VIBRIO AND *AEROMONAS* IN FRESHWATER: A FOCUS ON *V. CHOLERAE* AND *A. SALMONICIDA* TWO « ZOONOTIC » PATHOGENS?

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CONNAÎTRE, ÉVALUER, PROTÉGER

Why *Vibrio cholerae* & *Aeromonas salmonicida*?

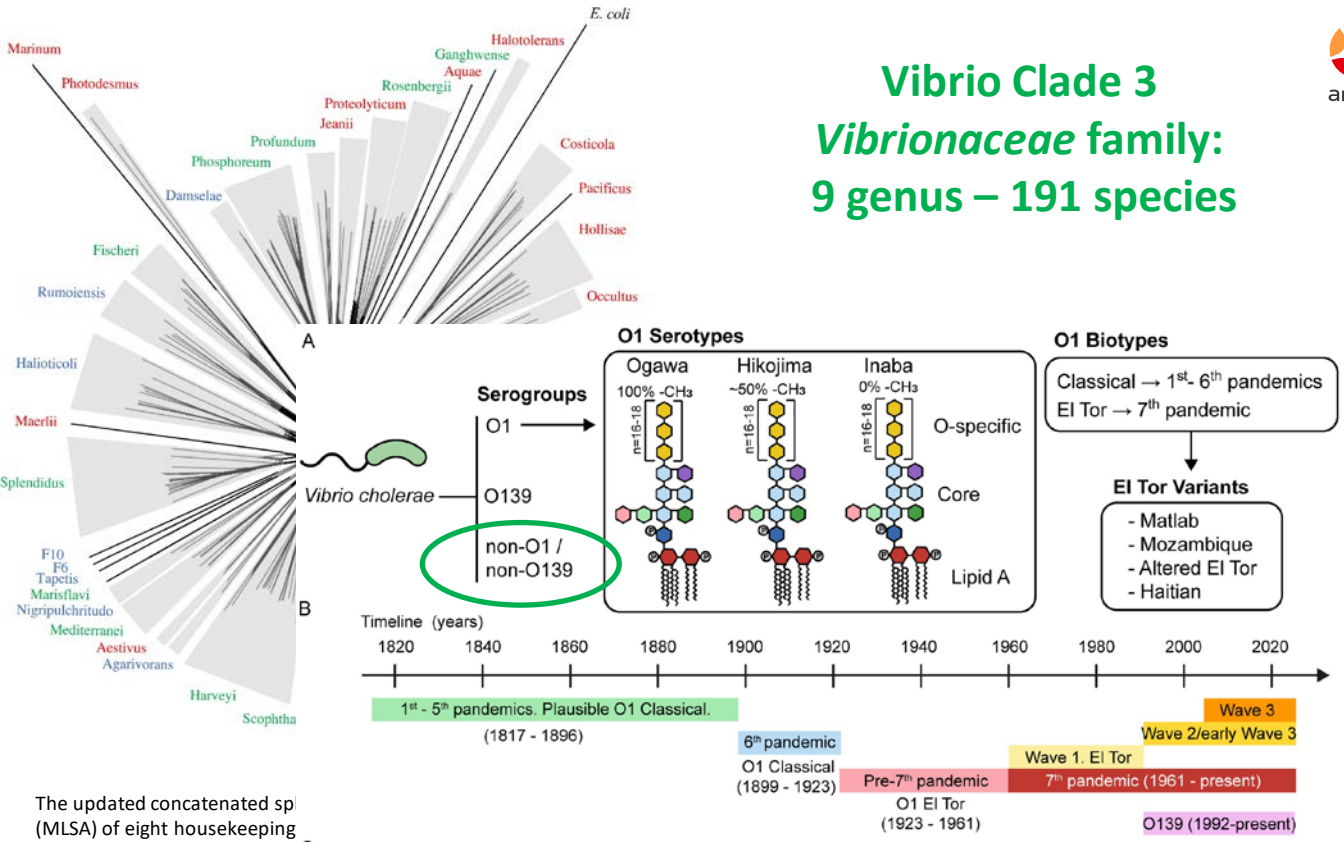
autochtonous of aquatic environment

Gram negative, oxydase +

La pisciculture – STEB (aquaculteurs-de-bretagne.fr)

Complex and constantly evolving taxonomy

Vibrio Clade 3
Vibrionaceae family:
9 genus – 191 species

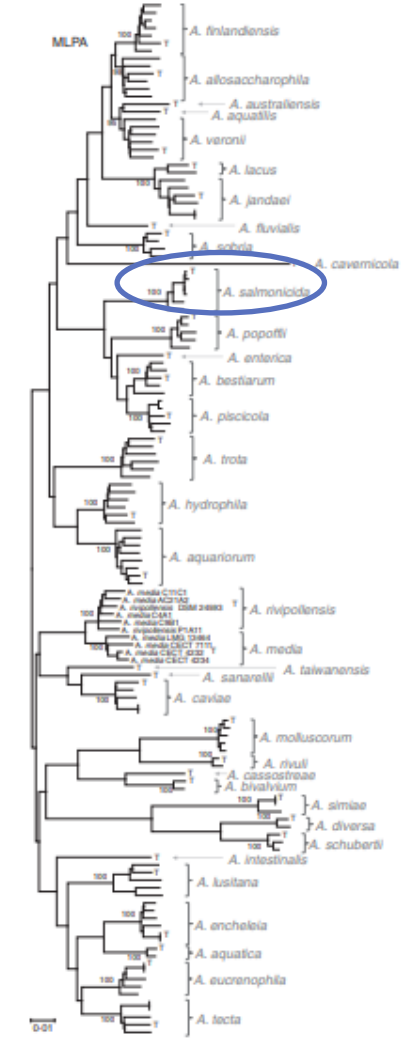


The updated concatenated sp
(MLSA) of eight housekeeping
species. The ftsZ, gapA, gyrB, mreB, pyrH, recA, rpoA, and topA gene sequences were concatenated and the tree was reconstructed using the SplitsTree4 ver. 4.14.8. Clades indicated by red, green, and blue represent the “new,” “emended,” and “unchanged” clades, respectively

Jiang C et al. Curr Microbiol. 2021

36 species of *Aeromonas*

anses



5 subspecies of *Aeromonas salmonicida* (*A. sal*):

*A. sal. subsp. salmonicida – psychrophilic**

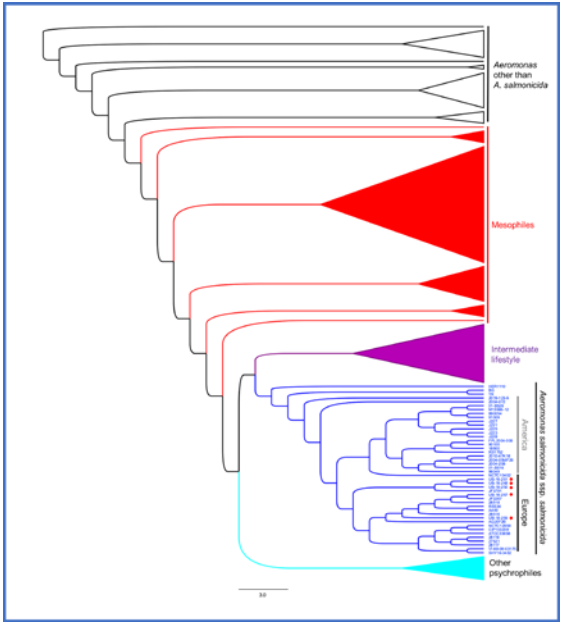
A. sal. subsp. achromogenes - psychrophilic ***

*A. sal. subsp. masoucida - psychrophilic***

A. sal. subsp. pectinolytica - mesophilic

*A. sal. subsp. smithia - psychrophilic***

\+ myriad of unclassified strains



**

Complex and constantly evolving taxonomy…

- Difficulties of identification
- Ecological meaning of the « new described » species?
- Impact in Human & Animal Health?

Ecotype (Fred Cohan 2002) ?

Eukaryote systematists have developed a universal concept of species: A species is a group of organisms whose divergence is capped by a force of cohesion; divergence between different species is irreversible; and different species are ecologically distinct. In the case of bacteria, these universal properties are held not by the named species of systematics but by ecotypes. These are populations of organisms occupying the same ecological niche, whose divergence is purged recurrently by natural selection.

Can *V. cholerae* and *Aeromonas* spp be considered as candidates to be potential indicators of antimicrobial resistance dissemination in the aquatic environment?

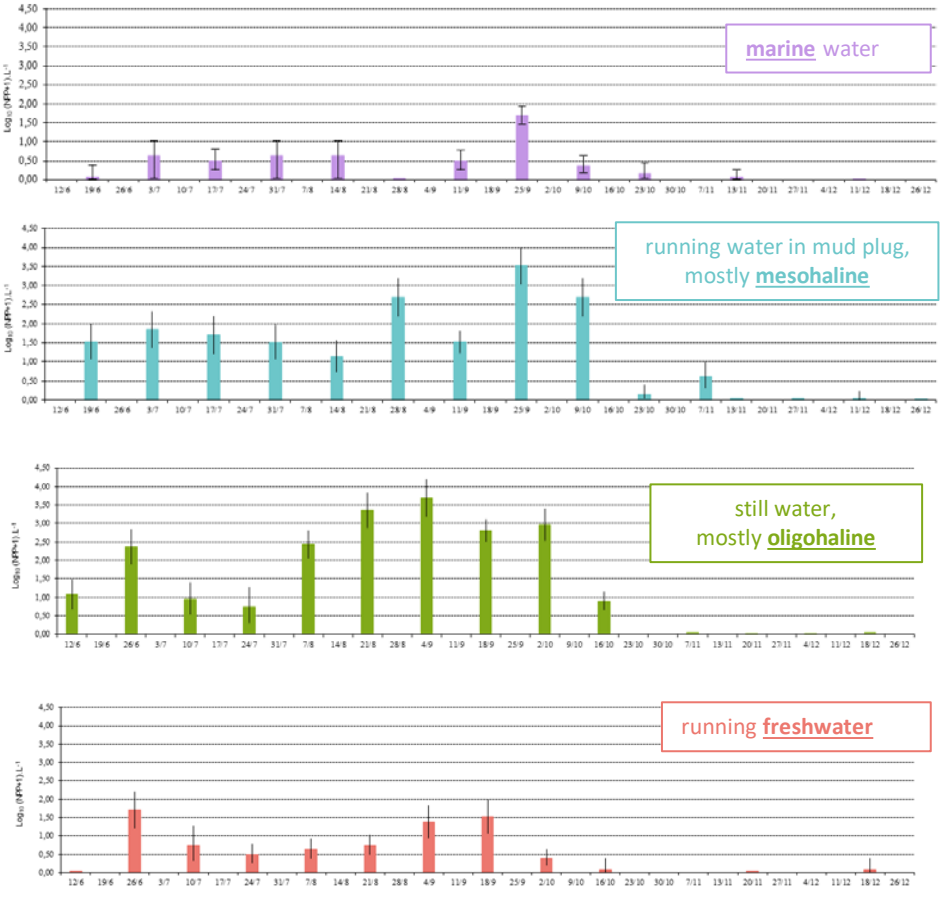
Criteria to be fulfilled:

- ❒ An easy and frequent detection in aquatic environment
- ❒ Accurate and rapid identification tools
- ❒ A capacity to acquire and exchange genes
- ❒ Cut-off values to categorize the isolates between wild type and non-wild type.

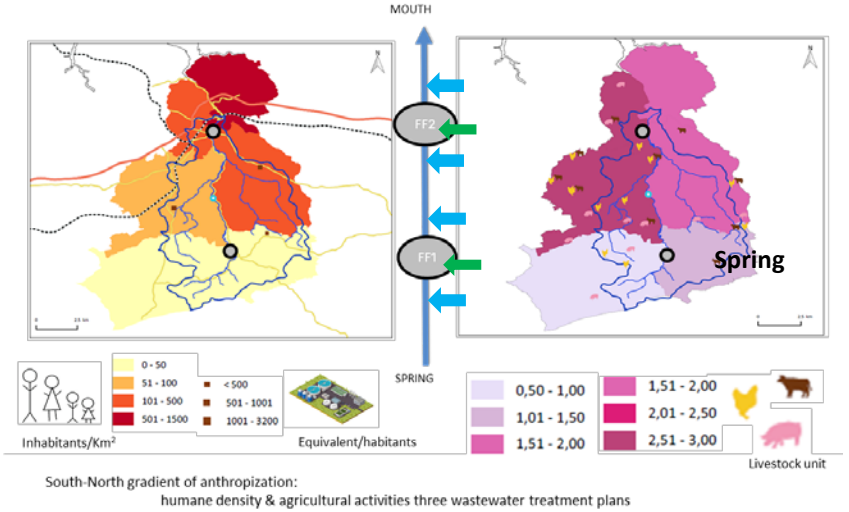
Vibrio cholerae – *Aeromonas spp.* ?

An easy and frequent detection in aquatic environment: *V. cholerae*

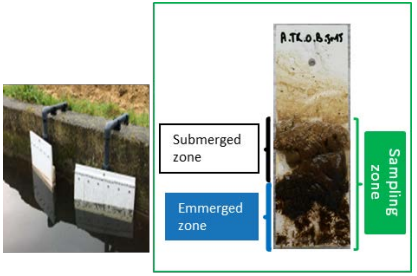
V. c abundances between June – December en $\log_{10}$.(MPN+1).L^{-1}



An easy and frequent detection in aquatic environment: Aeromonas spp.



South-North gradient of anthropization:
humane density & agricultural activities three wastewater treatment plans



Device for biofilm sampling in ponds

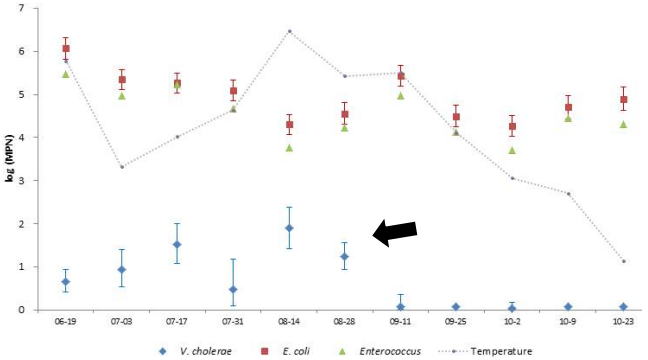
Aeromonas abundances during 17 months (CFU/mL)

	Nb samples	Prevalence[1]	Abundances[2]	
Up. FF1	36	100%	10^0	10^3
Biofilm FF1	36	100%	$>10^3$	$>10^6$
Down. FF1	36	100%	10^1	10^4
Up. FF2	36	100%	10^1	10^3
Biofilm FF2	36	100%	$>10^3$	$>10^6$
Down. FF2	36	100%	10^1	10^3

[1]positive samples, [2]Min – Max (CFU/mL), WW: wastewater, FF: Fishfarm

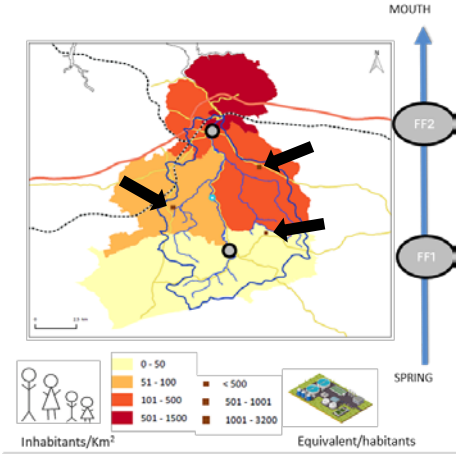
Detection in raw and treated wastewater ?

Vibrio cholerae (MPN/L): June-December



Aeromonas spp. (CFU/mL): January - June

	Nb samples	Prevalence	Abundances Min – Max	
Raw 1	3	100%	$>10^5$	$>10^5$
Treated 1	3	100%	$>10^3$	$>10^5$
Raw 2	5	100%	$>10^2$	$>10^5$
Treated 2	5	100%	$>10^3$	$>10^5$
Raw 3	5	100%	$>10^4$	$>10^5$
Treated 3	5	100%	$>10^2$	$>10^3$



An easy and frequent detection in aquatic environment

Vibrio cholerae: easy – period of no detection *Aeromonas* easy – detection all year long

Accurate and rapid identification tools

A capacity to acquire and exchange genes

Cut-off values to categorize the isolates between wild type and non-wild.

Accurate and rapid identification tools

	PCR	Maldi-Tof
Genus *Vibrio*	no	yes
Species of *Vibrio*	-	(no)
Vibrio cholerae	yes	yes
Genus *Aeromonas*	yes	yes
Species of *Aeromonas*	-	no
Aeromonas salmonicida	yes	no

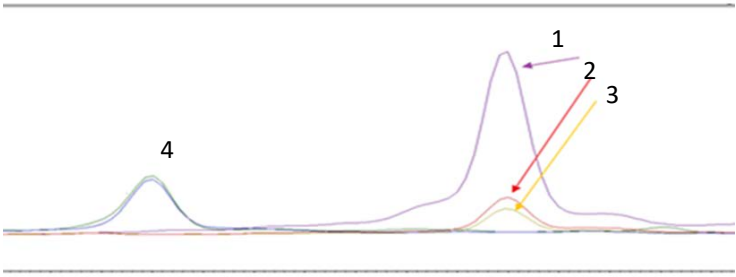
Collaboration with BFR

Almost ready …

Improvement of database (Maldi-ToF)

Species	n	Bruker database		anses database	
		DD	DA	DD	DA
Aeromonas bestiarum	68	91%	96%	99%	99%
Aeromonas popoffii	20	5%	0%	70%	80%
Aeromonas rivuli	1	0%	0%	100%	100%
A. salmonicida	78	87%	78%	83%	96%

Detection of biomarkers (Maldi-ToF)



1 *A. sobria*, **2** *A. bestiarum*,
3 *A. enterica*, **4** *A. salmonicida* & *A. enterica*

An easy and frequent detection in aquatic environment

Vibrio cholerae: ease - not detection period

Aeromonas spp: ease – detection all the year

Accurate and rapid identification tools

Vibrio cholerae: PCR & Maldi-ToF

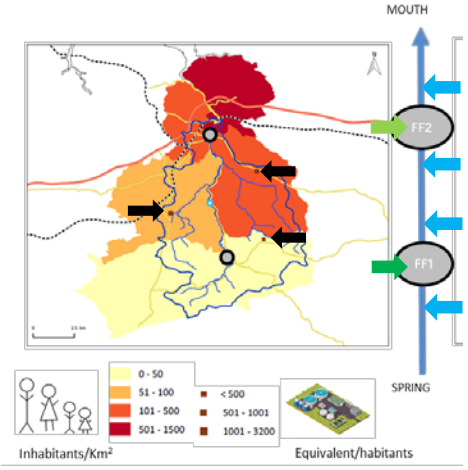
Aeromonas spp: PCR & Maldi-ToF

A capacity to acquire and exchange genes

Cut-off values to categorize the isolates between wild type and non-wild.

A capacity to acquire and exchange genes: mobile genetic element

Aeromonas spp. In water & biofilm of fishfarm: Integron



601 isolates – 98 harbored integron (16,3%)

	Nb samples	Positive samples	Nb isolates	% integron
S1	18	100%	85	1.2% (1)
S6	18	100%	87	27.6% (24)
Raw WW1	3	100%	30	3.3% (1)
Treated WW1	3	100%	30	10.0% (3)
Raw WW2	5	100%	49	2.0% (1)
Treated WW2	5	100%	49	6.1% (3)
Raw WW3	5	100%	48	25.0% (12*)
Treated WW3	5	100%	48	6.25% (3)
Biofilm FF1	18	61.1%	87	24.4% (21)
Biofilm FF2	18	66.7%	88	32.9% (29)

- Increase of integron in water from spring to mouth (X23)
- Average in raw wastewater: 11.6%, in treated wastewater: 12.6% (2% -25%)
- No difference in Biofilm

A capacity to acquire and exchange genes: mobile genetic element

Aeromonas & fish: Integron

Aquaculture 576 (2023) 739768

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Integrons from *Aeromonas* isolates collected from fish: A global indicator of antimicrobial resistance and anthropic pollution

Olivier Barraud [a,1], Lucie Laval [a,1], Laëtitia Le Devendec [b], Emeline Larvor [b], Claire Chauvin [c], Eric Jouy [b], Sophie Le Bouquin [c], Yann Vanrobaeys [b], Benoit Thuillier [d], Brigitte Lamy [e,f], Sandrine Baron [b,*]

Integrons detected from *Aeromonas* collections.

Fish category (number of isolates)	*Aeromonas salmonicida* ($n = 39$)		*Aeromonas* spp. non *salmonicida* ($n = 340$)		All *Aeromonas* ($n = 379$)	
	Integron positive	Integron negative	Integron positive	Integron negative	Integron positive	Integron negative
Wild Fish – WF ($n = 78$)	0	0	0	78	0	78
Farmed fish – FF ($n = 231$)	0	0	35	196	35	196
Diseased fish – DF ($n = 70$)	25*	14	9	22	34*	36
Total ($n = 379$)	25*	14	44	296	69*	310

*Includes the only class 2 integron.

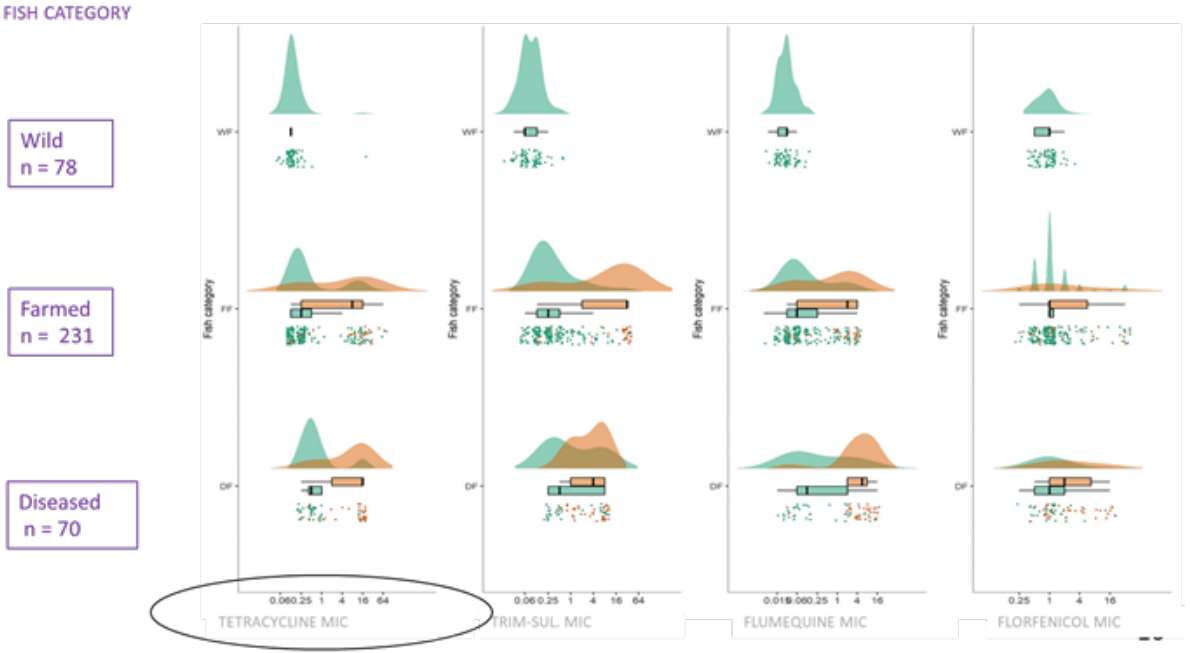
Highest frequencies of integron in *A. salmonicida* isolates

A. salmonicida isolates in diseased fish and farmed fish

A capacity to acquire and exchange genes: mobile genetic element

Aeromonas & fish: Integron

Minimum inhibitory concentration (MIC) probability density, box plot and scatter plot by fish category
WF: wild fish; FF: farmed fish; DF: diseased fish
- according to integrons presence (orange) or absence

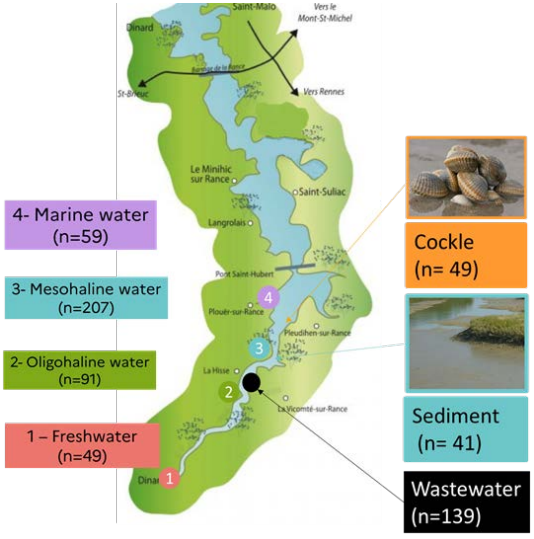


Integron negative | Integron positive

↳Lowest MIC for isolates without integron
↳ Difference between « fish » population

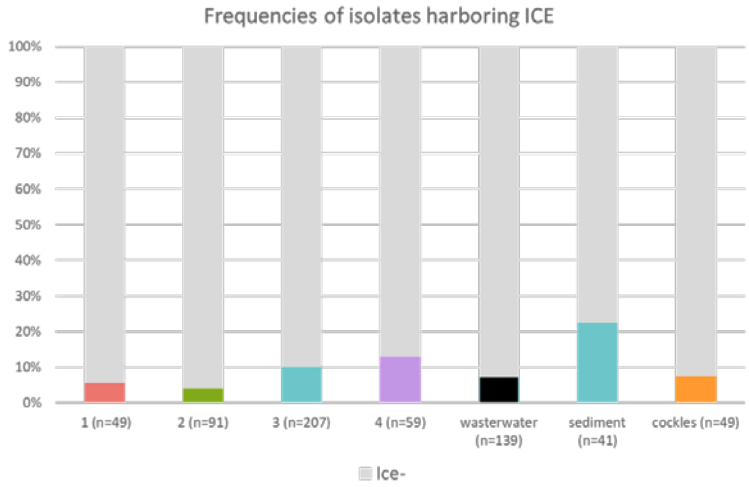
A capacity to acquire and exchange genes: mobile genetic element

Vibrio cholerae: Integrative Conjugative Element



V. cholerae isolation was conducted from water, sediment and cockles samples collected along a salinity gradient under a temperate climate (France) between June 2000 and September 2001

The 635 isolates were subjected to a molecular survey on the presence of ICE



ICE of the SXT/R391 family was detected in 10.9% of the analyzed strains (n=64)

isolates carrying ICE were further subjected to paired-end, short read whole-genome sequencing (WGS) for in depth characterization (BfR) …

An easy and frequent detection in aquatic environment

Vibrio cholerae: ease - not detection period

Aeromonas spp: ease – detection all the year

Accurate and rapid identification tools

Vibrio cholerae: PCR & Maldi

Aeromonas spp: PCR & Maldi

A capacity to acquire and exchange genes

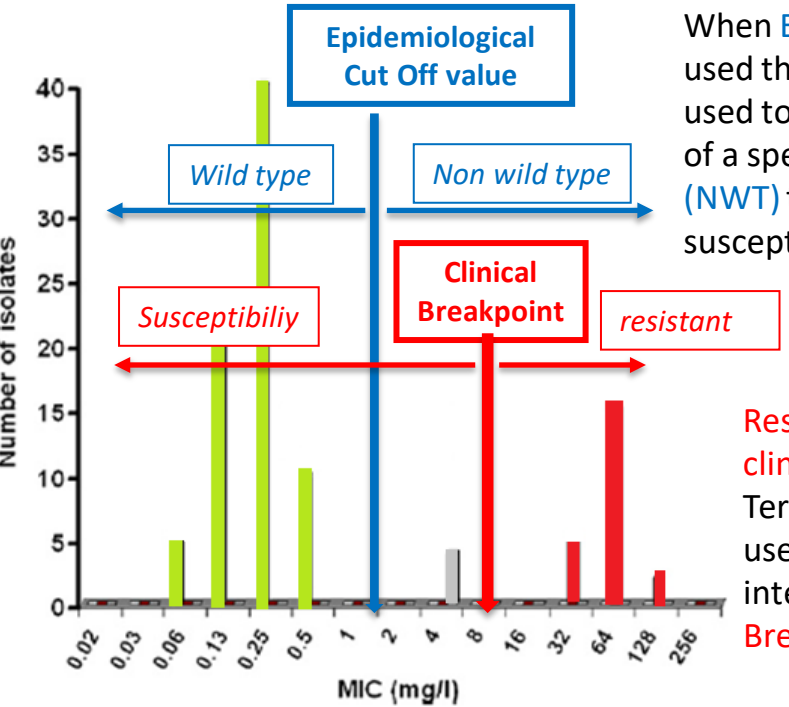
Vibrio cholerae: ICE

Aeromonas spp: INTEGRON

Cut-off values to categorize the isolates between wild type and non-wild.

Epidemiological Cut-off values to categorize the isolates between wild type and non-wild.

Clarification of the appropriate terminology by Silley (2012)



When Epidemiological Cut Off value are used the term Wild-type (WT) shoud be used to refer to fully susceptible members of a species and the term non-wild Type (NWT) to isolates that manifest a reduced susceptibility

Resistance should only be used to refer to clinical resistance.

Terms resistant and sensitive should only be used when susceptibility data has been interpreted by application of Clinical Breakpoint

Ecoff …

Vibrio cholerae non-O1/non-O139

Epidemiological cut-off values for non-O1/non-O139 *Vibrio cholerae* disc diffusion data generated by standardised methods

Peter Smith*, Laëtitia Le Devendec, Eric Jouy, Emeline Larvor, Jean Lesne, Alexander K. T. Kirschner, Carmen Rehm, Melanie Leopold, Sonja Pleininger, Florian Heger, Claudia Jäckel, Cornelia Göllner, Jonas Nekat, Jens Andre Hammerl, Sandrine Baron

Agent	CO_{WT} (mm)	S (mm)		Difference (CO_{WT} – CB)
		M45[a]	M100[b]	
Aminoglycosides				
Amikacin	≥18	≥17[c]		1
Gentamicin	≥16	≥15[c]		1
Streptomycin	≥13		≥15	-2
Aminopenicillins				
Ampicillin	≥19	≥17		2
Amoxicillin/ clavulanate	≥18	≥18[c]		0
Carbapenems				
Meropenem	≥25	≥23[c]		2
Imipenem	≥23	≥23[c]		0
Cephems				
Cefotaxime	≥31	≥26[c]		5
Ceftazidime	≥24	≥21[c]		3
Cefepime	≥27	≥25[c]		2
Folate pathway inhibitors				
Trimethoprim-sulfamethoxazole	≥24	≥16		8
Trimethoprim	≥23		≥16	7
Macrolides				
Erythromycin	≥16	na	na	
Phenicols				
Chloramphenicol	≥24	≥18		5
Florfenicol	≥27	na	na	
Quinolones				
Norfloxacin	≥28		≥17	11
Ciprofloxacin	≥31	≥21		10
Nalidixic acid	≥29		≥19	10
Tetracyclines				
Tetracycline	≥23		≥15	8

Publication in press

Aeromonas spp.


frontiers in Microbiology

ORIGINAL RESEARCH published: 28 March 2017 doi: 10.3389/fmicb.2017.00530

***Aeromonas* Diversity and Antimicrobial Susceptibility in Freshwater—An Attempt to Set Generic Epidemiological Cut-Off Values**

Sandrine Baron[1,2]*, Sophie A. Granier[1], Emeline Larvor[1,2], Eric Jouy[1,2], Maelan Cineux[1,2], Amandine Wilhelm[4], Benoit Gassilloud[4], Sophie Le Bouquin[2,3], Isabelle Kempf[1,2] and Claire Chauvin[2,5]

Provisional Ecoff for 12 antibiotics.

Aeromonas salmonicida (in press).

	IZ data (mm)			
Agent[a]	AMP	FLO	OXO	OXY
VET 04[b]	na	≥27	≥30	≥28
This work[c]	≥29	≥30	≥32	≥25

	MIC data (µg/ml)		
Agent[a]	FLO	OXO	OXY
VET 04[b]	≤4	≤0.125	≤1
This work[c]	≤4	≤0.125	≤1

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Aeromonas spp: ease – detection all the year

Accurate and rapid identification tools

Vibrio cholerae: PCR & Maldi

Aeromonas spp: PCR & Maldi

A capacity to acquire and exchange genes

Vibrio cholerae: ICE

Aeromonas spp: INTEGRON

Cut-off values to categorize the isolates between wild type and non-wil

Vibrio cholerae: 19 antibiotics

Aeromonas spp & A. salmonicida: 12 & 4 antibiotics

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Cut-off values to categorize the isolates between wild type and non-wild.

Vibrio cholerae: 19 antibiotics

Aeromonas spp & A. salmonicida: 12 & 4 antibiotics

Vibrio cholerae << *Aeromonas spp*

BUT - Intrinsic resistance in *Aeromonas:* C3G & carbapenem

- Diversity of species

AND *Vibrio cholerae* susceptible to C3G and carbapenem

Can they be considered as zoonotic pathogen?

V. cholerae - *Aeromonas salmonicida*: zoonotic pathogen?

With climate change:

increase of density in water

increase of humane cases

microorganisms

Article

Phenotypic and Genotypic Properties of *Vibrio cholerae* non-O1, non-O139 Isolates Recovered from Domestic Ducks in Germany

Nicola Hirsch [1], Eva Kappe [1], Armin Gangl [1], Keike Schwartz [2], Anne Mayer-Scholl [2], Jens Andre Hammerl [2] and Eckhard Strauch [2,*]

Infection due to:

V. c non-O1/non-O139

Also in goat, lambs, horse, american buffalo …

A. salmonicida

Mesophilic one* …

Detection in « healthy »...

V. c non-O1/non-O139 -VIM-1 and VIM-4 yellow-legged gull in southern France

Aquatic animal, terrestrial animal

A. salmonicida

Aquatic animal, pig**

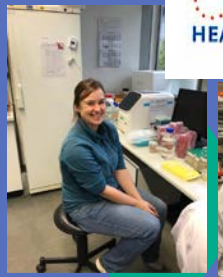
Contamination between animal species:

Great egret*

catfish tilapia

Great cormorant

Collaboration BfR-Anses
Vibrio spp – Aeromonas spp:
Phage & Aquaculture



In BfR
Nov 2022

In Anses
May 2023

Short-Term Mission

In 2024 STM in BfR and STM in Anses . . .

Short-Term Mission

anses

DANKE …

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