

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

M. Keppenne¹, C. Boland², M. Cargnel³, S. Lok Yee Tong⁵, P. Butaye^{4,5}, D. Thiry¹

1. Laboratory of Bacteriology, Department of Infectious and Parasitic Diseases, FARAH, Faculty of Veterinary Medicine, University of Liège, 4000 Liège, Belgium

2. Veterinary Bacteriology Service, Department of Infectious Diseases in Animals, Sciensano, 1050 Brussels, Belgium.

3. Coordination of Veterinary Activities and Veterinary Epidemiology, Infectious Diseases in Animals Department, Sciensano, 1050 Brussels, Belgium.

4. Department of Pathobiology, Pharmacology and zoological Medicine, Faculty of Veterinary Medicine, Ghent University, 9820 Merelbeke, Belgium.

5. Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, Kowloon, Hong Kong, China.

BACKGROUND

AMR in Belgian Aquaculture

- No fish-specific authorized vaccines in Belgium:**
Farmers rely on antimicrobials to treat fish diseases.
- No fish-specific authorized antibiotics in Belgium:**
Veterinarians use products intended for other species.
- No systematic AMR monitoring:**
Aquaculture lacks regular surveillance >< other livestock.

OBJECTIVES

Study goals

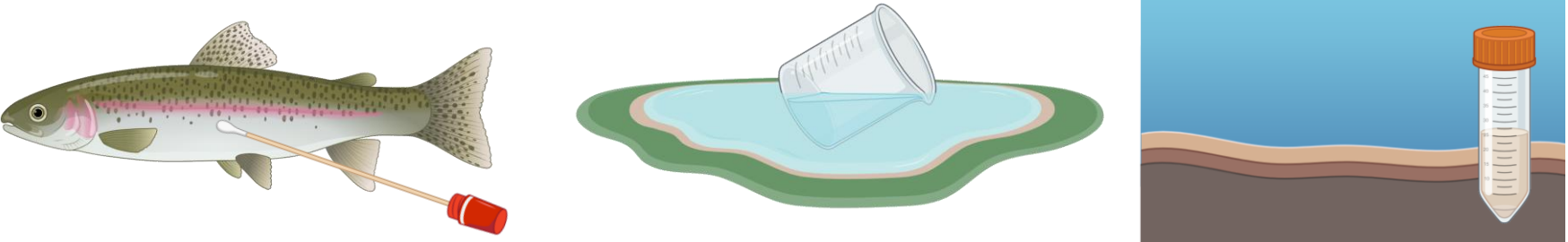
- ➔ Recruit participating fish farms across Belgium.
- ➔ Isolate *Aeromonas* spp. from fish mucus, water and sediment.
- ➔ Compare API 20NE vs. MALDI-TOF identification methods.
- ➔ Evaluate *Aeromonas* spp. as an AMR surveillance indicator.

METHODS - ISOLATION

Sampling protocol

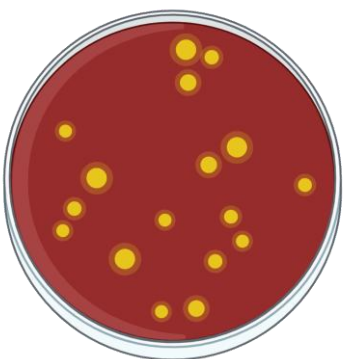
1

Sample collection (January-March 2025)
4 fish mucus (PBS), 4 water samples (1000 mL) and 4 sediments where possible · 22 farms



2

Selective plating on GSP
Mucus 20 µL PBS · Water 0.8 mL · Sediment 10 µL
Yellow colonies selected



3

Temperature incubation
18–72 h at 18 °C and 30 °C in parallel

4

Cryopreservation
LB broth 24 h → storage at –80 °C

18 °C
Selected temperature
Genetic stability preserved

30 °C
Comparative only
Parallel incubation

METHODS - IDENTIFICATION

Bacterial identification

- API 20NE:**
Biochemical identification of 53 isolates screened at 18 °C.
- MALDI-TOF MS:**
Identification of 426 isolates screened at 18 °C.
- Comparison:**
API 20NE vs. MALDI-TOF.

PERSPECTIVES

Upcoming analysis

- Antimicrobial susceptibility tests:**
EUAQUAN1 Sensititre™ panel (ThermoFisher)
- Whole-Genome Sequencing:**
Nanopore technology
- Resistome characterization:**
AMR gene detection & genetic architecture

RESULTS - RECRUITMENT

22 Fish farms enrolled

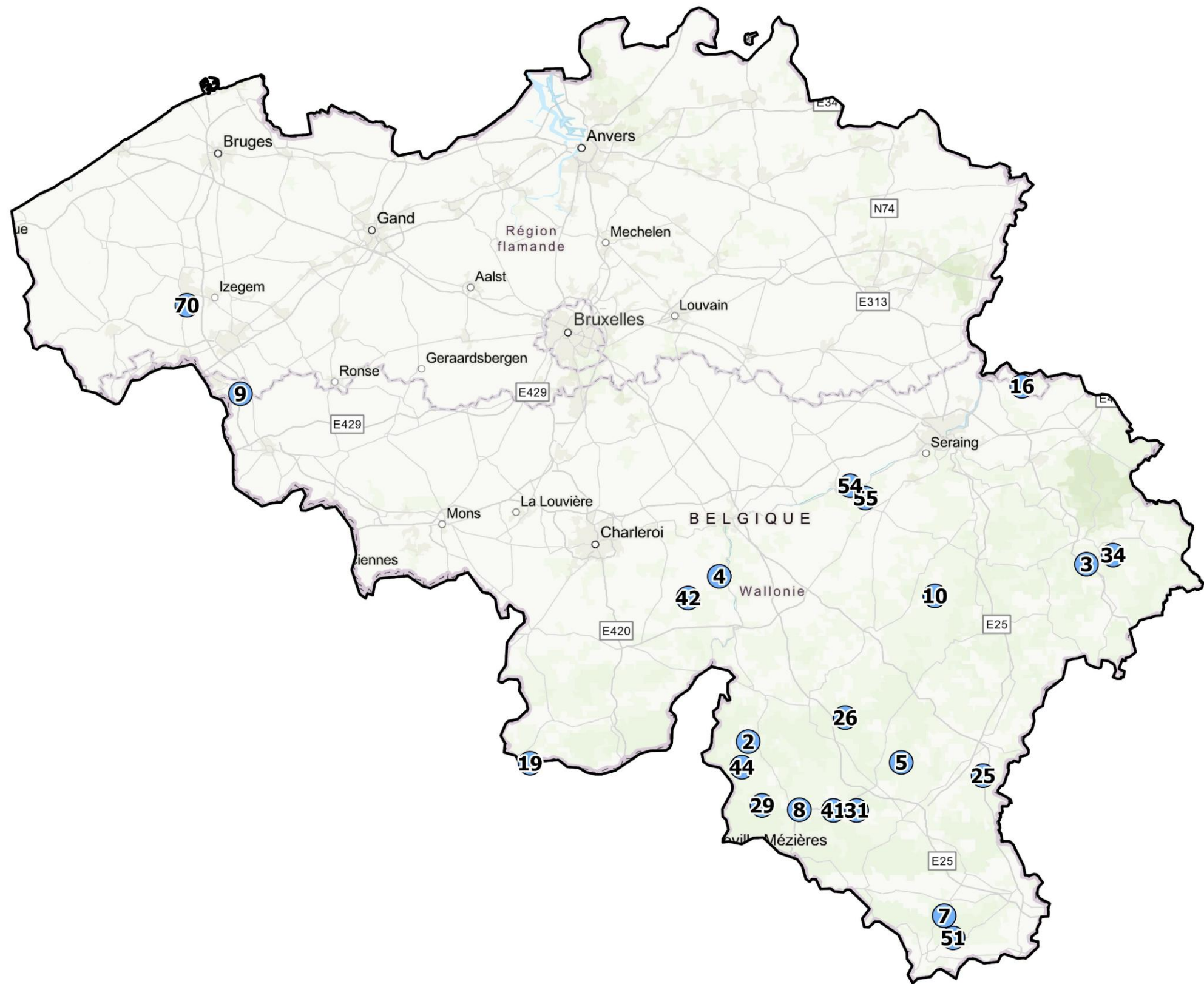


Figure 1. Geographic distribution of enrolled farms.

RESULTS - ISOLATION

886 Bacterial isolates

Table 1. Distribution of isolates by incubation temperature and sample type

Sample type	18 °C	30 °C	Total
Water	196	246	442
Fish	161	133	294
Sediment	69	81	150
Total	426	460	886

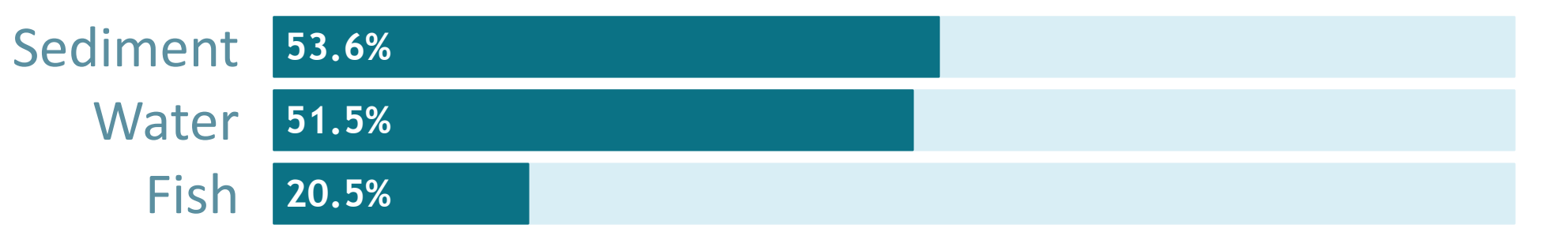
RESULTS –MALDI-TOF IDENTIFICATION

Aeromonas detection rate

171
Aeromonas spp. isolates confirmed by MALDI-TOF

40.1%
of 426 screened isolates confirmed as *Aeromonas* spp.

Detection rate by sample type



Aeromonas species detected

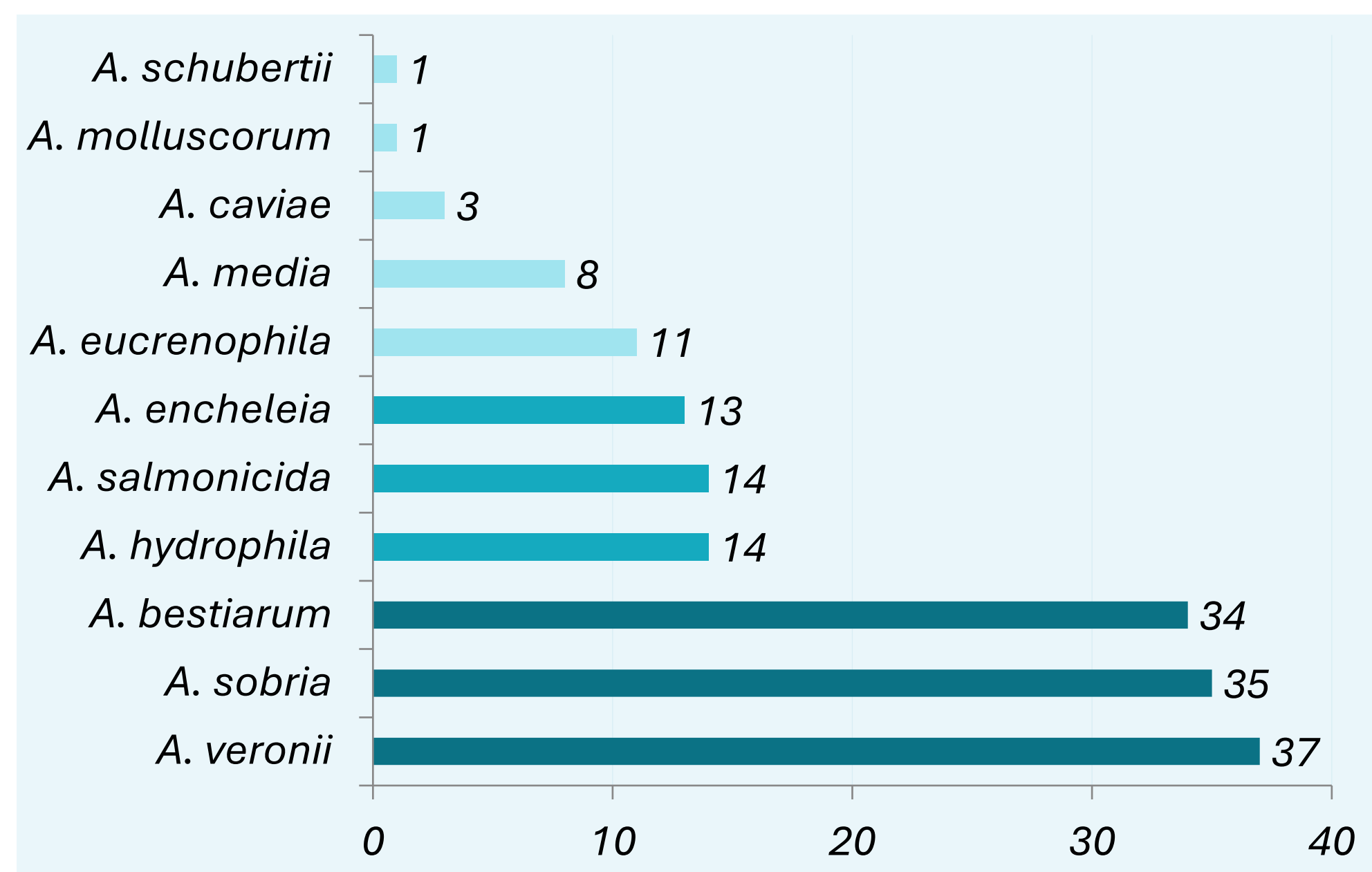


Figure 2. Distribution of *Aeromonas* species by MALDI-TOF

RESULTS - COMPARISON

API 20NE vs. MALDI-TOF (n = 53)

Table 2. Comparison of API 20NE and MALDI-TOF MS identifications for bacterial isolates recovered at 18 °C from 22 Belgian fish farms

Sample	API 20NE identification	MALDI-TOF identification	Agreement
70 F1	<i>Aeromonas hydrophila/caviae</i> (44.9%)	<i>Aeromonas sobria</i>	✓
70 W1	<i>Aeromonas hydrophila/caviae</i> (99.5%)	<i>Aeromonas bestiarum</i>	✓
70 S1	<i>Aeromonas hydrophila/caviae</i> (99.9%)	<i>Aeromonas veronii</i>	✓
55 F1	<i>Acinetobacter lwoffii</i> (91.7%)	<i>Acinetobacter modestus</i>	✓
55 W4	<i>Aeromonas hydrophila/caviae</i> (97.3%)	<i>Aeromonas hydrophila</i>	✓
55 S1	<i>Aeromonas hydrophila/caviae</i> (99.9%)	<i>Aeromonas hydrophila</i>	✓
54 F3	<i>Aeromonas sobria</i> (99.2%)	<i>Aeromonas veronii</i>	✓
54 W3	<i>Acinetobacter junii/johnsonii</i> (90.7%)	<i>Acinetobacter johnsonii</i>	✓
3 F2	<i>Brevundimonas vesicularis</i> (91.5%)	<i>Flavobacterium saccharophilum</i>	✗
3 W2	<i>Aeromonas hydrophila/caviae</i> (99.3%)	<i>Aeromonas hydrophila</i>	✓
3 S1	<i>Ralstonia pickettii</i>	<i>Pseudomonas corrugata</i>	✗
29 F1	<i>Aeromonas hydrophila/caviae</i> (90.4%)	<i>Aeromonas sobria</i>	✓
29 W1	<i>Burkholderia cepacian</i>	<i>Pseudomonas protegens</i>	✗
29 S1	<i>Pseudomonas putida</i>	<i>Pseudomonas thivervalensis</i>	✓
10 F1	<i>Sphingomonas paucimobilis</i>	<i>Massilia aurea</i>	✗
10 W1	<i>Pseudomonas fluorescens</i> (99.8%)	<i>Pseudomonas mandelii</i>	✓
10 S1	<i>Pseudomonas fluorescens</i> (85.3%)	<i>Pseudomonas rhodesiae</i>	✓
19 F1	<i>Burkholderia cepacia</i> (99.9%)	<i>Pseudomonas frederiksbergensis</i>	✗
19 W1	<i>Pseudomonas fluorescens</i> (98.6%)	<i>Pseudomonas fragi</i>	✓
19 S1	<i>Aeromonas hydrophila/caviae</i> (99.9%)	<i>Aeromonas hydrophila</i>	✓
41 F1	<i>Pseudomonas putida</i> (83.1%)	<i>Pseudomonas corrugata</i>	✓
41 W3	<i>Aeromonas hydrophila/caviae</i> (99.3%)	<i>Aeromonas bestiarum</i>	✓
41 S1	<i>Aeromonas hydrophila/caviae</i> (99.8%)	<i>Aeromonas salmonicida</i>	✓
26 F1	<i>Brevundimonas vesicularis</i>	<i>Flavobacterium plurextorum</i>	✗
26 W1	<i>Aeromonas hydrophila/caviae</i> (99.8%)	<i>Aeromonas veronii</i>	✓
26 S1	<i>Aeromonas hydrophila/caviae</i> (83.2%)	<i>Aeromonas eucrenophila</i>	✓
16 F1	<i>Aeromonas hydrophila/caviae</i>	<i>Aeromonas sobria</i>	✓
16 W1	<i>Aeromonas hydrophila/caviae</i> (91.6%)	<i>Aeromonas eucrenophila</i>	✓
16 S1	<i>Pseudomonas fluorescens</i> (99.9%)	<i>Pseudomonas extremorientalis</i>	✓
51 F1	<i>Sphingobacterium multivorum</i> (99.5%)	<i>Flavobacterium piscis</i>	✗
51 W1	<i>Aeromonas hydrophila/caviae</i> (48.3%)	<i>Aeromonas encheleia</i>	✓
9 F1	<i>Mannheimia haemolytica</i> (86.5%)	<i>Stenotrophomonas rhizophila</i>	✗
9 W1	<i>Aeromonas hydrophila/caviae</i> (99.9%)	<i>Aeromonas media</i>	✓
9 S1	<i>Aeromonas sobria</i> (94.8%)	<i>Aeromonas veronii</i>	✓
34 F2	<i>Aeromonas hydrophila/caviae</i> (99.9%)	<i>Aeromonas salmonicida</i>	✓
34 W2	<i>Vibrio alginolyticus</i> (70.0%)	<i>Aeromonas sobria</i>	~
5 F3	<i>Aeromonas sobria</i> (97.9%)	<i>Aeromonas sobria</i>	✓
5 W3	<i>Brevundimonas vesicularis</i> (91.5%)	<i>Flavobacterium araucanum</i>	—
42 F3	<i>Mannheimia haemolytica</i> (86.5%)	<i>Brevundimonas vesicularis</i>	✗
42 W3	<i>Aeromonas hydrophila/caviae</i> (99.9%)	<i>Aeromonas veronii</i>	✓
44 F1	<i>Pseudomonas fluorescens</i> (99.9%)	<i>Pseudomonas fluorescens</i>	✓
2 F1	<i>Aeromonas hydrophila/caviae</i> (99.8%)	<i>Aeromonas bestiarum</i>	✓
2 W1	<i>Mannheimia haemolytica</i> (86.5%)	<i>Flavobacterium piscis</i>	✗
8 F2	<i>Sphingobacterium multivorum</i> (99.5%)	<i>Flavobacterium araucanum</i>	✗
8 W2	<i>Sphingomonas paucimobilis</i> (97.9%)	<i>Flavobacterium gelidilacus</i>	✗
7 F1	<i>Sphingomonas paucimobilis</i> (98.8%)	<i>Flavobacterium oncorhynchi</i>	✗
7 W1	<i>Aeromonas hydrophila/caviae</i> (99.9%)	<i>Aeromonas veronii</i>	✓
31 F4	<i>Vibrio alginolyticus</i> (70.0%)	<i>Aeromonas sobria</i>	~
31 S2	<i>Mannheimia haemolytica</i> (86.5%)	<i>Iodobacter fluviatilis</i>	✗
25 F4	<i>Aeromonas hydrophila/caviae</i> (60.3%)	<i>Aeromonas sobria</i>	✓
25 W4	<i>Sphingomonas paucimobilis</i> (82.1%)	<i>Flavobacterium hibernum</i>	✗
4 F4	<i>Vibrio alginolyticus</i> (87.4%)	<i>Aeromonas sobria</i>	~
4 W4	<i>Aeromonas hydrophila/caviae</i> (91.5%)	<i>Aeromonas salmonicida</i>	✓

The leading number in each Sample ID corresponds to a randomly assigned fish farm identifier. The letter indicates sample type (F, fish mucus; W, water; S, sediment), followed by the replicate number. Agreement column: ✓ concordance at genus level; ~ reclassified as *Aeromonas*; ✗ discordance.

Key finding: All *Aeromonas* identified by API were confirmed by MALDI-TOF. Three *Vibrio* isolates (API) were reclassified as *Aeromonas* by MALDI-TOF, highlighting the limitations of biochemical identification methods.

CONCLUSIONS

Key takeaways

- 18 °C selected** for all subsequent isolations to preserve genetic stability and reduce stress-induced rearrangements.
- Water samples** appear to be the most reliable source of *Aeromonas* spp. for AMR surveillance in aquaculture.
- MALDI-TOF** provides faster, more accurate genus-level identification, though limited by incomplete reference databases

Acknowledgements

This study was supported by the Federal Public Service through the research project RT 24/01 AquAMR 2.



Contact: mado.keppenne@uliege.be