Current Microbiology (2026) 83:122
https://doi.org/10.1007/s00284-026-04722-7

REVIEW ARTICLE

Leptospira and Leptospirosis: A Review of Species Classifications, Genomes, Morphological Structures, Antimicrobial Resistances, Transmissions, and Clinical Manifestations

Lesley Maurice Bilung[1] · Ahmad Syatir Tahar[2] · Chai Fung Pui[3] · Muhammad Khairil Syamri Bakeri[1] · Lela Su'ut[4] · Romano Ngui[2] · Rosdi Kira[1] · Kasing Apun[1]

Received: 13 September 2025 / Accepted: 2 January 2026

Abstract

Leptospirosis, also known as "rat-urine disease", is a neglected zoonotic and waterborne disease that is caused by *Leptospira* spp. This disease is transmitted by direct and indirect exposure to the urine and stool of infected animals. The current estimate has highlighted that leptospirosis has caused at least one million cases and 60,000 deaths, with high endemicity in tropical regions. With climate change, urbanisation, and increasing human-animal interaction, the threat of leptospirosis and other zoonotic diseases will continue to emerge. Investing in multidisciplinary research, technology, and global collaboration is critical to anticipate, detect, and respond effectively to these evolving threats.

Introduction

Leptospira, a genus of spirochete bacteria, causes zoonotic and waterborne disease called Leptospirosis, a neglected but emerging tropical disease that poses a significant public health risk worldwide. *Leptospira* spp. can be classified based on the pathogenicity into three groups, namely pathogenic, intermediate, and saprophytic, in which each pathogenicity group contains several species [1]. Known to adapt to a broad range of hosts, *Leptospira* spp. are acquired from direct or indirect contact with the urine of infected animals, or water and soil contaminated by these bacteria. *Leptospira* spp. thrive in tropical and temperate climates and have caused at least one million cases with 60,000 deaths annually [2]. *Leptospira* spp. were classically grouped into pathogenic, intermediate, and saprophytic clades [1]; however, advances in molecular taxonomy now recognise more complex and phylogenetic relationships and numerous species within these lineages. The global burden of leptospirosis is substantial, especially in low-resource settings where environmental conditions and limited healthcare infrastructure facilitates disease transmission [2]. This scenario causes delayed treatment when the MAT, which is the gold standard in diagnosing leptospirosis, is not practical in remote settings [3]. Given the significant global burden of leptospirosis, a thorough understanding of *Leptospira* spp. epidemiology is needed for developing more effective diagnostic strategies and making treatment development more accessible. Additionally, the seasonal epidemiological patterns of leptospirosis, characterised by increased incidence following heavy rainfall and flooding events, underscore the need for enhanced surveillance and timely intervention during these high-risk periods. Management of leptospirosis requires a multifaceted approach; therefore, understanding every aspect of the bacterial agent and disease is important before appropriate actions can be taken. This review consolidates contemporary insights into the species classifications, genomes, transmissions, and clinical manifestations of *Leptospira* spp., while evaluating current knowledge

✉ Lesley Maurice Bilung
mblesley@unimas.my

[1] Resource Biotechnology Program, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Sarawak 94300, Kota Samarahan, Malaysia

[2] Department of Paraclinical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Sarawak 94300, Kota Samarahan, Malaysia

[3] Centre for Pre-University Studies, UCSI University Sarawak Campus, Kuching, Sarawak 93450, Malaysia

[4] Department of Pathology, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Sarawak 94300, Kota Samarahan, Malaysia

Published online: 05 January 2026

gaps and proposing new future research trajectories to more advanced evidence-based interventions for leptospirosis.

History of *Leptospira*

The history of *Leptospira* spp. dates back to Adolph Weil's description of Weil's disease in 1886 as an acute infectious disease with splenomegaly, jaundice, and inflammation of the kidneys [4]. Over the following decades, key discoveries included the first microscopic identification of the spirochete by Arthur Stimson in 1907, its isolation, and subsequent serovar classifications. He named the microorganism *Spirocheta interrogans* due to the hook at the end resembling a question mark. The microorganism was isolated by Inada and colleagues in Japan in 1915. They discovered this spirochete and found specific antibodies in the blood of miners who had contracted yellow fever [5, 6]. Almost simultaneously, two groups of doctors in Germany successfully transmitted the infection to guinea pigs and named as *Spirochaeta nodosa* and *Spirochaeta icterogenes* [7]. After detailed culture and microscopic observations, Hideyo Noguchi named it "*Leptospira*" (thin spirals) in 1918. From the 1920s to the 1950s, various serotypes of these spirochetes were discovered in Germany, Indonesia, and Japan. The detailed structure of *Leptospira* spp. was examined in the 1960s to 1970s with the aid of the electron microscope. Leptospirosis has been around for millennia, as some early disease outbreaks reported in ancient times were associated with leptospirosis. It was known before the typical aetiology was accepted. Names such as "rice field jaundice" in China, "seven-day fever" or "autumn fever" in Japan, and "schlammfieber (mud fever), "cane-cutter's disease" or "swine fever" in Europe, Australia, and elsewhere were used to indicate leptospirosis [8, 9].

Taxonomy and Classification of *Leptospira*

Taxonomically, *Leptospira* belongs to the family Leptospiraceae, within the order Spirochaetales, class Spirochaetia, and phylum Spirochaetota [10]. The family Leptospiraceae was introduced in 1979 [11] and comprises three genera, namely *Leptospira*, *Leptonema*, and *Turneriella*, which were elucidated based on 16 S rRNA gene sequences, DNA-DNA relatedness, and differences in G + C content. Thus, the G + C contents of *Leptospira*, *Leptonema*, and *Turneriella* are 33 to 43, 54, and 53.6 mol%, respectively [12]. The taxonomy and classification of *Leptospira* spp. are complex and controversial [13] and are primarily classified through three main approaches: historical methods that rely on morphological characteristics and growth conditions (based on phenotypic and serological characteristics), genotypic classification based on DNA sequence data, and phylogenetic classification to elucidate evolutionary relationships [12, 14].

a. Historical (Phenotypic and Serological) Classifications

Traditionally, the genus *Leptospira* was divided into two species based on phenotypic classification: the pathogenic *L. interrogans*, comprising strains found in animals and humans, and the saprophytic *L. biflexa*, comprising environmental isolates Click or tap here to enter text. *L. biflexa* was distinguished from *L. interrogans* by its ability to grow at 13 °C in the presence of 8-azaguanine, and by its failure to form spherical cells in 1 M of sodium chloride [5]. However, the lack of distinguishing features often hinders phenotypic identification in routine clinical microbiological laboratories [14]. Considering the limitations, serological classification was then used to divide the two species into at least 24 serogroups and more than 300 serovars based on the expression of surface-exposed lipopolysaccharide (LPS) [15].

The structural differences in the carbohydrate component of LPS determine the antigenic diversity of the different serovars, which serve as a basic classification [13, 16]. The definition of serovar was then changed in 1986 by the Taxonomic Subcommittee on *Leptospira*. It is assumed that two strains belong to different serovars if, after cross-absorption with appropriate amounts of heterologous antigens in repeated tests, 10% or mre of the homologous titre routinely remains in at least one of the two antisera [12]. Antigenically related serovars are further categorised into serogroups. For example, some common serovars can be summarised under the serogroups of *L. interrogans* [17]. Although the term serogroup has no taxonomic meaning, it has been used to define antigenically related serovars due to the shared surface LPS structures. These serovars can be identified with the microscopic agglutination test (MAT) and are therefore widely accepted due to their epidemiological importance [18].

With the development of modern molecular typing methods, relying solely on serovar designation may be insufficient for identifying epidemiologically important strains [19], especially when molecular classifications do not align with traditional serological classification, which has served clinical microbiologists for decades. Therefore, maintaining the serological classification remains relevant until a standardised molecular-based identification is developed [5].

b. Genotypic Classifications

Phenotypic classification was then superseded by genotypic classification, with the concept of "genomospecies"

were introduced to encompass the diverse serovars of *L. interrogans* and *L. biflexa*. This genomospecies was first established using DNA-DNA hybridisation in the late 20th century, which showed that serologically similar isolates were often genetically different, leading to the description of several new species [20]. While DNA-DNA hybridisation was foundational, 16S rRNA gene sequencing and multilocus sequence typing (MLST) emerged as more practical and widely adopted molecular tools for *Leptospira* genotyping [21].

16S rRNA gene sequencing targets 16S ribosomal RNA, often a highly conserved gene present in all bacteria, supporting a three-group model in which *Leptospira* spp. can be categorised as pathogenic (Group I), intermediate or opportunistic (Group II), and saprophytic or non-infectious (Group III) (Fig. 1) [21]. Group I causes diseases of varying severity in humans, ranging from asymptomatic infections to severe complications and death. Group II pathogens, on the other hand, survive better in culture and lead to a mild, self-resolving disease without causing death. They are considered opportunistic because of insufficient information on the involvement of these species in human leptospirosis, unclear pathogenicity, and the phenotypic characteristics do not match those of pathogenic *Leptospira* spp [22]. The pathogenicity status of Group II *Leptospira* remains controversial. A review highlighted that animal models have primarily been developed for pathogenic *Leptospira* and that chronic or subclinical infections may occur without severe clinical disease in some hosts, indicating the complexity of disease manifestations across species [23]. Whilst early animal studies, such as those involving hamsters inoculated with intermediate *L. inadai* and *L. licerasiae* did not result in clinical manifestations [24, 25]. However, more recent phylogenomic and epidemiological work has emphasised the ambiguous nature of these species (i.e. genetically closer to pathogenic clades yet associated with milder human disease, thereby blurring the lines of their classification) [26]. *Leptospira* spp. of Group III are free-living environmental microorganisms and do not cause disease in humans and animals [25].

However, the conserved nature of the 16S rRNA gene lacks the resolution to differentiate between very closely related species within the pathogenicity group and is unsuitable for discriminating serovars or strains. MLST was developed to overcome this limitation by sequencing multiple "housekeeping' genes (i.e. genes essential for basic cell function that are found in all strains). Due to a higher rate of evolution of these genes than the 16S rRNA gene, they can be used for better discrimination of the strains or serovars, allowing for intraspecies discrimination and clonal complex identification [20].

c. Phylogenetic Classifications

The phylogenomic classification of *Leptospira* spp. has evolved considerably with the advent of molecular and genome-wide analyses. The latest discovery through whole-genome sequencing (WGS) has significantly expanded the taxonomic landscape by identifying 74 validly described species (Table 1; Fig. 2) (instead of 21 species from genotypic classification) and enabling high-resolution comparisons of core and ancillary genomes [26]. Whole genome sequencing (WGS) has also revealed genomic signatures of virulence and ecological adaptation, highlighting the limitations of purely serological classification.

Subsequent application of WGS and phylogenomic analyses refined this taxonomy, dividing *Leptospira* spp. into four distinct clades: P1 (pathogenic, high virulence), P2 (intermediate pathogenicity) and the saprophytic clades S1 and S2 (Fig. 2) [26, 27]. S2 forms a distinct branch within the saprophytic lineage, separated from S1, and exhibits greater genetic divergence than S1 [26]. This four-class system is now widely accepted as the most robust representation of the evolutionary history of *Leptospira* spp., accounting for both genomic diversity and pathogenic potential, with approximately 74 validly described species distributed across the clades [27].

Genomic Features of *Leptospira* spp.

The genome of *Leptospira* is relatively large, as it normally ranges in size from approximately 4.6 to 5.1 megabases (Mb) (Table 2). One of the unique genomic structures of *Leptospira* is the presence of two circular chromosomes, which are rather unusual among spirochetes and most prokaryotes, reflecting the ecological and evolutionary complexity of *Leptospira* [16]. The two-chromosomal structure is most likely the result of genome plasticity and the retention of niche-associated characteristics, which may impact host interactions and virulence. Chromosome I is ~ 4.3 Mb in size and contains most housekeeping genes and many virulence-associated genes, whilst chromosome II is ~ 350–400 kilobases (kb) and behaves as a secondary chromosome (chromid) that carries genes related to accessory functions, environmental sensing, and host adaptation [29].

The role of some genes responsible for the pathogenesis of pathogenic *Leptospira* spp. is still not fully understood. For example, *L. interrogans* comprises 627 genes that are not found in the genome of *L. biflexa*. As the functions of more than 500 of these genes are unknown, it is hypothesised that additional genetic traits are required for survival in mammalian hosts and in the environment [37]. Although research on *Leptospira* spp. has entered the post-genomic

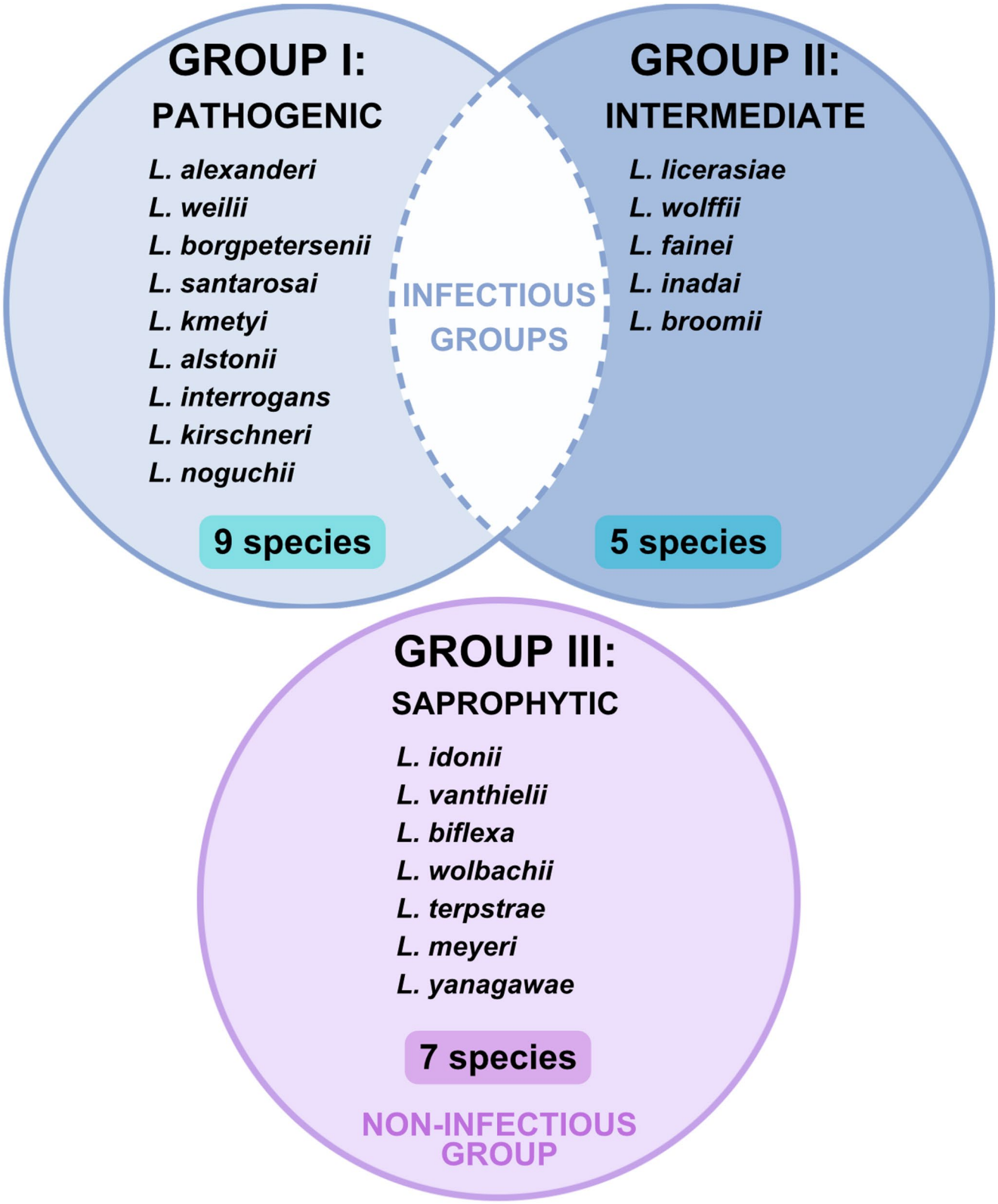

Fig. 1 Previous classification of *Leptospira* species based on the 16S rRNA gene sequences [12, 25]

Table 1 Updated list of 74 recognised *Leptospira* species classified into four established phylogenetic clades (P1, P2, S1, and S2) as described in [27]

Clade	Description	*Leptospira* species
P1 clade	Pathogenic, high-virulence, and responsible for the majority of severe human and animal leptospirosis cases	**21 species**: *L. adleri*, *L. ainazelensis*, *L. ainlah-djerensis*, *L. alexanderi*, *L. alstonii*, *L. barantonii*, *L. borgpetersenii*, *L. ellisii*, *L. gomenensis*, *L. gorisiae*, *L. interrogans*, *L. kirschneri*, *L. kmetyi*, *L. mayottensis*, *L. noguchii*, *L. santarosai*, *L. sanjuanensis*, *L. stimsonii (synonym of L. putramalaysiae)*, *L. tipperaryensis*, *L. weilii*, and *L. yasudae (synonym of L. dzianensis)*,
P2 clade	Intermediate pathogenic, capable of causing diseases but are generally associated with milder clinical outcomes.	**22 species**: *L. andrefontaineae*, *L. broomii*, *L. cinconiae*, *L. dzoumogneensis*, *L. fainei*, *L. fletcheri*, *L. fluminis*, *L. haakeii*, *L. hartskeerlii*, *L. inadai*, *L. johnsonii*, *L. koniamboensis*, *L. langatensis*, *L. licerasiae*, *L. neocaledonica*, *L. perolatii*, *L. saintgironsiae*, *L. sarikeiensis*, *L. selangorensis*, *L. semungkisensis*, *L. venezuelensis*, and *L. wolffii*
S1 clade	Non-pathogenic to humans and animals, free-living species that are common in water and soil. Widespread in soil and water. May have certain groups of genes that are not found in S2 clade.	**26 species**: *L. abararensis*, *L. bandrabouensis*, *L. biflexa*, *L. bourretii*, *L. bouyouniensis*, *L. brenneri*, *L. chreensis*, *L. congkakensis*, *L. ellinghausenii*, *L. harrisiae*, *L. iowaensis*, *L. jelokensis*, *L. kanakyensis*, *L. kemamanensis*, *L. levettii*, *L. meyeri*, *L.mgodei*, *L. milleri*, *L. montravelensis*, *L. mtsangambouensis*, *L. noumeaensis*, *L. perdikensis*, *L. terpstrae*, *L. vanthielii*, *L. wolbachii*, and *L. yanagawae*
S2 clade	Non-pathogenic to humans and animals. Smaller species group compared to S1 clade and may occupy more specialized niches.	**5 species**: *L. idonii*, *L. ilyithenensis*, *L. kobayashii*, *L. ognonensis*, *and L. ryugenii.*

era, genetic studies still lag behind other pathogenic bacteria [35]. Nevertheless, access to genome sequences and the development of mutagenesis systems, especially transposon mutagenesis, have enabled the elucidation of the phases of pathogenesis [36]. Comparative genomic analyses demonstrated that pathogenic strains have large repertoires of virulence determinants, including adhesins, hemolysins, CRISPR-Cas loci, and complex LPS biosynthesis clusters [16].

These virulence determinants are normally harboured in genomic islands and associated with insertion sequence (IS) mobilisation capability. Saprophytic *L. biflexa*, on the other hand, did not possess any of the pathogen-specific genes and had a lower number of pseudogenes and mobile elements [32]. Pathogenic *Leptospira* strains (P1 and P2 clades) also show a more open pan-genome structure indicative of frequent horizontal gene transfer, with elements such as vitamin B12 biosynthesis and sialic acid biosynthesis increasing host adaptation potential. In contrast, non-pathogenic strains (S1 and S2 clades) have the least open genome that corresponds to their stable environmental niches [16]. These genetic patterns reflect their evolutionary trajectory in which pathogenic strains evolve genome plasticity and specialisation through the acquisition of virulence encoding modules while saprophytic species maintain genome integrity to support their free-living lifestyle [34]. Recognising these distinctions is important for identifying potential diagnostic targets, vaccine antigens (i.e. Lig and LipL proteins), and further describing environmental versus host-adapted in *Leptospira* spp.

Comparative genomics is important in understanding the pathogenicity, resistance, and environmental adaptation in *Leptospira* spp [40]. By comparing the genomes of pathogenic versus saprophytic strains, several genomic regions have been identified as putative pathogenicity islands (PAls), revealing virulence-associated features that are predominantly found in highly pathogenic species such as *L. interrogans* and *L. kirschneri* [25]. These PAls have genes encoding surface-exposed adhesins (e.g., LipL32, LigA/B), as well as hemolysins, metalloproteases, and other proteins that interfere with the host's bactericidal pathways and facilitate immune invasion. These proteins are important for successful host-pathogen interactions [31]. One notable example is LIC11711, a gene characteristic of pathogenic *L. interrogans*. Heterologous expression of this gene in the saprophyte *L. biflexa* markedly increased bacterial adhesion to host laminin and plasminogen, suggesting a role of this gene in pathogenic strains in tissue colonisation and evasion of the immune responses [32].

Antimicrobial Resistance

Studies over several decades have investigated the antimicrobial susceptibility of *Leptospira* species using both culture-based and minimum-inhibitory concentration methods (Table 3), as well as *in vitro* and *in silico* methods (using CARD, RAST, PATRIC/BV-BRC for AMR detections) to understand the resistance mechanisms (Table 4). Early studies reported different resistance patterns: for example, *L. biflexa* showed resistance to streptomycin despite sensitivity to oxytetracycline, chloramphenicol, kanamycin, dihydrostreptomycin, and ampicillin [41], while *L. borgpetersenii* isolated from cattle was resistant to sulfamethazine but sensitive to penicillin, tetracycline, ampicillin, erythromycin, and streptomycin [42]. More comprehensive assessments

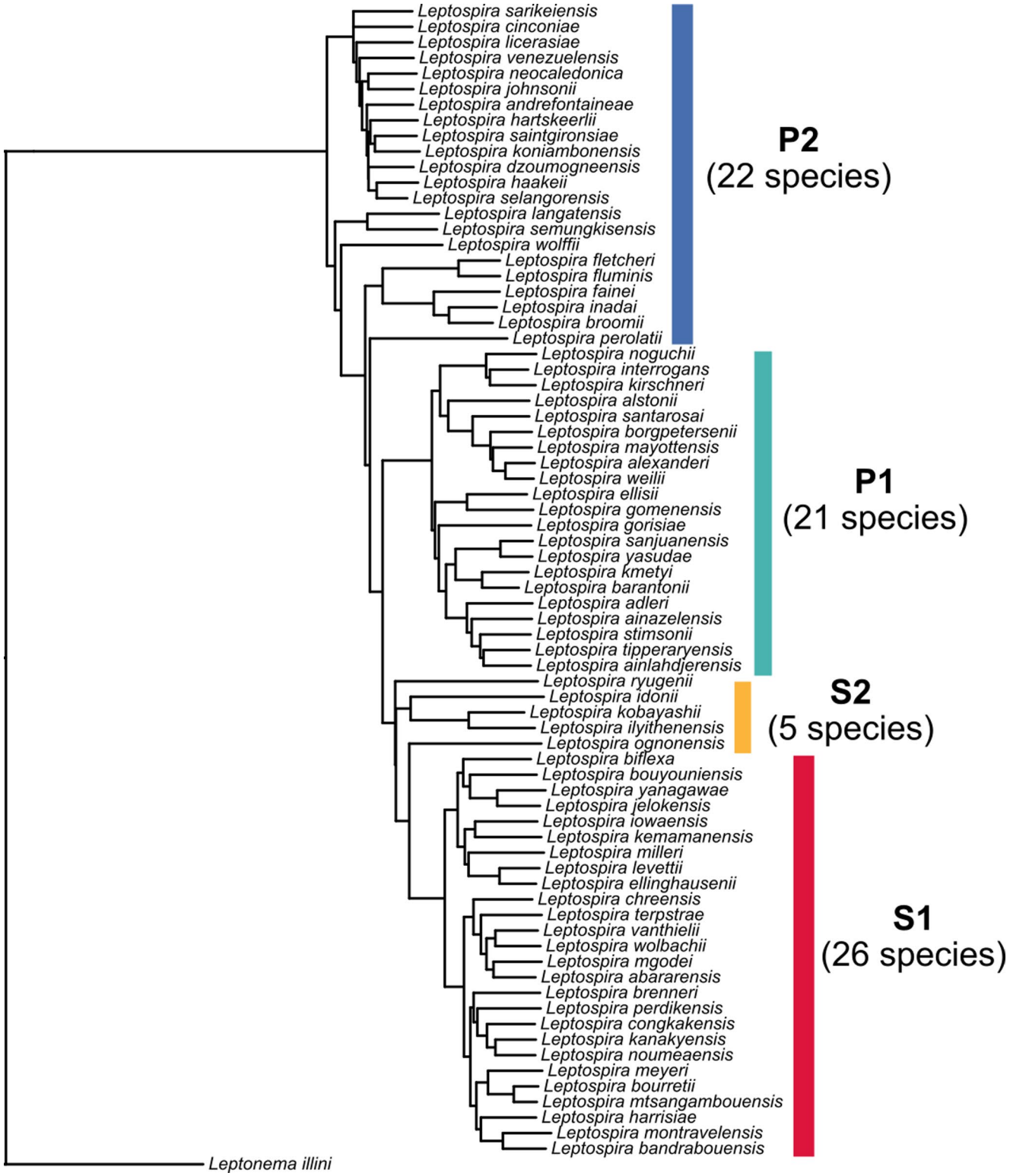

Fig. 2 The 74 currently recognised *Leptospira* species cluster into four subclades: P1 (pathogenic, high virulence), P2 (intermediate pathogenicity), S1 (non-pathogenic saprophytes), and S2 (non-pathogenic saprophytes). The phylogenetic tree was inferred from Mash distance matrices [28] using FastME [29]. Outgroup branch length (*Leptonema illini*) was shortened for visualisation purposes. The phylogenetic tree was constructed based on whole-genome assemblies retrieved from NCBI as provided in the Supplementary material

Table 2 Comparative genomic features of P1, P2, and S1/S2 *Leptospira* spp.

Genomic feature	Strains of P1 clade*	Strains of P2 clade*	Strains of S1 or S2 clades*	Reference
Genome size	~4.6–5.1 Mb	~4.0–4.3 Mb	~3.9–4.1 Mb	[5, 30]
Chromosome I (carries housekeeping and many virulence genes)	Present	Present	Present	[29]
Chromosome II (carries genes of accessory and essential functions)	Present	Present	Present	[30]
Pan-genome feature	Most open pan-genome	Open	Least open	[16, 26]
	Frequent gene acquisition via horizontal gene transfer.	Slower rate of gene acquisition via horizontal gene transfer than P1 clade.	Limited gene acquisition via horizontal gene transfer.	
Virulence factors	LipL32, LigA/B, hemolysins, adhesins, CRISPR-Cas	Partial presence	Absent	[16, 25, 31]
Genomic islands/pathogenicity islands	Abundant, encode virulence and immune evasion genes	Occasional	Absent	[16]
Mobile elements (insertion sequence and transposons)	The most abundance of mobile elements.	Fewer abundance of mobile elements than P1 clade.	Least abundance of mobile elements.	[32]
Antimicrobial resistance genes	At least 32 genes	Rare	Absent	[16]
Pseudogenes	Numerous, due to genome decay linked to host adaptation	Moderate, reflecting transitional ecology	Very few, stable genome needed for diverse environmental survival	[32]
Environmental adaptation genes	Osmoregulation, oxidative stress, nutrient uptake	Some	Basic core only	[33]
Host adaptation mechanisms	Plasminogen binding (e.g., LIC11711), sialic acid, B12 biosynthesis	Limited	Absent	[32]
Genomic plasticity	High; associated with ecological versatility	Moderate	Low; high stability	[34]
Genetic tools	Scarce; improved via transposon mutagenesis	Minimal, genetic manipulation remains poorly developed	Moderate, more tractable for genetic manipulation	[35, 36]
Unique genes	The most abundance of strain-specific genes. 627 genes not found in *L. biflexa*, 500 + uncharacterised, many linked to virulence and host adaptation	Fewer abundance of strain-specific genes than P1 clade. Partial overlap pathogenic strains; reduced virulence repertoire; transitional adaptation	The least abundance of strain-specific genes. Lacks pathogen-specific genes, enriched in metabolic and environmental survival functions	[37, 38]

*as representated by the *Leptospira* strains reported by Dellagostin et al. [39]

later confirmed that pathogenic species such as *L. interrogans*, *L. borgpetersenii*, *L. kirschneri*, *L. noguchii*, *L. santarosai*, and *L. weilii* generally remain sensitive to β-lactams, macrolides, tetracyclines, chloramphenicol, and fluoroquinolones, but show reduced sensitivity to aztreonam [38]. Recent surveys of clinical and environmental isolates show consistent susceptibility to β-lactams, doxycycline, and fluoroquinolones, although resistance to neomycin, fosfomycin, vancomycin, and especially sulfamethoxazole-trimethoprim has been increasingly reported in *L. interrogans* and *L. borgpetersenii* [43–46]. Regional studies have also shown differences in resistance, with some isolates showing lower susceptibility to polymyxin B, gentamicin, chloramphenicol, and rifampicin [52, 53]. A large-scale review of isolates collected between 1948 and 2016 confirmed that while penicillins, cephalosporins, tetracyclines, and fluoroquinolones remain largely effective, resistance to polymyxins is widespread in various *Leptospira* species and hosts [47].

Table 3 Antimicrobial susceptibility and resistance profiles of different *Leptospira* spp. from various sources

Leptospira species	Year	Main source	Method	Susceptible antibiotics	Resistant antibiotics	Reference
L. biflexia	1988	Culture strain	MIC	Oxytetracycline, chloramphenicol, kanamycin, dihydrostreptomycin, ampicillin	Streptomycin	[41]
L. borgpetersenii	1988	Cattle	MIC	Penicillin, tetracycline, ampicillin, erythromycin, streptomycin	Sulfamethazine	[42]
L. interrogans, *L. borgpetersenii*, *L. kirschneri*, *L. noguchii*, *L. santarosai*, *L. weilii*	2004	Culture strain	MIC	Penicillin and other β-lactams, macrolides, chloramphenicol, tetracycline, doxycycline, fluoroquinolones, telithromycin	Aztreonam	[38]
L. interrogans, *L. borgpetersenii*	2010	Rodent	MIC	Ampicillin, cefotaxime, fluoroquinolones, doxycycline, erythromycin, streptomycin	Neomycin, fosfomycin, sulfamethoxazole, trimethoprim, vancomycin	[43]
L. interrogans	2013	Human, dog, rodent, cattle	MIC	Penicillin and other β-lactams, fluoroquinolones, tetracyclines, gentamicin	Sulfamethoxazole-trimethoprim, neomycin	[44]
L. interrogans	2015	Rodent, dog	MIC	Amoxicillin, ceftriaxone, ciprofloxacin, clindamycin, doxycycline, erythromycin, imipenem, penicillin, polymyxin B	Polymyxin B, gentamicin, sulfamethoxazole-trimethoprim	[35]
L. interrogans, *L. borgpetersenii*, *L. kirschneri*, *L. weilii*	2015	Not available	Disc susceptibility test	Amoxicillin, azithromycin, cefoxitin, ceftazidime, ceftriaxone, chloramphenicol, ciprofloxacin, clindamycin, doripenem, doxycycline, gentamicin, linezolid, nitrofurantoin, penicillin, piperacillin/tazobactam, tetracycline	Sulfamethoxazole-trimethoprim, fosfomycin, nalidixic acid, rifampicin	[35]
L. interrogans, *L. borgpetersenii*, *L. meyeri*, *L. santarosai*	2016	Human, dog, rodent, cattle, swine	MIC	Penicillin, ampicillin	Sulfamethoxazole-trimethoprim	[45]
L. interrogans, *L. borgpetersenii*	2017	Dog, rodent, human, swine, water	MIC	Penicillin, ampicillin, doxycycline	Sulfamethoxazole-trimethoprim, chloramphenicol	[46]
L. borgpetersenii, *L. broomi*, *L. interrogans*, *L. kirschneri*, *L. noguchii*, *L. santarosai*	1948–2016	Various (swine, cattle, human, deer, and donkey)	MIC	Penicillin, amoxicillin, clavulanate, cephalexin, ceftriaxone, doxycycline, tetracycline, streptomycin, enrofloxacin, spectinomycin	Polymyxin	[47]

Taken together, these results show that while traditional first-line antibiotics remain largely effective against *Leptospira*, emerging resistance, particularly to aminoglycosides, folate pathway inhibitors, and polymyxins, warrants continued surveillance. However, there is no standardised clinical breakpoint for *Leptospira*. Resistance interpretations vary across the studies.

Comparing these phenotypic data with genomic features provides a basis for identifying mechanisms of AMR (Table 4). Several *in silico* and *in vitro* studies demonstrated that AMR in *Leptospira* is multifactorial and includes enzymatic inactivation, target site modification, efflux-mediated resistance, cell envelope changes, and metabolic evasion. Aminoglycoside-modifying enzymes such as acetyltransferases encoded by *N6'ac* prevent ribosomal binding of gentamicin and tobramycin, while β-lactamases, which can hydrolyse penicillins, cephalosporins, and carbapenems, render β-lactams ineffective [48, 50]. Resistance is also mediated by mutations in ribosomal proteins (*rpsL*, *rpsJ*), deletion of *gidB*, and alterations in *alr*, *ddl*, *murA*, *gyrA/gyrB*, and *rpoB/rpoC*, which impair the activity of aminoglycosides, streptomycin, cycloserine, fosfomycin, fluoroquinolones, and rifamycins, respectively. Rifamycins; modifications of the elongation factors EF-G and EF-Tu additionally impair the inhibition of protein synthesis [41, 49]. Efflux-mediated resistance is supported by transporters of the RND and MATE family as well as accessory proteins (TolC, AcrB, MacB, NodT), which excrete various antimicrobial substances and reduce intracellular drug levels [48]. Structural defence mechanisms include LPS modifications that limit polymyxin uptake, phospholipid alterations by *gdpD* and *pgsA* mutations that alter surface charge and confer cross-resistance to polymyxins and aminoglycosides, and glycopeptide resistance mediated by *vanT*, *vanW*, and *vanY* that remodel peptidoglycan termini [51]. Finally, intrinsic resistance to inhibitors of the folate pathway arises from changes in DHPS and DHFR or from the acquisition of alternative enzymes that bypass the inhibition induced by trimethoprim and sulfonamides and maintain folate biosynthesis despite drug exposure [49]. Plasmids and prophages are

Table 4 Antibiotic resistance mechanisms found across *Leptospira* spp. based on *in silico* and *in vitro* studies

Type of antimicrobial resistance	Resistance mechanism	Antibiotic class affected	Study type	Leptospira group/clade	Reference
Aminoglycoside-modifying enzyme	Resistant *Leptospira* produces aminoglycoside acetyltransferase (*N6'ac* gene) that inactivates aminoglycoside antibiotics (e.g. gentamycin, tobramycin) by acetylation, therefore preventing them from binding ribosomes that can interfere protein synthesis	Aminoglycosides	*In silico*	P1, S1	[48]
Folate-pathway bypass	Mutations of dihydropteroate synthase (DHPS) or dihydrofolate reductase (DHFR) or acquisition of alternative enzymes to bypass inhibition of folate synthesis induced by trimethoprim and sulfonamides. The reduced binding of trimethoprim and sulfonamides causes continued folate synthesis.	Trimethoprim and Sulfonamides	*In silico*	P1	[49]
Multidrug efflux pump system	Efflux pumps are natural transport proteins in bacterial membranes, responsible for exporting antibiotics out of bacterial cell. **This efflux pump system consists of**: - Resistance-Nodulation-Division (RND) pump - Multidrug And Toxic compound Extrusion (MATE) pump - Pump components: TolC, AcrB, MacB, NodT - Regulators (Reg)	Multiple classes	*In silico*	P2, S1, S2	[48]
Ribosomal target mutation	Mutations in the gene (encoding ribosomal protein S12) or the rpsJ gene (encoding ribosomal protein S10) reduce aminoglycoside binding, thereby preventing disruption of protein synthesis.	Aminoglycosides	*In vitro*	Saprophytic	[41]
			In silico	Pathogenic	[49]
Vancomycin resistance genes	*vanT*, *vanW*, *vanY* genes alter peptidoglycan precursors (D-Ala-DlLac instead of D-Ala-DlAla), reducing vancomycin binding	Glycopeptides (Vancomycin)	*In silico*	P1, P2, S1, S2	[48]
β-lactamase enzyme production	β-lactamase is an enzyme that hydrolyses the β-lactam ring of penicillins, cephalosphorins, and carbapenems, that can render them inactive.	β-Lactams	*In silico*	P1, P2, S1, S2	[48]
			In silico	Pathogenic	[50]
Cell-wall target alteration	Mutations in *alr* (alanine racemase) and *ddl* (D-Ala–D-Ala ligase) reduce binding of D-cycloserine, impairing inhibition of peptidoglycan biosynthesis	Cycloserine (cell-wall synthesis inhibitors)	*In silico*	P1	[51]
Cell-wall target alteration	Alteration in *murA* (UDP-N-acetylglucosamine enolpyruvyl transferase) reduces binding of fosfomycin, preventing inhibition of cell-wall precursor synthesis	Fosfomycin	*In silico*	P1	[51]
DNA gyrase/topoisomerase target mutation	Mutations in *gyrA* and *gyrB* reduce fluoroquinolone binding to DNA gyrase, impairing inhibition of DNA replication	Fluoroquinolones	*In silico*	P1	[51]
RNA polymerase target mutation	Mutations in *rpoB* and *rpoC* alter rifampicin binding to RNA polymerase β-subunits, preventing inhibition of transcription	Rifamycins	*In silico*	P1	[51]
Translation factor alteration	Alterations in *ef-G* (elongation factor G) and *ef-Tu* (elongation factor Tu) impair interaction with protein-synthesis inhibitors	Protein synthesis inhibitors (various)	*In silico*	P1	[51]
Loss of rRNA methyltransferase	Absence or inactivation of *gidB* (16 S rRNA methyltransferase) causes low-level streptomycin resistance by reducing ribosomal binding	Aminoglycosides (streptomycin)	*In silico*	P1	[51]
Cell-wall charge modification	Genes *gdpD* and *pgsA* modify outer-membrane lipopolysaccharides (LPS), altering surface charge and reducing uptake of cationic antibiotics	Polymyxins, Aminoglycosides	*In silico*	P1	[51]

also responsible in the horizontal gene transfer in bacteria, enhancing the spread of such resistance determinants [35].

Morphological Structures of *Leptospira* spp.

Leptospira spp. (from the Greek *leptos*, meaning "fine", and "*spira*", meaning "a spiral") are finely coiled, filamentous spirochetes. They are extremely thin, helically coiled spirochetes typically measuring 6–20 µm in length and approximately 0.1 µm (100 nm) in diameter [49, 54]. Certain species have been reported to reach lengths of about 15.1 µm and diameters of about 0.12 µm, with a wavelength of ~ 0.6 µm [55]. *Leptospira* spp. stain poorly with conventional aniline dyes, including Gram stain, and are too thin to be visible under a normal light microscope [4]. They are typically visualised using dark-field or phase-contrast microscopy. *Leptospira* spp. are corkscrew-shaped bacteria with "question mark"-hook-shaped ends that distinguish them from other spirochetes [22].

Leptospira spp. have a double-membrane envelope, similar to other Gram-negative bacteria but with distinct spirochetal features (Fig. 3A). The outer membrane contains numerous lipopolysaccharides (LPS) and a variety of outer-membrane proteins: LPS of many Gram-negative bacteria exhibits endotoxin activity that can activate TLR4; leptospiral LPS, however, exhibits low endotoxin activity and structurally unusual lipid A that signals through TLR2 instead of TLR4. This alters the innate immune recognition (whilst LPS of most Gram-negative bacteria usually triggers TLR4) and potentially enables the pathogen to partially evade typical endotoxin-driven responses while still inducing inflammation [55, 56]. The outer-membrane proteins include classical barrel porins (e.g., OmpL1), which contribute to nutrient exchange and host interaction [55], as well as many surface-exposed proteins involved in adhesion, immune evasion, and virulence. These include LipL41, LigA-LigC, LenA-LenF, and Lsa lipoproteins that bind a range of extracellular matrix (ECM) components such as fibronectin, laminin, collagen, and fibrinogen [57–60].

Within the periplasm, several internal lipoproteins of the outer membrane, such as LipL32 and LipL36, are located in the periplasmic face of the outer membrane; these proteins trigger inflammatory responses and are hypothesised to contribute to envelope stability [61]. Another periplasmic lipoprotein, LipL21, binds tightly to the peptidoglycan layer and protects it from enzymatic fragmentation, thereby preventing the release of muropeptides that would otherwise activate innate immune receptors [62]. The peptidoglycan layer itself lies unusually close to the inner membrane, is more reminiscent of a Gram-positive structural envelope, that plays an essential structural role in stabilising the elongated helical cell body, resisting mechanical deformation during motility, and providing an anchoring scaffold for the periplasmic endoflagella (Fig. 3A&B) [59].

A defining feature of *Leptospira* spp. is the presence of two periplasmic endoflagella, one located at each pole (Fig. 3C and F) [39]. The molecular architecture of these endoflagella is well characterised, consisting of a core (composed of FlaB1–FlaB4 subunits) surrounded by a sheath (composed of FlaA1, FlaA2, FcpA, and FcpB), which together regulate the stability, coiling, and mechanical properties of the filament [4, 5]. These periplasmic endoflagella are responsible for the motility of *Leptospira* spp. and are associated with pathogenicity, through coordinated rotation of both the endoflagella and the helical cell body, enabling the bacteria to switch between swimming in the liquid (undulatory or wave-like movement) and crawling on surfaces [58, 60, 61].

Isolation, Growth, and Cultivation of *Leptospira* spp.

The primary isolation of pathogenic *Leptospira* spp. from clinical samples (e.g. mammalian tissues, blood, or urine) is notoriously difficult. Cultures may require prolonged incubation of 6 to 13 weeks due to slow growth rate [5], low bacterial loads in clinical samples, and heightened risks of contamination [62, 63]. Environmental samples (e.g. water and soil) can be more challenging because saprophytic *Leptospira* naturally grow easily and rapidly compared to pathogenic *Leptospira*, affecting the downstream data analysis. For example, a study using Oxford Nanopore Technology (ONT) metagenomic sequencing on enriched cultures from soil and water found most sequencing reads (~ 98–99% reads) comprised saprophytic *Leptospira* compared to pathogenic species (~ 1–2% reads) [64, 76]. Once isolated, *Leptospira* typically grow more rapidly in subsequent in-vitro cultures, often reaching maximal cell density within 3 to 4 weeks under optimal conditions. In a semi-solid medium, growth concentrates in a distinct subsurface zone that progressively becomes more turbid with increasing incubation time. This growth is known as Dinger's ring or disc, an opaque region associated with optimal oxygen tension [5].

Outside the host, the most optimal conditions for the survival of *Leptospira* spp. are a humid environment with neutral pH and a temperature range of 20 to 32 °C [65]. In the environment, they can survive from a few weeks to almost a year in moist soil during the dry season or in surface water during the rainy season [17]. *Leptospira* spp. are obligate aerobes that require aeration for maximum growth [4]. When grown in suitably aerated culture media at pH 7.2–7.6 and temperatures of 28–30 °C, the generation time of *Leptospira* spp. varies considerably among species. Pathogenic *L. interrogans* typically exhibits a generation

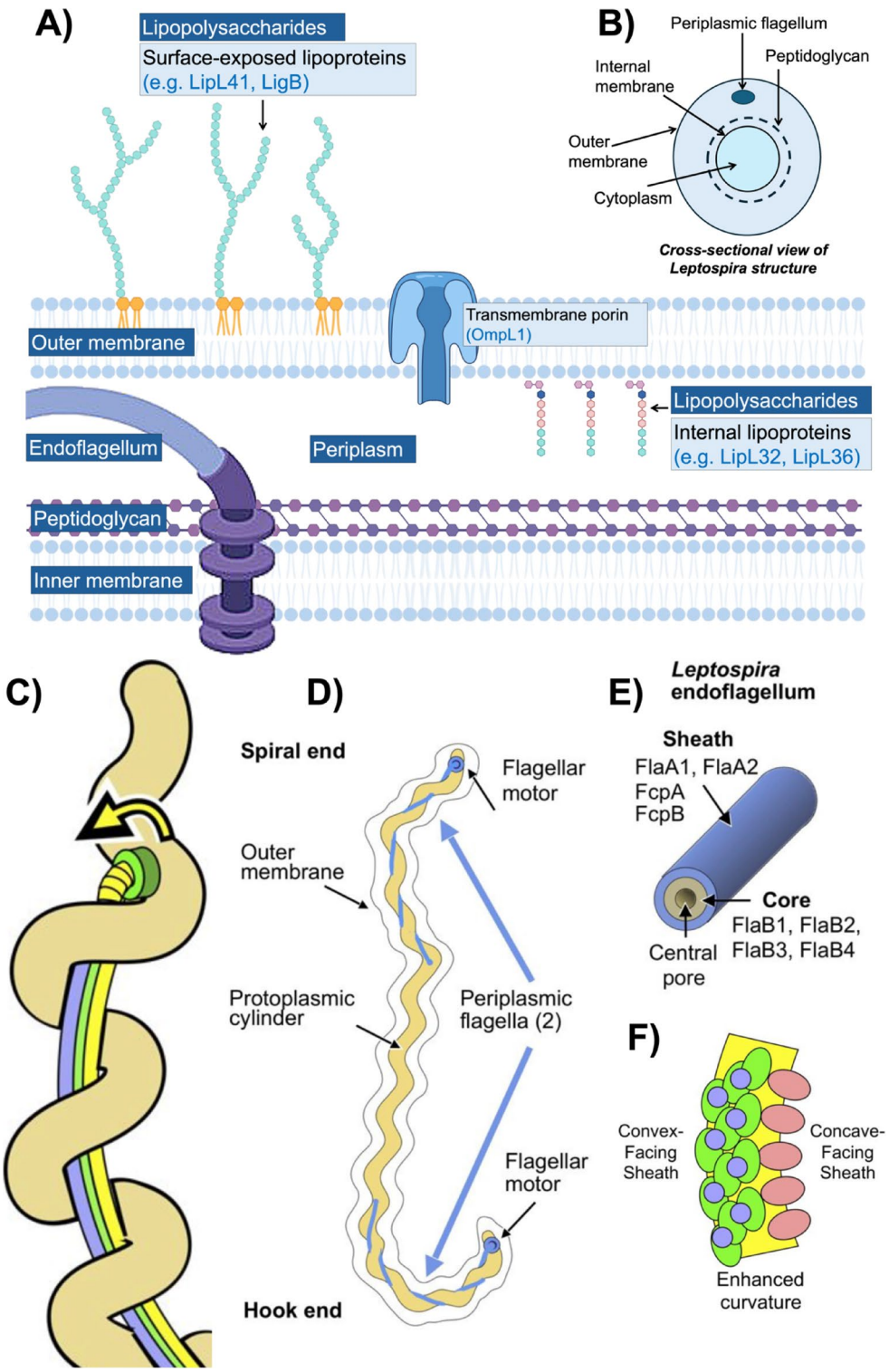

Fig. 3 **A** Schematic representation of the *Leptospira* cell envelope. The outer membrane contains surface-exposed and internal lipopolysaccharides (LPS), and the transmembrane porin OmpL1. Beneath it, the periplasm contains the endoflagellum, peptidoglycan layer. **B** Cross-sectional view showing the outer membrane, peptidoglycan layer, cytoplasm, and the distinctive periplasmic flagellum characteristic of *Leptospira*. **C** Schematic representation of the spiral *Leptospira* cell with the periplasmic endoflagella (green, purple, yellow) anchored to the cell pole and extending along the protoplasmic cylinder under the outer membrane. **D** Organisation of periplasmic endoflagella originating from flagellar motors located at both ends of the cell and spreading towards the centre of the cell, giving rise to the characteristic helix and hook morphology. **E** Molecular architecture of the endoflagellum, consisting of a core (FlaB1–FlaB4) surrounded by a sheath composed of the proteins FlaA1, FlaA2, FcpA, and FcpB, which together regulate the stability and function of the filament. **F** Model of the asymmetric organisation of the envelope, with convex and concave envelope proteins promoting curvature and helical cell shape (Fig. 3C–F were adapted from Gibson et al. [56] and used under the terms of the Creative Commons Attribution License (CC BY) license)

time of approximately 8–12 h [4], whereas many other species replicate more slowly under comparable conditions.

Growth and cultivation characteristics of *Leptospira* spp. also differ considerably based on their pathogenicity. Pathogenic *Leptospira* are more fastidious, with slower growth rates, and demonstrate limited ranges of temperature and pH tolerance [5], while saprophytic *Leptospira* grow more steadily and proliferate at lower temperatures [66]. Pathogenic *Leptospira* are susceptible to 8-azaguanine, whilst saprophytic species are resistant [67]. Taken together, these explain why screening and isolation of pathogenic species are more challenging.

Leptospira spp. can be cultivated using a range of liquid (broth), semi-solid (0.1–0.2% agar) [68], or fully solid media (1–1.2.2% agar) [69], which these physical forms are based on the type of media used, since some media are limited to certain forms only (e.g. only fully solid or only liquid and semi-solid). Liquid media are used for routine selective enrichment and maintenance, biomass production, metabolic studies, and preparation of antigens or nucleic acids. Semi-solid media [typically EMJH (Ellinghausen-McCullough-Johnson-Harris)] support primary isolation since motile leptospires form a characteristic subsurface Dinger's zone, enabling early detection from low inocula and separation from contaminants. Fully solid media [e.g. EMJH, *Leptospira* Vanaporn Wuthiekanun (LVW), and Hornsby-Alt-Nally (HAN) agars [47, 70, 71] enable visible colony formation that is essential for the selection of clonal isolates, genetic mutants, and antimicrobial susceptibility testing, as well as allow more rapid growth and long-term preservation.

Among these, the most widely used formulation is EMJH medium, supplied with bovine serum albumin or prepared in a protein-free form. EMJH medium is routinely used as a broth or as a semi-solid, for primary isolation and maintenance [72]. EMJH medium supplemented with selective antimicrobial agents has been reported to perform as well as or better than other commonly used media. Several comparative studies have justified that EMJH medium outperforms other media, including Gorman et al. [64] who found EMJH medium supplemented with antimicrobial agents outperforms other media in isolating and detecting *Leptospira* spp. from water and soil samples. Zarantonelli et al. [73] found that EMJH medium outperforms Fletcher's medium for isolating *Leptospira* in rodents. Steinparzer et al. mentioned that EMJH medium supplemented with STAFF antibiotics (sulfamethoxazole, trimethoprim, amphotericin, fosfomycin, and 5-fluorouracil) effectively enhances leptospiral isolation from swine urine. Since *Leptospira* spp. are intrinsically tolerant to 5-fluorouracil, this antibiotic is usually added to the culture media to prevent contamination and enhance the isolation of *Leptospira* spp [70]. Other than EMJH, there are alternative culture media: i) classical serum-rich formulations (e.g. Stuart, Korthof, Fletcher, and Noguchi media); and modern EMJH-derived formulations (e.g. EMJH-supplemented with pyruvate/serum, LVW, and HAN media) [70, 71, 74]. These media with improved modern EMJH-derived formulations have been developed to improve the isolation of fastidious or clinical strains. Therefore, the choice of media (considering the form and formulations of media) depends on the objectives of the laboratory procedure, the *Leptospira* species/serovars, and feasibility.

The nutritional requirements of *Leptospira* spp. are minimal, with long-chain fatty acids serving as the principal carbon and energy sources via β-oxidation, supplemented by vitamins B_1, B_{12} as growth factors, and ammonium salts as cellular nitrogen [75]. *Leptospira* spp. consistently exhibit catalase and oxidase activity, whereas urease production is restricted to only some species, and lipase activity is widely observed across the genus [71]. The cultures are usually preserved by repeated subculturing or storage in semi-solid agar with haemoglobin. Other than fully solid medium (LVW agar), liquid nitrogen is widely used for the long-term storage of *Leptospira* spp. because it preserves viability, motility, antigenicity, and virulence over extended periods [47].

Transmission Pathways of *Leptospira* Involving Animals and the Environment to Humans

Rodents play a central role as an important maintenance host of *Leptospira* spp. that can contaminate soil and surface water (Fig. 4) [1, 76, 77]. A wide range of domestic animals, such as dogs, cattle, pigs, goats, and horses, and diverse wild animals, such as bats, small mammals, primates, and ungulates, also serve as reservoirs or incidental hosts, contributing further to environmental contamination through urinary and faecal shedding [78]. Pathogenic *Leptospira* survive for a prolonged duration of weeks to months in warm, humid environments with neutral pH, and their persistence is enhanced by biofilm formation in water and soil [79, 80]. Human infection occurs predominantly through indirect exposure to contaminated water, mud, or soil during flooding, agricultural activities, or recreational freshwater contact, whilst direct exposure to infected animals remains important among high-risk occupational groups such as farmers, veterinarians, and sewage workers [77]. Humans are incidental hosts and rarely contribute to onward transmission, as person-to-person spread is extremely uncommon [81].

The most common entry points are cuts and abrasions on the skin, intact mucous membranes of the eyes, nose, or throat, and waterlogged skin. *Leptospira* spp. can also enter the body by inhaling aerosols containing *Leptospira* or through drinking water, especially in high-risk environments (e.g. contaminated water and animal facilities) [25, 82]. Once established in the renal tubules, particularly the proximal convoluted tubules, different animal hosts shed leptospires for markedly different durations. Rodents, especially rats, can shed large numbers of leptospires throughout their lifespan [83], whereas livestock such as cattle can shed organisms for months to years [84], and domestic pets, such as dogs, can shed intermittently even when clinically asymptomatic [85–87]. In the pathogenesis of leptospirosis, the first step is the penetration of tissue barriers to enter the human body [88, 89]. Environmental factors strongly

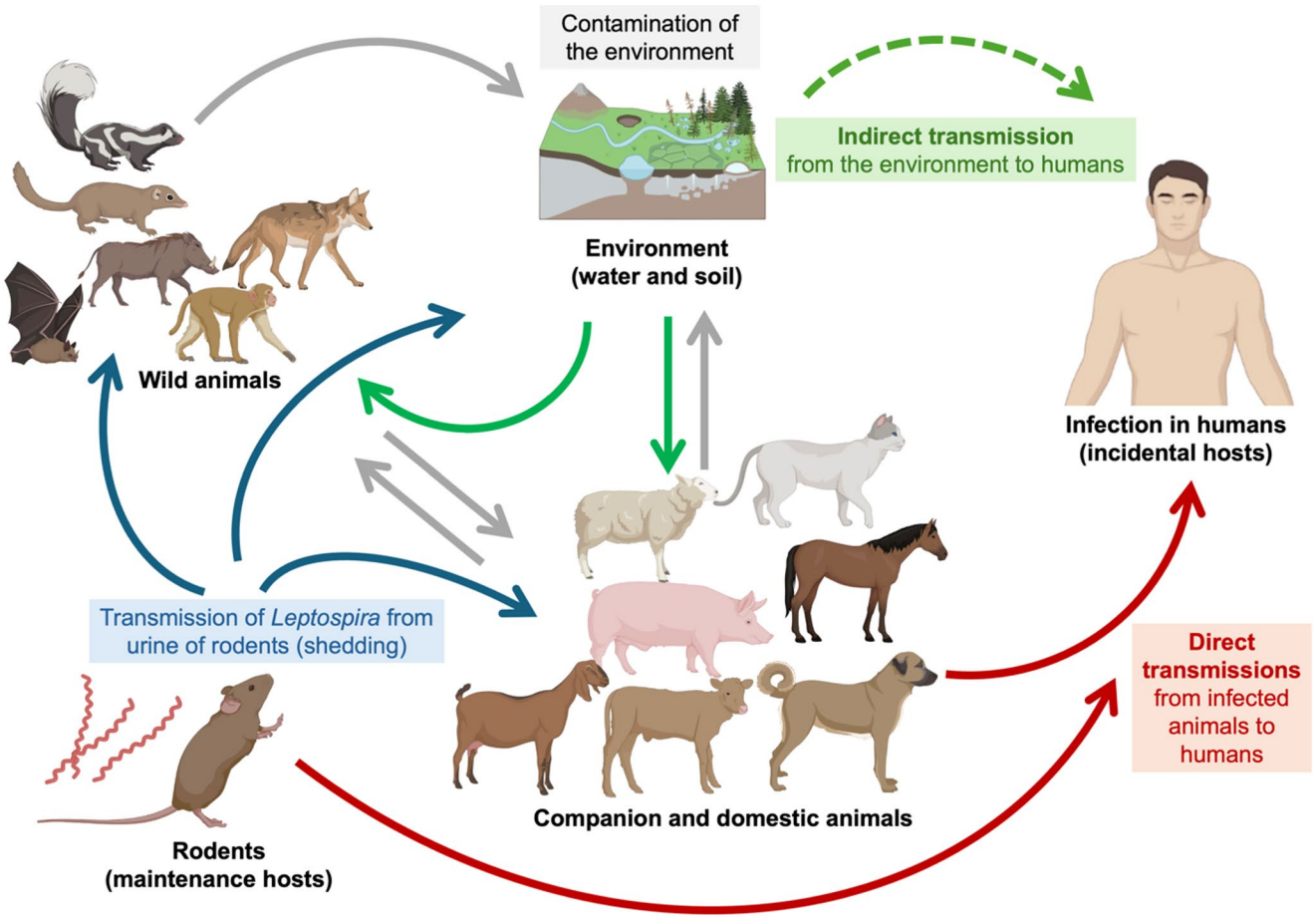

Fig. 4 *Leptospira* transmission occurs through a complex interplay of animal reservoirs, environmental persistence, and human exposure, with rodents serving as the primary maintenance hosts that chronically shed pathogenic *Leptospira* in urine and faeces, contaminating soil and surface water. Blue arrows indicate transmissions from rodents (the maintenance hosts) to other animals and the environment. Grey arrows indicate transmissions from animals to the environment, or bidirectional transmission between wild animals to domestic animals. Green arrows indicate environmental transmission to the animals. Dashed green arrows indicate indirect transmission from a contaminated environment to humans. Red arrows indicate direct transmissions from infected animals to humans

modulate transmission dynamics, such as heavy rainfall, flooding, and warm humid conditions, which enhance leptospiral survival in soil and water, facilitate their spread across wider areas, and amplify human exposure during outbreak periods [90]. Collectively, these ecological, host-related, and environmental factors interact to sustain the transmission cycle and drive leptospirosis emergence in endemic regions. The multi-host, multi-reservoir transmission system highlights the importance of a One Health approach integrating environmental management, rodent control, livestock vaccination, and improved sanitation to disrupt transmission pathways linking wildlife, domestic animals, contaminated environments, and human infection.

Clinical Manifestations of Leptospirosis

Leptospirosis is characterised by a broad spectrum of clinical manifestations that often mimic other infectious diseases (Table 5) [79]. In humans, it is usually misdiagnosed as influenza, aseptic meningitis, encephalitis, and dengue fever [14]. Jaundice (icterus) is a well-known symptom of leptospirosis that can also be observed in other liver-related diseases such as hepatitis [80]. The severity of leptospirosis is influenced by the size of the inoculum, strains of *Leptospira* spp., and the patient's health status and age [81]. One of the difficulties in eliminating the consequences of a *Leptospira* infection is the condition of the kidney carrier, which can last from months to years. Although uncommon, leptospirosis may progress to a fulminant and severe disease state, in which case mortality can range between 5 and 40% [91]. More than 90% of leptospirosis cases are mild and self-limiting, whilst multi-organ failure occurs in less than 10% of individuals [23].

As humans are a dead-end host for the spread of *Leptospira* spp., human-to-human transmission of leptospirosis is rare [84]. However, *Leptospira* spp. can be transmitted

Table 5 Clinical phases, symptoms, and key features of leptospirosis

Phase/form	Symptoms and clinical features	Notes
General/overall	- Broad spectrum, mimics influenza, dengue, aseptic meningitis, encephalitis - Jaundice (can mimic hepatitis) - Multi-organ failure (< 10% cases) - Mortality: 5–40% in severe cases	> 90% mild & self-limiting Mortality higher in fulminant cases
Acute phase (Septicaemic phase) (2–9 days)	- Sudden high fever - Headache - Chills - Myalgia (muscle pain) - Rash - Nausea, vomiting - Fatigue - Conjunctival suffusion (reddening without pus or discharged) - *Leptospira* in blood, cerebrospinal fluid, urine	Biphasic course (two distinct phase): fever → short afebrile phase → fever returns
Immune phase (Leptospiruric phase)		- Divided into anicteric and icteric forms - *Leptospira* appears in urine
a) Anicteric form (mild)	- Absence of jaundice - Severe headache - Neck stiffness (meningitis) - Uveitis (may occur months later)	- ~90% of cases - Usually self-limiting and resolve with proper treatment.
b) Icteric form (severe)	- Jaundice - Liver dysfunction - Renal insufficiency - Haemorrhagic episodes - Multi-organ failure (kidney, liver, lung, brain) - Severe pulmonary haemorrhage syndrome (> 50% mortality)	− 5–10% of cases - Weil's Disease - High risk of death (virulence linked to lipopolysaccharide hemolysins, outer membrane proteins of *Leptospira*)
Kidney involvement	- Non-oliguric nephropathy with potassium loss - Impaired sodium reabsorption - Oliguria if dehydrated → renal failure	Kidney is main target organ. Dialysis may be required
Liver involvement	- Jaundice (bilirubin increase) - Hepatocellular damage from intercellular junction disruption - Direct bilirubin ↑ (bile leakage) - Indirect bilirubin ↑ (haemolysis/breakdown of red blood cells)	Major organ target along with kidneys
Pregnancy-related	-Vertical transmission (breastfeeding, placenta invasion) - Miscarriage, fetal disorders	Human-to-human transmission is rare

vertically from infected mothers to susceptible infants through breastfeeding. Infection of pregnant women can cause various foetal disorders and even miscarriage through invasion of the placenta from the mother to the foetus [4]. On the other hand, infected livestock such as cattle, dogs and pigs are prone to stillbirths, miscarriages, mastitis and a reduced milk yield [85, 86]. Reproductive disorders in livestock represent a serious economic concern, with abortion-associated losses valued at USD 97 − 2,611 per case, and herd outbreaks capable of generating financial risks that may reach USD 150,000 per year [87].

Pregnant or lactating cows show a decrease in milk production as *Leptospira* spp. require a pregnant uterus and a lactating mammary gland to reproduce [71]. Leptospirosis typically has a biphasic course in humans, in which the first phase corresponds to the multiplication and spread

of *Leptospira* spp. in the body, while the second phase is characterised by the development of circulating antibodies and the shedding of *Leptospira* spp. in the urine [92]. The incubation period from exposure to the onset of symptoms is typically 7 to 12 days but can vary from 2 to 20 days [22].

a. Acute Phase (Septicaemic Phase)

The first phase of leptospirosis, known as the acute phase (septicaemic phase), typically lasts for about 3–7 days (Table 5). This phase is characterised by the appearance of *Leptospira* spp. in the bloodstream, in some cases, the cerebrospinal fluid (CSF), and is therefore also referred to as the leptospiraemic phase. Clinically, patients present with non-specific, flu-like symptoms, including sudden onset of high fever, headache, chills, myalgia, rash, nausea, vomiting, fatigue, and in many cases, conjunctival suffusion (reddening of the conjunctival vessels without purulent exudate) [6]. During this phase, *Leptospira* can be isolated from blood and CSF. Conjunctival suffusion is considered a particularly useful clinical clue because it is common in leptospirosis but uncommon in many other acute febrile illnesses [22]. The septicaemic phase is followed by a brief afebrile period (asymptomatic), after which fever returns, marking the onset of the second or "immune" phase of the disease. This classic biphasic pattern consists of the septicaemic phase of 3–7 days, followed by an immune phase characterised by the development of complications and recurrent fever [51].

b. Immune Phase (Leptospiruric Phase)

The second phase of leptospirosis is the immune phase (also known as the leptospiruric phase) in which *Leptospira* spp. can be isolated from the urine (Table 5). This phase can be divided into an anicteric and an icteric form. Clinically, most cases of leptospirosis (~ 90%) are anicteric and mild in nature, while the more severe icteric presentation, known as Weil's disease, is seen in approximately 5–10% of patients [93]. Accounting for approximately ~ 90% of cases, the anicteric form of leptospirosis is the predominant presentation, distinguished by the absence of jaundice and the possible occurrence of leptospiral meningitis [94]. It is also characterised by severe headaches and neck stiffness. Uveitis (inflammation inside the eyes) may develop at this time or two weeks to one year after the onset of the disease, with an average of six months [95].

Icteric leptospirosis, also known as Weil's syndrome, is the most severe manifestation of the *Leptospira* spp. infection. The symptoms can be triggered by the release of some virulent factors such as lipopolysaccharides, haemolysins, and outer membrane proteins [80]. It is usually characterised by jaundice, liver dysfunction, renal insufficiency, haemorrhagic episodes and multi-organ failure, including kidney, liver, lung, and brain, with a high risk of fatal outcome [6, 96]. Severe pulmonary haemorrhage syndrome (SPHS) leads to a mortality rate of over 50% and is th main cause of death [39, 97]. Leptospirosis leads to non-oliguric nephropathy with potassium loss, often characterised by impaired sodium reabsorption [98].

When inadequate oral intake leads to dehydration, patients are exposed to oliguria and renal failure, leading to death in areas where haemodialysis is not available. Pathogenic *Leptospira* primarily target the kidneys, especially the proximal convoluted tubules, where they can form biofilm-like aggregates that enable persistent colonisation and, in animals, lead to a chronic carrier state. Besides the kidney, another important target organ of leptospirosis is the liver. *Leptospira* spp. is known to preferentially attach in the perijunctional region between the hepatocytes. The disruption of the intercellular junctions between the hepatocytes and the damage to the liver cells lead to the leakage of bile. This leads to the higher levels of direct bilirubin seen in the icteric form. Sometimes the increase in indirect bilirubin levels can also occur in haemolysis caused by leptospirosis [22].

Research Gaps and Forward Directions

Despite substantial advances in our understanding of *Leptospira* spp., critical gaps remain in the effective management, diagnosis, and prevention of leptospirosis, particularly within veterinary and zoonotic contexts. Addressing these challenges requires a multidisciplinary, One Health approach. Key priorities include: (i) the expansion of *Leptospira* spp. diversity through discovery of new species and serovars highlights the need for more comprehensive taxonomic studies, particularly using state-of-the-art genomic approaches; (ii) In many countries, leptospirosis often goes unreported because the clinical symptoms are very similar to other communicable diseases [3]. Developing tools with sufficient sensitivity and specificity to detect and identify pathogenic *Leptospira* spp. strains in environmental samples are critical for risk assessment and targeting public health interventions. This includes multiplex PCR assays targeting clinically relevant species; (iii) Further studies are needed to fully discover the virulence mechanisms of *Leptospira* spp. and the specific role of different virulence factors, aligned with the evolving *Leptospira* genome, including genetic manipulation techniques such as CRISPRi which is powerful to study these mechanisms [99] (iv) Since leptospirosis is a neglected zoonotic disease, there is a need to improve diagnostic capabilities with consideration of rapid, sensitive and specific tests that can be readily deployed in marginal and resource-limited settings [3]. The use of machine learning algorithms, alongside traditional diagnostic methods,

is promising to improve diagnostic accuracy and identify high-risk populations; (v) Developing effective, broadly protective vaccines against leptospirosis remains a major challenge. Continued research on the immunology of *Leptospira* spp. and the development of new vaccine candidates are important. Research into the use of recombinant proteins and the development of DIVA strategies is essential to improve vaccine efficacy that does not cross-react with many diagnostic tests in vaccinated individuals; (vi) The combination of the lack of standardised diagnostic and harmonised reporting procedures further contributes to the difficulty in obtaining a reliable global estimate of leptospirosis mortality. The disparity in reported mortality rates underscores the urgent need for strengthened surveillance systems, including systematic monitoring of animal reservoirs and serogroup distribution at the regional level, alongside standardised case definitions, to generate more accurate and reliable estimates of the true global mortality burden of leptospirosis.

Conclusions

Leptospirosis is an example of the complexity and urgency of controlling emerging zoonotic diseases in a rapidly changing global health landscape. As this review highlights, advances in our understanding of *Leptospira* spp., from epidemiology and pathogenesis to diagnostics and genomics, have significantly deepened scientific insight, yet major challenges remain. Gaps in surveillance, diagnostic sensitivity, and vaccine development continue to hamper effective control, particularly in resource-poor settings where the disease burden is highest. A coordinated One Health approach is essential to reduce these gaps. Priorities include expanding genomic surveillance to capture the full diversity of *Leptospira* spp., improving the resolution and accessibility of diagnostic tools, deciphering virulence mechanisms with modern molecular tools, and developing next-generation vaccines that are both effective and compatible with surveillance systems. Equally important is the standardisation of reporting and case definitions to enable accurate estimates of the global burden. With climate change, urbanisation, and increasing human-animal interaction, the threat of leptospirosis and other zoonotic diseases will continue to emerge. Investing in multidisciplinary research, technology, and global collaboration is critical to anticipate, detect, and respond effectively to these evolving threats [100–117].

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00284-026-04722-7.

Acknowledgements Not applicable.

Authors' Contributions Lesley Maurice Bilung and Kasing Apun conceptualised the study. Ahmad Syatir Tahar, Chai Fung Pui, Muhammad Khairil Syamri Bakeri, and Rosdi Kira performed the literature search and interpreted relevant articles. Ahmad Syatir Tahar, Chai Fung Pui, and Muhammad Khairil Syamri Bakeri prepared the first manuscript draft. Lesley Maurice Bilung, Lela Su'ut, Romano Ngui, and Kasing Apun provided critical feedback. All authors read and approved the final manuscript.

Funding Open access funding provided by The Ministry of Higher Education Malaysia and Universiti Malaysia Sarawak. This work did not receive any funding.

Declarations

Conflict of interest All authors report no conflict of interest in this work.

Ethical Approval No applicable.

Consent To Participate No applicable.

Consent for Publication All authors agree to the publication of this work.

References

1. Pui CF, Bilung LM, Apun K, Su'ut L (2017) Diversity of *Leptospira* spp. in rats and environment from urban areas of Sarawak, Malaysia. J Trop Med 2017:3760674. https://doi.org/10.1155/2017/3760674
2. Costa F, Hagan JE, Calcagno J et al (2015) Global morbidity and mortality of leptospirosis: a systematic review. PLoS Negl Trop Dis 9:3898. https://doi.org/10.1371/journal.pntd.0003898
3. Pinto GV, Senthilkumar K, Rai P et al (2022) Current methods for the diagnosis of leptospirosis: issues and challenges. J Microbiol Methods 195:106438. https://doi.org/10.1016/j.mimet.2022.106438
4. Ningal SP, Kothule MB, Jadhav NY, Kadam SD (2015) A review on leptospirosis. World J Pharm Pharm Sci 4:1531–1543
5. Levett PN (2001) Leptospirosis. Clin Microbiol Rev 14:296–326
6. Dutta TK, Christopher M (2005) Leptospirosis—an overview. J Assoc Physicians India 53:545–551
7. Yaakob Y, Rodrigues KF, John DV (2015) Leptospirosis: recent incidents and available diagnostics– a review. Med J Malaysia 70:351–355

8. Adler B (2015) History of leptospirosis and leptospira. In: Adler B (ed) Current topics in microbiology and immunology. Springer, Heidelberg, pp 1–9
9. Bhat TS, Shah SAR, Nadeem M, Tak SF (2016) Pancytopenia: a rare presentation of weil's disease—case report. Int J Res Rev 3:96–98
10. Silva-Ramos CR, Matiz-González JM, Gil-Mora J et al (2024) Molecular characterization of *Leptospira* apecies among patients with acute undifferentiated febrile illness from the Municipality of Villeta, Colombia. Trop Med Infect Dis 9:168. https://doi.org/10.3390/tropicalmed9080168
11. Hovind-Hougen K (1979) Leptospiraceae, a new family to include leptospira Noguchi 1917 and leptonema gen. Nov. Int J Syst Bacteriol 29:245–251. https://doi.org/10.1099/00207713-29-3-245
12. Levett PN (2015) Systematics of leptospiraceae. In: Adler B (ed) Current Topics in Microbiology and Immunology. Springer, pp 11–20
13. Xiao D, Zhang C, Zhang H et al (2015) A novel approach for differentiating pathogenic and non-pathogenic *Leptospira* based on molecular fingerprinting. J Proteomics 119:1–9. https://doi.org/10.1016/j.jprot.2014.10.023
14. Wajjwalku W, Sukmak M, Amavisit P et al (2015) Molecular characterization of flab for leptospira identification. Southeast Asian J Trop Med Public Health 46:262–267
15. Benvin I, Paisie TK, Caetano Varanda I et al (2025) Whole genome characterization of leptospira kirschneri serogroup Pomona in croatia: insights into its diversity and evolutionary emergence. Pathogens 14:860. https://doi.org/10.3390/PATHOGENS14090860/S1
16. Fouts DE, Matthias MA, Adhikarla H et al (2016) What makes a bacterial species pathogenic?: comparative genomic analysis of the genus *Leptospira*. PLoS Negl Trop Dis 10:4403. https://doi.org/10.1371/journal.pntd.0004403
17. Chadsuthi S, Bicout DJ, Wiratsudakul A et al (2017) Investigation on predominant *Leptospira* serovars and its distribution in humans and livestock in Thailand, 2010–2015. PLoS Negl Trop Dis 11:5228. https://doi.org/10.1371/journal.pntd.0005228
18. Koizumi N, Morita M, Nuradji H et al (2022) Comparative genomic analysis of leptospira spp. Isolated from rattus norvegicus in Indonesia. Infect Genet Evol 102:105306. https://doi.org/10.1016/j.meegid.2022.105306
19. Bourhy P, Herrmann Storck C, Theodose R et al (2013) Serovar diversity of pathogenic *Leptospira* circulating in the French West Indies. PLoS Negl Trop Dis 7:2114. https://doi.org/10.1371/journal.pntd.0002114
20. Morey RE, Galloway RL, Bragg SL et al (2006) Species-specific identification of Leptospiraceae by 16S rRNA gene sequencing. J Clin Microbiol 44:3510–3516. https://doi.org/10.1128/JCM.00670-06
21. Wenderlein J, Zitzl T, Dufay-Simon N et al (2024) Detection and identification of pathogenic *Leptospira* spp. serogroups in Europe between 2017 and 2020 applying a novel gene-based molecular approach. Transbound Emerg Dis 2024:1101841. https://doi.org/10.1155/2024/1101841
22. Rajapakse S, Fernando N, Dreyfus A et al (2025) Leptospirosis. Nat Rev Dis Primers. https://doi.org/10.1038/s41572-025-00614-5. 11:
23. Gomes-Solecki M, Santecchia I, Werts C (2017) Animal models of leptospirosis: of mice and hamsters. Front Immunol 8:58. https://doi.org/10.3389/FIMMU.2017.00058
24. Matthias MA, Ricaldi JN, Cespedes M et al (2008) Human leptospirosis caused by a new, antigenically unique *Leptospira* associated with a *Rattus* species reservoir in the Peruvian Amazon. PLoS Negl Trop Dis 2:e213. https://doi.org/10.1371/JOURNAL.PNTD.0000213
25. Moreno LZ, Miraglia F, Loureiro AP et al (2018) Genomic characterisation of *Leptospira inadai* serogroup Lyme isolated from captured rat in Brazil and comparative analysis with human reference strain. Mem Inst Oswaldo Cruz 113:170444. https://doi.org/10.1590/0074-02760170444
26. Vincent AT, Schiettekatte O, Goarant C et al (2019) Revisiting the taxonomy and evolution of pathogenicity of the genus *Leptospira* through the prism of genomics. PLoS Negl Trop Dis 13:7270. https://doi.org/10.1371/journal.pntd.0007270
27. Hamond C, Tibbs-Cortes B, Fernandes LGV et al (2025) Leptospira gorisiae sp. nov, L. cinconiae sp. nov, L. mgodei sp. nov, L. milleri sp. nov and L. iowaensis sp. nov: five new species isolated from water sources in the Midwestern united States. Int J Syst Evol Microbiol 75:006595. https://doi.org/10.1099/IJSEM.0.006595/CITE/REFWORKS
28. Ondov BD, Treangen TJ, Melsted P et al (2016) Mash: fast genome and metagenome distance Estimation using MinHash. Genome Biol 17:132. https://doi.org/10.1186/S13059-016-0997-X/FIGURES/5
29. Lefort V, Desper R, Gascuel O (2015) FastME 2.0: a comprehensive, accurate, and fast distance-based phylogeny inference program. Mol Biol Evol 32:2798–2800. https://doi.org/10.1093/MOLBEV/MSV150
30. Chaemchuen S, Rungpragayphan S, Poovorawan Y, Patarakul K (2011) Identification of candidate host proteins that interact with LipL32, the major outer membrane protein of pathogenic Leptospira, by random phage display peptide library. Vet Microbiol 153:178–185. https://doi.org/10.1016/j.vetmic.2011.04.030
31. Di Azevedo MIN, Kremer F, Ezepha C et al (2024) Comparative genomics of leptospira Santarosai reveals genomic adaptations in bovine genital strains. Front Microbiol 15:1517151. https://doi.org/10.3389/fmicb.2024.1517151
32. Lehmann JS, Corey VC, Ricaldi JN et al (2016) Whole genome shotgun sequencing shows selection on leptospira regulatory proteins during in vitro culture attenuation. Am J Trop Med Hyg 94:302–313. https://doi.org/10.4269/ajtmh.15-0401
33. Picardeau M, Bulach DM, Bouchier C et al (2008) Genome sequence of the saprophyte Leptospira biflexa provides insights into the evolution of Leptospira and the pathogenesis of leptospirosis. PLoS One 3:1607. https://doi.org/10.1371/journal.pone.0001607
34. Kochi LT, Fernandes LGV, Nascimento ALTO (2020) Heterologous expression of the pathogen-specific LIC11711 gene in the saprophyte *L. biflexa* increases bacterial binding to laminin and plasminogen. Pathogens 9:1–16. https://doi.org/10.3390/pathogens9080599
35. Iraola G, Spangenberg L, Lopes Bastos B et al (2016) Transcriptome sequencing reveals wide expression reprogramming of basal and unknown genes in leptospira biflexa biofilms. mSphere 1:16–42. https://doi.org/10.1128/msphere.00042-16
36. Zhu W, Wang J, Zhu Y et al (2015) Identification of three extrachromosomal replicons in Leptospira pathogenic strain and development of new shuttle vectors. BMC Genomics 16:90. https://doi.org/10.1186/s12864-015-1321-y
37. Ko AI, Goarant C, Picardeau M (2009) *Leptospira*: the dawn of the molecular genetics era for an emerging zoonotic pathogen. Nat Rev Microbiol 7:736–747. https://doi.org/10.1038/nrmicro2208
38. Huete SG, Benaroudj N (2023) The arsenal of Leptospira species against oxidants. Antioxidants 12
39. Murray CK, Hospenthal DR (2004) Determination of susceptibilities of 26 leptospira sp. serovars to 24 antimicrobial agents by a broth microdilution technique. Antimicrob Agents Chemother 48:4002–4005. https://doi.org/10.1128/AAC.48.10.4002-4005.2004

40. Giraud-Gatineau A, Nieves C, Harrison LB et al (2024) Evolutionary insights into the emergence of virulent Leptospira spirochetes. PLoS Pathog 20:1012161. https://doi.org/10.1371/journal.ppat.1012161
41. Dellagostin OA, Grassmann AA, Rizzi C et al (2017) Reverse vaccinology: an approach for identifying leptospiral vaccine candidates. Int J Mol Sci 18:158. https://doi.org/10.3390/ijms18010158
42. Fukunaga M, Mifughi I (1988) Mechanism of streptomycin resistance in *leptospira biflexa* Dtrain Urawa. Microbiol Immunol 32:641–644. https://doi.org/10.1111/j.1348-0421.1988.tb01425.x
43. Prescott JF, Nicholson VM (1988) Antimicrobial drug susceptibility of *leptospira interrogans* serovar Hardjo isolated from cattle. Can J Vet Res 52:286–287
44. Chakraborty A, Miyahara S, Villanueva SYAM et al (2010) In vitro sensitivity and resistance of 46 *Leptospira* strains isolated from rats in the Philippines to 14 antimicrobial agents. Antimicrob Agents Chemother 54:5403–5405. https://doi.org/10.1128/AAC.00973-10
45. Miraglia F, Matsuo M, Morais ZM et al (2013) Molecular characterization, serotyping, and antibiotic susceptibility profile of *Leptospira interrogans* serovar Copenhageni isolates from Brazil. Diagn Microbiol Infect Dis 77:195–199. https://doi.org/10.1016/j.diagmicrobio.2013.08.003
46. Moreno LZ, Miraglia F, Lilenbaum W et al (2019) Profiling of leptospira interrogans, L. santarosai, L. meyeri and L. borgpetersenii by SE-AFLP, PFGE and susceptibility testing—a continuous attempt at species and serovar differentiation. Emerg Microbes Infect 5:1–7
47. Wuthiekanun V, Amornchai P, Paris DH et al (2013) Rapid isolation and susceptibility testing of *Leptospira* spp. using a new solid medium, LVW agar. Antimicrob Agents Chemother 57:297–302. https://doi.org/10.1128/AAC.01812-12
48. Santos JC, Handa S, Fernandes LGV et al (2023) Structural and biochemical characterization of *Leptospira interrogans* Lsa45 reveals a penicillin-binding protein with esterase activity. Process Biochem 125:141–153. https://doi.org/10.1016/j.procbio.2022.12.010
49. Pineda S, Martínez Garro JM, Salazar Flórez JE et al (2024) Detection of genes related to antibiotic resistance in *Leptospira*. Trop Med Infect Dis 9:203. https://doi.org/10.3390/tropicalmed9090203
50. Liegeon G, Delory T, Picardeau M (2018) Antibiotic susceptibilities of livestock isolates of leptospira. Int J Antimicrob Agents 51:693–699. https://doi.org/10.1016/j.ijantimicag.2017.12.024
51. Petakh P, Kamyshnyi O (2024) AMR mechanisms in L. interrogans serovars: a comprehensive study. Frontiers in Cellular and Infection Microbiology 14:1384427. https://doi.org/10.3389/fcimb.2024.1384427
52. Benacer D, Mohd Zain SN, Ooi PT, Thong KL (2017) Antimicrobial susceptibility of *leptospira* spp. isolated from environmental, human and animal sources in Malaysia. Indian J Med Microbiol 35:124–128. https://doi.org/10.4103/ijmm.IJMM_15_458
53. Suepaul SM, Carrington C, Campbell M et al (2015) Antimicrobial susceptibility of leptospira isolates from dogs and rats to 12 antimicrobial agents. Trop Biomed 32:1–10
54. Petakh P, Oksenych V, Kamyshnyi O (2024) Corticosteroid treatment for leptospirosis: a systematic review and meta-analysis. J Clin Med 13:4310. https://doi.org/10.3390/jcm13154310
55. Werts C, Tapping RI, Mathison JC et al (2001) *Leptospiral* lipopolysaccharide activates cells through a TLR2-dependent mechanism. Nat Immunol 2(4):346–352. https://doi.org/10.1038/86354
56. Gibson KH, Trajtenberg F, Wunder EA et al (2020) An asymmetric sheath controls flagellar supercoiling and motility in the leptospira spirochete. Elife 9:53672. https://doi.org/10.7554/eLife.53672
57. Nair N, Guedes MS, Hajjar AM et al (2021) Role of TLR4 in persistent leptospira interrogans infection: A comparative in vivo study in mice. Front Immunol 11:572999. https://doi.org/10.3389/FIMMU.2020.572999/BIBTEX
58. Takahashi MB, Teixeira AF, Nascimento ALTO (2022) Host cell binding mediated by *leptospira interrogans* adhesins. Int J Mol Sci 23:15550. https://doi.org/10.3390/IJMS232415550/S1
59. Souza NM, Vieira ML, Alves IJ et al (2012) Lsa30, a novel adhesin of *leptospira interrogans* binds human plasminogen and the complement regulator C4bp. Microb Pathog 53:125–134. https://doi.org/10.1016/J.MICPATH.2012.06.001
60. Stevenson B, Choy HA, Pinne M et al (2007) *Leptospira interrogans* endostatin-like outer membrane proteins bind host fibronectin, laminin and regulators of complement. PLoS One. https://doi.org/10.1371/JOURNAL.PONE.0001188
61. Lin YP, McDonough SP, Sharma Y, Chang YF (2011) *Leptospira* immunoglobulin-like protein B (LigB) binding to the C-terminal fibrinogen αC domain inhibits fibrin clot formation, platelet adhesion and aggregation. Mol Microbiol 79:1063–1076. https://doi.org/10.1111/J.1365-2958.2010.07510.X
62. Pinne M, Haake DA (2013) LipL32 is a subsurface lipoprotein of *leptospira interrogans*: presentation of new data and reevaluation of previous studies. PLoS One 8:e51025. https://doi.org/10.1371/JOURNAL.PONE.0051025
63. Ratet G, Santecchia I, Fanton d'Andon M et al (2017) LipL21 lipoprotein binding to peptidoglycan enables *leptospira interrogans* to escape NOD1 and NOD2 recognition. PLoS Pathog 13:e1006725. https://doi.org/10.1371/JOURNAL.PPAT.1006725
64. Evangelista KV, Coburn J (2010) *Leptospira* as an emerging pathogen: a review of its biology, pathogenesis and host immune responses. Future Microbiol 5:1413–1425. https://doi.org/10.2217/fmb.10.102
65. Fraga TR, Isaac L, Barbosa AS (2016) Complement evasion by pathogenic *Leptospira*. Front Immunol 7:623. https://doi.org/10.3389/fimmu.2016.00623
66. Tahara H, Takabe K, Sasaki Y et al (2018) The mechanism of two-phase motility in the spirochete Leptospira: Swimming and crawling. Sci Adv 4. https://doi.org/10.1126/SCIADV.AAR7975/
67. Nakamura S (2022) Motility of the zoonotic spirochete *Leptospira*: insight into association with pathogenicity. Int J Mol Sci 23(3):1859. https://doi.org/10.3390/IJMS23031859
68. Bergamo P, Le Guyader M, Hugonnard M et al (2024) Isolation of virulent Leptospira serogroup Australis field strains from symptomatic dogs for canine leptospiral vaccine development. Microorganisms 12(1946). https://doi.org/10.3390/MICROORGANISMS12101946/S1
69. Meny P, Menéndez C, Quintero J et al (2017) Characterization of *Leptospira* isolates from humans and the environment in Uruguay. Rev Inst Med Trop Sao Paulo 59:e79. https://doi.org/10.1590/S1678-9946201759079
70. Hornsby RL, Alt DP, Nally JE (2020) Isolation and propagation of leptospires at 37°C directly from the mammalian host. Sci Rep 10(1):9620. https://doi.org/10.1038/s41598-020-66526-4
71. Guedes IB, de Souza GO, de Castro JF P, et al (2022) Improvement of the enrichment used in the EMJH medium (Ellinghausen–McCullough–Johnson–Harris) for the cultivation of leptospira spp. Rev Argent Microbiol 54:95–99. https://doi.org/10.1016/J.RAM.2021.03.002
72. Johnson RC, Harris VG (1967) Differentiation of pathogenic and saprophytic Leptospires I. growth at low temperatures. J Bacteriol 94:27. https://doi.org/10.1128/JB.94.1.27-31.1967
73. Johson RC, Rogers P (1964) Differentiation of pathogenic Dan saprophytic leptospires with 8-azaguanine. J Bacteriol 88:1618. https://doi.org/10.1128/JB.88.6.1618-1623.1964
74. Wuthiekanun V, Amornchai P, Langla S et al (2015) Antimicrobial disk susceptibility testing of leptospira spp. Using leptospira

Vanaporn Wuthiekanun (LVW) agar. Am J Trop Med Hyg 93:241–243. https://doi.org/10.4269/AJTMH.15-0180/-/DC2/SD2.PDF
75. Thibeaux R, Girault D, Bierque E et al (2018) Biodiversity of environmental leptospira: improving identification and revisiting the diagnosis. Front Microbiol 9:360960. https://doi.org/10.3389/FMICB.2018.00816/BIBTEX
76. Gorman M, Xu R, Prakoso D et al (2022) *Leptospira* enrichment culture followed by ONT metagenomic sequencing allows better detection of *Leptospira* presence and diversity in water and soil samples. PLoS Negl Trop Dis 16:10589. https://doi.org/10.1371/journal.pntd.0010589
77. Zarantonelli L, Suanes A, Meny P et al (2018) Isolation of pathogenic *Leptospira* strains from naturally infected cattle in Uruguay reveals high serovar diversity, and uncovers a relevant risk for human leptospirosis. PLoS Negl Trop Dis 12:e0006694. https://doi.org/10.1371/JOURNAL.PNTD.0006694
78. Bierque E, Thibeaux R, Girault D et al (2020) A systematic review of leptospira in water and soil environments. PLoS ONE 15:1–22. https://doi.org/10.1371/journal.pone.0227055
79. Bilung LM, Pui CF, Tahar AS et al (2018) Occurrence of *Leptospira* species from rodents, soil and water from an oil palm plantation in Northern Sarawak. Asian J Anim Vet Adv 13:332–338. https://doi.org/10.3923/ajava.2018.332.338
80. Bradley EA, Lockaby G (2023) Leptospirosis and the environment: a review and future directions. Pathogens 12:1167. https://doi.org/10.3390/pathogens12091167
81. Apun K, Jalan J, Chai F et al (2018) Malaysian journal of microbiology biofilm forming ability of intermediate and saprophytic leptospira on abiotic and biotic surfaces. Malays J Microbiol 14:313–319. https://doi.org/10.21161/mjm.144183
82. Shruthi G, Balamurugan V, Prasad S et al (2017) Fatty acid metabolism in leptospira a key to its pathogenicity and evasion from host immune response leading to prolonged survival of the organism. Indian J Nat Sci 7:11939–11945
83. Stollberg K, Richter M, Windahl U et al (2025) Human-to-human transmission of leptospirosis: A global systematic review. medRxiv. https://doi.org/10.1101/2025.09.02.25334635
84. Sunil S, Jacob J, Varghese B (2016) Human leptospirosis—a review. World J Pharm Res 5:613–624
85. Boey K, Shiokawa K, Rajeev S (2019) Leptospira infection in rats: a literature review of global prevalence and distribution. PLoS Negl Trop Dis 13:e0007499. https://doi.org/10.1371/JOURNAL.PNTD.0007499
86. Hamond C, LeCount K, Putz EJ et al (2022) Bovine leptospirosis due to persistent renal carriage of leptospira borgpetersenii serovar Tarassovi. Front Vet Sci 9:848664. https://doi.org/10.3389/FVETS.2022.848664/BIBTEX
87. Miotto BA, Guilloux AGA, Tozzi BF et al (2018) Prospective study of canine leptospirosis in shelter and stray dog populations: identification of chronic carriers and different *Leptospira* species infecting dogs. PLoS One 13:e0200384. https://doi.org/10.1371/JOURNAL.PONE.0200384
88. Fentahun T, Alemayehu M (2012) Leptospirosis and its public health significance: A review. Eur J Appl Sci 4:238–244. https://doi.org/10.5829/idosi.ejas.2012.4.6.66162
89. Philip N, Garba B, Neela VK (2018) Long-term preservation of *leptospira* spp.: challenges and prospects. Appl Microbiol Biotechnol 102:5427–5435. https://doi.org/10.1007/s00253-018-9047-9
90. Boey K, Shiokawa K, Rajeev S (2019) Leptospira infection in rats: a literature review of global prevalence and distribution. PLoS Negl Trop Dis 13:7499. https://doi.org/10.1371/journal.pntd.0007499
91. Ristow P, Bourhy P, Kerneis S et al (2008) Biofilm formation by saprophytic and pathogenic leptospires. Microbiology 154:1309–1317. https://doi.org/10.1099/MIC.0.2007/014746-0
92. Rohilla P, Khurana R, Kumar A et al (2020) Detection of *Leptospira* in urine of apparently healthy dogs by quantitative polymerase chain reaction in Haryana, India. Vet World 13:2411. https://doi.org/10.14202/VETWORLD.2020.2411-2415
93. Zaidi S, Bouam A, Bessas A et al (2018) Urinary shedding of pathogenic *Leptospira* in stray dogs and cats, Algiers: a prospective study. PLoS One 13:e0197068. https://doi.org/10.1371/JOURNAL.PONE.0197068
94. Sykes JE, Reagan KL, Nally JE et al (2022) Role of diagnostics in Epidemiology, Management, Surveillance, and control of leptospirosis. Pathogens 11:395. https://doi.org/10.3390/PATHOGENS11040395/S1
95. Souza GM, Nascimento H, Belfort R (2025) Ocular leptospirosis: Report of a challenging 38 diagnosis. Ocul Immunol Inflamm 33:214–217.
96. Vries SG, Visser BJ, Nagel IM et al (2014) Leptospirosis in Sub-Saharan africa: A systemic review. Int J Infect Dis 28:47–64
97. Khaki P (2016) Clinical laboratory diagnosis of human leptospirosis. Int J Enteric Pathog 4:31859. https://doi.org/10.17795/ijep31859
98. Oliveira MAA, Leal ÉA, Correia MA et al (2017) Human leptospirosis: occurrence of serovars of *Leptospira* spp. in the state of Minas Gerais, Brazil, from 2008 to 2012. Braz J Microbiol 48:483–488. https://doi.org/10.1016/j.bjm.2016.12.010
99. Lane AB, Dore MM (2016) Leptospirosis: a clinical review of evidence based diagnosis, treatment and prevention. World Journal of Clinical Infectious Diseases 6:61. https://doi.org/10.5495/wjcid.v6.i4.61
100. Cilia G, Bertelloni F, Albini S, Fratini F (2021) Insight into the epidemiology of leptospirosis: a review of leptospira isolations from unconventional hosts. Animals 11:1–16. https://doi.org/10.3390/ani11010191
101. Lehmann JS, Matthias MA, Vinetz JM, Fouts DE (2014) Leptospiral pathogenomics. Pathogens 3:280–308. https://doi.org/10.3390/pathogens3020280
102. Fernandes LGV, Stone NE, Roe CC et al (2022) *Leptospira sanjuanensis* sp. nov., a pathogenic species of the genus *Leptospira* isolated from soil in Puerto Rico. Int J Syst Evol Microbiol 72:5560. https://doi.org/10.1099/IJSEM.0.005560
103. Gorman M, Xu R, Prakoso D et al (2022) *Leptospira* enrichment culture followed by ONT metagenomic sequencing allows better detection of *Leptospira* presence and diversity in water and soil samples. PLoS Negl Trop Dis 16:e0010589. https://doi.org/10.1371/JOURNAL.PNTD.0010589
104. Barragan V, Nieto N, Keim P, Pearson T (2017) Meta-analysis to estimate the load of *Leptospira* excreted in urine: beyond rats as important sources of transmission in low-income rural communities. BMC Res Notes 10:71. https://doi.org/10.1186/s13104-017-2384-4
105. Cosate MRV, Sakamoto T, Mendes TA, de O et al (2017) Molecular typing of leptospira interrogans serovar Hardjo isolates from leptospirosis outbreaks in Brazilian livestock. BMC Vet Res 13:177. https://doi.org/10.1186/s12917-017-1081-9
106. Aymée L, Mendes J, Lilenbaum W (2024) Bovine genital leptospirosis: an update of this important reproductive disease. Animals 14:322. https://doi.org/10.3390/ani14020322
107. Maruoka T, Nikaido Y, Miyahara S et al (2021) Correlation between renal distribution of leptospires during the acute phase and chronic renal dysfunction in a hamster model of infection with *leptospira interrogans*. PLoS Negl Trop Dis 15:9410. https://doi.org/10.1371/journal.pntd.0009410
108. McElroy J, Celik B, Ammakola Y et al (2024) Leptospirosis causing severe acute kidney injury, hyperbilirubinemia, and

thrombocytopenia: a case report. Cureus 16:74854. https://doi.org/10.7759/cureus.74854
109. Abdelrahim NA, Fadl-Elmula IM, Hartskeerl RA et al (2021) Are pathogenic *Leptospira* a possible cause of aseptic meningitis in suspected children in Sudan? Res Rep Trop Med 12:267–274. https://doi.org/10.2147/RRTM.S339058
110. Souza GM, Nascimento H, Belfort R (2025) Ocular leptospirosis: report of a challenging diagnosis. Ocul Immunol Inflamm 33:214–217. https://doi.org/10.1080/09273948.2024.2367651
111. Charan J, Saxena D, Mulla S, Yadav P (2013) Antibiotics for the treatment of leptospirosis: systematic review and meta-analysis of controlled trials. Int J Prev Med 4:501–510
112. Hoffmeister B, Peyerl-Hoffmann G, Pischke S et al (2010) Differences in clinical manifestations of imported versus autochthonous leptospirosis in Austria and Germany. Am J Trop Med Hyg 83:326–335. https://doi.org/10.4269/ajtmh.2010.10-0040
113. Herman HS, Mehta S, Cárdenas WB et al (2016) Micronutrients and leptospirosis: a review of the current evidence. PLoS Negl Trop Dis 10:4652. https://doi.org/10.1371/journal.pntd.0004652
114. Haake DA, Levett PN (2015) Leptospirosis in humans. Curr Top Microbiol Immunol 387:65. https://doi.org/10.1007/978-3-662-45059-8_5
115. Fernandes LGV, Hornsby RL, Nascimento ALTO, Nally JE (2021) Genetic manipulation of pathogenic *Leptospira*: CRISPR interference (CRISPRi)-mediated gene silencing and rapid mutant recovery at 37°C. Sci Rep 11:1768. https://doi.org/10.1038/s41598-021-81400-7
116. Galdino GS, de Sandes-Freitas TV, de Andrade LGM et al (2023) Development and validation of a simple machine learning tool to predict mortality in leptospirosis. Sci Rep 13:4506. https://doi.org/10.1038/s41598-023-31707-4
117. Fernandes LGV, Nascimento ALTO (2022) A novel breakthrough in *Leptospira* spp. mutagenesis: knockout by combination of CRISPR/Cas9 and non-homologous end-joining systems. Front Microbiol 13:915382. https://doi.org/10.3389/fmicb.2022.915382