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Prevalence of Legionella Pneumophila among Patients with Pneumonia at Al-Mak Nemer Hospital in Shendi City, Sudan

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Abstract

Background: Legionella pneumophila is becoming more widely acknowledged as a significant public health concern and a cause of community-acquired pneumonia (CAP). It must be well-known how vital different geographic locations are for Legionella species as CAP etiologic agents. The annual incidence is expected to be between 25,000 and 35,000 cases. In the summer, cases are more common when air conditioning is used. Five to thirty percent of people who contract legionnaires' disease pass away, and the untreated death rate can rise to eighty percent.

Aim of the study: To detect *Legionella pneumophila's* prevalence among pneumonia patients at Al-Mak Nemer Hospital in River Nile State, Shendi City, Sudan.

Patients and Methods: This study was a descriptive, cross-sectional, hospital-based study on 73 sputum samples meticulously collected from August to January 2022 at Al-Mak Nemer Hospital in River Nile State, Shendi City, Sudan. The following was done to all subjects: A sterile screw-top container was used to collect the sputum sample, which was then cultivated in a buffered charcoal yeast extract agar medium (BCYE). The plates were then incubated in a candle jar with 3-5% CO2 at 37ºC in a humidified atmosphere, and they were checked for Legionella spp. for 4–14 days. The growing isolates were identified by their colonial morphology, gram stain, and biochemical tests, ensuring the highest standards of accuracy.

Results: There were males (44.8%) and females (45.2%); out of Seventy-three sputum samples, only 3 (4.1%) were positive cultures for *Legionella pneumophila*. Their age ranged from under 65 (43; 58.9%), and only 30 (41.1%) were above 65. Cases with Legionella showed no significant statistical correlation between gender, residence, duration of CAP, and exposure risks (showers, air conditioning, swimming pools, and fountains). The study found that there was a significant statistical association between *Legionella pneumophila* and age (P value 0.03), with a higher frequency observed in older adults (≥65 years).

Conclusion: The spread of Legionella's impact on Community-Acquired Pneumonia is a global concern. Despite the lack of research from low- and middle-income nations, our study underscores the urgent need for more research in these regions to understand and mitigate the impact of this infection.

Keywords: Legionnaires' disease; Community-acquired Pneumonia; Sputum; Sudan

Abbreviations

CAP: Community-acquired Pneumonia; BCYE: Buffered Charcoal Yeast Extract Agar Medium; CT: Computerized Tomography; ELISA: Enzyme-linked Immune Sorbent Assays; IFA: Indirect Fluorescent Antibody.

Introduction

One definition of pneumonia is an infection of the parenchyma of the lung. Instead of treating pneumonia as a single illness, healthcare professionals must recognize that it is an umbrella term for a collection of syndromes caused by different organisms, leading to varied presentations and sequelae [1]. The lower respiratory tract below the larynx is sterile, so infections go to the lung by inhalation or aspiration, or they can be caused by direct injection (trauma or wound) or by being bloodborne [2]. Acute lung infections in a patient not recently admitted outside a hospital are called community-acquired pneumonia (CAP) [3]. Pneumonia, as commonly described in medical literature, is a new (though more accurately described as "newly recognized") pulmonary infiltration on chest X-ray or computerized tomography (CT). However, the diagnosis is not always straightforward. It requires vigilance and the identification of at least two of the following clinical symptoms: Leukocytosis or leukopenia; fever or hypothermia; oxygen desaturation; disorientation; a new or worsening cough, sputum output, or shortness of breath; pleuritic chest discomfort. This underscores the urgency and responsibility of medical professionals in identifying and managing pneumonia [3,4]. In the United States, there are 4 million cases of CAP annually, with an incidence of 5–10 cases per 1000 individuals [3]. This makes CAP the leading infectious disease-related cause of death [4]. Among the over 15 serogroups, Legionella pneumophila, particularly serogroup 1, is the most common cause of human illness [5]. Legionella species are fastidious gram-negative bacteria, ubiquitous, and associated with two distinct clinical syndromes: Legionnaire's disease and Pontiac fever. Legionnaires' disease is a severe multisystem disease comprised of fevers, rigors, headaches, myalgias, dyspnea, delirium, and diarrhea, whereas Pontiac fever is a self-limited influenza-like illness [5]. Since the disease was first described in 1977, Legionella pneumophila has gained recognition as a cause of both epidemic and sporadic community-acquired pneumonia (CAP) in healthy and immunocompromised hosts. Prompt initiation of antimicrobial therapy, including activity against these atypical pathogens, is essential because up to 20% of cases of community-acquired pneumonia are caused by atypical pathogens, and it has been demonstrated that delays in proper diagnosis and treatment of Legionella increase mortality [5,6]. Though lengthier incubation periods of up to two weeks can be required, thriving cultures typically take three to five days. Therefore, even when cultural results are accurate, they shouldn't be the only factor to inform therapeutic choices [6,7]. Urinary antigen testing for Legionella has essentially replaced serologic testing and increased the speed of diagnosis. It's essential to be aware that the sensitivity of serologic testing using enzyme-linked immune sorbent assays (ELISA) or indirect fluorescent antibody (IFA) can vary from 20 to 70%, while the specificity is 95%. However, after 8–12 weeks, acute and convalescent titers must increase fourfold by > 128 [7]. On the other hand, the urine antigen test is widely accessible and relatively easy to use; in many facilities, it may be completed in hours. However, the main drawback is that serogroup 1, which makes up about 70–80% of cases, is still the only one detected by commercial urine antigen assays [8]. The quality of the study and the severity of the condition determines sensitivity. Shimada et al. recently conducted a thorough meta-analysis that found that urine antigen testing had an excellent specificity of 0.99, providing a high confidence level in the test results and an overall pooled sensitivity of 0.74 [9]. In recent years, we have also seen the development of novel Legionella pneumonia diagnostic methods (such as the urine antigen test and polymerase chain reaction) and antibiotic treatments (such as third-generation fluoroquinolones and more current macrolides). However, the number of comprehensive clinical studies examining the problem remains low despite the potential impact of these advancements on case identification and case fatality rates. Passive monitoring systems provide the majority of data on trends in Legionnaires disease [10,11]. Therefore, we must conduct more thorough clinical research. This is not only to enhance patient outcomes but also to deepen our understanding of Legionella pneumonia, and it is a task that cannot be delayed. The present study aimed to detect Legionella pneumophila prevalence among pneumonia patients at Al-Mak Nemer Hospital in River Nile State, Shendi City, Sudan.

Methodology

This observational cross-sectional hospital-based study, conducted with meticulous attention to detail, aimed to determine *Legionella pneumophila* among hospitalized patients from August to January 2022 at AL-Mak Nemer Teaching Hospital in Shendi locality, a significant medical Centre in River Nile State, Sudan. Shendi is a town in northern Sudan situated on the east bank of the Nile (150 km) northeast of Khartoum. Shendi is also about 45 km southwest of the ancient cities of Meroawi and Napata, 250 km to the northwest. Patients attending the study area during the period of study diagnosed as community-acquired pneumonia patients in this study included males and females aged 18 years and older. Patients who agreed to fill out the informed consent form were included in the study according to their age. Patients admitted to the

hospital before the previous 15 days were excluded, as were those who disagreed to participate or refused to complete informed consent.

Sample Size

A total of 73 convenience non-probability probability samples for sputum were meticulously collected from study participants. These participants were randomly selected from all patients who met the specific criteria for hospitalization with CAP during the four months. The requirements included gastrointestinal symptoms, especially diarrhea; neurological symptoms, especially confusion; fever up to 40°C; hyponatremia; and hepatic dysfunction, all of which were critical indicators for the study. The thoroughness of this study ensures the reliability of the findings.

Data Collection

Personal and clinical data was meticulously collected using a comprehensive, structural, closed-ended questionnaire after the verbal consent of each patient or his relative to participate in the study. This included age, sex, residence, exposure risks, and duration of CAP, ensuring a thorough understanding of each participant's condition.

Collection of Specimens

The sputum sample is collected with utmost care in a sterile plastic cup in the morning. Before eating or drinking anything, the patient must brush their teeth and rinse their mouth with water. They then take an intense breath, hold the air for 5 seconds, slowly breathe, take another deep breath, and cough hard until some sputum enters their mouth. The sputum is then carefully spat into the sterile plastic cup, ensuring it is not saliva. The date and number of the patient are then written on the cup, maintaining the sample's integrity. After being cultivated in buffered charcoal yeast extract agar medium under aseptic conditions (near a Bunsen burner), all samples were incubated in a candle jar with 3-5% CO2 at 37ºC in a humidified atmosphere for 4–14 days to check for the presence of Legionella spp.

Indirect Gram Stain

You are responsible for preparing a dry smear as a student or professional in microbiology or laboratory sciences. This involves emulsifying a colony in a drop of physiological saline and spreading it evenly on a clean, dry slide. Then, allow it to dry and fix the smear by passing over the flame for seconds. Crystal violet is added to the fixed smear for one minute and then washed with tap water. Lugol's iodine is added for one minute and washed off with a tab of water, then decolorized by using acid alcohol for 15-20 seconds and also washed with a tab of water. Finally, saffron is added for 2 minutes, washed off with tap water, and the back of the slide is carefully wiped and left to dry before being examined under a microscope with an oil immersion lens (X100).

Interpretation of Cultural Growth

Aliquots of 0.1 ml were carefully plated onto BCYE Agar medium (Difco Laboratories, Detroit, Mich., USA) supplemented with L. cysteine from all concentrated and treated samples. After that, the plates were kept in a candle jar with 3–5% CO2 at 37ºC in a humidified environment for 4–14 days to check for Legionella Spp. Colonies. This extended incubation period ensures the detection of even the most elusive Legionella Spp.

Colonies exhibiting traits of Legionella species, such as glossy, greyish-white colonies, were chosen following incubation. The thin, weakly pigmented filamentous Gram-negative morphology was demonstrated using a gram stain. For verification, suspected colonies were subcultured on non-selective mediums such as sheep-blood agar, McConkey agar, and BCYE agar with and without L-cysteine. L. cysteine-containing isolates that developed on BCYE agar but not on the other media were regarded as suspected Legionella. The comprehensive nature of the identification process is further underscored by the use of biochemical assays to identify Legionella species.

Statistical Analysis

A thorough statistical analysis was conducted to ensure the validity of the results. Some tables were constructed and calculated manually, while others were done by Statistical Package for Social Science (SPSS) version 26, providing a comprehensive understanding of the data.

Results

Seventy-three sputum samples were obtained from hospitalized patients diagnosed with pneumonia in AL-Mak Nemer Hospital in Shendi town. In this study, the participants were classified into two groups according to their ages: most of them were under 65 years old (43; 58.9%), and only 30 (41.1%) were above 65 years old (Table 1). The study participants were also classified according to gender into males (44.8%) and females (45.2%) (Table 2). In this study, about 49 (67.1%) of the participants were from rural areas, and only 24 (32.9%) were from urban areas (Table 3). In this study, the exposure risk of showers was 8 (11%), air conditioners were 20 (27.4%), both showers and air conditioners were 18 (24.6%), fountains were 0%, swimming pools were 0%, and 27 (37%) of study populations showed no exposure to the selected risk factors (Table 4). The duration

of CAP from 1–7 days (69.9%) was higher than 8–14 days (30.1%) (Table 5). The study revealed that the frequency of *L. pneumophila* among patients with pneumonia was 4.1% (Table 6) and found that the positive cases of *L. pneumophila*in among males were 1 (2.5%) and 2 (6.0%) among females. There was a significant statistical correlation between the *L. pneumophila* infection and patients' age (*P-value* = 0.035) (Table 7). The study denoted that there is no significant statistical correlation between *L. pneumophila* and gender, residence, exposure risks, and duration of CAP (*P-value* = 0.453) (Table 8), (*P-value* = 0.145) (Table 9), (*P-value* = 0.886) (Table 10), (*P-value* = 0.251) (Table 11), respectively.

Age Groups	Frequency	Percent %
Less than 65 years	43	58.90%
More than 65 years	30	41.10%
Total	**73**	**100%**

Table 1: The distribution of the study population according to age.

Gender	Frequency	Percentage
Male	40	54.80%
Female	33	45.20%
Total	**73**	**100%**

Table 2: The distribution of the study population according to gender.

Residence Area	Frequency	Percent %
Rural	49	67.10%
Urban	24	32.90%
Total	**73**	**100%**

Table 3: The distribution of the study population according to the area of residence.

Exposure Risks	Frequency	Percent %
Shower	8	11%
Air condition	20	27.40%
Both	18	24.60%
Swimming pool	0	0%
Fountains	0	0%
No	27	37%
Total	**73**	**100%**

Table 4: The distribution of the study population according to exposure risks.

Duration of CAP	Frequency	Percent %
1 -7 days	51	69.90%
8 -14 days	22	30.10%
Total	**73**	**100%**

Table 5: Distribution of study population according to duration of CAP.

Legionella Pneumophila	Frequency	Percent %
Positive	3	4.10%
Negative	70	95.90%
Total	**73**	**100%**

Table 6: The frequency of *Legionella pneumophila* in patients with CAP.

Age Group	*Legionella Pneumophila*		*P value*
	Frequency	Percent %	
Under 65 years (n=43)	0	0%	
Above 65 years (n=30)	3	10%	**0.035**

Table 7: The correlation of *Legionella pneumophila* infection with age.

Gender	*Legionella pneumophila*		*P value*
	Frequency	Percent %	
Male (n=40)	1	2.50%	
Female ((n=33)	2	6.10%	**0.453**

Table 8: The correlation of *Legionella pneumophila* infection with gender.

Residence Area	*Legionella pneumophila*		*P value*
	Frequency	Percent %	
Rural (n=49)	3	6.10%	
Urban (n=24)	0	0%	**0.145**

Table 9: The correlation of *Legionella pneumophila* infection with residence area.

Exposure Risks	*Legionella pneumophila*		*P value*
	Frequency	Percent %	
Shower (n=8)	1	12.50%	
Air condition (n=20)	2	10%	
Both (n=18)	0	0%	**0.886**
Fountains (n =0)	0	0%	
Swimming pool(n=0)	0	0%	
No =27	0	0%	

Table 10: The correlation of *Legionella pneumophila* infection with exposure risks.

Duration of CAP	*Legionella Pneumophila*		*P value*
	Frequency	Percent %	
1 -7 days (n=51)	2	3.90%	0.251
8 -14 days(n=22)	1	4.54%	

Table 11: The correlation of *Legionella pneumophila* infection with duration of CAP.

Discussion

It is becoming more widely acknowledged that Legionella species can induce occasional and severe CAP that necessitates hospitalization. 95% of cases of Legionnaires disease in the US and Europe are caused by L. pneumophila [12]. A 2008 article [13] used advanced diagnostic techniques on CAP patients to find that 3.8% of hospitalized patients had *L. pneumophila*. This pioneering study, which utilized cutting-edge diagnostic methods, is a crucial reference point for our current investigation. Only three (4.1%) of the 73 sputum specimens taken from individuals with community-acquired pneumonia in the current investigation demonstrated positive growth for Legionella pneumophila. Our research supports a 2008 study by von Baum H. and associates in Germany that examined the prevalence of legionella pneumonia, which was shown to affect 3.8% of patients [13]. This finding disagreed with Rabih and his co-workers in 2014, who revealed that about 68 (22.7%) of 300 cases showed positive growth for *Legionella pneumophila* [14]. However, the result is lower than that reported by Elsanousi in 2017, who indicated that about 222 (42.3%) were positive for *Legionella pneumophila growth* [15]. Also, Mustafa and his colleagues in 2019 found that 53.8% were positive for *L. pneumophila* [16]. These variations in studies have been attributed to the differences in study populations and variations in sample sizes. In this study, 43 (59.1%) participants were under 65, and only 30 (41.9%) were over 65. The study revealed a significant statistical association between infection with *legionella pneumonia* and age (*P. value* = 0.035), in which the infection rate was higher in those over 65 years old compared to those under 65 years old. This finding was agreed upon by a study conducted in Iran by Goodarzi in 2020, in which the frequency of *L. pneumophila was* significantly higher among patients over 60 years old *(P. value = 0.03)* [17]. The higher frequency among older adults may be due to their weak immunity. In this study, *L. pneumophila* was isolated from 2 (6.1%) females and only 1 (2.5%) males, with no significant statistical correlation between *Legionella pneumophila* infection and gender (*P. value =0.453).* This finding disagreed with results obtained by Mustafa and his colleagues in 2019, who indicated the frequency of *L. pneumophila was* higher among males (18.5%) than females (13.2%) [16]. The study revealed no significant statistical correlation (*P. value* = 0.145) between *L. pneumophila* infection in the residence area, in which the frequency of infection among rural patients was 3 (6.1%) and 0% among urban patients. In this study, the frequency of *L. pneumophila infection among* those exposed to air conditioners was 2 (10%), 1 (12.5%) in those exposed to showers, and 0% for those exposed to both air conditioners and showers, fountains, or swimming pools, with no significant statistical correlation with these risk exposures (*P. value* = 0.886). The current study showed that the frequency of *L. pneumophila* infection among those with a duration of community-acquired pneumonia of 1–7 days was 2 (3.9%) and about 1 (4.54%) among those with a duration of 8–14 days. The study revealed no significant correlation between *L. pneumophila* infection and the duration of pneumonia (*P. value* = 0.251). While not definitive, these findings provide valuable insights into the prevalence and factors associated with Legionella pneumophila infection in patients with community-acquired pneumonia, highlighting the need for further research.

Conclusion

Legionella's spread and impact on Community-Acquired Pneumonia are global concerns. Despite the lack of research from low—and middle-income nations, our study underscores the urgent need for more research in these regions to understand and mitigate the impact of this infection.

Sources of Funding

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Conflict of Interest

The authors have declared that no competing interests exist.

Author Contribution Statement

Saria Hamza Elhassan Mohamed and Waseem Sameer Kwami were responsible for Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, and Supervision. Leila Mohamed A. Abdelgader, Tibyan Abd Almajed Altaher, and Ghanem Mohammed Mahjaf are responsible for Writing - reviewing, and editing the manuscript. All authors read and approved the final manuscript.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available upon reasonable request from the corresponding author.

Ethical Approval

The study was approved by the Department of Medical Microbiology in Medical Laboratory Sciences at Shendi University; the study was matched to the ethical review committee board. Sample collection was done after signing a written agreement with the participants. Permission for this study was obtained from the local authorities in the area. This study's aims and benefits were explained with the assurance of confidentiality. All protocols in this study were done according to the Declaration of Helsinki (1964).

Consent

The patient's written consent has been collected.

Consent to Publish

Not applicable.

Acknowledgment

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