Genomic and virulence insights of Western European *Aeromonas salmonicida* subsp. *salmonicida* and development of *Galleria mellonella* infection assay

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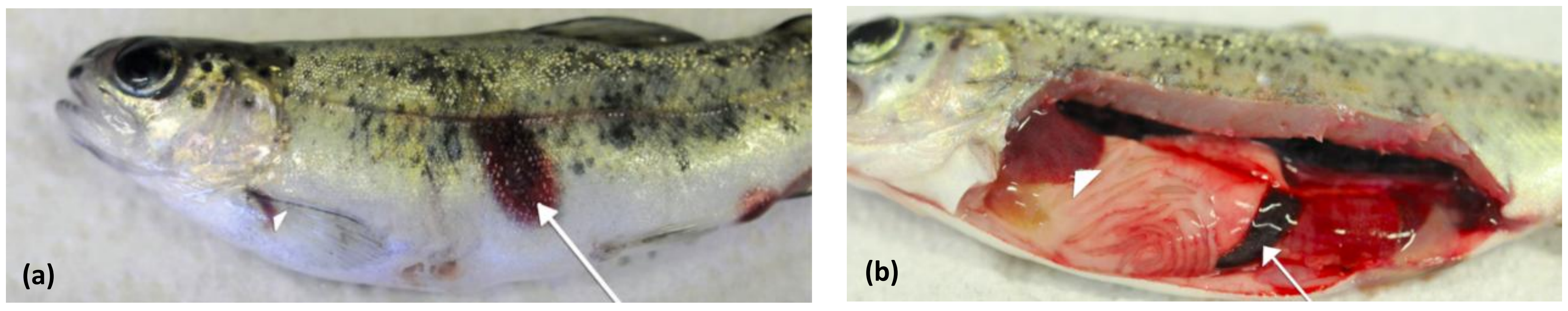
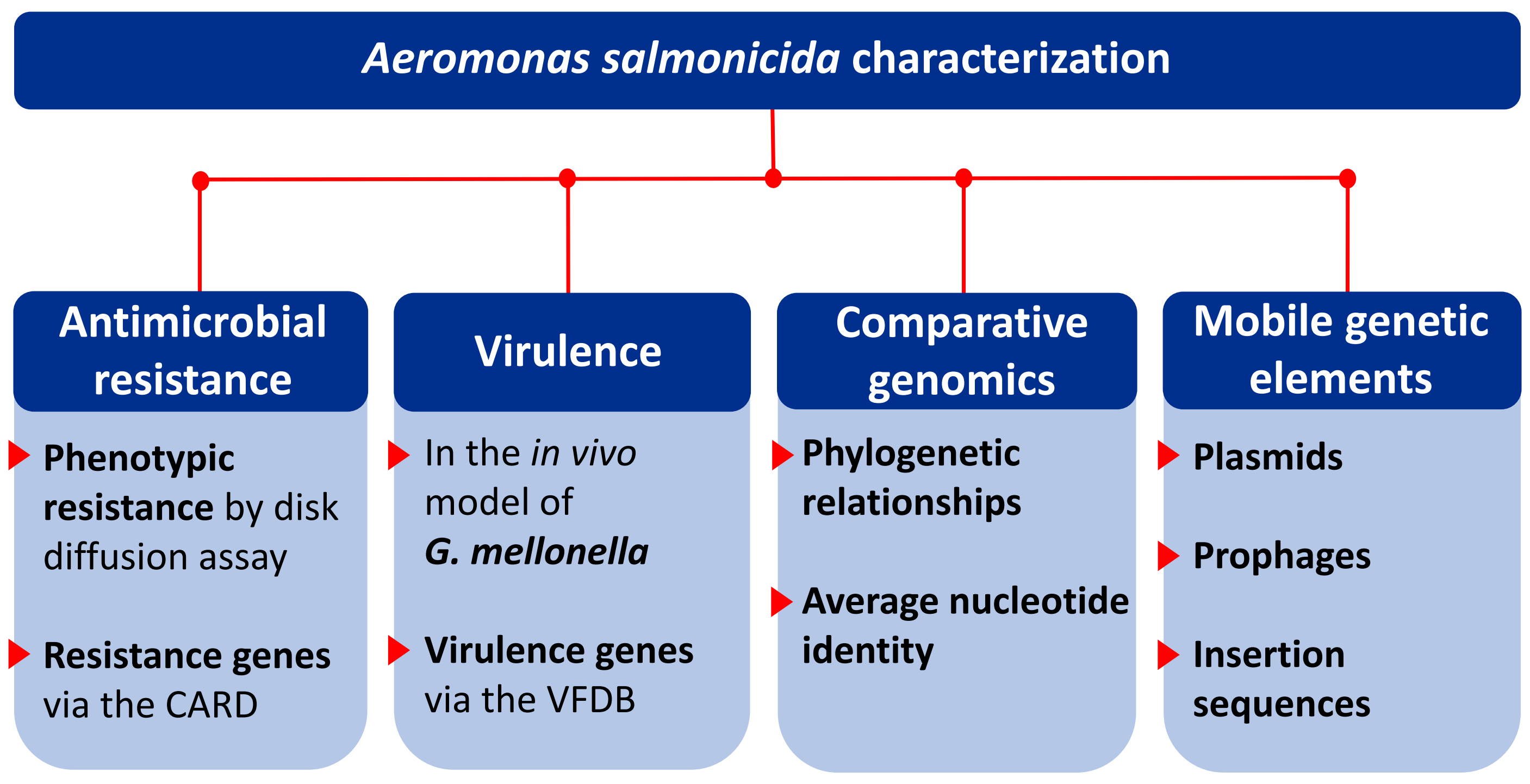
BACKGROUND *Aeromonas salmonicida* subsp. *salmonicida* is the etiological agent of the **furunculosis**, a fish **disease highly aggressive for salmonids** and responsible for significant economic losses in aquaculture industry worldwide. This study aimed to explore **genomic** and **antimicrobial resistance traits** of three Western European *A. salmonicida* subsp. *salmonicida* strains and to develop an **adapted infection model** using larvae of the greater wax moth ***Galleria mellonella*** to assess the pathogenic potential of this psychrophilic subspecies.



External ulcerative lesions **(a)**, splenomegaly and widespread systemic hemorrhages **(b)** on a rainbow trout *Oncorhynchus mykiss* (from Menanteau-Ledouble et al., 2016).

METHODS



***Aeromonas salmonicida* characterization**

Antimicrobial resistance
- **Phenotypic resistance** by disk diffusion assay
- **Resistance genes** via the CARD

Virulence
- In the *in vivo* model of ***G. mellonella***
- **Virulence genes** via the VFDB

Comparative genomics
- **Phylogenetic relationships**
- **Average nucleotide identity**

Mobile genetic elements
- **Plasmids**
- **Prophages**
- **Insertion sequences**

RESULTS Two *A. salmonicida* subsp. *salmonicida* strains exhibited a **multidrug resistance profile** to commonly used antibiotics for the anti-furunculosis treatment (**Figure 1**). Comparative genomic analyses revealed that the chromosomes of the three strains were closely related to the European reference strain A449 of *A. salmonicida* belonging to the **typical psychrophilic group** (**Figure 2**). Virulence assays in *G. mellonella* showed that this subspecies was **lethal at doses as low as 10^1 CFU/larva** (**Figure 3**), and that a fully functional Type III secretion system (T3SS) is not essential for the infection of *G. mellonella* (**Figure 4**).

FIGURE 1.

Antibiotic resistance phenotypes observed in the *A. salmonicida* CER1, CER2, and CER3 strains against frequently used antibiotics to control furunculosis in fish **(a)** along with **antibiotic resistance gene compositions** of the CER genomes and nine other *A. salmonicida* subsp. *salmonicida* complete genomes **(b)**.

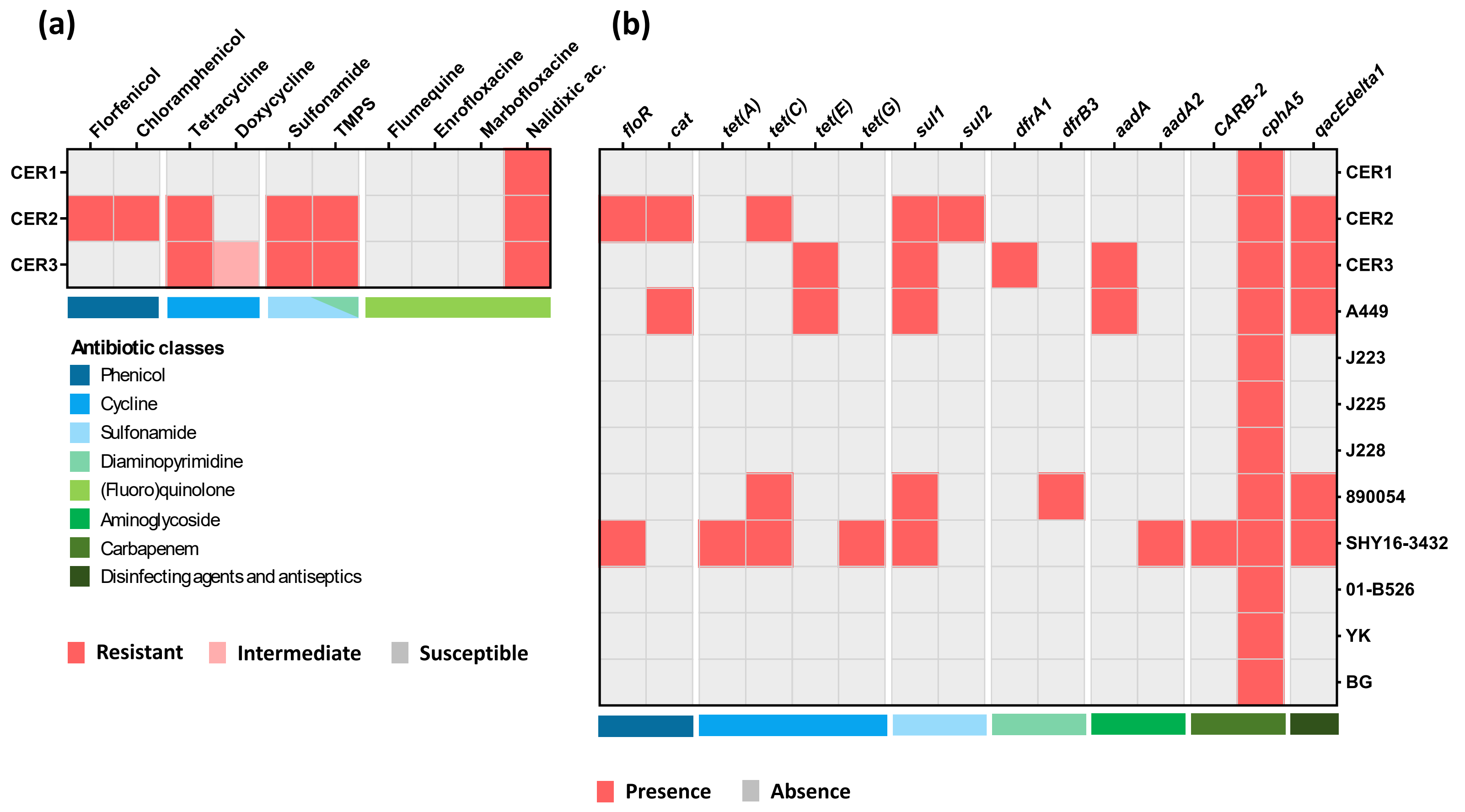


FIGURE 2.

Phylogenetic relationships among the CER1, CER2, CER3, and 32 *A. salmonicida* complete genomes based on their core genes, and heatmap representing the **average nucleotide identity** values.

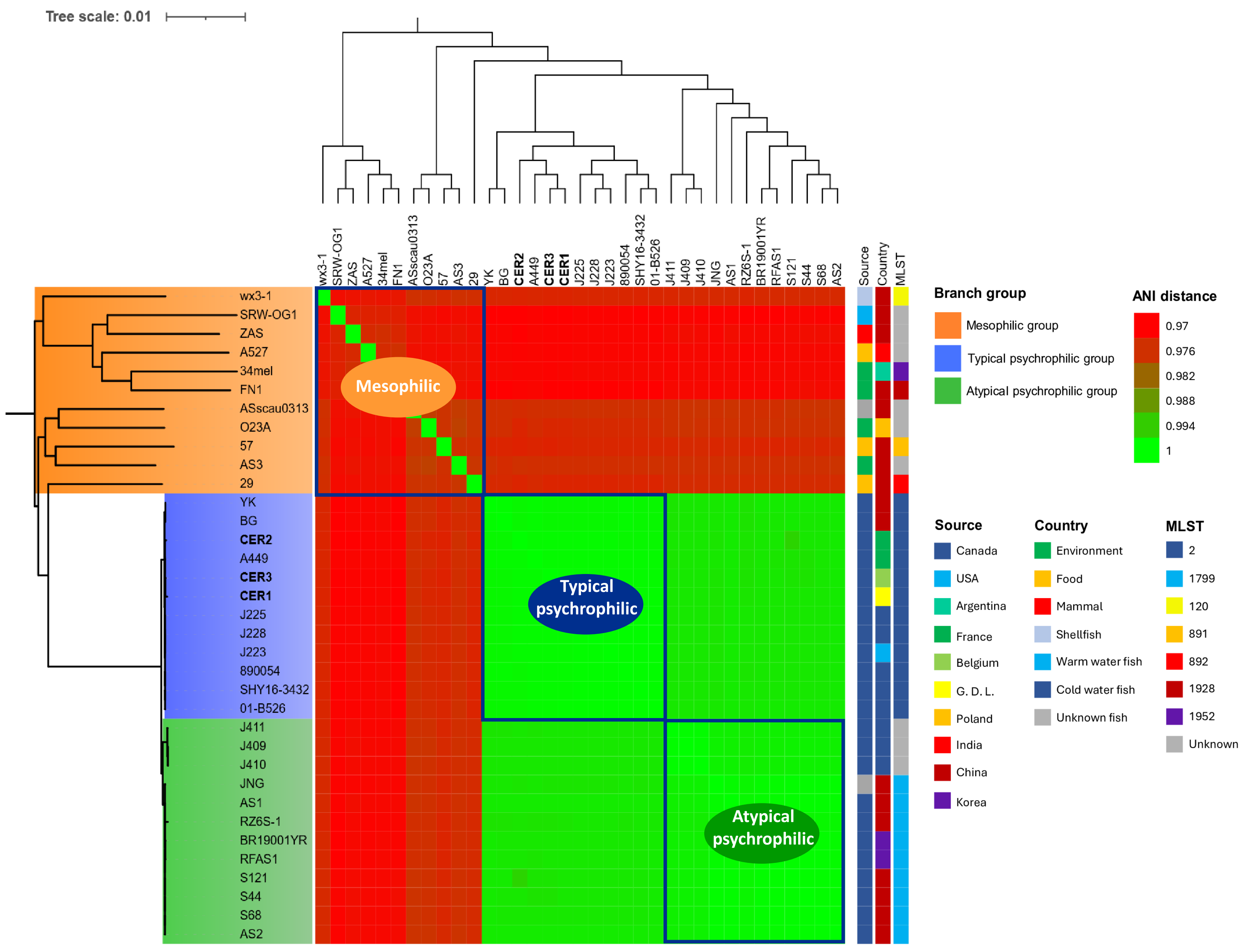


FIGURE 3.

Kaplan-Meier **survival curves of *Galleria mellonella*** larvae groups inoculated at 18°C with PBS or with *A. salmonicida* subsp. *salmonicida* 01-B526 control strain at six different concentrations **(a)**, 01-B516 control strain at six different concentrations **(b)**, 01-B526, 01-B516, CER1, CER2, and CER3 strains at the highest and lowest concentrations **(c)**. Each group contained 20 larvae. HPI: hours post-inoculation. p-value (*) ≤ 0.05.

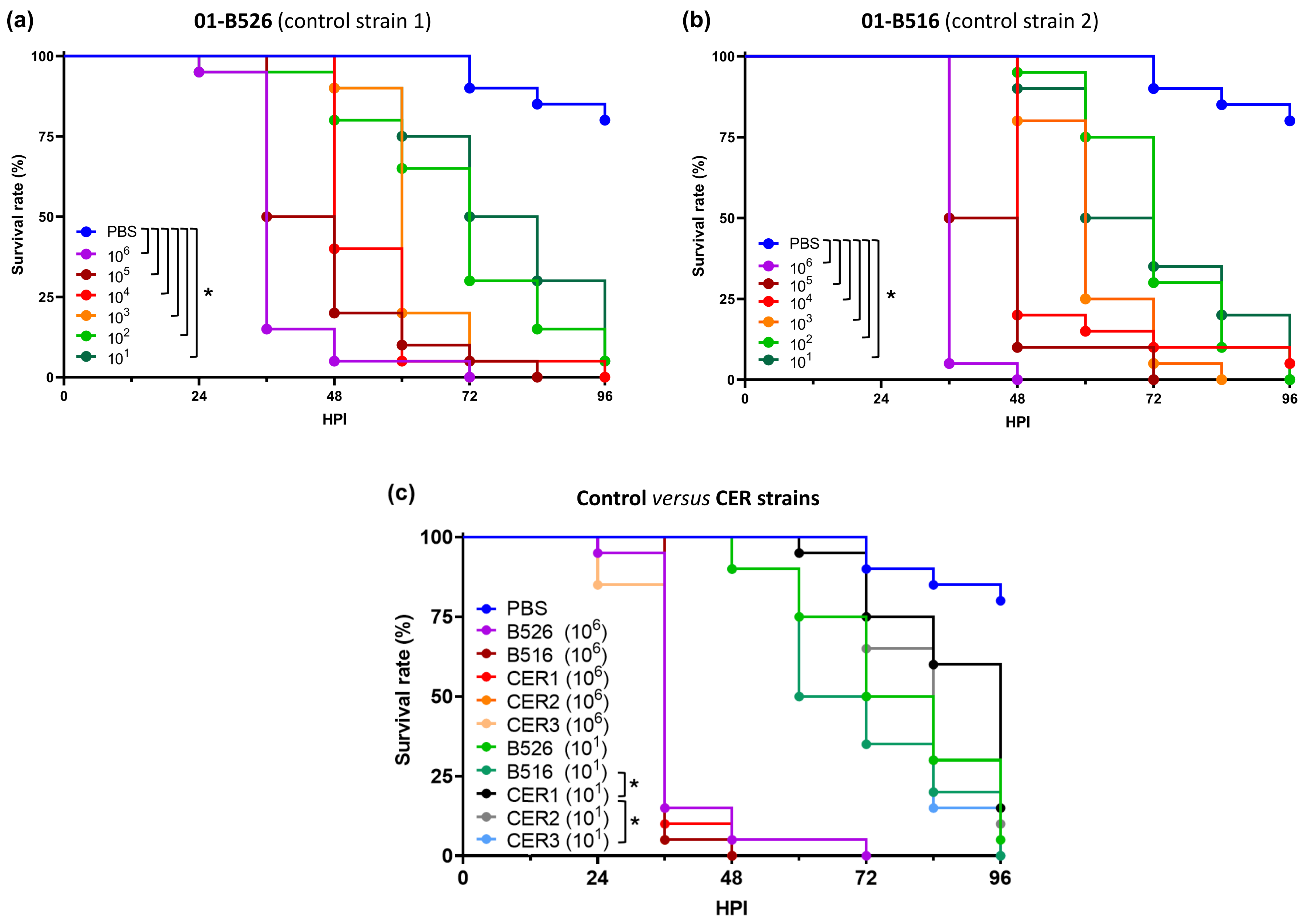
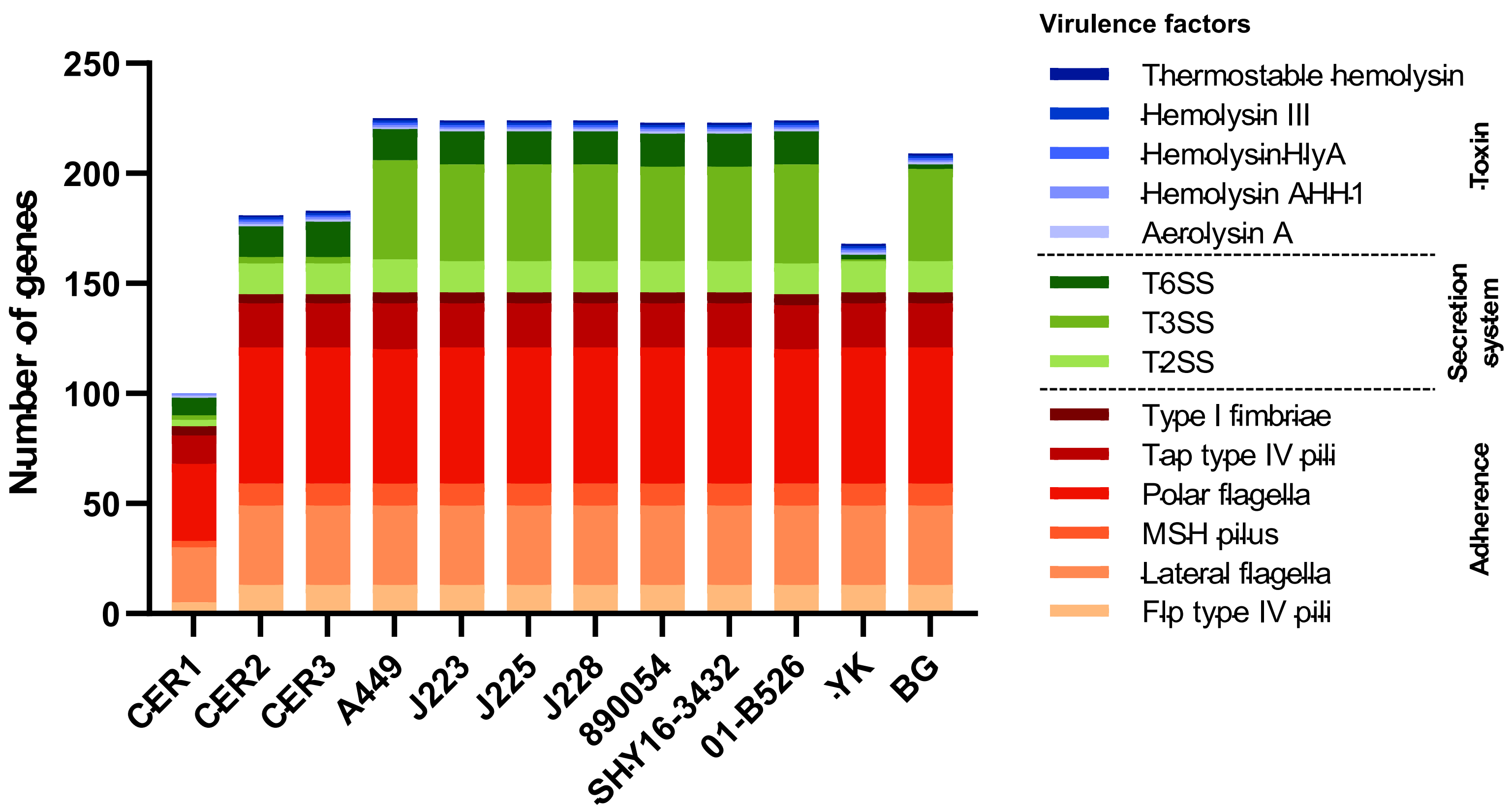


FIGURE 4.

Distribution of virulence genes per virulence factor in the CER1, CER2, CER3, and nine other *A. salmonicida* subsp. *salmonicida* genomes.



CONCLUSIONS This study highlights the **genomic complexity of *Aeromonas salmonicida* subsp. *salmonicida*** and the **circulation of multidrug-resistant strains in Western Europe**. Our results also **validate the use of *Galleria mellonella*** larvae as a **relevant alternative infection model** to study the pathogenicity of the *salmonicida* subspecies. Overall, these findings provide valuable insights into the epidemiology, antimicrobial resistance, and pathogenic potential of this psychrophilic fish pathogen.

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