Laurens Tuts

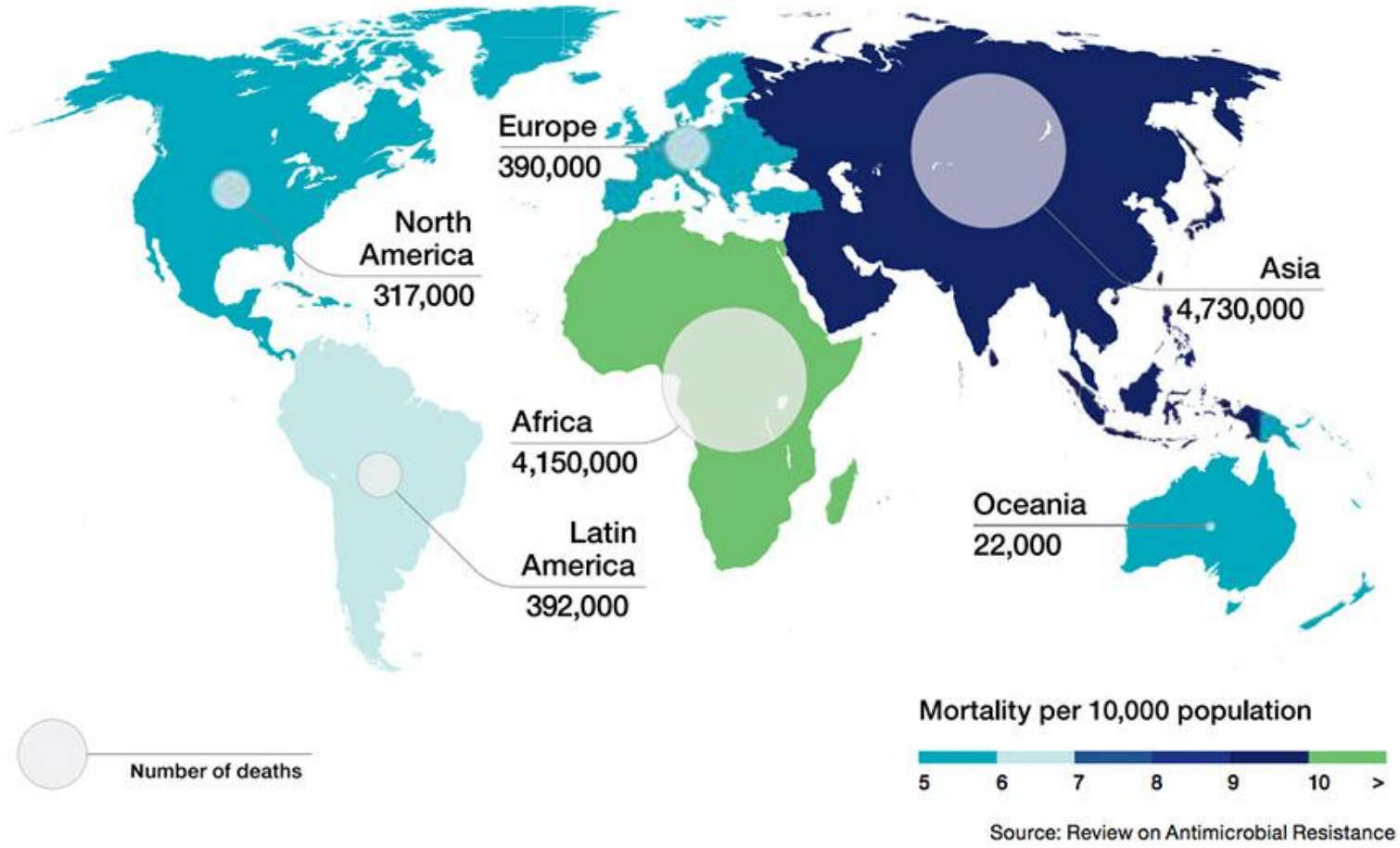
ANTIBIOTIC RESIDUES AND RESISTANCE IN AQUATIC ENVIRONMENTS

Manure application and marine sediments increase antibiotic residues and drive resistance in aquatic environments

ILVO belspo GHENT UNIVERSITY Water JPI JPI OCEANS jpiamr Joint Programming Initiative on Antimicrobial Resistance Aquatic Pollutants

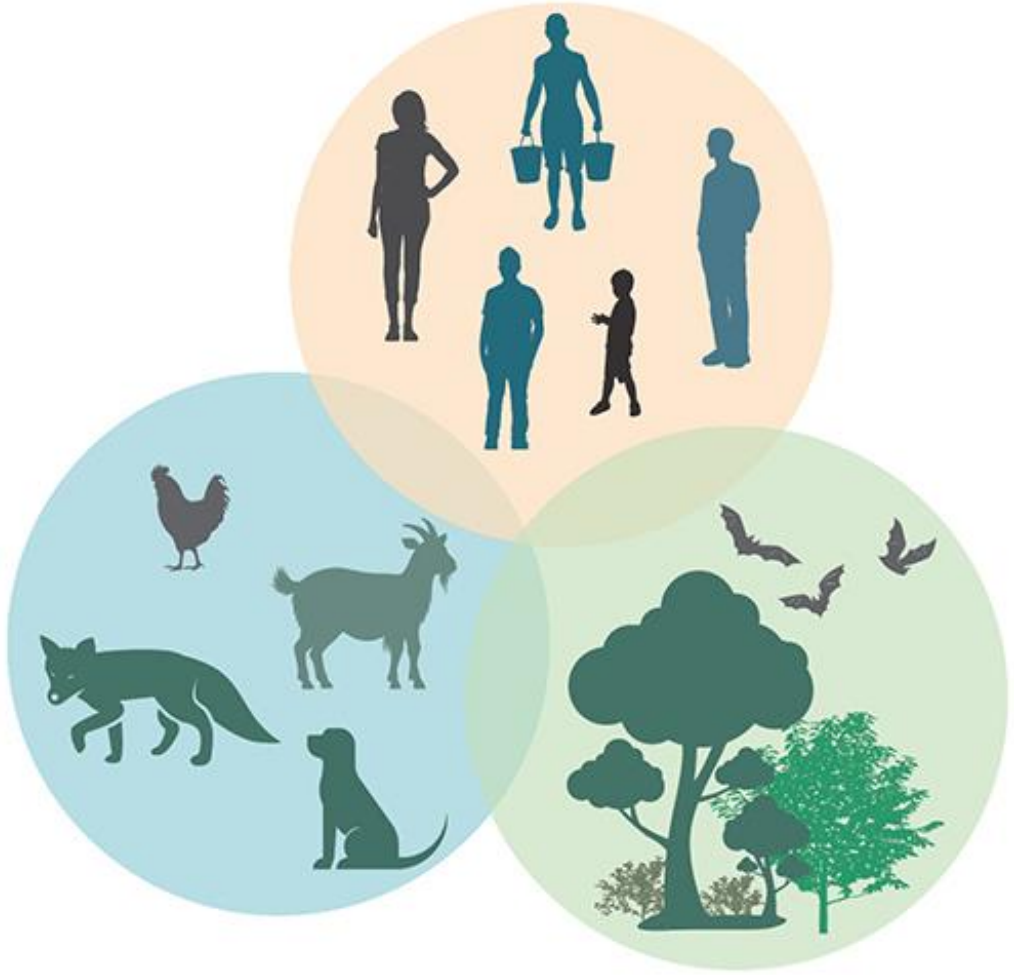
BACKGROUND

PROJECTIONS OF DEATHS FROM ANTIMICROBIAL RESISTANCE (2050)



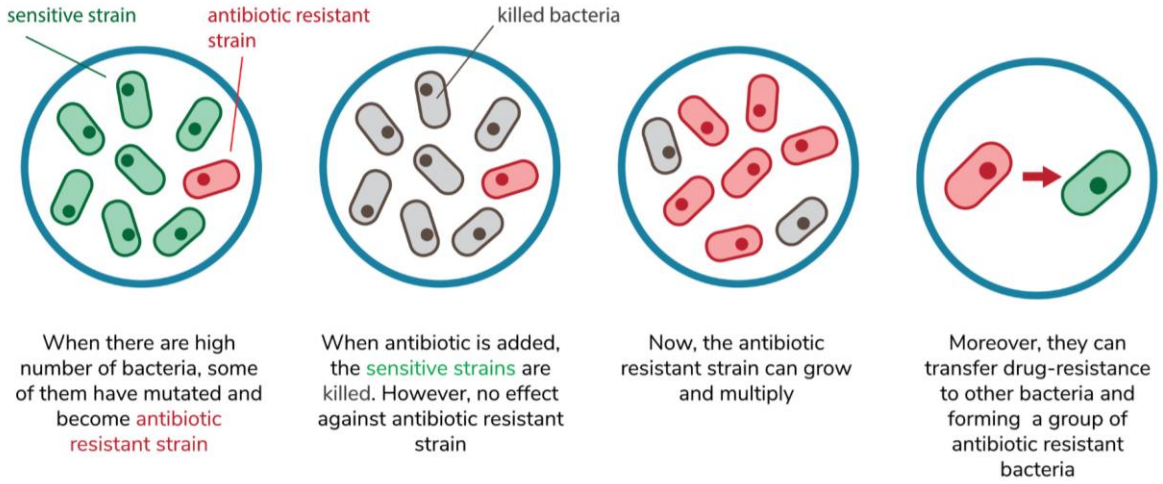
BACKGROUND

ONE HEALTH is the idea that the health of people is connected to the health of animals and our shared environment



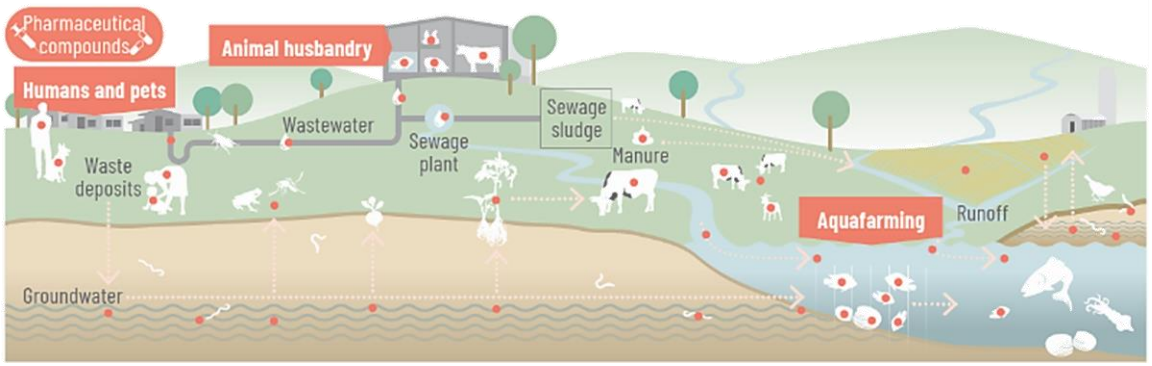
Center for disease control & prevention (2021)

HOW DO BACTERIA BECOME RESISTANT TO ANTIBIOTICS?



SPARTHA Medical, 2023

PATHWAYS OF ANTIBIOTICS FOR HUMAN AND VETERINARY USE



Berkner S, Konradi S, Schönfeld J. (2014). Antibiotic resistance and the environment--there and back again: Science & Society series on Science and Drugs.

OVERVIEW OF STUDIES

AIMS

- Antibiotic residues and antibiotic resistant bacteria in aquatic environments
- **Freshwater (agricultural)** field study
  - Surface water and groundwater
  - *Does antibiotic residues and antibiotic resistance increase after fertilization with manure?*
- **Harbor** field study
  - Water and sediments
  - *Where are hotspots of contamination? What is the relation between antibiotic residues and the detected antibiotic resistance?*

METHODOLOGY

ANTIBIOTIC RESIDUES LC-MS/MS ANALYSIS

ANTIBIOTIC RESIDUES: UHPLC-MS/MS analysis of water samples

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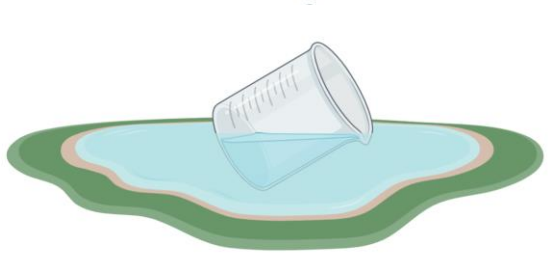
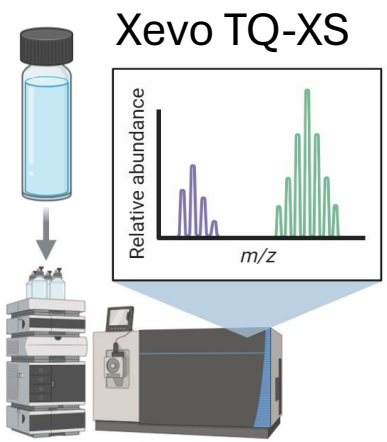
METHODOLOGY



Surface water, groundwater, marine water (100 mL)

Solid Phase Extraction
(SPE)
OASIS HLB cartridges (6 cc, 500 mg)



78 antibiotics
10 antibiotic classes

Limit of detection:
0.01 – 1 µg/L
Validated EU 2021/808

Chemosphere 352 (2024) 141455

Contents lists available at ScienceDirect

Chemosphere

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

Detection of antibiotic residues in groundwater with a validated multiresidue UHPLC-MS/MS quantification method

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[c] Ghent University, Faculty of Veterinary Medicine, Department of Pathobiology, Pharmacology and Zoological Medicine, Salisburylaan 133, 9820, Merelbeke, Belgium
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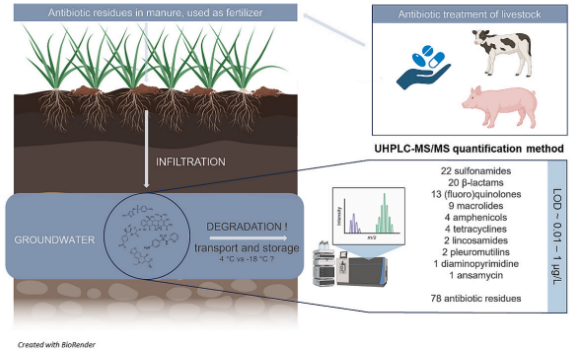
HIGHLIGHTS

- Intensive livestock farming leads to antibiotics entering the environment.
- Validated, UHPLC-MS/MS method for quantification of 78 antibiotics in water samples.
- β-lactams and macrolides are most subjected to degradation during storage.
- Groundwater contains antibiotic residues (<0.1 µg/L).

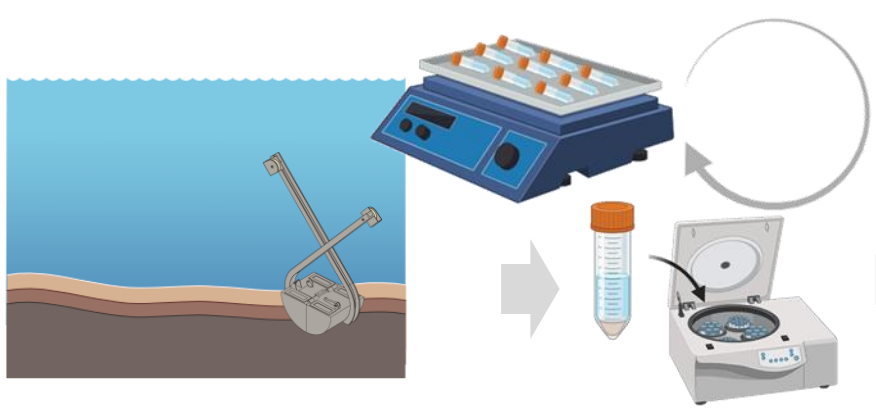
GRAPHICAL ABSTRACT



ANTIBIOTIC RESIDUES: UHPLC-MS/MS analysis of sediment samples

METHODOLOGY

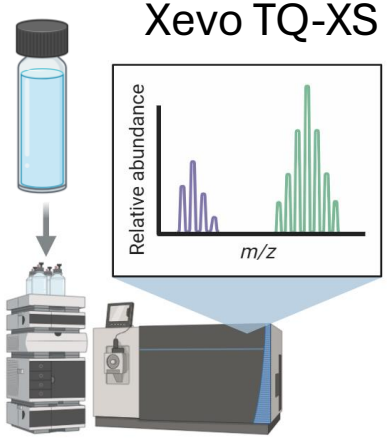
+ QUECHERS absorbents:
K_2PO_4, C18, PSA



Sediment
(freeze-dried, sieved)

Extraction

Xevo TQ-XS



59 antibiotics
9 antibiotic classes

Limit of detection:
0.1 – 10 µg/kg
Validated EU 2021/808

β-lactams

ampicillin
benzylpenicillin
cefadroxil
cefalexin
cefalonium
cefapirin
cefazolin
cefoperazone
cefquinome
ceftiofur
nafcillin
oxacillin
phenoxymethyl penicillin

Lincosamides

lincomycin
pirlimycin

(Fluoro) quinolones

cinoxacin
ciprofloxacin
difloxacin
enoxacin
enrofloxacin
flumequine
nalidixic acid
norfloxacin
ofloxacin
oxolinic acid
sarafloxacin

Phenicols

florphenicol
thiamphenicol
chloramphenicol

Macrolides

clarithromycin
erythromycin
josamycin
tylosin
tylvalosin

Diaminopyridine deriv.

trimethoprim

Pleuromutulines

tiamulin
valnemulin

Tetracyclines

oxytetracycline
tetracycline
chloortetracycline
doxycycline

Sulfonamides

dapsone
sulfabenzamide
sulfachloropyridazine
sulfadiazine
sulfadimethoxine
sulfadoxine
sulfamerazine
sulfameter
sulfamethazine
sulfamethizole
sulfamethoxazole
sulfamethoxypyridazine
sulfamonomethoxine
sulfaphenazole
sulfapyridine
sulfaquinoxaline
sulfathiazole
sulfisoxazole

METHODOLOGY

ISOLATION OF INDICATOR ORGANISM

E. coli

ANTIBIOTIC RESISTANCE: ISOLATION OF INDICATOR ORGANISMS

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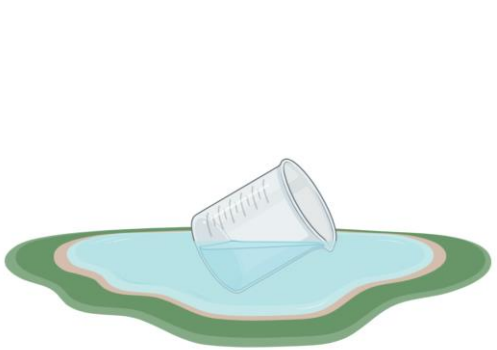
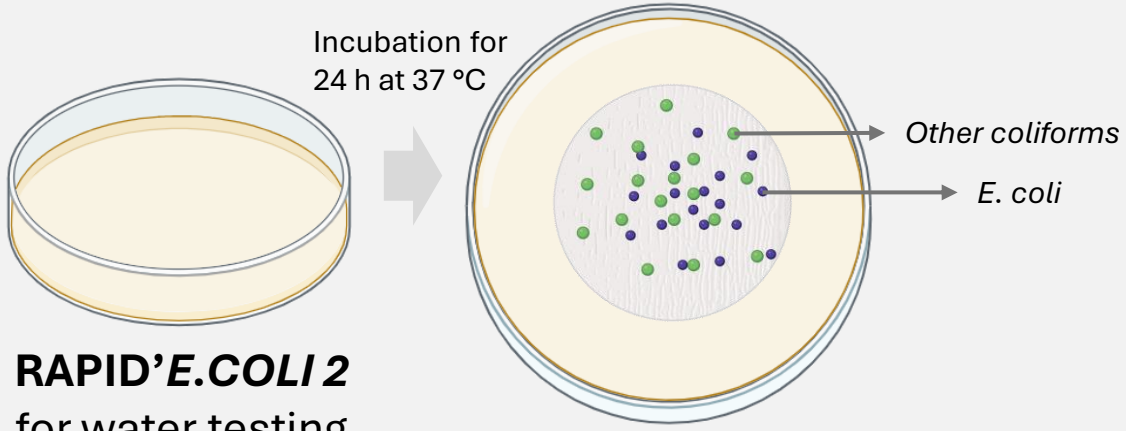
METHODOLOGY



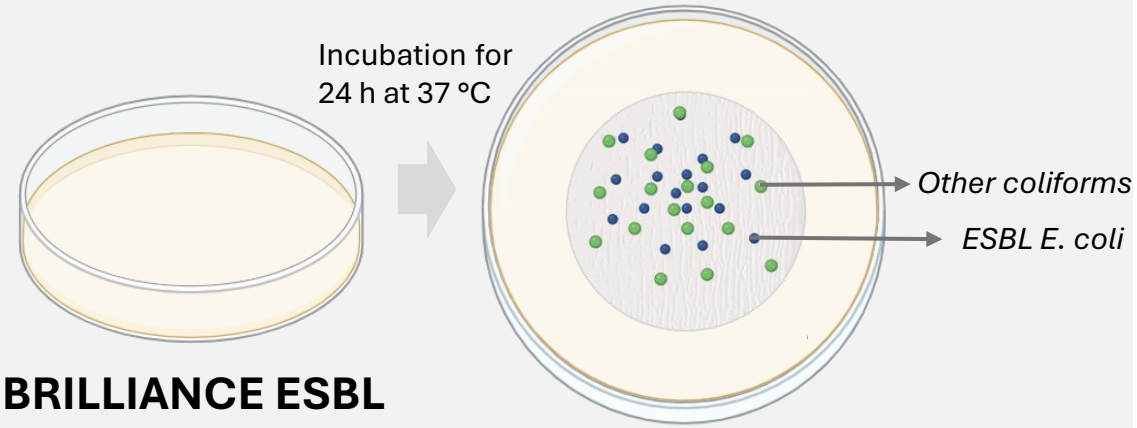
Filtration

E. coli



RAPID'*E.COLI 2*
for water testing

Extended Spectrum β-lactamase producing *E. coli*



BRILLIANCE ESBL

METHODOLOGY

ASSESSMENT OF ANTIBIOTIC RESISTANCE

ANTIBIOTIC RESISTANCE: ANTIBIOTIC SUSCEPTIBILITY TESTS

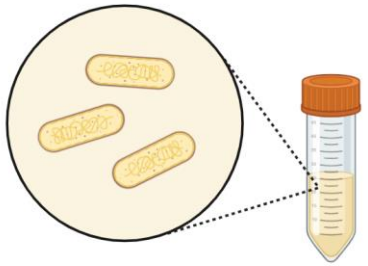
ILVO

METHODOLOGY

E. coli

Sensititre EU Surveillance *E. coli*
EUVSEC Plate (ThermoFisher)

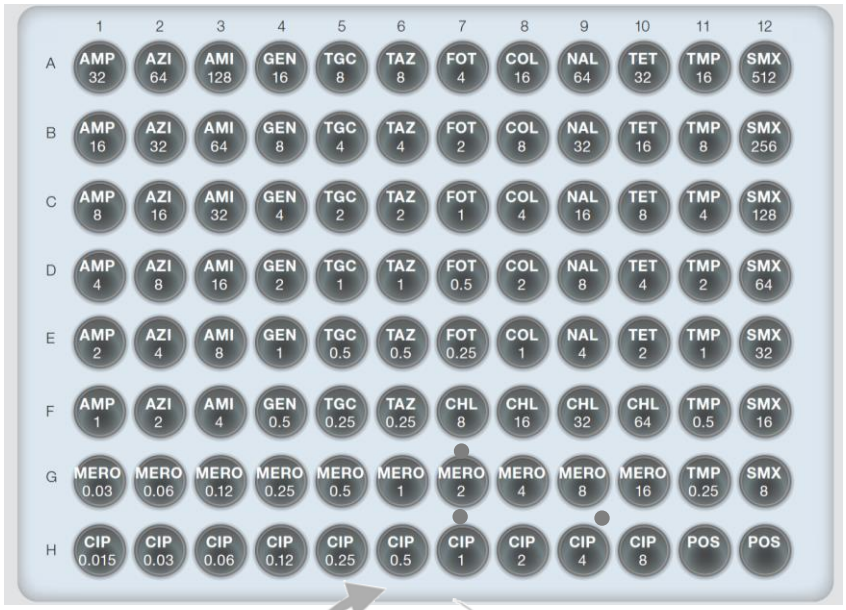
Inoculation



Cation Adjusted Mueller Hinton Broth

CONCENTRATION

ANTIBIOTIC



Minimal inhibitory concentration

Positive control

Incubation
24h at 37 °C

ANTIBIOTIC RESISTANCE: ANTIBIOTIC SUSCEPTIBILITY TESTS

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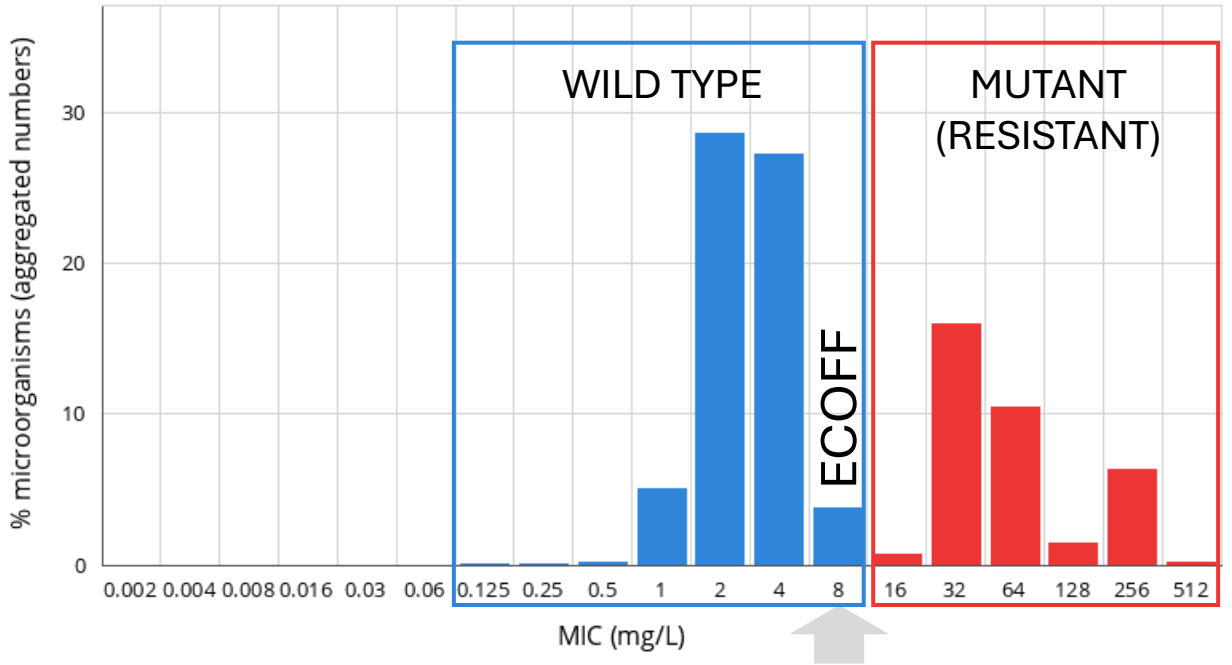
METHODOLOGY

MIC distributions for ampicillin resistance of *E. coli*



MIC
Epidemiological cut-off (ECOFF): 8 mg/L
Wildtype (WT) organisms: ≤ 8 mg/L

Confidence interval: 4 - 16
105483 observations (53 data sources)

EUCAST EUROPEAN COMMITTEE ON ANTIMICROBIAL SUSCEPTIBILITY TESTING
European Society of Clinical Microbiology and Infectious Diseases

Minimum inhibitory concentration
>
Epidemiological cut-off value
=
isolate is **resistant**

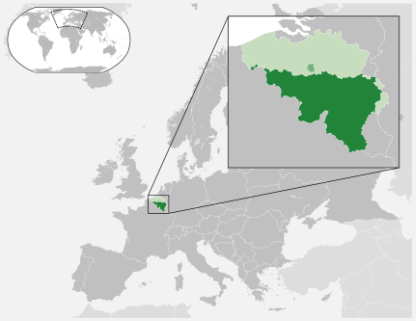
FRESHWATER

SAMPLING

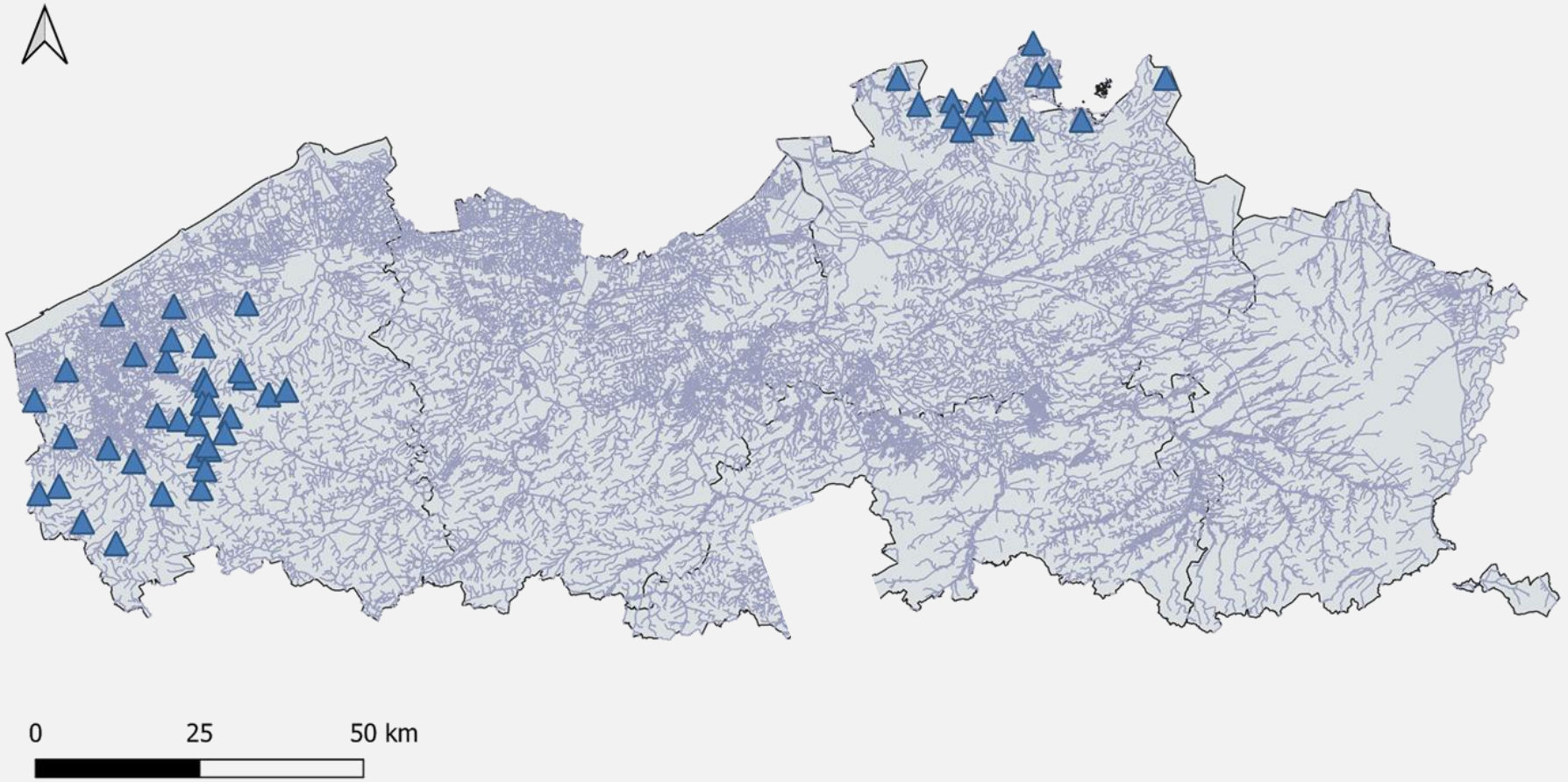
STUDY AREA

ILVO

- 50 sampling locations ~ EU Nitrate Directive → more polluted areas
- Before and after fertilization → manure = entry of antibiotics and antibiotic resistant bacteria?



Flanders, Belgium



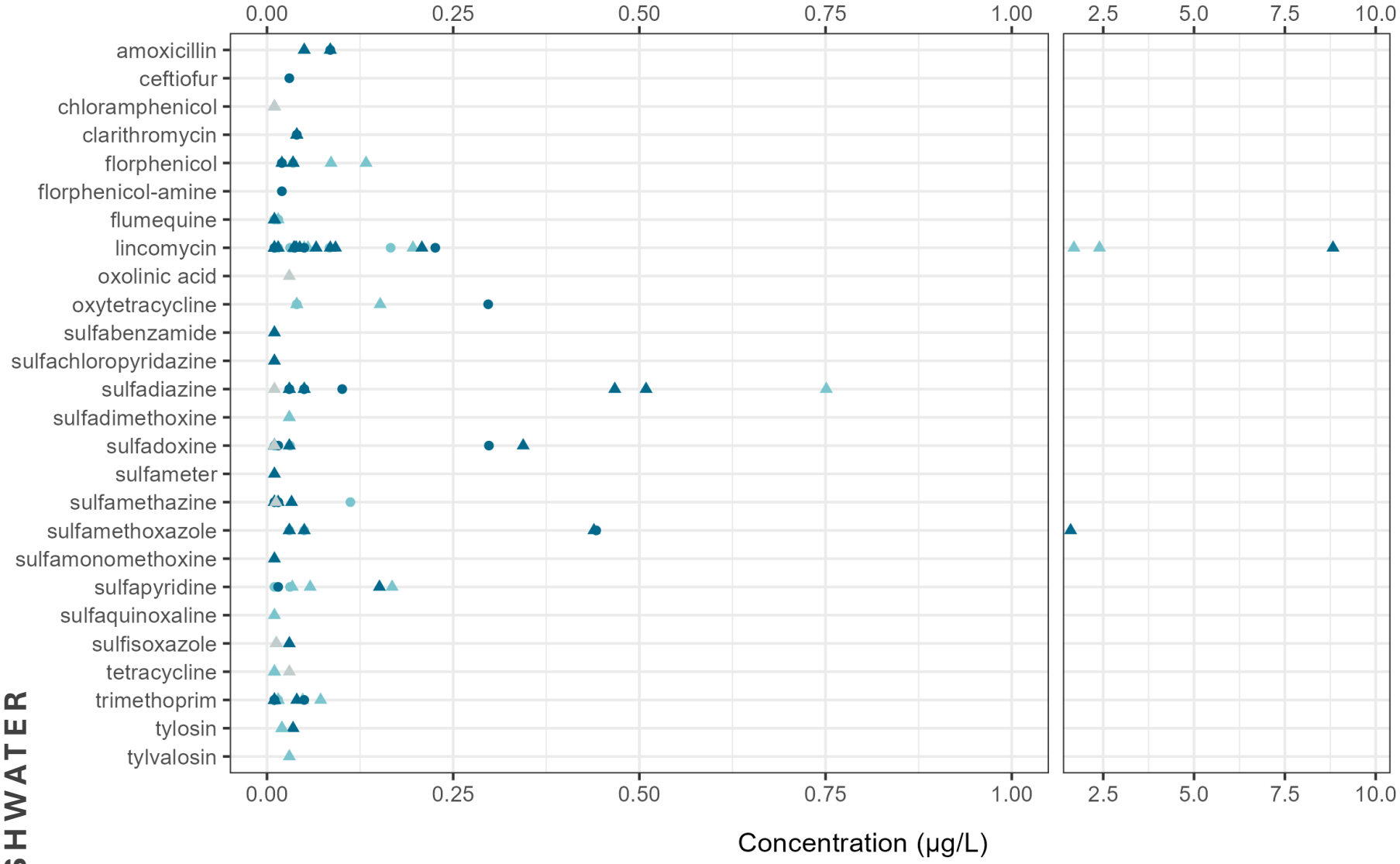
FRESHWATER

FRESHWATER

ANTIBIOTIC RESIDUES

ANTIBIOTIC RESIDUES

n = 99



- 60% of surface water contaminated with residues
- 25 different antibiotic residues
- Low concentrations
  - Maximum concentration = 8.8 µg/L (lincomycin)
- Lincomycin and sulfonamides

NO significant INFLUENCE of the fertilization period

FRESHWATER

LINK BETWEEN ANTIBIOTICS AND ANTIBIOTIC RESIDUES

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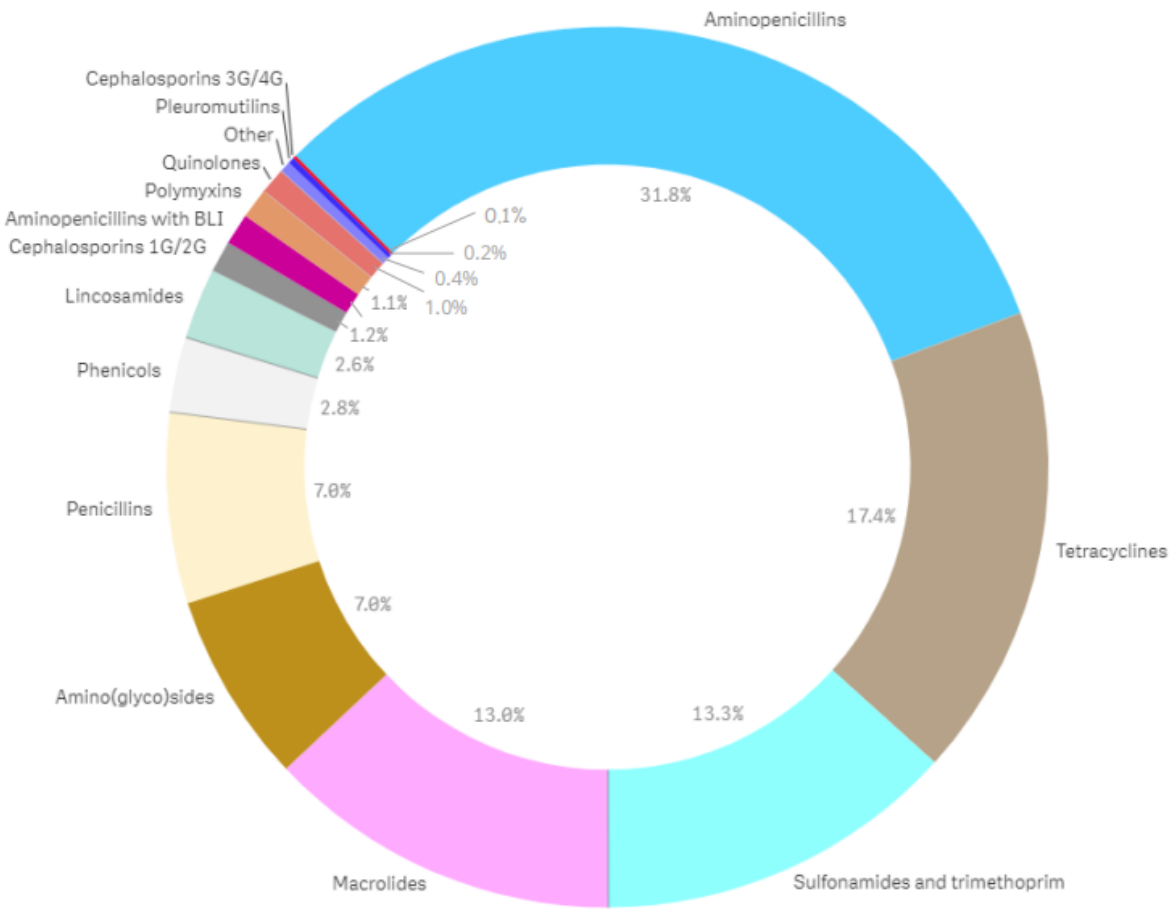
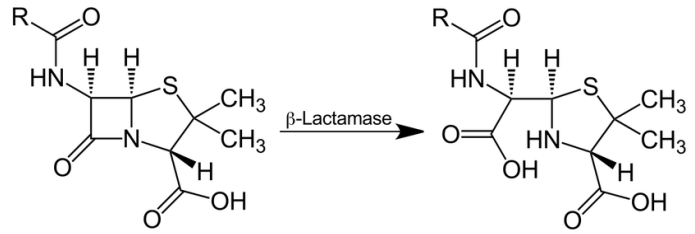
VETERINARY USAGE (BelVetSac 2023)

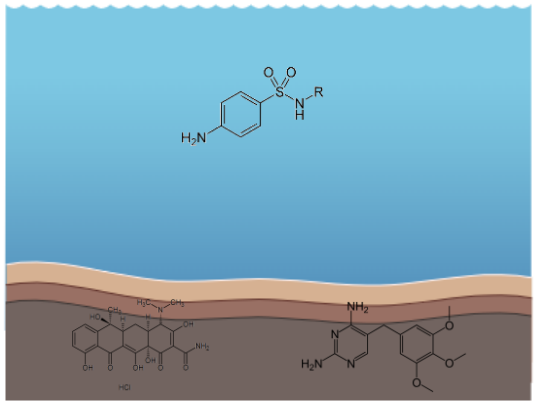


PHYSICO-CHEMICAL PROPERTIES



Enzymatic hydrolysis of β-lactam antibiotics in water

MATRIX PROPERTIES



FRESHWATER

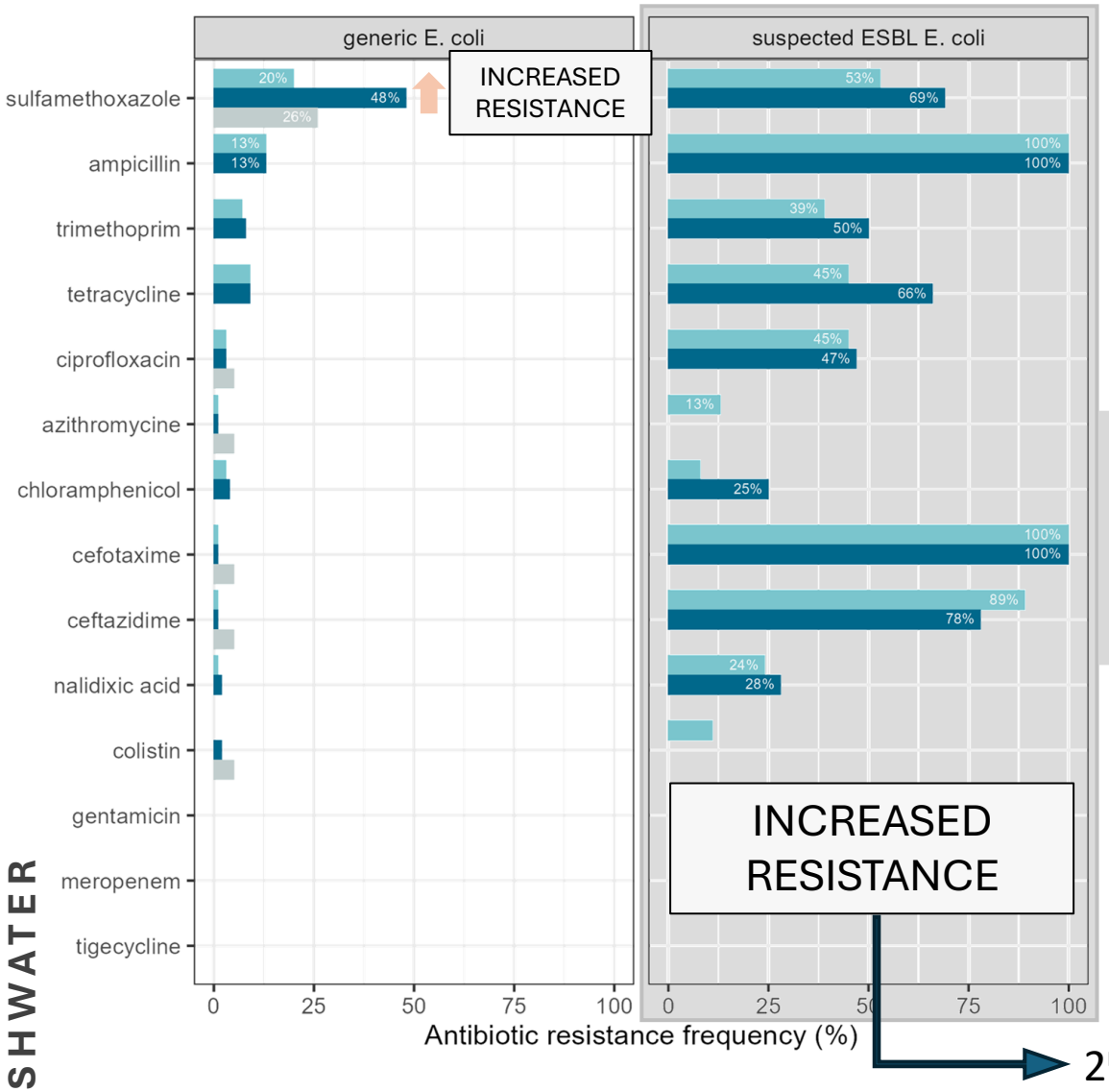
FRESHWATER

ANTIBIOTIC RESISTANCE

ANTIBIOTIC SUSCEPTIBILITY TESTS

ILVO

FRESHWATER



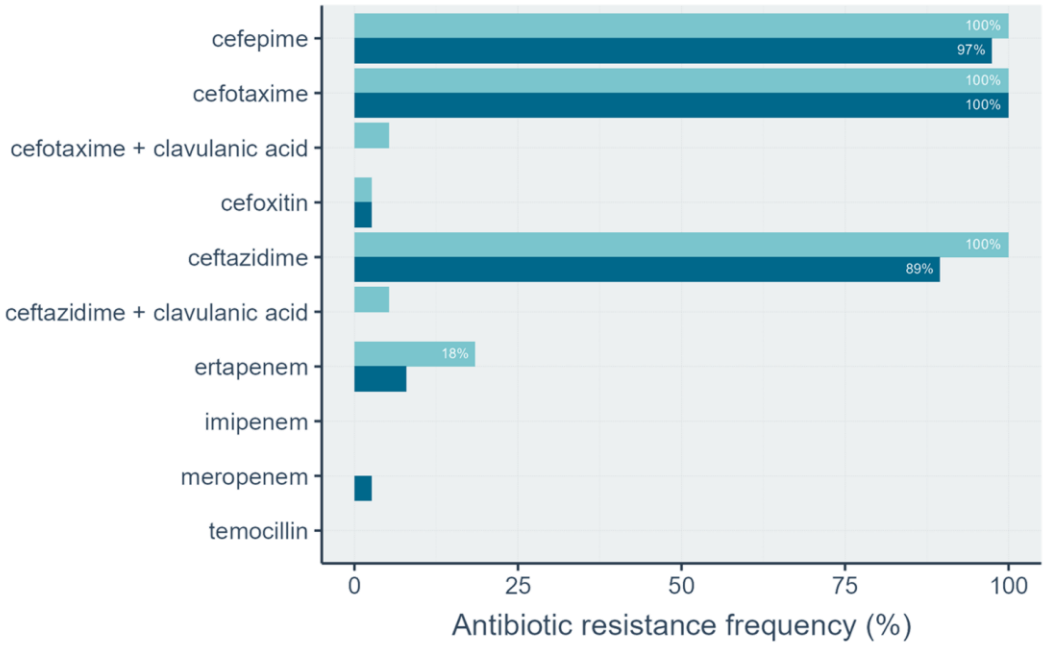
INCREASED RESISTANCE

EUVSEC 3

- **sulfamethoxazole**
  - ↑ Increase after fertilization
- ampicillin
- trimethoprim
- tetracycline

2nd field study: only ESBL *E. coli*

ESBL-producing *E. coli*



EUVSEC 2

- 3rd and 4th generation cephalosporines
- carbapenems
- β-lactams + inhibitor

CONCLUSION: ASSOCIATION BETWEEN RESIDUES AND RESISTANCE

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- Minimum selective concentration < detected concentration < minimum inhibitory concentration?

$$\text{RISK} = \frac{\text{Measured concentration}}{\text{Predicted no-effect concentration for antibiotic resistance selection}}$$

  - RISK > 1 (sulfadiazine, lincomycin): 5 times

- Sulfonamides frequently present

⇔

- Sulfamethoxazole resistance widespread in the environment

  + increased resistance after the fertilization period

FRESHWATER

HARBOR SAMPLING

STUDY AREA

STUDY AREA + SAMPLING PLAN

ILVO

Water + sediment

2023

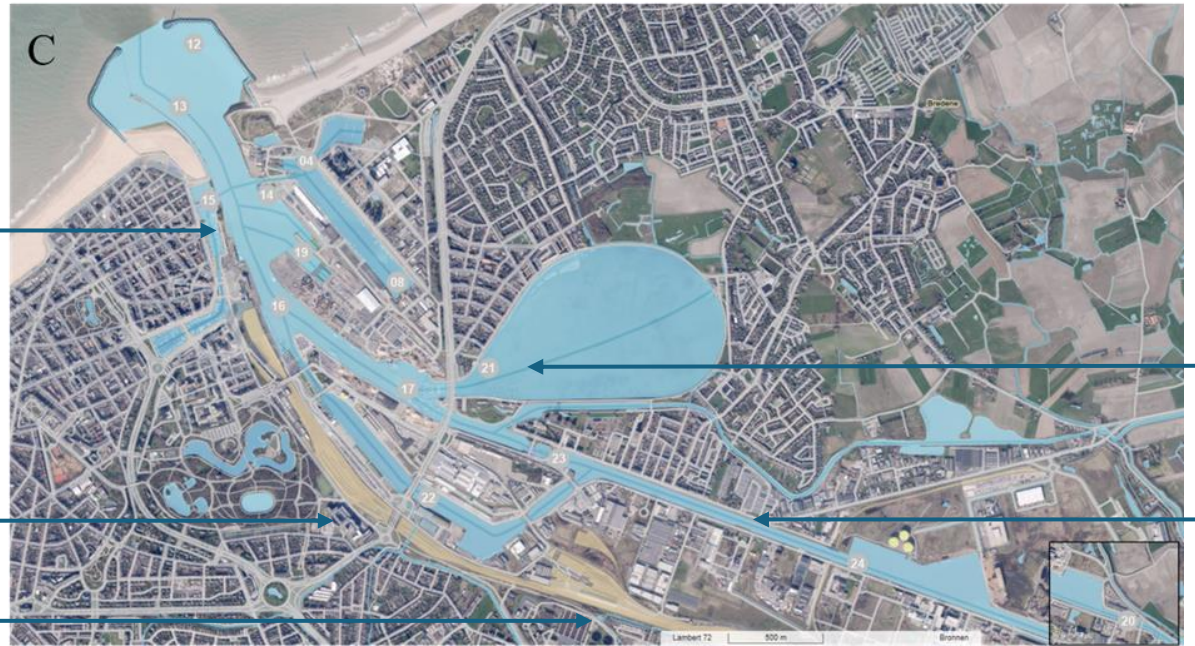
- 9 locations in Nieuwpoort (B)
- 10 locations in Oostende (C)

2024

- 14 locations in Oostende (C)

Marina

IJzer



Marina

Recreation

Hospital

Brugge-Oostende

WWTP

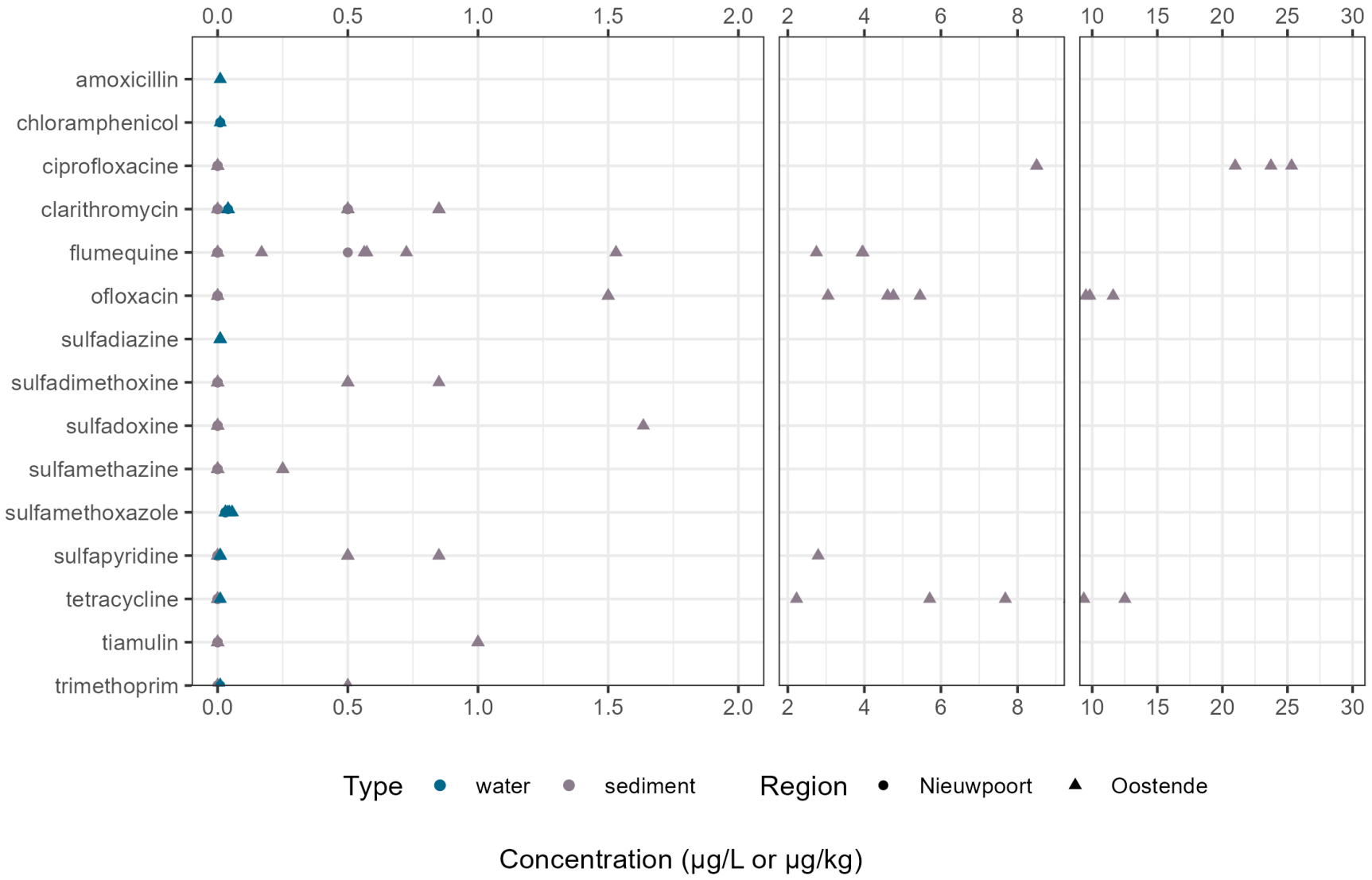
HARBOR SAMPLING

HARBOR SAMPLING

ANTIBIOTIC RESIDUES

ANTIBIOTIC RESIDUES

ILVO



- 15 residues from 8 classes
- More antibiotic residues and higher concentrations in **sediments**
- **ciprofloxacin**, **tetracycline** and **ofloxacin** were reported at the highest concentrations in sediments
- concentrations were higher in **Oostende**
- sulfonamides were most frequently detected in water

~ Use in human medicine, physicochemical properties, matrix properties

HARBOR SAMPLING

HARBOR SAMPLING

ANTIBIOTIC RESISTANCE

ANTIBIOTIC SUSCEPTIBILITY TESTING: n = 59 ESBL *E. coli*

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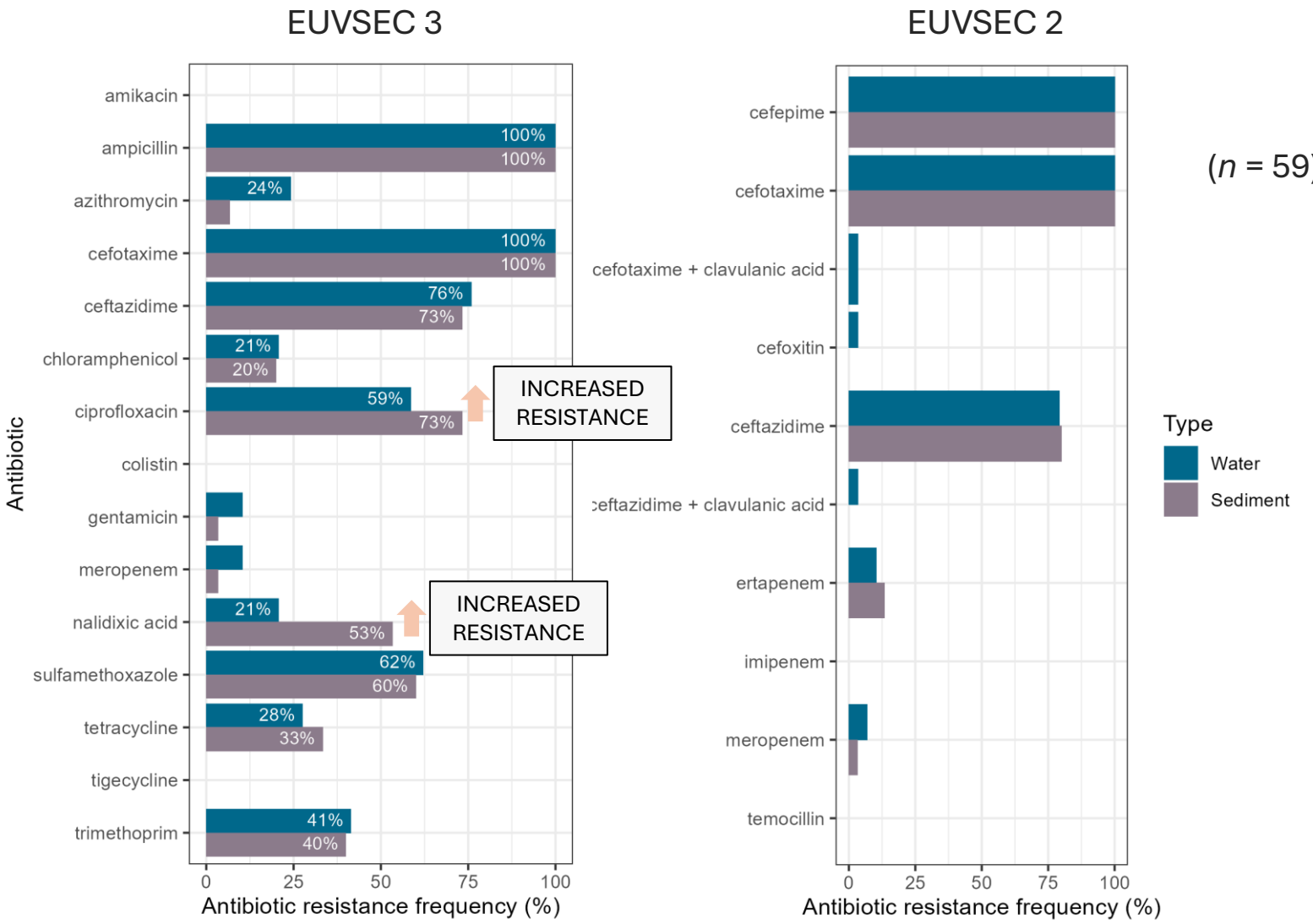


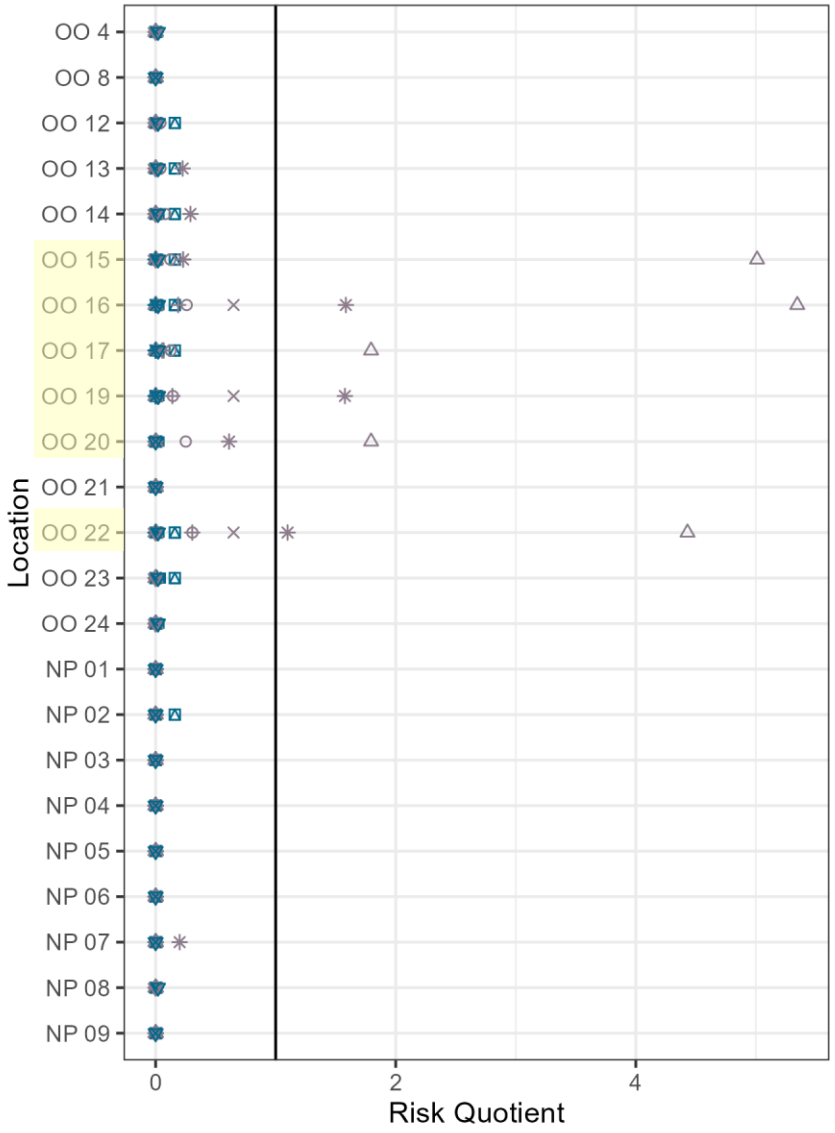
(*n* = 59)

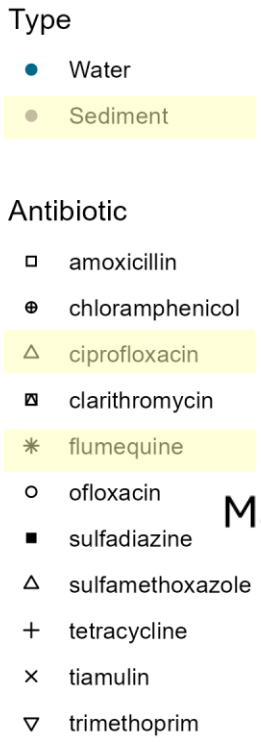
- High resistance levels for ESBL *E. coli* (=fecal indicator)
  - 70 % MDR (> 3 AB classes)
- ! Resistance to carbapenems (=critical antibiotic (WHO))
- Significant differences between water and sediment samples ($p < 0.05$)
  - ↑ ciprofloxacin and nalidixic acid resistance in sediments
  - ! Sediment = sink for antibiotics → resistance selection

HARBOR SAMPLING

RISK ASSESSMENT: CONCENTRATION / PNEC

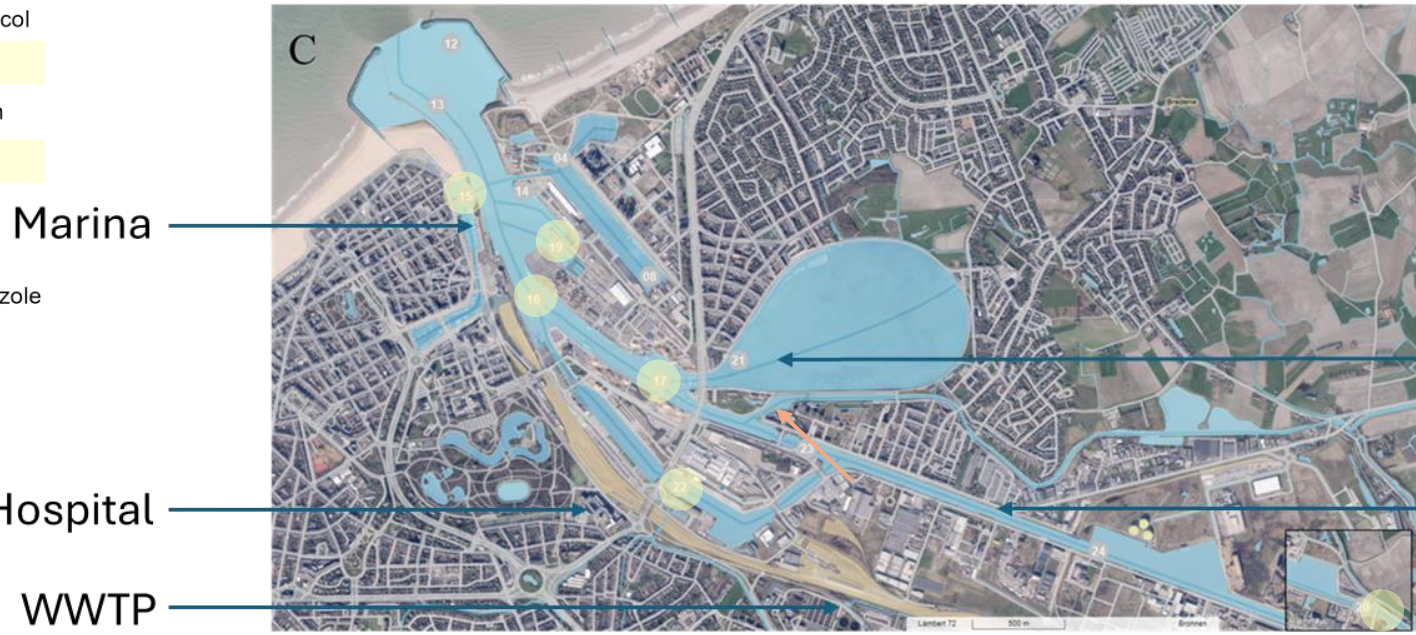
ILVO





$$\text{RISK} = \frac{\text{Measured concentration} * \text{Kd}}{\text{Predicted no-effect concentration}}$$

1) Sediments
2) Quinolones
3) WWTP & marina hotspots = entry of antibiotics and antibiotic resistance



HARBOR SAMPLING

CONCLUSIONS

ILVO

- Need for responsible antibiotic use and effective manure/water treatment
- Need for continuous surveillance, including:
  - Antibiotic resistance
  - Critical antibiotics
  - Sediment/soils

WHAT'S NEXT?

ILVO

NUTRITIVE

Innovative decision-making tool for defining the most suitable manure management strategies to achieve a sustainable livestock farming system during the whole value chain

AIM: reducing air, water and soil pollutants produced by livestock manure management throughout its chain.

https://nutritive.es/

THANK YOU

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Sunniva Hoel
Jonatan Rodriguez
Enrique Joffré
Annie Justh
Kartik Baruah
María Del Mar Tavío Pérez

belspo ILVO GHENT UNIVERSITY

Water JPI jpiamr Joint Programming Initiative on Antimicrobial Resistance

JPI OCEANS Aquatic Pollutants

QUESTIONS?