Hybrid Sequencing Resolves Complete Genome and Methylome to Uncover Virulence Mechanisms of *Legionella pneumophila* in Hong Kong

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Background

Legionella pneumophila is a ubiquitous aquatic bacterium, yet only specific clones successfully transition to infect human hosts. To elucidate the mechanisms driving this pathogenicity, we investigated the genomic and epigenetic landscape of clinical and environmental isolates.

Methods

We collected 116 *L. pneumophila* isolates (18 clinical, 98 environmental) in Hong Kong between 2016 and 2025. Strains were isolated from diverse water sources, biofilms, and patient sputum. Identities were confirmed using PCR and MALDI-TOF. Complete genome assembly and methylome analysis utilized hybrid nanopore long read and next generation sequencing reads.

Results

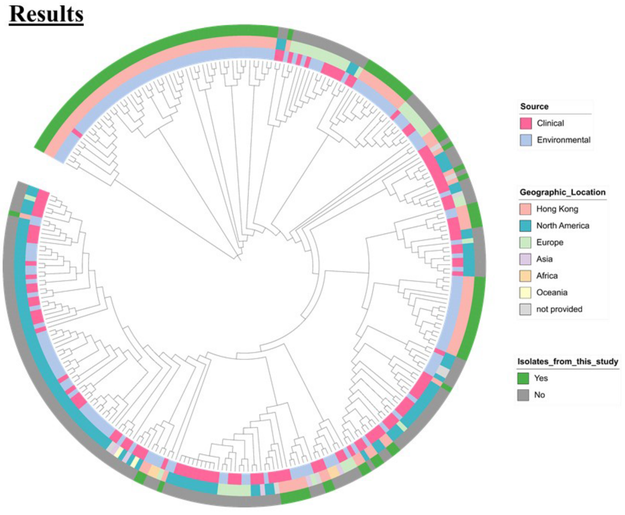

Figure 1. Source and genetic diversity of *L. pneumophila* (n=291). Comparing 116 Hong Kong genomes to 175 global references with complete genome reveals distinct regional dynamics. The HK cohort is dominated by ST1 (52.5%), with local expansions of ST1317 and ST2254. Conversely, global strains are driven by ST36 and ST378. Overall regional overlap is minimal, with only 6 shared STs identified between the local and global cohorts.

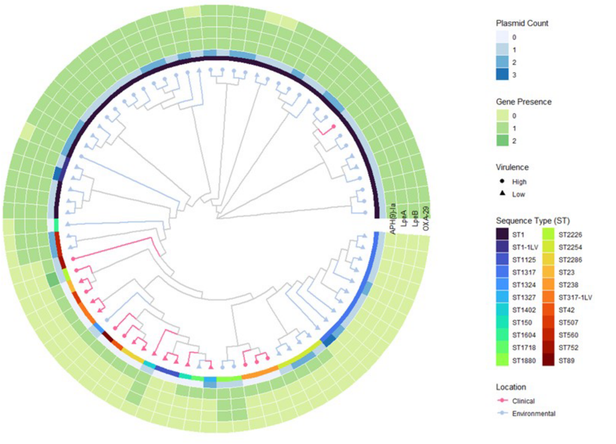

Figure 2. Phylogenetic distribution and accessory genomic profiles of *L. pneumophila* isolates in Hong Kong. Hybrid assemblies revealed Sequence Type 1 (ST1) and ST1317 are well colonized across Hong Kong, with ST1 dominating. Only ST1 appeared in both clinical and environmental samples. Maximum-likelihood phylogenetic tree based on core-genome analysis of *L. pneumophila* isolates. High virulence phenotypes were intrinsically linked to ST1 subclades harbouring antimicrobial resistance (AMR) genes *APH(9)-Ia*, *LpeA*, *LpeB*, and *OXA29*. The beta lactamase *OXA29* is carried on a unique plasmid. Tip points are coloured by isolation source and shaped by predicted virulence phenotype.

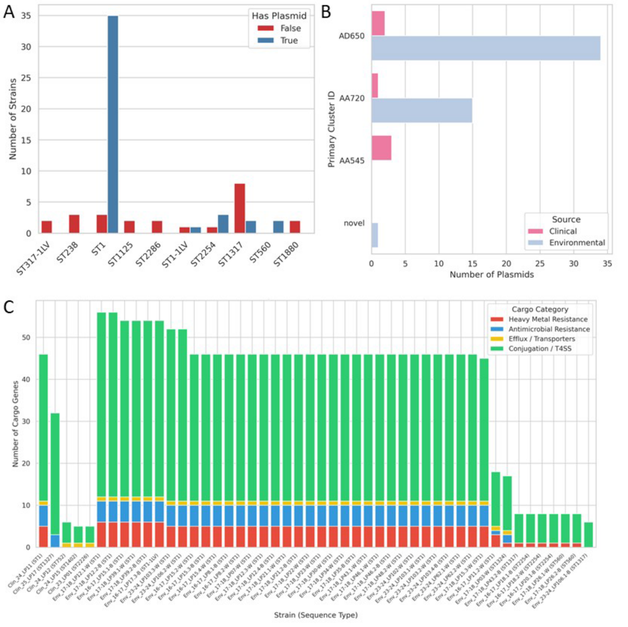

Figure 3. Lineage-specific plasmid carriage and resistance determinants in *L. pneumophila*. Integration of plasmid profiles with core-genome phylogeny reveals that plasmid distribution is highly restricted by ST (A). High-virulence clinical lineages lack plasmids, relying instead on core chromosomal virulence (B). Conversely, the environmental ST1 clade ubiquitously harbours a highly conserved, high-molecular-weight conjugative plasmid (C). This plasmid stably maintains heavy metal (*copG*, *merR*) and antimicrobial (*OXA29*) resistance genes within the environment. Notably, the clinical ST1 infection shares this exact primary plasmid cluster, demonstrating the direct transmission of the intact, multidrug-resistant mobile element from the environment to the patient.

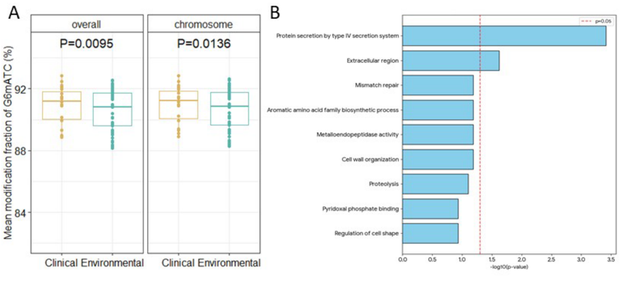

Figure 4. Epigenetically, clinical isolates exhibited significantly higher chromosomal 6mA methylation fractions than environmental strains. Variable promoters revealed distinct niche specific methylation patterns. Hypomethylation in clinical strains was enriched in Dot/Icm Type IV secretion system (T4SS) and cell wall biosynthesis genes.

Conclusions

L. pneumophila is colonized in Hong Kong water systems, with ST1 and ST1317 as major sequence types. While the core genome remains conserved, targeted epigenetic plasticity modulates essential virulence factors. This study identifies specific markers, particularly the ST1 associated AMR profile spanning chromosomal and plasmid borne genes, as critical targets for environmental surveillance and risk assessment.

References

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2. Wee, Bryan A., et al. *Nature communications* 12.1 (2021): 7165.
3. Romanov, Katerina A., and Tamara J. O'Connor. *Journal of bacteriology* 206.12 (2024): e00324-24.

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