National Institute for Public Health
and the Environment
*Ministry of Health, Welfare and Sport*

# Antibiotikaresistenzen im Abwasser- Berufsrisiken oder Datenquelle?

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# 1

# Wastewater as occupational risk: The AWARE study

# AWARE project: Occupational impact of exposure to antibiotic resistant bacteria in WWTPs

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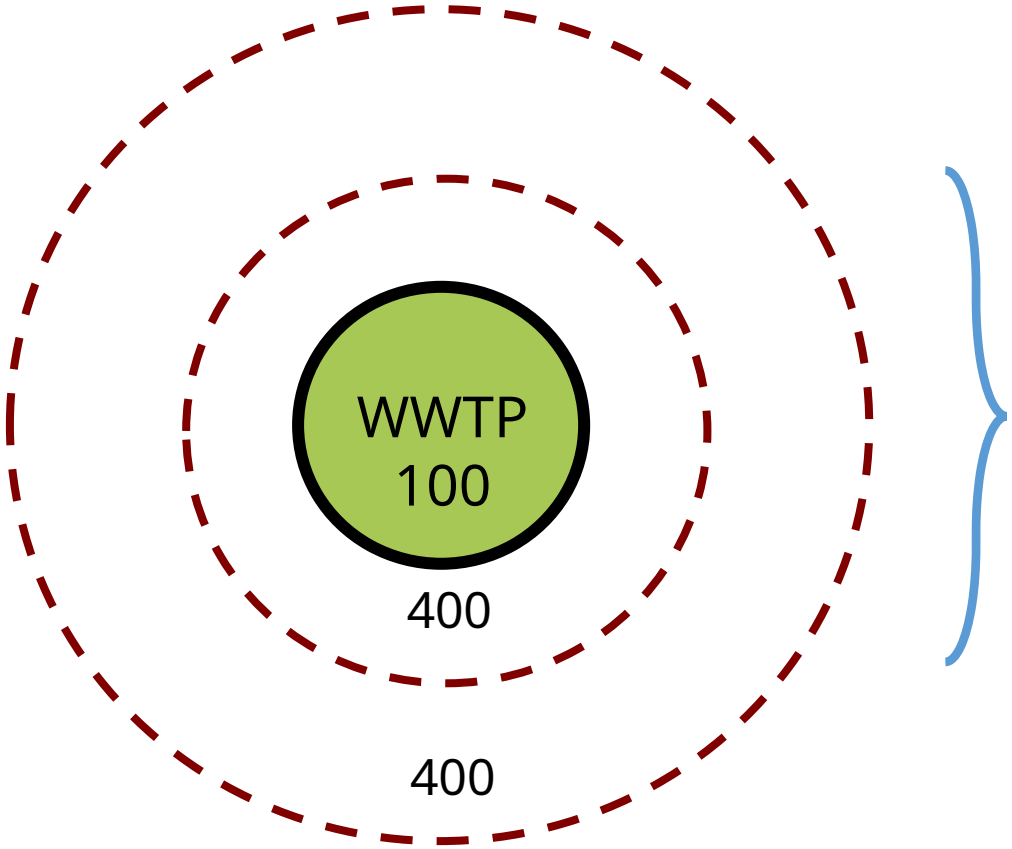
# Sampling campaign




Wengenroth et al. (2021) AB

# Analysis parameters

- ESBL *E. coli* (important class of bacteria resistant to beta-lactam antibiotics)
- Resistance genes, metagenomes
- Antibiotics

Wengenroth et al. (2021) AB

# Occupational and residential health effects

Recruitment
by i.a. letters

Participant questionnaires and fecal sample ➔ ESBL *E. coli* detection

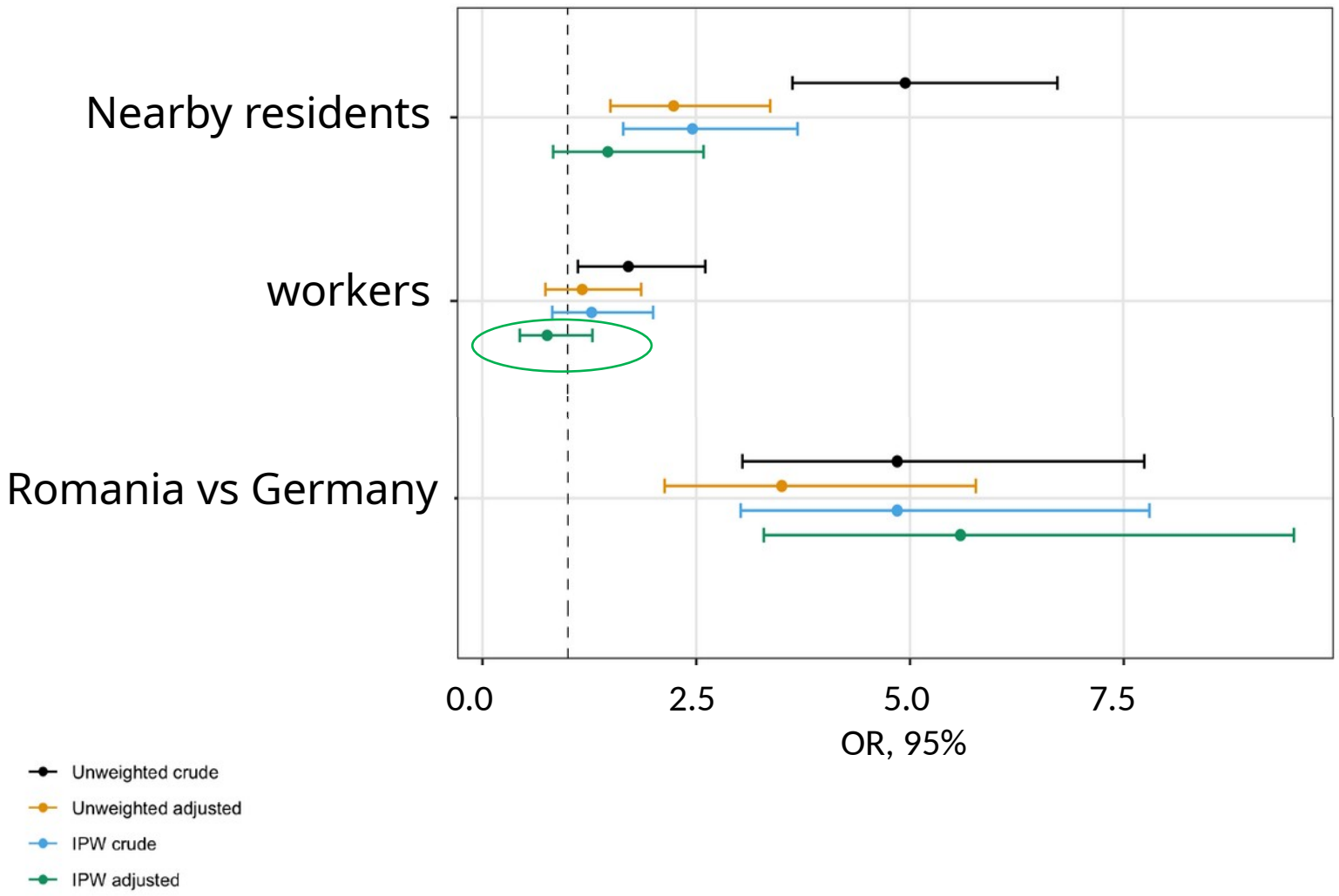
Statistical modelling: logistic regression of ESBL *E. coli* prevalence

# Results: ESBL *E. coli* carriage

| | n | Overall | Workers | Nearby residents | Distant residents |
|---|---|---|---|---|---|
| | 1940 | 13% | 11% | 29% | 7% |
|  | 482 | 7% | 0% | 6% | 8% |
|  | 828 | 6% | 4% | - | 6% |
|  | 608 | 28% | 23% | 36% | 12% |

Rodríguez-Molina et al. (2021) EJCMID

# Results: statistical modelling




- No significantly elevated carriage of ESBL *E. coli* in workers or residents when adjusted for other risk factors and response differences
- Higher ESBL *E. coli* prevalence in Romania
- Significantly higher prevalence in Romanian workers and residents as compared to controls
  residents: socioeconomic factors

Rodríguez-Molina et al. (2021) EJCMID

# Occupational and residential health effects - conclusion

- Effect of exposure in WWTP on ESBL *E. coli* carriage differs per country (work habits?) – no effect across all countries together
- Effects in nearby residents in RO: related to socioeconomic factors
- Power issues – even with large studies (63% for OR 1.7)

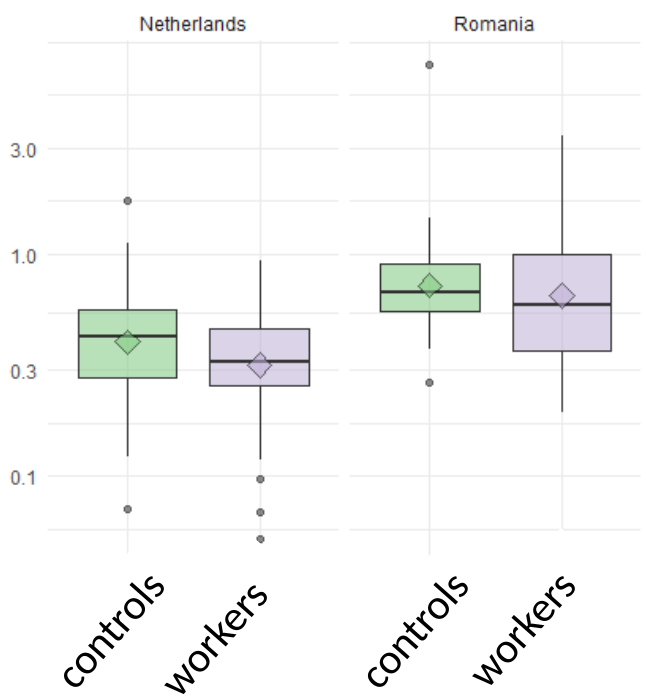
# Metagenomic analysis of human feces and wastewater

n=174
Matched
workers and
controls

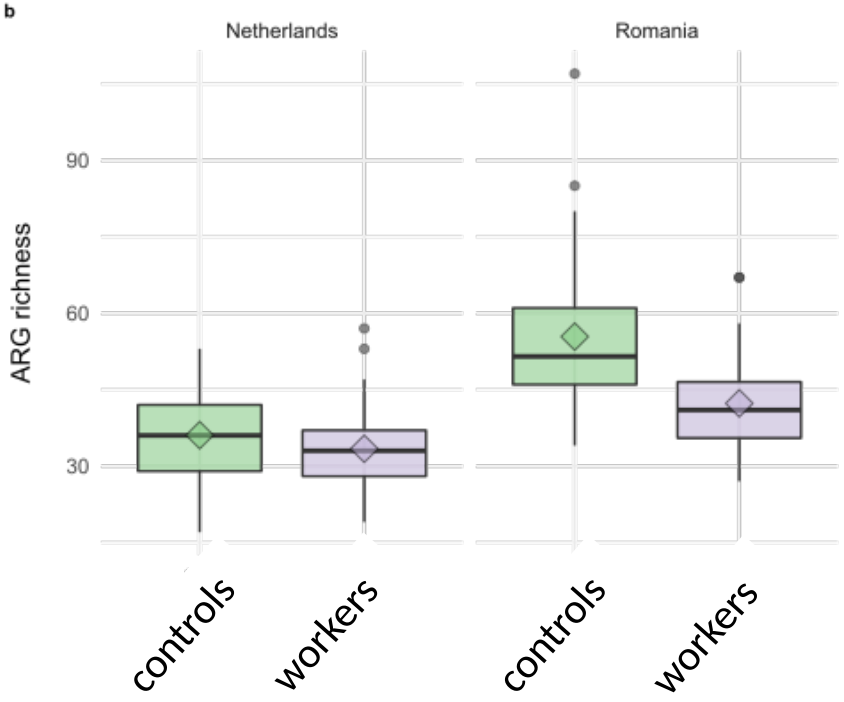
Metaxa-2
Resfinder
Normalisation
GLM modelling
DeSeq

# Antibiotic resistance genes – workers versus controls

Relative abundance




Richness




- Country effect: RO
- Lower relative abundance and diversity of ARG in workers (significant for NL / all)

Berglund (in preparation)

# Metagenomics - conclusion

- WWTP exposure: Subtle effects, in opposite direction than expected
- General agreement of resistome and bacteriome findings
- Confirmation of known risk factors (antibiotic use)

- Overall, occupational risks to encounter resistant bacteria or resistance genes in wastewater treatment plants appear moderate
- Modeled WWTP exposure: overall low, but highly variable – role of protective equipment

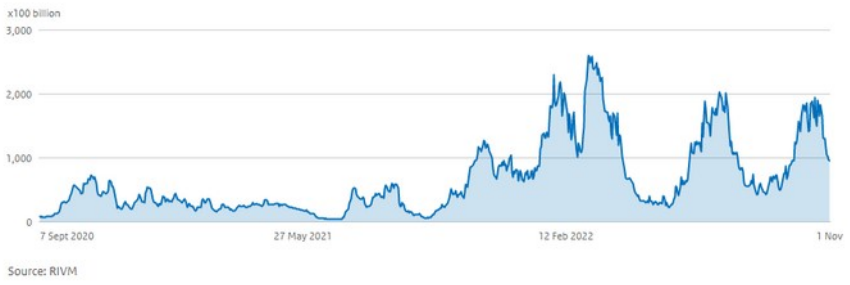
# 2

# Wastewater as data source: Wastewater-based AMR surveillance

# Why Wastewater based surveillance (WBS)?

- Human feces and urine collected in sewers and wastewater treatment plants (wwtp)
- Fecal matter in wwtp predominantly from humans
  - Including pathogens and antibiotic resistant bacteria
- Wastewater can be used to gain insight in the prevalence of fecal pathogens in the human population

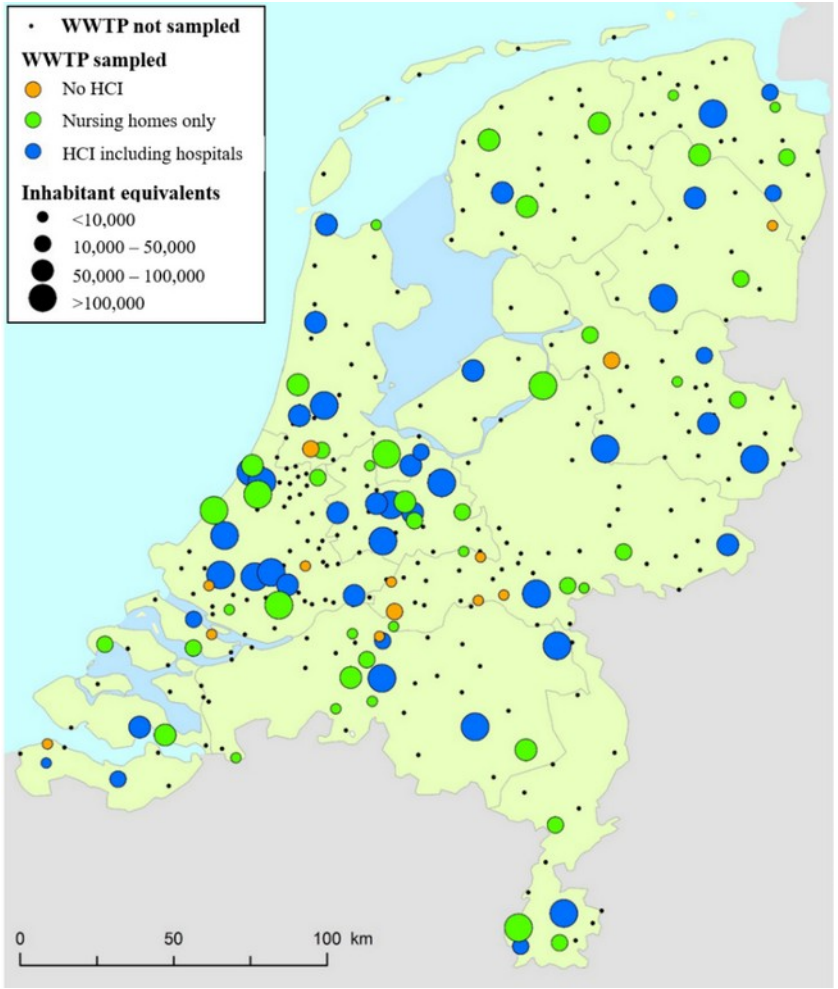





# AMR in wastewater in the Netherlands studied since 2016

- One of the activities resulting from the National ‘approach to antibiotic resistance’, next to:
  - human exposure to AMR in recreational water,
  - animal manure as source of AMR

- 100 WWTP
  - Distributed across the NL and all provinces
  - Large and small wwtp
  - With/without healthcare institutions
  - In regions differing with respect to urbanization




*Blaak et al. (2021) Sci Tot 776*
*RIVM report 2022-0008*

# Target for culture: CPE

- Low prevalence in NL in general population (<1:10.000 persons, mainly known for 2016)
- Carriage associated with international travel and international hospital admission
- Next to ESBL, *E. coli*

**Figure 3.** ***Escherichia coli*****. Percentage of invasive isolates resistant to carbapenems (imipenem/meropenem), by country, EU/EEA, 2023**

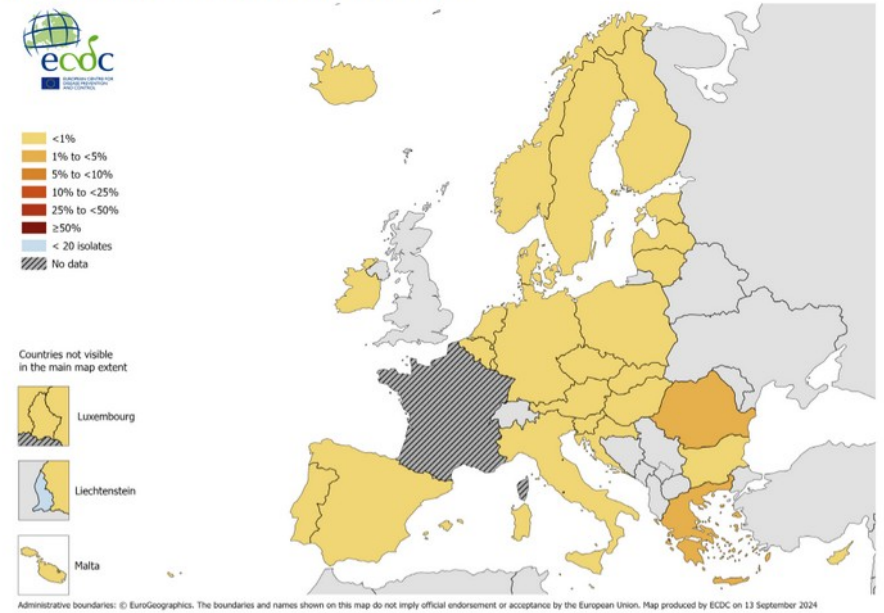
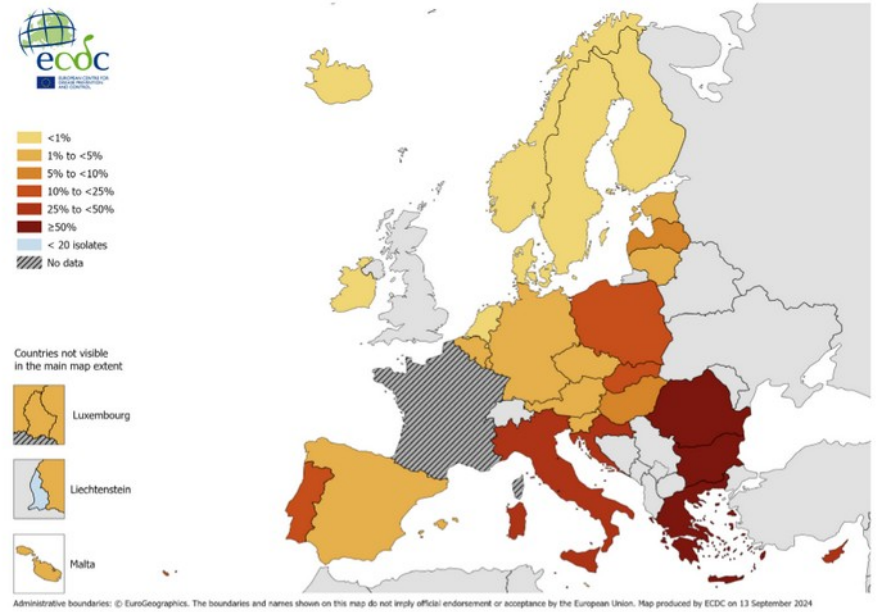
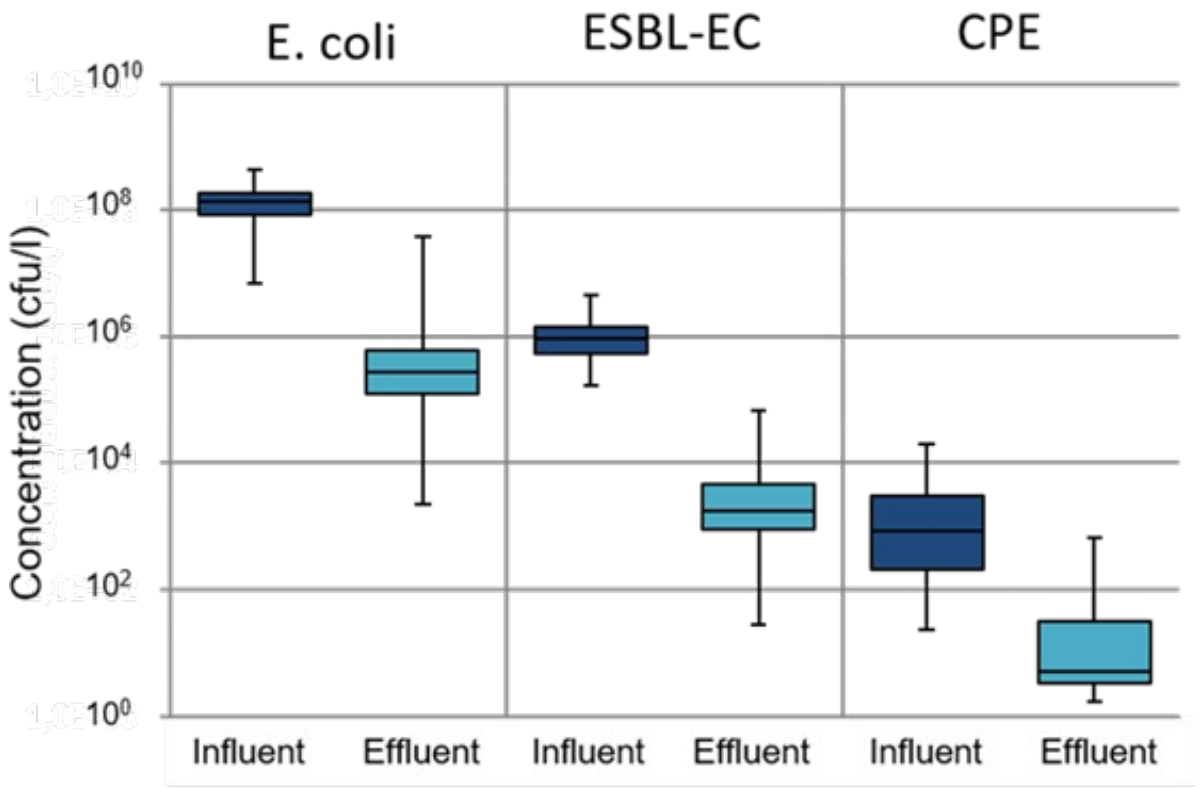



**Figure 5.** ***Klebsiella pneumoniae*****. Percentage of invasive isolates resistant to carbapenems (imipenem/meropenem), by country, EU/EEA, 2023**



# Results – concentrations of resistant bacteria in wwtp

- ESBL-EC well measurable in all effluents
- Removal of ESBL similar to that of *E. coli*
- CPE found in ~90% of all WWTP in influent
- Spurred analyses of wastewater-based surveillance for AMR

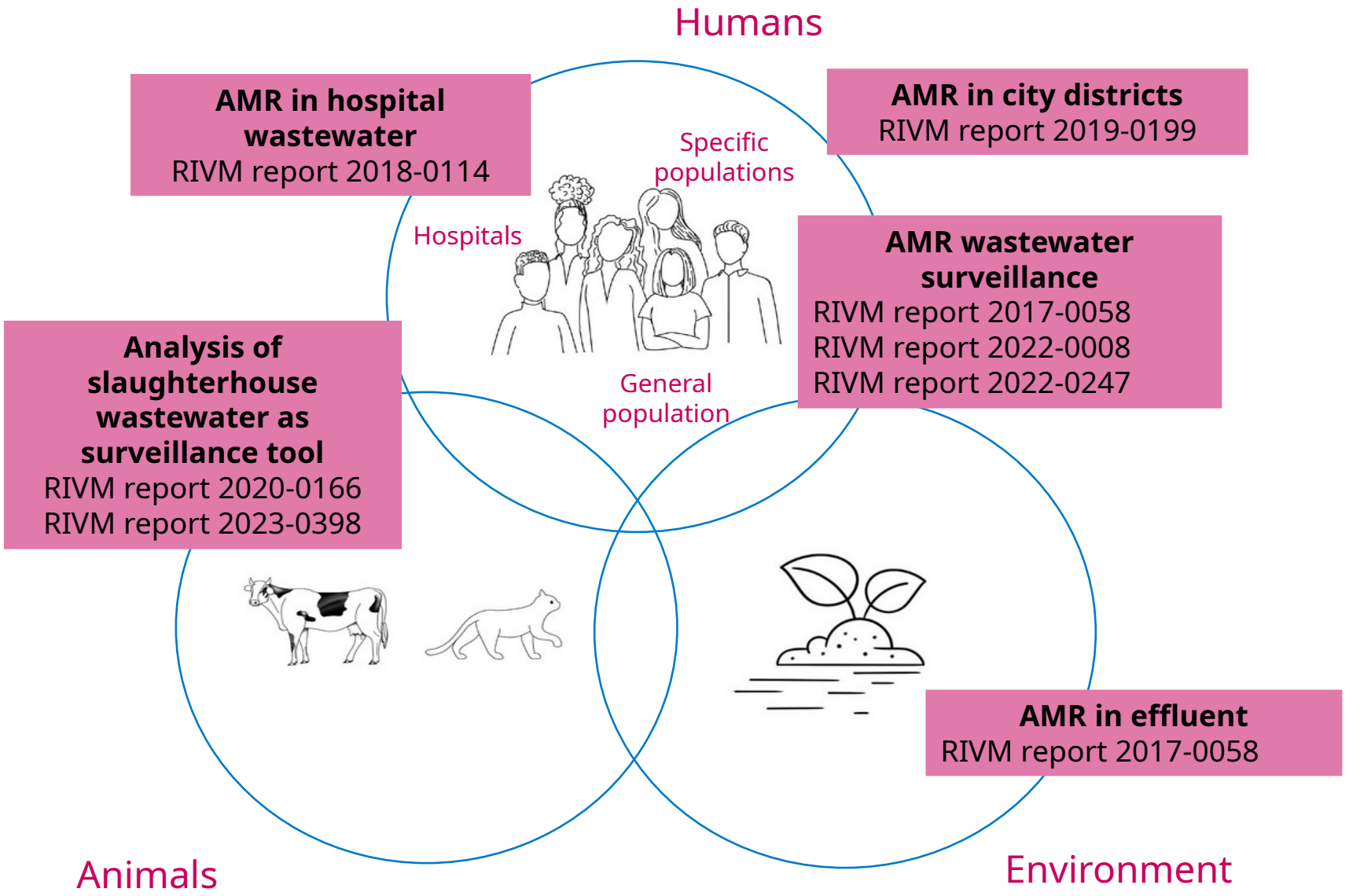



*Blaak et al. (2021) SciTot 776*

# Studies since then

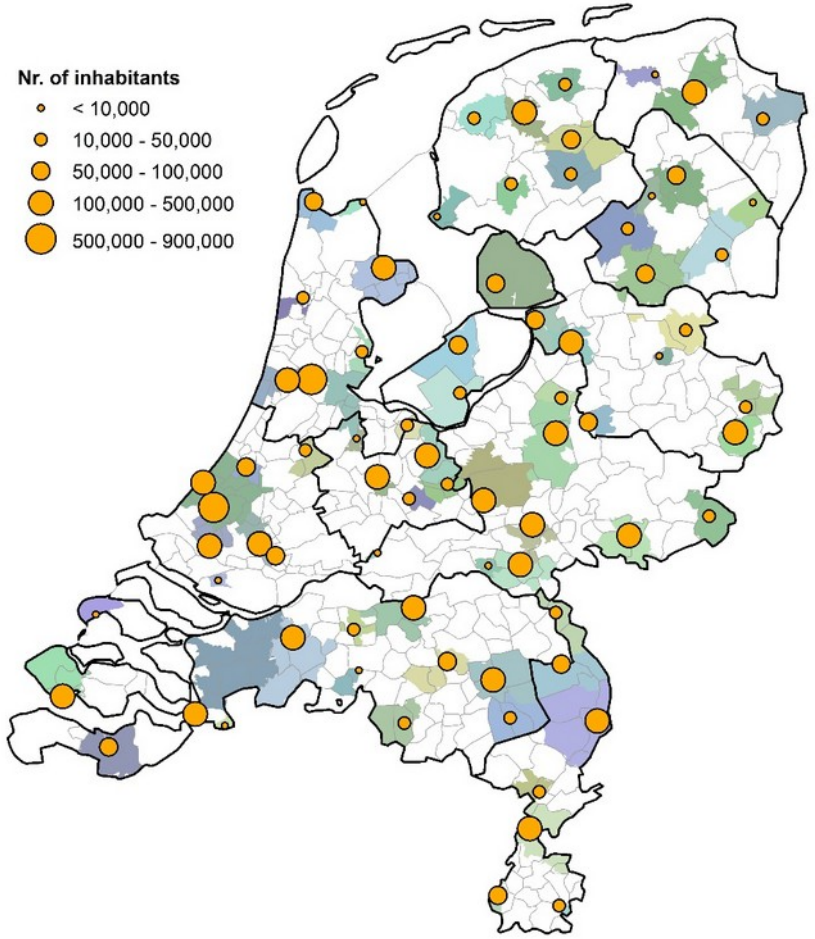
Objective:

- Evaluate wastewater based surveillance as complementary surveillance tool to determine trends in carriage of highly public health relevant bacteria in the human (animal) population



# National wastewater based surveillance since 2020

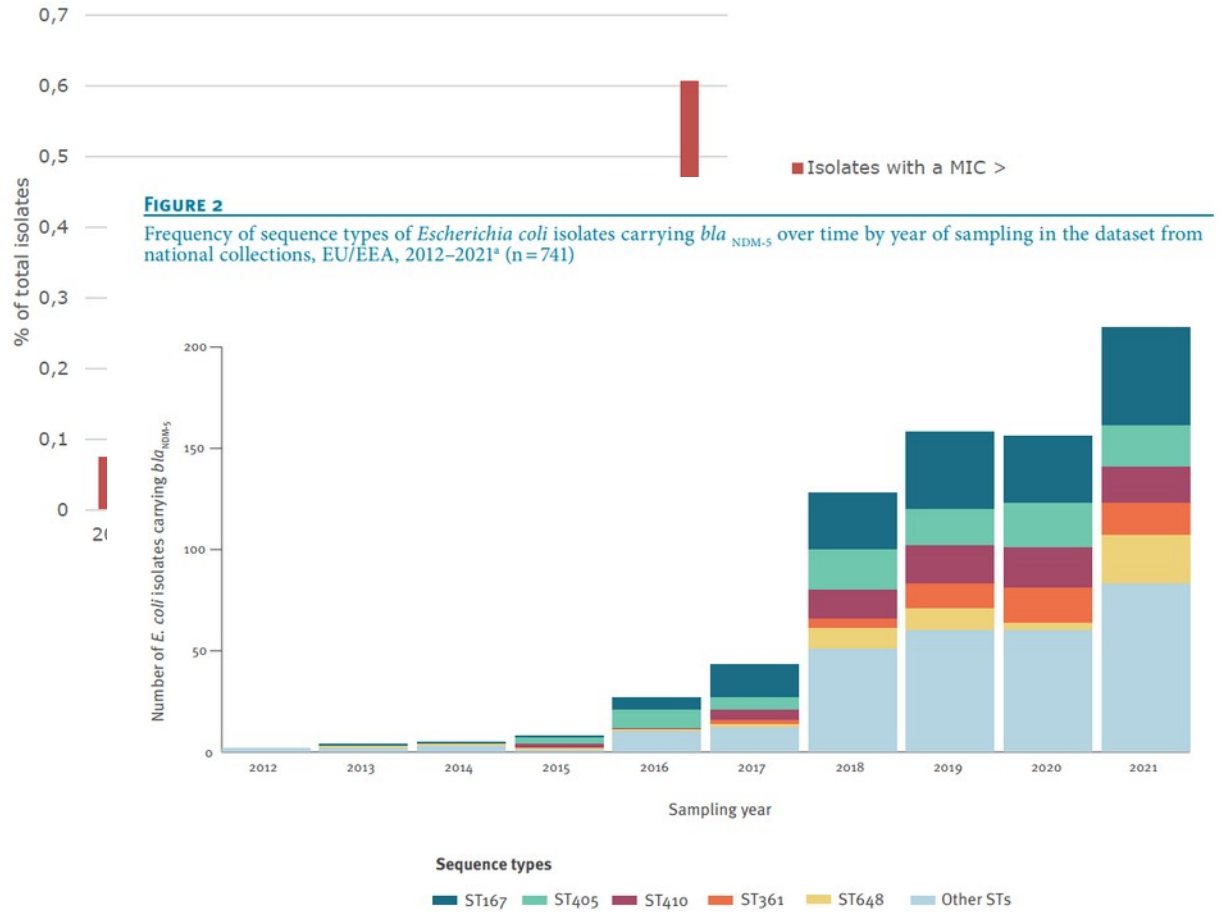
- 2020: 76 of 313 RWZIs
  - Serve 43% of the population
  - Distributed across the NL and provinces
  - Large and small wwtp
  - In regions differing with respect to urbanisation
  - All 12 province capitals
- Since 2020: longitudinal sampling
  - All 12 provincial capitals
  - 12 reference wwtp without hospitals
  - 2-5x per year



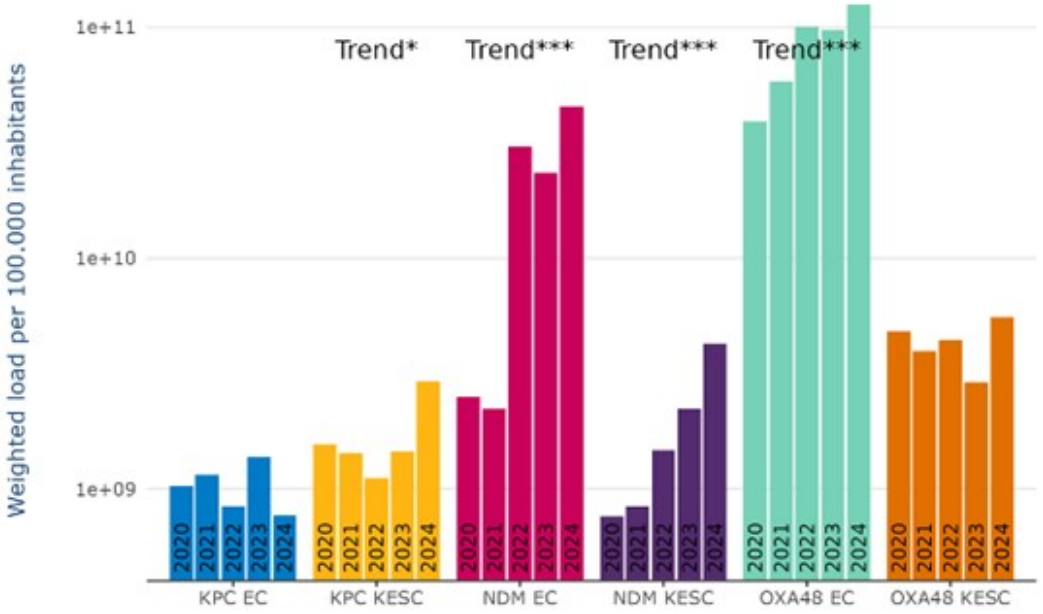

*Blaak et al. (2021) Sci Total Environ 776: 145925*
*RIVM report 2022-0008*

# CPE – trends over time in humans and wastewater

***Figure 5.2.2.1a*** *Proportion of isolates with elevated MIC and proportion of CRE isolates (%) in E. coli and K. pneumoniae by year, in 36 laboratories, ISIS-AR 2020-2024*




*Nethmap One Health 2025, Linkevicius 2023 Eurosurveillance*



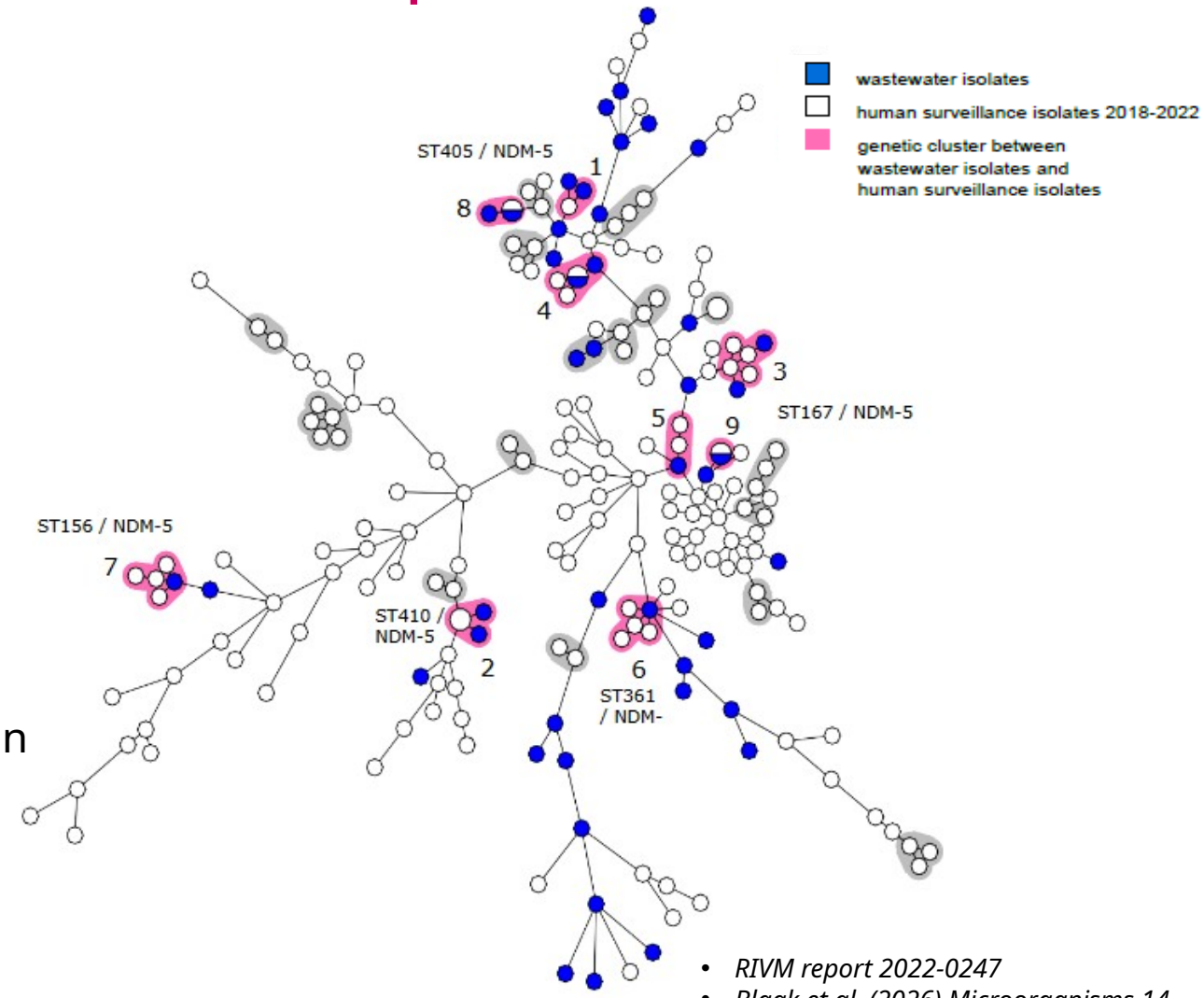

*KPC, NDM and OXA E. coli and Klebsiella*

*Unpublished*

# Can we get more insight in the makeup of these CPE?

Do we find similar resistant bacteria in humans and in wastewater?

- Whole genome sequencing of all human CPE (n=414) and 2016 ww CPE (NDM, KPC)
- Complementarity expected
  - Limited WWTP sampling (100/300 wwtp, 1x)
  - More CPE carriers than clinical patients / isolates
- Highly similar bacteria found for *E. coli* / *Klebsiella* (grey clusters), next to dissimilar bacteria
- 2020: Clusters of wastewater and human isolates were found in 4 out of 28 known carriers that were related to a sampled wwtp in time (±6 mo) and location



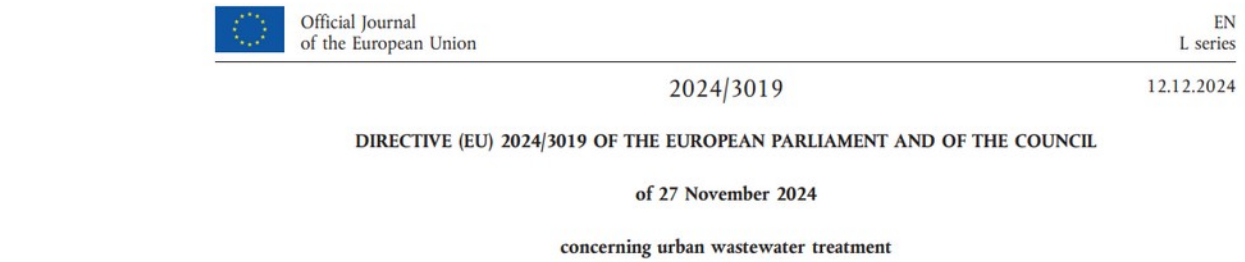

- *RIVM report 2022-0247*
- *Blaak et al. (2026) Microorganisms 14*

# Under discussion: Usage of wastewater data in surveillance

- Main discussion points: reflection of human population?
  - Possible contributions from animals – at least for CPE, assumed to be small
  - Growth of *E. coli* / Enterobacterales in sewers – for *E. coli,* only survival likely
  - Horizontal gene exchange between sewer microbiome and monitored species
  - No calibration with recent human population studies (requires some 10.000 individuals)
  - Data from general population
  - No patient-level information for empirical treatment
  - Interventions arising from changes in prevalence
- Role of WBE in surveillance landscape

# EU Urban wastewater treatment directive (2024)

- Mandatory monitoring of AMR in WWTP of large agglomerations by 2028
- Most likely parameters:
  - ESBL, CPE by culture
  - Panel of resistance genes by qPCR
  - Metagenomics on a voluntary base
- Implementing act detailing measurement obligations this fall (comitology)
- So far, little information on surveillance objectives



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DIRECTIVE (EU) 2024/3019 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL

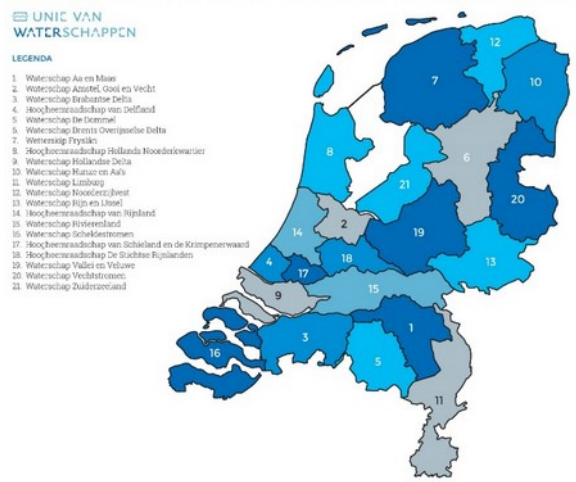
of 27 November 2024

concerning urban wastewater treatment

3. For agglomerations of 100 000 p.e. and above, Member States shall, by the last day of the second year from the date of adoption of the implementing act in the second subparagraph, ensure that antimicrobial resistance is monitored in urban wastewater.

## Personal outlook –
## AMR wastewater surveillance

- Information from wastewater is – and, to be useful, has to be - complementary to clinical surveillance
- Most promising application for routine surveillance: prevalence / emergence of ‘rare’ antibiotic resistant bacteria in the population and their geographical distribution
- Position in current EU surveillance under discussion, yet mandatory according to European directive
- Open questions: in-sewer processes (persistence, HGT) and large-scale ecological agreements of human data and wastewater data
- Other objectives (emissions to surface water, ww treatment efficiency) yet to be explored

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