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

Wastewater based surveillance of AMR in the Netherlands

Hetty Blaak, Merel Kemper, Ana Maria de Roda Husman, Heike Schmitt

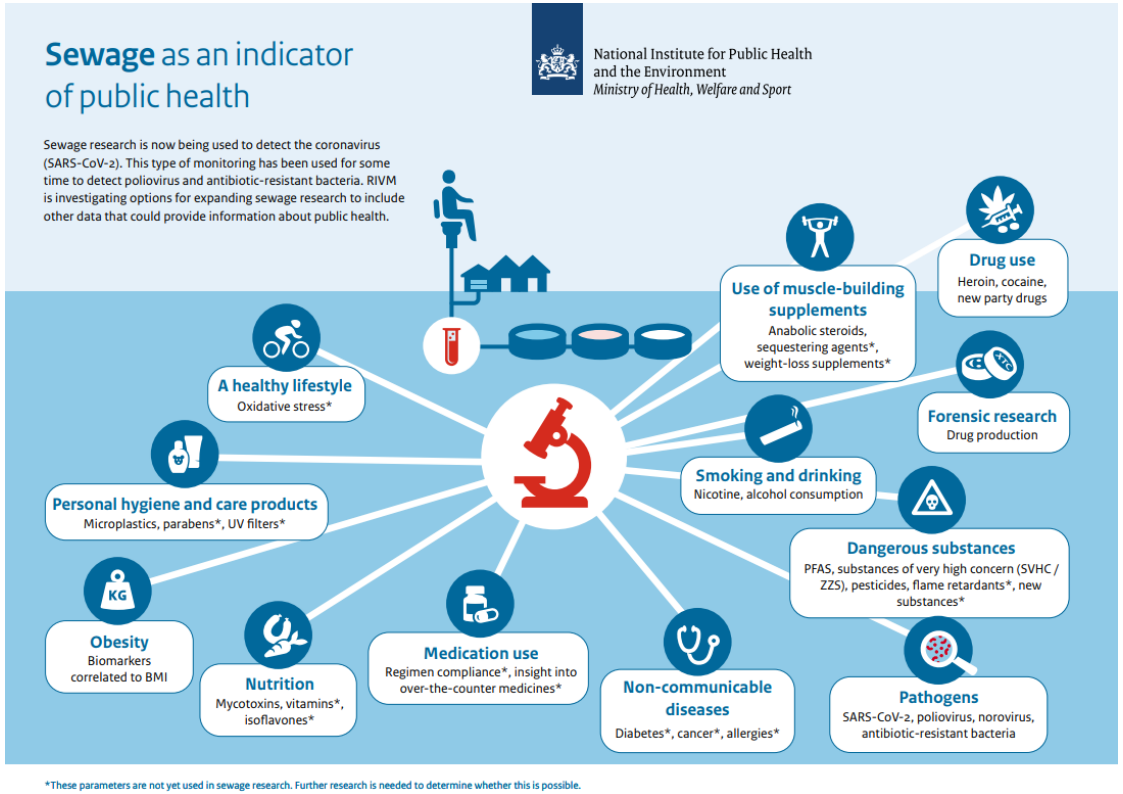
Environmental surveillance

- Human feces and urine ends up in sewers
- Sewage consists of wastewater from:
  - households (71%)
  - industry and institutions (29%)
  - among which health care institutions (3.3%)
  - and agricultural, hunting and foresting industries (0.6%)
- Sewage is collected and treated in wastewater treatment plants (WWTP)
- Effluent is released to surface water
- Wastewater can be used to gain insight in the prevalence of fecal pathogens in the general population and their geographical distribution

Environmental surveillance – now and further

- AMR surveillance since 2016 within Aanpak Antibioticaresistentie

Source: RIVM



https://coronadashboard.government.nl/landelijk/rioolwater

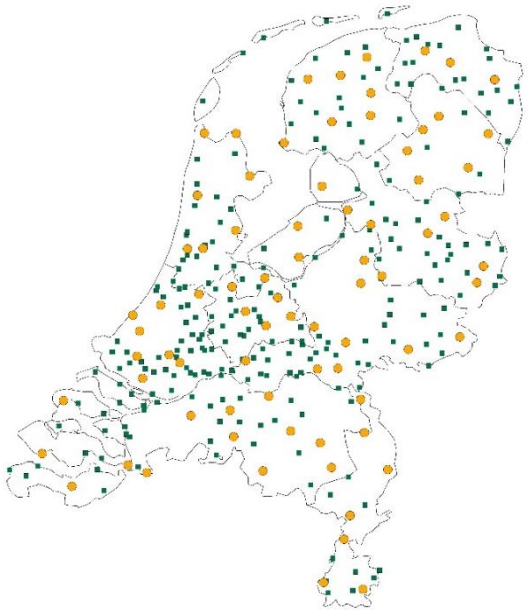
Environmental AMR surveillance – current goals

1. Prevalence of BRMO and specific BRMO types
2. Distribution across the country
3. Trends in time
4. Estimates of carriage
5. Evaluation of usage for local healthcare institutions

..This for the case of specific rare BRMO, such as carbapenemase producing Enterobacterales

Surveillance of antibiotic resistance - method

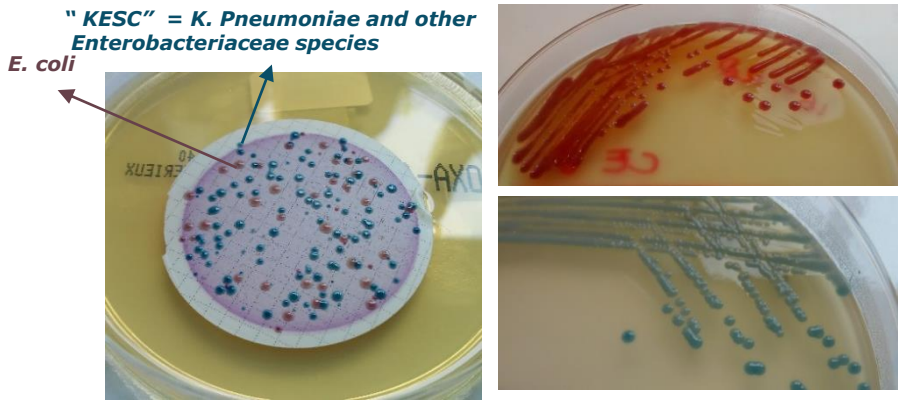
RWZI selection



Sampling

Filtration

Selective culture



CPE isolates:

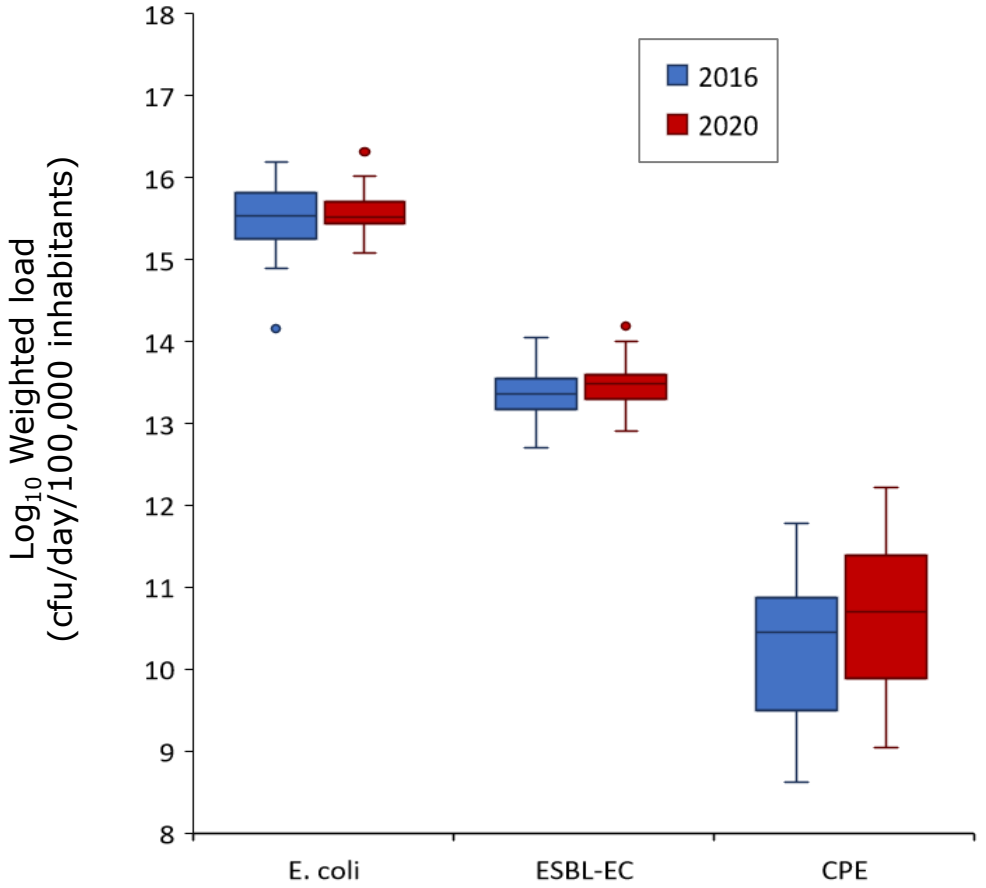
Identification of carbapenemase-gen by PCR: OXA-48, NDM, KPC, IMP, VIM, GES

Genotyping: sanger sequencing (GES, OXA-48) and WGS (KPC en NDM)

CPE (carbapenemase producing Enterobacterales)

- Low prevalence in NL (~1:10.000 persons)
- Carriage associated with international travel and international hospital admission
- Resistance encoded by different genes (OXA-48, NDM, KPC, VIM)

CPE load– trends over time



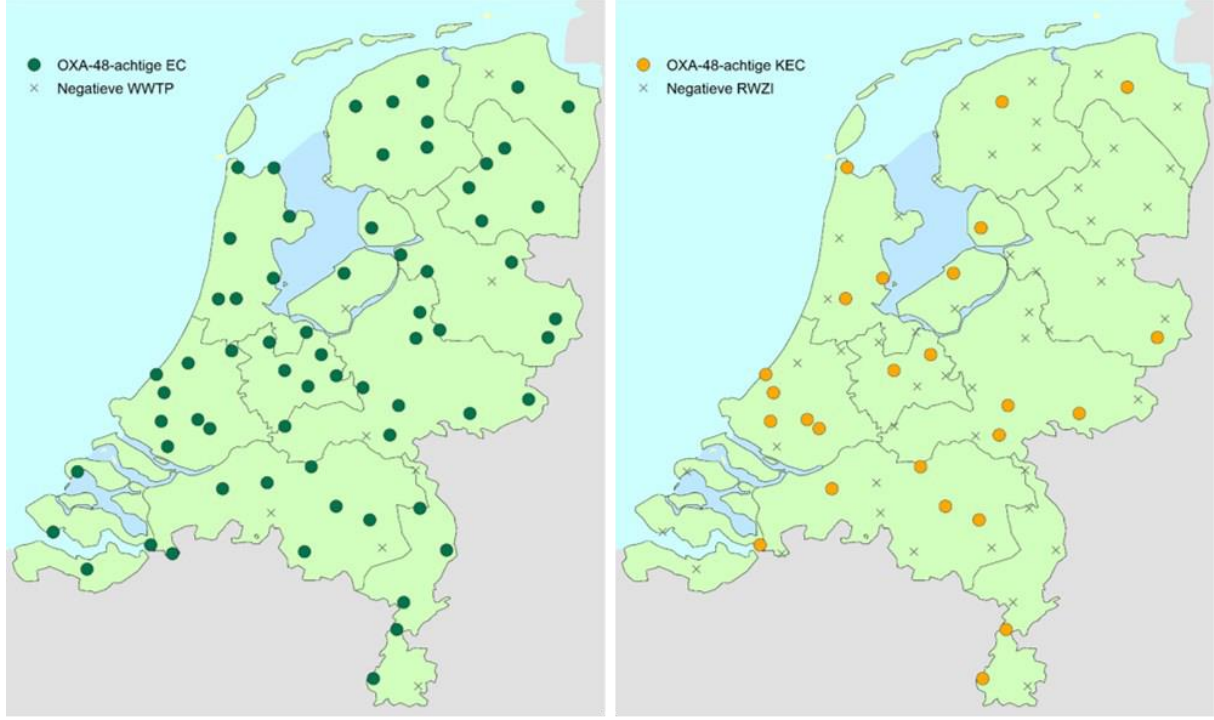
- CPE load 10^2-10^3 times lower than ESBL-EC, and 10^4-10^5 times lower than *E. coli* load
- Constant loads for *E. coli* and ESBL-EC between 2016 and 2020
- Slight increase in CPE load (2.5-fold difference in geomean), p=0.093

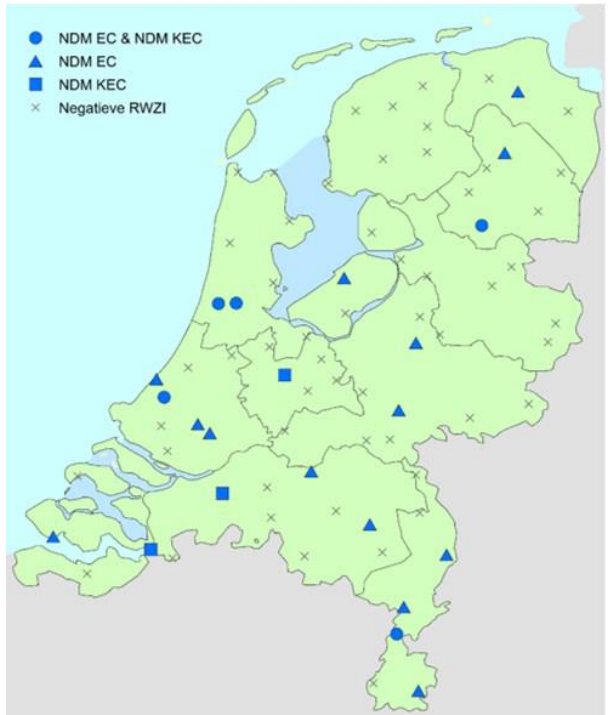
Load = concentration x 24h volume of influent (cfu/day)

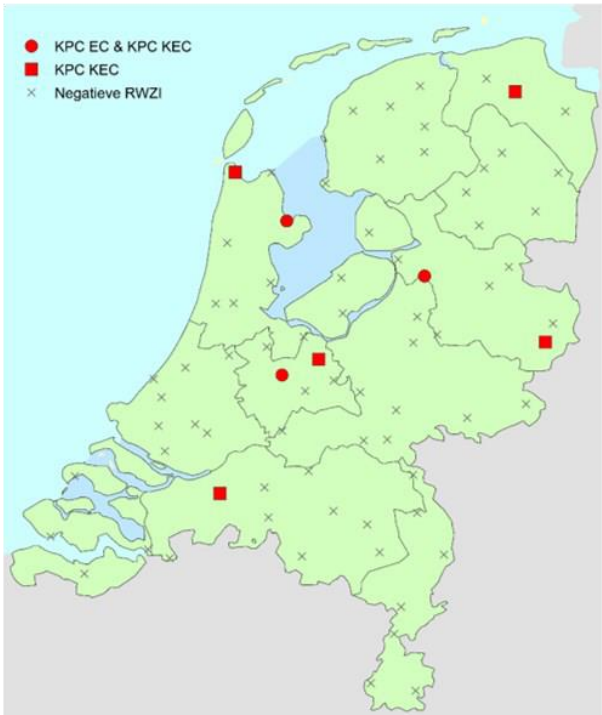
Blaak (2021) RIVM rapport

Regional distribution

- No signs of difference in regional distribution of 4 CPE genotypes

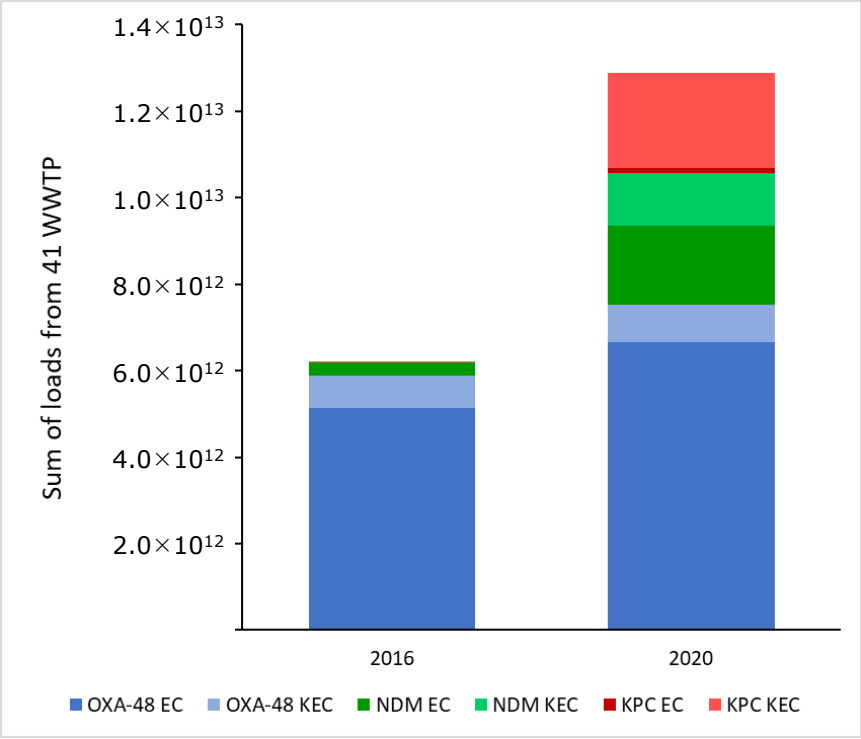






Population carriage estimates

Sum of CPE loads of 41 WWTP - 4.4×10^6 inhibitants



Point estimate of CPE carriage

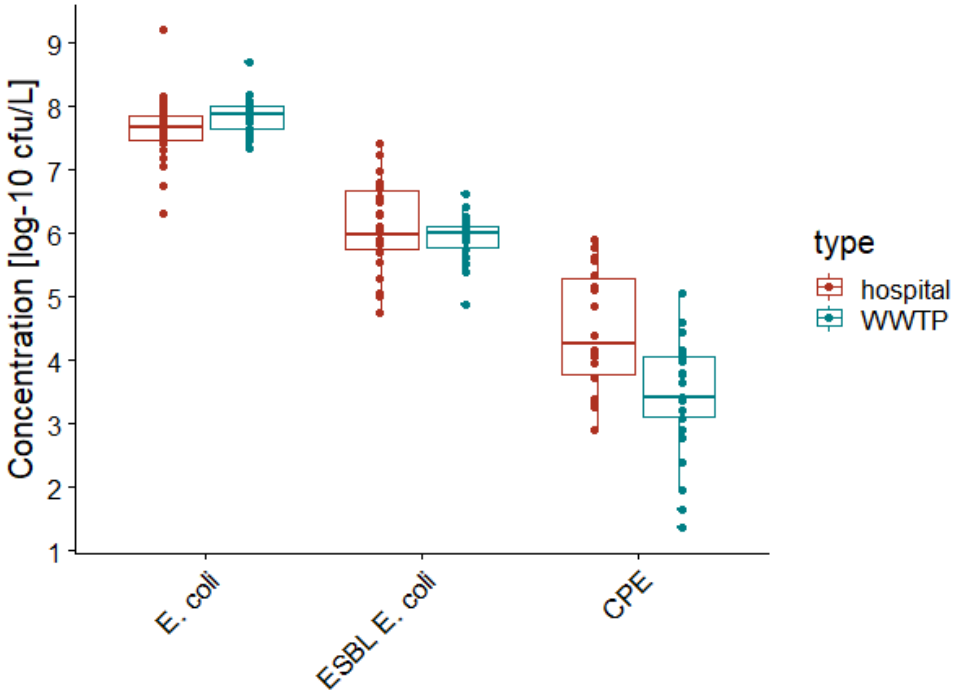
	2016	2020
CPE	1043 (0.02%)	2078 (0.05%)
NDM/KPC CPE	55 (0.001%)	860 (0.02%)

- Based on ΣCPE-load relative to ΣESBL-load (1.3×10^{15}),
- Based on 5% ESBL-carriers in 2014-2016 (vd Bunt et al. 2019) ,
- Assumed: same fecal load/carrier for ESBL and CPE carriers

Blaak (2021) RIVM rapport

In-depth studies: hospitals as source of CPE

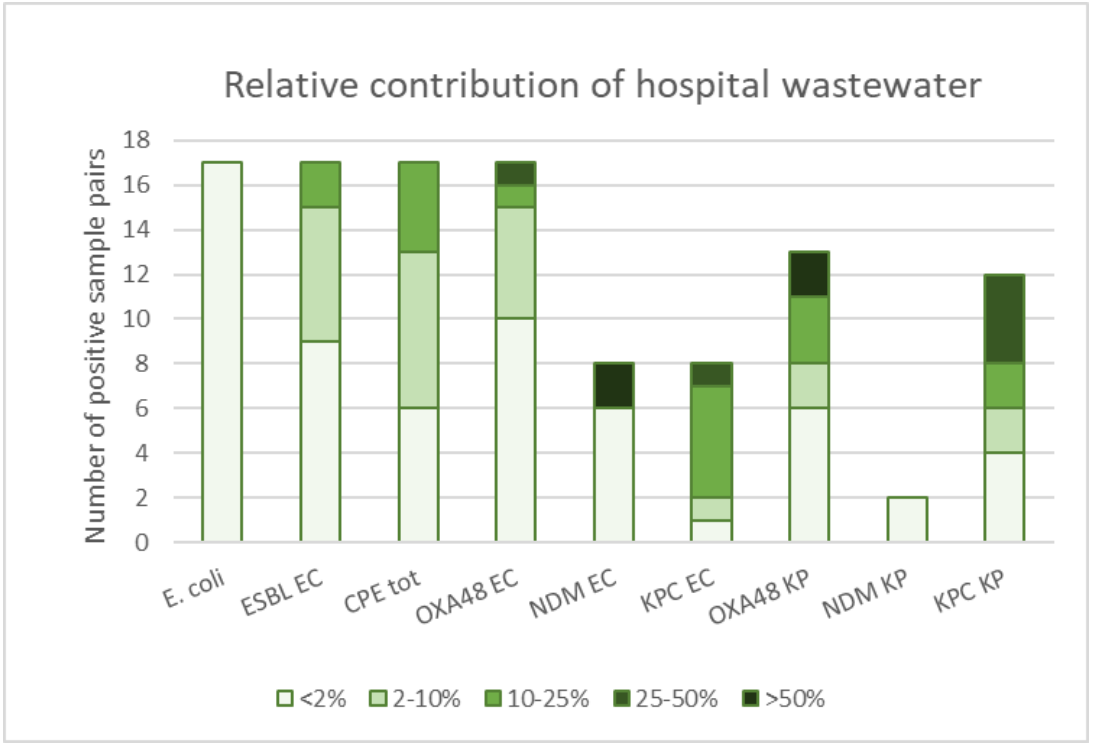
- 8 hospitals, sampled 3-4 times, paired samples from hospital and WWTP
- Significantly higher concentrations of CPE found in hospital effluent



Schmitt et al. 2018 RIVM Briefrapport 2018-0114

Contribution of hospitals to total WWTP - load

- Hospitals on average 1% of the total wastewater volume
- Load: total number of bacteria (=concentration * wastewater volume)
- Hospitals do contribute to CPE load
- But mostly much less than 25%
  - Apart from single gene types

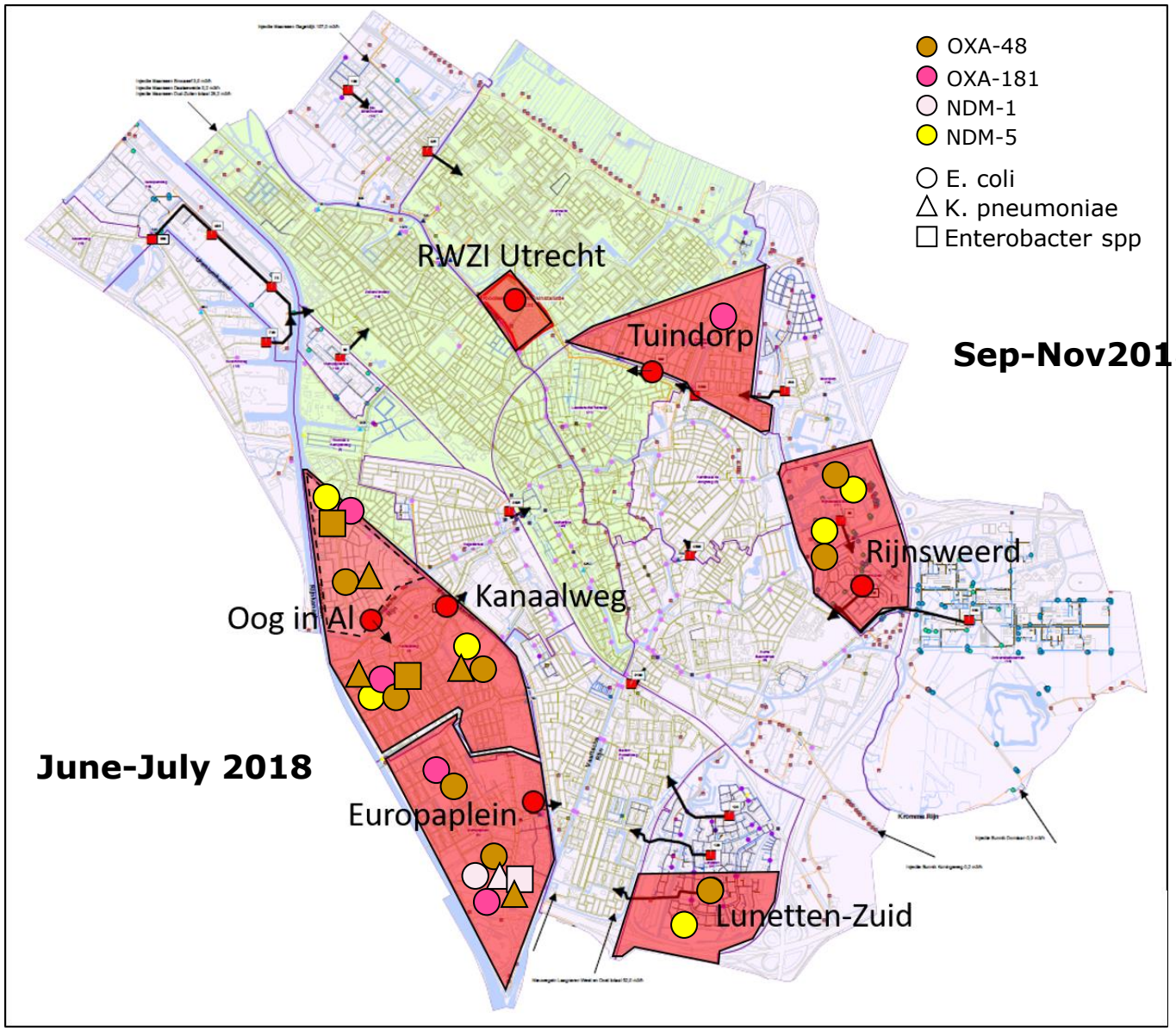


Healthcare institutions and wastewater

- No major point source for emissions to the environment
- Wastewater treatment at institutions: no major contribution to emission reduction

Contribution of environmental surveillance to human surveillance?

- Outbreak detection
- Overall prevalence in HCI



CPE in district sewers

- CPE are detected in district sewers, further substantiating carriage in the community
- Multiple CPE types are found in different districts of the same city
- Detection of CPE in small districts suggests high sensitivity (likely restricted numbers of carriers)

Blaak et al. 2019 RIVM rapport 2019-0199

Other BMRO

Colistin resistant *E. coli*

- presence/absence analyses rather than concentrations

CPE method applicable to global analysis

- JPE TRIuMPH

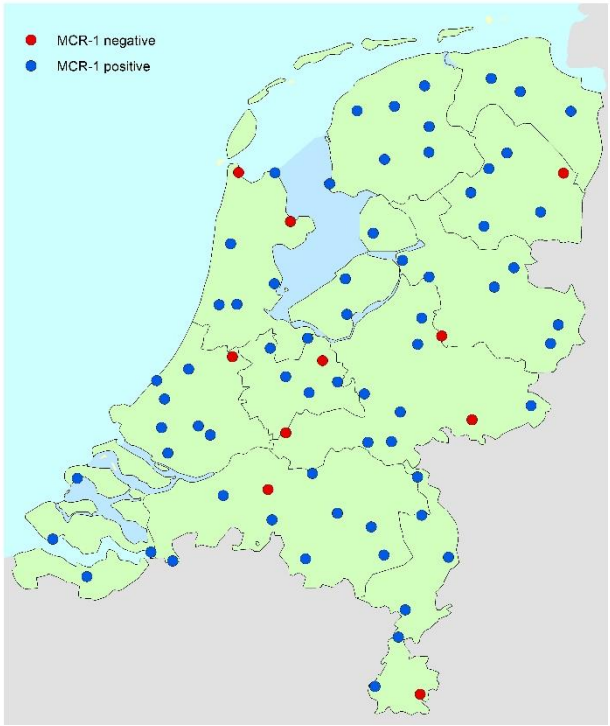
Carbapenem resistant *Pseudomonas aeruginosa*

- JPE SAMPAN

Metagenome, *Acinetobacter baumannii*

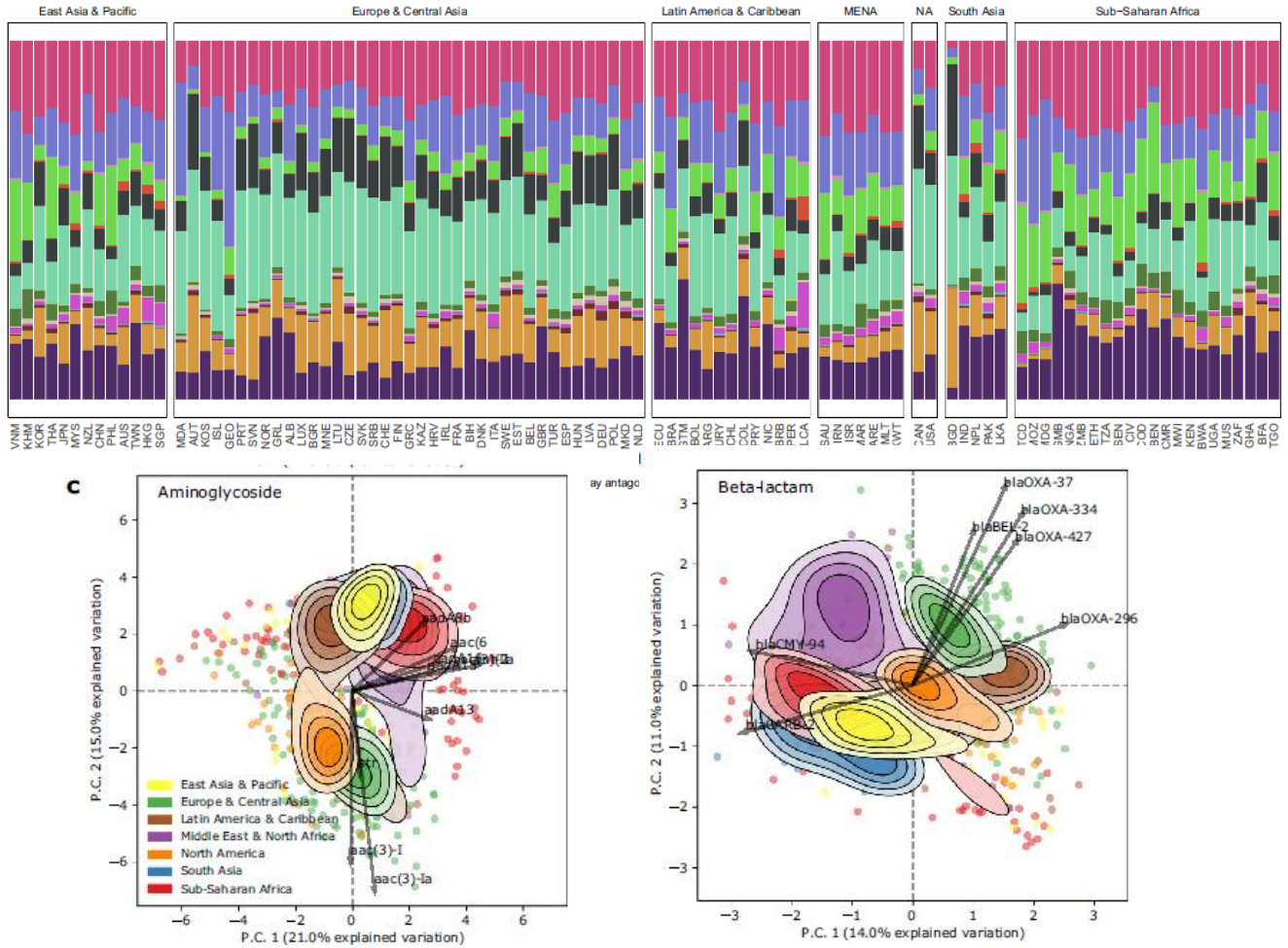
- Wish list

	Samples	Isolates
Total	76	296
MCR-1 pos	66	203
	87%	69%



Global Sewage Surveillance Project

- Metagenomic analyses of sewage samples from >100 countries
- Differences in types of resistance genes across continents
- Correlation not only with antibiotic usage, but also health spendings / sanitation



Munk (2022) Nature communications

Conclusions

Wastewater based surveillance (WBE)

- Most promising application: prevalence of rare antibiotic resistant bacteria in the population and their geographical distribution
- Trends in prevalence of CPE: No decrease despite measures to control Covid-19 pandemic (longitudinal measurements still ongoing)
- Single resistotypes: Overlap of wastewater isolates and isolates from human surveillance - WBE provides additional information
- WBE can also be used at the level of districts or institutions

Thanks to

- Hetty Blaak, Merel Kemper, Ka Yin Leung, Ana Maria de Roda Husman
- Many people at all Dutch waterboards, Waternet, Delfluent, Aquon, Aqualysis, WBL
- CBS
- Min VWS