Aeromonas spp. as candidate indicator for antimicrobial resistance surveillance in Belgian aquaculture.

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AMR and AMU in Belgian aquaculture

- **No vaccine for fish:** Farmers rely on antimicrobials to treat fish diseases.
- **No authorized antibiotics in Belgium:** Veterinarians use products intended for other species.
- **No systematic AMR monitoring:** Unlike other livestock sectors, aquaculture lacks regular antimicrobial resistance surveillance.

Objectives

- **Recruit participating fish farms**
- **Isolate *Aeromonas* spp.** from fish, water, and sediment.
- Evaluate *Aeromonas* spp. as an **AMR surveillance indicator.**

Recruitement of the fish farms

- **22 fish farms** agreed to participate in the study (Fig.1).
- **Sample collection:** Conducted biannually for two years.

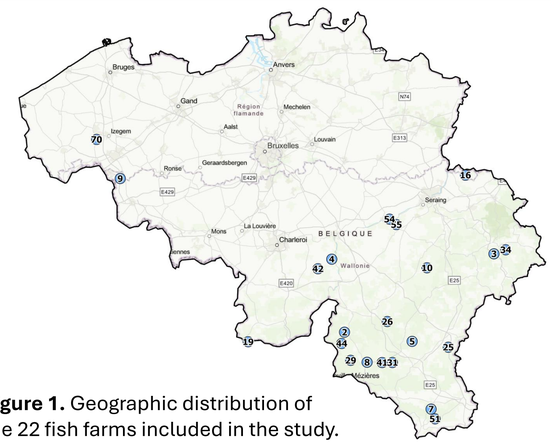
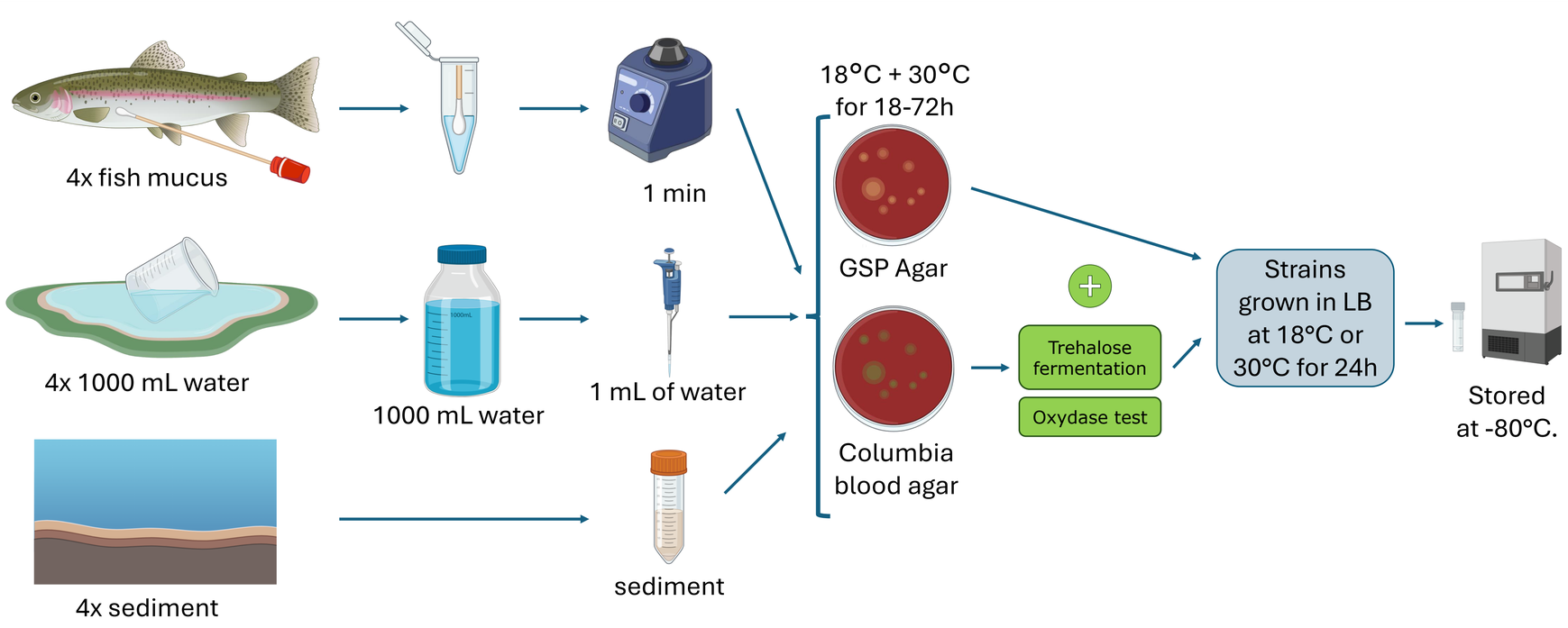


Figure 1. Geographic distribution of the 22 fish farms included in the study.

Isolation of *Aeromonas* spp.



Methodology:

1. **Sample collection (Jan–Mar 2025):** 22 farms, 88 fish mucus swabs, 88 water samples, 29 sediment samples.
2. **Isolation of Aeromonas spp.**: Swabs placed in PBS and vortexed. Water (1 mL) and sediment (10 µL) plated directly. Samples plated on GSP and blood agar. Incubated at 18°C & 30°C (18–72h) (Fig.2).
3. **Colony selection**: Yellow colonies from GSP grown in LB broth. If absent, beta-hemolytic colonies from blood agar used. Preliminary ID: trehalose fermentation + oxidase test.

Figure 2. Protocol for the isolation of *Aeromonas* spp.

Identification of the isolated strains

Methodology:

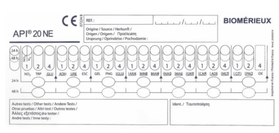
1. **Selection of Isolates**: Preference given to isolates from fish, water and sediment from the same tank, incubated at 18°C. Alternative samples used when needed.
2. **Identification:** 35 isolates were identified using API® 20NE test strips (BioMérieux, France)(Table 1):
   - *Aeromonas hydrophila/caviae* (n = 19)
   - *Aeromonas sobria* (n = 2)
   - *Vibrio alginolyticus* (n = 3)
   - *Mannheimia haemolytica* (n = 4)
   - *Brevundimonas vesicularis* (n = 2)
   - *Burkholderia cepacia* (n = 2)
   - *Pseudomonas putida* (n = 1)
   - *Pseudomonas fluorescens* (n = 1)
   - *Ralstonia pickettii* (n = 1)

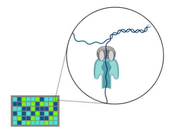
Table 1: Bacterial identification results obtained using the API® 20NE test strips from samples collected at different fish farms. Isolates were derived from three sample types (fish, water, and sediment), with indication of the sampling date, sample code, and bacterial identification result. The symbol "/" indicates no isolate was recovered. In red: fish farms that import fish. In orange: fish farms that reproduce fish. In red and orange with hatching: fish farms that both import and reproduce fish.

Date	Fish farm	Fish	API20NE identification	Water	API20NE identification	Sediment	API20NE identification
03-12-24	70	70F1	*Aeromonas hydrophila/caviae (44,9%)*	70W1	*Aeromonas hydrophila/caviae (99,5%)*	70S1	*Aeromonas hydrophila/caviae (99,9%)*
08-01-25	55	55F4	*Not conclusive*	55W4	*Aeromonas hydrophila/caviae (97,3%)*	55S	*Aeromonas hydrophila/caviae (99,9%)*
08-01-25	54	54F3	*Aeromonas sobria (99,2%)*	54W3	*Not conclusive*	/	/
13-01-25	3	3F2	*Brevundimonas vesicularis (91,5%)*	3W2	*Aeromonas hydrophila/caviae (99,3%)*	3S1	*Ralstonia pickettii*
14-01-25	29	29F1	*Aeromonas hydrophila/caviae (90,4%)*	29W1	*Burkholderia cepacia*	29S	*Pseudomonas putida*
15-01-25	10	10F1	*Not yet done*	10W1	*Not yet done*	10S1	*Not yet done*
20-01-25	19	19F1	*Burkholderia cepacia (99,9%)*	19W1	*Pseudomonas fluorescens (98,6%)*	19S1	*Aeromonas hydrophila/caviae (99,9%)*
23-01-25	41	41F3	*Not yet done*	41W3	*Not yet done*	41S1	*Aeromonas hydrophila/caviae (99,8%)*
24-01-25	26	26F1	*Brevundimonas vesicularis (91,5%)*	26W1	*Aeromonas hydrophila/caviae (99,8%)*	26S1	*Aeromonas hydrophila/caviae (83,2%)*
30-01-25	16	16F1	*Aeromonas hydrophila/caviae*	16W1	*Aeromonas hydrophila/caviae (91,6%)*	16S1	*Not yet done*
03-02-25	51	51F1	*Not yet done*	51W1	*Aeromonas hydrophila/caviae (48,3%)*	/	/
04-02-25	9	9F1	*Mannheimia haemolytica (86,5%)*	9W1	*Aeromonas hydrophila/caviae (99,9%)*	9S1	*Aeromonas sobria (94,8%)*
10-02-25	34	34F2	*Aeromonas hydrophila/caviae (99,9%)*	34W2	*Vibrio alginolyticus (70,0%)*	/	/
14-02-25	5	5F1	*Not yet done*	5W1	*Not yet done*	0	*0*
24-02-25	42	42F3	*Mannheimia haemolytica (86,5%)*	42W3	*Aeromonas hydrophila/caviae (99,9%)*	/	/
27-02-25	44	44F1	*Not yet done*	0	*0*	/	/
10-03-25	2	2F1	*Aeromonas hydrophila/caviae (99,8%)*	2W1	*Mannheimia haemolytica (86,5%)*	/	/
10-03-25	8	8F1	*Not yet done*	8W1	*Not yet done*	/	/
12-03-25	7	7F1	*Not yet done*	7W1	*Not yet done*	/	/
29-03-25	31	31F4	*Vibrio alginolyticus (70,0%)*	31W4	*Not conclusive*	31S2	*Mannheimia haemolytica (86,5%)*
29-03-25	25	25F1	*Not yet done*	25W1	*Not yet done*	25S1	*Not yet done*
31-03-25	4	4F4	*Vibrio alginolyticus (87,4%)*	4W4	*Aeromonas hydrophila/caviae (91,5%)*	/	/

Conclusion and perspectives

- *Aeromonas* spp. were successfully isolated at both 18°C and 30°C; however, incubation at 18°C was selected for the remainder of the project to preserve the genetic stability of the strains and reduce the risk of stress-induced genetic rearrangements.
- *Aeromonas* spp. were isolated from all three sample types (fish mucus, water, and sediment) with water samples appearing to be the most reliable source for AMR surveillance in aquaculture.
- Future work will include further identification by comparing API® 20NE and MALDI-TOF MS to determine the optimal sample type for *Aeromonas* isolation. In addition, phenotypic and genomic analyses are planned: antimicrobial susceptibility testing using Sensititre™ MIC plates and whole-genome sequencing (WGS) with Nanopore technology to characterize resistance profiles and genetic features.





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