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Policy Analysis

Monitoring *Legionella* in Drinking Water: Should We Focus on *L. pneumophila* or All Species to Effectively Protect Public Health?

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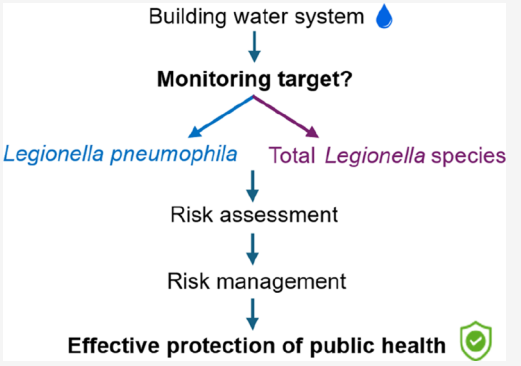
ABSTRACT: *Legionella pneumophila* is responsible for the majority of reported Legionnaires' disease cases worldwide. However, environmental monitoring of building plumbing systems often targets a broad range of *Legionella* species, raising the question of whether monitoring should focus exclusively on *L. pneumophila* or include all *Legionella* species. This review examines the policy and public health implications of both strategies by assessing case attribution data for Legionnaires' disease, the environmental prevalence of *Legionella* species, and the validity of using non-*pneumophila* counts as indicators for *L. pneumophila*. Although over 30 species can cause illness, *L. pneumophila* dominates culture-confirmed cases despite the frequent detection of *L.* non-*pneumophila* species in building plumbing and other known sources. Ecological differences between species, including growth temperatures and disinfection resistance, arguably limit the suitability of *L.* non-*pneumophila* species as reliable indicators for *L. pneumophila*. As a result, using all *Legionella* species counts to inform risk management may lead to excessive interventions without proportional public health benefits. We conclude that routine monitoring should prioritize *L. pneumophila* to ensure targeted, cost-effective, and health-relevant risk management. Broader monitoring may be warranted in high-risk settings or where local epidemiological data justify a more inclusive approach. These findings support risk-based regulatory frameworks that align monitoring targets with public health outcomes.



KEYWORDS: *Legionella pneumophila, Legionella species, disease burden, quantitative microbial risk assessment, public health policy*

1. INTRODUCTION

Since the first identified outbreak of Legionnaires' disease at the American Legion convention in Philadelphia in 1976, significant advances have been made in understanding *Legionella* biology, transmission modes, diagnostic approaches, and risk management strategies.[1] However, critical gaps in prevention and control measures persist, undermining efforts to reduce the rise in the global incidence of Legionnaires' disease.[2]

Legionnaires' disease is an acute, pneumonia-like illness often presenting with fever, cough, and muscle aches, accompanied by nausea, diarrhea, and confusion.[3] In addition to Legionnaires' disease, *Legionella* species can cause Pontiac fever, a milder, influenza-like illness. Transmission of pathogenic *Legionella* species primarily occurs through inhalation of contaminated aerosols generated by engineered water systems such as cooling towers, wastewater treatment systems, hot and cold water distribution systems, spas, and hot tubs. Soil-derived potting mixes and composts have also been implicated. Transmission can also occur through aspiration of water, although this is less common.[4]

The causative agents are Gram-negative bacteria within the genus *Legionella*. Approximately 30 of the more than 60 *Legionella* species described and named to date are considered pathogenic, and unnamed species that have yet to be cultured have been identified through sequencing technologies.[5] Common *Legionella* species often found in building drinking water systems include *Legionella pneumophila*, and *Legionella* non-*pneumophila* species such as *L. anisa*, *L. taurinensis*, *L. rubrilucens*, and *L. londiniensis*.[6−11] Their detection typically relies on culture-based methods (e.g., ISO 11731), using selective media like buffered charcoal yeast extract (BCYE) agar supplemented with antimicrobial agents to inhibit the growth of other microorganisms. These selective media were initially designed to selectively grow *L. pneumophila* from engineered water systems, but they also support the growth of various *Legionella* non-*pneumophila* species.[12] Adjustments to incubation conditions, including temperature and pH, can improve selectivity for *L. pneumophila* by inhibiting the growth

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Table 1. Attribution of Culture-Confirmed or PCR-Presumptive Clinical Legionnaires' Disease Cases to *Legionella* Species and Serogroups

			L. pneumophila (%)							
Region	Period	Culture or PCR cases[a]	All Sgs	Sg 1	Sg 2–16	Other[b]	*L. longbeachae*	Other species	Nonspeciated	Study
Culture-confirmed cases[a]										
Denmark	2017–2023	640	97.9	55.7	39.5	2.7	<0.1	1.6	–	SSI[34], SSI[35], SSI[36], SSI[37], SSI[38], SSI[39], SSI[40]
EU/EEA	2019–2021	3166	95.1	78.8	8.0	8.3	1.6	2.0	0.1	ECDC[33], ECDC[41], ECDC[42]
Japan	2008–2016	427	98.0	87.1	10.7	0.2	0.7	1.1	–	Amemura-Maekawa et al.[43]
Netherlands	2013–2022	748	92.5	79.6	5.6	7.3	5.3	2.0	0.0	Reukers et al.[44]
Sweden	2011–2021	77	89.4	58.4	29.8	1.2	3.8	6.4	–	Wikén et al.[45]
USA	2014–2019	1397	64.0	35.5	4.1	24.4	1.2	3.7	27.1	CDC[46], CDC[47], CDC[48]
PCR-presumptive cases[a]										
Sweden	2011–2021	197	73.0	–	–	73.0	1.5	2.0	23.3	Wikén et al.[45]
Culture-confirmed or PCR-presumptive cases[a]										
New Zealand	2000–2020	2675	31.2	20.3	7.4	3.5	51.0	13.5	4.3	Graham et al.[49]
Scotland	2017–2023	242	82.1	71.4	3.7	7.0	5.3	12.3	–	PHS[50], PHS[51]

[a]Method used for reported laboratory cases. Culture: Isolation of *Legionella* species from a clinical lower respiratory tract specimen. Polymerase Chain Reaction (PCR): Detection of *Legionella* species nucleic acid in a lower respiratory tract specimen. [b]Serogroup mixed, nonserogroup 1, or serogroup unknown.

of almost all *L.* non-*pneumophila* species.[13] A well-known limitation ofculture-based methods is that they can underestimate viable *Legionella* counts because the bacteria may enter a viable-but-not-cultivable (VBNC) physiological state[14−16] and intracellular replication within protozoa can hinder their recovery by direct plating.[17,18] Although alternative detection methods for the detection of *Legionella pneumophila* are available (see Section 5), culture-based methods are currently the regulatory standard for monitoring in most countries. A key consideration for environmental monitoring using culture-based methods is whether health risk assessments should focus on *L. pneumophila*—the species primarily associated with Legionnaires' disease—or the broader category of *Legionella* species. Some authors have argued that focusing only on *L. pneumophila* might excessively underestimate risks posed by pathogenic *L.* non-*pneumophila* species.[19,20] In contrast, others have argued that using results from routine testing of all *Legionella* species to guide risk management may incur disproportionate costs and interventions, especially in low-risk settings, because infections attributable to *L.* non-*pneumophila* species only occur sporadically in immunocompromised people.[21,22] Diagnostic limitations complicate this issue, as the widely used urine antigen test typically detects only *L. pneumophila* serogroup 1, limiting the accurate assessment of the disease burden posed by *L.* non-*pneumophila* species and *L. pneumophila* nonserogroup 1.[23] Some researchers also suggested that the presence of *L.* non-*pneumophila* species can indicate favorable conditions for *L. pneumophila* growth,[20,24] and can, therefore, be used as an indicator organism for *L. pneumophila*. However, limited scientific evidence supports this approach, and differences in the ecology and disinfection resistance across *Legionella* species may challenge its applicability. In fact, numerous studies have reported *L. nonpneumophila* detection without any *L. pneumophila* and vice versa.[6,8,9,20,25,26] This lack of consensus creates uncertainty in the selection of a monitoring target for effective *Legionella* risk assessment, leading to inconsistent and potentially ineffective public health interventions.

This review evaluates whether results for culturable *L. pneumophila* or all culturable *Legionella* species are more effective for health risk assessments of building drinking water systems. First, we examine the clinical and public health relevance of *L. pneumophila* compared to *L.* non-*pneumophila* species. Second, we investigate the prevalence and ecological characteristics of *L.* non-*pneumophila* species in hot- and cold-water systems.Third, we assess whether *L. nonpneumophila* species meet established criteria as reliable indicator organisms for *L. pneumophila*. Our findings aim to inform evidence-based decisions regarding risk assessment practices and the prioritization of resources to mitigate Legionnaires' disease.

2. WHAT IS THE RISK ASSOCIATED WITH *L.* NON-*PNEUMOPHILA* SPECIES?

2.1. Diagnostic Methods for Legionnaires' Disease.

Legionnaires' disease is likely underdiagnosed in regions with limited routine testing, in part because its nonspecific symptoms are often not recognized by clinicians.[27] Empirical treatment of pneumonia without pathogen identification is common, and mild cases of Legionnaires' disease often go undetected unless clinical deterioration necessitates further diagnostic investigations.[28] Beyond empirical treatment itself, clinicians can begin antibiotics promptly (as guidelines advise) and obtain sputum or bronchoalveolar lavage only afterward; this sequence can further reduce the probability of *Legionella* isolation by culture.[1] Widespread reliance on the urine antigen test, though simple and rapid, further contributes to underdiagnosis, as the test primarily detects *L. pneumophila* serogroup 1, limiting the identification of infections caused

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by other *L. pneumophila* serogroups or *L.* non-*pneumophila* species.[23,29]

Lower respiratory tract samples (e.g., sputum or bronchoalveolar lavage) are required for culturing, but these can be difficult to obtain because patients are often too ill to produce them.[1] Consequently, most Legionnaires' disease cases are detected by urine antigen testing. However, when respiratory samples are available, culture is preferred because it can detect *Legionella* species and serogroups other than *L. pneumophila* serogroup 1, in addition to providing the actual strain suitable for whole genome sequencing. Culturing, though, is labor-intensive, requires specialized culture media, and may take several days of incubation.[30] Moreover, many *L.* non-*pneumophila* species are difficult to culture because selective media were initially designed to selectively grow *L. pneumophila*,[12] further complicating identification. To help overcome some of these limitations, PCR-based methods are increasingly used to analyze lower respiratory tract samples as they can detect DNA sequences from all known *Legionella* species and all serogroups (1−16) of *L. pneumophila*.[30] PCR is generally regarded as a highly sensitive and specific method for detecting *Legionella* DNA. The main limitation is the lack of a single, universally adopted reporting standard for PCR results across laboratories. Some agencies, like the European Centre for Disease Prevention and Control (ECDC), remain cautious in classifying PCR-only diagnoses as "confirmed" and classify them as "presumptive" cases.[31] Meanwhile, other agencies, including the U.S. Centers for Disease Control and Prevention (CDC), accept PCR as confirmatory, reflecting growing confidence in the high specificity and performance of modern molecular assays.[32] In practice, the ECDC reported, for 2021, that 89% of cases in Europe were reported to be diagnosed with a urine antigen test, 11% of cases were reported having been diagnosed with a culture test, and the use of PCR method test was reported for 12% of the cases.[33] To align with European surveillance practices, this paper adopts the ECDC case definition and refers to PCR-only diagnoses as "presumptive" cases.

2.2. Attribution of Culture-Confirmed or PCR-Presumptive Legionnaires' Disease Cases to *Legionella* Species and Serogroups. The distribution of culture-confirmed or PCR-presumptive Legionnaires' disease cases by *Legionella* species and serogroups varies regionally (Table 1). *L. pneumophila* accounts for most culture-confirmed cases globally, with serogroup 1 dominating in Denmark (2017−2023, n = 640, 55.7%),[34−40] EU/EEA (2019−2021, n = 3166, 78.8%),[33,41,42] Japan (2008−2016, n = 427, 87.1%),[43] and The Netherlands (2013−2022, n = 748, 79.6%).[44] Culture-confirmed cases caused by other *L. pneumophila* serogroups are generally less than 20%, except in Denmark and Sweden, where they represent, respectively, 39.5% and 29.8% of all culture-confirmed cases.[34−40]

In Australia and New Zealand, *L. longbeachae* is endemic[1] and is likely associated with exposure to potting soils and compost.[52,53] In other Western countries where Legionnaires' disease is notifiable, *L. longbeachae* accounts for a much smaller proportion of cases. Culture-confirmed or PCR-presumptive *L. longbeachae* cases represent around 5% of reported infections in some countries, such as the Netherlands and Scotland. Culture-confirmed cases attributed to other *L.* non-*pneumophila* species, however, accounting for less than 4% of cases in Japan, the USA, and the majority of countries from the European Economic Area. A study from Sweden reported a higher proportion of culture-confirmed cases attributed to other *L.* non-*pneumophila* species than *L. longbeachae*, with 6.4% (n = 5/77) attributed to *L. bozemanii* (three cases) and *L. micdadei* (two cases).[45] In contrast, among PCR-presumptive cases, the proportion attributed to non-*pneumophila* species was lower, at 2.0% (n = 7/197). These low percentages of other culture-confirmed *L.* non-*pneumophila* cases may be due to the lower virulence of *L.* non-*pneumophila* species, which typically cause disease in severely immunocompromised patients.[3,54] Differences in environmental abundance and exposure may also contribute, although data on environmental abundance for *L.* non-*pneumophila* is generally missing to test this hypothesis.

Culture- or PCR-based tests of respiratory specimens have been reported alongside urine antigen test results in studies from Denmark, New Zealand, Scotland, and Sweden.[45,49,51,55] The inclusion of PCR-presumptive results in higher proportions of cases (17.6%) attributed to *L.* non-*pneumophila* species in Scotland.[50,51] Where the exact *Legionella* species was identified in 2023 in Scotland, 37.5% of the *L.* non-*pneumophila* Legionnaires' disease cases related to *L. longbeachae*, whereas the other *Legionella* species belonged to yet unknown *Legionella* species for which no proof is available whether these unknown species are truly pathogenic. In New Zealand, the following *L.* non-*pneumophila* species were observed in more than 1% of the cases: *L. longbeachae* (51.0%), *L.micdadei* (3.2%), *L. dumoffii* (3.0%), *L. bozemanii* (2.0%), *L. sainthelensi* (1.5%), *L. gormanii* (1.3%) and unknown *Legionella* species (4.3%).[49] The routine culture-based testing in Denmark showed low percentages of *L.* non-*pneumophila* cases (1.6%),[34−40] which is comparable to percentages observed in countries that do not routinely perform culture-based testing of sputum samples. It, however, showed much higher percentages of culture-confirmed cases attributed to *L. pneumophila* SG2−15 than in countries that rely mainly on the urine antigen test. This difference may be attributed to regional variations as well as reporting practices, as Scotland and New Zealand combined culture-confirmed cases and PCR-presumptive cases in their reporting, whereas Denmark officially reported culture-confirmed and PCR-presumptive cases separately.[34]

In summary, surveillance data from peer-reviewed articles and up-to-date reports from governmental or public health agencies indicate that the vast majority of culture-confirmed and PCR-presumptive Legionnaires' disease cases are attributed to *L. pneumophila*, predominantly serogroup 1, except in regions like Australia and New Zealand, where *L. longbeachae* (likely associated with potting soil and compost) accounts for a significant proportion. Only a few countries reported comprehensive results from culture- or PCR-based testing of respiratory samples, revealing that *L. pneumophila* SG2−15 and *L. longbeachae* are mostly missed when relying only on urine antigen testing. Broader reporting of culture- and PCR-based test results from available respiratory samples in more countries would provide a more reliable view of *L. pneumophila* serogroups or *L.* non-*pneumophila* species involved in Legionnaires' disease.

2.3. Using Quantitative Microbial Risk Assessment (QMRA) to Predict Risks from *Legionella* Species. QMRA can complement epidemiological surveillance by providing quantitative estimates of health risks based on exposure estimates and dose−response relationships.[1,56] Dose−response models quantify the probability of infection, illness, or death as

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Table 2. Prevalence of *Legionella* Species in Building Drinking Water Systems across Europe

Study	Location	Period	Site	Method[a]	Total sample	Positive sample	Positive samples (%)			
							Lpn[b] only	*L. anisa* only	Co-isolates *Lpn*, n-*Lpn*	Other species
Hot-water systems										
De Giglio et al.[25] (1)	Italy	2021–2022	Hospital	MALDI-TOF	277	59	94.9	5.1	0.0	–
Dilger et al.[6]	Germany	N/S	Residential & public buildings	MALDI-TOF	76,200	15,300[c]	83.8	10.5	–	5.7
Girolamini et al.[7]	Italy	2013–2019	Hospital	Agglut. test, *mip* seq.	307	191	35.0	17.2	43.4	4.4
Kruse et al.[8]	Germany	2013–2014	Residential & public buildings	Agglut. test	3630	771	93.4	4.8	1.8	–
Mazzotta et al.[9] (1)	Italy	N/S	Hospitals	Agglut. test, *mip* seq.	N/S	156	22.4	25.0	–	52.6
Mazzotta et al.[9] (2)	Italy	N/S	Public buildings	Agglut. test, *mip* seq.	N/S	84	3.5	20.2	–	76.3
Salinas et al.[68] (1)	Spain	2014	Domestic systems	Agglut. test 16S rDNA	993	183	77.0	–	0.0	23.0[d]
Cold-water systems										
De Giglio et al.[25] (2)	Italy	2021–2022	Hospital	MALDI-TOF	69	69	48.1	44.5	7.4	–
Salinas et al.[68] (2)	Spain	2014	Domestic systems	Agglut. test, 16S rDNA	639	77	62.2	–	3.9	33.7[d]
Hot- and cold-water systems										
Arrigo et al.[26]	Italy	2018–2021	Hospital	MALDI-TOF	251	123	61.0	4.9	34.1	0.0
Crook et al.[20]	UK	2020–2021	Hospital	MALDI-TOF	613	322	49.0	37.5	–	13.5
van der Lugt et al.[69]	Netherlands	2011–2015	Health care facilities	MALDI-TOF & PCR	6171	998	3.1	–	–	96.9[d]

[a]MALDI-TOF: Matrix-assisted laser desorption ionization time-of-flight mass spectrometry. [b]*Lpn*: *Legionella pneumophila* [c]Species identification from all grown colonies, including multiple colonies per sample. [d]This percentage can include *L. anisa* as results were reported as *L.* non-*pneumophila* only.

a function of the pathogen dose (inhaled or ingested, for example).[57] For *Legionella* species, inhalation and aspiration can lead to a wide range of clinical outcomes, from asymptomatic seroconversion and mild fever to severe illness requiring medical intervention and, in extreme cases, death.

Experimental studies using guinea pigs (*Cavia porcellus*) as surrogate models provided the basis for developing dose–response models for *L. pneumophila*. guinea pigs were chosen because of their physiological similarities to humans in macrophage uptake and replication of *L. pneumophila*.[58] Controlled aerosol exposures of guinea pigs to viable *L. pneumophila* serogroup 1[59,60] generated data for the development of two single-hit dose–response models: the subclinical infection end point and the clinical severity infection end point.[58] The subclinical infection end point model predicts the probability of fever in guinea pigs as a function of dose, reflecting mild infections that typically do not require medical intervention,[61,62] whereas the clinical severity infection end point model quantifies the relationship between dose and mortality in guinea pigs, predicting severe health outcomes such as pneumonia and death associated with Legionnaires' disease.[61,62]

A dose–response model for *L. longbeachae*, predicting the probability of mortality as a function of dose, has been developed based on intratracheal inoculation studies in mice.[63,64] Notably, the predicted LD_{50} values (median lethal doses) for both *L. longbeachae* and *L. pneumophila* are in the range of $\sim 5 \times 10^4$ colony-forming units (CFUs). However, no published dose–response models exist for other *Legionella* species. The absence of detailed dosing studies or validated experimental models represents a critical research gap, limiting the ability to assess the public health risks from *L.* non-*pneumophila* species and define appropriate interventions or responses to their detection. A study where *L. anisa* was dosed to guinea pigs demonstrated that infected animals can develop transient fever and weight loss.[65] However, these symptoms resolved quickly, indicating the low virulence of *L. anisa*. The normal guinea pigs' body temperature and fever-associated body temperature of humans of approximately 39 °C align with *L. anisa*'s inability to grow at 40 °C,[13] suggesting that the limited virulence of *L. anisa* in guinea pigs and humans is linked to its poor or absent growth at 40 °C.

The disability-adjusted life years (DALY) metric can be used to quantify healthy years lost to disability, illness, or death associated with *Legionella* infections. This approach allows benchmarking against population health-based targets, such as the 10^{-6} DALY/person-year threshold deemed acceptable for pathogens in drinking water,[66] and facilitates direct comparisons of DALY outcomes across different opportunistic pathogens using available dose–response models and DALY factors. For *L. pneumophila*, infection risks predicted by the clinical severity infection end point model have been converted into DALYs using a factor of 0.97 DALYs per case of legionellosis, derived from Dutch surveillance data.[62,67] However, these health risk estimates are subject to significant uncertainty due to variability in disease surveillance data, differences in population characteristics, and assumptions inherent in animal dose–response models. Validation of these models with human outbreak data could improve their accuracy and reliability.

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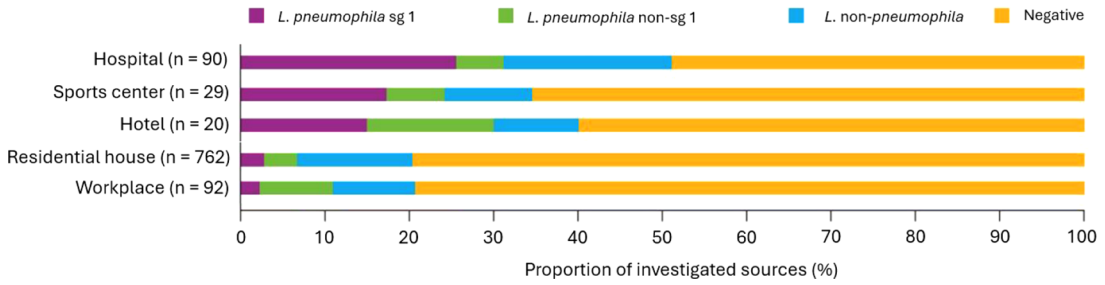

Figure 1. Sampling results of 993 investigated potential sources of Legionnaires' disease cases by building types in the Netherlands from 2002 to 2012. Source: Den Boer et al.[74]

In summary, dose−response models show that quantification (not just detection) is essential for proportional risk management. Concentration data should be interpreted together with species identity; extrapolating an action level established for *L. pneumophila* to other *Legionella* species is not scientifically justified because dose−response models are lacking for most non-*pneumophila* species. To avoid mischaracterizing risks, monitoring should aim to determine whether specific pathogenic species are present and likely to exceed concentration thresholds derived from published dose−response models and health-based targets such as the 10^{-4} annual infection risk or the 10^{-6} DALY.

3. HOW COMMON ARE *L.* NON-*PNEUMOPHILA* SPECIES IN THE ENVIRONMENT?

3.1. Prevalence in Building Drinking Water Systems. Studies across Europe demonstrate that *L.* non-*pneumophila* species are frequently isolated from building drinking water systems, either alone or co-occurring with other *Legionella* species (Table 2).

In hot-water systems from German private homes, Dilger and Melzl[6] identified *L. pneumophila* for 83.8% of isolates. They found that reduced hot-water temperatures were associated with an increased prevalence of *L. anisa*. A similar temperature shift toward *L. anisa* was reported in an Italian hospital by De Giglio et al.[25] who observed an increase in its prevalence from 5.1% in hot-water samples to 44.5% in cold-water samples. Salinas and Fenoy[68] also reported a higher prevalence of *L.* non-*pneumophila* species in cold-water systems in Spain. Nonetheless, hot-water systems are not always dominated by *L. pneumophila*. In hospital hot-water systems, Mazzotta et al.[9] reported a high proportion of *L.* non-*pneumophila* isolates (>75%), dominated by *L. taurinensis*. These findings indicate the role of temperature in the ecology of *Legionella* species in water systems, as discussed in more detail in Section 4.

The high prevalence of *L. anisa* has been reported across multiple countries. In the UK, Crook et al.[20] found a prevalence of 37.5% of *L. anisa* in drinking water systems from a hospital, and in The Netherlands, van der Lugt et al.[69] observed a high prevalence of *L.* non-*pneumophila* species (96.9%), likely due to *L. anisa*, based on earlier regional data.[70,71] Co-isolations of *L. pneumophila* with other *Legionella* species are also notable, occurring in over 30% of positive samples in some studies.[7,26]

Together, these findings indicate that some *L.* non-*pneumophila* species, like *L. anisa* and *L. taurinensis*, can (i) individually colonize hot- and cold-water systems, (ii) be more dominantly present than *L. pneumophila* in these systems, and (iii) co-occur with *L. pneumophila*. However, the mechanisms by which different *Legionella* species compete or exhibit commensal interactions are still poorly understood.[7] Hence, understanding these dynamics represents an important area for future research.

3.2. Relationship between Environmental and Clinical Distributions of *Legionella* Species. Despite the widespread presence of *L.* non-*pneumophila* species in drinking water systems (Table 2), their contribution to Legionnaires' disease cases is low (Table 1). For example, *L. anisa*, although common in cold-water systems, was implicated in only four cases of Legionnaires' disease in Europe between 2019 and 2021[33,41,42] and in only three cases of Legionnaires' disease in the USA between 2018 and 2019.[46] This difference aligns with earlier studies showing that *L. anisa* is more prevalent in the environment than in the clinical samples, as observed in Denmark[72] and France.[73] This disparity is likely due to the low virulence of *L. anisa*,[65] making only severely immunocompromised persons vulnerable to disease. As described in Section 2, Scotland, New Zealand, and Sweden routinely monitor cases by PCR diagnostics of sputum samples, which makes it possible to address cases to specific *Legionella* species.[45,49,51] None of the *Legionella* cases in Scotland (2023) and Sweden (2021) were related to *L. anisa*, whereas only five (0.2%) cases in the period 2000−2020 in New Zealand were attributed to *L. anisa*. Consequently, this more extensive monitoring of Legionnaires' disease cases using PCR on sputum samples from patients did not result in finding more cases related to *L. anisa*. This indicates that there is currently no underreporting of cases of *L. anisa* due to the diagnostic method used.

A study in the Netherlands investigated the presence of *Legionella* species in sources in order to link environmental strains to strains from patients with Legionnaires' disease[74] (Figure 1). Results for building drinking water systems show overall that sources tested mostly negative for cultivable *Legionella* species, and if sources tested positive, they had a higher prevalence of *L.* non-*pneumophila* species. Notably, even though *L.* non-*pneumophila* species were frequently detected in sources linked to patients; most patients were infected with *L. pneumophila*. A link between a *L.* non-*pneumophila* case and the drinking water environment could not be made in the Netherlands.

These findings highlight a significant discrepancy between the environmental prevalence and clinical impact of *L.* non-*pneumophila* species. The high prevalence of *L. anisa* in drinking water systems across Europe may lead to unnecessary interventions if monitoring efforts focus on controlling the risk of Legionnaires' disease. An exception to this, however, might be buildings where immunocompromised persons gather, such as healthcare facilities.

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4. CAN CULTURABLE *L.* NON-*PNEUMOPHILA* SPECIES BE RELIABLE "INDICATOR ORGANISMS" FOR *L. PNEUMOPHILA*?

Studies have been published claiming that *L.* non-*pneumophila*, such as *L. anisa*, might be an indicator for *L. pneumophila*. van der Mee-Marquet et al.[75] concluded that "the detection of *L. anisa* in water samples should be considered an indication that the water system was colonized by *Legionella species*, including *L. pneumophila*." In addition, Crook et al. (2024) concluded "the role of *L. anisa* should not be underestimated··· as an indicator of the need to intervene to control *Legionella* colonization". However, contrary to their conclusion, both studies show data suggesting that *L. anisa* is a poor indicator for *L. pneumophila*. Van der Mee-Marquet et al. (2006) observed that when heat-treatment with water at 70 °C was applied to the premise plumbing system of a hospital, *L. anisa* was eradicated, whereas *L. pneumophila* could still be observed. This indicates that the two *Legionella* species respond differently to heat treatment, which could result in false negative results when *L. anisa* is used as an indicator for *L. pneumophila*. Crook et al. observed that prolonged stagnation during the COVID-19 pandemic resulted in a decrease in *L. anisa* positive samples and an increase in *L. pneumophila* samples. These results also demonstrate that both *Legionella* species behave differently to conditions in building plumbing systems. Consequently, based on these two studies, one should be cautious in using *L.* non-*pneumophila* species, such as *L. anisa*, as indicators of *L. pneumophila*.

To examine whether culturable *L.* non-*pneumophila* species (excluding *L. pneumophila*, so not total culturable *Legionella* spp.) can be used as a reliable indicator for culturable *L. pneumophila*, we evaluated whether culturable *L.* non-*pneumophila* meets the criteria for an indicator organism in general. In this evaluation, the term "indicator organism" refers to a microbial surrogate measured as a substitute for directly analyzing a sample for a pathogenic microorganism.[76−79] The desirable characteristics of an indicator organism can be divided into two categories: the biological attributes of the organism itself and the attributes of the methods used to detect it.[79]

This section evaluates whether culturable *L. nonpneumophila* species meet the biological and methodological criteria for serving as reliable indicators of *L. pneumophila*, namely:

(i) The indicator organism must be present in higher numbers than the target organisms.
(ii) The ecology of the indicator organism must closely align with that of the pathogen.
(iii) The indicator organism must be more resistant to disinfection than the pathogen.
(iv) The indicator organism must grow independently of other organisms when inoculated on artificial media.

4.1. The Indicator Organism Must Be Present in Higher Numbers Than the Target Organisms. Numerous studies have examined the concentrations of *L. pneumophila* and *L.* non-*pneumophila* in water samples from drinking water systems using the traditional culture method.[6,8,10,71,80−90] Most studies show that *L. pneumophila* is regularly cultivated, whereas *L.* non-*pneumophila* species are less frequently detected. *L.* non-*pneumophila* may remain undetected because of their lower concentrations relative to *L. pneumophila*. Consequently, culturable *L.* non-*pneumophila* species do not meet this first criterion. Their concentrations are not consistently higher than those of the target organism *L. pneumophila*, and, therefore, they do not reliably signal its presence or abundance.

4.2. The Ecology of the Indicator Organism Must Closely Align with That of the Pathogen. Over 60 *Legionella* species can grow on traditional culture media according to ISO 11731[1] but the ecology of most *L.* non *pneumophila* species has not been studied yet and, therefore, is not well understood. The comparison herein primarily focuses on the ecology of *L. anisa* and *L. pneumophila* because both species are most often detected in drinking water systems (Table 2). However, studies on the ecology of *L. anisa* are limited, which makes it possible to compare only a few ecological conditions.

It has been demonstrated that *L. pneumophila* multiplies within protozoa that graze on biofilm in drinking water systems.[1,91] Laboratory studies indicate that *L. anisa* also replicates within protozoa.[65,92,93] It remains unknown whether *L. anisa* also proliferates mainly in protozoan hosts, as is the case for *L. pneumophila,* or if it can grow freely in biofilms in drinking water systems. Furthermore, *L. pneumophila* has been confirmed to replicate in protozoan vacuoles, whereas *L. anisa* could not be observed in these vacuoles.[94]

Studies have shown that *L. pneumophila* concentrations increase in areas with elevated iron concentrations[95−97] because iron is a critical nutrient for the growth of *L. pneumophila*.[98] One study investigated the influence of iron on *L. anisa* growth and showed that iron rust particles stimulate the growth of *L. anisa*.[99] This implies that both *L. pneumophila* and *L. anisa* have the same response to iron in the environment.

Temperature plays a critical role in determining the growth and distribution of *Legionella* species in drinking water systems. A pilot study showed different temperature preferences for *L. anisa* and *L. pneumophila*, with *L. anisa* multiplying more effectively at lower water temperatures while *L. pneumophila* multiplies more efficiently at higher temperatures.[100] Below 30 °C, only *L. anisa* was detected in the biofilm, whereas above 38 °C, only *L. pneumophila* was found. This finding is consistent with *L. pneumophila* requiring temperatures above 30 °C to multiply in protozoa.[17]

As discussed in Section 3, field studies also suggest that drinking water temperatures affect the growth of *L.* non-*pneumophila* and *L. pneumophila* differently: at lower temperatures (cold-water samples), *L.* non-*pneumophila* species are more frequently detected,[25,68,99,101] whereas at higher temperatures (hot-water samples), *L. pneumophila* is most frequently detected.[6,8,10,25, 68,85,89] As a result, relatively high concentrations of culturable *L.* non-*pneumophila* species have been observed on BCYE agar for water temperatures of 20−25 °C.[69,71,87,99,102−104] Keeping cold-water systems below 20 °C could limit the growth of these species, but this is not practical because (i) distributed treated water from drinking water treatment plants supplied by surface water sources often exceeds 20 °C in the summer period, (ii) water can heat up during the distribution of drinking water (e.g., in urban heat hot spots), and (iii) distribution of water within buildings can raise temperatures (e.g., in building hot spots such as heating pipes in floors or shafts). This is particularly relevant when interpreting total *Legionella* concentrations, as the presence of most *L. nonpneumophila* species at moderate temperatures does not pose the same health risk as *L. pneumophila*.

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Other ecological factors, such as nutrient concentrations, water quality, and pipe material, also influence the growth of *L. pneumophila*,[1,105] but studies on their impact on *L.* non-*pneumophila* species, including *L. anisa*, have not been found in the scientific literature.

This synthesis of the ecological differences between *L.* non-*pneumophila,* especially *L. anisa*, and *L. pneumophila*, particularly regarding their growth temperature, indicates that "all *Legionella* species" is not a reliable indicator for *L. pneumophila*. Therefore, the presence of *L.* non-*pneumophila* species does not necessarily also indicate favorable conditions for *L. pneumophila* growth in drinking water systems.

4.3. The Indicator Organism Must Be More Resistant to Disinfection Than the Pathogen. To determine whether *L.* non-*pneumophila* species are equal or more resistant to *L. pneumophila*, it is relevant to compare the degree of resistance of *L. pneumophila* and *L.* non-*pneumophila* species to various disinfection strategies.

Few laboratory-controlled studies have directly compared the disinfection sensitivities of *L. pneumophila* and *L.* non-*pneumophila* species. One such investigation found no significant differences in the efficacy of thermal and chlorine disinfection on protozoan-borne *L. pneumophila* and *L. erythra*.[94] In other studies, *L. longbeachae* was reported to be less resistant than *L. pneumophila* serogroup 1 to ultraviolet irradiation, free chlorine, and thermal inactivation.[106,107] However, water is not the primary reservoir of *L. longbeachae*, and, therefore, those findings are less relevant for this comparison. In a pilot-scale study using a UV-A light-emitting diodes system, *L. dumoffii* (2.1-log inactivation) showed greater resistance than *L. pneumophila* (3.0-log inactivation) at a dose of 1,700 mJ/cm^2.[108]

Field studies in hospital water systems have demonstrated that copper−silver ionization, thermal management, hydrogen peroxide with silver ions, and hyperchlorination effectively reduced both *L. pneumophila* and *L.* non-*pneumophila* species (*L. anisa* and/or *L. rubrilucens*) to below detectable concentrations.[109−112] Nevertheless, some studies report that *L. anisa* and other *L.* non-*pneumophila* species are more sensitive to thermal disinfection than *L. pneumophila*.[8,75,85] Conversely, in one study hydrogen peroxide proved effective against *L. pneumophila*, but certain *L.* non-*pneumophila* species were less sensitive.[113] Notably, the introduction of chemical disinfection may lead to a shift in *Legionella* speciation. A shift from *L. pneumophila* serogroup 1 to *L. bozemanii* was observed after monochloramine was introduced in a hospital hot water system.[114]

Taken together, current evidence suggests that *L.* non-*pneumophila* species and *L. pneumophila* can have different disinfection sensitivities under some disinfection regimes. Therefore, these findings indicate that the quantification of all *Legionella* species partially meets this criterion for serving as an indicator of the inactivation of *L. pneumophila*. Nevertheless, if the goal of the disinfection is to control *L. pneumophila,* higher initial concentrations of total *Legionella* species than *L. pneumophila* can result in the implementation of excessive treatment requirements.

4.4. The Indicator Organism Must Grow Independently of Other Organisms When Inoculated on Artificial Media. *Legionella* legislation often requires cultivation according to ISO 11731:2017, which can recover *L. pneumophila* and various *L.* non-*pneumophila* species, such as *L. anisa*. However, several *L.* non-*pneumophila* species demonstrate only marginal growth on BCYE agar.[12,13] For species such as *L. anisa*, *L. bozemanii*, and *L. dumoffii*, optimal recovery occurs at a pH of 6.5,[13] which is lower than the ISO-recommended pH of 6.9 ± 0.1.[115] Still, *L. anisa* or *L. dumoffii* in drinking water samples can be regularly observed using the ISO 11731:2017 method, indicating that the ISO-recommended pH does not completely inhibit the growth of these two *Legionella* species. Moreover, the ubiquitous presence of not-yet-culturable *L.* non-*pneumophila* species in drinking water[116] further complicates the reliable enumeration of all *Legionella* species with culture-based methods.

Therefore, it can be concluded that culturable *L.* non-*pneumophila* species do not consistently grow independently on artificial media, making them unreliable as indicator organisms of culturable *L. pneumophila*.

4.5. Overall Assessment. Overall, we conclude that culturable *L. nonpneumophila* species are not suitable indicators for culturable *L. pneumophila* because they (i) are not consistently more abundant, (ii) occupy different ecological (e.g., temperature) niches, (iii) differ in disinfection sensitivity, and (iv) have inconsistent recovery on standard media.

5. ALTERNATIVE METHODS FOR TARGETED MONITORING OF PATHOGENIC *LEGIONELLA* SPECIES

Routine plate culture (ISO 11731) remains the standard in most regulations, but its 10- to 15-day turnaround time can delay the detection of *Legionella* growth events and the implementation of corrective actions. Faster, species-targeted methods can address this gap and support the selection of the most relevant monitoring target for pathogenic *Legionella* species.

Quantitative PCR (qPCR) detects genome copies of either all *Legionella* species or selected species, including *L. pneumophila*.[117−122] As with culture-based methods, comparison studies show that *L. pneumophila* can be present when *L.* non-*pneumophila* species are not, and vice versa.[123,124] These differences can be amplified by qPCR's ability to detect unculturable *Legionella* species. The short turnaround of qPCR (hours) can enable early screening and prioritization of system zones for intervention. In high-risk settings such as hospitals, running both a *L. pneumophila*-specific assay and one targeting high-risk *L.* non-*pneumophila* species may be warranted to protect immunocompromised patients who are susceptible to less virulent *L.* non-*pneumophila* pathogens. A key limitation is that standard qPCR quantifies genome copies rather than colony-forming units (CFU), capturing DNA from intact, injured, viable-but-nonculturable, and dead cells. This complicates the translation of results into exposure doses for use in dose−response models for QMRA.[125] Viability PCR[120] and RNA-based assays[119] can help narrow the difference between genome copies and CFUs, but requires further standardization for regulatory use and can introduce additional cost, workflow complexity, and methodological biases. Despite these challenges, qPCR, particularly when species-specific, can support faster and more targeted interventions.

In addition to molecular tools, alternative growth-based methods offer practical advantages. Enzyme substrate-based most probable number (MPN) culture methods (e.g., ASTM D8429-21)[126] and Diamidex MICA assay[127,128] enable quantification of viable *L. pneumophila* alone, without relying on conventional plate culture, and typically do so within 2−7 days. Although these methods differ from traditional culture,

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they express results in CFU or MPN, making them easy to interpret within existing risk assessment frameworks.

Using methods that selectively detect and quantify pathogenic *Legionella* species keeps control efforts focused on the species responsible for the greatest disease burden. Further research is needed to define when and how high-risk *L.* non-*pneumophila* species should be included in monitoring strategies.

6. DISEASE BURDEN ASSOCIATED WITH *LEGIONELLA* VERSUS OTHER WATER-RELATED OPPORTUNISTIC PATHOGENS

In addition to *Legionella* species, other opportunistic pathogens can also be present in drinking water systems. These include certain species of nontuberculous mycobacteria (NTM), *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Aspergillus fumigatus*, *Acanthamoeba* spp., *Naegleria fowleri*, and *Waddlia chondrophila*.[129−131] While transmission routes vary, drinking water is considered a significant exposure pathway for many of these organisms.

A structured expert-judgment study estimated that 52% of *Legionella* species cases, 67% of NTM cases, and 16% of *Pseudomonas* septicemia cases were attributable to drinking water.[132] Using these estimates, Gerdes et al.[133] calculated that the USA experienced approximately 5,760 cases of Legionnaires' disease, 46,400 cases of NTM infection, and 929 cases of *Pseudomonas* septicemia annually, resulting in 520, 2,560, and 112 deaths, respectively. Similarly, in Ontario, Canada, NTM and *Pseudomonas* species were linked to higher hospitalization and death rates than *Legionella* species.[134]

Dutch surveillance data show that 93.5% of culture-confirmed cases were caused by *L. pneumophila* and only 6.5% by *L.* non-*pneumophila*.[135] This translates to mean annual incidences of 6.3 and 0.4 hospital cases per 100,000 persons, respectively. By comparison, estimates for pathogenic NTM and *A. fumigatus* range from 4.1 to 7.2 per 100,000[136,137] (Figure 2). *P. aeruginosa*, detected in 2.2% of community-acquired pneumonia hospital cases, corresponds to an estimated 30.8 cases per 100,000, significantly higher than any *Legionella* species.[138,139] These comparisons indicate that *L.* non-*pneumophila* species contribute far less to the overall disease burden than other waterborne opportunistic pathogens. Therefore, focusing monitoring and control efforts on *L. pneumophila* and potentially other high-burden opportunistic pathogens may be more effective and resource-efficient for public health protection.

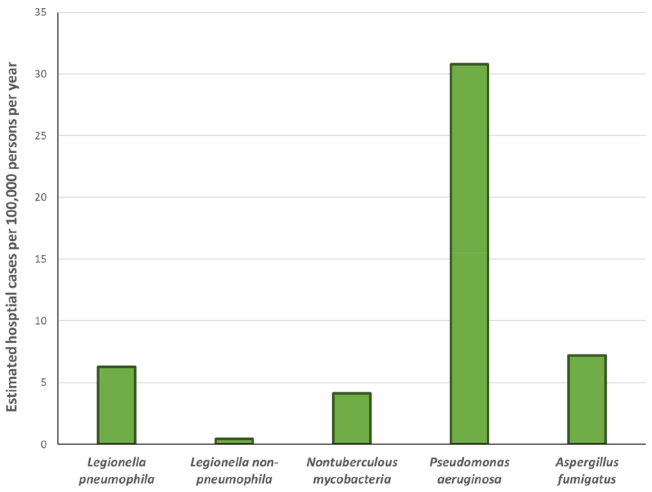

Figure 2. Incidence of estimated hospital cases per 100,000 persons per year in the Netherlands for *L. pneumophila*, *L.* non-*pneumophila*, pathogenic nontuberculous mycobacteria, *P. aeruginosa*, and *A. fumigatus*. (Based on data from ref 140; van der Wielen et al.[135]; Schildkraut et al.[136]; Buil et al.[137]; Wiersinga et al.[138]; and Postma et al.[139]).

7. IMPLICATIONS

Based on the results from our review, we conclude that culturable *L. pneumophila* is a more effective target than the quantification of all culturable *Legionella* species to mitigate Legionnaires' disease cases associated with building drinking water systems for the following reasons:

- Global surveillance shows that *L. pneumophila* causes the vast majority of waterborne cases of Legionnaires' disease.
- Despite the widespread presence of *Legionella* non-*pneumophila* species in drinking water systems, they rarely cause disease. Consequently, blanket regulations (e.g., "all *Legionella* species" monitoring) will not yield greater public health benefits than those targeting *L. pneumophila*. Such regulations could result in much higher costs for subsequent control measures compared to focusing only on *L. pneumophila*.
- Ecological differences between *L. anisa* and *L. pneumophila*, particularly in growth temperatures, make "all *Legionella* species" an unreliable indicator of *L. pneumophila* in drinking water systems.
- In high-risk settings such as healthcare facilities, monitoring both *L. pneumophila* and high-risk *L.* non-*pneumophila* species may be necessary to protect immunocompromised patients susceptible to less virulent *L.* non-*pneumophila* pathogens. In these cases, the choice of target opportunistic pathogens for risk assessment could be based on a conservative approach or informed by disease burden studies.

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Notes

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