2010 to 2015. Euro Surveill. 2018;23(50).
28. Hung YP, Wu CJ, Chen CZ, Lee HC, Chang CM, Lee NY, et al., Comparisons of clinical characters in patients with pneumococcal and Legionella pneumonia. J Microbiol Immunol Infect. 2010; 43 (3): 215-21.
29. Almirall J, Serra-Prat M, Bolibar I, Palomera E, Roig J, Hospital I, et al., Passive smoking at home is a risk factor for community-acquired pneumonia in older adults: a population-based case-

control study. BMJ Open. 2014; 4(6): e005133.
30. Almirall J, Blanquer J, Bello S. Community-acquired pneumonia among smokers. Archivos de bronconeumologia. 2014;50(6):250-4.
31. Anbumani S, Gururajkumar A, Chaudhury A. Isolation of Legionella pneumophila from clinical & environmental sources in a tertiary care hospital. The Indian journal of medical research. 2010;131:761-4.
32. Maniwa K, Taguchi Y, Ito Y, Mishima M, Yoshida S. Retrospective study of 30 cases of Legionella pneumonia in the Kansai region. J Infect Chemother. 2006; 12 (5): 272-6.
33. Prevotat A, Bure M, Bergoin C, Tavernier JY, Van Grunderbeeck N, Yazdanpanah Y, et al., [Factors predicting mortality during an outbreak of Legionnaire's disease in the north of France]. Rev Mal Respir. 2008; 25(3):285-94.
34. Jespersen S, Sogaard OS, Schonheyder HC, Fine MJ, Ostergaard L. Clinical features and predictors of mortality in admitted patients with community- and hospital-acquired legionellosis: a Danish historical cohort study. BMC infectious diseases. 2010;10:124.