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Review Article

Microbiology Section

Review Article Legionellosis: An update

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ABSTRACT

Legionella is an important cause of adult pneumonia and it must be actively considered in both community-acquired and nosocomial pneumonia. The incidence of legionellosis is underestimated for a variety of reasons, which include a lack of clinical awareness, a non-classical presentation, extrapulmonary infections, a delayed seroconversion and a lack of specialized culture facilities or urinary antigen detection tests. More illness is usually found in the summer and in early fall (June to September), but it can occur at any time of the year. It is uncommon in our country, because we are not looking for it hard enough. Unless the doctor looks out and tests for Legionella, these cases are invariably missed. Physicians need to familiarize themselves with the disease, as a prompt recognition and an early treatment can considerably curtail the total outcome in the affected cases and prevent additional cases. Although many aspects about this disease are clear, there are some dark areas regarding the vaccine development, that need to be further explored and understood. The present article details out almost everything which is known about this disease, along with the review of the recent literature.

Key Words: Legionella, Legionellosis, Pneumonia, Treatment

INTRODUCTION

In 1976, an explosive outbreak of pneumonia occurred among the persons who attended an American Legion convention in Philadelphia [1]. A total of 221 persons became ill with pneumonia, and 34 of them died. Later, in January 1977, Dr.Joseph McDade of the Centers for Disease Control isolated the bacterium which caused the outbreak [2].

Legionella pneumophila, the agent of this outbreak, became the first named member of the family, Legionellaceae. Although this bacterium was found before 1976, more illness from the Legionnaires' disease is being detected now.

This is because we are looking for this disease whenever a patient has pneumonia. Currently, the family, Legionellaceae, comprises of 48 species with 70 serogroups [3]. Legionella pneumophila contains 14 serogroups; the serogroups [1,4] and 6 are the most commonly implicated ones in human infections [4]. To date, 20 species other than L. pneumophila have been associated with human infections [Table/Fig-1], among which L. micdadei, L. bozemani, L. dumoffii and L. longbeachae are the most common ones [5,6]. Legionellosis refers to the two clinical syndromes (Pontiac fever and Legionnaires' disease) which are caused by the bacteria of the genus, Legionella. New diagnostic, therapeutic and preventive modalities are being developed to tackle this fatal disease of mankind.

THE ORGANISM

Identification

Legionellae appear as lightly staining, pleomorphic, and thin gram-negative rods. They grow on BCYE agar, but not on media which does not have L-cysteine. Their growth is slow (3 to 5 days) and the colonies show the characteristic "ground glass" appearance. Specific staining with fluorescein-labeled antibodies can confirm the identity of the organisms. Legionellae stain much more readily in the tissues with the Diff-Quik, Giemsa or the Gram-Weigert stains than they do with the traditional Gram staining.

Legionella are strictly aerobic and nutritionally fastidious. They require media which are supplemented with L-cysteine and iron for their primary isolation. They can be grown on Buffered Charcoal Yeast-Extract (BCYE) agar [7] and Mueller- Hinton medium which contains 1% haemoglobin and 1% Isovitalex [8].

These organisms are non-fermentative and they derive energy from the metabolism of amino acids [9]. Most of the species are motile and catalase-positive, they liquefy gelatine, and they do not reduce nitrate or hydrolyze urea. The sodium hippurate hydrolysis test is positive for L. Pneumophila [10] and negative for a majority of the other Legionella species.

Legionellae are asaccharolytic and weakly catalase or oxidase-positive. In contrast to the identification of the genus, the species classification requires the help of reference laboratories. Although the biochemical tests and the ability of the rods to fluoresce under long-wave ultraviolet light are useful for differentiating the species, the species can be identified definitively only through an analysis of the major branched-chain fatty acids in the cell wall and by nucleic acid genetic studies [42].

Initially, legionellae were identified to the serogroups level by using polyclonal or monoclonal antisera. But now, the L. pneumophila serogroup 1 can be divided into a number of subtypes by various molecular techniques. The various techniques include pulsed-field gel electrophoresis (PFGE), arbitrarily primed PCR (AP-PCR), electrophoretic alloenzyme typing, plasmid typing, restriction fragment length polymorphism, ribotyping and amplified fragment length polymorphism. Subtyping is used to match the environmental isolates with the patient isolates which are obtained during investigations on the legionellosis outbreak.

Species	No. of serogroups associated with disease
L. pneumophila	15
L. bozemanii	2
L. dumoffii	1
L. micdadei	1
L. longbeache	2
L. jordanis	1
L. wadsworthii	1
L. hackeliae	2
L. feeleii	2
L. maceachernii	1
L. birmingamensis	2
L. cincinnatiensis	1
L. gormanii	1
L. sainthelensi	2
L. tucsonensis	1
L. anisa	1
L. lansingensis	1
L. erythra	1
L. parisiensis	1
L. oakridgensis	1

[Table/Fig-1]: Legionella species and serogroups associated with human disease

DIFFERENTIAL DIAGNOSIS

The Legionnaires' disease is often included in the differential diagnosis of "atypical pneumonia" along with infections which are caused by Chlamydia pneumoniae, C.psittaci, Mycoplasma pneumoniae, Coxiella burnetii and some viruses. The clinical similarities among these types of pneumonia include a relatively non-productive cough and a low incidence of grossly purulent sputum. However, the clinical manifestations of Legionnaires' disease are usually more severe than those of most cases of the "atypical" pneumonia. Diarrhoea and hyponatraemia occur significantly more often in Legionnaires' disease than in other forms of pneumonia. The findings on chest radiography do not serve to distinguish Legionnaires' disease from pneumonias of other aetiologies [38].

Depending upon their growth on the BCYE agar, legionellae should be differentiated from Francisella tularensis, Bordetella pertussis and certain thermophilic spore-forming bacilli. In contrast to the Francisella spp., which produce acid from carbohydrates, the Legionella spp. neither ferment nor oxidize carbohydrates. The Legionella spp. produce the characteristic branched- chain fatty acids in their cell wall, which help in differentiating them from other organisms [39].

LABORATORY DIAGNOSIS

The diagnosis of Legionnaires' disease requires special microbiological tests [Table/Fig-3]. Various abnormalities which have been noted in Legionella pneumonia are hyponatraemia, thrombocytopaenia, haematuria, hypophosphataemia and abnormal liver function tests. Saline or sodium salt-based buffers should not be used in processing or transporting the specimens which contain legionellae because of the inhibitory effects of sodium on the Legionella spp. The culture of the respiratory secretions is the most specific and the most sensitive test. When the sputum is cultured, it is best to pretreat the sample by either acidification or heat. Bahl et al., [40] from Delhi used culture sensitivity, direct fluorescence and ELISA for the lower respiratory tract infections. Chaudhary et al., [41] reported the role of ELISA in the detection of L.pneumophila. PCR of the serum has been used for the identification of Legionella.

EPIDEMIOLOGY

Most of the members of Legionellaceae are found worldwide and they occur naturally in aquatic sources such as lakes, rivers, hot springs, ground water and mud [11]. L. longbeachae has been isolated from soil [12,13]. Legionellae resist the usual chlorination of the water treatment facilities and they subsequently gain entry into and colonize the human-made water supplies [14,15]. Hot water systems, cooling towers, showers,[16] evaporative condensers, ornamental fountains, whirlpools [17] and humidifiers are their artificial reservoirs. These have been implicated frequently in the outbreaks of the Legionnaires' diseases [Table/Fig-2]. A large outbreak at a flower show in the Netherlands was traced to the whirlpool spas which were used in the exhibits [18]. Legionellae can grow and proliferate in human-constructed aquatic reservoirs. The factors which are known to enhance the colonization and the amplification of legionellae include warm temperatures (25^{0}C to 42^{0}C), stagnation, and the presence of biofilms on the surfaces of pipes which contain commensal bacteria, ciliated protozoa, algae and amoebae [19]. The biofilms protect Legionella from the direct exposure to ultraviolet (UV) light, desiccation, and the chemicals which are used to control its growth.

Rowbotham was the first to report that the Legionella species multiply in close association with the free-living amoebae of the genera, Acanthamoeba and Naegleria [20]. Others have confirmed and extended these observations, not only with Acanthamoeba and Naegleria, but also with other amoebae such as Hartmanella and the ciliate Tetrahymena [21,22]. The amoebae may protect legionellae within their cysts against the effects of chlorine [23].

The Centres for Disease Control and Prevention (CDC) has estimated that between 10,000 and 20,000 cases of the Legionnaires' disease occur each year in the United States [24]. Although sporadic outbreaks of the disease occur throughout the year, most of the epidemics of its infection occur in late summer and autumn, presumably because the organism proliferates in water reservoirs during the warm months [25]. The most common risk factors for the Legionnaires' disease are cigarette smoking, chronic lung disease, malignancy, advanced age, and immunosuppression [26]. Elderly persons are at a greatest risk of the disease because of their decreased cellular immunity and compromised pulmonary function. Almost 25% of the reported cases are acquired in hospitals, presumably because of the predominance of the high-risk patients. Surgery is an prominent predisposing factor in nosocomial infections, with transplant recipients being at the highest risk.

Although the disease has been under-reported, travel, hotel, and resort related outbreaks are being reported each year [27]. In the outbreaks of Legionnaires' disease, the attack rates for the exposed high-risk population are usually low (<5%) as opposed to Pontiac fever (>90%). Numerous prospective studies have found Legionella to rank among the top four microbial causes of community - acquired pneumonia, accounting for 3 to 15% cases. Between 1980 and 2002, 4402 cases of the Legionnaires' disease were identified in England and Wales [28].

Year	Area	Source	Cases	Death
1976	Philadelphia	Air conditioner	221	34
1977	Nottingham	Air conditioner	41	*
1978	Memphis	Cooling tower	44	Nil
1980	Burlington	Cooling tower	85	*
1981	Denver	Potable water supply	3	*
1985	Stafford	Air conditioner	101	28
1986	Glasgow	Air conditioner	16	*
1987	Burlington	Air conditioner	17	6
1988	Adelaida	Air conditioner	9	*
1989	Barcelona	Cooling tower	56	*
1990	Stafford	Air conditioner	68	*
1991	Varnamo	Cooling water	28	3
1992	Sydney	Air conditioner	24	*
1993	Loni siana	Air conditioner	128	*
1994	Delaware	Air conditioner	29	*
1995	Michigan	Cooling tower	24	*
1996	Turkey	Air conditioner	17	4
1997	Netherlands	Sauna	6	2
1998	Melbourne	Cooling tower	16	Nil
1999	Netherlands	Whirlpool spa	242	28
1999	Belgium	Hot water system	4	1
2000	Melbourne	Cooling water	101	4
2000	South Wales	Food display humidifier	5	2
2001	Spain	Cooling water	420	*
2001	Norway	Cooling tower	26	7
2002	Britain	Cooling tower	13	4
2003	UK	Cooling tower	28	2
2004	Spain	Cooling water	27	7
2005	South Dakota	Ornamental fountain	17	1
2006	Netherlands	Coooling tower	30	2
2007	Russia	Hot water system	150	4
2007	Spain	Cooling tower	15	1

[Table/Fig-2]: Outbreaks of Legionnaires' disease

The Legionellae species are transmitted to the human hosts from environmental sources, primarily via aerosolized particles. Most of the outbreaks of this disease originate from potable water distribution contamination. The other means of transmission include aspiration of the contaminated water or secretions, direct inoculation into the lung by respiratory therapy equipment [29,30] and surgical wound contamination with tap water [31]. Nasogastric tubes have been linked to the nosocomial Legionnaires' disease [32]. Aerosolization of the legionellae by devices which are filled with tap water, which include nebulizers and humidifiers, has caused cases of the Legionnaires' disease [33].

Pontiac fever occurs in epidemics. The high attack rate (>90%) reflects an airborne transmission. A person-to-person transmission or an animal reservoir has not yet been demonstrated. There is no convincing evidence of a carrier state in humans.

PATHOGENESIS

Legionellae enter the lungs through aspiration or direct inhalation. The alveolar macrophages readily phagocytose the legionellae. Within the macrophages, the legionellae inhibit the phagolysosomal fusion and acidification of the phagosome [34]. They continue to multiply inside the macrophages and ultimately the cells rupture, thus releasing the organisms, which can then infect other phagocytic cells.

Cell-mediated immunity is the primary mechanism of host defence against Legionella. The Legionnaires' disease is more common and the disease manifestations are more severe in patients with a depressed cell-mediated immunity, which include transplant recipients, patients who are infected with HIV [35] and patients who are receiving glucocorticoids.

Legionella usually produces a lobar, segmental or a patchy pulmonary infiltration. Acute purulent pneumonia which involves the alveoli is present, with dense intra-alveolar exudates of macrophages, neutrophils and fibrin. There is little interstitial infiltration. The organisms are facultative intracellular pathogens and they may be found within the macrophages and the neutrophils or extracellularly.

THE CLINICAL SPECTRUM

The clinical manifestations of the Legionella infections include febrile disease with pneumonia (Legionnaires' disease) or without a pulmonary involvement (Pontiac fever) and an extrapulmonary in-

Nature of test	Test	Comment	Specimens
Detection of whole organism	**Microscopy** Gram stain Gimenez's stain Dieterle's silver stain Giemsa stain Direct fluorescent antibody (DFA) test Modified kinyoun acid-fast stain **Culture** BCYE agar Blood culture **DNA** Probe	Lightly staining gram-negative bacilli. Little value if the specimens are contaminated with normal oral bacteria. The most convenient laboratory test for confirming a suspected Legionella isolate. Test is specific but sensitivity is low varying from 25% to 75%. Some species of Pseudomonas, Bacteriodes, Corynebacterium, and other bacteria may cross-react with the polyvalent conjugates. L. micdadei is weakly acid-fast in clinical specimens. Gold standard and diagnostic test of choice. Cut-glass colonies may appear after 3 to 5 days. Examine all culture under UV before discard. Isolates are identified by use of specific antisera in an immunofluorescence test. Not rewarding. Blood is processed with the lysis- centrifugation tube system. Highly specific but sensitivity varies from 70% to 75%. The test can be used as a substitute for DFA test.	Sputum, bronchial aspirate or washings, bronchoalveolar lavage, transtracheal aspirate, pleural fluid, lung biopsy or autopsy material.
Detection of soluble antigen	ELISA Rapid immune-chromatographic assay	Easy, rapid and specific. Sensitive for L.pneumophila serogroup 1 but have poor sensitivity for other serogroups and species. Antigen is detectable 3 days after the onset of clinical disease and persist for several weeks.	Urine
Detection of anitbody in paired sera taken 3 weeks apart	Fluorescent antibody test (FTA) ELISA Rapid microagglutination test (RMAT)	Mainly of epidemiological interest. Antibodies are detectable after 1 week and may persist for months or years.	Surum

[Table/Fig-3]: Diagnostic tests for Legionellosis

fection. Pontiac fever is an acute, self-limiting, flulike illness with a 1 to 2 days incubation period. Fever, headache, malaise, fatigue and myalgia are the most frequent symptoms [36]. A complete recovery takes place only within a few days without antibiotic therapy. The name "Pontiac" has been derived from a city with this name in Michigan, that was the site of an outbreak in 1968.

The Legionnaires' disease occurs both sporadically, in the form of community-acquired pneumonia, and in epidemics. The incubation period for the Legionnaires' disease is 2 to 10 days and the patients usually present with fever (high grade), myalgia, arthralgia and non-productive cough. Pneumonia, dyspnoea and respiratory distress are the clinical features of the progressing disease. Abdominal pain and gastrointestinal symptoms occur in many patients. There may be confusion, delirium, renal failure and encephalopathy [37]. The Legionellae may be disseminated to other organs via the blood stream and the lymphatics. So, they may involve the liver, kidney, spleen, heart, bone marrow, lymph nodes, GIT and the central nervous system.

ANTIMICROBIAL SUSCEPTIBILITY AND TREATMENT

The susceptibility testing of the Legionella species is not standardized or performed routinely. The newer macrolides (especially azithromycin) and the quinolones are now the antibiotics of choice. The Quinolones are the preferred antibiotics for transplant recipients. For the severely ill patients with Legionnaires' disease, the combination of rifampin plus a macrolide or a quinolone can be used for the initial treatment. However, the use of rifampin is not recommended by the Infectious Disease Society of America [43]. Initially, the therapy (3 to 5 days) should be given by the intravenous route, after which an oral therapy can be substituted. The total duration of the therapy in an immunocompetent host is 10 to 14 days and 3 weeks may be required for immunosuppressed patients and those with advanced disease [44]. The β-lactam antibiotics are ineffective because most of the isolates produce β-lactamases, and these antibiotics do not penetrate the macrophages. With an appropriate and a timely antibiotic treatment, the mortality which results from the community-acquired Legionnaires' disease among the immunocompetent patients varies from 0 to 11 %. Without a treatment, the figure may be as high as 31% [45]. Pontiac fever requires only symptomatic treatment. Supportive treatment in the form of oxygen, intravenous fluids, and chest physiotherapy is the same as for any other pneumonia.

PREVENTION

No current vaccine is available. The prevention of legionellosis requires identification of the environmental source of the organism

and minimizing the production of aerosols in public places from water that may be contaminated with Legionella. There have been outbreaks which have been associated with mists which were used in supermarkets to make the vegetables look fresh and shiny. Legionellae can be eradicated from water by hyperchlorination and by superheating (>700C) of the water before its distribution [46] If the above measures are not successful, then continuous copper-silver ionization of the water supply may be necessary. Monochloramine is attractive for the municipal water system, because it penetrates better into the biofilms than the free chlorine [47] and results in the reduced production of the disinfection by-products. Its action is slower than that of free chlorine but it is more stable and less corrosive and it provides a better residual disinfectant over a long distribution system. The control of the biofilm- associated legionellae may lead to the most effective control measures which may help in preventing legionellosis. For the hospitals with an outbreak or a hyperendemic Legionella problem, a periodic microbiologic surveillance of the environment, combined with ongoing or repetitive control measures should be considered, unless it can be shown that no new cases of legionellosis have occurred [48]. The guidelines for the prevention of nosocomial legionellosis have been published by the Allegheny County Health Department [49]. This approach emphasizes an environmental monitoring for the Legionella species. However, the CDC advocates an intensive clinical surveillance without a routine environmental surveillance because L. pneumophila is “ubiquitous” in hospital water systems [50].

CONCLUSION

Legionellosis is a potentially fatal disease if treatment is not taken in time. The clinically suspected cases of legionellosis should be reported immediately to the epidemiological centres to facilitate a laboratory confirmation of their diagnoses. There is a need for the evaluation of new methods which include ultraviolet light sterilization, ozonation, and the addition of amoebicidal agents for the decontamination of the water supplies. Future studies are required, which should include the development of new diagnostic modalities, biocides and vaccine development.

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