

Genomic characterization and virulence assessment of three *Aeromonas salmonicida* subsp. *salmonicida* strains isolated from salmonids in western Europe

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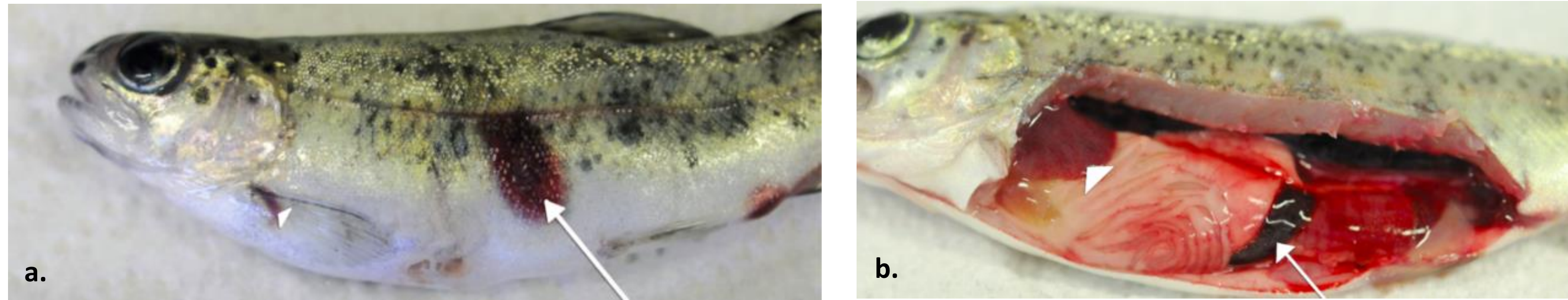
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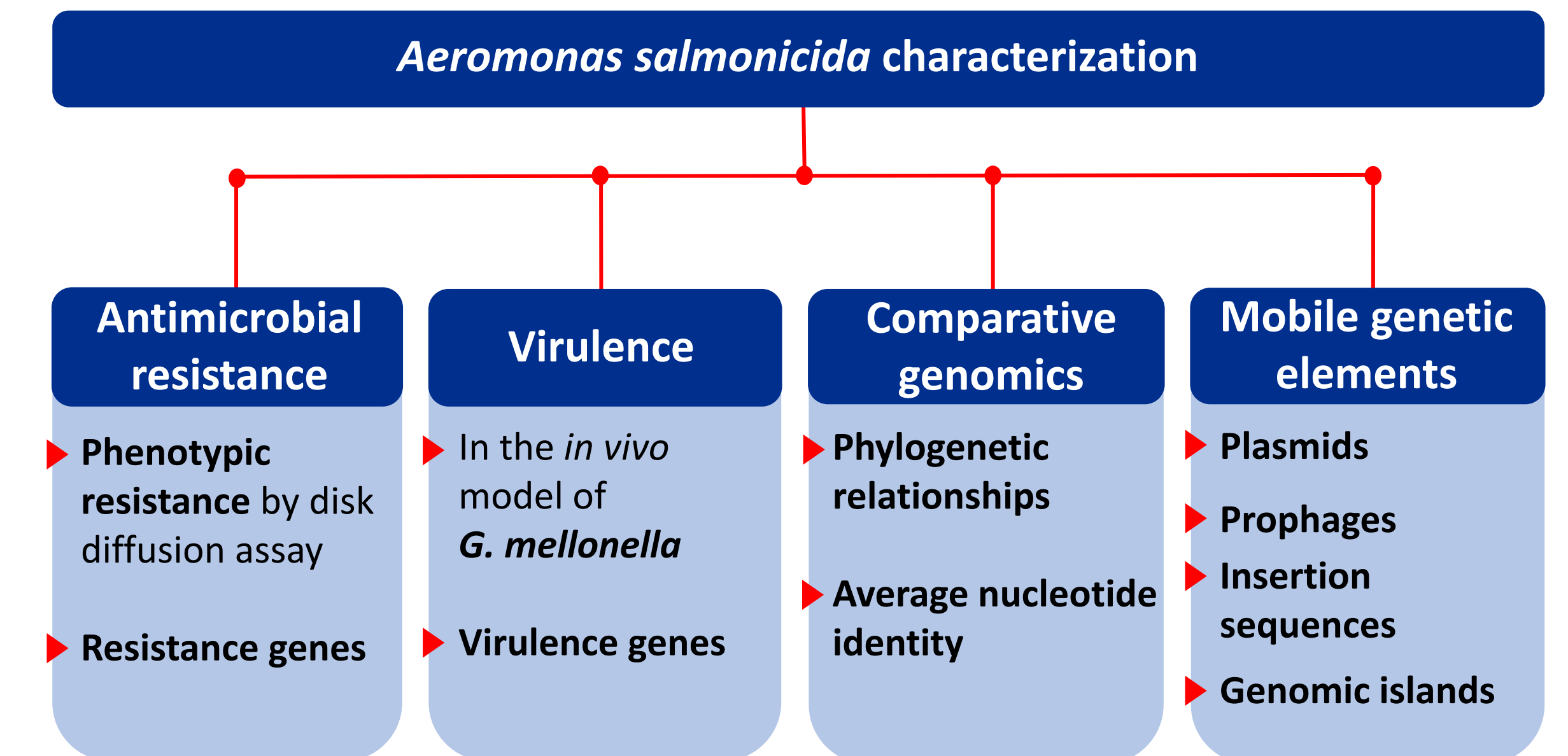
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BACKGROUND *Aeromonas* (*A.*) *salmonicida* subsp. *salmonicida* is the etiological agent of the furunculosis, a fish disease highly aggressive for salmonids and responsible for significant economic losses in aquaculture industry worldwide. This work aimed to study the antimicrobial resistance profile, perform an in-depth genomic characterization, and assess the bacterial virulence in the *Galleria* (*G.*) *mellonella* larval model of three European isolates of this subspecies originating from salmonids displaying clinical signs of furunculosis.



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

METHODS



RESULTS Two *A. salmonicida* subsp. *salmonicida* strains, named CER1 and CER3, were isolated from brown trout (*Salmo trutta*) coming from fish farms in Luxembourg and Belgium, respectively. A third strain, named CER2, was isolated from a parr (*Salmo salar*) coming from France. The CER2 and CER3 isolates exhibited a multi-drug resistance profile to commonly used antibiotics for the anti-furunculosis treatment (Table 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 1). They harbored multiple plasmids identified to display antibiotic resistance genes, such as the pRAS3.5, pAB5S9b and the pAsa4b (Table 2). The three strains showed a marked virulence from a dose of 10² CFU/10μl in the *G. mellonella* model (Figure 2).

Antibiotic class	Antibiotic	Disc content (μg)	Inhibition zone diameter (mm) ¹		
			CER1	CER2	CER3
Penicillin	Amoxicillin	20	30 (S)	30 (S)	30 (S)
	Ampicillin	20	20 (S)	17 (S)	26 (S)
	Amoxicillin/clavulanic acid	30	30 (S)	30 (S)	30 (S)
Cephalosporin	Cefoxitin	30	36 (S)	30 (S)	36 (S)
	Cephalothin	30	18 (I)	18 (I)	18 (I)
	Cefquinome	30	34 (S)	36 (S)	34 (S)
Carbapenem	Meropenem	10	38 (S)	34 (S)	36 (S)
	Aztreonam	30	38 (S)	40 (S)	42 (S)
Glycopeptide	Vancomycin	5	6 (R)	6 (R)	6 (R)
	Colistin	20	20 (S)	20 (S)	20 (S)
Polymyxin	Gentamycin	10	20 (S)	20 (S)	20 (S)
	Kanamycin	30	20 (S)	22 (S)	20 (S)
Macrolide	Erythromycin	15	20 (I)	24 (S)	24 (S)
	Clindamycin	2	6 (R)	6 (R)	6 (R)
Cycline	Tetracycline	30	32 (S)	8 (R)	13 (R)
	Doxycycline	30	38 (S)	24 (S)	24 (S)
Phenicol	Florfenicol	30	36 (S)	6 (R)	32 (S)
	Chloramphenicol	30	36 (S)	40 (S)	40 (S)
Quinolone	Nalidixic acid	30	6 (R)	6 (R)	6 (R)
	Flumequine	30	34 (S)	32 (S)	32 (S)
Fluoroquinolone	Enrofloxacin	5	20 (S)	36 (S)	30 (S)
	Marbofloxacin	5	44 (S)	40 (S)	44 (S)
Sulfonamide	Sulfonamide	200	6 (R)	6 (R)	6 (R)
	TMPS	25	28 (S)	20 (S)	6 (R)

TABLE 1. Antibiotic resistance and susceptibility of *A. salmonicida* subsp. *salmonicida* CER strains.

¹ With S = susceptible; I = intermediate; R = resistant that were designated using breakpoints described by EUCAST (2023), CASFM (2023) or CASFM VET (2023).

TABLE 2. Mapping coverage of sequencing reads of the three *A. salmonicida* subsp. *salmonicida* CER strains on reference genomic elements in relation to their composition in prophage-derived sequences, virulence and antibiotic resistance genes.

¹ Absence of gene coverage in read mappings for the three CER strains.
² Partial gene coverage in read mappings for the three CER strains.

Genomic elements	Sequencing breadth of coverage (%)			# of prophage derived sequences	Virulence genes	Best tblastn matches of antimicrobial resistance genes
	CER1	CER2	CER3			
A449 strain chromosome	99.8	99.8	99.9	2	<i>vapA, aspA, satA, pla, aerA, aerB, aexT</i>	Vancomycin (<i>vanE, vanH, vanR, vanS, vanT</i>), Clindamycin (<i>cplR, cfr, erm</i>), Nalidixic acid (<i>farB, bonA, fusA, fusE, gyrB</i>), Sulfonamide (<i>sul4, folP</i>), Florfenicol (<i>cfr, optra, estDL136, poxtA</i>), Tetracycline (<i>otr, tet(B), tet(T), tcr3, acrB, adel, lon, mexB, rpsI, soxR, tmxD</i>), Trimethoprim (<i>dfr, dut, mexT, yciV</i>), Sulfamethoxazole (<i>dut, yciV</i>), Erythromycin (<i>lmrS, msr, acrB</i>)
AsaGEI2d	0	100	0	1		
pAsa1	100	100	100	-		
pAsa2	100	100	100	-		
pAsa3	28.3	98.8	15.5	-		
pAsa4b	0.5	8.2	100	-		Tetracycline (<i>tet(E)</i>)
pAsa5	52.8	52.5	53.1	-	<i>aopO, (ati2, ascC, ascV, exsD, acrV, aopN, ascU, aopH)¹, aopH²</i>	
pAsa6	54.6	54.3	77.8	-		
pAsa7	12.8	100	16.0	-		
pAsal1D	100	99.9	100	-	<i>aopP</i>	
pAsal5	48.3	47.8	48.2	-	<i>aopH²</i>	
pJF4097	38.5	62.8	30.0	-	<i>exoY¹</i>	
pRAS3.5	0	98.8	0	-		Tetracycline (<i>tet(C)</i>)
pAB5S9b	0	79.8	0	-	<i>virD2</i>	Sulfonamide (<i>sul2, sul3</i>), Florfenicol (<i>floR</i>), Tetracycline (<i>tet(H)</i>) ²
pAER-f909 ³	0	83.6	3.3	-		Sulfonamide (<i>sul1</i>)

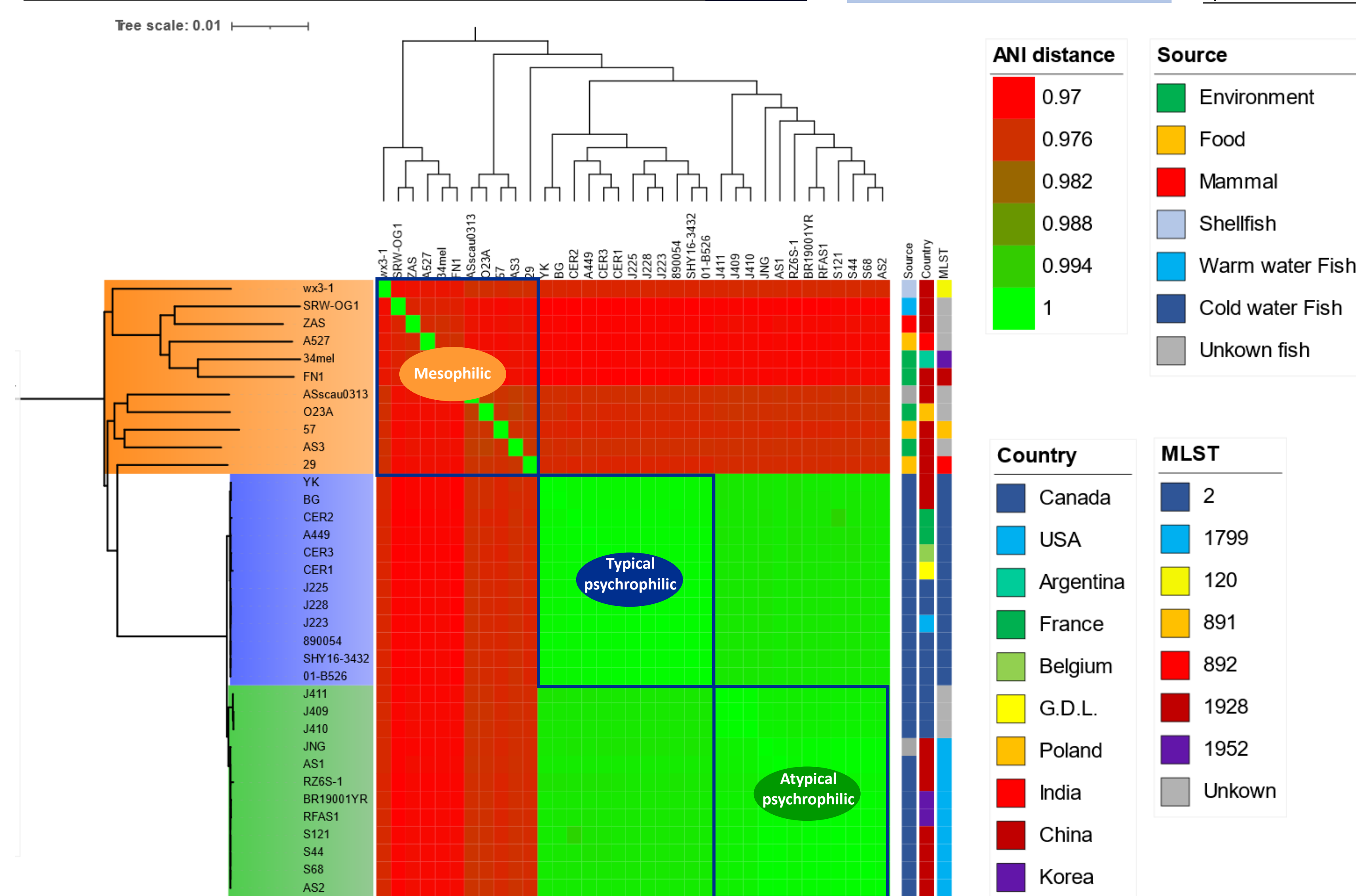
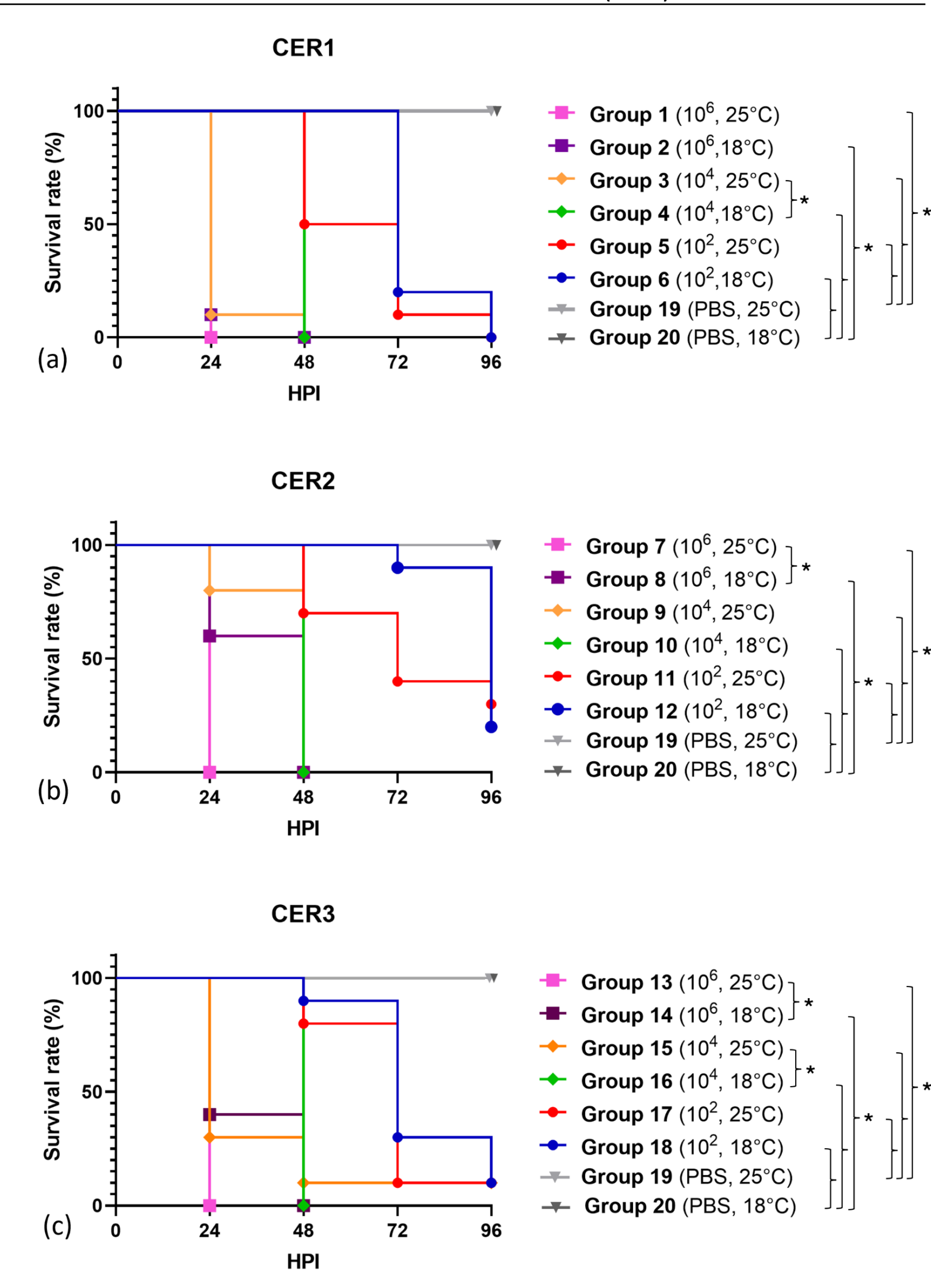


FIGURE 1. Phylogenetic relationship among 35 *A. salmonicida* complete genomes based on core genome and heatmap representing the average nucleotide identity values.

FIGURE 2. Kaplan-Meier survival curves of *G. mellonella* larvae groups inoculated with PBS or with three different doses of *A. salmonicida* (a) CER1, (b) CER2, and (c) CER3 strains at 25°C and 18°C. Each group contained 10 larvae. HPI: hours post-inoculation. p-value (*) ≤ 0.05.



CONCLUSIONS In this study, the genomes of three multi-drug resistant strains of *A. salmonicida* subsp. *salmonicida* were sequenced to explore their phylogenetic relationship and their composition in mobile genetic elements. These analyses revealed the presence of multiple plasmids harboring resistance genes against commonly used anti-furunculosis treatment in salmon industry in Europe. The bacterial virulence of this subspecies was assessed in the *G. mellonella* larval model and showed that these isolates were virulent from a dose of 10² CFU/10μl. Overall, these findings contribute to enrich the European collection of characterized genomes of *A. salmonicida* subsp. *salmonicida* and support the use of *G. mellonella* larvae as a new infection model to study this pathogenic subspecies causing furunculosis in salmonids.

