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Legionelloses Caused by Non-Pneumophila *Legionella* Species and Their Diagnosis: A Narrative Review

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Abstract

The genus *Legionella* is comprised of over 60 different species including the first described and most notorious one, *L. pneumophila*. However, although less well-known, other species of this genus also have a pathogenic potential. This review investigates 226 reports of legionellosis caused by Non-Pneumophila *Legionella* species retrieved. Among those reports, at least one instance of human infection was found for 27 Non-Pneumophila *Legionella* species. Infection of the respiratory tract was the most frequent clinical presentation, with over 75% of the reports. Cardiac and skin/soft tissue infections ranked second and third (7% and 6% of the reports, respectively). The top three species were *L. longbeachae* (24.2% of the reports), *L. micdadei* (23.2%) and *L. bozemanae* (13.2%). *L. micdadei* was the prominent species involved in pneumonia and Pontiac fever outbreaks. *L. longbeachae* distinguished itself by its peculiar reservoir which was identified as soil/potting mix, as opposed to water for all of the other Non-Pneumophila species. Historically, infections caused by Non-Pneumophila *Legionella* species were first identified in immunocompromised subjects. However, over the last decade, reports of infections in immunocompetent subjects have outnumbered those in immunocompromised ones. This observation coincides with the development and wider use of non-targeted molecular biology diagnostic methods such as broad range PCR and metagenomic next-generation sequencing. If these methods are progressively included in the routine workflow of microbiology labs, a better picture of the real contribution of Non-Pneumophila *Legionella* species to the overall legionellosis burden could be expected.

Keywords: Non-Pneumophila *Legionella*; Legionellosis; Diagnosis; Sequencing; MALDI-TOF

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Abbreviations

AFLP: Amplified Fragment Length Polymorphism; BCYE: Buffered Charcoal Yeast Extract; DFA: Direct Fluorescence Antigen Assay; FTIR: Fourier-Transform Infrared; LD: Legionnaires' Disease; MALDI-TOF: Matrix-Assisted Laser Desorption Ionization Time-Of-Flight; NGS: Next-Generation Sequencing; NPL: Non-Pneumophila *Legionella;* PCR: Polymerase Chain Reaction; PF: Pontiac Fever; UAG: Urinary Antigen Detection Test; WGS: Whole Genome Sequencing

Introduction

Legionellosis can be defined as any type of infection caused by bacteria belonging to the genus *Legionella*. In humans, the most famous and most frequently diagnosed legionellosis is a severe form of pneumonia mainly caused by the species *Legionella pneumophila*. This kind of pneumonia was first described in Philadelphia in 1976 and termed "Legionnaires' Disease" (LD) [1-3]. Another less frequent and distinct clinical picture of legionellosis is a self-limiting flu-like disease without pulmonary involvement and termed "Pontiac Fever" (PF) [4]. Other infections caused by *Legionella* spp. are even rarer and include, but are not limited to, skin and soft tissue infections, joint and bone infections as well as cardiac ones. While *L. pneumophila* has been reported to account for over 90% of reported pneumonia cases worldwide and is also the leading species in PF cases [5, 6], it is not the sole species within the genus *Legionella* to cause infections in humans. According to the latest list of prokaryotic names with standing in nomenclature [7, 8], this genus is as of now comprised of 67 species of Gram-negative, intracellular, non-spore forming, rod-shaped, aerobic bacteria. And among those species, over 20 have been described at least once as involved in human infections with varying clinical presentations. Moreover, the actual contribution of *Legionella* species other than *Legionella pneumophila* to the overall legionellosis burden is thought to be underestimated, primarily because the common testing strategy for legionellosis is biased towards the detection of *Legionella pneumophila* serogroup 1 [6, 9-10]. The aim of this narrative review was therefore to

Table 1: Non-Pneumophila *Legionella* species, reported clinical presentations and number of cases.

Legionella species	Infection type				
	Pontiac fever	Skin & Soft tissues*	Joints & Bones$	Heart & blood vessels†	Miscellaneous
L. anisa	151[a] [37, 38]	-	2 [39, 40]	2 [41, 42]	-
L. bozemanae	-	1 [43]	2 [44, 45]	2 [46, 47]	1 unspecified [48] 1 lymphadenitis [49]
L. cardiaca	-	-	-	2 [50, 51]	-
L. cincinnatiensis	1 [52]	1 [53]	1 [54]	-	1 encephalomyelitis [52]
L. dumoffii	-	-	1 [55]	1 [56]	1 unspecified [48]
L. feeleii	317 [57]	2 [58, 59]	-	2 [60, 61]	1 unspecified [48]
L. jordanis	-	-	-	2 [46, 62]	-
L. londiniensis	-	-	-	-	1 unspecified [48]
L. longbeachae	18 [63, 64]	3 [65-67]	2 [68, 69]	5 [46, 62, 70, 71]	1 pancreatitis [72] 1 ocular infection [73] 1 lymphadenitis [74]
L. maceachernii	-	3 [75-77]	-	-	-
L. micdadei	192 [78-82]	3 [83-85]	2 [86, 87]	3 [88-90]	1 febrile illness [91] 1 diarrhea [92] 2 brain abscesses [90, 93] 1 laryngeal lesion [94]
L. rubrilucens	-	-	-	-	1 unspecified [48]
L. sainthelensi	-	-	1 [95]	-	1 lymphadenitis [96]

Includes *: wounds, cellulitis, submandibular infection, neck mass; $: septic arthritis, osteomyelitis, tenosynovitis; †: endocarditis (native & prosthetic valves), pericarditis, mycotic aneurysm. [a]: number of cases.

summarize the available information on published clinical infections linked with Non-Pneumophila *Legionella* (NPL) species and the diagnostic techniques used for their identification.

Human Infections Caused by Non-Pneumophila *Legionella* Species

Methods

The PubMed database was searched in April 2026 for individual NPL species names. Taxonomical synonyms such as *L. bozemanii*, *Fluoribacter bozemanae* or *Tatlockia micdadei* were also included in the search. Results were then screened on paper titles and abstracts to identify those reporting on extra-pulmonary human infections. Papers for which the language was different from English and French were excluded from the analysis as well as those for which the abstract and/or the full paper could not be retrieved from the internet. Duplicate reports, retrospective studies overlapping with previously reported cases as well as reviews with no new cases described were also excluded from the analysis.

Distributions were compared using Yates's Chi-square test and a p-value below 0.05 was considered as significant.

Clinical presentations of Non-Pneumophila *Legionella* infections

The search retrieved 27 NPL species for which various clinical infections were reported. All those species were identified in classical LD and pulmonary infections. Pulmonary infection was the only clinical presentation reported for 14 out of these 27 NPL species (~52%): *L. birminghamensis* [11], *L. cherrii* [12], *L. clemsonensis* [13], *L. gormanii* [14-23], *L. hackeliae* [24, 25], *L. lansingensis* [26], *L. nagasakiensis* [27], *L. oakridgensis* [28, 29], *L. parisiensis* [15, 30], *L. quinlivanii* [31, 32], *L. tucsonensis* [33], *L. wadsworthii* [5, 34, 35], *L. waltersii* [36], *L. worsleiensis* [31]. Extra-pulmonary infections caused by the other NPL species are listed in Table 1.

L. longbeachae (24.2% of the reports), *L. micdadei* (23.2%) and *L. bozemanae* (13.2%) were the most frequently cited species in the 226 retrieved publications.

A vast majority (174/226, 77%) of reported NPL infections were pulmonary ones. They ranged from mild symptoms to typical LD, but also included lung abscesses, nodules and empyema. Most were published as single cases or small case series, with only 8 outbreaks reported. Among those, LD was the prominent clinical feature and species at the root of outbreaks were: *L. longbeachae* [97-99], *L. micdadei* [100, 101], *L. bozemanae* [102], *L. dumoffii* [103] and *L. sainthelensi* [104]. Five of these eight outbreaks were definitely identified as nosocomial [100-104]. A source of infection involving water was pinpointed in four of them [101-104]. A further outbreak was thought to be of nosocomial origin but *L. longbeachae* could not be retrieved from environmental water samples [97]. However, a possible environmental origin related to soil was not investigated for this outbreak, even though it is thought to be the main contamination source for this peculiar species. The two last and most recently reported outbreaks were community-acquired [98, 99]. Both implied *L. longbeachae* and in one instance Amplified Fragment Length Polymorphism (AFLP), a molecular biology technique comparing the length of DNA fragments yielded by enzymatic cleavage, identified similar strains in both potting soil and clinical samples [98].

The second most frequent clinical presentation of NPL species infections was cardiac and vascular ones (15/226, ~7%). These mainly included endocarditis on either native [42, 46, 50] or prosthetic valves [47, 51, 61, 71, 88-90] (Table 1). Pneumonia-associated pericarditis was also identified [60, 62]. Regarding endocarditis and pericarditis, it should be highlighted that early reports based their diagnosis on antibody titers and that significant titers against NPL species could be found for more than one of them in a given patient, hinting at either a co-infection and/or a cross-reactivity between species [46, 62]. The death rate for cardiac and vascular infections with NPL was of 4/16

(25%) [50, 60, 61, 70].

Infections of skin/soft tissues ranked third with 14 reports (6%). The outcome of this type of infections was by and large favorable with no associated death reported. However, a necrotizing cellulitis caused by *L. micdadei* led to the amputation of a left arm [84]. Of note, most of these infections were diagnosed after the year 2000 (12/14, 86%), mainly using broad range 16S rDNA PCR amplification followed by sequencing and/or metagenomic Next-Generation Sequencing (NGS) (10/14, 71%).

PF and infections of joint/bone roughly accounted for the same number of published papers (10 and 12/226, respectively) and represented around 5% of the reports each. All joint/bone infections were published after the year 2000 while no publication dealing with PF was found after 2010. Once more, a vast majority (9/12, 75%) of the joint/bone infections were diagnosed through broad-range PCR or NGS. The mentioned outcome in case of PF was always recovery while only one death was related in a case of combined pneumonia & osteomyelitis in a patient suffering from systemic lupus erythematosus [68].

Cardiac infections as well as infections of skin/soft tissues and joints/bones were primarily available as case reports/case series. PF was the only exception with 90% of the reports available as community-acquired outbreaks (9/10). The sole PF case report was a case of combined encephalomyelitis and PF caused by *L. cincinnatiensis* [52]. Otherwise, 56% (5/9) of NPL PF outbreaks were caused by *L. micdadei* [78-82] while *L. anisa* accounted for two PF outbreaks and *L. feelei*, *L. longbeachae* for one each (Table 1).

Immune status and Non-Pneumophila *Legionella* infections

The immune status of subjects infected with NPL species was also assessed. When individual information was available, subjects were classified as either immunocompetent or immunocompromised (175 individuals). A subject was considered as immunocompromised when at least one of the following criteria was met: (i) transplant recipient; (ii) on-going immunosuppressive therapy at the time of infection; (iii) underlying solid or hematologic cancer and/or (iv) other underlying condition triggering a defect in immune response (e.g. HIV infection). On this basis, immunocompromised patients represented around 59% (103/175) of the studied subjects. Interestingly, when the last ten-year period (2016-2026) was compared to the previous one (1981-2016), a significant difference in the distribution of immunocompromised vs. immunocompetent subjects was noticed (43% *vs.* 69%, $p<0.002$, Yates's Chi-square test) (Figure 1).

This difference might be due to a better diagnostic of NPL in immunocompetent subjects over the last decade. Indeed, non-targeted molecular biology approaches such as broad-range 16S rDNA PCR and/or metagenomic NGS can contribute to the detection of NPL cases in immunocompetent patients because they are applied whatever the immune status of the patient while these species were previously more specifically looked for in immunocompromised patients, which is known as a risk factor for infections involving these species [105].

Identified contamination sources

A good number of published papers did not report investigating the potential origin of infections caused by NPL species (97/226, ~43%). The remaining 129 publications either stated that patients were questioned as to potential sources of *Legionella* in their

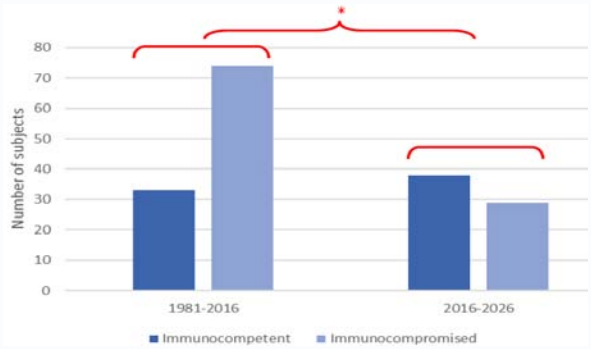

Figure 1: Number of reported immunocompromised and immunocompetent subjects with Non-Pneumophila *Legionella* infections according to the time period.
*: significant difference between the two periods ($p<0.002$, Yates's Chi-square test).

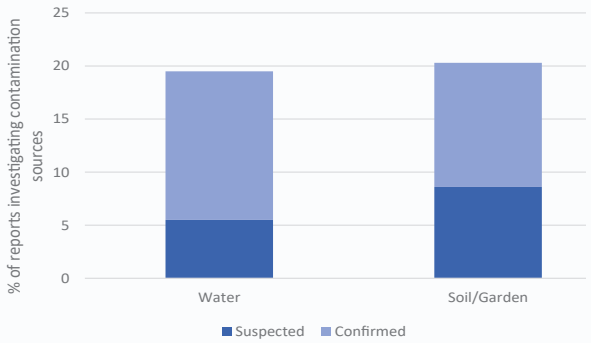

Figure 2: Suspected and confirmed sources of contamination in Non-Pneumophila *Legionella* infections.

environment or that a bona fide environmental investigation was performed. Among those, 78 (60%) did not identify a potential source of contamination. For the remaining publications, involved/suspected sources were water and soil/gardening (~19% and ~20% of the reports investigating a potential source of contamination, respectively) (Figure 2). A source was considered as confirmed when undistinguishable strains of an NPL species were identified in clinical and environmental samples, whatever the laboratory technique used (Figure 2).

According to the authors, infections with NPL species were community-acquired in over 86% of the cases (70 cases) and nosocomial in 14% (11 cases). Reports where a community/nosocomial origin was not explicitly mentioned by the authors were excluded from this calculation.

Diagnosis and Identification of Non-Pneumophila *Legionella* Species Involved in Human Infections

Pneumonias caused by NPL species are virtually undistinguishable from one another and clinically difficult to differentiate from other pathogenic agents involved in atypical pneumonias [105, 106]. Laboratory diagnostic for *Legionella* is usually not undertaken routinely but most frequently after an initial unfavorable course with empiric treatment using broad-range antibiotics covering the most frequent pathogenic agents found in pneumonia. Moreover, it remains challenging as there is no quick and easily accessible tool

permitting the detection of all *Legionella* species and serogroups [107]. Culture on Buffered Charcoal Yeast Extract (BCYE) with cysteine still is the gold standard for the diagnosis of legionellosis [9, 15, 106]. NPL *Legionella* diagnostic was confirmed by a positive culture sample in 147 of the 226 reports analyzed in this study (~65%). However, culture is hampered by the slow growth of *Legionella*, which can take up to 14 days [9]. Moreover, NPL species do not always grow steadily on commercially available media [108, 109].

Prior to the 2000s', to increase detection rates and shorten the time-to-diagnostic, several alternatives for a quicker detection of legionellosis were developed such as the Direct Fluorescent Antibody Assay (DFA) allowing detection on clinical samples or the detection of antibodies against various *Legionella* species *via* indirect fluorescence (i.e. serology). DFA was used in 28/226 of the reports (~12%) and serology in 70/226 (~31%). Serology was the sole diagnostic technique used in 31 cases, including 8/31 (~26%) outbreak reports, mostly PF ones. Both DFA and indirect fluorescence can take advantage of species-specific antibodies, hence enabling identification of the *Legionella* species against which they were produced. Nevertheless, limits to these techniques were rapidly made apparent such as cross-reactions between *Legionella* species for some of the used antibodies [25, 110, 111] or with other respiratory pathogens such as *Pseudomonas aeruginosa* [112]. Therefore, false positive results or misleading identification could occur depending on the used antibodies. DFA was also shown to lack sensitivity and its performances for species other than *L. pneumophila* have not been thoroughly investigated [109, 113]. Additionally, the detection of specific antibodies is also not always reliable, as a good number of NPL patients are immunocompromised and, even in immunocompetent subjects, there is a mandatory delay before antibodies reach the detection level.

Urinary antigen detection tests (UAGs) were then developed but their usefulness in case of infections with NPL species is limited as most of these tests only target and therefore properly detect *L. pneumophila* serogroup 1 [10, 114]. Thus, a negative UAG test should not rule out a legionellosis diagnostic and/or lead to the cessation of an empiric therapy covering *Legionella* spp. if the clinical suspicion of LD is high [115, 116]. Indeed, even though the use of UAG tests was commonly described in the 226 selected reports, only 2 publications dealing with *L. longbeachae* infections related a positive result [117, 118]. One of those two papers specified that the UAG used was specific for *L. longbeachae* [118].

The technical limitations of the above-mentioned diagnostic methods have led to their progressive replacement by molecular biology ones which, through the amplification of the pathogen's nucleic acid and its sequencing, allow for both its detection and identification with a better reliability. Nowadays, PCR targeting the 16S rDNA and metagenomic NGS are the most frequently used of those methods. Sequencing of the partial or, even better, full-length 16S rDNA resolves the identification of most *Legionella* species with huge confidence, including Non-Pneumophila ones [15, 119, 120]. Similarly, amplification and sequencing of the macrophage infectivity potentiator gene (*mip*) has proved useful [121]. Metagenomic NGS allows for an even better detection and confidence in identification as it permits Whole Genome Sequencing (WGS) and comparison to reference sequences deposited in freely accessible databases [122]. In the 226 papers gathered in this review, the first mention of a PCR detection of NPL species was found in 1998 [52] and the first NGS mention in 2019 [87]. Overall, PCR and NGS detections were used in

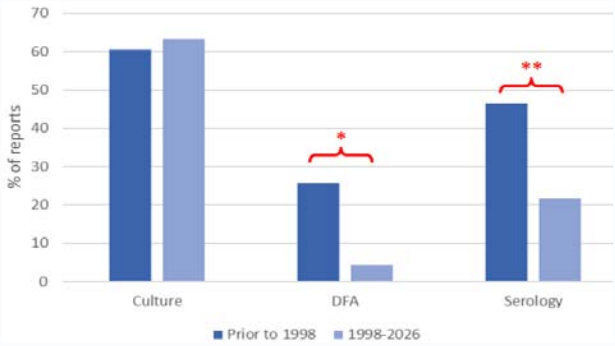

Figure 3: Percentage of reports relating the use of the mentioned diagnostic technique (DFA: Direct Fluorescent Antigen assay) prior to and after the introduction of PCR detection of Non-Pneumophila *Legionella* species. Significant difference in distributions between the two time periods (Yates's Chi-square test at *: $p<0.0001$; **: $p<0.0002$).

25% (56/226) and 5% (12/226) of the reports, respectively. Since the introduction of molecular biology techniques in 1998, a significant decrease in the use of DFA ($p<0.0001$, Yates's Chi-square test) and serology ($p<0.0002$, Yates's Chi-square test) has been observed while the use of culture remained stable (Figure 3).

Of note, a positive PCR test triggered a renewed attempt at culture on BCYE which was successful in 19 cases. An available cultural isolate is important as it enables further diagnostic and epidemiologic investigations such as the use of Matrix-Assisted Laser Desorption Ionization Time-Of-Flight mass spectrometry (MALDI-TOF) for identification [123] or Fourier-Transform Infrared (FTIR) spectroscopy for identification and comparison of isolates [124, 125]. The latter method has two main advantages in case of an outbreak investigation: a quicker turn-around time and a better affordability than WGS.

Conclusion

It should be pointed out that, with only a minority of retrieved retrospective or prospective studies, the real import of NPL infections is certainly underestimated as not all such cases are bound to be published as case reports or case series. Additionally, legionellosis surveillance systems worldwide, when they exist, operate under varying conditions [9] and do not always implement mandatory reporting of NPL species. The bias towards underreporting is therefore amplified for those species. This situation is compounded by the overall underdiagnosis of legionellosis and the bias towards *L. pneumophila* generated by the wide use of UAG tests specific for *Legionella pneumophila* serogroup 1, the negative results of which are not often challenged. Therefore, the predominance of *L. pneumophila* over other *Legionella* species might not be as overwhelming as it has previously been reported. Improved diagnostic testing with molecular biology methods such as PCR-based or NGS assays combined with improved identification methods such as MALDI-TOF are gradually increasing the recognition of NPL as well as their detection in immunocompetent subjects. If these tools become more widely included in the routine workflow of microbiology laboratories, a better picture of the real contribution of NPL species to the overall legionellosis burden is expected.

Conflicts of Interest

The author declares that they have no conflicts of interest.

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