Review Article

Diagnostic methods and therapeutics strategies of *Legionella* infection in postbone marrow transplantation

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Organ transplantation represents a critical therapeutic intervention for patients with end-stage organ failure or hematological malignancies, often serving as a last-resort treatment. Among these, bone marrow transplantation (BMT) is vital but complex, as it induces profound and long-lasting immunosuppression. Patients undergoing BMT are highly vulnerable to opportunistic infections due to concurrent chemotherapy, radiation, and immunosuppressive therapies. *Legionella* infections emerge as a significant threat, accounting for considerable morbidity and mortality in hospitalized immunocompromised individuals. These infections often progress rapidly to severe pneumonia, with high mortality rates compared to those infecting immunocompetent people. Early and accurate diagnosis remains challenging due to nonspecific clinical presentations and limitations of conventional microbiological methods. Consequently, timely detection using advanced diagnostic tools and therapeutic intervention is necessary. This comprehensive review critically observes the epidemiology, risk factors, diagnostic methods, clinical manifestations, and treatments of *Legionella* in BMT recipients. It emphasizes the need for institutional prevention protocols to alleviate the exposure risks to reduce the burden of *Legionella*-related complications in high-risk BMT recipients.

Key words: Bacterial infections, bone marrow transplantation, legionellosis, therapeutics

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INTRODUCTION

Legionella and legionnaires' disease

Legionella infections represent a critical public health concern, serving as a leading cause of hospital-acquired pneumonia and associated mortality. First identified as a pathogenic agent in 1976 during an outbreak linked to the American Legion contract in Philadelphia, *Legionella* has since emerged as a major threat to immunocompromised populations, particularly recipients of bone marrow, cardiac, and kidney transplants.[1-4]

In the United States alone, an estimated 8000–18,000 cases of Legionnaires' disease (LD) are reported annually. Of these, approximately 23% are classified into healthcare-associated infections, according to data from the Centers for Disease Control and Prevention. Hospital outbreaks have frequently been linked to contamination of domestic hotwater systems, affecting transplant recipients. While less common, transmission through cooling towers or tap water used for respiratory therapy equipment has also been documented.[5]

Legionella, a Gram-negative, obligate aerobic bacterium, exhibits complex nutritional requirements. Among its species, *Legionella pneumophila* is most frequently implicated in human disease. This pathogen may cause asymptomatic colonization or mild, self-limiting

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illnesses such as Pontiac fever, a flu-like syndrome resolving within 2–5 days. Collectively, these manifestations are termed legionellosis. However, in high-risk populations, including the elderly, individuals with chronic pulmonary conditions, and immunocompromised hosts, *Legionella* poses a significant risk for severe, life-threatening pneumonia.[6] As opportunistic pathogens, *Legionella* species are globally distributed and capable of inducing both pulmonary and extrapulmonary infections, primarily through inhalation of contaminated aerosols.[7]

In total, 21 species of *Legionella* are known to be harmful to humans, particularly affecting patients with chronic lung disease in the hospital environments.[6] LD is a pneumonia illness with a mortality rate nearing 10%, caused by *Legionella* that can be found in aquatic and terrestrial environments, and in patients with legionellosis.[6,8]

Transmission occurs through inhalation or microaspiration of aerosolized bacteria from the contaminated water sources. Environments favorable to *Legionella* proliferation include stagnant water systems with temperatures between 25°C and 42°C. Common reservoirs include hospital hotwater networks, nebulizers, and showers. Notably, person-to-person transmission has not been documented.[6]

Patients with weakened immune systems, particularly those receiving hematopoietic stem cell transplants or solid organ transplants, are particularly vulnerable to *Legionella* infections, including those caused by nonpneumophila *Legionella* species. Ever since the pathogenic properties of *Legionella* species were identified over 30 years ago, there have been many reported cases and hospital outbreaks of *Legionella* infections within transplant communities.[7]

TRANSMISSION, NATURAL HISTORY, AND RISK FACTORS

Legionella spp. can be isolated from the water temperatures ranging from 6°C to 60°C, with optimal growth occurring between 25°C and 42°C, particularly in stagnant water. The *Legionellaceae* family includes only one genus, *Legionella*, which comprises 52 species, more than 20 of which are known to be pathogenic to humans. These species belong to more than 70 serogroups. In humans, infections caused by *Legionella* spp. can lead to pontiac fever, a self-limiting flu-like illness, and LD, a severe form of pneumonia accompanied by multisystem dysfunction.[9]

Hospital-acquired *Legionella* infections are commonly spread through contaminated aerosols or aspiration of contaminated water, and the major sources of these aerosols include medical devices and outlets within the water distribution system. Some methods to eliminate *Legionella* from the hospital water distribution systems include superheating, hyperchlorination, ultraviolet light treatment, and the application of copper and silver electrodes for water.[10]

This dataset (9 cases) does not directly address environmental controls but underscores the importance of integrating measures to reduce infection risks in BMT units. To reduce *Legionella* transmission risks in immunocompromised populations, particularly bone marrow transplant (BMT) recipients, we propose actionable measures aligned with legal authorities' guidelines. A structured approach to water system management includes monthly sampling using buffered charcoal yeast extract (BCYE) agar for reliable quantification of *Legionella* (≥1 CFU/mL) and biannual polymerase chain reaction (PCR) testing for rapid genus-level detection in the resource-limited settings. If ≥30% of samples exceed contamination thresholds, interventions such as hyperchlorination, thermal eradication, or temporary system shutdown should be implemented. Sustained safety requires annual water system checks, routine cleaning of surfaces with 70% alcohol, and staff education to recognize contamination signs. These protocols offer a plan to align clinical practice with public health standards, safeguarding vulnerable patients in high-risk environments.

Regular monitoring of water systems for *Legionella* spp. is strongly recommended before admitting patients into hospital units and should continue at regular intervals thereafter to ensure ongoing safety and effective risk management. In cases where outbreaks of waterborne pathogens have occurred, documented responses offer valuable insights into practical control measures. These strategies typically involve avoiding the use of tap or shower water for high-risk individuals, establishing a scheduled replacement of showerheads, conducting routine cleaning and disinfection protocols, maintaining storage tanks through periodic decontamination, and applying biocides as part of a comprehensive water treatment plan.[11]

Both adult and pediatric populations have demonstrated susceptibility to *Legionella*-induced pneumonia, particularly among those with compromised immune function, such as individuals undergoing corticosteroid therapy, cancer patients, and organ transplant recipients.[12] Immunocompromised patients are especially vulnerable to a range of waterborne pathogens, including not only *Legionella* spp. but also other Gram-negative bacteria and opportunistic fungal agents.[11]

Among the recognized risk factors for legionellosis, hematologic malignancies and various forms of immunodeficiency remain prominent. *L. pneumophila* which is responsible for approximately 90% of reported human

infections. Within *Legionella* species, serogroup 1 accounts for more than 84% of LD cases worldwide.[9]

LEGIONELLOSIS IN TRANSPLANTATION

Despite considerable progress in immunosuppression, preventive measures, and management before and after transplantation, both candidates and recipients of solid organ and hematopoietic stem cell transplants remain at an elevated risk for healthcare-related infections compared to other patients. Extended waiting periods and new systems cause some patients to depend on resident devices such as central venous catheters or ventilators before and following transplantation, while the hospital surroundings can also act as a potential source of infections caused by opportunistic pathogens. Apart from invasive fungus infections, transplant recipients and other immunocompromised individuals face a greater risk of infections from waterborne pathogens. Pathogen transmission occurs through direct and indirect contact, digestion, aspiration, and/or aerosolization.[13]

L. pneumophila ranks among the three most common causative agents of community-acquired pneumonia. The primary defense against *Legionella* relies on intact cell-mediated immunity; consequently, LD is notably more prevalent and severe in individuals with compromised immune function, such as transplant recipients or those undergoing immunosuppressive therapy.[14]

The clinical manifestations of *Legionella* infections can closely resemble those caused by other opportunistic pathogens, particularly in severely immunocompromised patients. Diagnosing these infections in transplant recipients presents particular challenges. For instance, conventional urinary antigen tests (UATs) are limited in their ability to detect nonpneumophila species, despite the susceptibility of immunocompromised individuals to such strains. Changes in transplant management practices, alongside shifts in *Legionella* epidemiology, suggest that the number of transplant recipients at risk for *Legionella* exposure may be on the rise.[7] While *Legionella* is most commonly associated with pulmonary infections, it can also cause extrapulmonary manifestations, which are relatively more frequent in immunocompromised hosts. The typical incubation period for *Legionella* infections ranges from 2 to 10 days.[7] Although pneumonia remains the most recognized clinical presentation of *Legionella* spp., atypical presentations such as pulmonary nodules have been reported. In some cases, these nodules may progress to cavitary lesions or abscesses.[15]

Patients undergoing bone marrow or solid organ transplantation are especially vulnerable to *Legionella* infections, primarily due to the extended periods of neutropenia and dysfunction in cell-mediated immunity. To reduce the risk of nosocomial infections, many healthcare institutions have implemented standardized protocols for *Legionella* eradication from the hospital water systems.[16]

LEGIONELLA INFECTIONS IN A BONE MARROW TRANSPLANT

Patients undergoing bone marrow transplantation (BMT) experience profound immunosuppression, making them highly susceptible to a broad spectrum of infectious agents. The pattern and timing of posttransplant infections are closely linked to the duration since transplantation and the level of immune reconstruction. Cell-mediated immunity remains impaired during the first 3 months following BMT and can be further compromised by severe graft-versus-host disease and its associated treatments. Given the crucial role of cellular immunity in defending against *Legionella*, it is not surprising that cases of pneumonia caused by *L. pneumophila* have been reported among BMT recipients.[17]

Therapeutic interventions often result in mucosal damage, disruption of physical barriers, and diminished cell-mediated and humoral immunity function. Consequently, these patients are prone to infections originating from their endogenous microbiota and environmental pathogens, particularly those present in air, water, medical equipment, and hospital surfaces.[11]

Implementing effective water management strategies to alleviate the conditions favorable for *Legionella* growth is crucial in reducing bacterial proliferation and preventing transmission to high-risk individuals. One widely used disinfection approach in healthcare settings is copper-silver ionization, which has demonstrated efficacy in decreasing *Legionella* colonization in water systems. This method may also impact other Gram-negative bacteria by disrupting cell membranes and eradicating biofilms that serve as microbial reservoirs.[13]

Another commonly employed disinfectant is monochloramine, a compound formed by combining chlorine and ammonia, which is utilized to control the growth of opportunistic pathogens such as *Legionella* in the hospital water supplies. However, both disinfection strategies have limitations. Despite the application of copper-silver ionization, *Legionella* species have still been isolated from hospital water systems. Moreover, monochloramine-based disinfection may accidentally promote the proliferation of mycobacteria within these environments.[13] Patients undergoing BMTs are mainly vulnerable to *Legionella* infections due to extended periods of neutropenia and disruptions in the cell-mediated immunity. As a result, *Legionella* infections in immunocompromised individuals can easily become severe and lead to high mortality rates.[10]

A notable example of nosocomial *Legionella* transmission occurred during an outbreak of pneumonia caused by *Legionella micdadei* among kidney and heart transplant recipients in a U. S. hospital. Epidemiological investigations outlined the source of contaminated hot water systems. Subsequent decontamination measures included thermal shock followed by chlorination. While national surveillance data indicate an overall mortality rate of approximately 25% for LD, specific statistics for transplant recipients remain undefined.[13]

Evidence indicates that transplant recipients encounter a higher risk of infections caused by non-pneumophila *Legionella* species. In several studies, almost 60% of transplant patients experienced non-pneumophila *Legionella* infections. Nonpneumophila species are rarely documented in healthy individuals and in other immunocompromised hosts who are not transplant recipients. This might be due, however, to the prevalent use of the *Legionella* UAT, which does not identify species other than *L. pneumophila* serotype 1, such as non-pneumophila *Legionella* species.[7] Individuals who have undergone hematopoietic stem cell transplantation are required to stay in "reverse isolation wards" (bone marrow transplant units) during the initial weeks posttransplantation to ensure their survival.[9]

LEGIONELLOSIS IN BMT: PATHOGENIC MECHANISMS AND IMMUNE FAILURE

L. pneumophila causes LD by inhaling contaminated aerosols. Its pathogenesis relies on intracellular survival within macrophages. Upon phagocytosis, *Legionella* employs a type IV secretion system (Dot/Icm) to inject effector proteins into host cells, disrupting phagosome-lysosome fusion and creating a replication-permissive niche. These effectors disrupt cellular processes, including immune signaling, enabling bacterial proliferation.[18]

In immunocompetent individuals, innate immunity controls *Legionella* via macrophage activation. Toll-like receptors recognize bacterial components like lipopolysaccharide, triggering proinflammatory cytokines (e.g., tumor necrosis factor-α and interleukin [IL]-12). IL-12 stimulates natural killer cells, and T-helper 1 (Th1) cells to produce Interferon-gamma (IFN-γ), which enhances macrophage bactericidal activity through reactive oxygen species and nitric oxide[19] [Figure 1].

However, patients with bone marrow transplantation (BMT) face serious risks. Defective Th1 responses and reduced IFN-γ production impair macrophage activation, allowing uncontrolled bacterial replication. In addition, suppressed phagocytic function and cytokine signaling increase

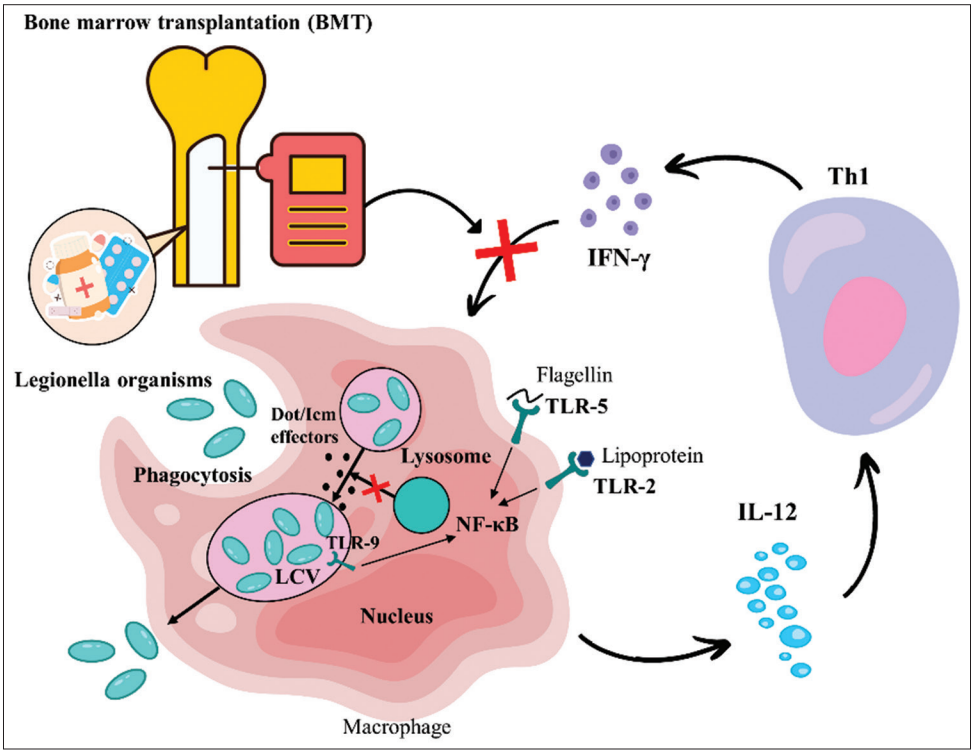

Figure 1: *Legionella pneumophila* Dot/Icm T4SS disrupts phagosome-lysosome fusion, enabling intracellular replication. In immunocompetent hosts, toll-like receptor recognition triggers Interferon-gamma (IFN-γ) production by natural killer/T-helper 1 (Th1) cells, enhancing macrophage bactericidal activity. Patients with bone marrow transplantation exhibit impaired Th1 responses, reduced IFN-γ, and phagocytosis defects, permitting uncontrolled bacterial growth and extrapulmonary spread. IFN-γ: Interferon-gamma, TLR: Toll-like receptor, IL: Interleukin, BMT: Bone marrow transplantation, LCV: Leukocytoclastic vasculitis, NF-kB: Nuclear factor kappa B

susceptibility to extrapulmonary dissemination. *Legionella* also evades immune detection by downregulating surface antigens and secreting effectors that inhibit apoptosis and antigen presentation. In immunocompromised individuals, these mechanisms compound existing immune deficiencies, leading to delayed clearance and higher mortality. Targeted therapies, such as cytokine supplementation (e.g. IFN-γ) or antimicrobial agents disrupting Dot/Icm function, are critical for improving the outcomes in this vulnerable population.[20]

UNDERLING MALIGNANCY

Bone marrow transplantation (BMT) represents a critical therapeutic intervention for various hematologic malignancies, particularly in cases where conventional treatments are ineffective or the disease exhibits aggressive behavior. Among these conditions, acute lymphoblastic leukemia (ALL) is recognized as the most prevalent form of cancer in pediatric populations and predominantly affects younger individuals [Table 1]. In contrast, chronic lymphocytic leukemia (CLL) is characterized by a slow progression and primarily affects older adults. BMT is generally considered for patients with high-risk disease or those who have experienced relapse following initial therapy. Chronic myelogenous leukemia (CML), identified as the second most common hematologic malignancy in this review, is distinguished by the excessive proliferation of myeloid cells. For patients with advanced-stage CML or those resistant to standard therapies, BMT remains a potentially curative option. Aplastic anemia (AA), another major indication for BMT, is defined by bone marrow failure leading to inadequate production of blood cells. In severe cases, particularly among younger patients, BMT is often regarded as the primary therapeutic approach. Furthermore, emerging evidence supports the role of BMT in managing thalassemia major (TM), especially when performed at earlier stages of the disease. Each hematologic disorder presents distinct clinical challenges that necessitate individualized treatment strategies aimed at optimizing the patient outcomes. The decision to continue with BMT involves a comprehensive evaluation of multiple factors, including the patient's age, overall health status, and specific disease characteristics. In the context of this review, the majority of underlying conditions requiring BMT were hematologic disorders. ALL accounted for 30% ($n = 3$) of cases, followed by chronic myeloid leukemia (CML) at 20% ($n = 2$). Additional indications included AA, CLL, TM, and non-Hodgkin lymphoma.[10,12,14,15,17,21-24]

Early and precise diagnosis of *Legionella* infection in patients with postbone marrow transplantation (BMT) remains difficult. Clinical symptoms are often nonspecific, and traditional microbiological methods have significant limitations. Therefore, using advanced diagnostic technologies for rapid identification and new effective treatment strategies is crucial. This review analyzes the epidemiology, risk factors, diagnostic methods, clinical features, and therapeutic options for *Legionella* infection in individuals who have undergone bone marrow transplantation (BMT). Furthermore, it underscores the critical importance of establishing institutional preventive measures to lower exposure risks and lessen the impact of *Legionella*-associated complications in this vulnerable patient group.

AGE AND SEX

While both age and sex have a significant impact on the risk and severity of *Legionella* infections in bone marrow transplant recipients, the infection occurs in patients of varying ages and sexes. More than 50% of BMT patients who catch *Legionella* infection are children, and it seems it happens among men more than women.[10,12,14,15,17,21-23]

SIGNS AND SYMPTOMS

Legionella infections can exhibit the various symptoms that may overlap with those of other illnesses. In patients with BMT, the symptoms might be more noticeable or unusual due to their compromised immune systems. The most common symptoms include a high fever, cough, chest discomfort, and gastrointestinal issues such as nausea, vomiting, and diarrhea,[25] but the typical symptoms that were observed are fever and a dry cough [Table 1].

Clinical presentation was universally characterized by fever (≥38.5°C) across all cases. Respiratory symptoms were prominent, including nonproductive cough (almost 60% of cases) and pleuritic chest pain (almost 50%). Gastrointestinal manifestations, such as diarrhea, nausea, and vomiting, were reported in 40% of cases, while symptoms like weight loss, malaise, and headache occurred nonspecifically.

A low-grade fever, as seen by Schindel *et al*.,[14] might suggest a milder form or an early phase of the illness; however, Scerpella *et al*.[22] observed severe respiratory distress and pleuritic pain point to a more advanced pneumonia stage. Considering gastrointestinal symptoms, Gonzalez and Martin[12] reported nausea along with fever, which aligns with common manifestations of LD, while Larru *et al*.[21] reported chest pain and cough without gastrointestinal symptoms, indicating a more localized respiratory condition. Certain reports, such as one carried out by Erat *et al*.,[10] revealed systemic effects due to the presence of both headache and abdominal pain, though they may not be as severe as others. Lee *et al*.[23] reported jaundice and a rash in addition to respiratory symptoms, suggesting possible extrapulmonary manifestations or complications from the

Table 1: Legionella infection after bone marrow transplantation in different cases

Author	Underlying malignancy	Transplantation	Age	Sex	Sign	Diagnosis	Organism	Chest radiograph and chest CT scan	Initial therapy	Treatment after diagnosis	Outcome
Miller *et al.*[15]	ALL	Related T-cell-depleted HSCT	12	Male	Weight loss, anorexia, malaise, mild rhinorrhea, nonproductive cough, and fever	Growth on BCYE after 4 days, PCR and DNA sequencing	*L. bozemanii*	Right-sided cavitary pulmonary lesion, abscess, numerous bilateral pulmonary nodules, and a small, round, hypodense lesion in the spleen	First, with meropenem, vancomycin, azithromycin, and liposomal amphotericin B, and then trimethoprim-sulfamethoxazole with continuation of meropenem	Azithromycin	Recovered
Gonzalez and Martin[12] (February 2005)	ALL	BMT	9	Female	Nausea and fever (39.5°C)	Performing a BAL and positive culture 3 days later	*L. pneumophilia*	Right upper lobe consolidation	Vancomycin, piperacillin-tazobactam, and Tobramycin	A 21-day course of levofloxacin	Recovered
Gonzalez and Martin[12] (August 2005) Recurrence	ALL	Cord blood stem cell transplant	9	Female	Fever (39.4°C) and pain beneath the left scapula	Performing a BAL, culture, and negative Legionella DFA	*L. pneumophilia* serogroup 1	Left lower lobe infiltrate and a nodule with peripheral enhancement and ventral low attenuation consistent with an abscess	-	Treated with levofloxacin	Recovered
Larru *et al.*[21]	AA	Unrelated donor BMT	18	Male	Fever, left-sided chest pain, and a nonproductive cough	Performing BAL, gram stain, molecular identification, 16S rRNA sequencing, culture on BCYE. Positive DFA and positive urine antigen	*L. pneumophila* serogroup 1	A new, cavitating, wedge-shaped area of consolidation within the left upper lobe	Acyclovir, gentamicin, amoxicillin, and trimethoprim/sulfamethoxazole	Ciprofloxacin and azithromycin for a total of 21 days	Recovered
Schindel *et al.*[14]	CML	BMT	45	Male	Low-grade fever (38.5°C)	Performing BAL and a positive urine test	*L. pneumophilia* serotype 1	Infiltrates in the right lower and middle lobes accompanied by a small pleural effusion and central abscess formation in the follow-up	Amphotericin	Imipenem, rifampicin, and roxithromycin and then imipenem was replaced by clindamycin thoracic surgery	Recovered
Scerpella *et al.*[22]	CLL	Allogeneic BMT	55	Male	Fever, chest pain, severe respiratory distress, and hypoxemia	Performing BAL, and a positive DFA	*L. pneumophila* serogroup 1	Ill-defined infiltrate in the left lower lobe and blunting of the left costophrenic angle, and then extensive bilateral pulmonary infiltrates and bilateral pleural effusions	Vancomycin, ceftazidime, amphotericin B, and oral itraconazole	Trimethoprim-sulfamethoxazole and then changed to oral clarithromycin	Recovered

Contd...

Table 1: Contd...

Author	Underlying malignancy	Transplantation	Age	Sex	Sign	Diagnosis	Organism	Chest radiograph and chest CT scan	Initial therapy	Treatment after diagnosis	Outcome
Schwebke *et al*.[17] (Case 1)	ALL	BMT	17	Male	Fever, diarrhea, nausea, vomiting, and a nonproductive cough	Performing BAL, colony on BCYE after 7 days of incubation	*L. micdadei*	Ill-defined infiltrate in the right lower lobe	Broad-spectrum antibiotics (ceftizoxime, mezlocillin, and vancomycin) and amphotericin B	Oral erythromycin	Recovered
Schwebke *et al*.[17] (Case 2)	CML	Allogeneic BMT	5	Female	Fever (39°C–40°C), pleuritic left-sided chest pain, diarrhea, nausea, and vomiting	No culture, DFA staining for tissue sections, and of the isolate from the mycobacterial broth	*L. micdadei*	Small pulmonary nodule in the lower lobe of the left lung subsequently enlarged into an infiltration with a small effusion	Ceftizoxime, mezlocillin, vancomycin, and amphotericin B	Erythromycin	Died
Erat *et al*.[10]	TM	Second allogeneic HSCT	7	Female	Fever (39°C), nonproductive coughing, abdominal pain and headache	Negative BAL fluid, negative urine antigen and positive PCR	*L. pneumophila*	Pneumonic infiltration in the lower lobe of the right lung and a consolidated area in the right lung's lower lobe superior segment and minimal pleural effusion	Meropenem and teicoplanin and then liposomal amphotericin B	Levofloxacin and azithromycin	Recovered
Lee *et al*.[23]	Non-Hodgkin's lymphoma	Allogeneic BMT	69	Male	Jaundice, diarrhea, a generalized macular rash, fever (39°C), productive cough, and dyspnea	Negative urinary antigen and DFA testing and Histopathological examination of the lung tissue (numerous bacilli)	*L. feeleii*	A focal consolidation in the left hilar region and an extensive area of consolidation with air bronchograms in the left upper lobe and a left-sided pleural effusion	Imipenem and liposomal amphotericin B, and then Imipenem was discontinued and intravenous cipro£oxacin was started	Ciprofloxacin (3 weeks)	Recovered

CT=Computed tomography; ALL=Acute lymphoblastic leukemia; AA=Aplastic anemia; CML=Chronic myelogenous leukemia; CLL=Chronic lymphocytic leukemia; TM=Thalassemia major; HSCT=Hematopoietic stem cell transplant; BMT=Bone marrow transplantation; BCYE=Buffered charcoal-yeast extract medium; PCR=Polymerase chain reaction; BAL=Bronchoalveolar lavage; DFA=Direct immunofluorescent antibody; L. *pneumophilia=Legionella pneumophila*; UAT=Urinary antigen test; *L. feeleii=Legionella feeleii; L. micdadei=Legionella micdadei; L. bozemanae=Legionella bozemanae*

infection. Lastly, it is worth noting that the symptoms of *Legionella* infection usually emerge 2–10 days postexposure and can vary greatly among different patients.

LEGIONELLA SPECIES IN BMT

Legionella infections, particularly among bone marrow transplant (BMT) recipients, can be caused by a variety of *Legionella* species. The specific strain involved may significantly influence both the clinical severity and the prognosis of the infection.[26]

L. pneumophila is the most frequently reported species associated with LD, as indicated in epidemiological studies and summarized in Table 1. It is commonly linked to outbreaks that originate from contaminated water systems.[2] The severe pneumonia caused by *L. pneumophila* has high mortality rates among immunocompromised patients. Among various serotypes, *L. pneumophila* serotype 1 is the most prevalent, often related to severe disease and implicated in hospital-related outbreaks.[1] *Following L. pneumophila*, *L. micdadei* is another species known to cause pneumonia, although it generally exhibits lower virulence. Nevertheless, it remains a notable pathogen among immunocompromised patients. Other less commonly reported species include *L. bozemanii* and *L. feeleii*, which, despite their relative rarity, are still capable of causing pneumonia in individuals with weakened immune systems.[15,23]

Their clinical relevance is still under investigation, particularly in the context of BMT. Sometimes we can see footprints of some microorganisms, making the situation worse. In Gonzalez and Martin[12] report, the immune-suppressing effects of cytomegalovirus may have played a role in increasing susceptibility to *Legionella*. In Schindel *et al*.[14] case, there was also a *Prevotella* infection, an anaerobic bacterium typically found in the oral cavity and often associated with lung abscesses. The authors propose that this superinfection could have contributed to the development of the abscess. Therefore, the primary *Legionella* species associated with infections postbone marrow transplantation are mainly *L. pneumophila*, followed by *L. micdadei*.

DIAGNOSIS

Legionella infections, particularly in the post-BMT period, show considerable diagnostic challenges due to their nonspecific clinical presentation and variability in laboratory methodologies.

Bronchoalveolar lavage (BAL) is a commonly used procedure for evaluating pulmonary pathology, as it allows for sampling of the lower respiratory tract. In numerous studies, differential cell counts obtained from BAL fluid have been associated with specific pulmonary conditions. Furthermore, BAL has been frequently incorporated into diagnostic protocols for *Legionella* pneumonia, as evidenced by its inclusion in several reported cases [Table 1].[27]

Diagnostic strategies for *Legionella* infection have varied across studies. BAL was employed in approximately 70% of documented cases. Culture on BCYE medium confirmed *Legionella* infection in nearly 40% of these instances. Notably, approximately 20% of cases (n = 2) yielded false-negative results using direct fluorescent antibody (DFA) testing, while approximately 10% (n = 1) demonstrated false-negative outcomes with the urinary antigen assay. These findings highlight the complexities and limitations associated with diagnosing *Legionella* infections in immunocompromised individuals.

Gonzalez and Martin[12] reported a case in which a BAL was conducted, and cultures tested positive after 3 days. This underscores the importance of prompt specimen collection and processing. Another BAL reported by Larru *et al*.[21] showed thin Gram-negative rods, needing 8 days of incubation to allow adequate colony growth for molecular identification using 16S rRNA sequencing.

BCYE agar is a novel bacteriological medium that promotes robust growth of *Legionella*. Cultures have been carried out in various cases.

For instance, Miller *et al*.[15] detected a growth starting 4 days after inoculation on BCYE, a selective medium for *Legionella* species. This medium enhances recovery by inhibiting competing flora. In the first patient reported by Schwebke *et al*.[17] a culture from lung tissue produced one colony of *Legionella* after 7 days of incubation without any prior direct immunofluorescent antibody (DFA) testing, indicating that cultures can sometimes provide results even in the absence of rapid tests.

Like coming from Miller *et al*.[15] report, correctly diagnosing infections caused by *Legionella* spp. demands using the specific media such as BCYE or targeted nucleic acid tests, highlighting the importance of considering this pathogen during clinical evaluations. Clinicians should incorporate *Legionella* spp. into their differential diagnosis when addressing the cases of cavitary lung disease, especially when dealing with organisms that test positive in acid-fast stains.

The *Legionella* DFA has been previously established as a specific rapid test for the diagnosis of legionellosis. In cases reported by Scerpella *et al*.[22] and Schwebke *et al*.,[17] DFA was positive after BAL, demonstrating a quick identification

method that can serve to complement culture results. However, in a study done by Lee *et al*.,[23] the DFA testing for *Legionella* was negative, highlighting the necessity for other laboratory methods alongside DFA. Larru *et al*.[21] and Schindel *et al*.[14] indicated a positive urine test for *Legionella* antigen following BAL, showing the effectiveness of non-invasive testing methods in combination with more invasive techniques. Nonetheless, the urine test for *L. pneumophila* antigen was negative in Erat *et al*.[10] case report, showing the requirement for further tests to detect *Legionella*.

PCR is among the most sensitive assays conducted in these cases. In Erat *et al*.'s[10] case report, while the urine was negative for *L. pneumophila* antigen and BAL fluid yielded negative results, PCR returned positive, illustrating the sensitivity of molecular methods in identifying *Legionella* DNA even when antigen tests are inconclusive. Diagnosing *Legionella* infections after BMT requires a multifaceted strategy that integrates culture methods (particularly on BCYE), molecular technologies such as PCR, and serological assessments such as urine antigen detection. Each case report showed distinct timelines and approaches that aid the successful identification of *Legionella* species, emphasizing the importance of comprehensive diagnostic strategies to enhance the patient outcomes in immunocompromised individuals.

In Larru *et al*.'s[21] report, observations indicate that pediatric caregivers and clinicians often do not follow diagnostic testing for *Legionella* pneumonia, leading to the underestimation of its true incidence. Consequently, available epidemiological data derived primarily from case reports may be subject to sampling bias. *Legionella* infections are harder to diagnose because the bacteria have particular growth needs, frequently necessitating specialized culture media. In certain instances, such as the case described here, the pathogen was identified incidentally due to poor growth on conventional media. This highlights the critical importance of timely specimen collection for *Legionella* testing and the necessity of considering atypical pathogens in the differential diagnosis of high-risk pediatric patients.

It is strongly recommended to employ a combination of detection methods to enhance diagnostic accuracy. Each diagnostic assay has unique strengths and limitations; therefore, the selection of appropriate tests should be guided by clinical context, patient status, and the availability of laboratory resources.

INITIAL THERAPY

Antibiotics play a critical role in managing pneumonia caused by *L. pneumophila*, particularly due to its intracellular lifecycle. Antibiotic selection is primarily guided by their ability to effectively target intracellular bacteria and achieve therapeutic concentrations within host cells. In the situation of infections following bone marrow transplantation (BMT), the choice of antimicrobial agents is further complicated by the patient's immunocompromised state and the need for prophylactic and therapeutic interventions against a broad spectrum of potential pathogens.

Among the most commonly utilized agents, vancomycin and amphotericin B are frequently administered, as detailed in Table 1. Vancomycin is predominantly employed for its efficacy against Gram-positive organisms, including multidrug-resistant strains such as methicillin-resistant *Staphylococcus aureus*. Amphotericin B, on the other hand, is widely used for antifungal prophylaxis or treatment, reflecting the susceptibility of posttransplant patients to invasive fungal infections. These agents are often combined into experimental and targeted therapeutic strategies to address the complex microbial challenges faced by immunocompromised individuals. In cases reported by Miller *et al*.[15] and Larru *et al*.,[21] Trimethoprim-Sulfamethoxazole (TMP-SMX) has been utilized. TMP-SMX is essential for the prevention of *Pneumocystis pneumonia*, a common opportunistic infection in patients who have undergone BMT. In a study conducted by Larru *et al*.,[21] alongside TMP-SMX, acyclovir and amoxicillin have been prescribed to prevent viral infections such as herpes simplex and varicella-zoster, while also providing treatment coverage for various bacterial infections. As revealed by Scerpella *et al*.,[22] a combination of vancomycin, ceftazidime, amphotericin B, and oral itraconazole effectively addresses bacterial and fungal infections. Employing broad-spectrum antibiotics (ceftizoxime, mezlocillin, and vancomycin) in conjunction with amphotericin B is critical due to the diverse infection risks faced by these patients.

The selection of antibiotics in the post-BMT period reflects a strategic approach informed by the complex infection risks associated with profound immunosuppression. These regimens are formulated to target a broad spectrum of potential pathogens while concurrently modifying challenges such as multidrug-resistant organisms and opportunistic infections.

TREATMENT OF *LEGIONELLA* INFECTION AFTER DIAGNOSIS IN BMT

Different antibiotic regimens have been employed to manage *Legionella* infections, each distinguished by its distinct pharmacokinetic and pharmacodynamic properties. The selection of appropriate therapy for *Legionella*-related pneumonia in bone marrow transplant (BMT) recipients

necessitates careful consideration of multiple variables, including patient-specific factors (e.g., drug tolerance), infection severity, potential pharmacokinetic interactions, and local antimicrobial resistance patterns.[28,29]

Clinical outcomes from reported cases underscore notable differences in therapeutic efficacy. Nearly all survivors (90%, $n = 8$) were treated with newer macrolides, such as azithromycin (30% of cases), or fluoroquinolones, including levofloxacin (30% of cases). Conversely, the single mortality event was associated with erythromycin therapy, an older macrolide known for suboptimal intracellular penetration and higher rates of resistance emergence, particularly in immunocompromised populations. Survivors were typically administered 21-day courses of azithromycin or levofloxacin, whereas prolonged or intensified regimens (e.g., ciprofloxacin combined with surgical intervention) were reserved for complex or refractory cases.

Azithromycin and levofloxacin are increasingly prioritized in clinical practice due to their demonstrated efficacy against *Legionella* and favorable safety profiles [Table 1]. While combination therapies may enhance outcomes in high-risk settings, they require cautious monitoring to mitigate the risk of resistance development. Azithromycin, as a typical macrolide, exhibits broad activity against intracellular pathogens, including *Legionella* species, making it a cornerstone in infection management.

It is typically utilized alone, as reported by Miller *et al*.,[15] or in combination with other antibiotics, such as in the case reported by Erat *et al*.,[10] where it is paired with levofloxacin for severe infections.

Research indicated that high-dose levofloxacin can effectively treat atypical pneumonia, including those caused by *Legionella*. Gonzalez and Martin[12] required an extended treatment period of 21 days, which might be for serious cases or those with complications.

Another combination therapy, as illustrated by Larru *et al*.,[21] can involve ciprofloxacin and azithromycin. This dual approach capitalizes on the advantages of both fluoroquinolone (ciprofloxacin) and macrolide (azithromycin) antibiotics, proving effective against a wide variety of pathogens, including resistant ones. Nonetheless, monitoring resistance patterns is essential.

Schindel *et al*.[14] selected a combination of antibiotics such as imipenem, rifampicin, and roxithromycin.

This combination has effects on a broad spectrum of bacteria, including resistant strains. Imipenem, a carbapenem antibiotic, has a strong activity against Gram-negative bacteria, while rifampicin improves efficacy against intracellular pathogens such as *Legionella*. However, patient tolerance or resistance patterns influence switching from imipenem to clindamycin.

Scerpella *et al*.[22] to enhance coverage against atypical pathogens, changed the antibiotic from TMP-SMX to oral clarithromycin. TMP-SMX is effective against several pathogens, including *Legionella* spp.

Utilizing erythromycin in the first patient reported by Schwebke *et al*.[17] was effective, but it has largely been displaced by azithromycin and clarithromycin due to their superior tolerability and easier dosing, and it was ineffective for the second patient of course we know that the first patient experienced a mild infection with a low bacterial count, while second patient suffered from a severe infection with a high bacterial load.

Employing intravenous and oral formulations can ensure prompt control while moving to outpatient therapy. Erat *et al*.[10] used levofloxacin and oral azithromycin to enable quick initial treatment, followed by continued oral consumption.

The continued use of ciprofloxacin in the Lee *et al*.[23] case underscores the significance of maintaining antibiotic exposure to manage persistent infections. Long-term use of ciprofloxacin may lead to resistance; hence, monitoring is crucial.

In the certain clinical scenarios, alternative therapeutic approaches may be advisable when conventional antibiotic regimens prove insufficient. Notably, in Case 4, despite adherence to evidence-based antimicrobial therapy, the abscess failed to resolve, which was a potential consequence of suboptimal antibiotic penetration within the compromised tissue environment. In this context, surgical intervention emerged as critical for infection control. These findings underscore the necessity of modifying treatment strategies to balance therapeutic efficacy with patient safety.

NEW THERAPEUTIC STRATEGIES FOR *LEGIONELLA* INFECTION

L. pneumophila antibiotic resistance demands new therapeutic approaches beyond conventional antibiotics. One of the most promising strategies is antisense therapy, which can restore the sensitivity of pathogens by targeting the vesicle trafficking pathway in *Legionella*. This approach works by interfering with the intracellular transport system, thereby blocking the fusion of phagosomes and lysosomes within macrophages. As a result, bacteria trapped in

lysosomes are efficiently destroyed by lysosomal enzymes. In addition, ongoing research is investigating the use of recombinant DNA vaccines targeting specific virulence factors, such as peptidoglycan-associated lipoprotein and PilE. Studies in mice have shown that these vaccines can activate cellular and humoral immune responses, leading to quicker recovery from infection. Collectively, these findings highlight the importance of monitoring *Legionella* strains, particularly in the hospital water systems, to promptly identify shifts in antibiotic resistance and prevent outbreaks caused by resistant bacteria.[30]

The key clinical implications emerged from this analysis. First, *Legionella* should be considered in the differential diagnosis for febrile pediatric BMT recipients presenting with respiratory symptoms, given its 50% incidence in this subgroup. Second, diagnostic reliance on DFA or urine antigen tests alone may lead to false-negative results, necessitating confirmatory PCR or culture on BCYE media. Third, experimental therapy with azithromycin or fluoroquinolones appears superior to erythromycin in this high-risk population.

Limitations of this analysis include the small sample size and heterogeneity in diagnostic and therapeutic approaches, which restricts the generalizability. Future studies should prioritize systematic reviews or multicenter registries to validate these observations and refine evidence-based guidelines for managing *Legionella* infections in immunocompromised hosts.

CONCLUSION

The susceptibility of bone marrow transplant (BMT) recipients to *Legionella* infections highlights the critical interaction between profound, prolonged immunosuppression and environmental pathogen exposure. This review provides evidence highlighting the challenges in diagnosing and managing *Legionella*-related complications in this high-risk population, emphasizing the urgent need for multifaceted interventions to decrease morbidity and mortality. Key findings reveal that *Legionella* infections in BMT patients often present with nonspecific clinical features, combined with the limitations of conventional diagnostic tools such as UATs, which fail to detect non-pneumophila species. Advanced molecular techniques and specialized culture methods emerge as essential tools for timely and accurate identification, particularly in pediatric cases. Therapeutic strategies must balance efficacy, safety, and resistance management. While azithromycin and levofloxacin demonstrate robust efficacy, combination therapies may enhance outcomes in severe cases. Notably, novel approaches, such as antisense therapies targeting intracellular bacterial pathways or recombinant vaccines, offer promising ways for future research to address evolving antibiotic resistance. Beyond individual patient management, institutional prevention protocols are essential. Water system disinfection methods reduce *Legionella* proliferation in healthcare settings, yet their limitations necessitate continuous monitoring. In conclusion, addressing *Legionella* infections in BMT recipients requires a different effort, such as early diagnosis, optimized antimicrobial strategies, and institutional prevention frameworks. By mixing advanced diagnostics, tailored therapies, and environmental controls, healthcare systems can reduce the burden of these infections and improve outcomes for immunocompromised populations. Future studies should focus on refining diagnostic procedures, evaluating emerging therapeutics, and standardizing institutional protocols to ensure reasonable protection for all high-risk patients.

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There are no conflicts of interest.

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