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

- **Recruitement of the fish farms.**
- **Isolation and identification of *Aeromonas* spp.** from fish, water, and sediment samples.

Recruitement of the fish farms

- **22** out of 44 Belgian fish farms agreed to participate in the study (Fig.1).
- **Sample collection:** Conducted biannually for two years.

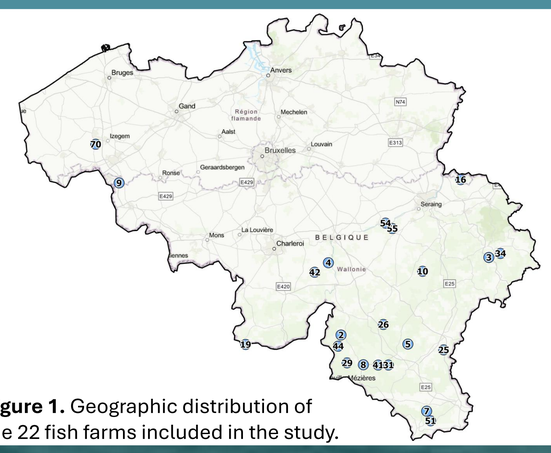

Figure 1. Geographic distribution of the 22 fish farms included in the study.

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.**: 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.

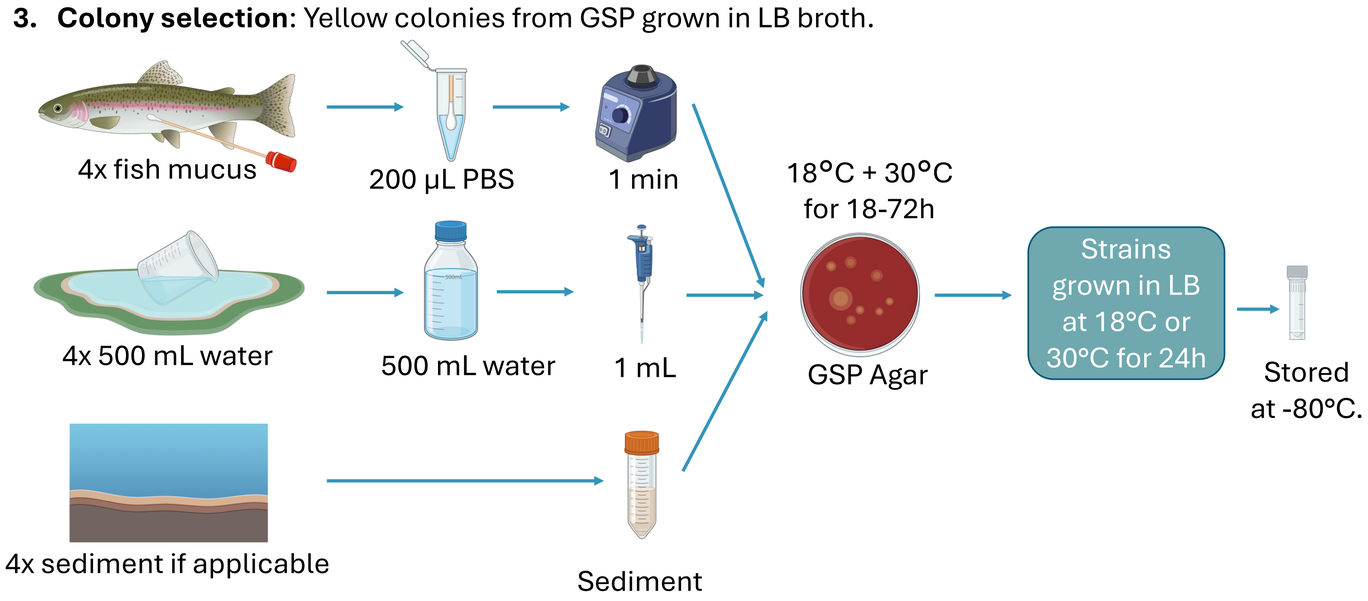

Figure 2. Protocol for the isolation of *Aeromonas* spp.

Results:

Distribution analysis showed water samples yielded the most *Aeromonas* spp. isolates, followed by fish and 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 *Aeromonas* spp.

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

Sample ID	API 20NE	MALDI-TOF
70 F1 1	*Aeromonas hydrophila/caviae*	*Aeromonas sobria*
70 W1 1	*Aeromonas hydrophila/caviae*	*Aeromonas bestiarum*
70 S1 1	*Aeromonas hydrophila/caviae*	*Aeromonas veronii*
55 W4 1	*Aeromonas sobria*	*Aeromonas hydrophila*
55 S1 2	*Aeromonas hydrophila/caviae*	*Aeromonas hydrophila*
54 F3 4	*Aeromonas sobria*	*Aeromonas veronii*
3 W2 1	*Aeromonas hydrophila/caviae*	No organism identification possible
29 F1 2	*Aeromonas hydrophila/caviae*	*Aeromonas sobria*
19 S1 3	*Aeromonas hydrophila/caviae*	*Aeromonas hydrophila*
41 S1 1	*Aeromonas hydrophila/caviae*	*Aeromonas salmonicida*
26 W1 1	*Aeromonas hydrophila/caviae*	*Aeromonas veronii*
26 S1 1	*Aeromonas hydrophila/caviae*	*Aeromonas eucrenophila*
16 F1 2	*Aeromonas hydrophila/caviae*	*Aeromonas sobria*
16 W1 1	*Aeromonas hydrophila/caviae*	*Aeromonas eucrenophila*
51 W1 2	*Aeromonas hydrophila/caviae*	*Aeromonas encheleia*
9 W1 1	*Aeromonas hydrophila/caviae*	*Aeromonas media*
9 S1 1	*Aeromonas sobria*	*Aeromonas veronii*
34 W2 2	*Vibrio alginnolyticus*	*Aeromonas sobria*
42 W3 3	*Aeromonas hydrophila/caviae*	*Aeromonas veronii*
2 F1 1	*Aeromonas hydrophila/caviae*	*Aeromonas bestiarum*
31 F4 3	*Vibrio alginnolyticus*	*Aeromonas sobria*
4 F4 1	*Vibrio alginnolyticus*	*Aeromonas sobria*
4 W4 1	*Aeromonas hydrophila/caviae*	*Aeromonas salmonicida*

Methodology:

- Identification of bacterial isolates was performed using **API 20NE** (35 isolates) and **MALDI-TOF MS** (376 isolates).
- Comparison of the identification was performed for 20 *Aeromonas* spp. and 3 *Vibrio* isolates identified with API 20NE.

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

Three 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.

Acknowledgements

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