

# 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.
- ➔ Compare identification methods (**API 20NE** and **MALDI-TOF**)
- ➔ Evaluate *Aeromonas* spp. as an **AMR surveillance indicator**.

## Recruitment of the fish farms

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

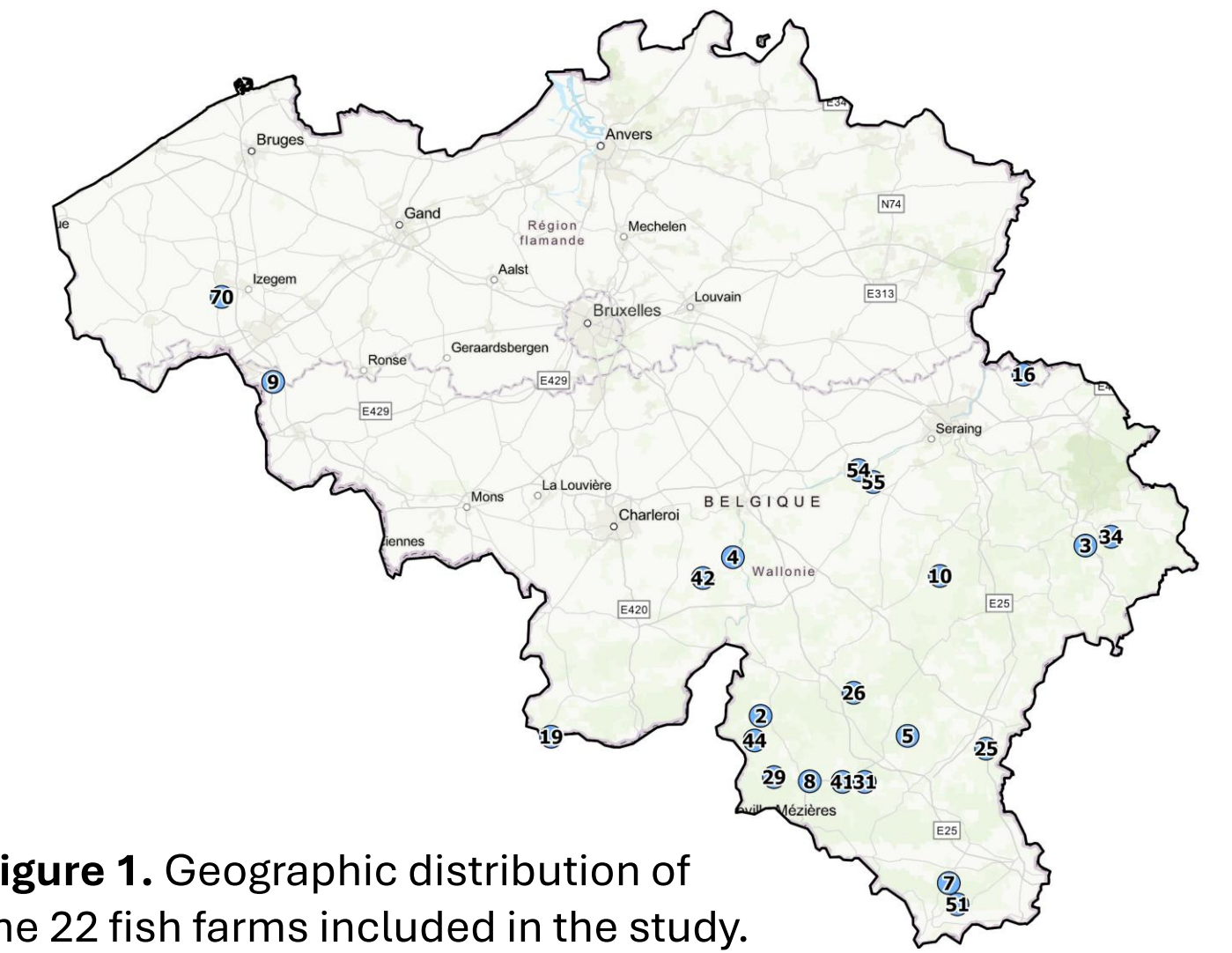


Figure 1. Geographic distribution of the 22 fish farms included in the study.

## Isolation of *Aeromonas* spp.

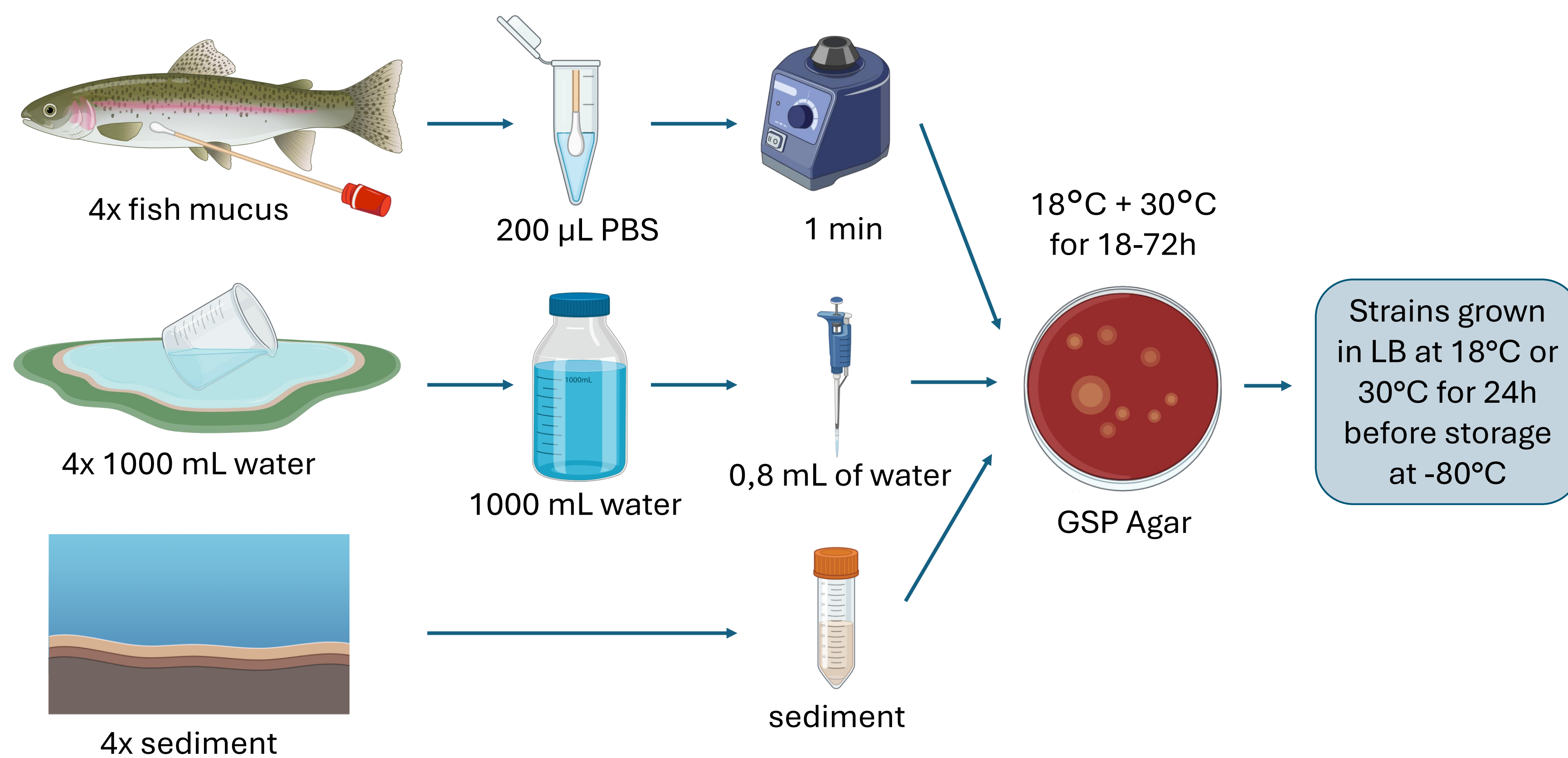


Figure 2. Protocol for the isolation of *Aeromonas* spp.

### Methodology:

88 fish mucus swabs, 88 water, 29 sediment samples from 22 farms (Jan–Mar 2025). Swabs in PBS; water (0.8 mL) & sediment (10 µL) plated on GSP agar. Incubated 18–72 h at 18°C & 30°C. Yellow colonies cultured in LB broth.

### Results:

Distribution analysis showed water samples yielded the most *Aeromonas* spp. isolates, followed by sediment, highlighting water as the main source (Table 1).

Table 1. Distribution of the total number of bacterial isolates obtained according to incubation temperature (18 °C and 30 °C) and sample type (fish, water, sediment).

T°	Fish	Water	Sed.	Total
18 °C	168	204	70	442
30 °C	133	246	81	460
Total	301	450	151	902

## Identification of the isolated strains

Table 2. Comparison of bacterial identification results obtained using API 20NE and MALDI-TOF<sup>®</sup> mass spectrometry for selected isolates. W = Water, S = Sediment.

Sample ID	API 20NE identification	MALDI-TOF identification
70 F1 1	<i>Aeromonas hydrophila/caviae</i> (44,9%)	<i>Aeromonas sobria</i>
70 W1 1	<i>Aeromonas hydrophila/caviae</i> (99,5%)	<i>Aeromonas bestiarum</i>
70 S1 1	<i>Aeromonas hydrophila/caviae</i> (99,9%)	<i>Aeromonas veronii</i>
55 W4 1	<i>Aeromonas hydrophila/caviae</i> (97,3%)	<i>Aeromonas hydrophila</i>
55 S1 2	<i>Aeromonas hydrophila/caviae</i> (99,9%)	<i>Aeromonas hydrophila</i>
54 F3 4	<i>Aeromonas sobria</i> (99,2%)	<i>Aeromonas veronii</i>
3 W2 1	<i>Aeromonas hydrophila/caviae</i> (99,3%)	No organism identification possible
29 F1 2	<i>Aeromonas hydrophila/caviae</i> (90,4%)	<i>Aeromonas sobria</i>
19 S1 3	<i>Aeromonas hydrophila/caviae</i> (99,9%)	<i>Aeromonas hydrophila</i>
41 S1 1	<i>Aeromonas hydrophila/caviae</i> (99,8%)	<i>Aeromonas salmonicida</i>
26 W1 1	<i>Aeromonas hydrophila/caviae</i> (99,8%)	<i>Aeromonas veronii</i>
26 S1 1	<i>Aeromonas hydrophila/caviae</i> (83,2%)	<i>Aeromonas eucrenophila</i>
16 F1 2	<i>Aeromonas hydrophila/caviae</i>	<i>Aeromonas sobria</i>
16 W1 1	<i>Aeromonas hydrophila/caviae</i> (91,6%)	<i>Aeromonas eucrenophila</i>
51 W1 2	<i>Aeromonas hydrophila/caviae</i> (48,3%)	<i>Aeromonas encheleia</i>
9 W1 1	<i>Aeromonas hydrophila/caviae</i> (99,9%)	<i>Aeromonas media</i>
9 S1 1	<i>Aeromonas sobria</i> (94,8%)	<i>Aeromonas veronii</i>
34 F2 1	<i>Aeromonas hydrophila/caviae</i> (99,9%)	ND
34 W2 2	<i>Vibrio alginolyticus</i> (70,0%)	<i>Aeromonas sobria</i>
42 W3 3	<i>Aeromonas hydrophila/caviae</i> (99,9%)	<i>Aeromonas veronii</i>
2 F1 1	<i>Aeromonas hydrophila/caviae</i> (99,8%)	<i>Aeromonas bestiarum</i>
4 F4 1	<i>Vibrio alginolyticus</i> (87,4%)	<i>Aeromonas sobria</i>
4 W4 1	<i>Aeromonas hydrophila/caviae</i> (91,5%)	<i>Aeromonas salmonicida</i>
31 F4 3	<i>Vibrio alginolyticus</i> (70,0%)	<i>Aeromonas sobria</i>

### Methodology:

- Identification of bacterial isolates was performed using **API 20NE** and **MALDI-TOF MS**.
- Comparison of the identification was performed for 21 *Aeromonas* spp. and 3 *Vibrio* isolates identified with API 20NE.

All *Aeromonas* spp. identified by API were confirmed by MALDI-TOF.

Tree isolates misidentified as *Vibrio* by API were reclassified as *Aeromonas* spp. by MALDI-TOF.

## 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 of *Aeromonas* spp. for AMR surveillance in aquaculture.
- ➔ MALDI-TOF provides a faster and more accurate alternative for genus-level identification, though its full potential is currently restricted by incomplete reference databases.
- ➔ Future work will include phenotypic and genomic analyses: 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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