THE PREVALENCE OF ANTIMICROBIAL RESISTANCE IN THE WATER CYCLE

Leal, HF

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National Water and Wastewater Conference, 2023

WHAT IS ANTIMICROBIAL RESISTANCE?

Amoxicillin

Ciprofloxacin

Cephalexin

Bactrim

Penicillin
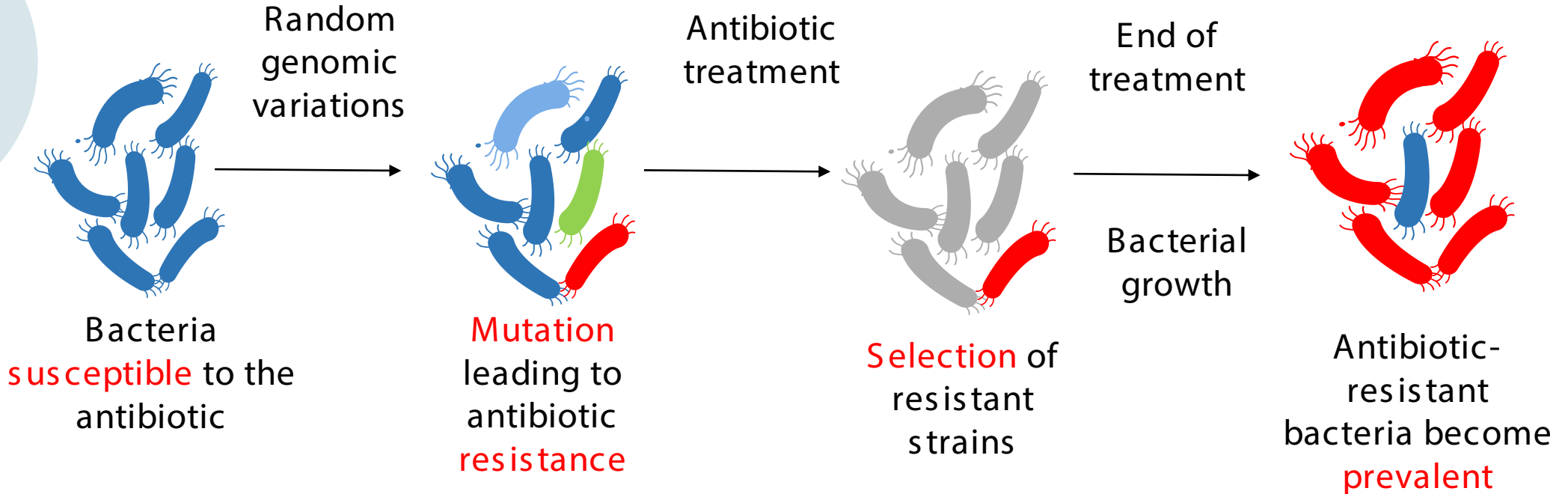
2

Image source: icons and shapes available on the SlidesGo website

WHAT IS ANTIMICROBIAL RESISTANCE?

Ability to survive the effects of substances that are supposed to kill or control bacterial growth

Random genomic variations

Antibiotic treatment

End of treatment

Bacteria **susceptible** to the antibiotic

Mutation leading to antibiotic **resistance**

Selection of resistant strains

Bacterial growth

Antibiotic-resistant bacteria become **prevalent**

One infection every 11 seconds
One death every 15 minutes

WHO, Antimicrobial Resistance Fact sheet, Updated September 2016
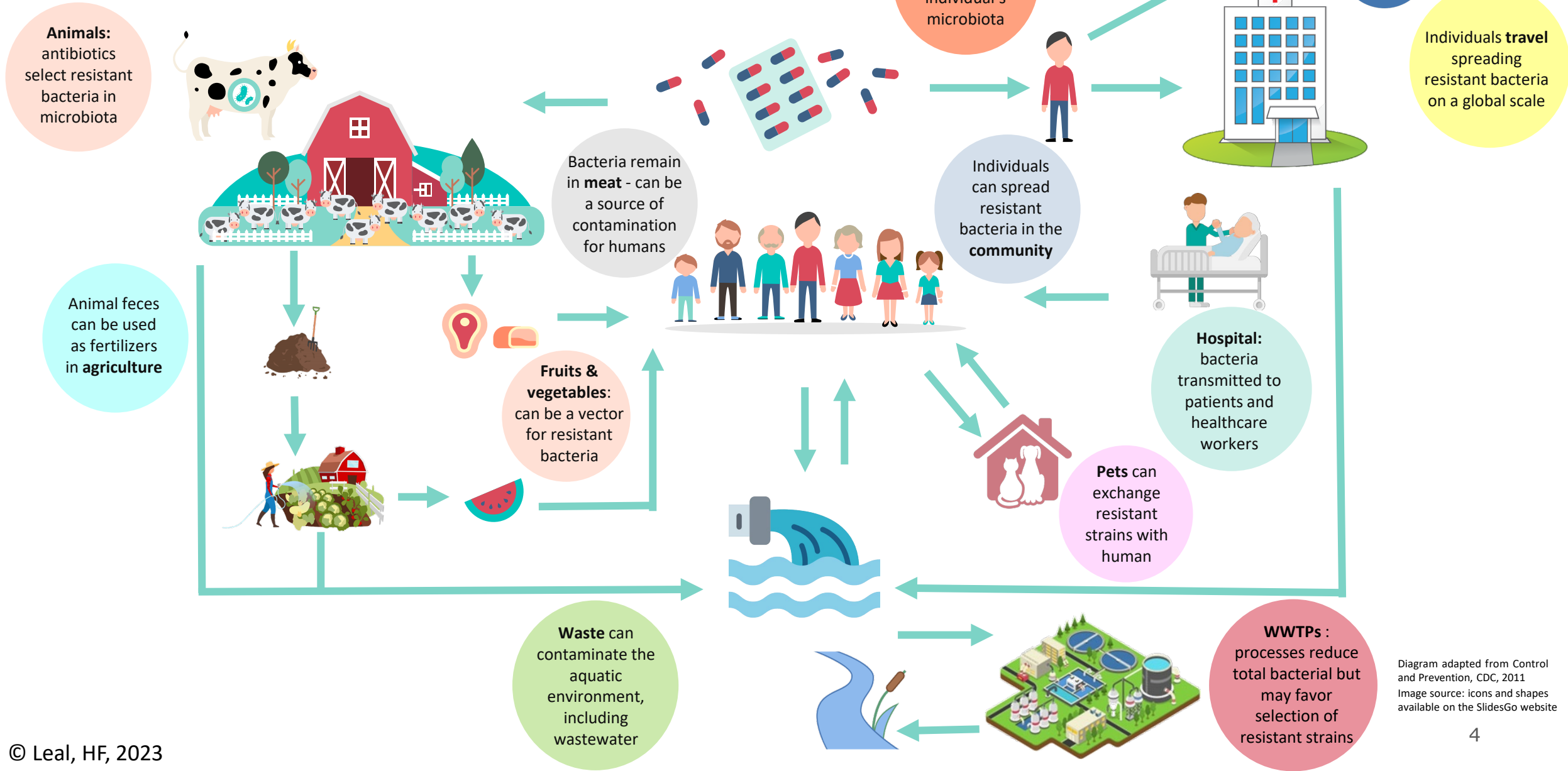Image source: icons and shapes available on the SlidesGo website

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3

BEYOND THE HOSPITAL

Animals: antibiotics select resistant bacteria in microbiota

Antibiotics select resistant bacteria in individual's microbiota

Individuals **travel** spreading resistant bacteria on a global scale

Bacteria remain in **meat** - can be a source of contamination for humans

Individuals can spread resistant bacteria in the **community**

Animal feces can be used as fertilizers in **agriculture**

Fruits & vegetables: can be a vector for resistant bacteria

Hospital: bacteria transmitted to patients and healthcare workers

Pets can exchange resistant strains with human

Waste can contaminate the aquatic environment, including wastewater

WWTPs: processes reduce total bacterial but may favor selection of resistant strains

Diagram adapted from Control and Prevention, CDC, 2011
Image source: icons and shapes available on the SlidesGo website

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4

WHY STUDY THE WATER CYCLE ?

Population-based surveillance studies → laborious and expensive

Water cycle → reflects of what happens at community level

Combine this research with an analysis of lifestyle habits

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6
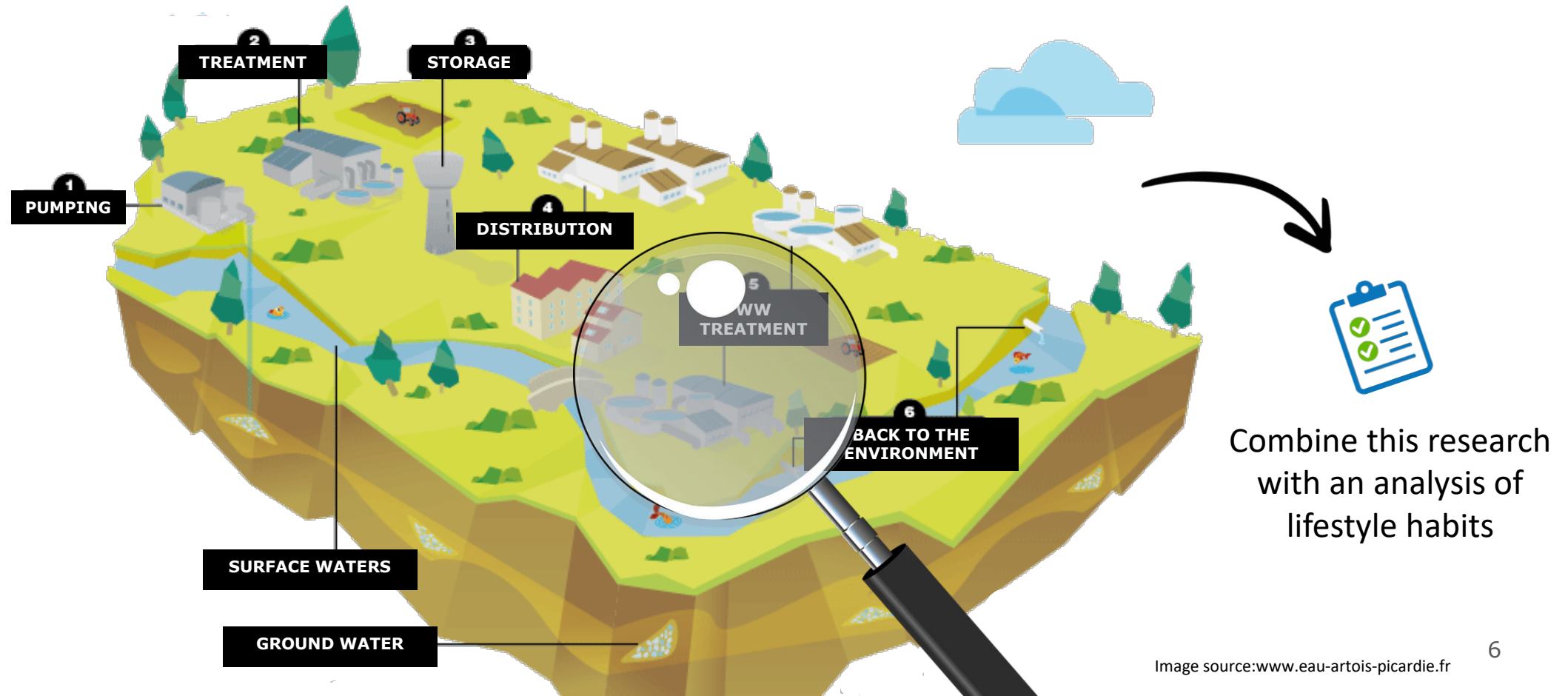
OBJECTIVES

Correlate the prevalence of resistance genes in the water cycle with population behaviors to estimate the impact of peoples' lifestyle habits on AMR evolution

QUESTIONS

Which kind of bacteria?

What type of resistance?

How does AMR vary in time and space?

Impact of lifestyle habits

ANALYSES

Microbial diversity
(16s rRNA)

ARGs detection

ARGs and 16s quantification

Epidemiological and spatial analysis

METHODOLOGY

Sep 2020 March 2021 Dec 2021 Aug 2022
Dec 2020 June 2021 April 2022 Oct 2022

2 case studies

Wastewater influent

Water cycle

River A

WWTP B

WTP A

WWTP A

River B

WTP B

WTP C

Hospital

WWTP C

Water cycle

Surface waters

Treatment

Treatment

Wastewater

Distribution

Usage
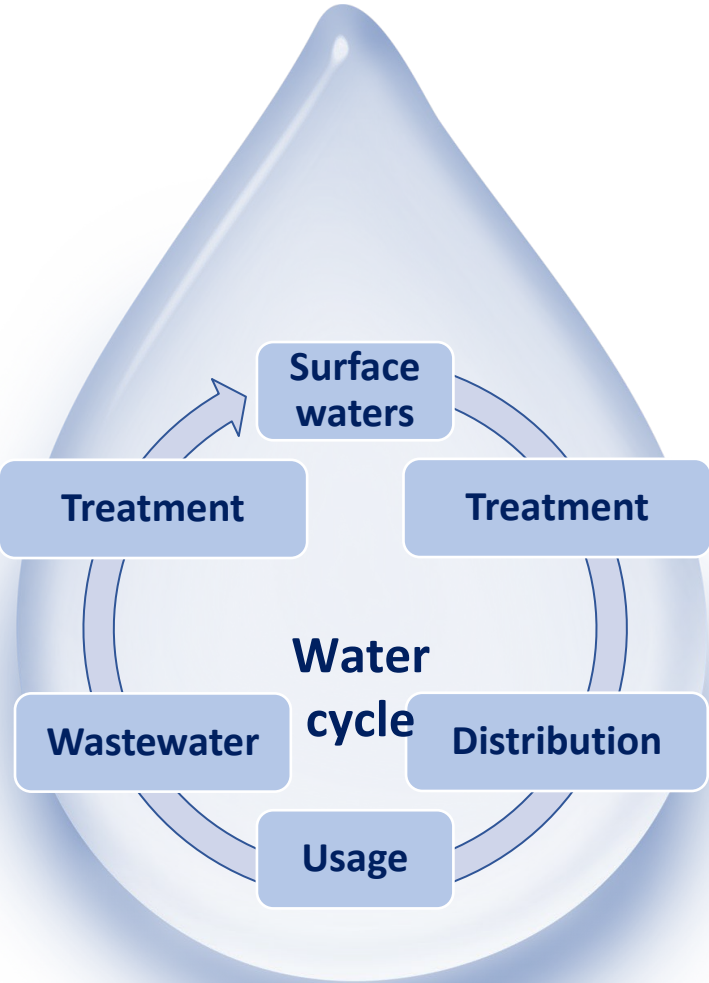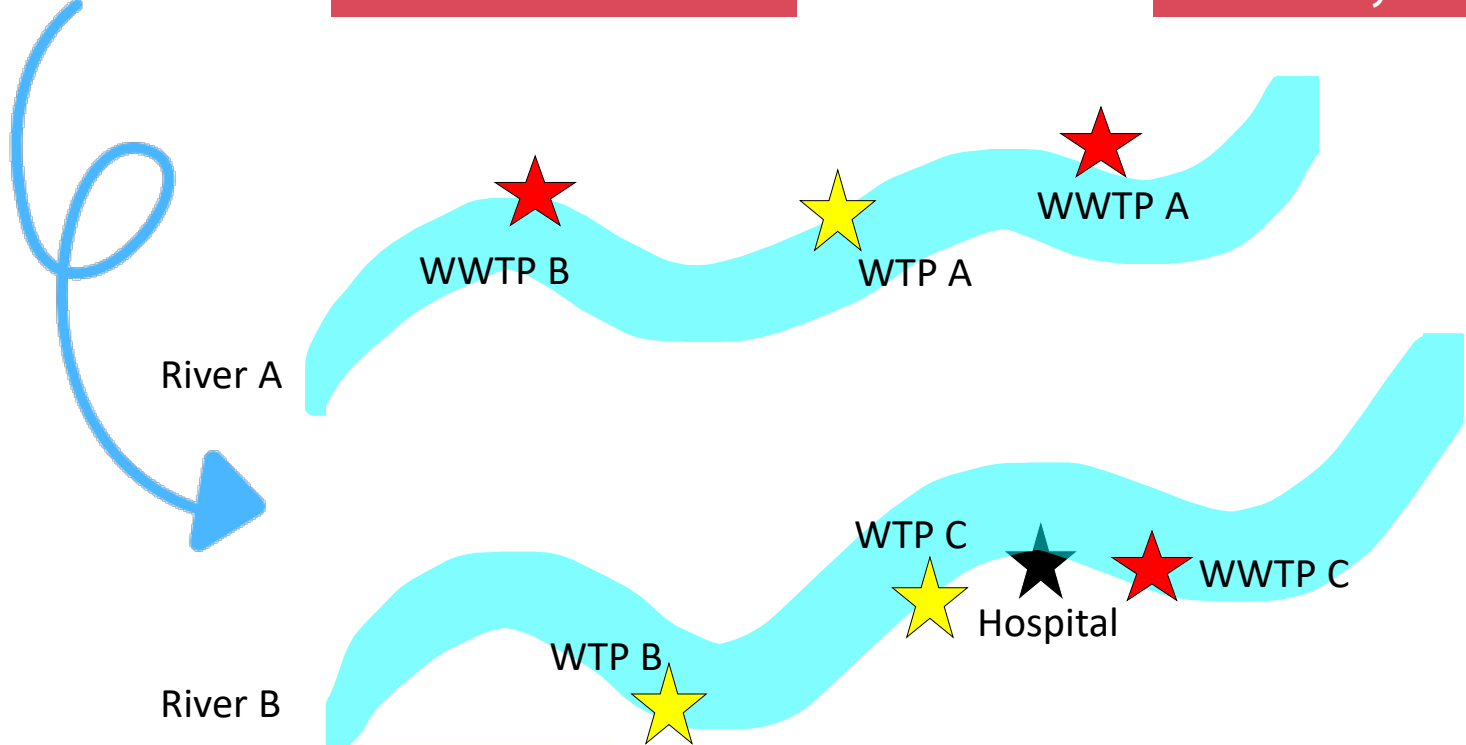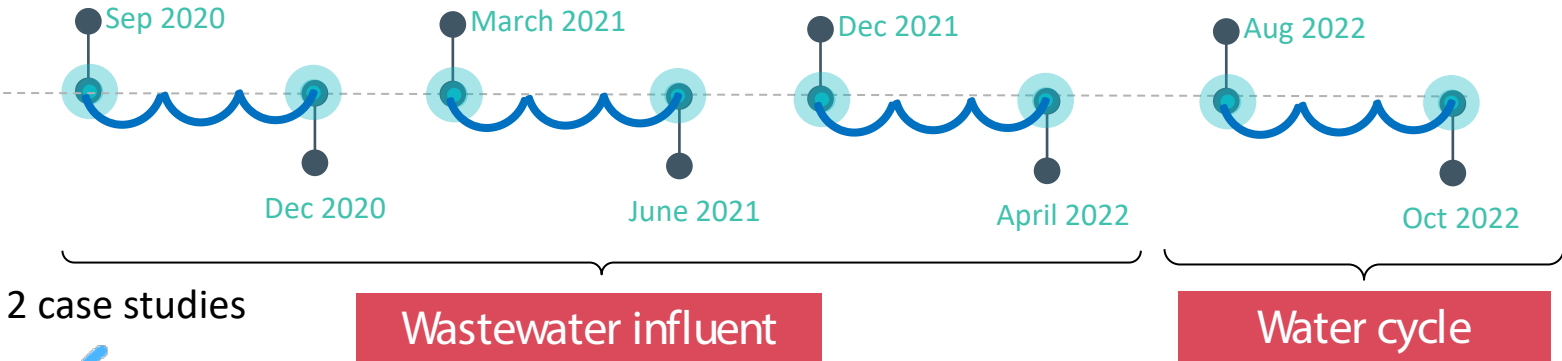
★ Raw water
Drinking water

★ Hospital water intake
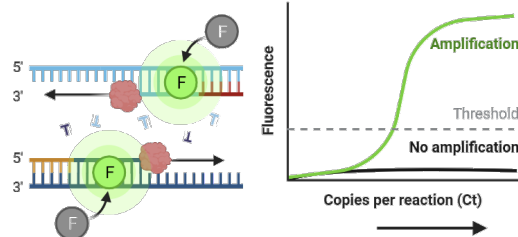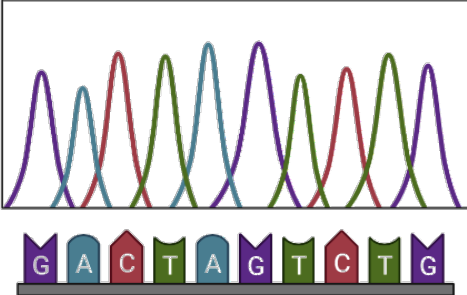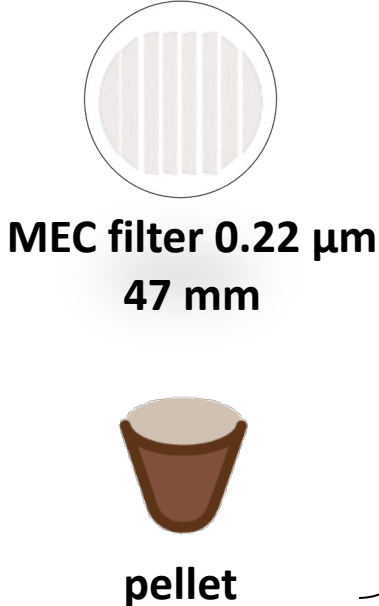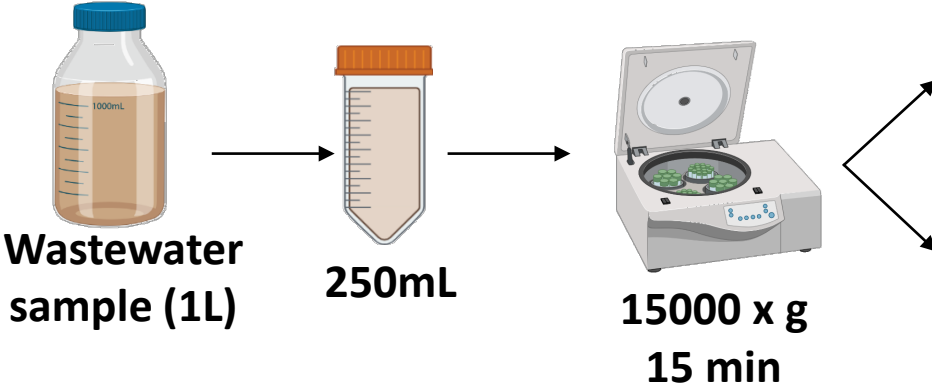Hospital effluent

★ Influent, effluent and sludge from WWTPs

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8

METHODOLOGY

Image source: created with Biorender

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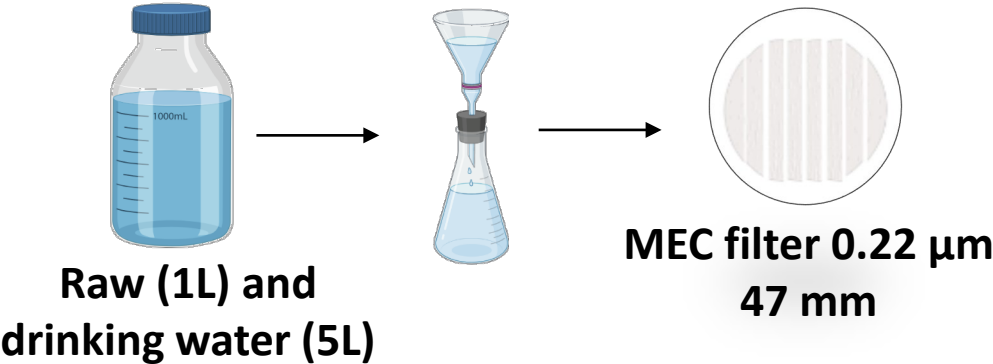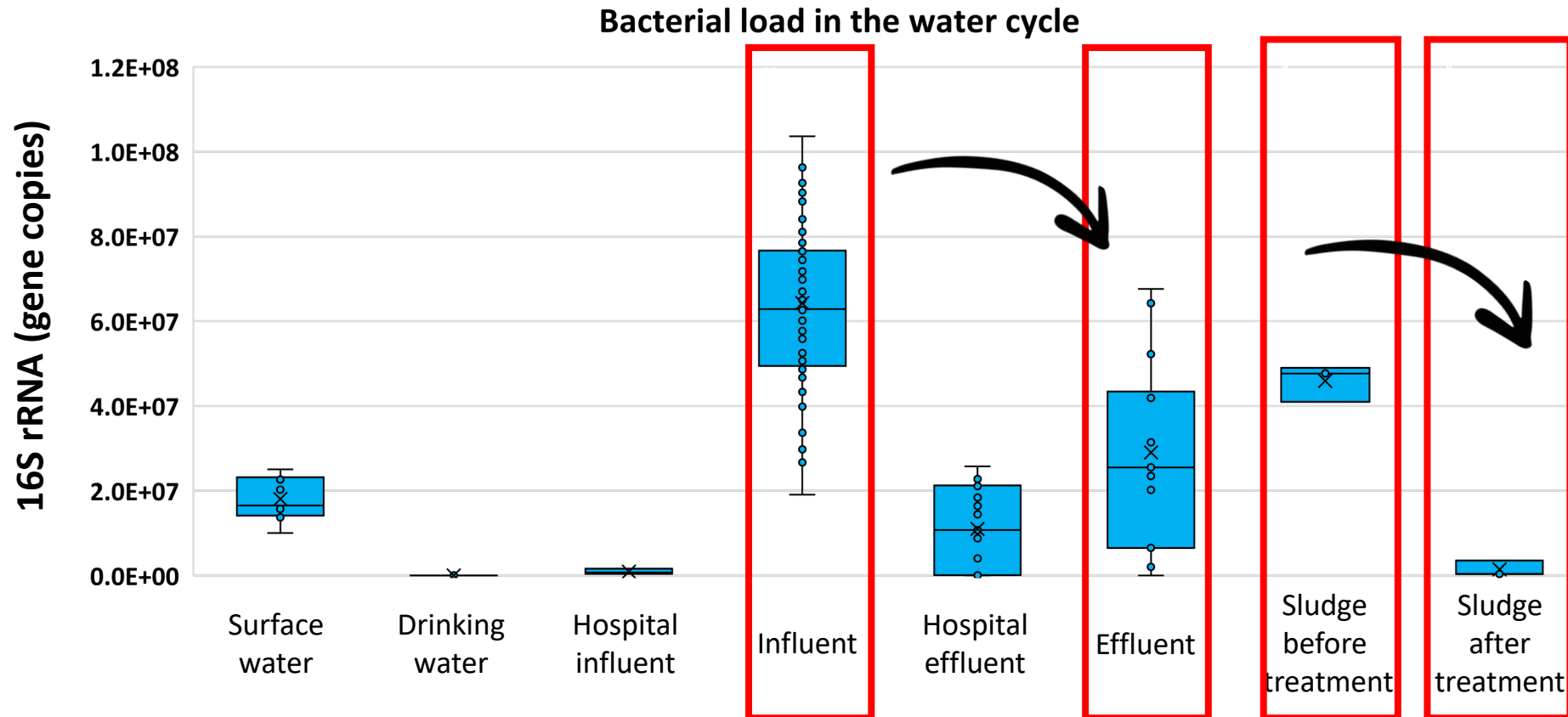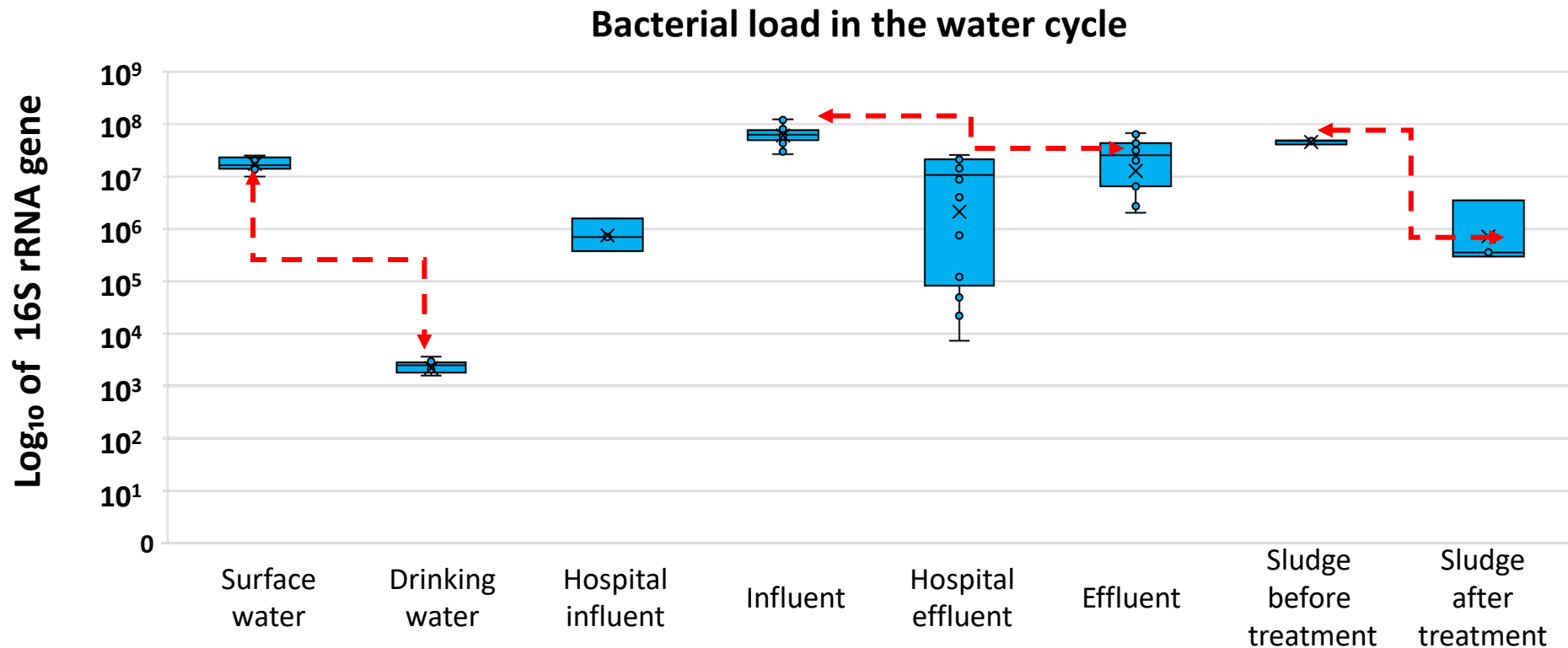
9

RESULTS

Total: 162 samples recovered

There is a reduction in the bacterial load between the influent and effluent, as well as the sludge before and after treatment


Bacterial load in the water cycle

RESULTS

Total: 162 samples recovered

From a water cycle standpoint, this reduction is less significant
(greater reduction between surface waters and drinking water)

Bacterial load in the water cycle

Log$_{10}$ of 16S rRNA gene

Surface water · Drinking water · Hospital influent · Influent · Hospital effluent · Effluent · Sludge before treatment · Sludge after treatment

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RESULTS

Total: 162 samples recovered

All WWTPs show a reduction between influent and effluent, but WWTP 3 is the one where this reduction is least significant
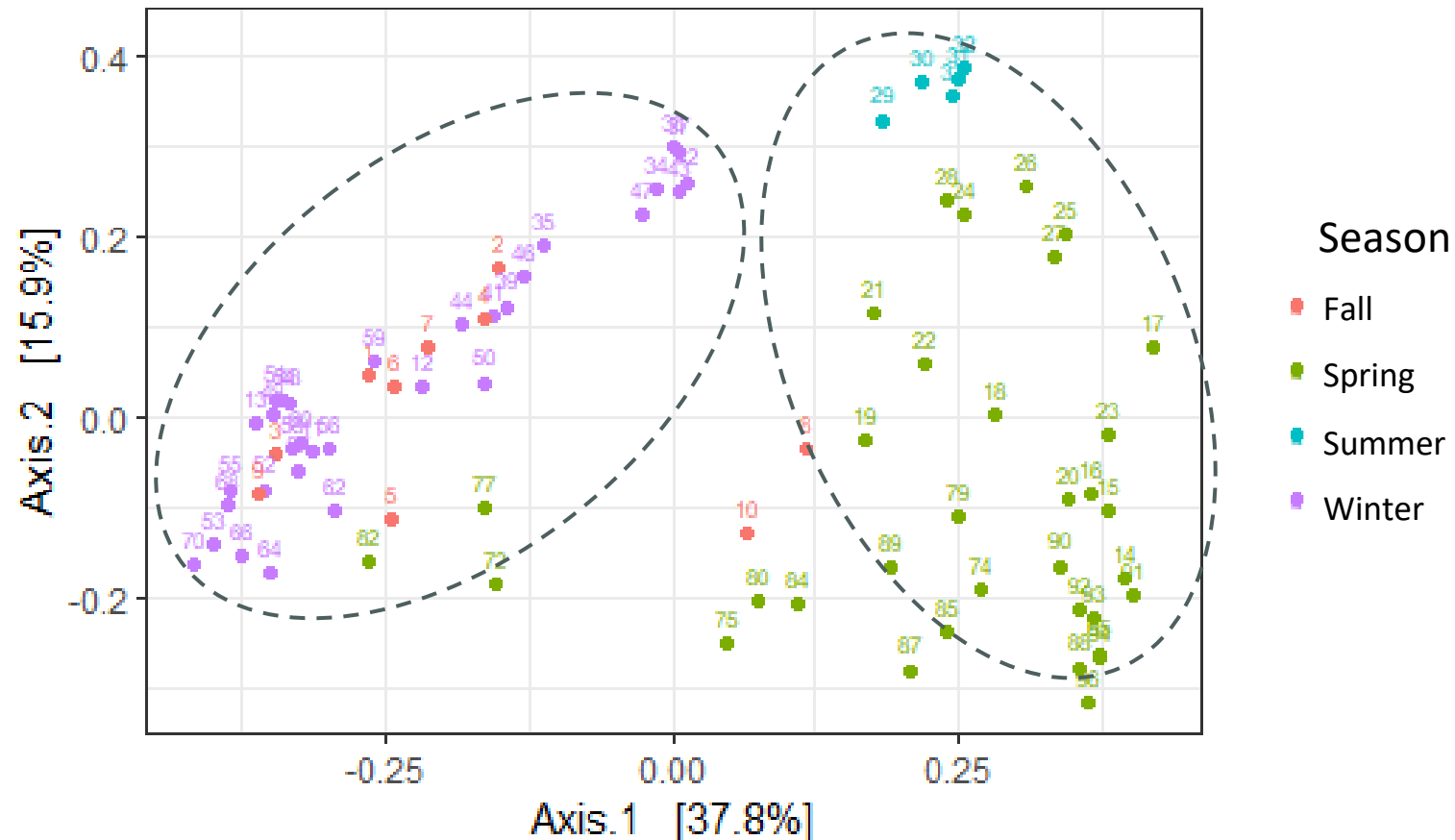(it is also the WWTP the highest initial concentration)

Influent (16s – gene copies)

Effluent (16s – gene copies)

12
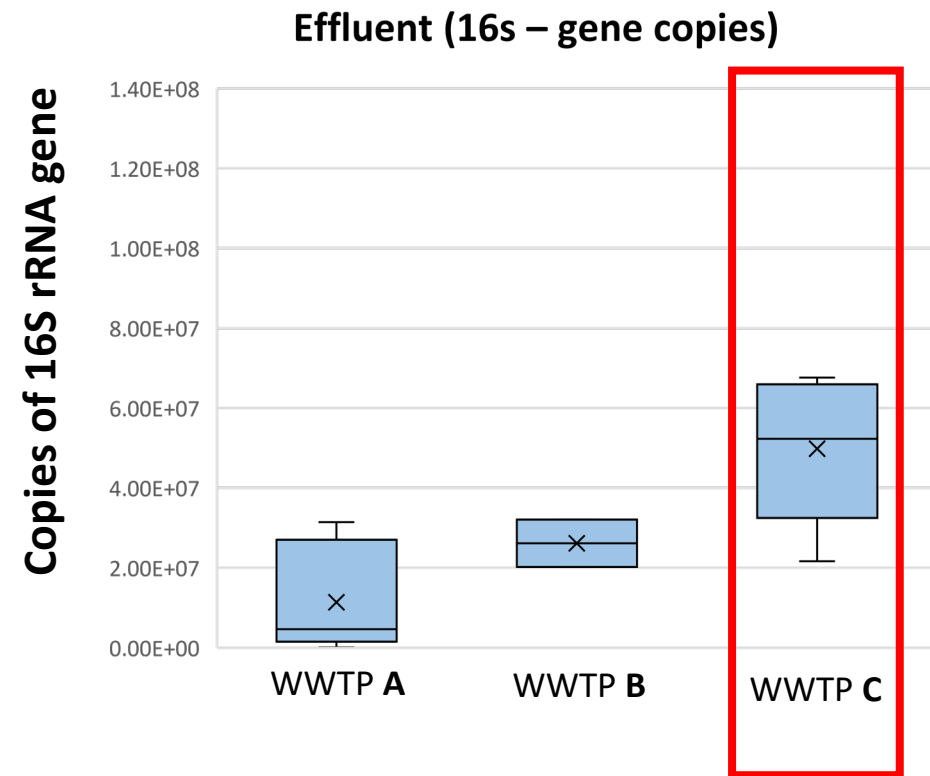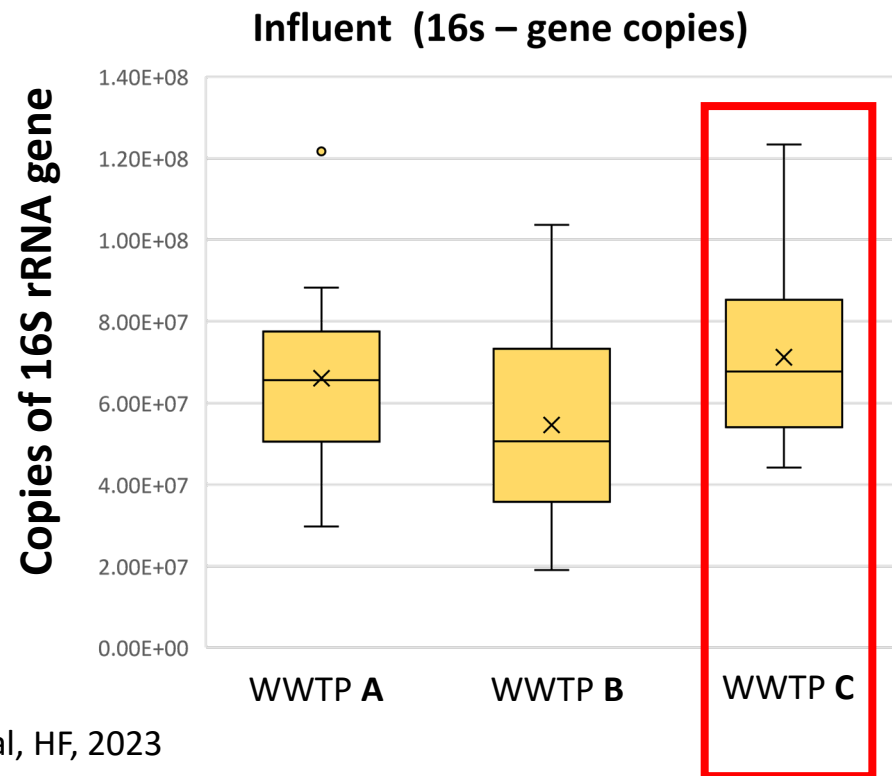
RESULTS

Total: 162 samples recovered

1st phase of environmental analysis → **Influents** (*n*=96)

PCoA analysis reveals that seasonal variations have a significant impact on diversity
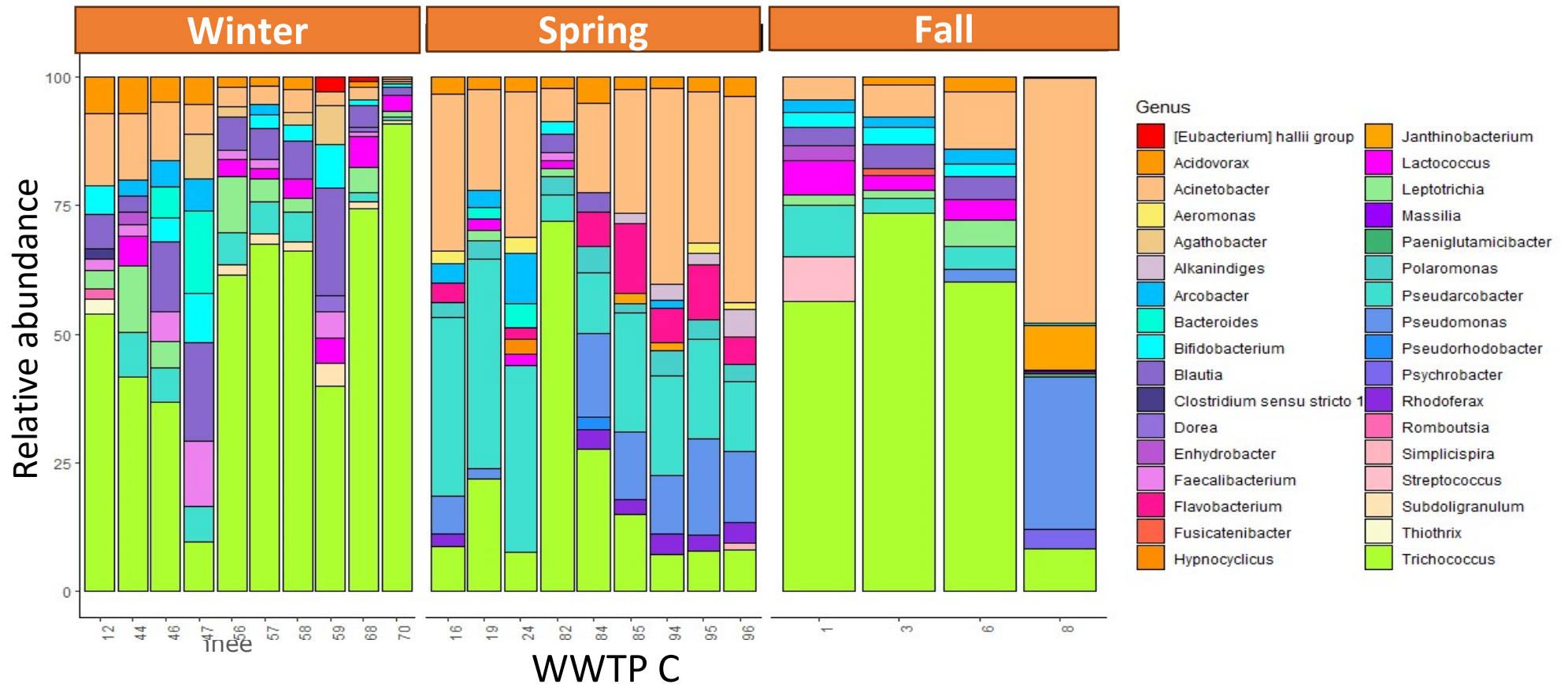
13

RESULTS

Total: 162 samples recovered

1st phase of environmental analysis → **Influents** (*n*=96)

Environmental conditions seen in colder seasons favor the growth of *Trichococcus spp.* while in spring, more balanced conditions allow a diverse range of bacteria to coexist
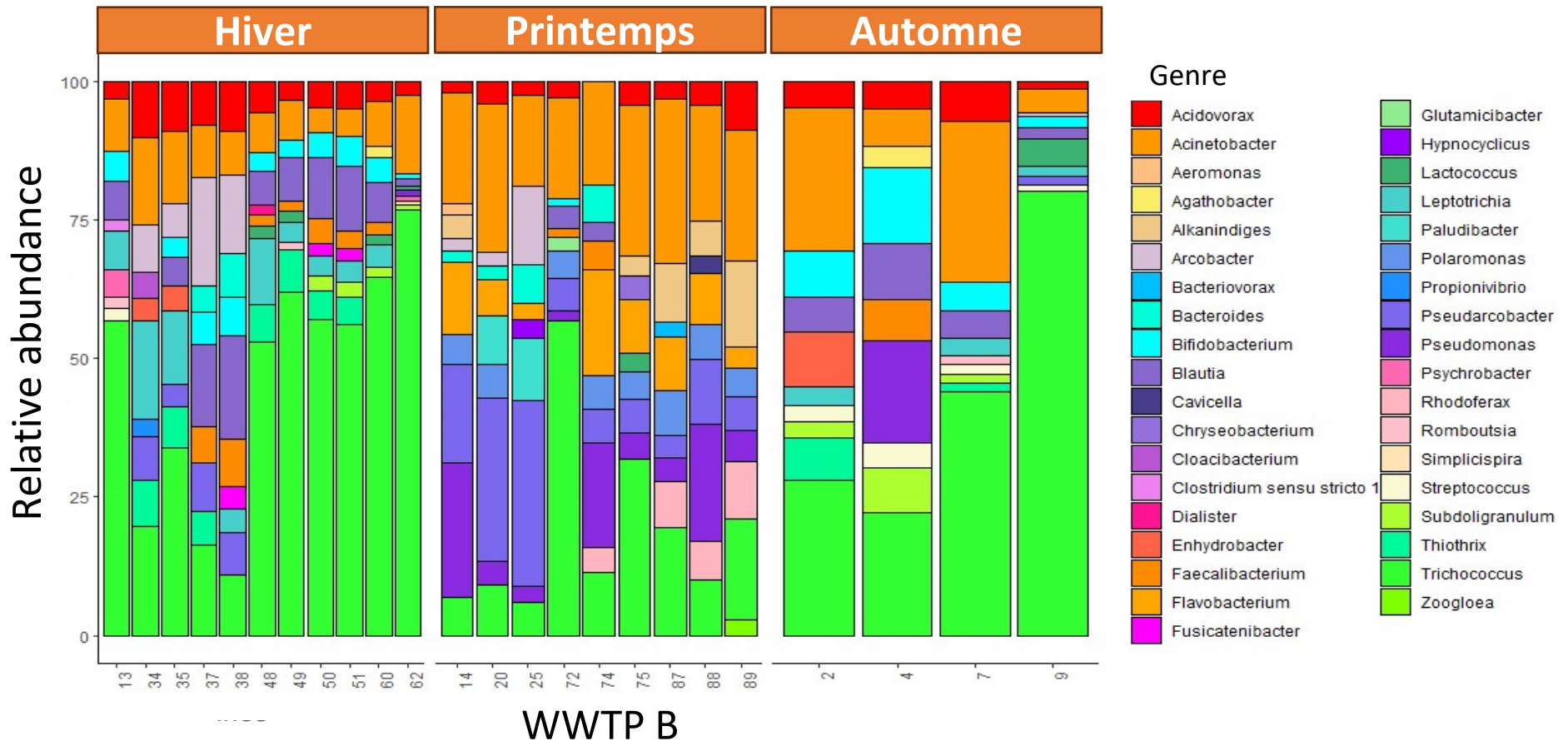

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14

RESULTS

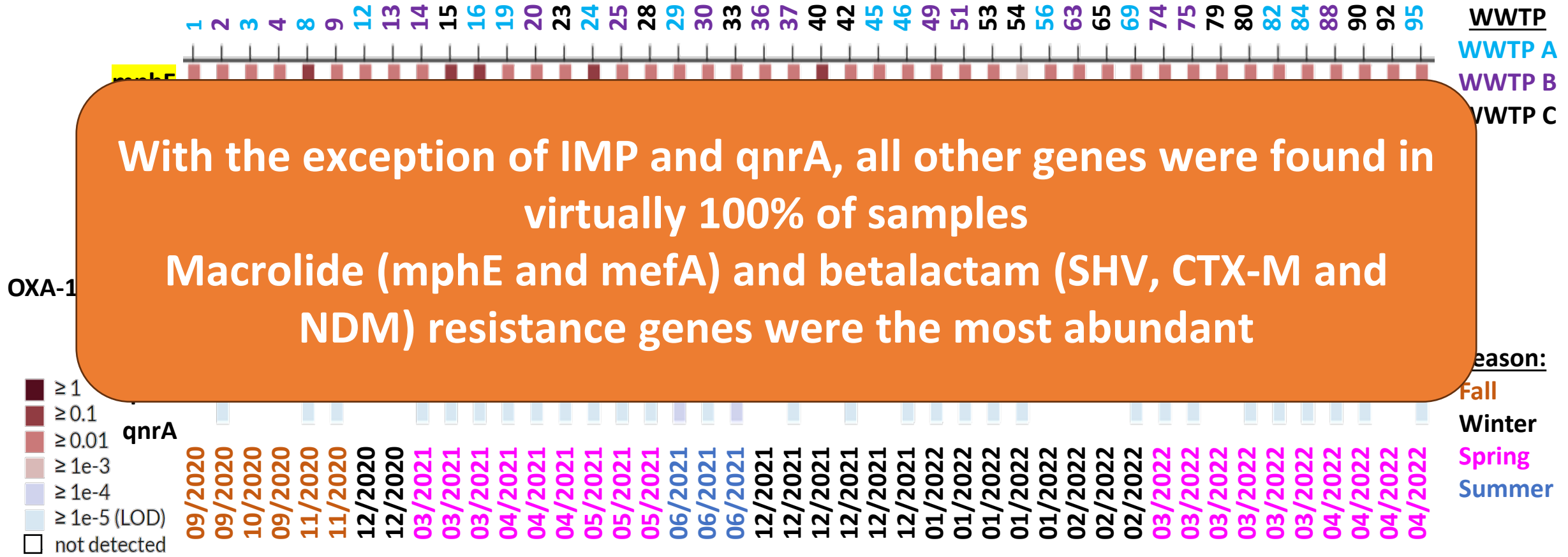Total: 162 samples recovered

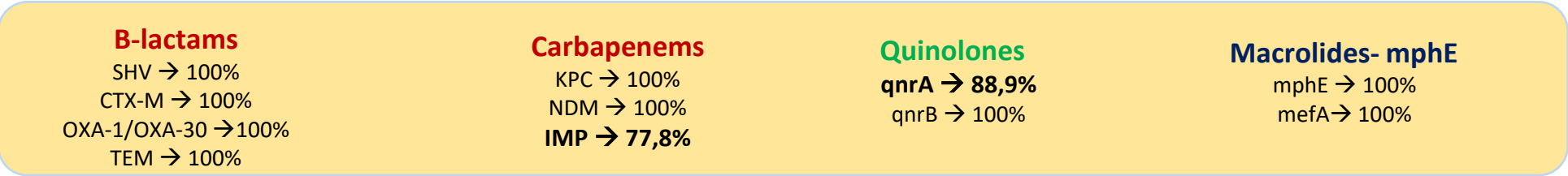1st phase of environmental analysis → **Influents** (*n*=96)

15

RESULTS

B-lactams
SHV → 100%
CTX-M → 100%
OXA-1/OXA-30 → 97,7%
TEM → 100%

Carbapenems
KPC → 100%
NDM → 100%
IMP → 54,5%

Quinolones
qnrA → 75%
qnrB → 100%

Macrolides- mphE
mphE → 100%
mefA → 100%

1 2 3 4 8 9 12 13 14 15 16 19 20 23 24 25 28 29 30 33 36 37 40 42 45 46 49 51 53 54 56 63 65 69 74 75 79 80 82 84 88 90 92 95   **WWTP**

WWTP A
WWTP B
WWTP C

mphE

With the exception of IMP and qnrA, all other genes were found in virtually 100% of samples

Macrolide (mphE and mefA) and betalactam (SHV, CTX-M and NDM) resistance genes were the most abundant

OXA-1

Season:
Fall
Winter
Spring
Summer

≥ 1
≥ 0.1
≥ 0.01
≥ 1e-3
≥ 1e-4
≥ 1e-5 (LOD)
not detected

qnrA

09/2020 09/2020 10/2020 09/2020 11/2020 11/2020 12/2020 12/2020 03/2021 03/2021 03/2021 04/2021 04/2021 04/2021 05/2021 05/2021 05/2021 06/2021 06/2021 06/2021 12/2021 12/2021 12/2021 12/2021 12/2021 01/2022 01/2022 01/2022 01/2022 02/2022 02/2022 02/2022 03/2022 03/2022 03/2022 03/2022 03/2022 04/2022 04/2022 04/2022

Colors in the heatmap indicate gene abundance relative to the 16S rRNA gene

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RESULTS

Influent

B-lactams
SHV → 100%
CTX-M → 100%
OXA-1/OXA-30 →100%
TEM → 100%

Carbapenems
KPC → 100%
NDM → 100%
IMP → 77,8%

Quinolones
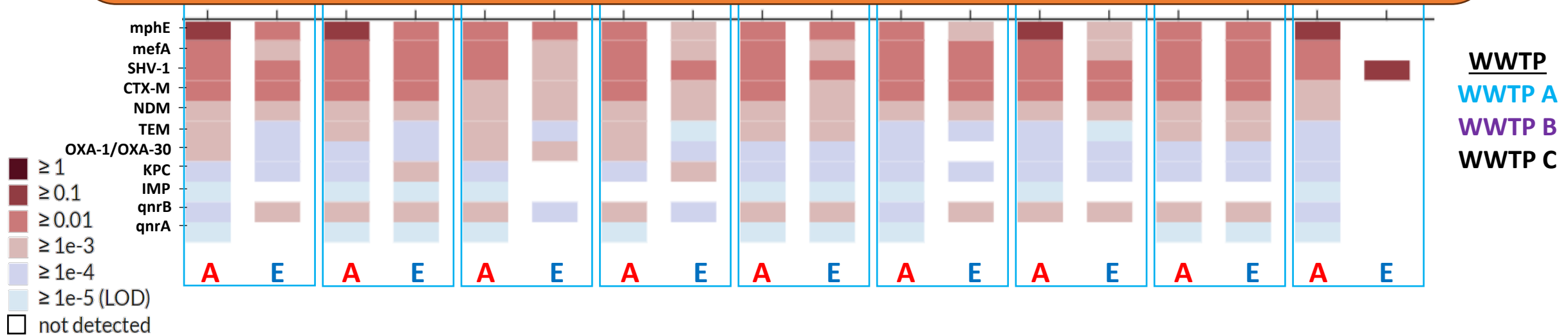**qnrA → 88,9%**
qnrB → 100%

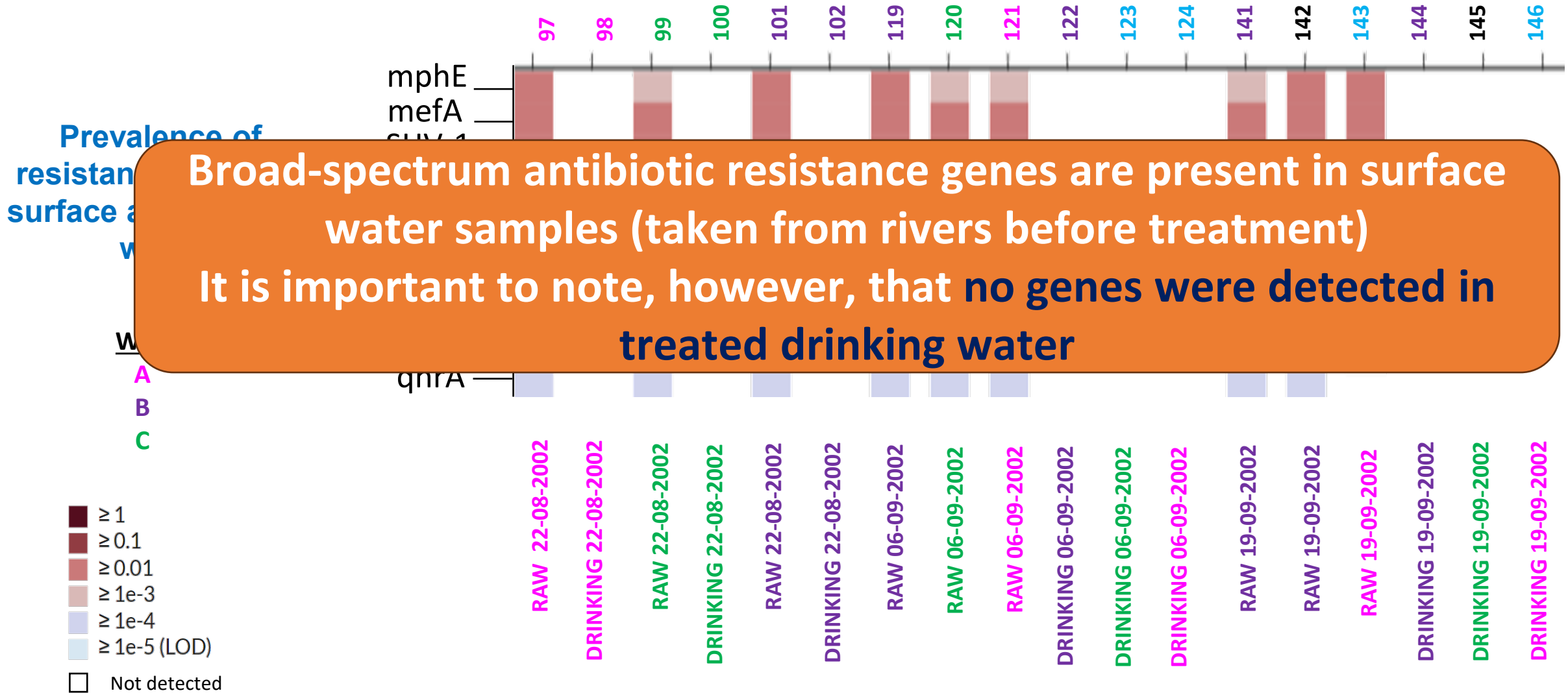**Macrolides- mphE**
mphE → 100%
mefA→ 100%

Effluent

Although the abundance of resistance genes decreases between influent and effluent, in most cases resistance genes are still present in detectable concentrations

Heatmap rows: mphE, mefA, SHV-1, CTX-M, NDM, TEM, OXA-1/OXA-30, KPC, IMP, qnrB, qnrA

Legend:
≥ 1
≥ 0.1
≥ 0.01
≥ 1e-3
≥ 1e-4
≥ 1e-5 (LOD)
not detected

Column labels: A / E (repeated per WWTP)

WWTP
WWTP A
WWTP B
WWTP C

Colors in the heatmap indicate gene abundance relative to the 16S rRNA gene

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RESULTS

Prevalence of resistance in surface and drinking water

Broad-spectrum antibiotic resistance genes are present in surface water samples (taken from rivers before treatment)
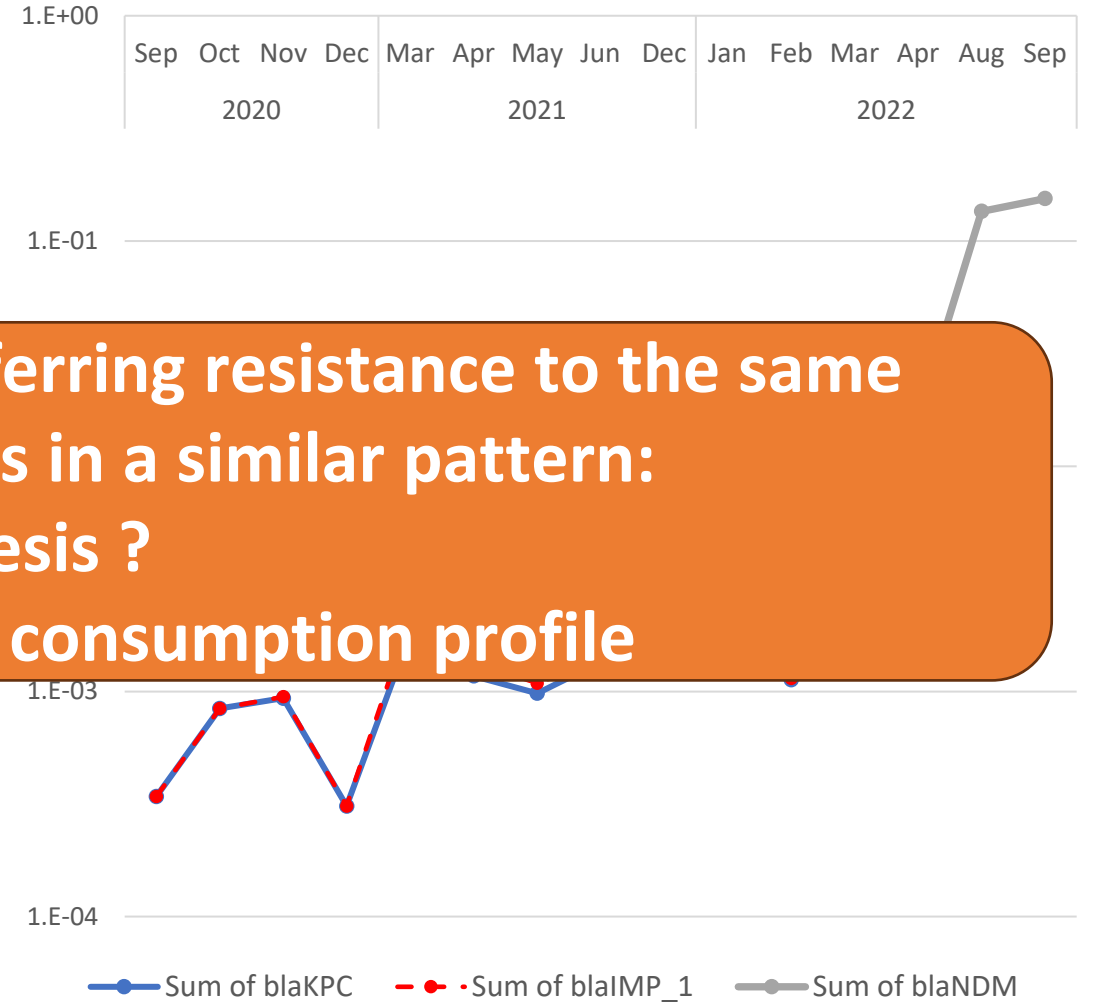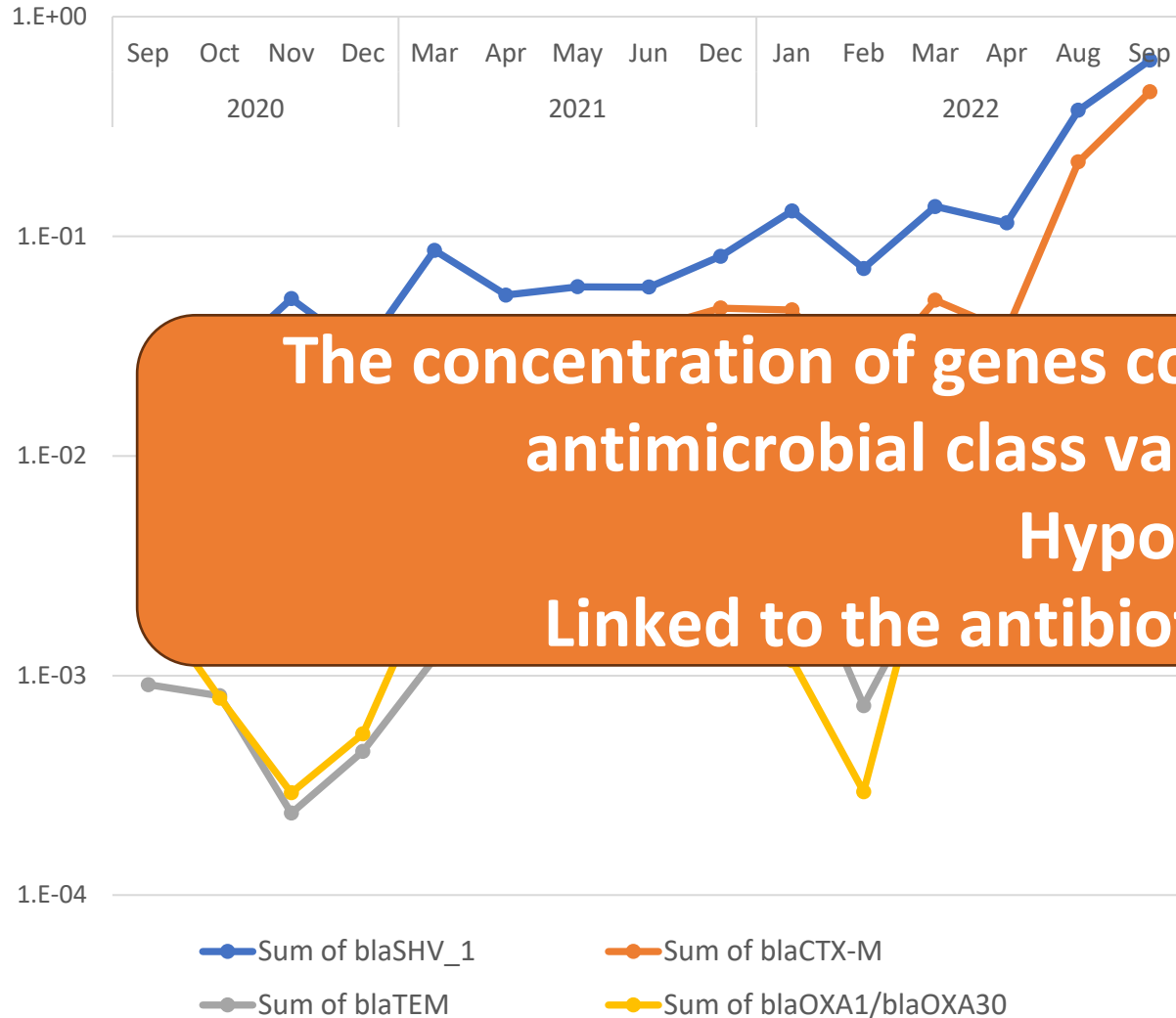**It is important to note, however, that no genes were detected in treated drinking water**

Colors in the heatmap indicate gene abundance relative to the 16S rRNA gene
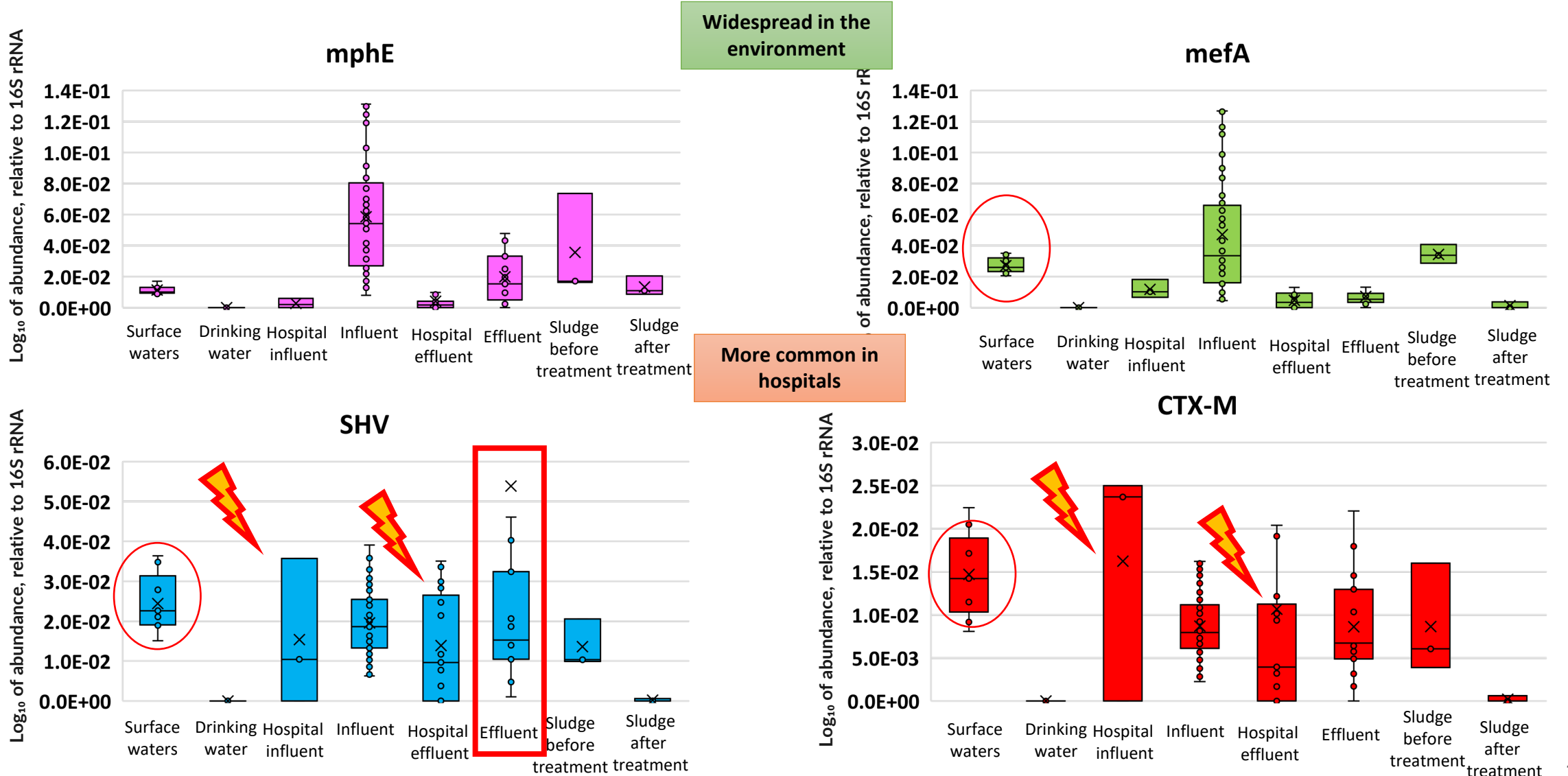
RESULTS

Variation **in time** and space

The concentration of genes conferring resistance to the same antimicrobial class varies in a similar pattern:
Hypothesis ?
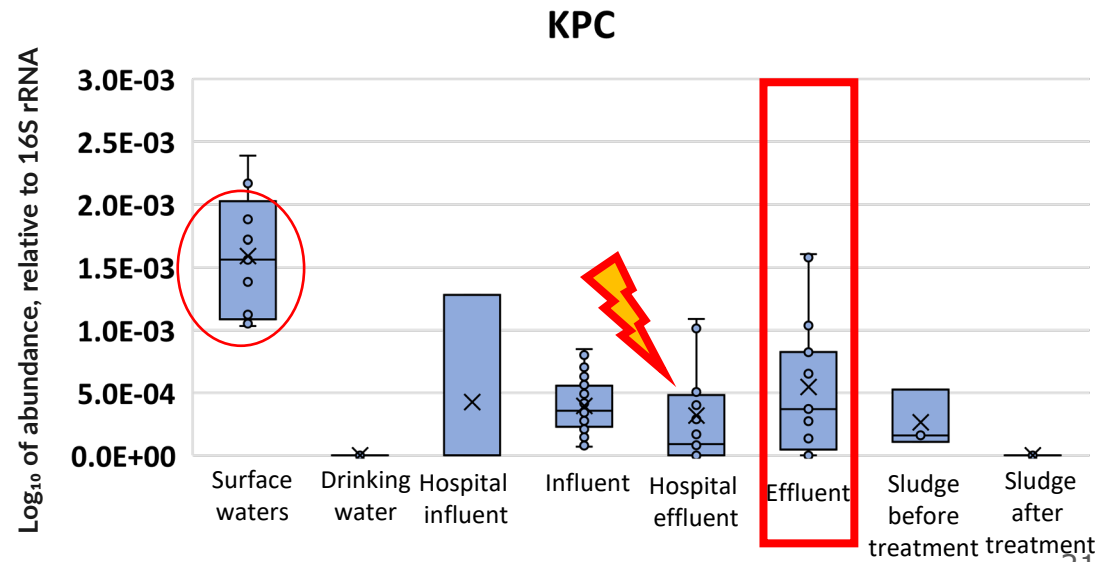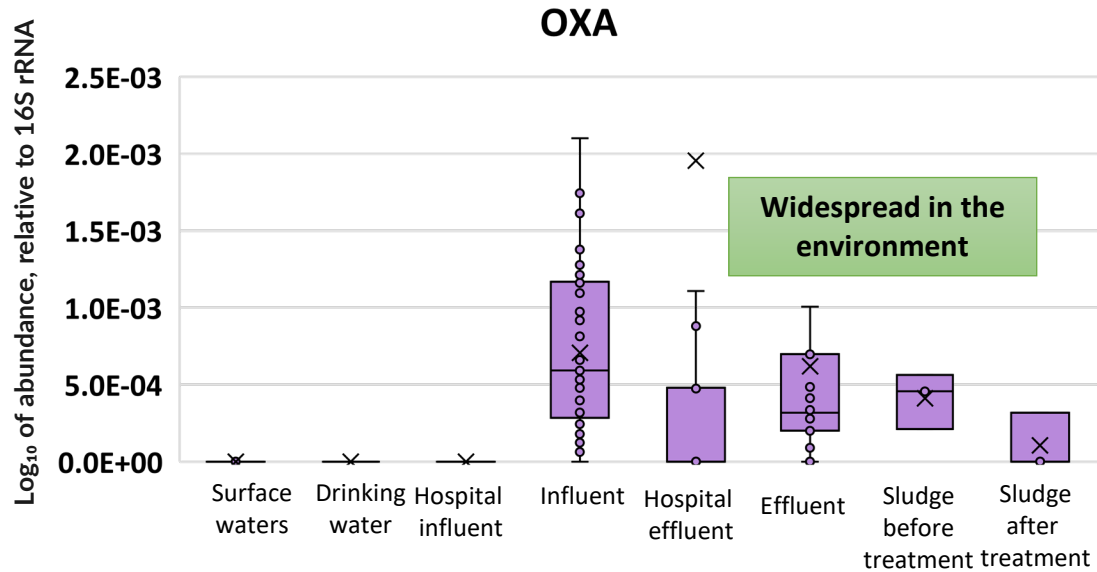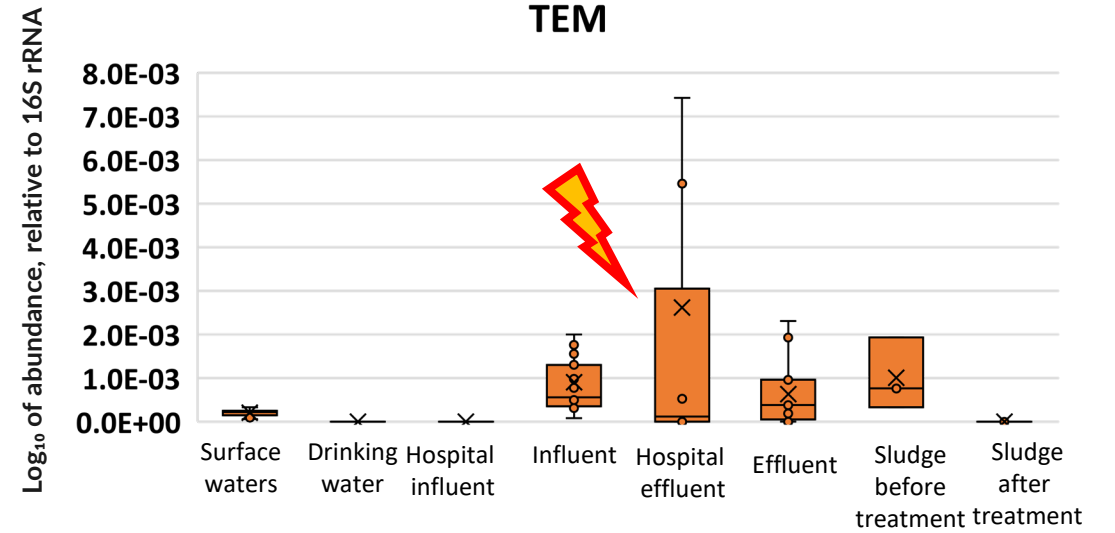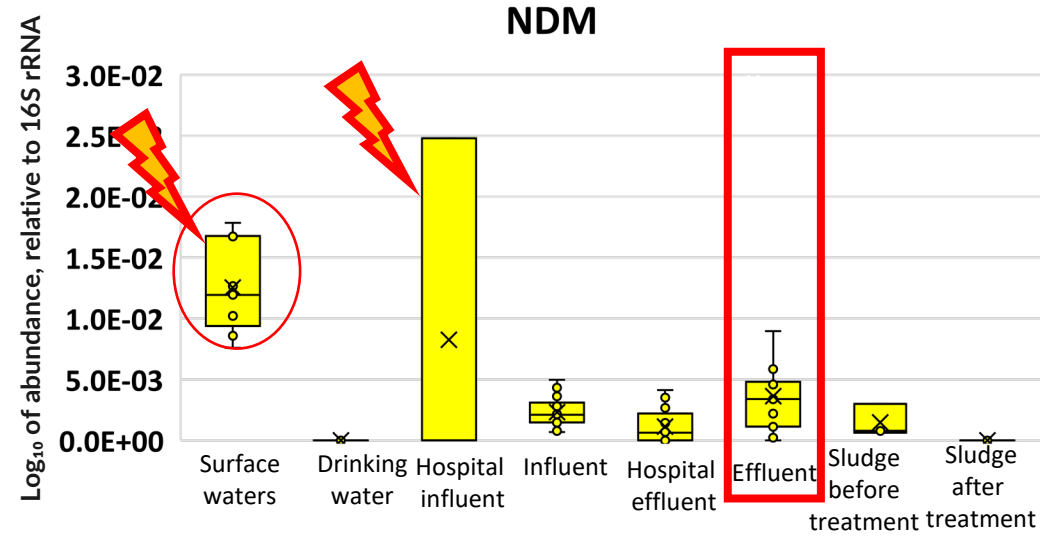Linked to the antibiotic consumption profile

19

RESULTS
Variation in time and **space**

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20

RESULTS
Variation in time and **space**

NDM

Log$_{10}$ of abundance, relative to 16S rRNA

3.0E-02
2.5E-02
2.0E-02
1.5E-02
1.0E-02
5.0E-03
0.0E+00

Surface waters | Drinking water | Hospital influent | Influent | Hospital effluent | Effluent | Sludge before treatment | Sludge after treatment

TEM

Log$_{10}$ of abundance, relative to 16S rRNA

8.0E-03
7.0E-03
6.0E-03
5.0E-03
4.0E-03
3.0E-03
2.0E-03
1.0E-03
0.0E+00

Surface waters | Drinking water | Hospital influent | Influent | Hospital effluent | Effluent | Sludge before treatment | Sludge after treatment

OXA

Log$_{10}$ of abundance, relative to 16S rRNA

2.5E-03
2.0E-03
1.5E-03
1.0E-03
5.0E-04
0.0E+00

Widespread in the environment

Surface waters | Drinking water | Hospital influent | Influent | Hospital effluent | Effluent | Sludge before treatment | Sludge after treatment

KPC

Log$_{10}$ of abundance, relative to 16S rRNA

3.0E-03
2.5E-03
2.0E-03
1.5E-03
1.0E-03
5.0E-04
0.0E+00

Surface waters | Drinking water | Hospital influent | Influent | Hospital effluent | Effluent | Sludge before treatment | Sludge after treatment

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21

EPIDEMIOLOGICAL STUDY

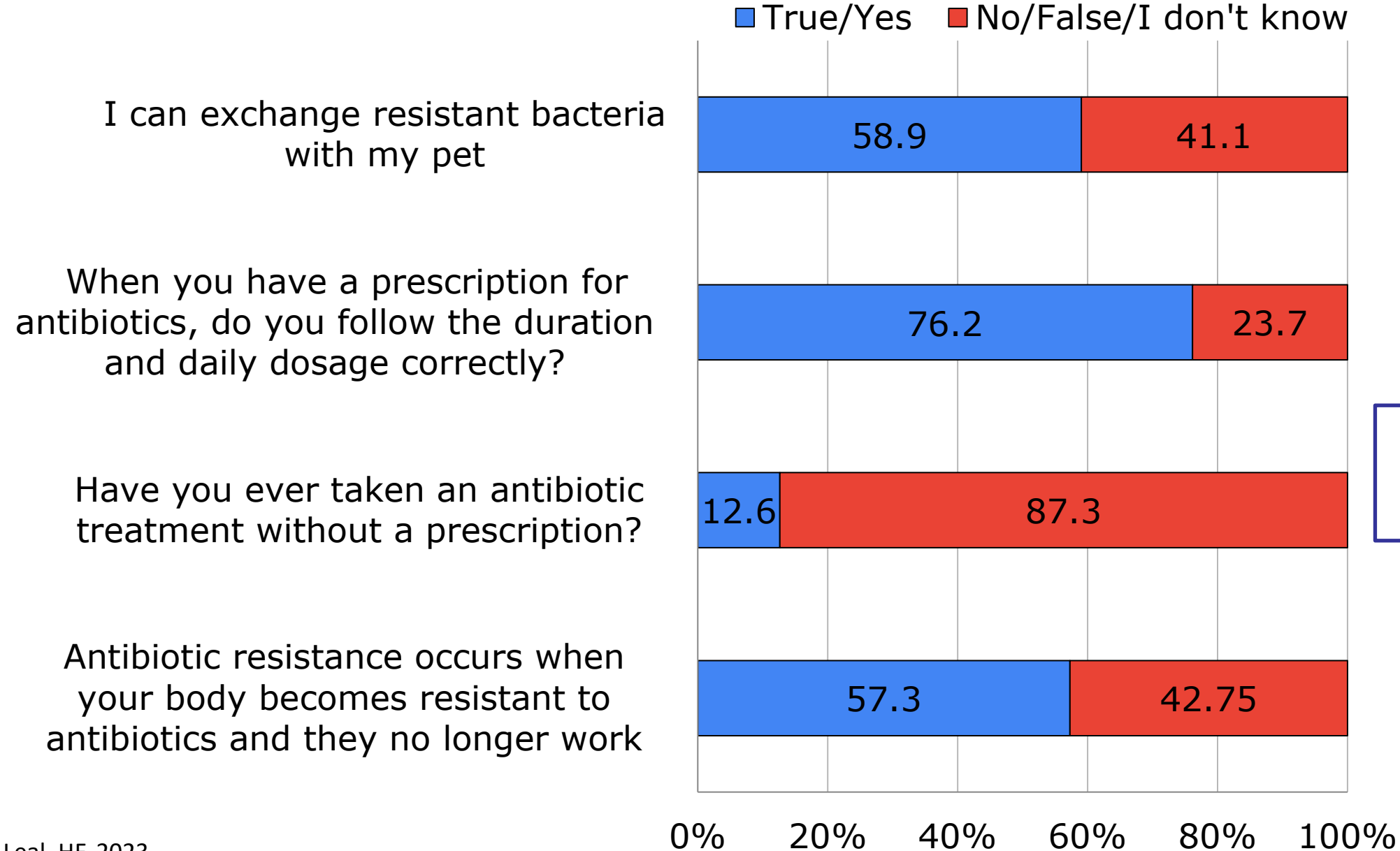Assessment of population lifestyle habits and behaviors

Type of study: Observational, cross-sectional and descriptive

Study population: Residents two large Canadian cities, 18 yr-old or older, with no gender restrictions

Data collection: Questionnaire adapted from the standardized survey of WHO (Simple Survey)

Data analysis: Data was managed and analyzed using RStudio and Microsoft Excel

RESULTS

Legend: ■ True/Yes ■ No/False/I don't know

Question	True/Yes	No/False/I don't know
I can exchange resistant bacteria with my pet	58.9	41.1
When you have a prescription for antibiotics, do you follow the duration and daily dosage correctly?	76.2	23.7
Have you ever taken an antibiotic treatment without a prescription?	12.6	87.3
Antibiotic resistance occurs when your body becomes resistant to antibiotics and they no longer work	57.3	42.75

The bacteria become resistant, not our bodies!

Analysis performed on Microsoft Excel 2010

CONCLUSIONS → TAKE HOME MESSAGES

Water cycle analysis
Useful for obtaining a picture of the dissemination of resistance in the environment

Wastewater influent samples have **the highest bacterial load** in the water cycle

The reduction in bacterial load is less significant for wastewater treatment than for drinking water treatment

Seasons have a significant impact on bacterial diversity

Antimicrobial resistance genes are widely present in wastewater samples →
abundance of some genes increases after treatment

CONCLUSIONS → TAKE HOME MESSAGES

Some broad-spectrum antibiotic resistance genes are detected in surface water, **but not in treated drinking water**

Preliminary analysis of lifestyle habits reveals some misconceptions about antimicrobial resistance

PERSPECTIVES
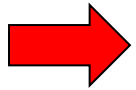
→ Resistance genes vary in time and space → influenced by antibiotic consumption?
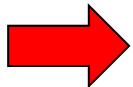
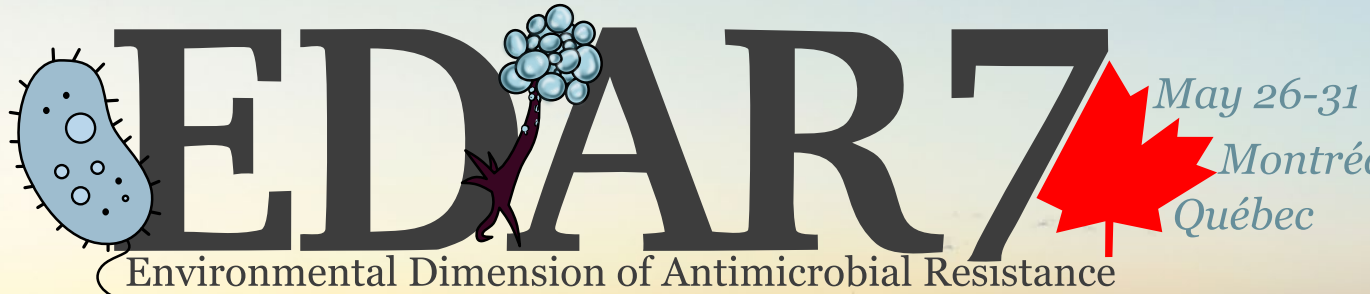→ Association between the prevalence of resistance genes and lifestyle habits

25

ACKNOWLEDGMENTS

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Amina Barka
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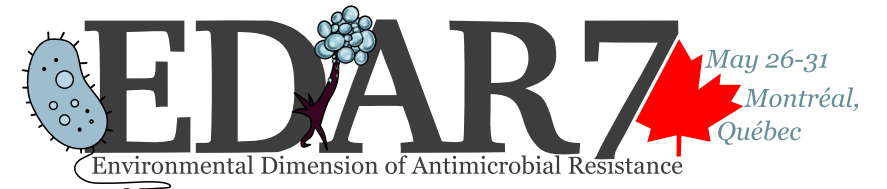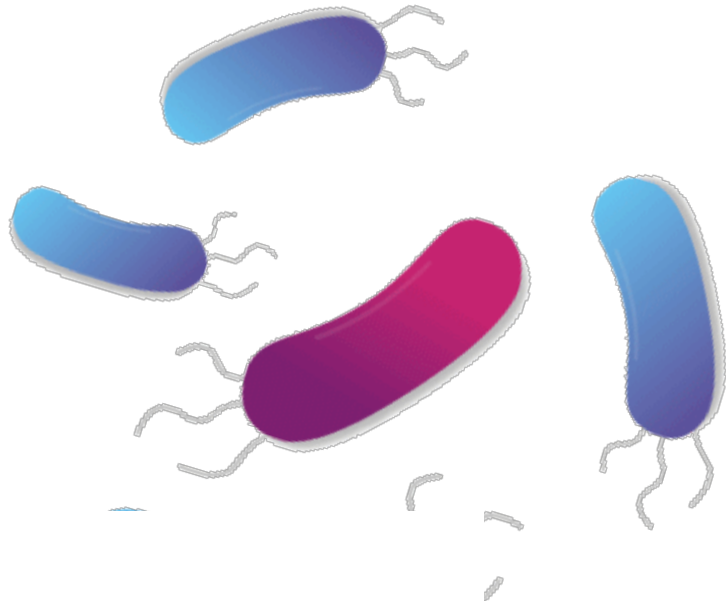Loan Nguyen
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Thank you!

Questions?

Comments?

EDAR 7
May 26-31 Montréal, Québec
Environmental Dimension of Antimicrobial Resistance

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