# ESTABLISHING INTERPRETATION CRITERIA FOR ANTIMICROBIALS USED IN FARMED FISH

## – the background of the problem

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NVRI, Puławy

# Ichtioxan


Withdrawal period depends on the water temperature and fish species:

- carp: 35 days (water temp. 12 - 20°C)
  30 days (water temp. abowe 20°C);
- rainbow trout: 60 days (water temp. belowe 7°C)
  50 days (water temp. 8 - 12°C)
  40 days (water temp. 13 - 20°C)

# If there is no veterinary medicinal product intended for use in an animal in a given indication

Is a veterinary medicinal product authorized in the country, and intended for another animal species or for the same species, but for a different indication?

↓ YES

↓

Apply

↓ NO

Has a veterinary medicinal product for the same or a different animal species, for the same or other indications, been authorized in another Member State?

↓ YES

↓

Apply

↓ NO

# Prudent use of antimicrobials in veterinary medicine

The extensive use of antimicrobials has accelerated the emergence and spread of resistant microorganisms.

This situation has been worsened by the lack of investment in developing new effective antibiotics.

⬇

**COMMISSION NOTICE**

**Guidelines for the prudent use of antimicrobials in veterinary medicine**

**(2015/C 299/04)**

**SCOPE AND PURPOSE : Relates to the prudent use of antimicrobials in animals, and, in particular, how prudent usage can contribute to containing the development of AMR**

# Interpretation criteria for antimicrobials used in treatment of pathogenic bacteria for fish are not available

- **CLSI** - Clinical and Laboratory Standards Institute;
- **EUCAST** - European Committee on Antimicrobial Suscepitbility Testing

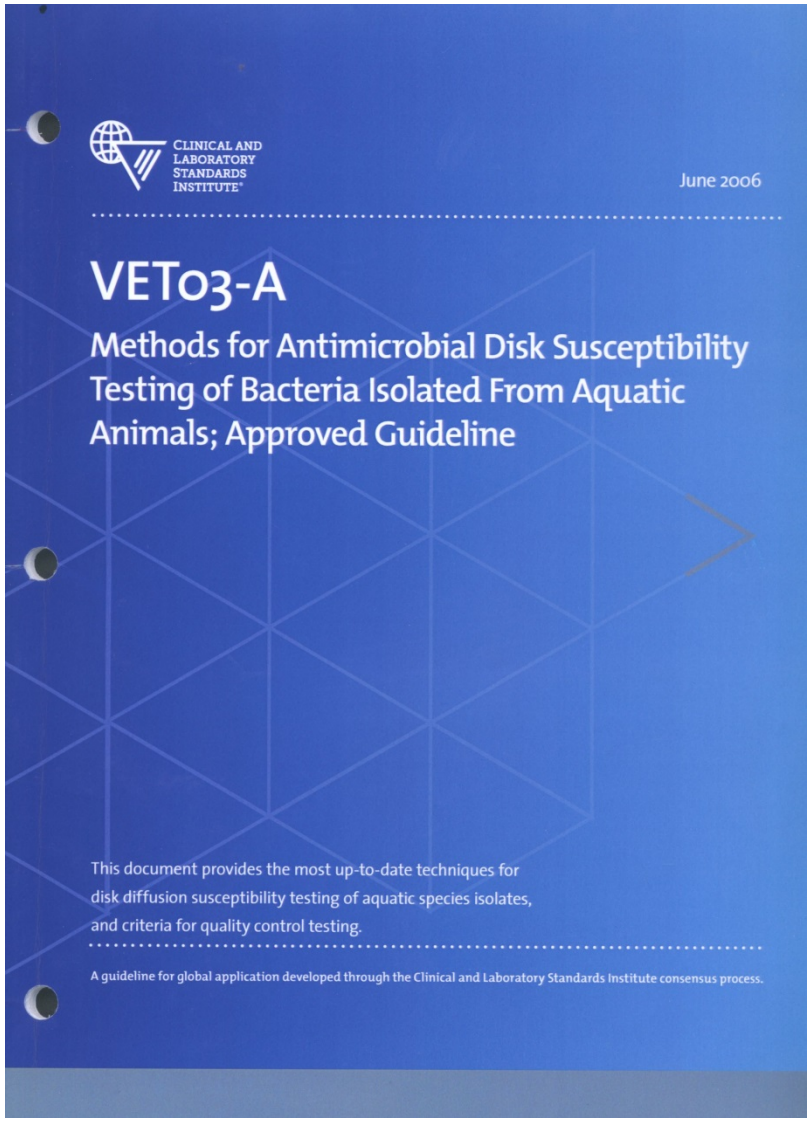





**Table 1. Frequently Isolated Bacterial Pathogens of Fish**

| Bacterial Pathogen | Disease |
|---|---|
| *Aeromonas hydrophila*<br>*Aeromonas caviae*<br>*Aeromonas sobria* | Motile Aeromonad septicemia |
| *Aeromonas salmonicida* | Furunculosis, Ulcer disease, Carp erythrodermatitis |
| *Aerococcus viridans* | Gaffkemia |
| *Carnobacterium maltaromaticum* | |
| *Corynebacterium* spp. | |
| *Edwardsiella ictaluri* | Enteric septicemia of catfish |
| *Edwardsiella tarda* | Red pest disease, *Edwardsiella* septicemia |
| *Flavobacterium branchiophilum* | Bacterial gill disease |
| *Flavobacterium columnare* | Columnaris disease |
| *Flavobacterium psychrophilum* | Cold-water disease, Rainbow trout fry syndrome |
| *Lactococcus garvieae*<br>*Lactococcus piscium* | Lactococcosis, *Lactococcus* septicemia |
| *Moritella viscosa* | Winter ulcer disease |
| *Mycobacterium* spp. | Mycobacteriosis |
| *Photobacterium damselae* subsp. *damselae* | Vibriosis |
| *Photobacterium damselae* subsp. *piscicida* | Photobacteriosis, Fish pasteurellosis, Pseudotuberculosis |
| *Piscirickettsia salmonis* | Piscirickettsiosis, Salmonid piscirickettsial septicemia |
| *Plesiomonas shigelloides* | Winter disease |
| *Pseudomonas* spp. | Pseudomoniasis |
| *Pseudomonas anguilliseptica* | Red spot disease |
| *Renibacterium salmoninarum* | Bacterial kidney disease |
| *Shewanella putrefaciens* | |
| *Streptococcus iniae* | Streptococcosis |
| *Streptococcus difficilis* | Group B streptococcosis |
| *Streptococcus dysgalactiae* | Group C streptococcosis |
| *Tenacibaculum maritimum* | Salt-water columnaris, marine flexibacteriosis |
| *Vagococcus salmoninarum* | Cold-water streptococcosis |
| *Vibrio salmonicida* | Cold-water vibriosis, Hitra disease |
| *Vibrio* spp. | Vibriosis |
| *Yersinia ruckeri* | Enteric redmouth disease |

## Table 3. Standard Methods for Disk Diffusion Susceptibility Testing of Aquatic Bacterial Pathogens

| Organisms | Medium | Incubation |
|---|---|---|
| **Group 1: Nonfastidious bacteria**[a] | | |
| Enterobacteriaceae | MHA | 22 °C (24-28 h and/or 44-48 h) or 28 °C (24-28 h) |
| *Aeromonas salmonicida* (nonpsychrophilic strains) | | |
| *Aeromonas hydrophila* and other mesophilic aeromonads | | |
| *Pseudomonas* spp. | | |
| *Plesiomonas shigelloides* | | |
| *Shewanella* spp. | | |
| Vibrionaceae and related bacteria (nonobligate halophilic strains) | | |

**Footnote**

a. Only Group 1 organisms have a standardized disk diffusion susceptibility testing method.

# *Aeromonas salmonicida* subsp. *salmonicida*

| | Concentration | S | I | R |
|---|---|---|---|---|
| OT | 30µg | ≥28 | 22 - 27 | 21≤ |
| OA | 2µg | ≥30 | 25 - 29 | 24≤ |

PIWet

## *Pseudomonas* spp.

**EUCAST Clinical Breakpoint Tables v. 8.0, valid from 2018-01-01**

**MIC determination (broth microdilution according to ISO standard 20776-1 except for fosfomycin where agar dilution is used)**
**Medium:** Mueller-Hinton broth
**Inoculum:** $5x10^5$ CFU/mL
**Incubation:** Sealed panels, air, 35±1ºC, 18±2h
**Reading:** Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth.
**Quality control:** *Pseudomonas aeruginosa* ATCC 27853. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor combinations, see EUCAST QC Tables.

**Disk diffusion (EUCAST standardised disk diffusion method)**
**Medium:** Mueller-Hinton agar
**Inoculum:** McFarland 0.5
**Incubation:** Air, 35±1ºC, 18±2h
**Reading:** Unless otherwise stated, read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
**Quality control:** *Pseudomonas aeruginosa* ATCC 27853. For agents not covered by this strain and for control of the inhibitor component of beta-lactam inhibitor-combination disks, see EUCAST QC Tables.

| Penicillins | MIC breakpoint (mg/L) | | Disk content (µg) | Zone diameter breakpoint (mm) | | Notes |
|---|---|---|---|---|---|---|
| | S ≤ | R > | | S ≥ | R < | Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method. |
| Benzylpenicillin | - | - | | - | - | **1.** Breakpoints are based on high dose therapy, see table of dosages ~~(4 g x 4, with or without tazobactam)~~. |
| Ampicillin | - | - | | - | - | **2.** For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L. |
| Ampicillin-sulbactam | - | - | | - | - | **3.** Breakpoints are based on high dose therapy, see table of dosages. |
| Amoxicillin | - | - | | - | - | **4.** For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L. |
| Amoxicillin-clavulanic acid | - | - | | - | - | |
| Piperacillin[1] | 16 | 16 | 30 | 18 | 18 | |
| Piperacillin-tazobactam[1] | 16[2] | 16[2] | 30-6 | 18 | 18 | |
| Ticarcillin[3] | 16 | 16 | 75 | 18 | 18 | |
| Ticarcillin-clavulanic acid[3] | 16[4] | 16[4] | 75-10 | 18 | 18 | |
| Temocillin | - | - | | - | - | |
| | | | | | | |
| Phenoxymethylpenicillin | - | - | | - | - | |
| | | | | | | |
| Oxacillin | - | - | | - | - | |
| Cloxacillin | - | - | | - | - | |
| Dicloxacillin | - | - | | - | - | |
| Flucloxacillin | - | - | | - | - | |
| | | | | | | |
| Mecillinam (uncomplicated UTI only) | - | - | | - | - | |

PIWet

## *Aeromonas* spp.

**EUCAST Clinical Breakpoint Tables v. 8.0, valid from 2018-01-01**

**MIC determination (broth microdilution according to ISO standard 20776-1)**
**Medium:** Mueller-Hinton broth
**Inoculum:** $5x10^5$ CFU/mL
**Incubation:** Sealed panels, air, 35±1ºC, 18±2h
**Reading:** Unless otherwise stated, read MICs at the lowest concentration of the agent that completely inhibits visible growth.
**Quality control:** *Pseudomonas aeruginosa* ATCC 27853. For agents not covered by this strain, see EUCAST QC Tables.

**Disk diffusion (EUCAST standardised disk diffusion method)**
**Medium:** Mueller-Hinton agar
**Inoculum:** McFarland 0.5
**Incubation:** Air, 35±1ºC, 18±2h
**Reading:** Unless otherwise stated, read zone edges as the point showing no growth viewed from the back of the plate against a dark background illuminated with reflected light.
**Quality control:** *Pseudomonas aeruginosa* ATCC 27853. For agents not covered by this strain, see EUCAST QC Tables.

| Cephalosporins | MIC breakpoint (mg/L) | | Disk content (µg) | Zone diameter breakpoint (mm) | | Notes |
|---|---|---|---|---|---|---|
| | S ≤ | R > | | S ≥ | R < | Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method. |
| Cefepime | 1 | 4 | 30 | 27 | 24 | |
| Ceftazidime | 1 | 4 | 10 | 24 | 21 | |

| Monobactams | MIC breakpoint (mg/L) | | Disk content (µg) | Zone diameter breakpoint (mm) | | Notes |
|---|---|---|---|---|---|---|
| | S ≤ | R > | | S ≥ | R < | Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method. |
| Aztreonam | 1 | 4 | 30 | 29 | 26 | |

| Fluoroquinolones | MIC breakpoint (mg/L) | | Disk content (µg) | Zone diameter breakpoint (mm) | | Notes |
|---|---|---|---|---|---|---|
| | S ≤ | R > | | S ≥ | R < | Numbered notes relate to general comments and/or MIC breakpoints. Lettered notes relate to the disk diffusion method. |
| Ciprofloxacin | 0.25 | 0.5 | 5 | 27 | 24 | |
| Levofloxacin | 0.5 | 1 | 5 | 27 | 24 | |

**The aim of our research**
**is to start**
**establishing interpretation criteria**
**for antimicrobials used for treatment**
**of bacterial infections**
**in farmed fish**

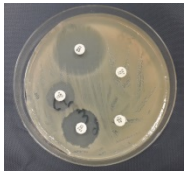
# Methods

## Bacterial species used:

- *Aeromonas hydrophila*
  n = 100 isolates
- *Aeromonas sobria*
  n = 39 isolates
- *Pseudomonas fluorescens*
  n = 63 isolates
- *Shewanella putrefaciens*
  n = 59 isolates

**Quinolones**
**Phenicols**
**Sulfonamides**
**Trimethoprim**
**Tetracyclines**

## Disk-diffusion method



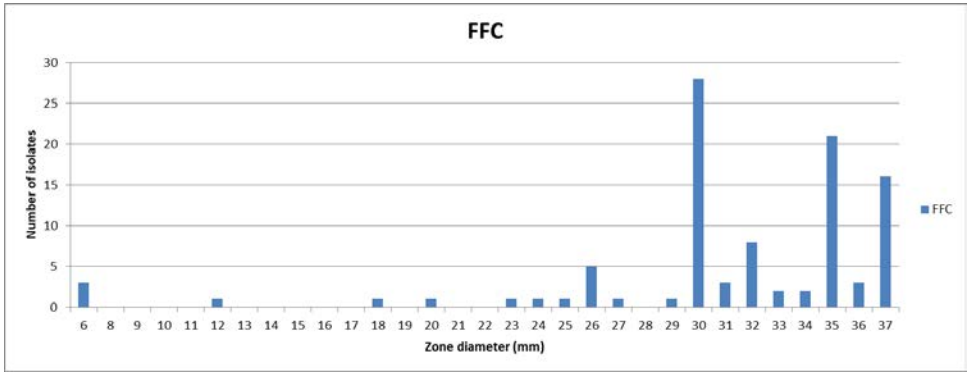
CLSI: VET03-A „Methods for Antimicrobial Disk Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Approved Guideline” (2006)

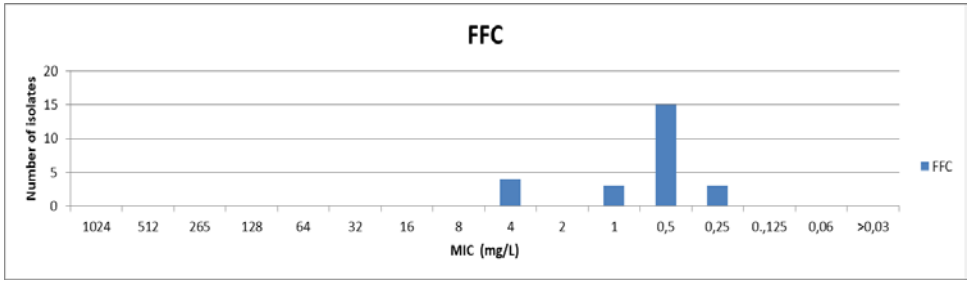
## Minimal inhibitory concentrations (MICs)

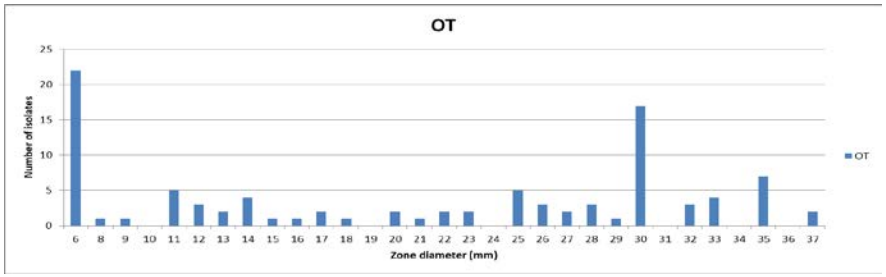
User-defined POLARGEN Sensititre plates (Thermo Fischer Scientific)

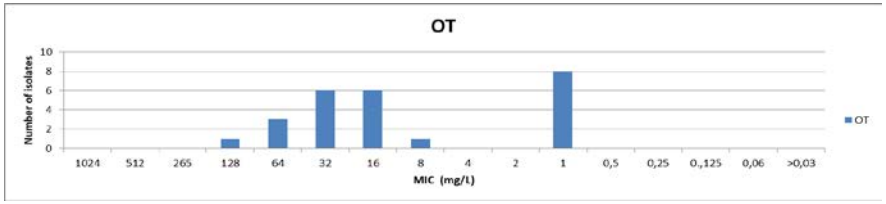
# Results

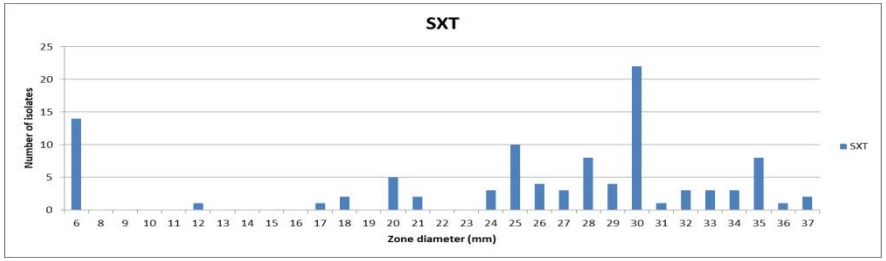
# *Aeromonas hydrophila*

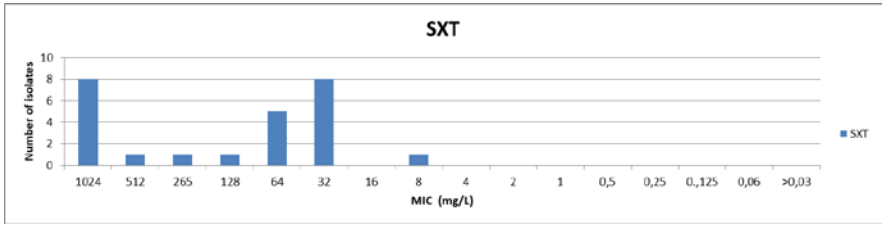


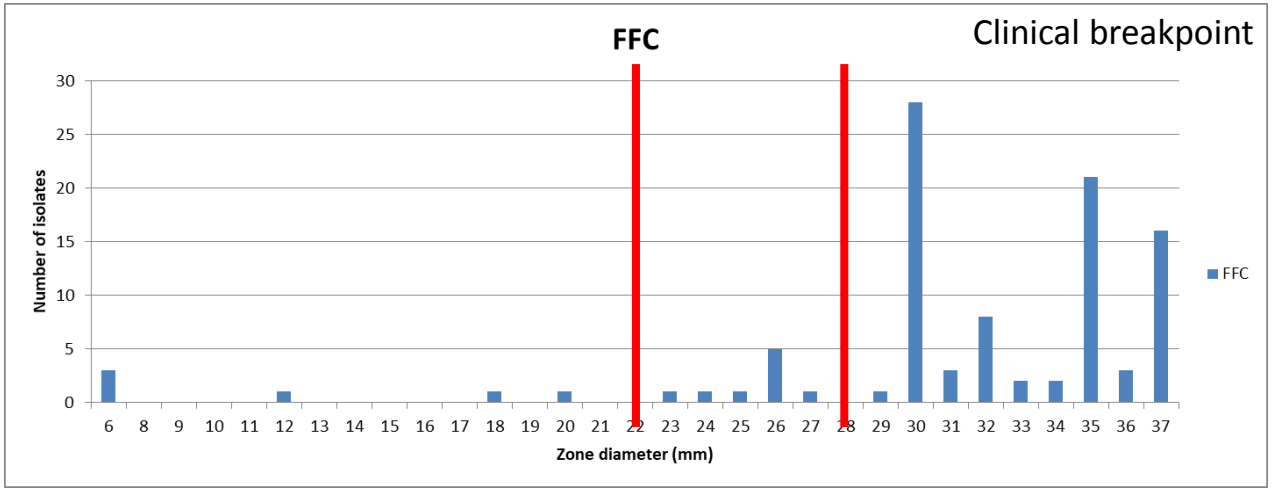



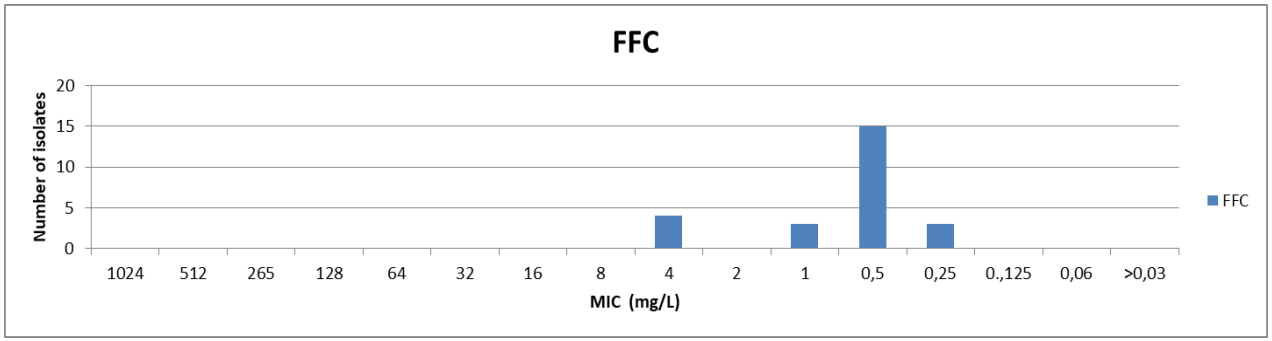



# Results

# *Aeromonas hydrophila*

Interpretation criteria



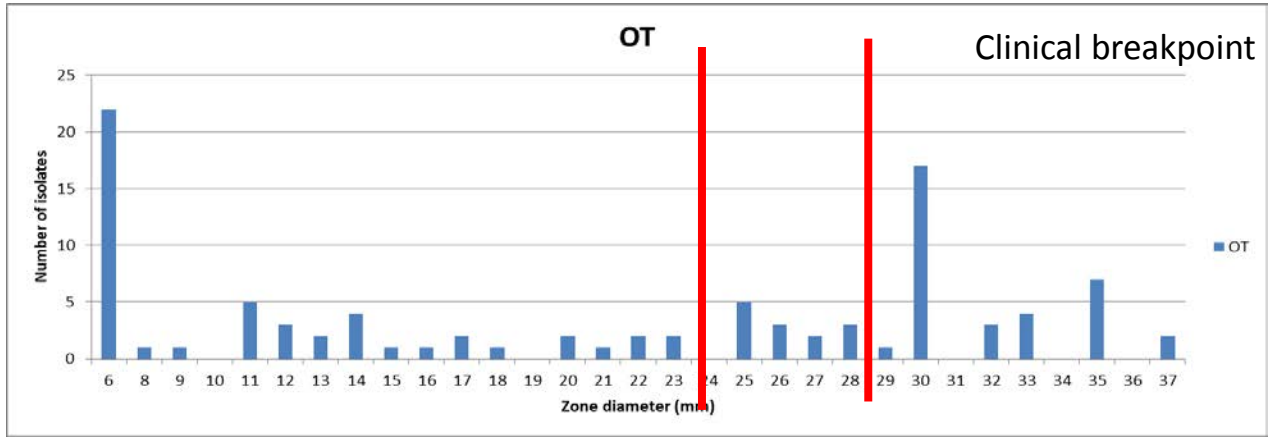

**FFC**

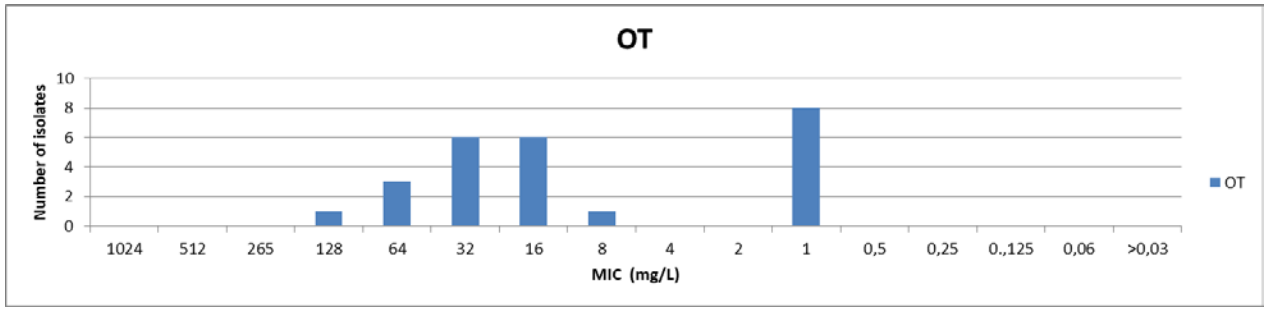
| S | I | R |
|---|---|---|
| ≥28 | 22 - 27 | 21≤ |

# Results

# *Aeromonas hydrophila*

Interpretation criteria




**OT**

| S | I | R |
|---|---|---|
| ≥29 | 23 - 28 | 24≤ |

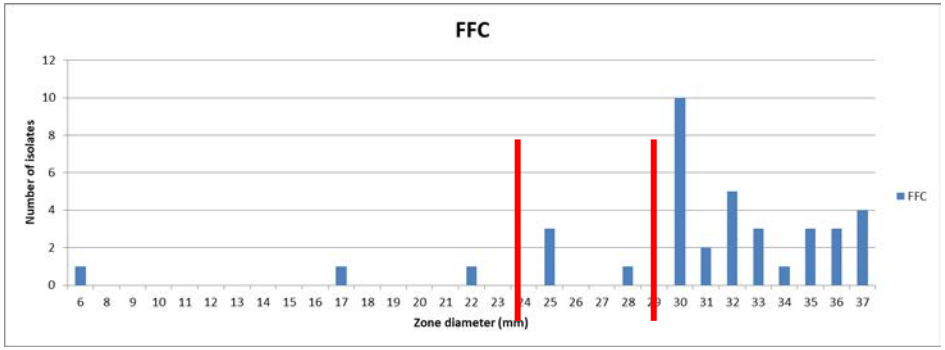
# *Aeromonas hydrophila*

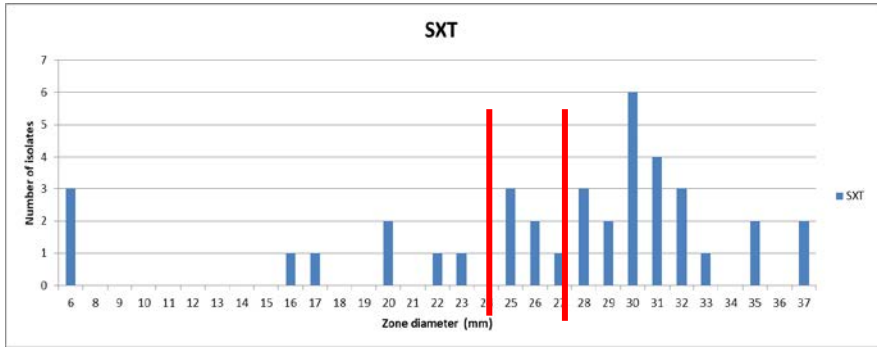
Interpretation criteria

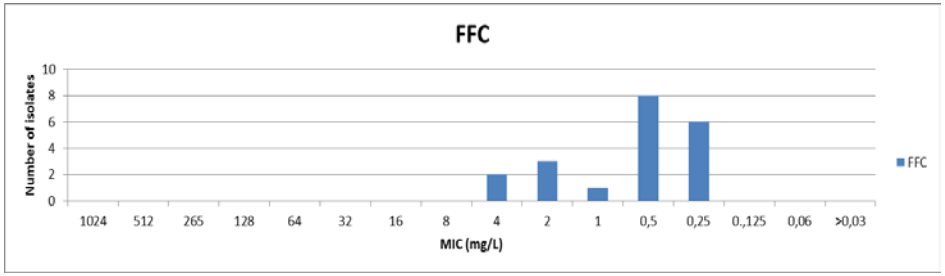
| | S | I | R |
|---|---|---|---|
| FFC | ≥28 | 22 - 27 | 21≤ |
| OT | ≥29 | 25 - 28 | 24≤ |
| ENR | ≥28 | 25 - 27 | 24≤ |
| UB | ≥28 | 24 - 27 | 23≤ |
| S | ≥28 | 25 - 27 | 24≤ |
| SXT | ≥29 | 23 - 28 | 22≤ |

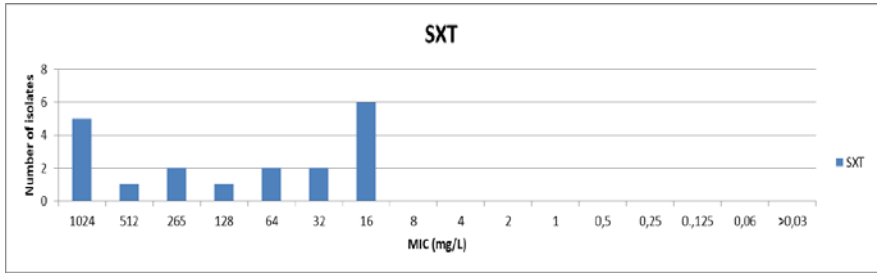
# Results

## *Aeromonas sobria*



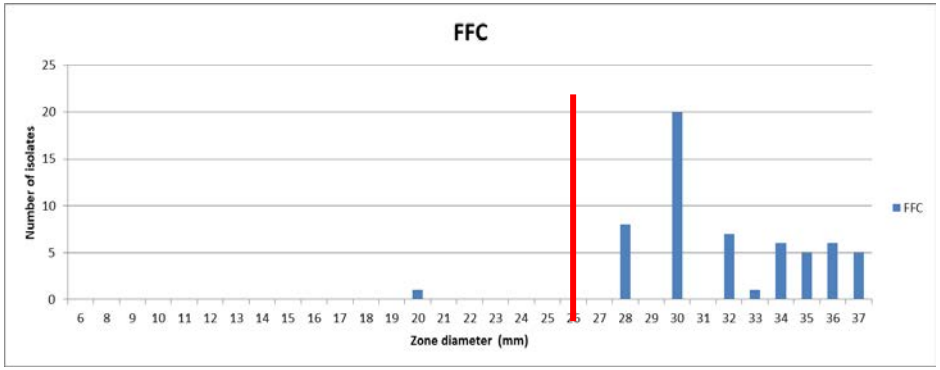



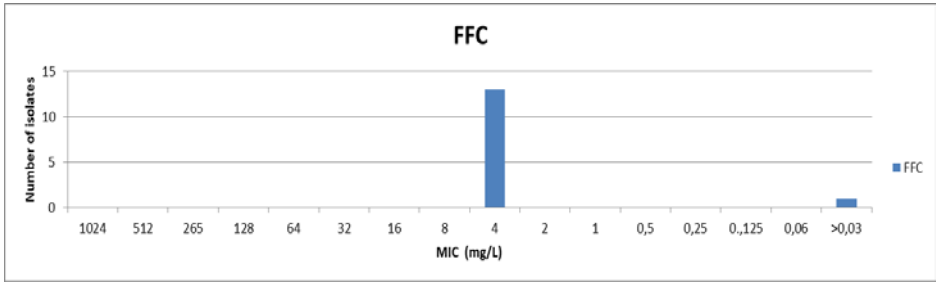



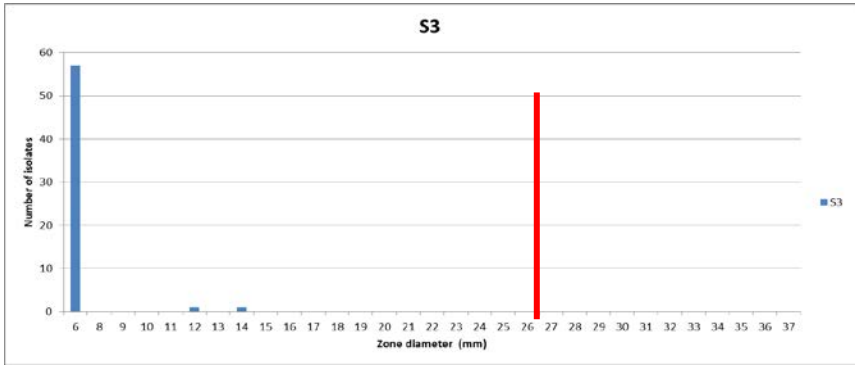



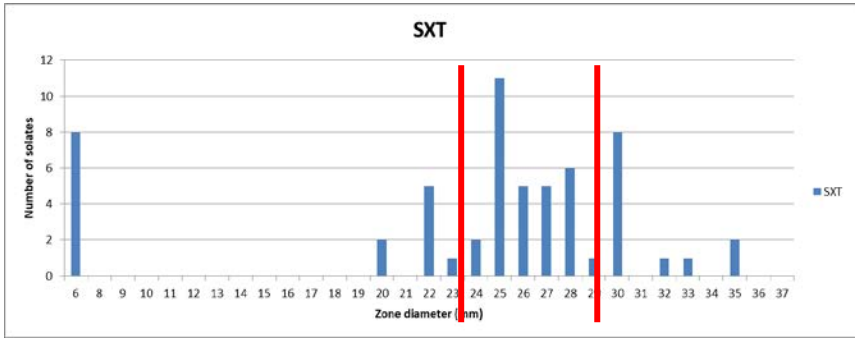
# Results

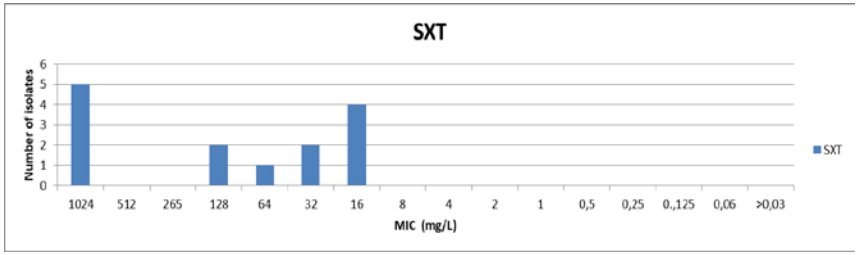
## *Shewanella putrefaciens*



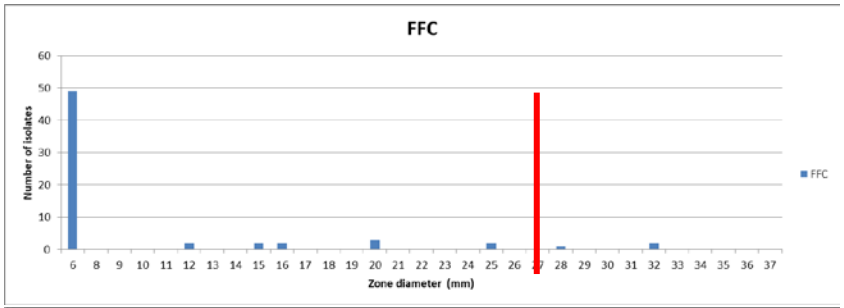



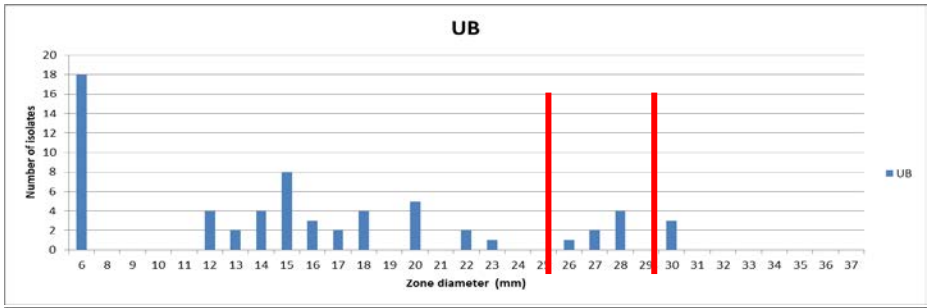



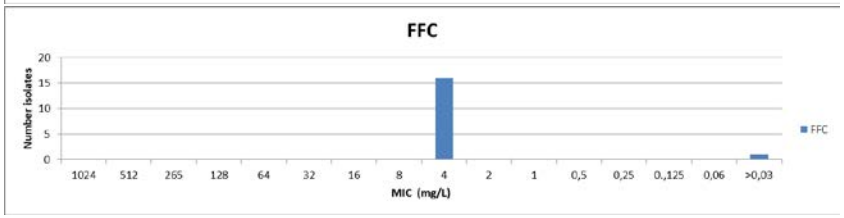



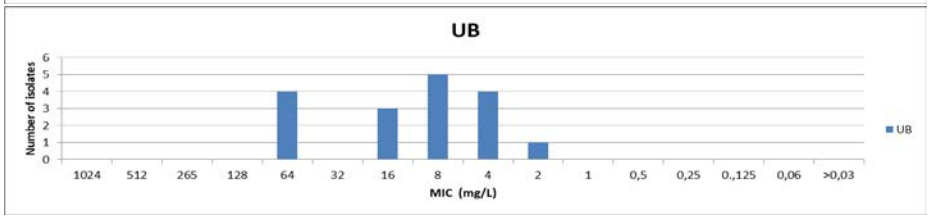
# Results

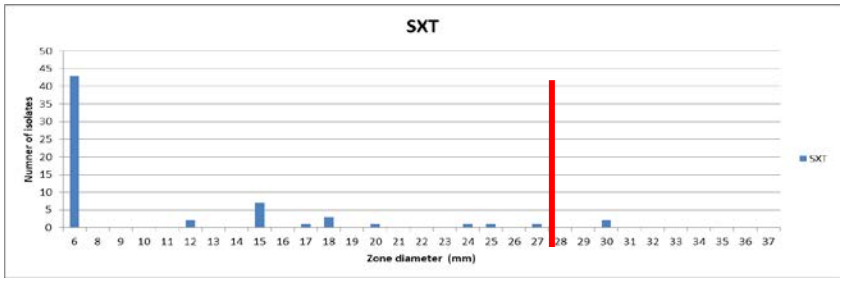
## *Pseudomonas fluorescens*

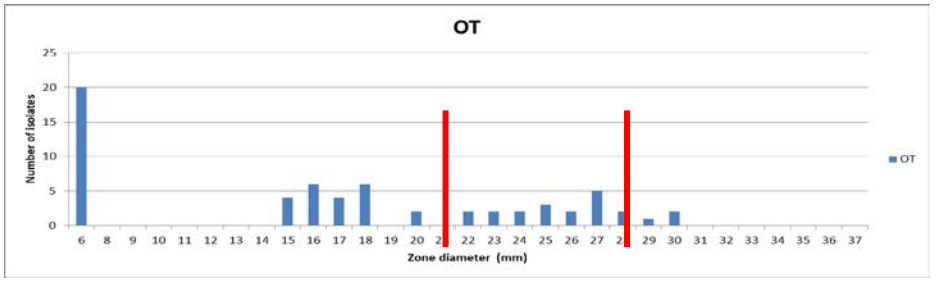


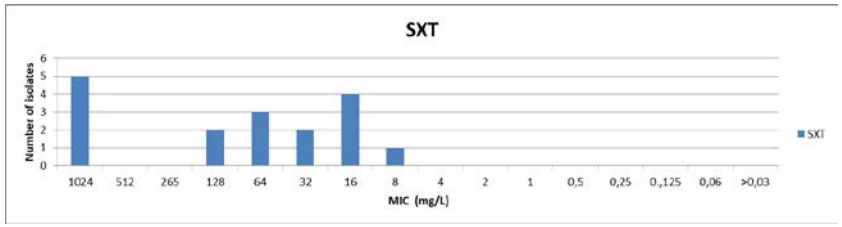



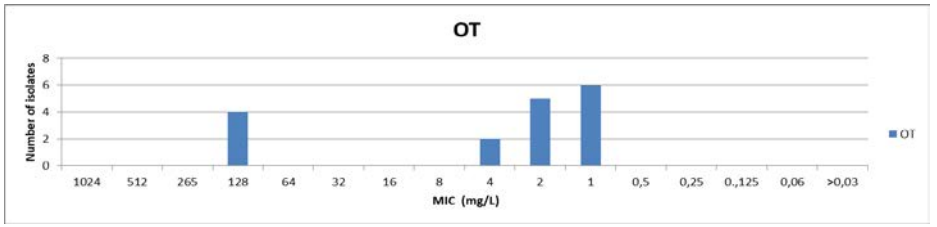



# Conclusion

- Our study indicate that antimicrobials resistance in ichthyopathology is present
- Testing of collection of bacterial strains is a first step towards the development of interpretation criteria for antimicrobials used in combating bacterial infections in farmed fish

DUBLIN, MAY 22ND- 24TH 2019

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