Communication

Exploring Leptospira Interrogans FDAARGOS_203: Insights into AMR and Anti-phage Defense

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Exploring *Leptospira interrogans* FDAARGOS_203: Insights into AMR and Anti-phage Defense

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Abstract: Leptospira, which are known to be important disease-causing agents transmitted between animals and humans, results in significant illness and, in some cases, significant death in human populations. This study aims to examine the genomic structure of *Leptospira interrogans* serovar Copenhageni strain FDAARGOS_203 to identify the specific genetic factors that contribute to antimicrobial resistance (AMR) and defense against phages. The genome, consisting of two contigs totaling 4,630,574 base pairs, underwent thorough examination for protein-coding sequences, transfer RNA genes, and ribosomal RNA genes. A total of twenty-two antibiotic resistance genes that specifically target essential cellular processes such as cell wall synthesis, DNA replication, and protein synthesis have been identified. Significant among these were gidB, GdpD, and PgsA, each involved in separate aspects of antibiotic resistance. In addition, the investigation explored the defense mechanisms of bacteriophages, revealing the presence of defense islands that contain a range of anti-phage systems, including RM_Type_IV, PrrC, Borvo, CAS_Class1-Subtype-IC, and CAS_Class1-Subtype-IB. This comprehensive genomic analysis enhances our understanding of the molecular mechanisms that determine Leptospira's ability to adapt to various environments. The identified genetic factors linked to AMR and defense against phages not only enhance our scientific comprehension but also provide a basis for focused interventions to reduce the impact of leptospirosis.

Keywords: leptophages; Cas system; bacteriophages; Leptospira interorgans; antibiotic

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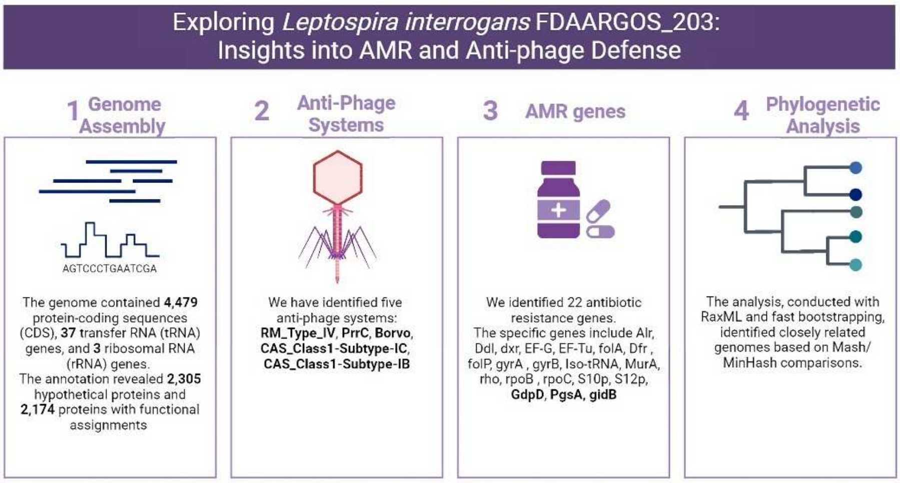
Graphical Abstract



The genome contained **4,479** protein-coding sequences (CDS), **37** transfer RNA (tRNA) genes, and **3** ribosomal RNA (rRNA) genes.
The annotation revealed **2,305** hypothetical proteins and **2,174** proteins with functional assignments

We have identified five anti-phage systems: **RM_Type_IV**, **PrrC**, **Borvo**, **CAS_Class1-Subtype-IC**, **CAS_Class1-Subtype-IB**

We identified 22 antibiotic resistance genes.
The specific genes include Alr, Ddl, dxr, EF-G, EF-Tu, folA, Dfr , folP, gyrA , gyrB, Iso-tRNA, MurA, rho, rpoB , rpoC, S10p, S12p, **GdpD**, **PgsA**, **gidB**

The analysis, conducted with RaxML and fast bootstrapping, identified closely related genomes based on Mash/MinHash comparisons.

1. Introduction

Leptospira, major agents of zoonotic disease, cause considerable morbidity and, in some instances, significant mortality in humans [1–6]. The genus *Leptospira* comprises over 20 species based on DNA relatedness, with more than 350 serovars identified based on surface agglutinating lipopolysaccharide antigens [7]. These species are broadly categorized into three groups. Saprophytic species like *Leptospira biflexa* are not associated with disease. Pathogenic species such as *Leptospira interrogans* and *Leptospira borgpetersenii* cause leptospirosis globally, ranging from mild or asymptomatic infection to severe forms resulting in multiple organ failure and death. An intermediate group, including *Leptospira fainei* and *Leptospira licerasiae*, may be associated with infection and mild disease.

Despite the clinical significance of leptospirosis, there is a notable lack of comprehensive data regarding the protective mechanisms employed by leptospires against antibiotics and phages. *Leptospira* spp. exhibit intrinsic resistance to various antimicrobial agents, though the specific mechanisms responsible remain unidentified [8,9]. Nevertheless, resistance to sulfonamides, neomycin, actidione, polymyxin, nalidixic acid, vancomycin, and rifampicin has facilitated the development of selective media for isolating leptospires [10].

Current recommendations for treating human leptospirosis involve penicillin, ampicillin, ceftriaxone, or cefotaxime [1,11]. Alternatives, particularly for those with allergies or in non-hospital settings, include oral doxycycline or azithromycin. In veterinary settings, a penicillin-streptomycin combination is the preferred therapy for acute leptospirosis, although ampicillin, amoxicillin, tetracyclines, tulathromycin, and third-generation cephalosporins have also been utilized [12]. Tilmicosin presents an additional alternative [13].

Renewed interest in bacteriophages as alternatives to antibiotics and their role in bacterial evolution has emerged, yet little is known about phage diversity within the *Leptospira* genus [14,15].

Saint Girons et al. first isolated bacteriophages from *Leptospira* species in 1990, but their exploration remains limited [16]. Schiettekatte et al. demonstrated that leptophages utilize lipopolysaccharides (LPS) as receptors on bacterial cells [15]. Bacteria engage in a continuous arms race, evolving defence mechanisms against the expanding arsenal of phage weapons [17]. These defence systems, discovered in recent years, protect against phage through various molecular mechanisms. Anti-phage defence systems exhibit a non-random distribution in microbial genomes, often forming "defence islands" where multiple systems cluster together [18–20].

The strain FDAARGOS_203, being a reference strain, provides a unique opportunity to explore the genetic basis of antibiotic and phage resistance in *Leptospira interrogans*. Through a comprehensive examination of the genome, we aim to contribute valuable insights into the genetic factors governing AMR and anti-phage defence, enhancing our understanding of leptospirosis and paving the way for more effective therapeutic interventions.

2. Materials and Methods

2.1. Data

The genome of *Leptospira interrogans* serovar Copenhageni strain FDAARGOS_203 was downloaded in FASTA format files from the Bacterial and Viral Bioinformatics Resource Center (BV-BRC) database (GenBank: GCA_002073495.2) [21]. Leptospiral genome was annotated using RAST tool kit (RASTtk) [22].

2.2. Detection of AMR Genes

The genomes were then analyzed using the PATRIC tool from the BV-BRC to identify antimicrobial resistance genes [23]. The Genome Annotation Service in PATRIC uses k-mer-based AMR genes detection method, which utilizes PATRIC’s curated collection of representative AMR gene sequence variants, and assigns to each AMR gene functional annotation, broad mechanism of antibiotic resistance.

2.3. Detection of Antiviral Systems

DefenseFinder was used to identify anti-phage defense systems [24]. DefenseFinder utilizes MacSyFinder27, a program dedicated to the detection of macromolecular systems, functioning with one model per system [25]. This approach involves a two-step process: first, the detection of all proteins involved in a macromolecular system through a homology search using Hidden Markov Model (HMM) profiles; second, the application of decision rules to retain only the HMM hits that satisfy the genetic architecture of the system of interest. Genomic features such as phage and genomic island sequences were recognized using online bioinformatic tools such as Island Viewer.

2.4. Phylogenetic Analysis

The closest reference and representative genomes were identified by Mash/MinHash [26]. PATRIC global protein families (PGFams) were selected from these genomes to determine the phylogenetic placement of this genome [27]. The protein sequences from these families were aligned with MUSCLE, and the nucleotides for each of those sequences were mapped to the protein alignment [28]. The joint set of amino acid and nucleotide alignments were concatenated into a data matrix, and RaxML was used to analyze this matrix, with fast bootstrapping was used to generate the support values in the tree [29].

2.5. Figures and Statistical Analysis

Statistical analysis and visualization were performed using SRplot and jvenn [30,31].

3. Results

3.1. Genome Assembly and Annotation

The Leptospira interrogans serovar Copenhageni strain FDAARGOS_203 genome was assembled and analyzed for its genetic content. The assembly consisted of 2 contigs, totaling 4,630,574 base pairs, with an average G+C content of 35.05% (Table 1).

Table 1. Assembly Details.

Feature	Value
Contigs	2
GC Content	35.05
Plasmids	0
Contig L50	1
Genome Length	4,630,574 bp
Contig N50	4,280,403
Chromosomes	0

Quality control measures, such as removal of low-quality reads and trimming of adapters, were performed prior to assembly. The genome was then annotated using RAST tool kit (RASTtk) and assigned a unique genome identifier of 173.581. The genome contained 4,479 protein-coding sequences (CDS), 37 transfer RNA (tRNA) genes, and 3 ribosomal RNA (rRNA) genes. The annotation revealed 2,305 hypothetical proteins and 2,174 proteins with functional assignments (Table 2 and Table 3).

Table 2. Annotated Genome Features.

Feature	Value
CDS	4,479
Repeat Regions	485
tRNA	37

rRNA	3

The genome exhibited a variety of proteins with Enzyme Commission (EC) numbers, Gene Ontology (GO) assignments, and proteins mapped to KEGG pathways, contributing to the overall functional diversity (Table 3).

Table 3. Protein Features.

Feature	Value
Hypothetical proteins	671
Proteins with functional assignments	556
Proteins with EC number assignments	517
Proteins with GO assignments	4,061
Proteins with Pathway assignments	4,160
Proteins with PATRIC genus-specific family (PLfam) assignments	671
Proteins with PATRIC cross-genus family (PGfam) assignments	556

A circular graphical representation displayed the genome annotations, including contigs, CDS on the forward and reverse strands, RNA genes, and features related to antimicrobial resistance and virulence factors (Figure 1).

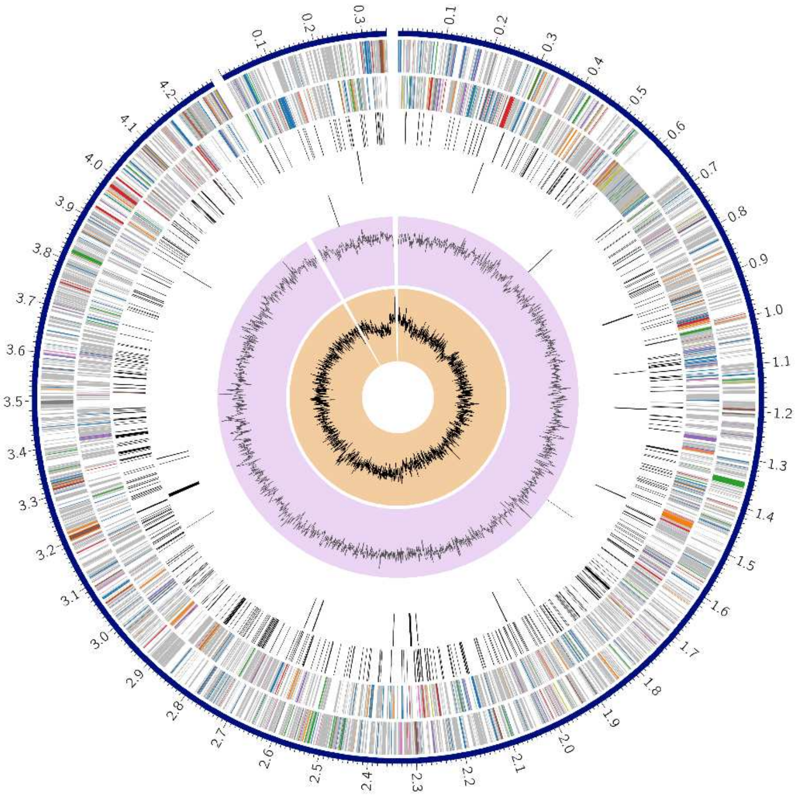


Figure 1. Circular Genome Display. From outer to inner rings, the contigs, CDS on the forward strand, CDS on the reverse strand, RNA genes, CDS with homology to known antimicrobial resistance genes, CDS with homology to known virulence factors, GC content, and GC skew. The colors of the CDS on the forward and reverse strands indicate the subsystems to which these genes belong.

The distribution of subsystems unique to this genome was illustrated, providing an overview of its functional organization (Figure 2).

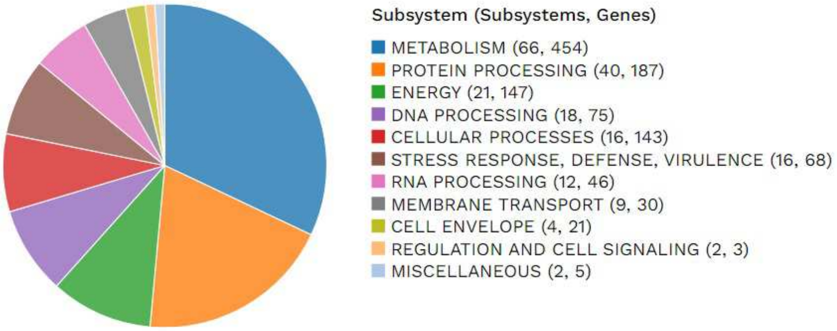


Figure 2. Subsystem overview. Distribution of subsystems, molecular pathways and processes, are indicated by a color code (**left**), and named (**right**). Number of subsystems and genes are indicated in parentheses.

3.2. Specialty Genes

Several genes annotated in the genome demonstrated homology to known transporters, virulence factors, drug targets, and antibiotic resistance genes. Specifically, 22 antibiotic resistance genes were identified using the PATRIC database, along with one drug target and 67 transporter genes (Table 4). The antibiotic resistance genes targeted various essential cellular functions, such as cell wall synthesis, DNA replication, and protein synthesis (Table 4).

Table 4. Specialty Genes.

Type	Source	Genes
Antibiotic Resistance	4,479	22
Drug Target	485	1
Transporter	37	67

3.3. Phylogenetic Analysis

The phylogenetic placement of the Leptospira interrogans serovar Copenhageni strain FDAARGOS_203 genome was determined using reference and representative genomes. The analysis, conducted with RaxML and fast bootstrapping, identified closely related genomes based on Mash/MinHash comparisons. The resulting tree (Figure 3) provides insights into the evolutionary relationships of this strain within the broader context of Leptospira species.

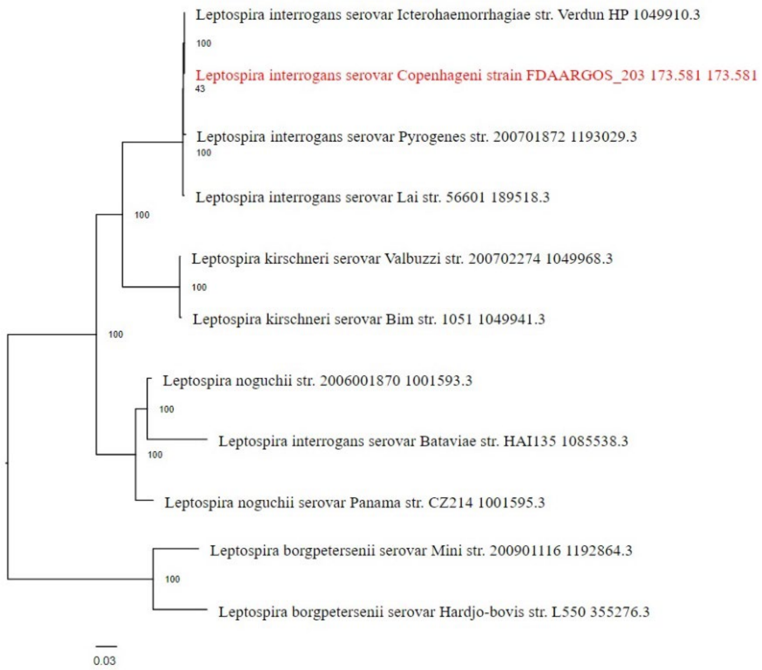


Figure 3. Phylogenetic Tree. The tree shows the evolutionary position of *Leptospira interrogans* FDAARGOS_203 using Mash/MinHash for reference genome selection and PATRIC global protein families. MUSCLE-aligned protein sequences, integrated with nucleotide data, were analyzed by RaxML with fast bootstrapping. Node support values indicate reliability.

3.4. Anti-Phage Systems

The genome analysis also revealed the presence of various anti-phage defense systems. Multiple defense islands, housing systems such as RM_Type_IV, PrrC, Borvo, CAS_Class1-Subtype-IC, CAS_Class1-Subtype-IB. These defense mechanisms likely play a crucial role in protecting the bacterium from phage attacks and contribute to its survival in various environments.

3.5. AMR Genes

A study of the genome of the Leptospira interrogans serovar Copenhageni strain FDAARGOS_203 showed a group of genes that are resistant to antibiotics. These genes target essential cellular functions, including protein synthesis, DNA replication, and cell wall synthesis. Notably, the gidB gene was identified, suggesting its role in conferring resistance through absence. Additionally, GdpD and PgsA genes were associated with altering cell wall charge, contributing to antibiotic resistance (Table 5).

Table 5. AMR genes.

Type	Gene Names
Antibiotic target in susceptible species	Alr, Ddl, dxr, EF-G, EF-Tu, folA, Dfr, folP, gyrA, gyrB, Iso-tRNA, MurA, rho, rpoB, rpoC, S10p, S12p
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	GdpD, PgsA

4. Discussion

Our study was conducted to analyze for the first time the genome of a reference strain of *Leptospira* for the presence of anti-phage systems and mechanisms of resistance to antibiotics. This study provides a solid foundation for initiating new research in this field.

We identified only two studies that investigated *Leptospira* anti-phage systems, both of which focused solely on Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) and their subtypes [32,33]. CRISPR Types I and III are considered dominant for *Leptospira*. However, our discovery revealed additional methods of protection against leptophages, specifically RM_Type_IV, PrrC, and Borvo.

The detection of Type IV restriction-modification (R-M) system is particularly interesting. R-M systems, the most studied class of defense systems since their discovery in the 1960s, recognize specific DNA motifs and are categorized into four broad types (I–IV) [34]. Type IV R-M systems have only a restriction endonuclease (REase) that cleaves foreign DNA with methylation at the same site as the recognition motif [35]. The finding of Type IV requires further research.

If the R-M system is compromised by a phage inhibitor as the primary defense, PrrC can still provide a secondary line of defense [36]. PrrC is an anticodon nuclease that specifically targets tRNALys, inhibiting protein synthesis and leading to cell death [37–39]. Borvo, which possesses a CHAT protease domain protein, results in cell death despite its unknown mechanism of immunity [40].

Leptospires have evolved several defense mechanisms against bacteriophages, and CRISPR is just one of them. Our findings make a significant contribution to future research, particularly for the development of potential drugs for treating leptospirosis in animals or humans.

Additionally, we identified 20 genes responsible for leptospirosis resistance to antibiotics. The apparent absence of significant antimicrobial resistance emergence in *Leptospira* raises the question of why this has not occurred (18). Speculatively, in the environment, leptospires coexist with numerous bacterial species, but the lack of therapeutically useful antimicrobial agents results in minimal selective pressure (19). Leptospiral infections are typically monomicrobial, limiting opportunities for horizontal resistance gene acquisition. Moreover, there is no experimental evidence of foreign DNA uptake by *Leptospira* spp., although genomic analyses support this notion. Finally, human leptospirosis is a dead-end infection, with human-to-human transmission being extremely rare.

Author Contributions: Conceptualization, O.K. and P.P.; software, P.P.; validation, O.K. and P.P.; formal analysis, O.K.; investigation, P.P.; data curation, P.P.; writing—original draft preparation, P.P. and O.K.; writing—review and editing, V.O.; visualization, P.P.; supervision, O.K. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflicts of interest.

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