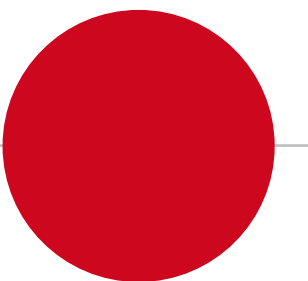


Major Bacterial Pathogens and Antimicrobial Susceptibility Testing (AST) in Aquaculture

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● Outline

Major bacterial disease of freshwater aquaculture

Diseases caused by Gram positive bacteria:

- Piscine Streptococcosis

Diseases caused by Gram negative bacteria:

- Mobile Aeromonas Septicaemia
- Flavobacteriosis
- Edwardsiellosis
- Yersiniosis

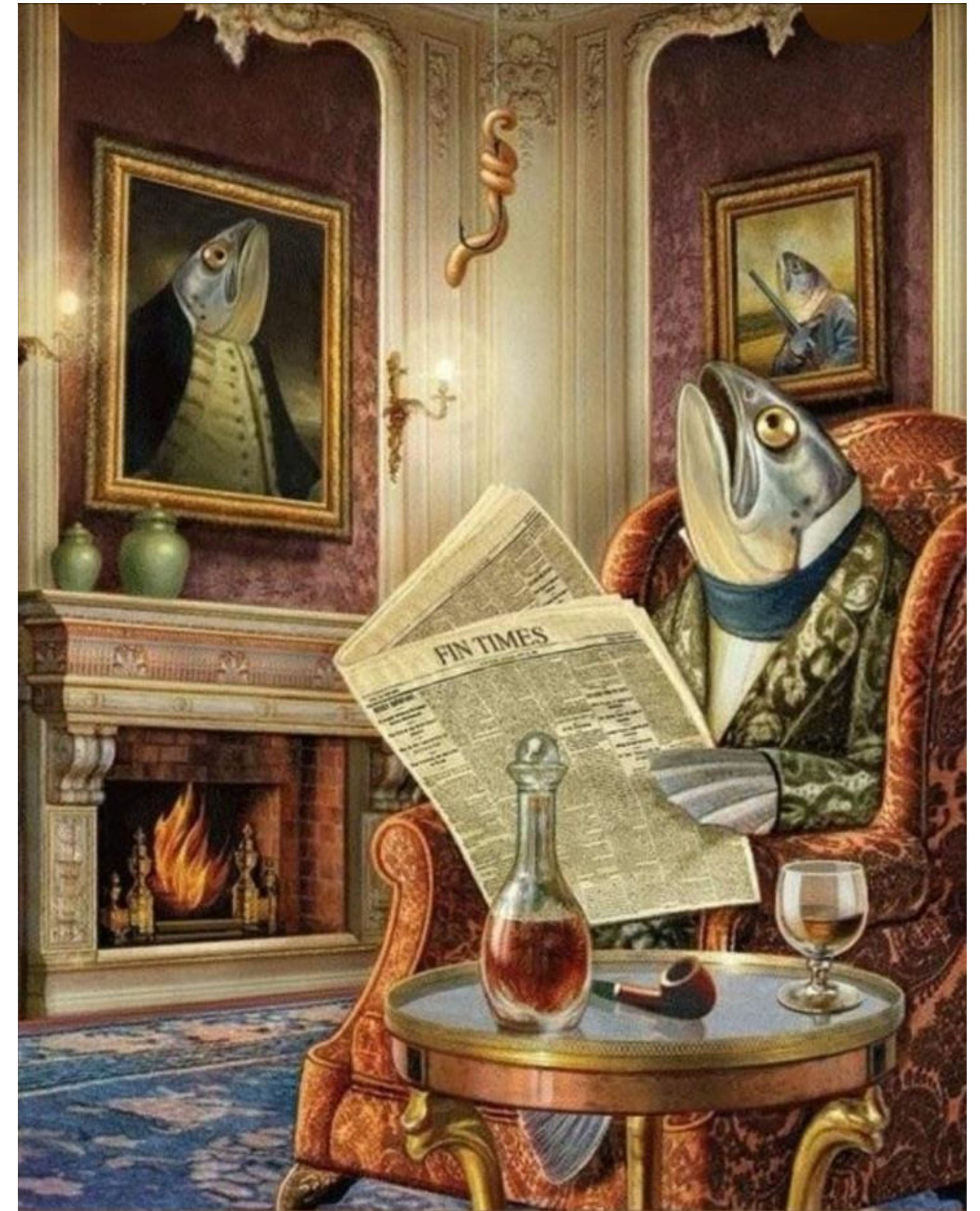
Antimicrobial Susceptibility testing

Bacterial isolation

Bacterial identification

Antimicrobial Susceptibility Testing

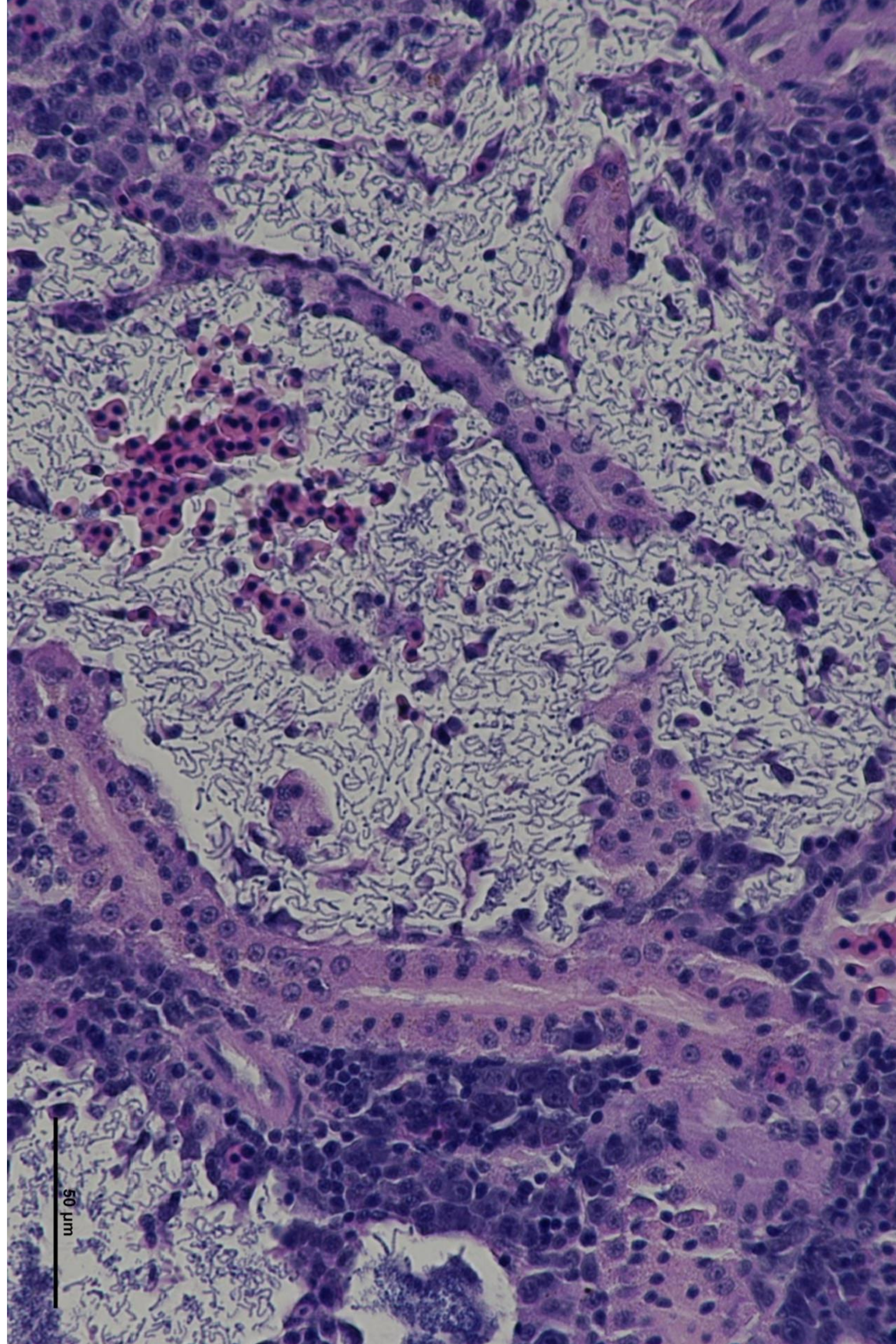
- Antibigram
- Minimum Inhibitory Concentration (MIC)



Credit: Pinterest



Diseases caused by Gram positive bacteria



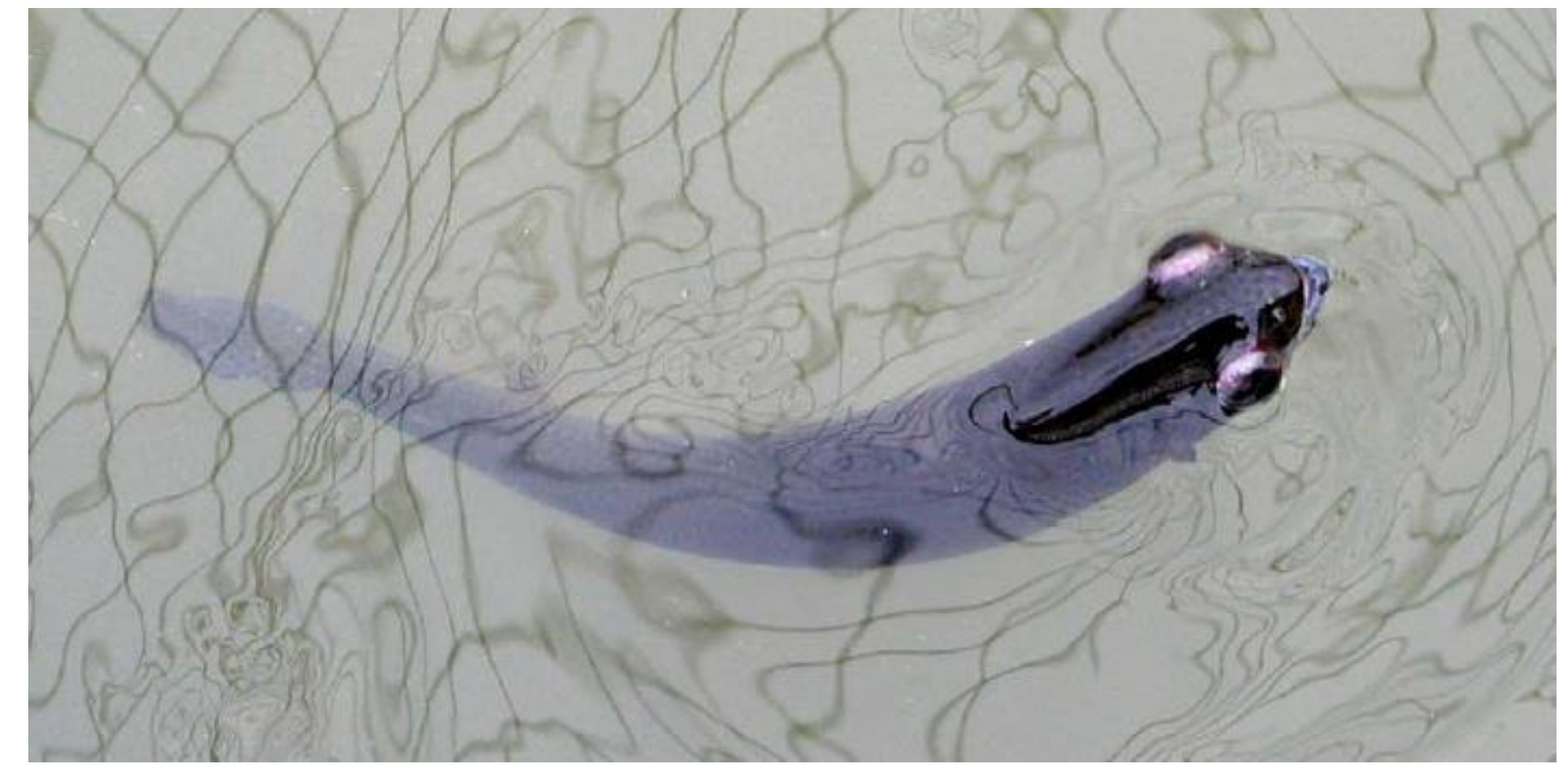
Piscine Streptococcosis: haemorrhagic septicaemia by Gram + cocci

What to expect?

Exophthalmia (pop-eye)

Anorexia

Skin darkening

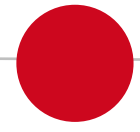


Internally: multiple **haemorrhages** and organic **megalia, epicarditis**

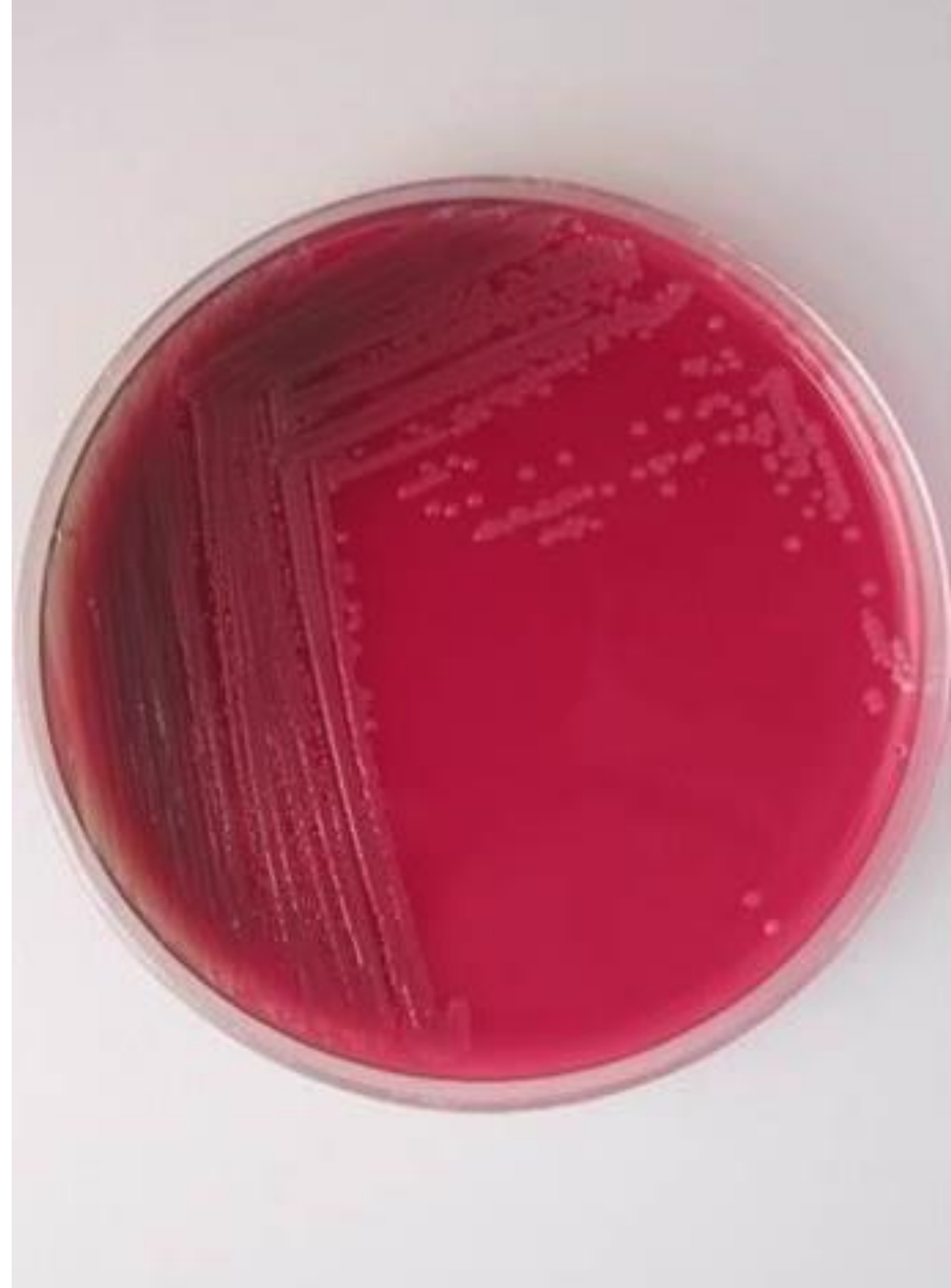
Cold-water (water temp $<15^{\circ}\text{C}$) -> **Vagococcosis** by ***Vagococcus salmoninarum***:
disease of salmonid broodstocks after spawning or manipulation

Warm-water (water temp $>15^{\circ}\text{C}$)-> **Lactococcosis** by ***Lactococcus garvieae*, *L. petauri*, *L. formosensis***
-> **Streptococcosis** by ***Streptococcus iniae*, *S. agalactiae*, *S. dysgalactiae***

Diseases of over 30 fresh and saltwater finfish species worldwide.
Highly important in trout, sturgeon and tilapia farming



Diseases caused by Gram negative bacteria



● Motile Aeromonas septicemia (MAS)

What to expect?

Skin reddening and haemorrhages

Ulcers and erosions



Internally: multiple **haemorrhages** and organic **megalania**

Aeromonas hydrophila, A. caviae A. dhakensis, A. schubertii, A. veronii and others

Important disease of carp, tilapia and snakehead aquaculture.

The *Aeromonas* species are ubiquitous in the aquatic environment and are commonly found in the guts of healthy fish. However, under stressors such as poor water quality or overcrowding, or in the presence of injuries, these bacteria can spread to internal tissues, causing septicemia.

● Flavobacteriosis

What to expect?

Skin ulcers

Erosion of dorsal and caudal fins

Gills erosions



Internally: **splenomegaly and colliquation of the organ**

Flavobacterium psychrophilum -> Rainbow trout fry syndrome, systemic flavobacteriosis

Cold water disease (temp. < 15°C)

Susceptible hosts: salmonids, sturgeon species

Flavobacterium columnare complex - warm water disease (water temperature > 20°C)

- ***F. columnare*** -> salmonids, ciprinids
- ***F. covaie*** -> channel catfish, bass species, salmonids
- ***F. davisii*** -> channel catfish, bass species, salmonids
- ***F. oreochromis*** -> *Oreochromis spp.*

● Edwardisellosis

What to expect?

Skin reddening

Skin ulcers (hole in the head)

Mild exophthalmia

Swollen abdomen



Internally: **mottled** appearance of the **liver**, **splenomegaly**, severe **enteritis and gut dilatation**

- ***Edwardsiella tarda*** -> channel catfish, eel, tilapia, carp, hybrid snakehead
- ***E. ictaluri*** -> channel catfish, striped catfish, hybrid catfish (**Enteric septicaemia of catfish**), tilapia
- ***E. anguillarum*** -> eel, sea bream, hybrid catfish, tilapia
- ***E. piscicida*** -> barramundi, hybrid catfish, tilapia, largemouth bass

● Yersiniosis - Enteric Redmouth disease (ERM)

What to expect?

Reddening and erosion of mouth

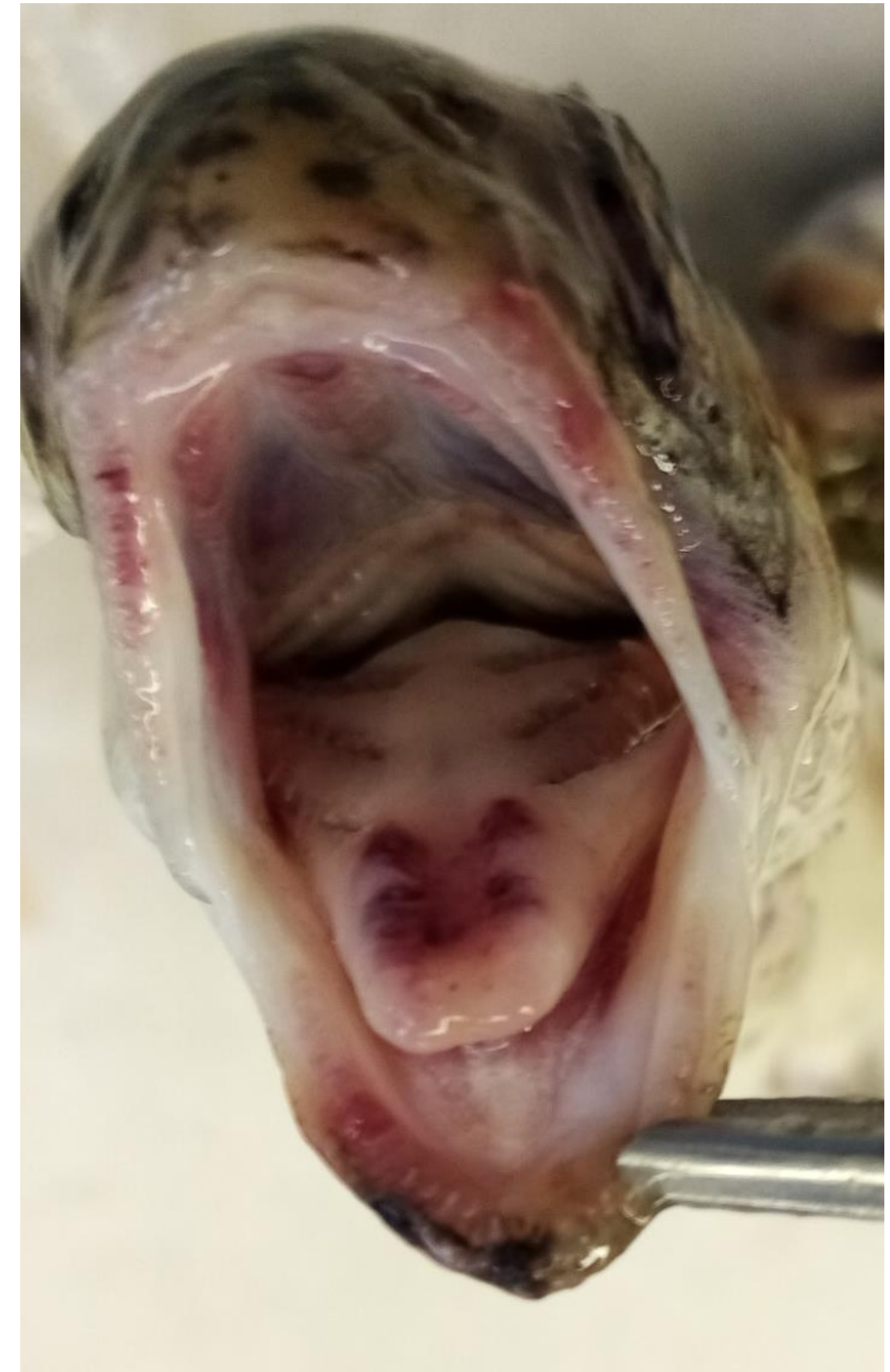
Skin reddening

Internally: **haemorrhages**, **splenomegaly**, severe **enteritis**

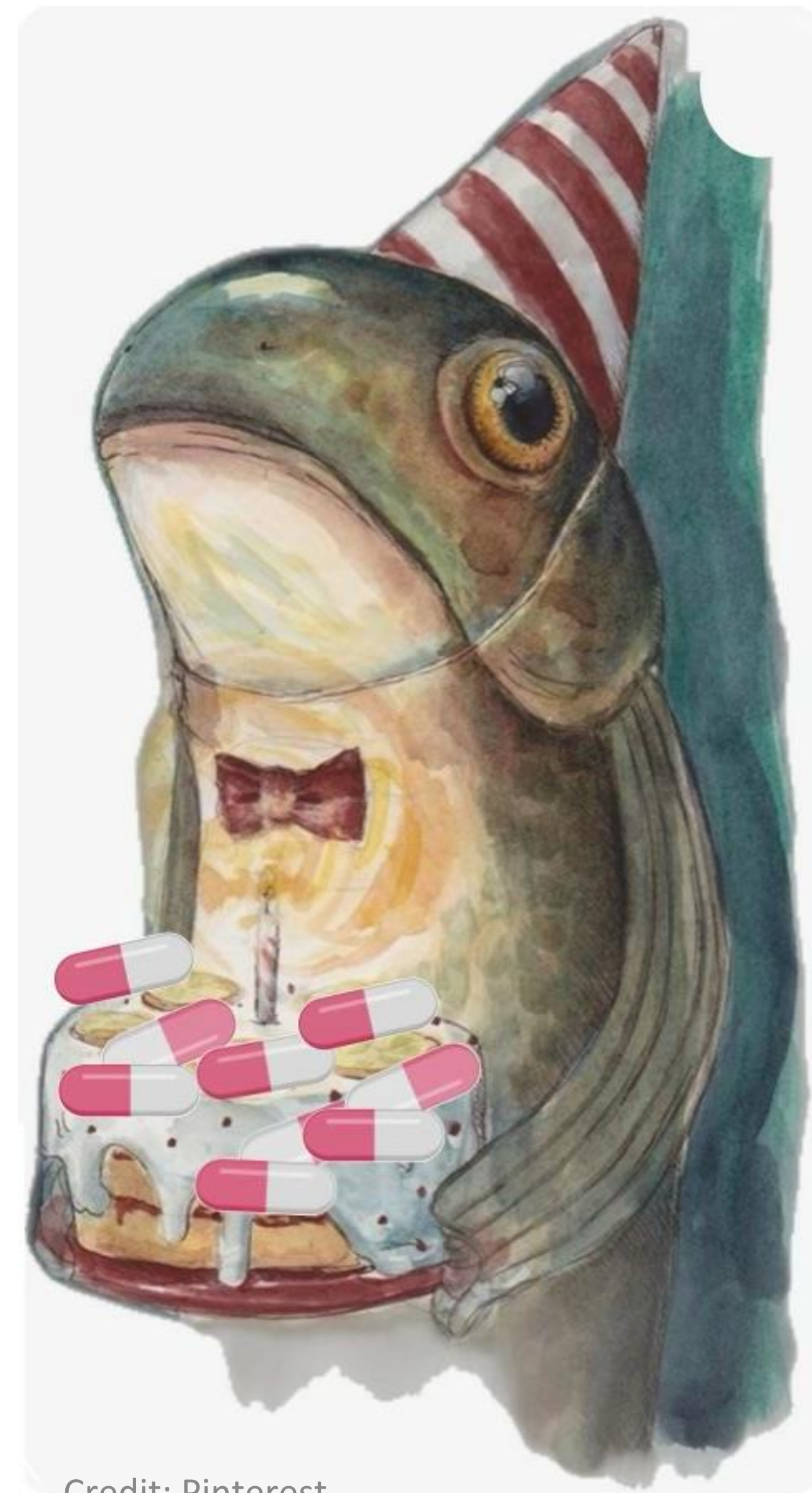
Disease caused by ***Yersinia ruckeri***

Susceptible hosts: **salmonids**, mainly rainbow trout

High mortalities in juveniles and adults



Antimicrobial Susceptibility Testing AST



Credit: Pinterest

● Antimicrobial Susceptibility Testing (AST)

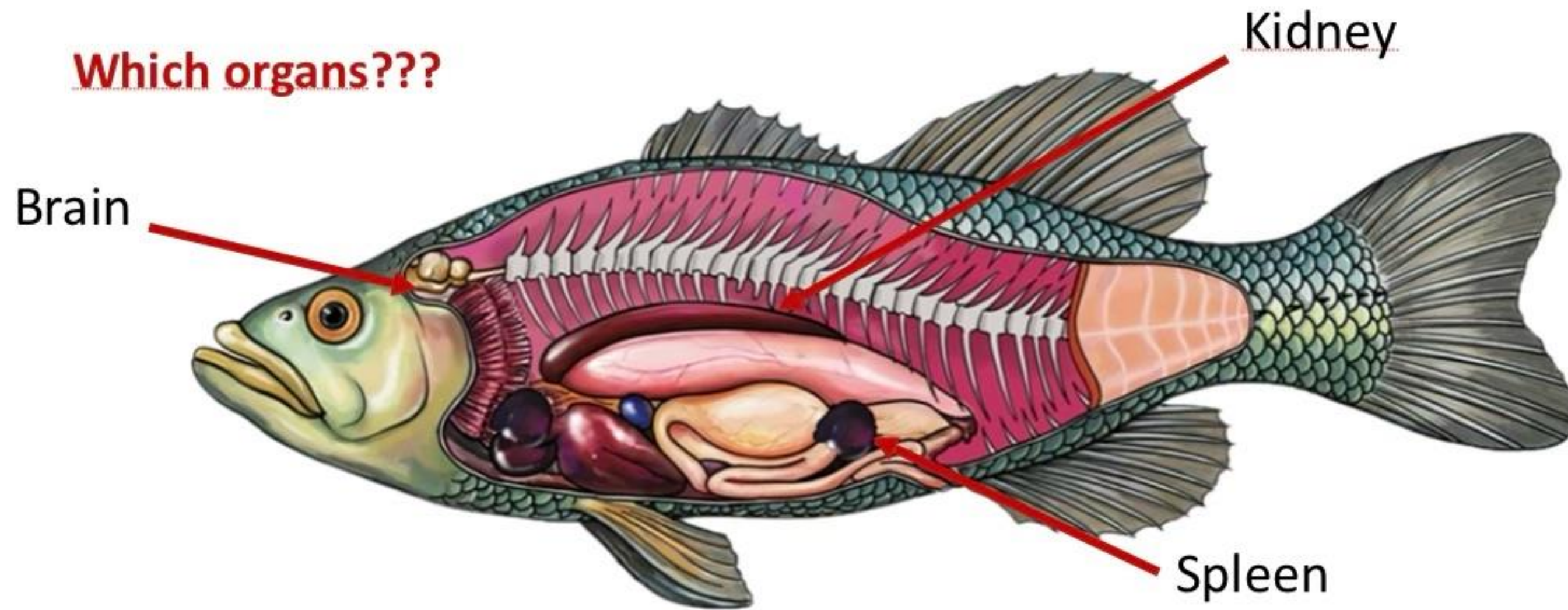
Antimicrobial Susceptibility Testing is a powerful tool that **supports the selection of the correct treatment** to solve an outbreak of bacterial disease.

With this umbrella term are included several laboratoristic techniques capable of investigating the **phenotypic expression** of antimicrobial resistance of bacterial isolates

Steps:

- 1) Bacterial isolation
- 2) Bacterial identification
- 3) AST analysis

● Bacterial isolation



And all the other relevant lesions!!!

Which growth media???

Freshwater species

Anacker and Ordal
Enriched Agar (AOAE)

Necessary for the
isolation of
Flavobacterium spp.

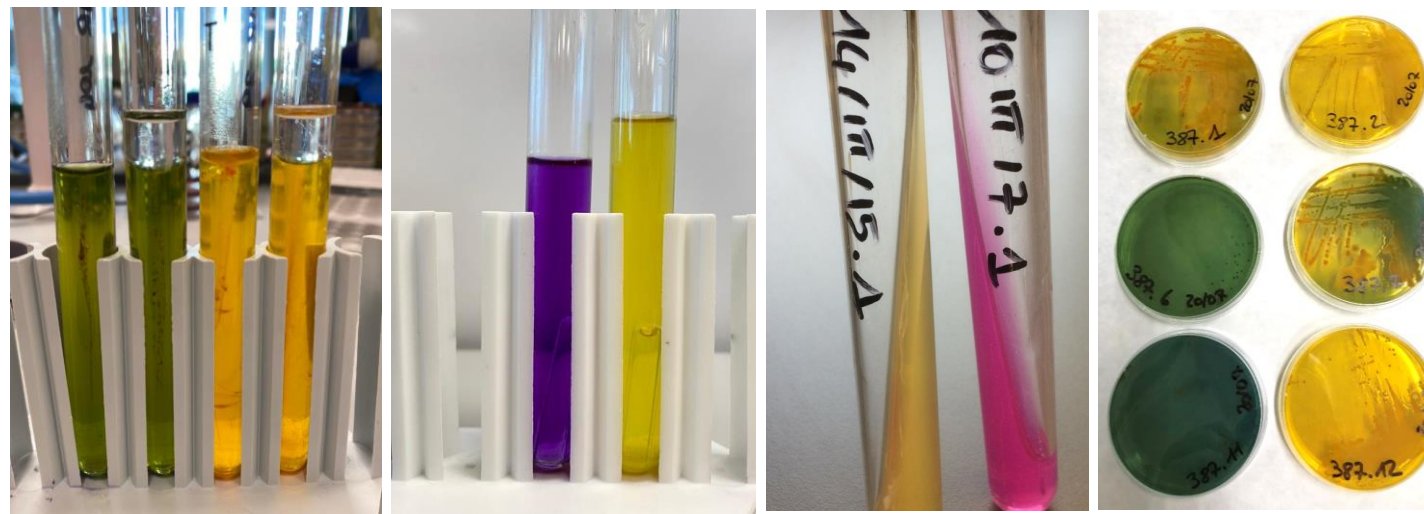
ALWAYS BLOOD AGAR
Multipurpose permissive
medium

Bacterial identification

Bacterial identification is a multi-step process that combines classical microbiological methods with advanced analytical techniques.

Biochemical identification

- Colony morphology; haemolysis; Gram/KOH; oxidase; catalase
- Macromethod

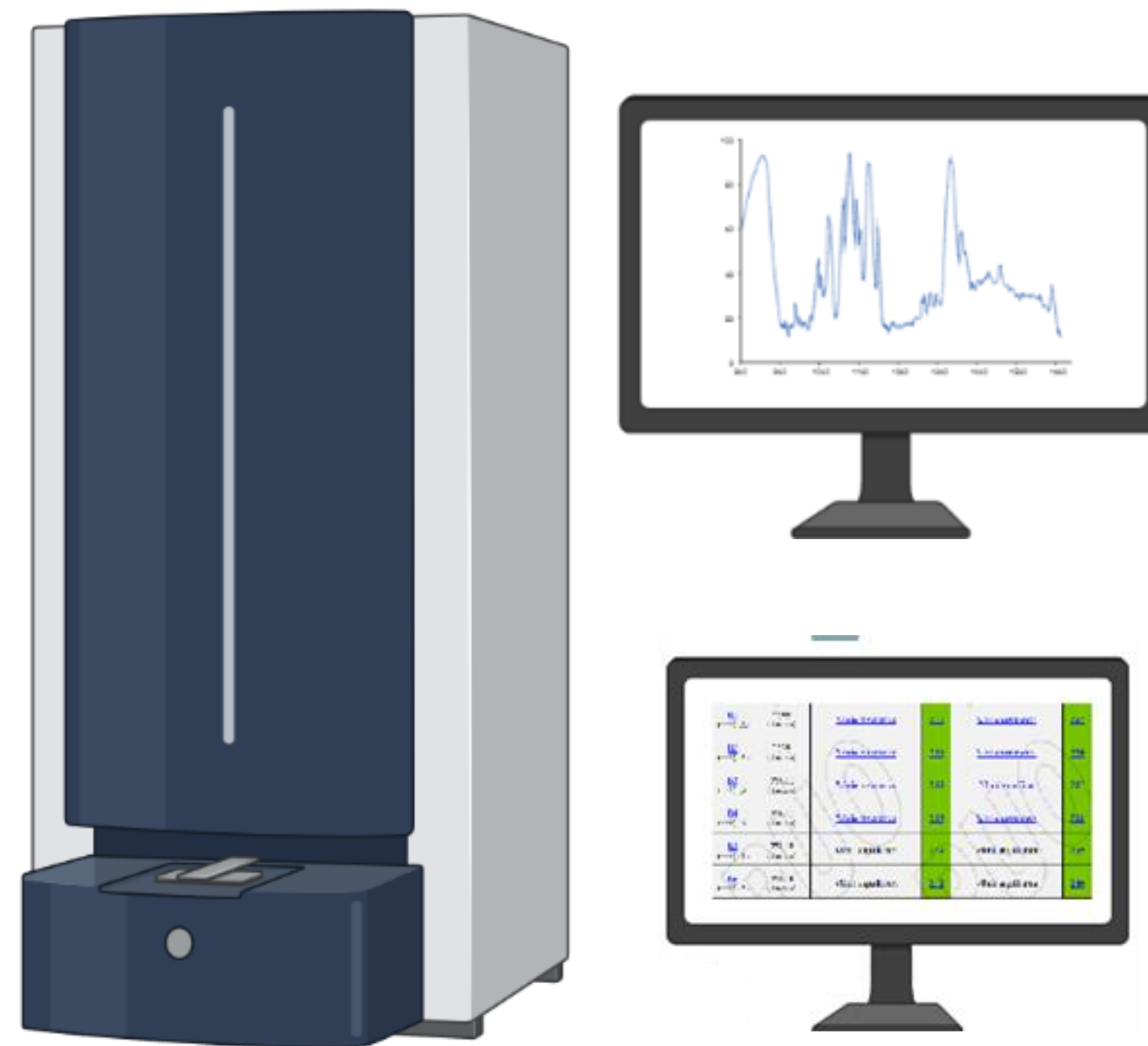


- Micromethod



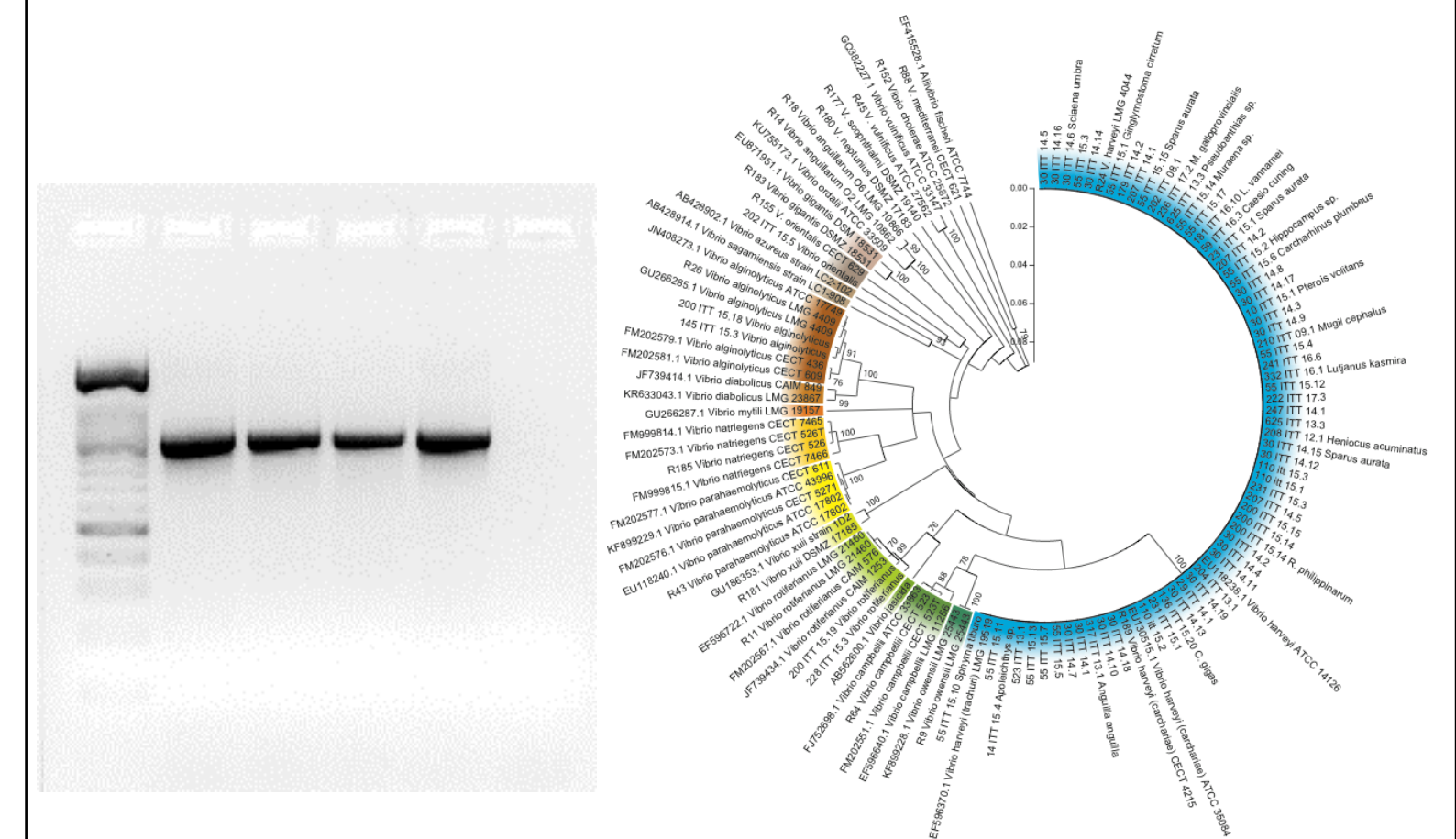
Proteomic identification

- MALDI-tof



Molecular identification

- End-point PCR
- Real-time PCR
- Sequencing
- Phylogeny

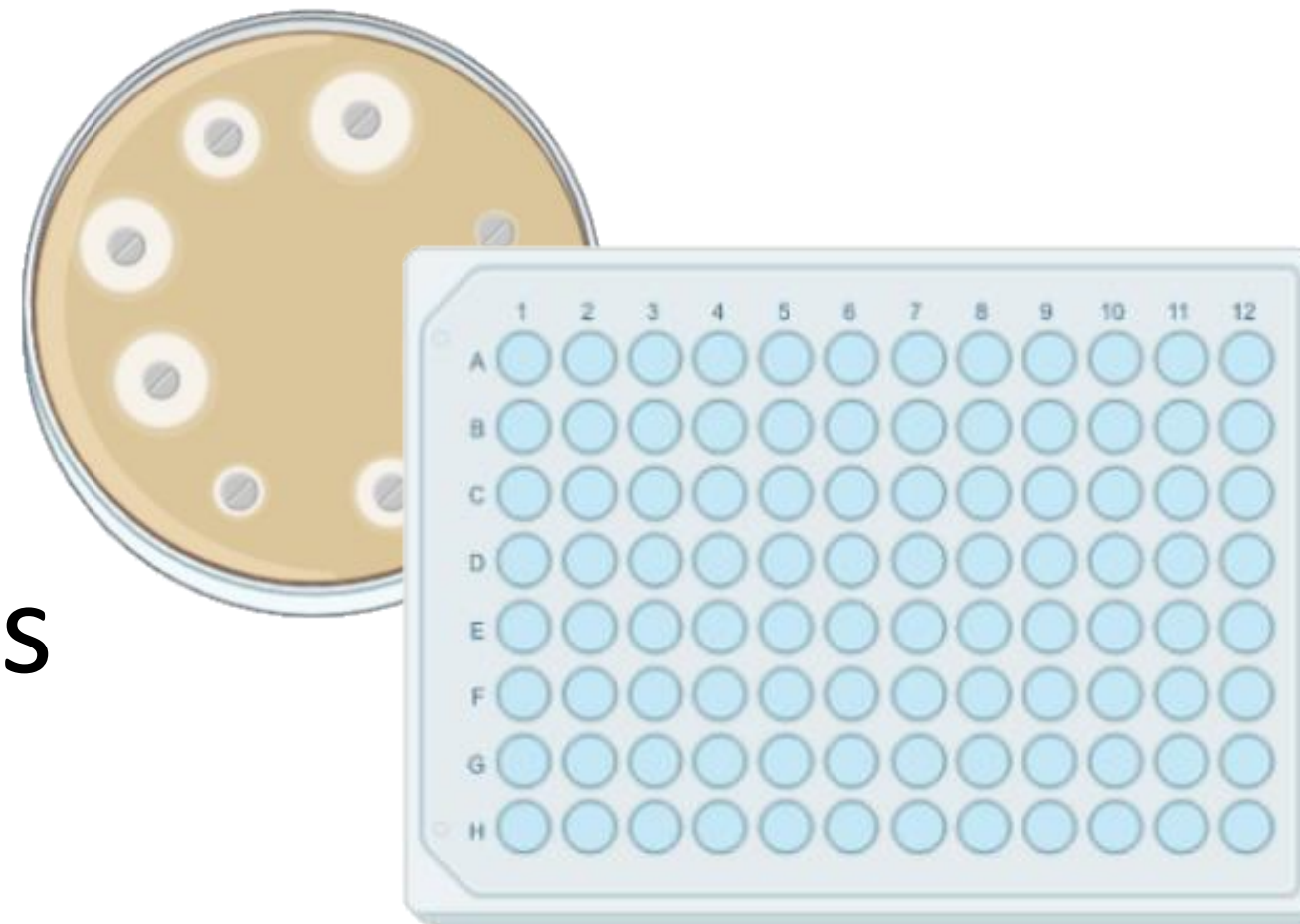


● Antibiotic sensitivity testing-phenotypic methods

Disk diffusion method
or Kirby-Bauer



«QUALITATIVE»
(but only in results
expression)



Determination of
MIC value



QUANTITATIVE

- Broth macrodilution
- Broth microdilution
- E-test

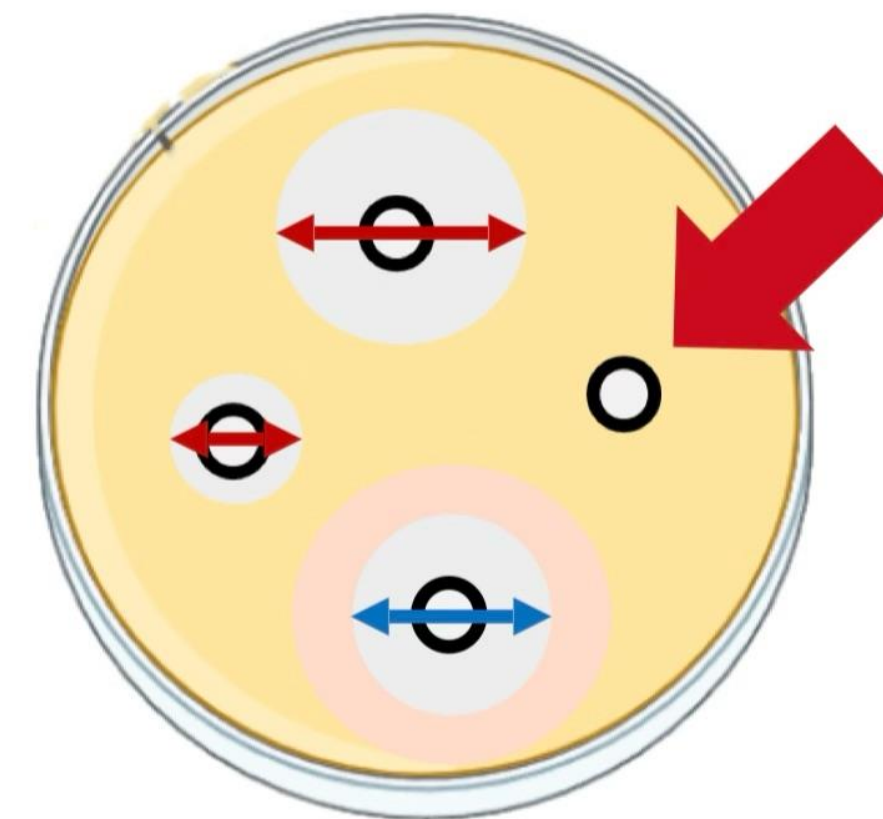
The technical aspects (growth medium, time and temperature of incubation) of these tests may vary according to the bacterial species under investigation.

● Antibigram (Kirby Bauer Method; agar-diffusion method)

- It consists of exposing a bacterial isolate streaked onto an agarized medium (Mueller Hinton Agar) to discs containing known concentrations of antimicrobial molecules.
- It is based on the concept that the size of the **halo of growth inhibition** of a given bacterial strain is proportional to its sensitivity to the molecule under investigation

“SEMIQUALITATIVE” METHOD: based on the size in mm of the inhibition halo, the strain can be classified as:

- **SENSITIVE,**
- **INTERMEDIATE**
- **RESISTANT**

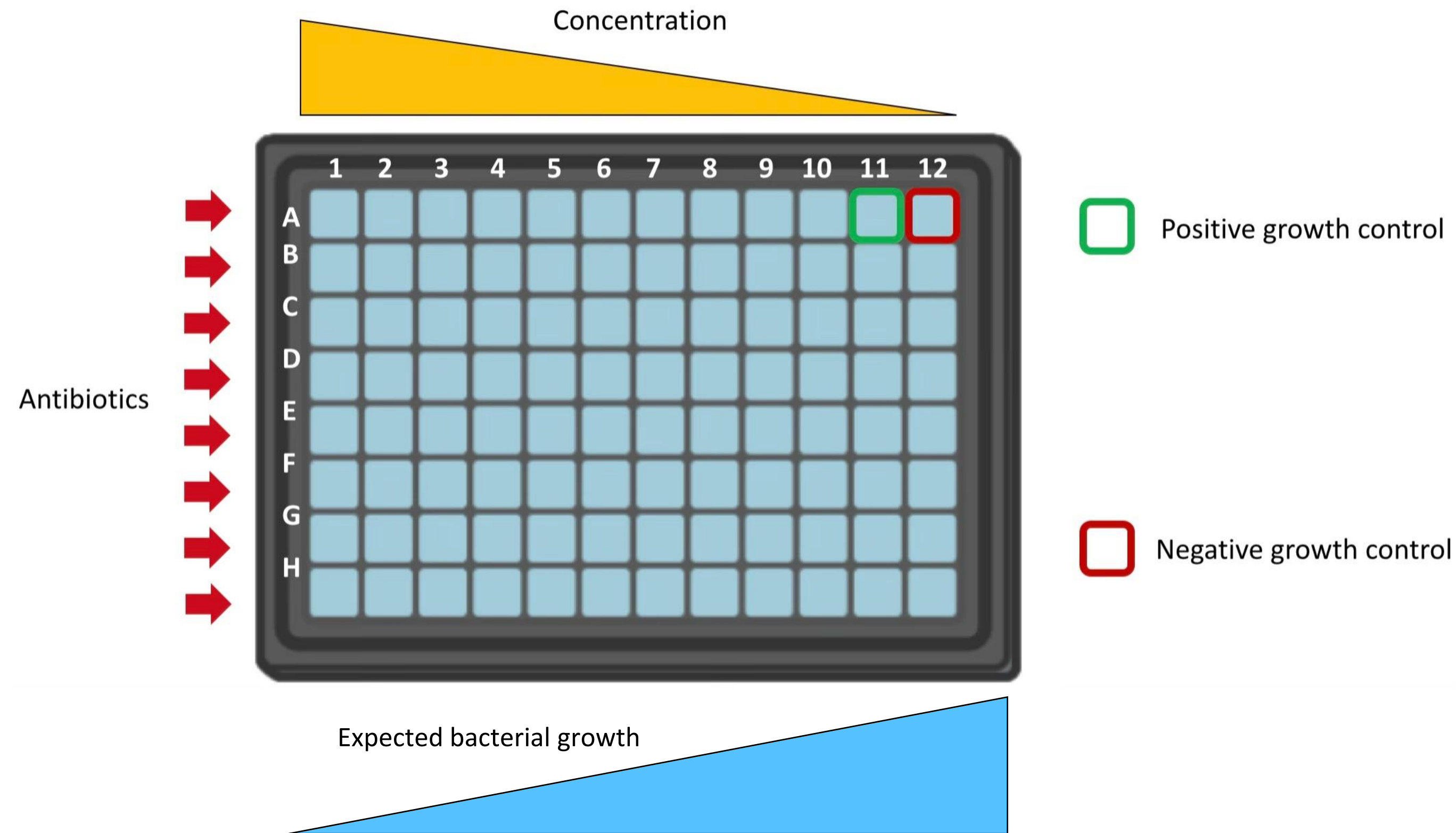


If no halo is present, the isolate is totally resistant to the antimicrobial tested

It's important to have interpretative criteria (nowdays scarce for aquatic bacteria)

● Minimum Inhibitory Concentration (MIC)

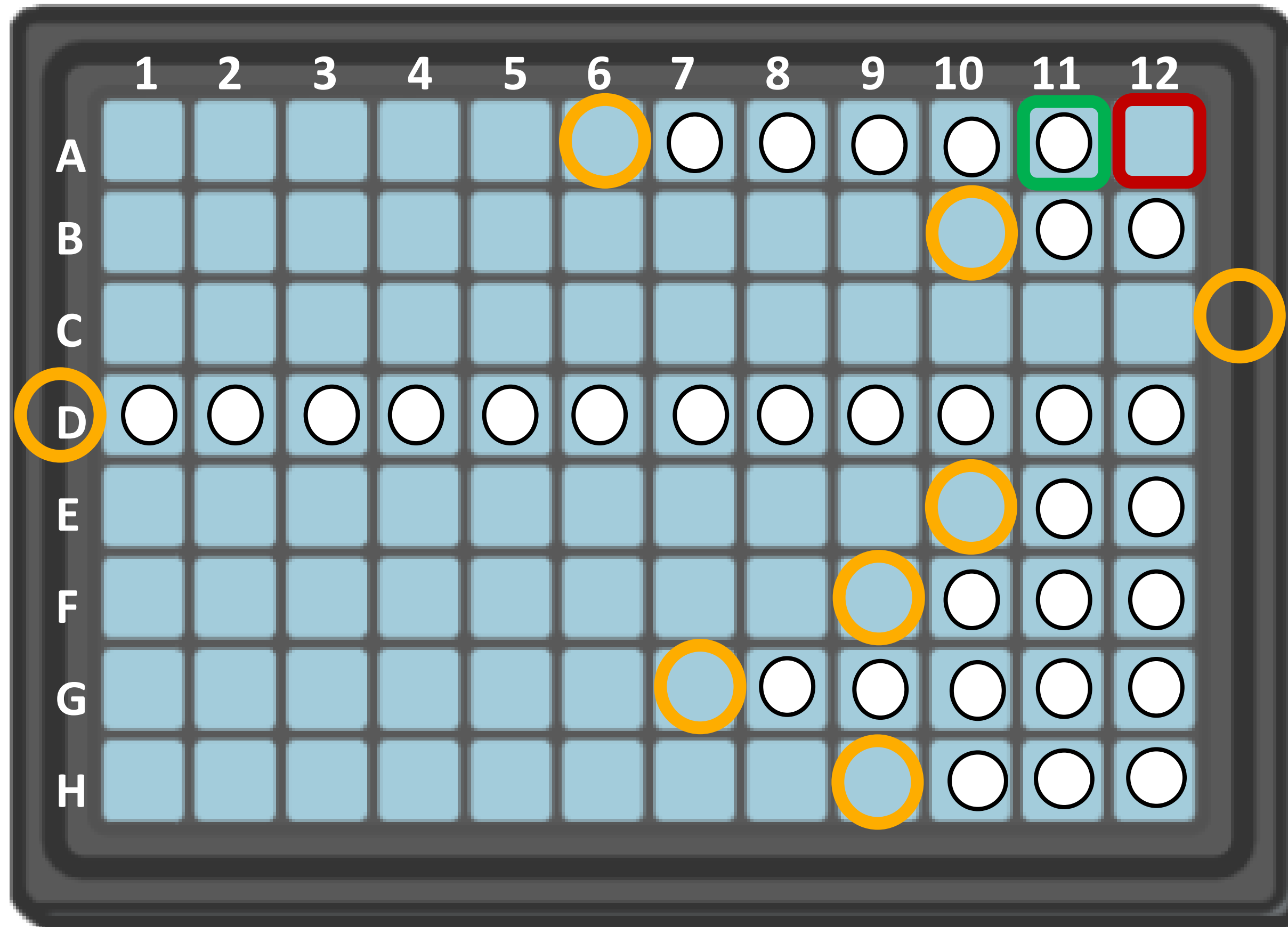
DEFINITION: a quantitative value expressing the **minimum concentration** of a given active substance **capable to inhibit in vitro**, under given conditions of timing and incubation temperatures, **the growth of a given bacterial isolate**.



The determination of MIC value can be performed in macro or micromethod

Results are expressed as a concentration value (e.g. $\mu\text{L/mL}$)

MIC testing – Reading



- Negative growth control: sterile
- Positive growth control: positive
- No charging anomalies



Result: the plate can be read

○ = bacterial growth

○ = MIC value

● Practical example of MIC results

	1	2	3	4	5	6	7	8	9	10	11	12
A	ERY 32	ERY 16	ERY 8	ERY 4	ERY 2	ERY 1	ERY 0,5	ERY 0,25	ERY 0,125	ERY 0,063	GC+	GC-
B	ENR 8	ENR 4	ENR 2	ENR 1	ENR 0,5	ENR 0,25	ENR 0,125	ENR 0,063	ENR 0,031	ENR 0,016	ENR 0,008	ENR 0,004
C	FLM 32	FLM 16	FLM 8	FLM 4	FLM 2	FLM 1	FLM 0,5	FLM 0,25	FLM 0,125	FLM 0,063	FLM 0,031	FLM 0,016
D	AMX 32	AMX 16	AMX 8	AMX 4	AMX 2	AMX 1	AMX 0,5	AMX 0,25	AMX 0,125	AMX 0,063	AMX 0,031	AMX 0,016
E	FLL 64	FLL 32	FLL 16	FLL 8	FLL 4	FLL 2	FLL 1	FLL 0,5	FLL 0,25	FLL 0,125	FLL 0,063	FLL 0,031
F	OXY 16	OXY 8	OXY 4	OXY 2	OXY 1	OXY 0,5	OXY 0,25	OXY 0,125	OXY 0,063	OXY 0,031	OXY 0,016	OXY 0,008
G	DOX 16	DOX 8	DOX 4	DOX 2	DOX 1	DOX 0,5	DOX 0,25	DOX 0,125	DOX 0,063	DOX 0,031	DOX 0,016	DOX 0,008
H	T/S 8/152	T/S 4/76	T/S 2/38	T/S 1/19	T/S 0,5/9,5	T/S 0,25/ 4,75	T/S 0,125/ 2,375	T/S 0,063/ 1,188	T/S 0,031/ 0,594	T/S 0,016/ 0,297	T/S 0,008/ 0,148	T/S 0,004/ 0,074

Aeromonas veronii biovar *sobria* (pangasius)

	1	2	3	4	5	6	7	8	9	10	11	12
A	ERY 32	ERY 16	ERY 8	ERY 4	ERY 2	ERY 1	ERY 0,5	ERY 0,25	ERY 0,125	ERY 0,063	GC+	GC-
B	ENR 8	ENR 4	ENR 2	ENR 1	ENR 0,5	ENR 0,25	ENR 0,125	ENR 0,063	ENR 0,031	ENR 0,016	ENR 0,008	ENR 0,004
C	FLM 32	FLM 16	FLM 8	FLM 4	FLM 2	FLM 1	FLM 0,5	FLM 0,25	FLM 0,125	FLM 0,063	FLM 0,031	FLM 0,016
D	AMX 32	AMX 16	AMX 8	AMX 4	AMX 2	AMX 1	AMX 0,5	AMX 0,25	AMX 0,125	AMX 0,063	AMX 0,031	AMX 0,016
E	FLL 64	FLL 32	FLL 16	FLL 8	FLL 4	FLL 2	FLL 1	FLL 0,5	FLL 0,25	FLL 0,125	FLL 0,063	FLL 0,031
F	OXY 16	OXY 8	OXY 4	OXY 2	OXY 1	OXY 0,5	OXY 0,25	OXY 0,125	OXY 0,063	OXY 0,031	OXY 0,016	OXY 0,008
G	DOX 16	DOX 8	DOX 4	DOX 2	DOX 1	DOX 0,5	DOX 0,25	DOX 0,125	DOX 0,063	DOX 0,031	DOX 0,016	DOX 0,008
H	T/S 8/152	T/S 4/76	T/S 2/38	T/S 1/19	T/S 0,5/9,5	T/S 0,25/ 4,75	T/S 0,125/ 2,375	T/S 0,063/ 1,188	T/S 0,031/ 0,594	T/S 0,016/ 0,297	T/S 0,008/ 0,148	T/S 0,004/ 0,074

Aeromonas veronii biovar *sobria* (frog)

MIC value in µl/mL

Thank you for
your attention!

Do you
Have any question?

