International Journal of Basic, Applied and Innovative Research
IJBAIR, 2017, 6(3): 72 - 80
ISSN: 2315 - 5388 www.arpjournals.com; www.antrescentpub.com E-ISSN: 2384 - 681X

RESEARCH PAPER

DETECTION OF LEGIONELLA ANTIGEN IN URINE BY ELISA FOR DIAGNOSIS OF LEGIONNAIRES' DISEASE IN PARTS OF SOUTH EAST, NIGERIA

***Mgbachi, N. G[1]., Onyemelukwe N. F[2]., Mgbakogu R. A[3]., and Ohotu, E. O[4].**
[1]Department of Microbiology, University of Nigeria Teaching Hospital, Enugu, Nigeria. [2] Department of Medical Laboratory Sciences, Faculty of Health Sciences, University of Nigeria, Enugu Campus. [3] College of Health Sciences and Technology, Obosi, Anambra State, Nigeria. [4] Department of Haematology and Immunology, University of Nigeria Teaching Hospital, Enugu, Nigeria.
Corresponding Author: nnemgbachi@yahoo.com

Published: 30th September, 2017

***Endorsed By**: Innovative Science Research Foundation (**ISREF**) and International Society of Science Researchers (**ISSCIR**).*
***Indexed By**: African Journal Online (AJOL); Texila American University; Genamics; Scholarsteer; EIJASR; CAS-American Chemical Society; and IRMS Informatics India (J-Gate)*

ABSTRACT

Diagnosis of Legionnaires' disease by urinary antigen detection has been shown to be specific and timely. This study is designed to evaluate the presence of *Legionella* urinary antigen in mid-stream urine of individuals with respiratory tract infections. A total of 90 samples were collected from 38 males and 52 females. The samples were processed by Enzyme- Linked Immunosorbent Assay (ELISA) technique within 24 hours of collection or preserved at 2 – 8 ^{O}C for not more than 14 days. The results showed 12.2% prevalence of Legionnaires' disease in the subjects. A higher prevalence of the disease was observed more in males 63.6% than in females 36.4%. All age groups were at risk for the disease. Hospital admission duration and type of water for bathing were statistically significant ($p<0.5$) risk factors to Legionnaires' disease infection. Other risk factors like, level of education, type of artificial air source, smoking habit, alcohol consumption and location of residence did not show statistical significance ($p>0.5$). It is therefore recommended that *Legionella* urinary antigen test be included as a primary test for all respiratory tract illnesses since it permits early diagnosis, which will enable prompt treatment of the disease.

INTRODUCTION

Legionnaires' disease (LD) is pneumonia responsible for 1 – 5 % of cases of community – acquired pneumonia (CAP) requiring hospital admission (Dominguez *et al.*, 1998). In most cases, the disease is severe and a fatal form of bacterial pneumonia. (Mercante and Winchell, 2015). *Legionella pneumophila* causes 91% of all reported cases of Legionnaires' disease with sero group 1 being the most predominant (Plouffe *et al.*, 1995). *Legionella pneumophila* has shown to cause nosocomial and community-acquired pneumonia (Lieberman *et al.*, 1996). Patients on admission are particularly at risk from a nosocomial source (Kurtz and MacFarlane, 1996). Risk factors of *Legionella* infection include age (people over 60 years), smoking, excessive drinking, chronic bronchitis, emphysema, steroid therapy, cancer chemotherapy and diabetes mellitus (Brooks *et al.*, 2010).

There is a poor notification rate of Legionnaires' disease even though it is a severe form of pneumonia (Jacquinet *et al*, 2015). In several surveys Legionnaires' disease accounted for 3% - 8% of cases of community-acquired pneumonia (Fang *et al*, 1990). However, the proportion of these cases that are diagnosed and reported to the Centers for Disease

Control and Prevention (CDC) are very small. Inability to diagnose Legionnaires' disease early leads to increased morbidity and mortality (Plouffe *et al*., 1995).

Detection of soluble *Legionella* antigen in urine sample is a rapid method that provides an early diagnosis for *Legionella* infection (Murdoch, 2003). Urinary antigen testing has enabled the recognition of outbreaks of Legionnaires' disease and enhanced a rapid public health response (Lepine *et al.*, 1998) especially for the initiation of appropriate antibiotic therapy (Kashuba and Ballow, 1996).

Urinary antigen test detection of *Legionella pneumophila* sero group 1 show sensitivities in the range of 70 - 100% and specificities up to 100% (Kashuba and Ballow, 1996). *Legionella* antigenuria can be detected as early as 1 day after onset of symptoms and persists for weeks (Kohler *et al*., 1984). The advantages of these methods include ease of specimen collection, the ability to obtain large quantities of specimen for concentration, the ability to detect antigen even after initiation of antibiotic therapy, and the ability to obtain results quickly (Williams and Lever, 1995).

Urinary antigen detection was found to be the most useful test when compared with Direct fluorescent antibody (DFA) and culture (Ramirez and Summersgil, 1994). Though, Radioimmunoassay (RIA) showed great sensitivity in urinary antigen detection but the difficulty involved in the handling and disposal of radioisotopes required in performing RIA, it was replaced by an ELISA in the mid-1980s (Hackman *et al.*, 1996).

There is paucity of information on the presence of the disease in Nigeria. In developed countries, the disease has shown high mortality in untreated cases hence, this study was borne out of a yearning need to establish the presence of Legionnaires' disease using ELISA technique for *Legionella* urine antigen detection in parts of south East, Nigeria.

MATERIALS AND METHODS

Study Area: The study was carried out in parts of South East, Nigeria. The test subjects were recruited from University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu State Teaching Hospital (Park lane), Annunciation Specialist Hospital, Emene, all in Enugu State, Federal Teaching Hospital Abakaliki, Ebonyi State and Federal Medical Center, Owerri, Imo State, all in South East, Nigeria.

Sample Collection: Ninety mid-stream urine samples were collected from 38 males and 52 females that had signs and symptoms of respiratory tract infections. Questionnaires were used to source for vital information (age, Gender, Hospital admission history, Alcohol consumption habit, location of residence, Types of water for bath, Cigarette smoking habit, Level of education, Type of artificial air source). Random urine specimens were collected in standard sterile containers without preservatives. The samples were stored at room temperature and assayed within 24 hours of collection. Alternatively, specimens were stored at 2 - 8 °C for up to 14 days or frozen (-70 °C) for longer periods before testing. All samples were ensured to be at room temperature before performing the assay.

Inclusion and Exclusion Criteria: Only persons with signs and symptoms of lower respiratory tract infections were included for this study. Each person recruited was after an informed consent has been duly signed.

Ethical Consideration: Ethical clearance approval was obtained from the Medical Research Ethics Committee of the University of Nigeria Teaching Hospital, Ituku-Ozalla, Enugu. After critical study of the research proposal, a written approval was signed by the Committee. Informed Consent was obtained from all enlisted subjects. The concept of the research was explained to all the subjects and having understood the scope of the work, granted their consent.

Sample Analysis: This assay is a double antibody (sandwich) ELISA using an anti-*Legionella pneumophilia* antibody to capture the antigen from the urine. A second antibody, conjugated to peroxidase is then added which binds to the complex. This reaction is visualized by the addition of the chromogen tetramethylbenzidine (TMB). The resulting blue color development indicates the presence of *Legionella pneumophilia* antigens being bound by the anti-*Legionella pneumophilia* antibodies. Urine samples were tested in the Diagnostic Automation Inc. Legionella urinary

antigen coated plates according to the manufacturer's instruction. Controls were included each time the kit was run. The results were read as recommended by the Kit manufacturer.

RESULTS

The results showed that the prevalence of Legionnaires' disease in the subjects with signs and symptoms of respiratory tract disease was 12.2% (n = 11). The results also showed that the prevalence of the disease according to gender was higher in males 63.6% (n = 7) than in females 36.4% (n = 4). For age group prevalence, all the age groups had 1.1% (n = 1) except age group 61 – 70 years which had 3.3% (n = 3). Age group difference was not statistically significant in this study (p>0.05). (See Table 1).

Table 1: Age group and sex distribution of positive subjects for urine antigen test

Age group (years)	No. of subjects	No. of positives	Males	Females
1 - 20	13	2	2	0
21 – 40	34	2	1	1
41 – 60	29	2	1	1
61 – 80	12	4	3	1
81 – 100	2	1	0	1
Total	90	11	7	4

Also, hospital admission of subjects with signs and symptoms of respiratory tract infection was found to be statistical significant factor from this study ($p < 0.05$). The prevalence of legionnnaires' disease in subjects admitted above 7 days was highest 36.0% (n = 4), followed by 4 – 7 days' admission (14.3%; n = 1) and least prevalence of 8.3% (n = 6) for subjects that were not on hospital admission. (See Table 2).

Table 2: Distribution of positive subjects for *Legionella* urine antigen test according to hospital admission duration.

	Not admitted	1 – 3 days	4 – 7 days	Above 7 days
No. of subjects	72	0	7	11
Positive subjects	6	0	1	4

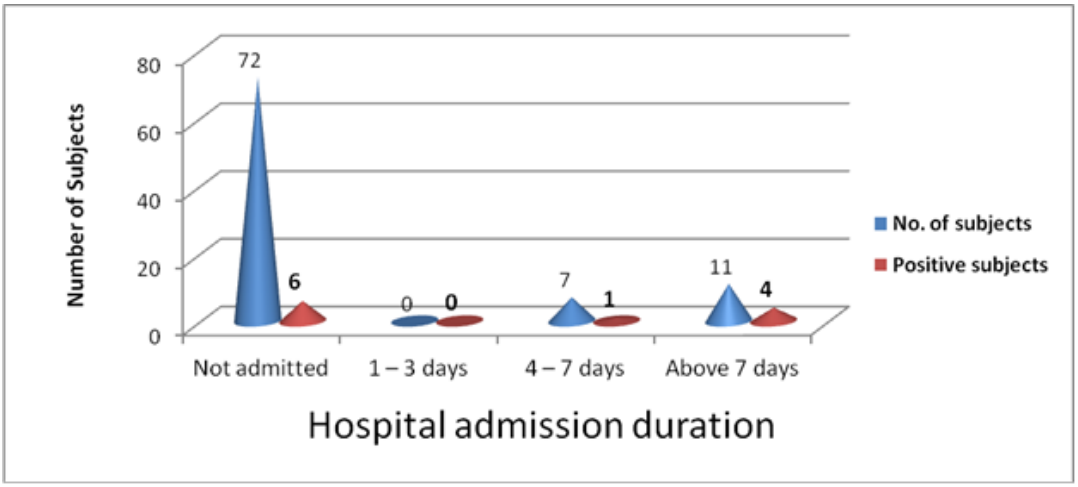

Fig. 1: Distribution of positive subjects for *Legionella* urine antigen test according to hospital admission duration.

Again, from this study, alcohol consumption was not found statistically significant as a prerequisite for *L. pneumophila* infection as detectable in urine ($p > 0.05$). The prevalence of Legionnaires' disease was highest in the non-alcoholic consuming subjects 63% (n = 7), followed by 27.3% (n = 3) from those with average daily alcoholic consumption of 1 - 4 bottles and 9.1% (n = 1) from consumers of 5 – 10 bottles of daily alcohol. See Figure 2.

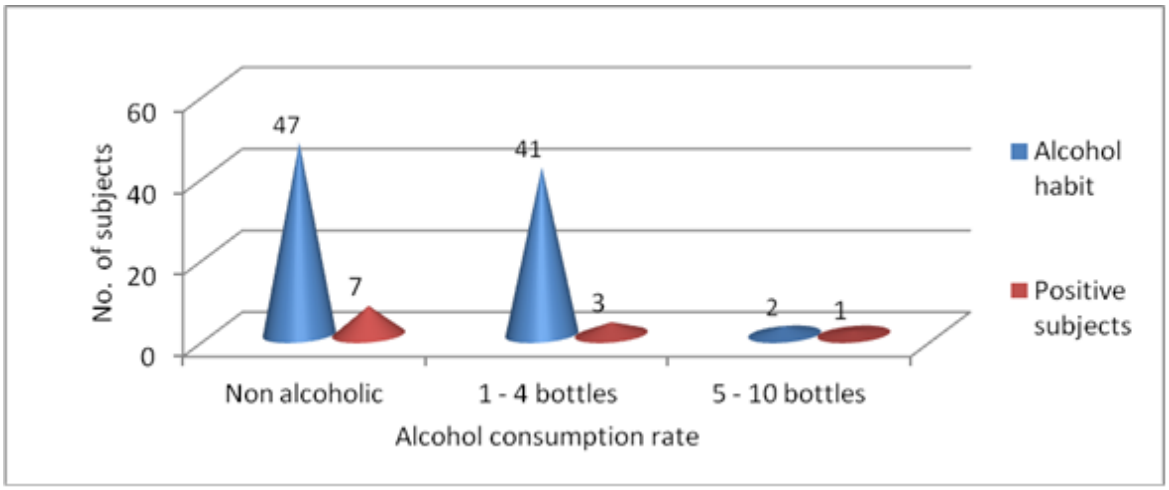

Fig. 2: Distribution of positive subjects for *Legionella* urine antigen test according to alcohol consumption habit.

Furthermore, the distribution of positive subjects for *Legionella* urine antigen test according to location of residence showed that the highest prevalence was seen in urban dwellers 45.6% (n = 5), then the rural dwellers 36.3% (n = 4), the least were semi urban dwellers 18.1% (n = 2). (See Figure 3).

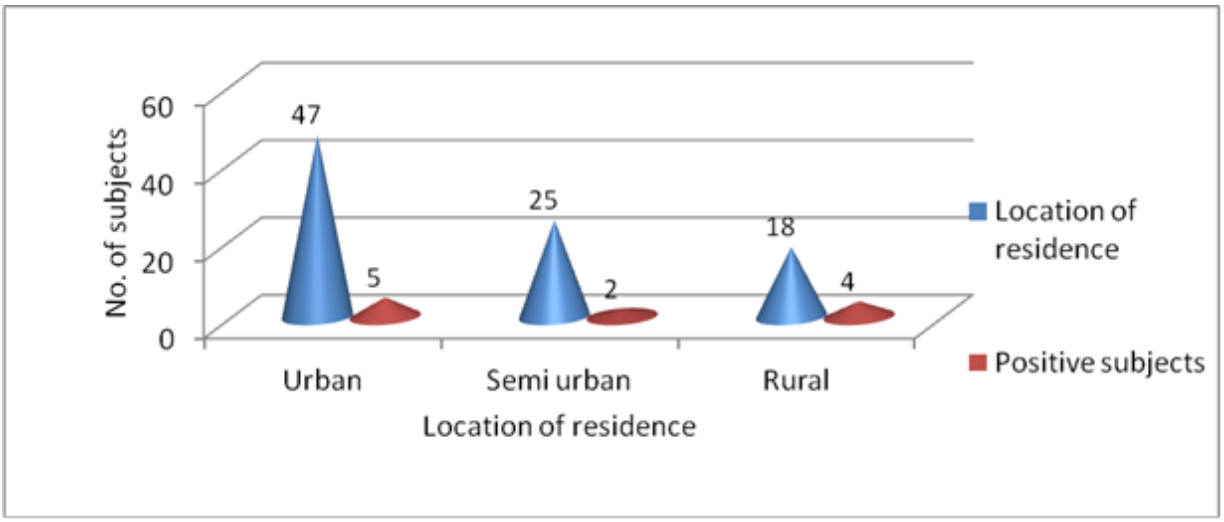

Fig. 3: Distribution of positive subjects for *Legionella* urine antigen test according to location of residence.

Again, as regards to the type of water for bathing, the highest prevalence of the disease was observed in subjects that use Well water for bathing 63.7% (n = 7) followed by subjects that use bore-hole water and stream water for bathing

18.2% (n = 2) respectively. Statistically, type of water for bathing proved to be a significant factor ($p<0.05$) for Legionnaires' disease presence. (See figure 4).

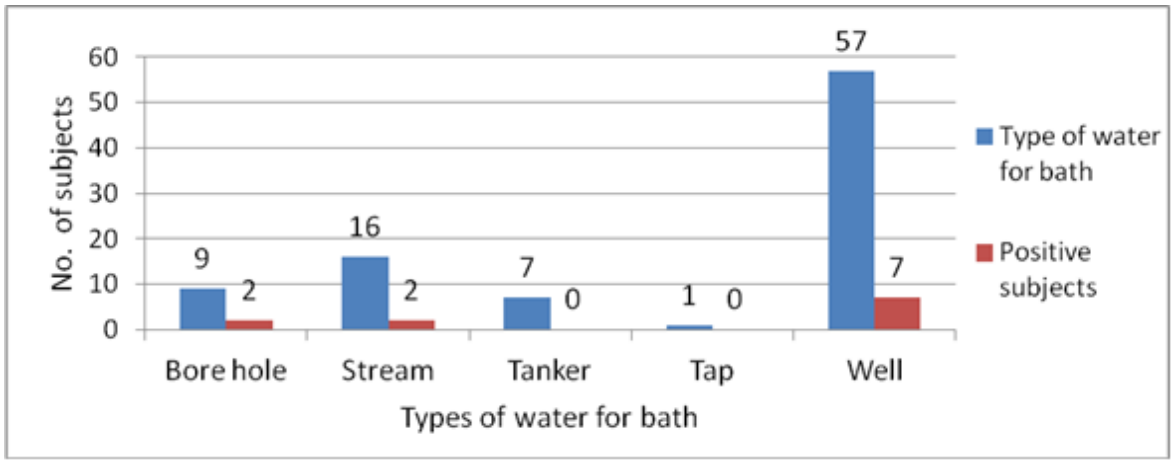

Fig.4: Distribution of positive subjects for *Legionella* urine antigen test according to the type of water for bath.

A high prevalence of 90.9% (n = 10) were from non-smokers while only 1.1% (n = 1) was from a mild smoker that consumed 1 – 4 sticks daily. From this study, smoking was not found to be a statistical significant factor ($p>0.05$) for Legionnaires' disease infection. (See figure 5).

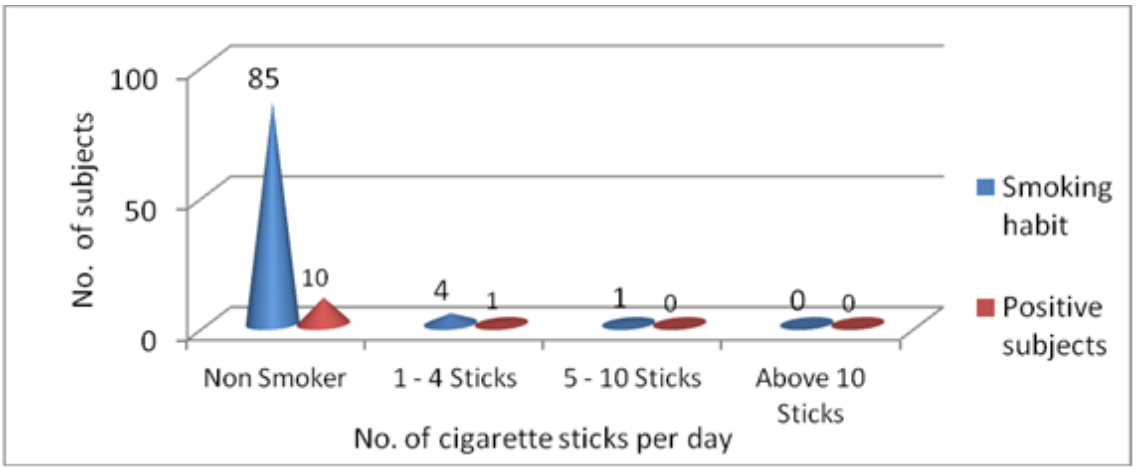

Fig. 5: Distribution of positive subjects for *Legionella* urine antigen test according to smoking habit.

This study showed that the type of artificial air source used by a person does not predispose the individual to Legionnaires' disease infection. A prevalence of 81.8% (n = 9) was observed in subjects that used fan as their artificial source of air, while 18.2% (n = 2) was observed in those that used only natural air. The wide margin in prevalence of the observed was not found to be statistically significant ($p>0.05$). (See figure 6).

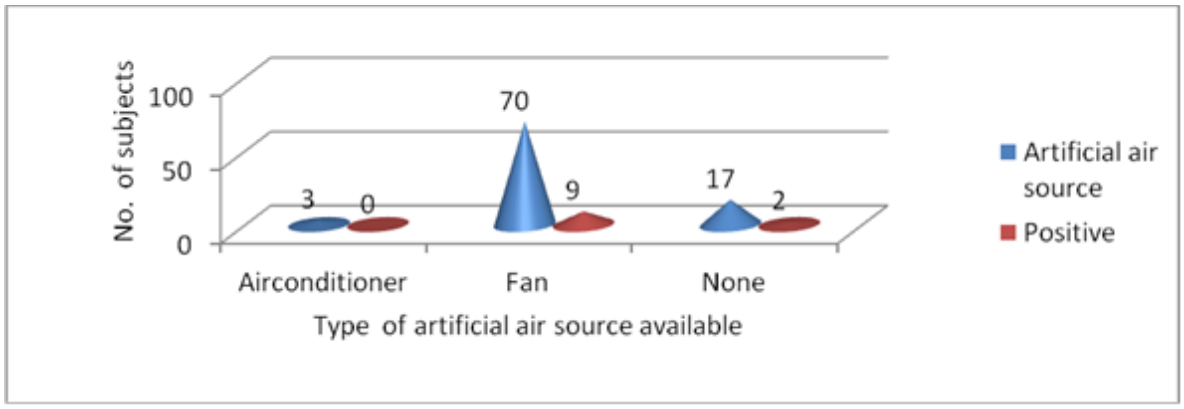

Fig. 6: Distribution of positive subjects for *Legionella* urine antigen test according to type of air source available.

Furthermore, considering the educational level attained by subjects diagnosed with legionnaires' disease, a prevalence of 36.4% (n = 4) was observed in those that had neither formal nor primary education respectively, followed by those that attained secondary 18.2% (n = 2) while a prevalence of 9.1% (n = 1) was from a subject that attained a tertiary level of education. (See figure 7).

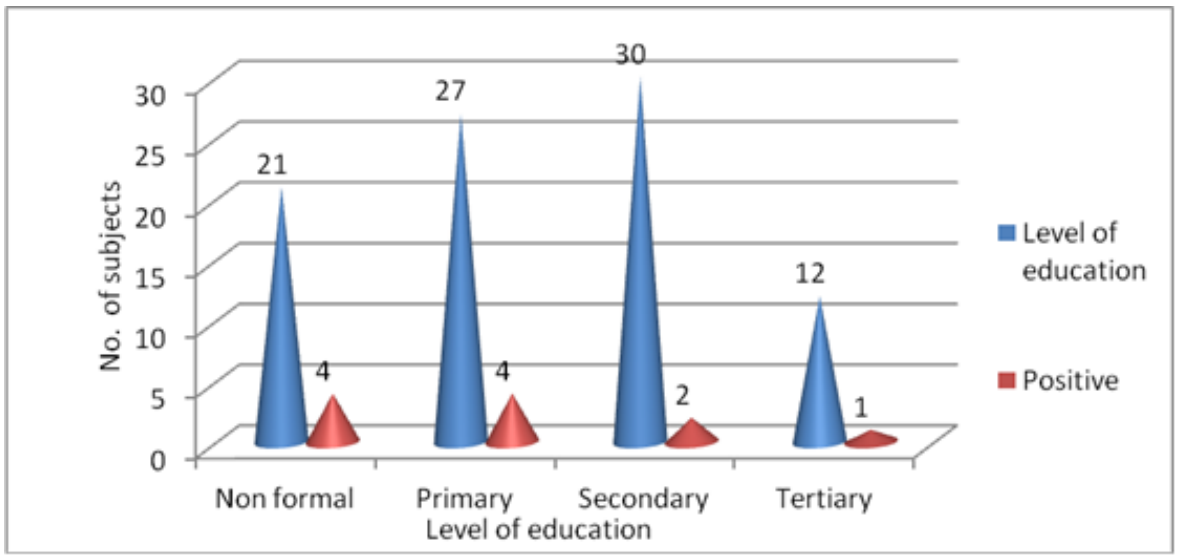

Fig. 7: Distribution of positive subjects for *Legionella* urine antigen test according to level of education.

DISCUSSION

The positive values of *Legionella pneumophila* presence obtained from this study 11 (12.2%) was found to be lower than 35 (66%) obtained by Lindsay *et al.,* (2004) at Scotland and also far much lower than 93 (77.5%) by Birtles *et al.* (1990) in London. These higher values obtained in these developed countries could be due to their weather condition and the availability of hot water systems as well as aerosol producing systems such as cooling towers. The

observed low value could also be due to the documentation by Bhopal (1993) that ecological niches that support *Legionella* are not as common in developing countries, so their incidence may be comparatively low in these countries.

From this study, infection was observed more in males 7 (63.6%) than females 4 (36.4%) as also observed at Metropolitan France by Santa-Olala *et al.* (2005). In theirs, out of the identified cases confirmed by detection of soluble *Legionella* antigen in urine, they observed 13.0% higher prevalence of the infection in males than in females. Legionnaires' disease is most commonly found in subjects over the age of 40 years, with a peak in the 60 – 70-year age group. Deterioration in the body defenses associated with aging is also an important factor, Greenwood *et.al.* (2010). From this study, subjects within the age group 61 – 70 years had the highest prevalence 33.3% (n = 3). This finding resemble the result obtained by Silk *et. al.*, (2013) in Baltimore city. The age range of patients in their study was 62 – 77 years. This could also be due to the fact that Legionnaires' disease most frequently occurs in the elderly, Stout and Yu, (1997), Neil and Berkelman, (2008). In conclusion, *Legionella* urinary antigen test is an important test in diagnosis of Legionnaires' disease infection due to its specificity and timeliness. Subjects on hospitalization for more than 7 days are at a greater risk of the infection. *Legionella pneumophila* can infect all age groups though more in the elderly. Also more males are infected than females. Legionnaires' disease prevalence is lower in developing countries than in developed countries. It is not possible to clinically distinguish patients with Legionnaires' disease from patients with other types of pneumonia and *Legionella* urinary antigen test permits its early diagnosis. It is therefore recommended that *Legionella* urinary antigen test be included as a primary test for all respiratory tract illness symptoms.

ACKNOWLEDGEMENT

On behalf of other contributors, I wish to appreciate the following; my beloved husband, Mr. Ernest Mgbachi for his financial and moral support throughout the research work. All my subjects and staff of UNTH Ituku-Ozalla, Enugu State Teaching Hospital (Park lane) and Annunciation Specialist Hospital, Emene, all in Enugu State, Federal Teaching Hospital Abakaliki, Ebonyi State and Federal Medical Center, Owerri, Imo State, all in South East, Nigeria.

REFERENCES

Bhopal, R. S., (1993). Geographical Variation of Legionnaires' Disease: A Critique and Guide to Future Research. *International Journal of Epidemiology*; 22(6):1127 - 1136.

Birtles, R. J., Harrison, T. G., Samuel, D., and Taylor, A. G., (1990). Evaluation of urinary antigen ELISA for diagnosing *Legionella pneumophila* serogroup 1 infection. *Journal of Clinical Pathology*, 43: 685 – 690.

Chang, F. Y., Stout, J. E., and Yu, V. L., (1996). Assessment of enzyme immunoassay versus dioimmunoassay for detection of *Legionella pneumophila* serogroup 1 antigen in frozen urine specimens. *Journal of Clinical Microbiology*; 34**:** 2628 - 2629.

Dominguez, J. A., Gali, N., Pedroso, P., Fargas, A., Padilla, E., Manterola, J. M., Matas, L., (1998). Comparison of the Binax *Legionella* urinary antigen enzyme immunoassay (EIA) with the Biotest Legionella Urine antigen EIA for detection of *Legionella* antigen in both concentrated and non-concentrated urine samples. *Journal of Clinical Microbiology*; 36: 2718 – 2722.

Fang, G. D., Stout, J. E., Yu, V. L., Goetz, A., Rihs, J. D., Vickers, R. M., (1990). Community- acquired pneumonia caused by *Legionella dumoffii* in a patient with hairy cell leukemia. *Infection*; 18: 383 – 385.

Greenwood, D., Slack, R., Peutherer, J., Barer, M., (2010). *Legionella* in Medical Microbiology: a guide to microbial infections. 17th Ed. Pg. 332 – 335. Churchill Livingstone.

Hackman, B. A., Plouffe, J. F., Benson, R. F., Fields, B. S., Breiman, R. F., (1996). Comparison of Binax Legionella urinary antigen EIA kit with Binax RIA urinary antigen kit for detection of *Legionella pneumophila* serogroup 1 antigen. *Journal of Clinical Microbiology*; 34: 1579 - 1580.

Joseph, J. A., and The European Working Group for *Legionella* Infections. (2004). Legionnaires' disease in Europe 2000 – 2002. *Epidemiology of Infections*; 132**:** 417 – 424.

Kashuba, A. D., and Ballow, C. H., (1996). *Legionella* urinary antigen testing: potential impact on diagnosis and antibiotic therapy. *Diagnostic Microbiology and Infectious Diseases*; 24(3):129-139.

Kohler, R. B., Winn, W. C., Wheat, L. J., (1984). Onset and duration of urinary antigen excretion in Legionnaires' disease. *Journal of Clinical Microbiology*; 20:605-607.

Kurtz, J. B., and Macfarlane, J. T., (1996). Legionellosis and legionnaires' disease. Oxford Text book of Medicine. Oxford University Press Inc. New York (13th ed.) pp 722 – 728.

Lepine, L. A., Jernigan, D. B., Butler, J. C., Pruckler, J. M., Benson, R. F., Kim, G., Hadler, J. L., Cartter, M. L., Fields, B. S., (1998). A recurrent outbreak of nosocomial Legionnaires' disease detected by urinary antigen testing: evidence for long – term colonization of a hospital plumbing system. *Infection Control Hospital Epidemiology*; 19**:** 905 - 910.

Lieberman, D., Porath, A., Schlaeffer, F., Lieberman, D., Boldur, I., (1996). *Legionella specie* community-acquired pneumonia. A review of 56 hospitalized adult patients. *Chest* 109: 1243 – 1249.

Lindsay, D. S. J., Abraham, H., William, F., Christie, P., Johnston, F., Edwards, G. F. S., (2004). Laboratory diagnosis of legionnaires' disease due to *Legionella pneumophila* serogroup 1: comparison of phenotypic and genotypic methods. *Journal of Medical Microbiology* 53: 183 – 187.

Murdoch, D. R., (2003**).** Diagnosis of *Legionella* Infection. *Clinical Infectious Diseases*; 36: 64 – 69.

Neil, K., and Berkelman, R., (2008). Increasing incidence of legionellosis in the United States, 1990 – 2005: changing epidemiologic trends. *Clinical Infectious Diseases*; 47: 591 – 599.

Plouffe, J. F., File, T. M. Jr., Breiman, R. F., Hackman, A. B., Salstrom, S. J., Marston, B. J., Fields, B. S., **(**1995). Reevaluation of the definition of Legionnaires' disease: use of the urinary antigen assay. *Clinical Infectious Diseases*; 20**:** 1286 – 1291.

Ramirez, J. A., and Summersgill, J. T., (1994). Rapid tests for the diagnosis of *Legionella* infections. *Journal of Kentucky Medical Association* 92: 62 – 65.

Santa – Olala, P., Campese, C., Jacquier, G., Bitar, D., Che, D., (2005). Host related and environmental risk factors associated with sporadic and community- acquired legionnaires' disease: a matched case – control study. 20th Annual Meeting European Working Group for *Legionella Infections*: *Abstract book* 37 – 78.

Stout, J. E., and Yu, V. L., (1997). Legionellosis. *New England Journal of Medicine*; 337: 682 - 687.

Silk, B. J., Foltz, J. L., Ngamsnga, K., Brown, E., Munoz, M. G., Hampton, L. M., Jacobs – Slifka, K., Kozak, N. A., Underwood, J. M., Krick, J., Travis, T., Farrow, O., Fields, B. S., Blythe, D., Hicks, L. A., (2013). Legionnaires' disease case finding algorithm, attack rates, and risk factors during a residential outbreak among older adults: an environmental and cohort study. *Bio Med Central Infectious Diseases*: 13: 291.

Williams, A., and Lever, M. S., (1995). Characterization of *Legionella pneumophila* antigen in urine of guinea pigs and humans with Legionnaires' disease. *Journal of Infection*; 30: 13 - 16.

AUTHORS' CONTRIBUTIONS

All the authors contributed significantly towards this research that enabled the actualization of the objectives of this work