National Surveillance of Antimicrobial Resistance in Wastewater and Air

Towards an Affordable One Health Monitoring Framework

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The University of Queensland

Next Water 2025
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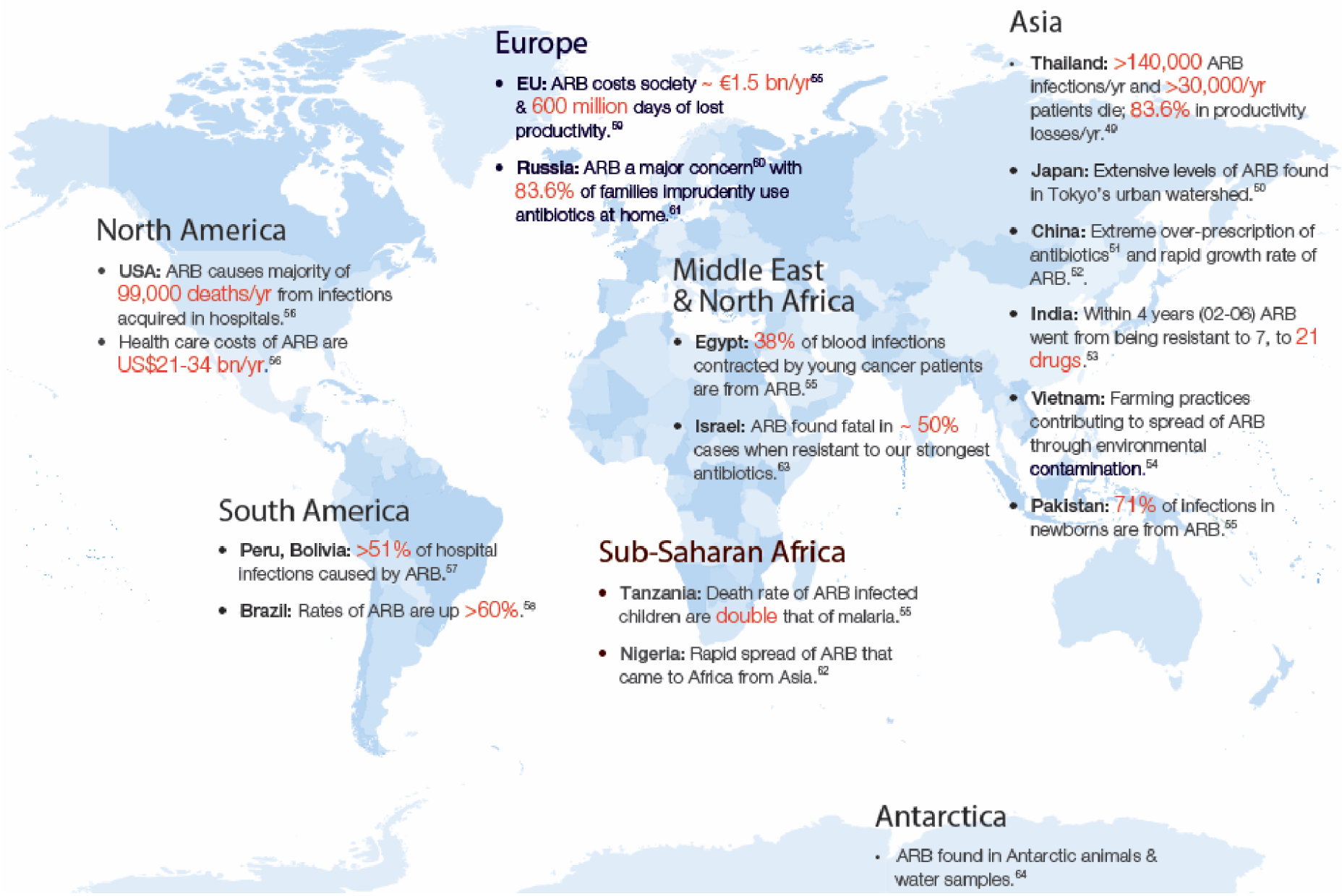
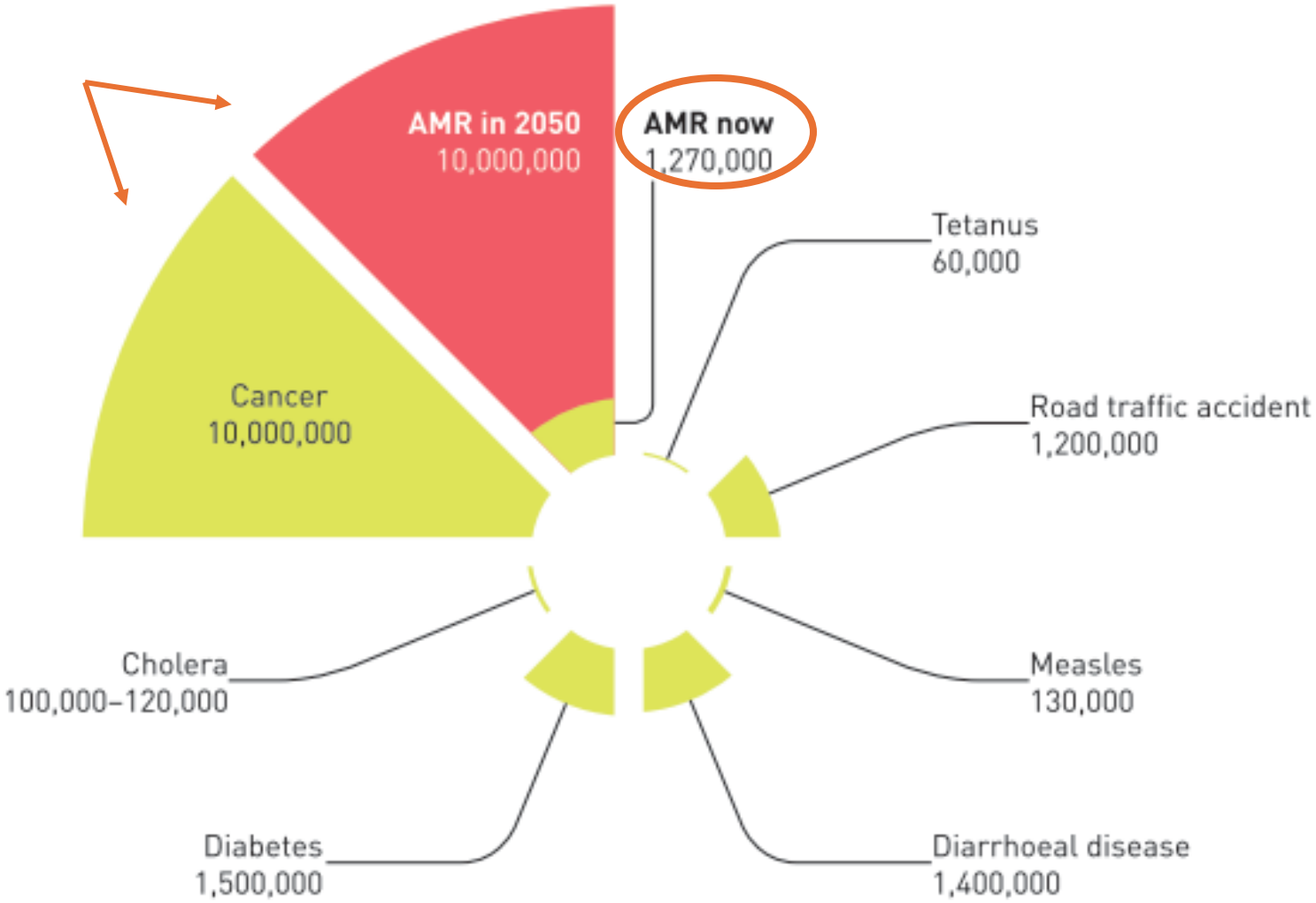
Background



Cost, impact, and burden of antimicrobial resistance bacteria

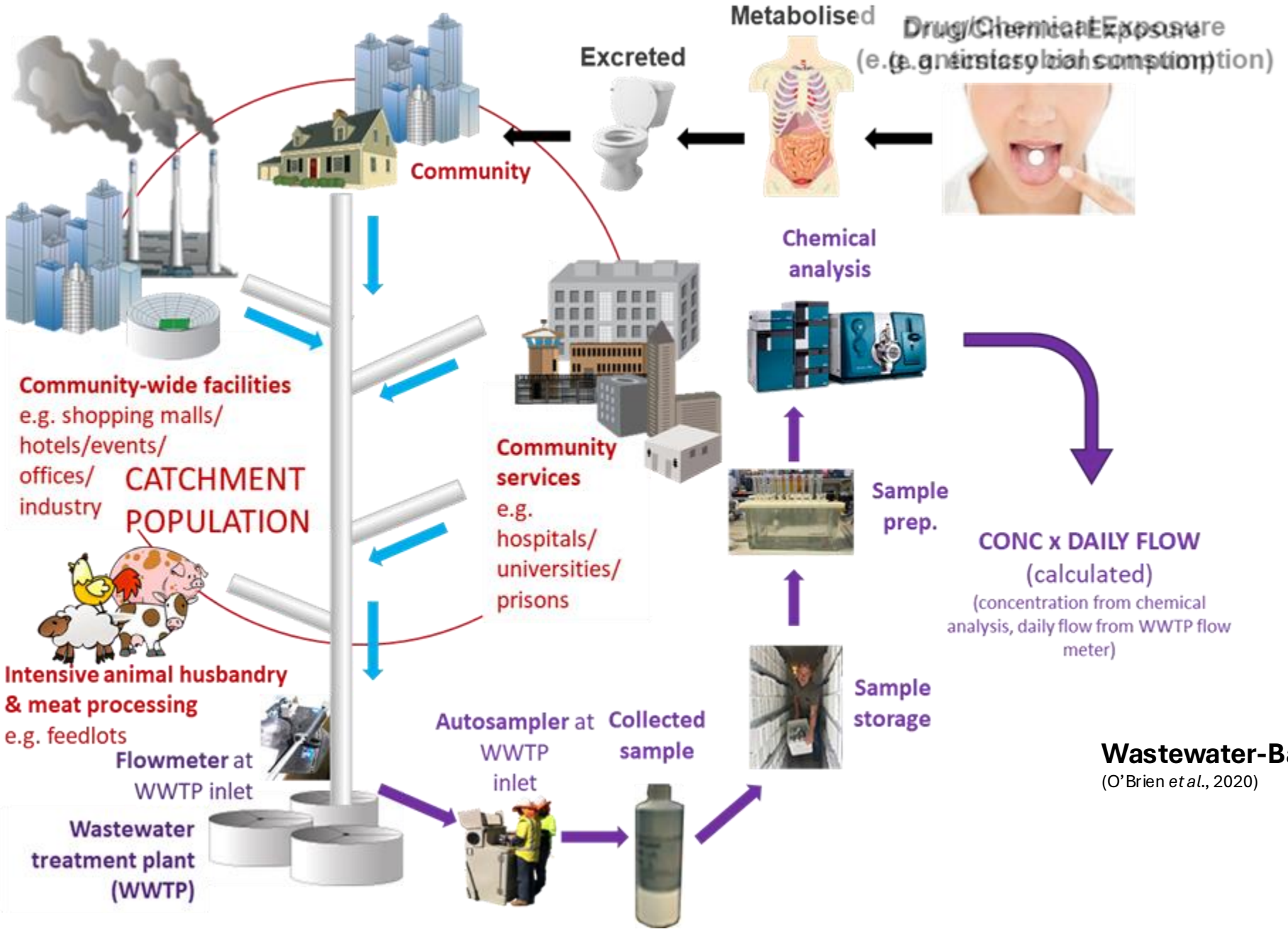
(Guo *et al.*, 2017)

Background

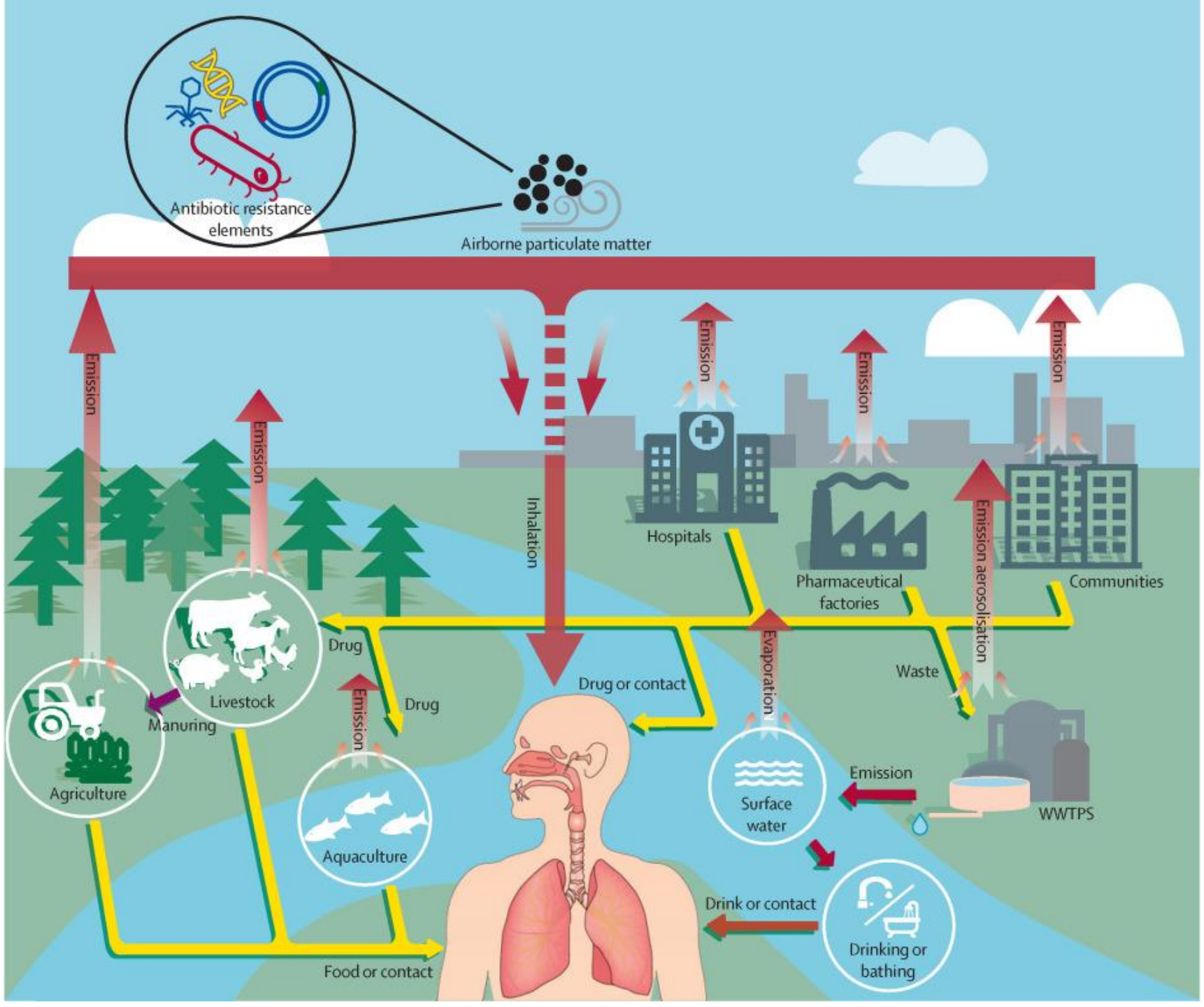


Predicted mortality from AMR compared to common causes of death today
(adapted from O'Neill 2016)

Wastewater-Based Epidemiology (WBE)



Wastewater-Based Epidemiology (WBE)
(O'Brien *et al.*, 2020)



Association between particulate matter (PM)2·5 air pollution and clinical antibiotic resistance: a global analysis
Zhou, Zhenchao et al.
The Lancet Planetary Health, Volume 7, Issue 8, e649 - e65

Workflow: Sampling → Analysis → Outputs

Sampling

- Water: influent, effluent, rivers
- Air: headworks, aeration,
- Soil: banks, sediments,

Processing

- Concentrate / filter → DNA or
- Field & travel blanks
- Record metadata

Analyses

- Shotgun metagenomics
- qPCR panels (ARG targets)

Bioinformatics

- ARGs • Hosts • Mobility (MGEs/plasmids/integrons)
- Link ARG↔host; evidence across W–A–S
- Statistics and QC

Outputs

- ARG abundance
- ARG diversity
- Resistance classes
- Pathogen taxa
- Hosts (host profiling)
- Mobility risk assessment (MGEs/plasmids/integrons)
- Homology analysis

Metagenomic profiling of wastewater from major Australian cities reveals the resistome, host range, and mobilization potential of antibiotic resistance genes.

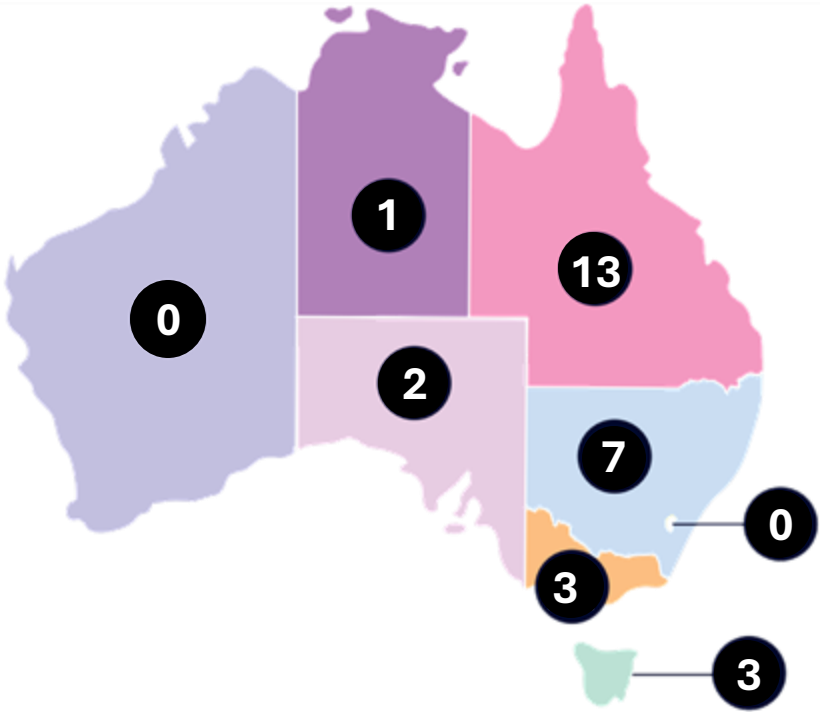
- Develop a national metagenomic surveillance framework for WWTP influent, including pipelines to profile the resistome, link ARGs to hosts, and assess mobility (MGEs/plasmids/integrons).
- Apply this framework to map spatial patterns of ARG abundance, diversity, host range, and mobilization potential across Australian cities and WWTPs.

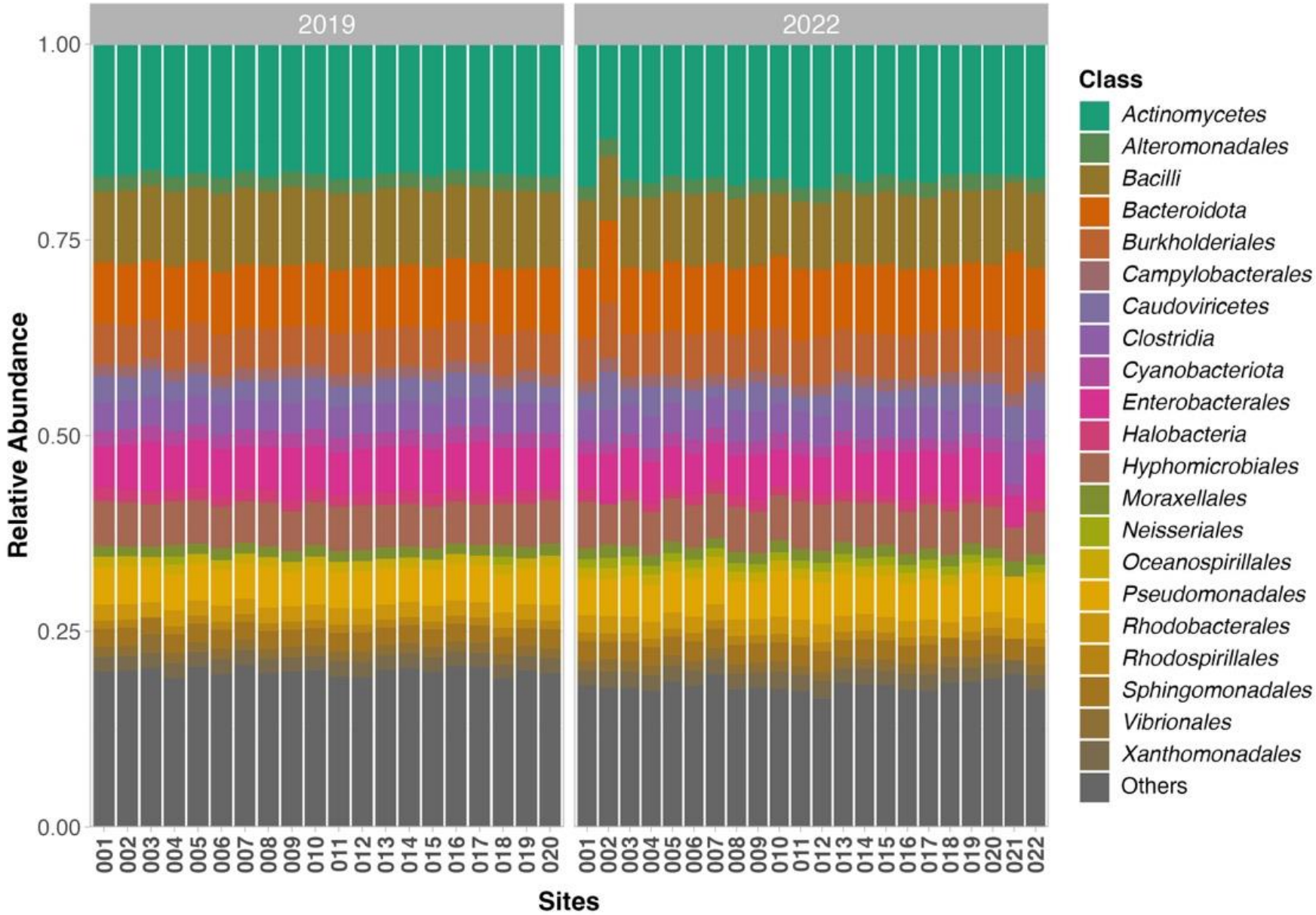
Antimicrobial Reconnaissance in Australian Wastewater and their Socioeconomic Correlates

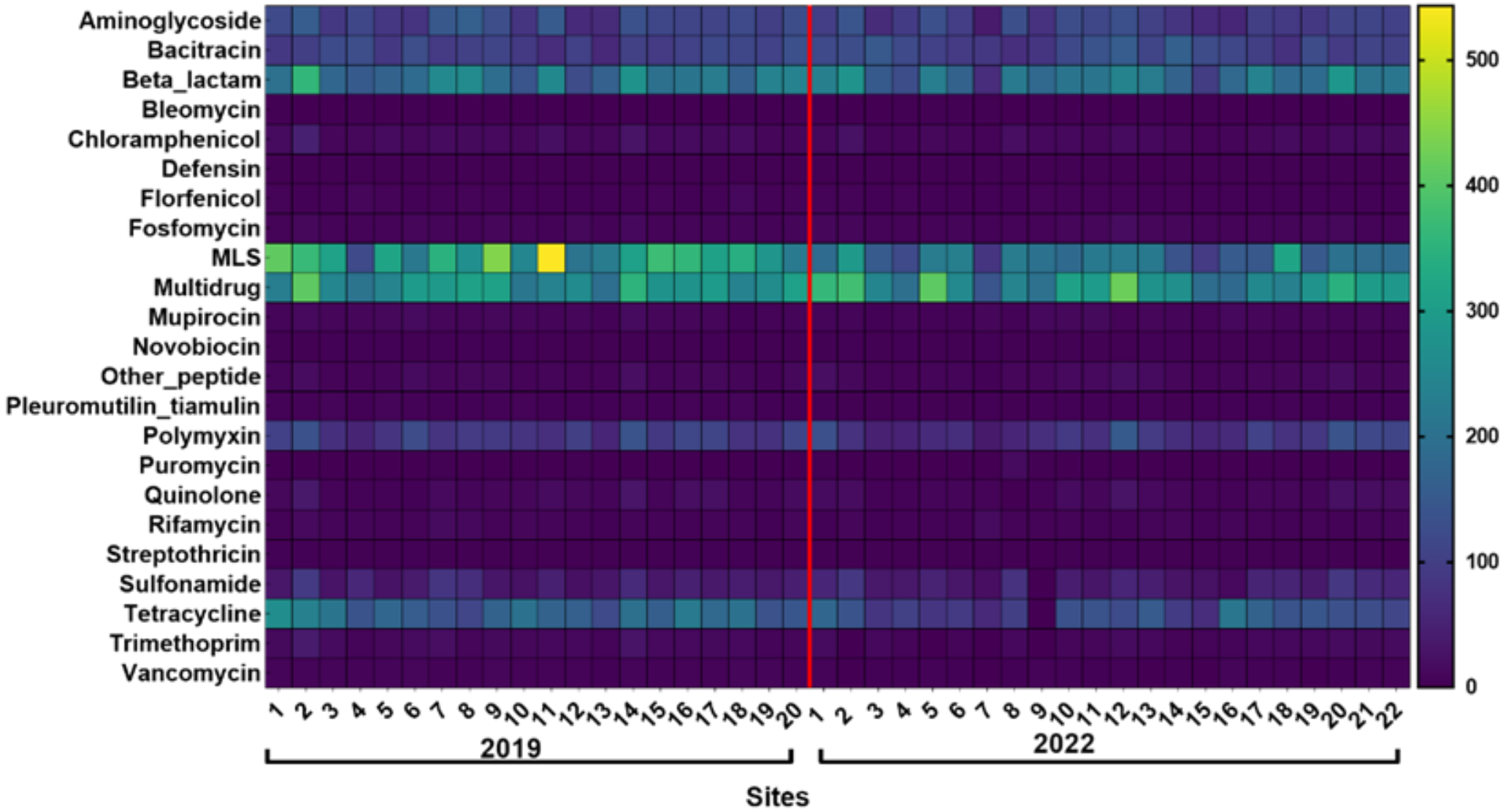
The University Of Queensland AUSTRALIA

Wastewater sampling strategy:

- 29 sites
- 2019 and 2022 samples
- ~50% of the Australian population
- **Raw Wastewater** sampled on Census Day




2019
2022
1.00
0.75
0.50
0.25
0.00
Relative Abundance
001 002 003 004 005 006 007 008 009 010 011 012 013 014 015 016 017 018 019 020
001 002 003 004 005 006 007 008 009 010 011 012 013 014 015 016 017 018 019 020 021 022
Sites
Class
Actinomycetes
Alteromonadales
Bacilli
Bacteroidota
Burkholderiales
Campylobacterales
Caudoviricetes
Clostridia
Cyanobacteriota
Enterobacterales
Halobacteria
Hyphomicrobiales
Moraxellales
Neisseriales
Oceanospirillales
Pseudomonadales
Rhodobacterales
Rhodospirillales
Sphingomonadales
Vibrionales
Xanthomonadales
Others



macrolide–lincosamide–streptogramin (MLS)

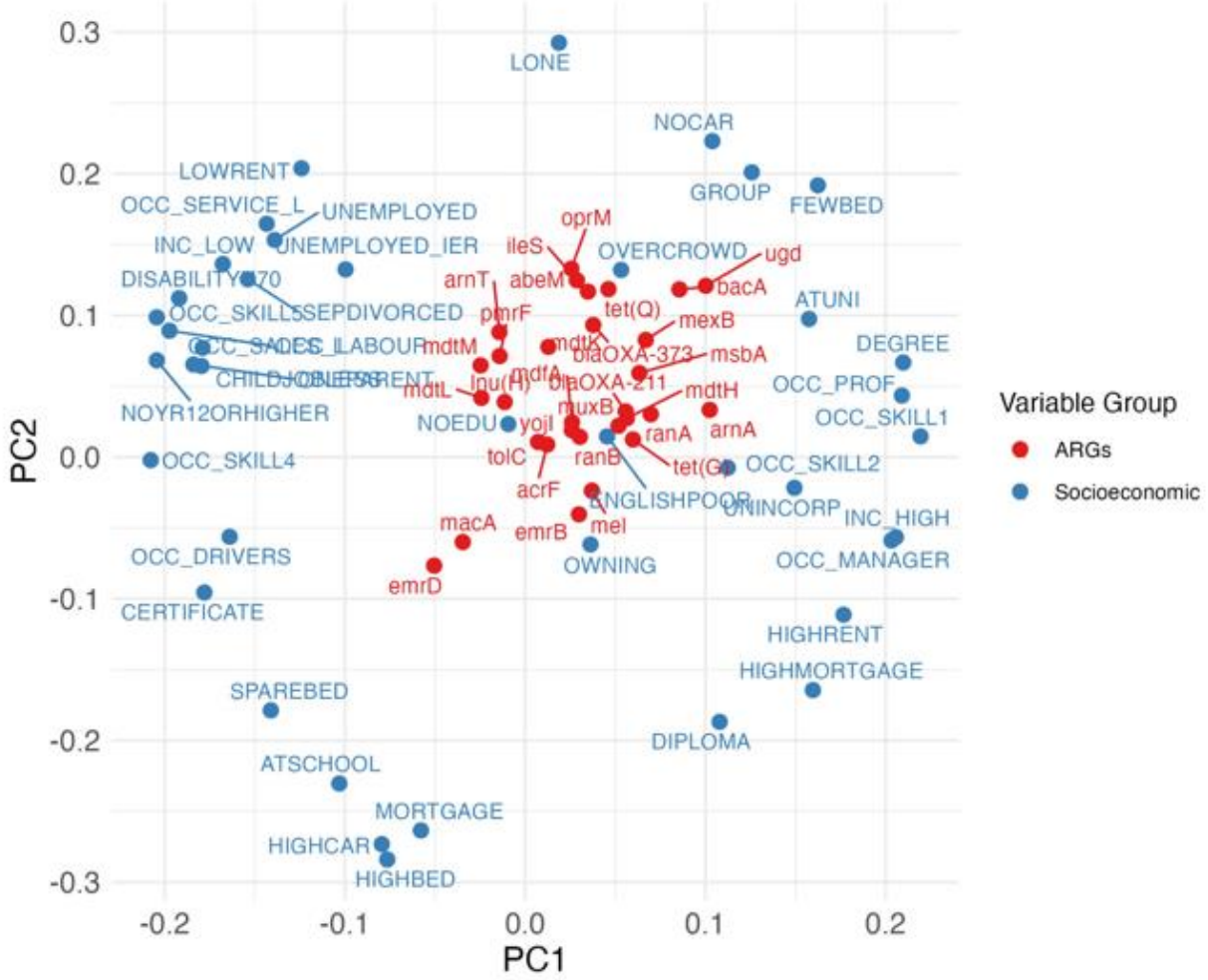
Table A2.1 lists resistant pathogens by level.

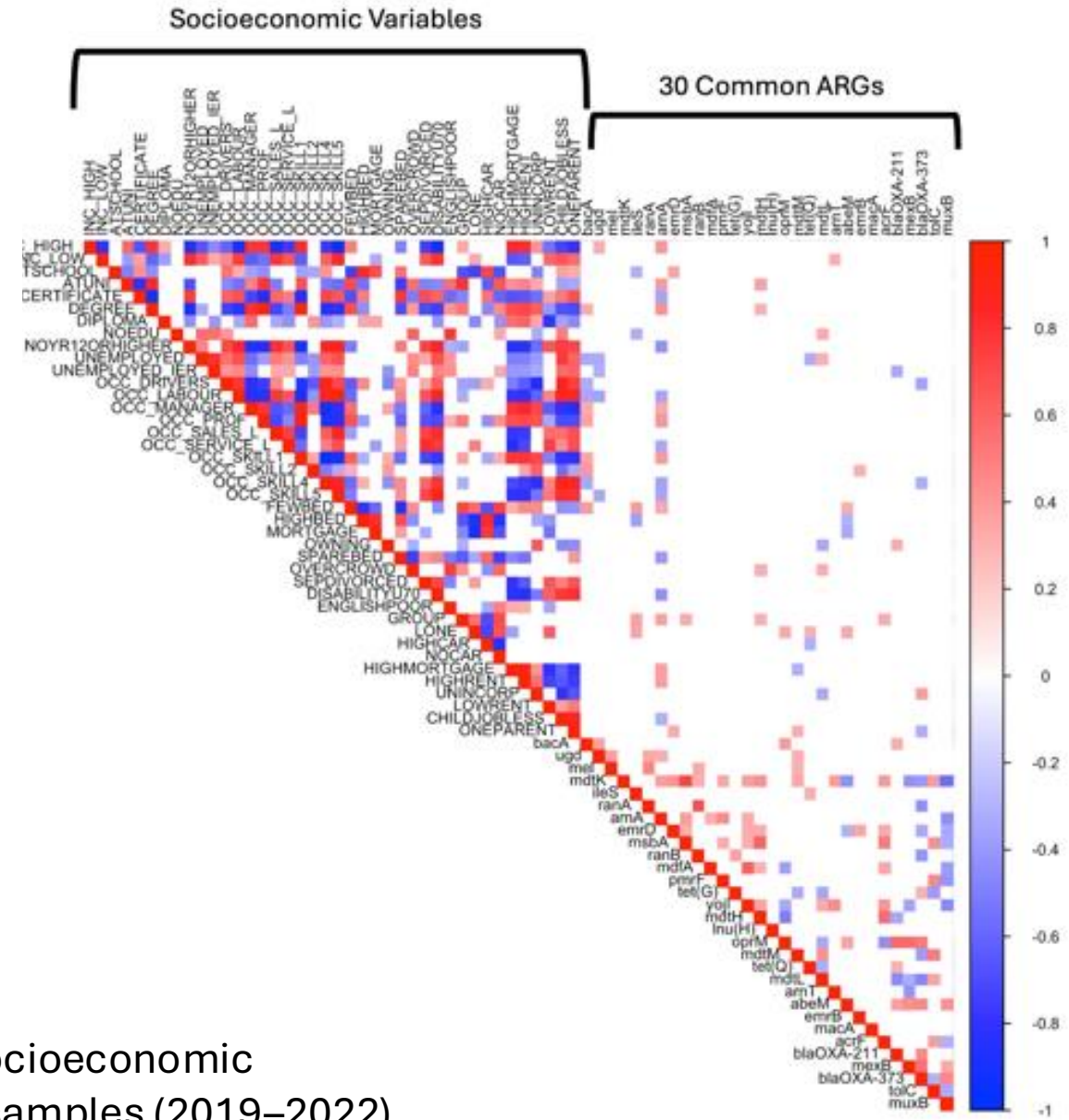
Also, in our risk 1 bacteria list

Table A2.1. Resistant pathogens by level of resistance

Low (< 5%)	Low-medium (5–10%)	Medium (11–20%)	Medium-high (21–30%)	High (> 30%)
FQR nontyphoidal *Salmonella*	FQR *Shigella* spp.	3GCR *E. coli*	CR *E. coli*	CR *K. pneumoniae*
FQR *N. gonorrhoeae*	3GCR *Morganella* spp.	FQR *Salmonella* Typhi	3GCR *K. pneumoniae*	CR *A. baumannii*
3GCR *N. gonorrhoeae*	Pen-R Group B Streptococci	3GCR *Enterobacter* spp.	VR *E. faecium*	CR *P. aeruginosa*
Macro-R Group A Streptococci		3GCR *Citrobacter* spp.	MR *S. aureus*	CR *Enterobacter* spp.
		3GCR *Proteus* spp.		
		3GCR *Serratia* spp.		
		Macro-R *S. pneumoniae*		
		Ampi-R *H. influenzae*		

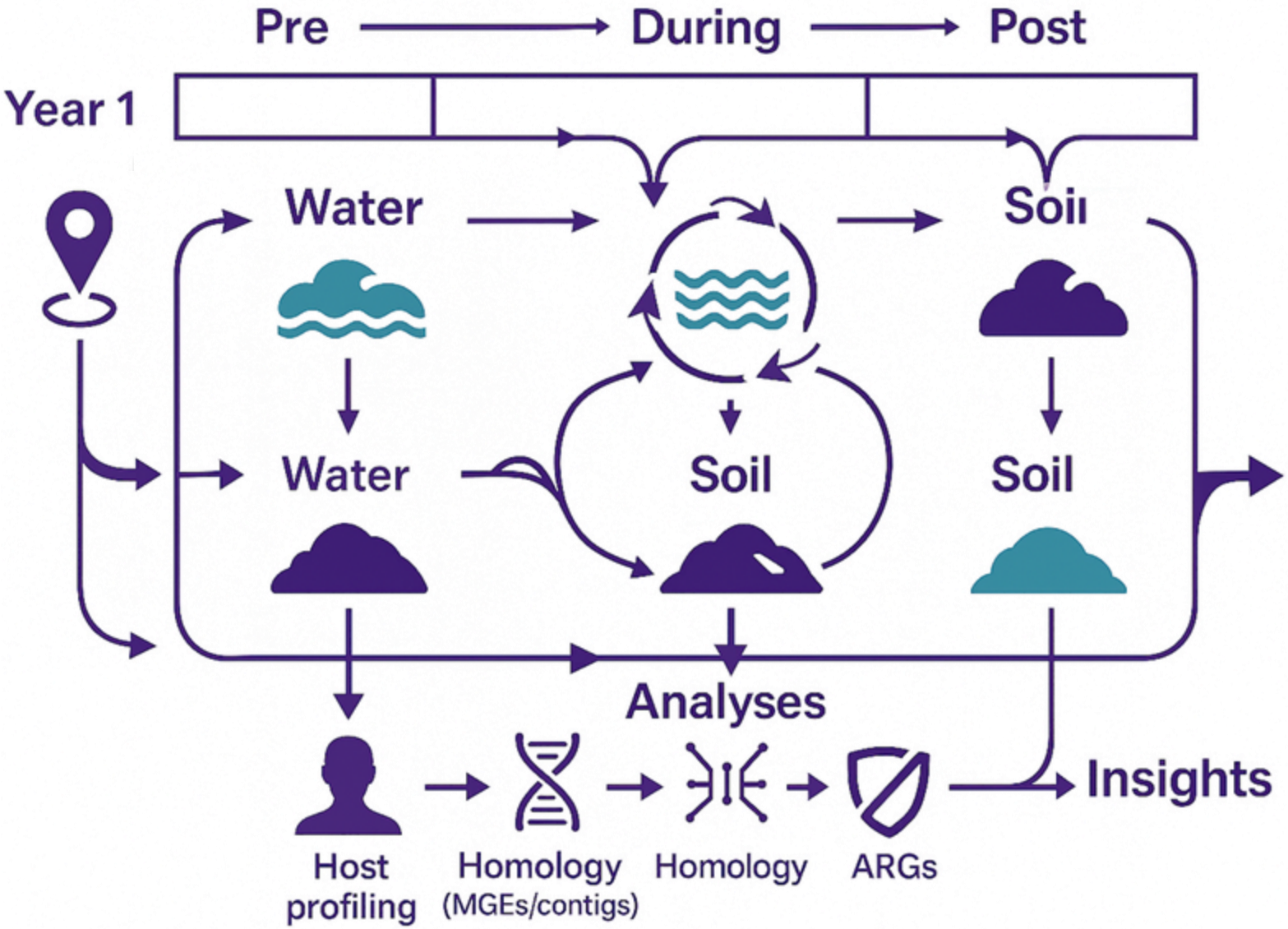
FQR, fluoroquinolone-resistant; 3GCR, third-generation cephalosporin-resistant; CR, carbapenem-resistant; Pen-R, penicillin-resistant; VR, vancomycin-resistant; Macro-R, macrolide resistant; Ampi-R H.influenzae, ampicillin-resistant





PCA and correlation analysis showing associations between socioeconomic indicators and ARG abundance across Australian wastewater samples (2019–2022).

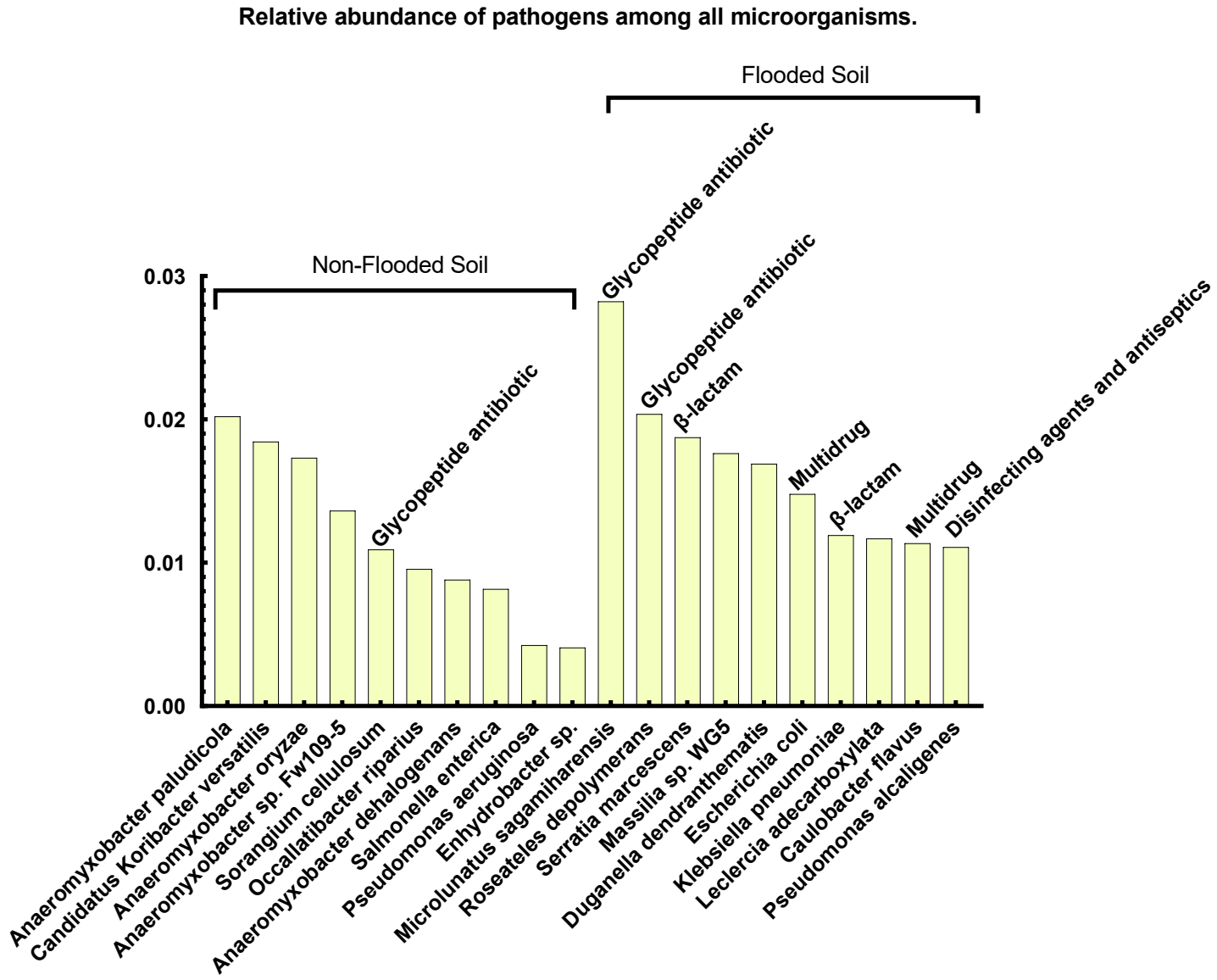
Longitudinal Flood Study: Water–Soil ARG Dynamics at One Site



- Same-site, multi-year sampling across pre/during/post flood
- Paired water & soil metagenomes to track ARG dynamics

Flood Reshapes Host–ARG Correspondence in Soils

Shifted pathogen and shifted ARG classes in flooded cores



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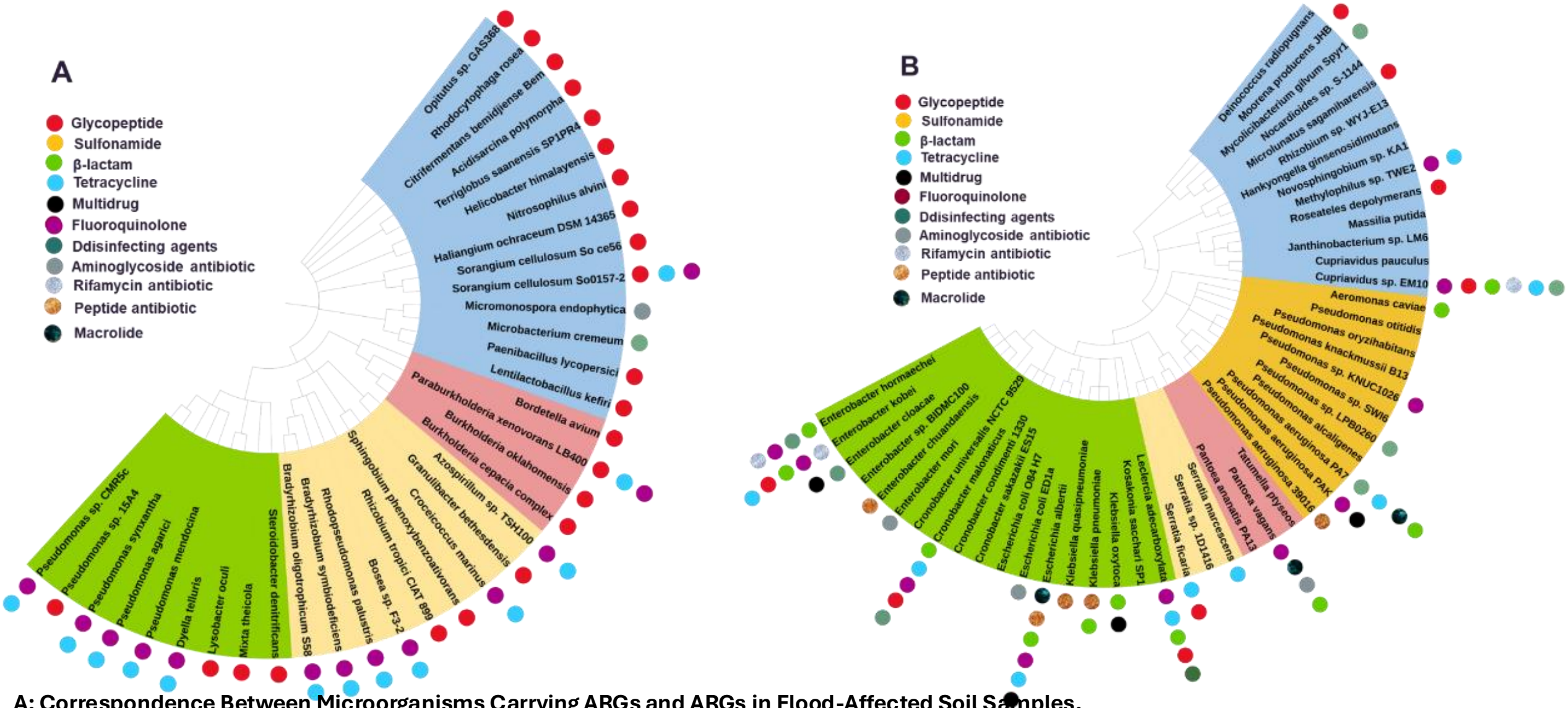


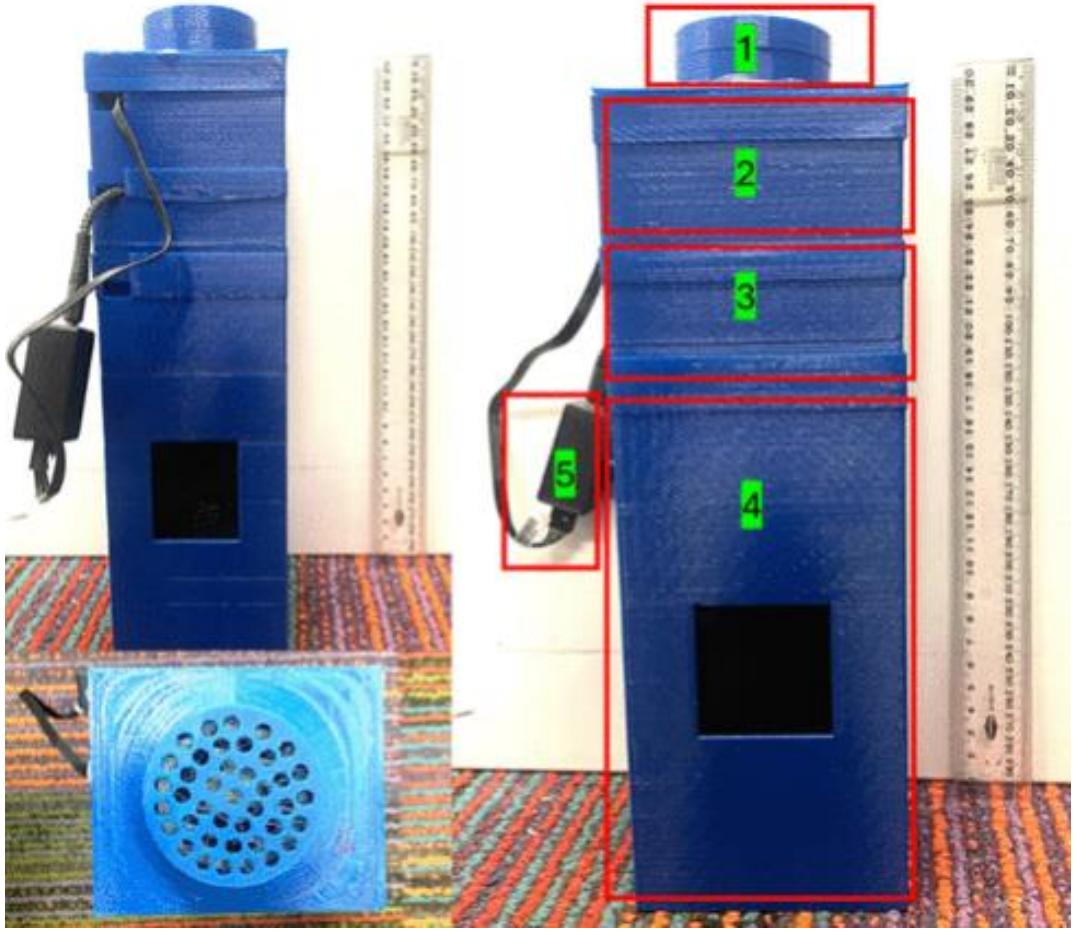
A: Correspondence Between Microorganisms Carrying ARGs and ARGs in Flood-Affected Soil Samples.

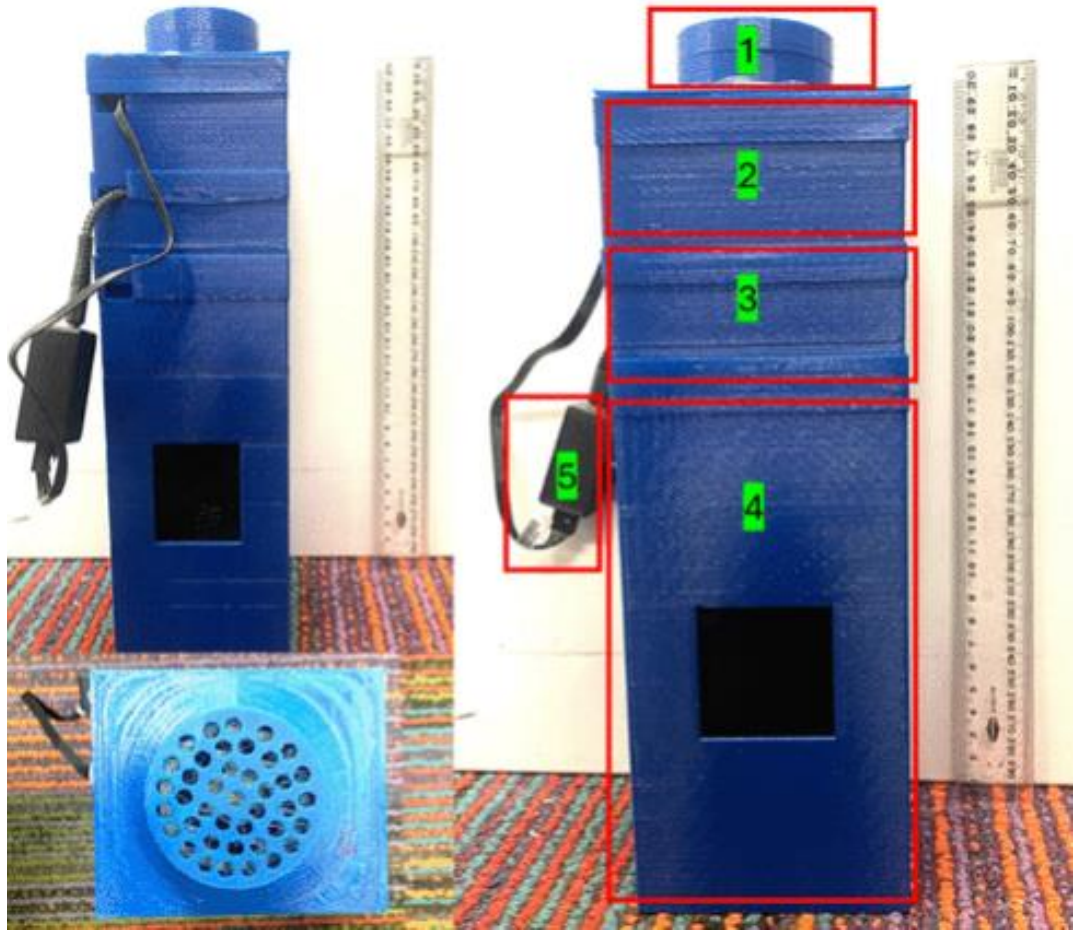
B: Correspondence Between Microorganisms Carrying ARGs and ARGs in Non-Flooded Soil Samples

Custom Air Sampler for WWTP Bioaerosols Monitoring Airborne Pathogens and ARGs

- Design and validate a low-cost active air sampler for WWTP bioaerosols, quantifying LOD, recovery, and repeatability for airborne pathogens and ARGs, and benchmarking signals against co-sampled wastewater resistomes and mobility markers (MGEs/plasmids/integrons).
- Deploy the sampler across WWTP operational zones to resolve spatial gradients and temporal dynamics, linking airborne ARG abundance, diversity, host range, and mobilization potential to influent/effluent profiles and inferring transmission pathways.

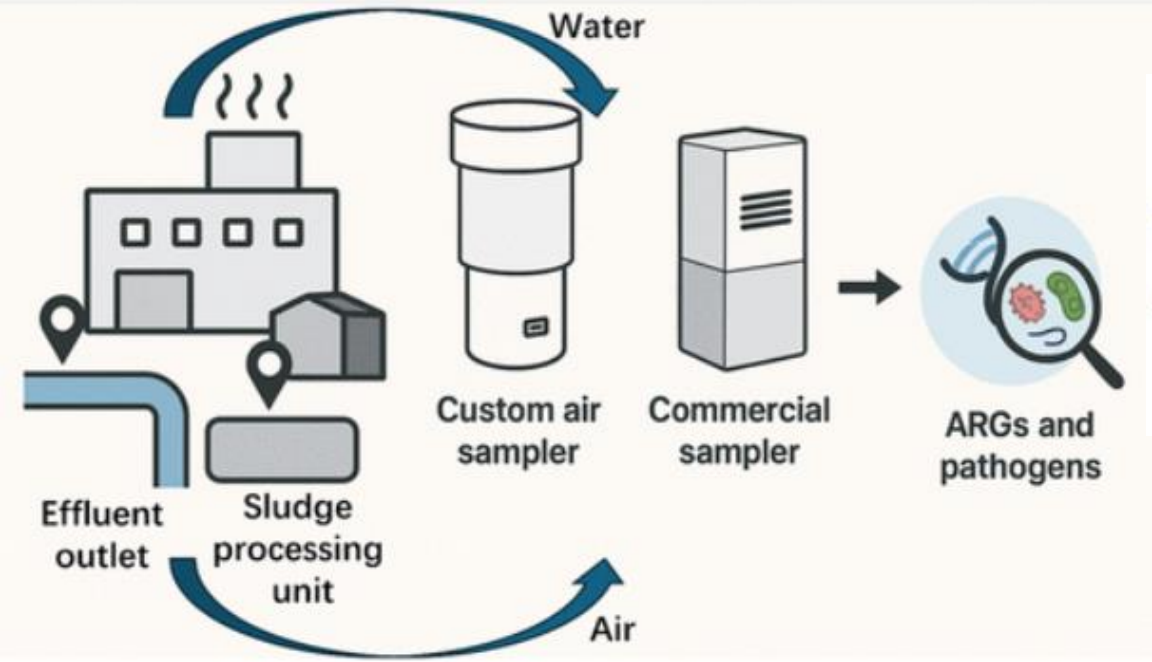
Development of a Self-Designed Air Sampler

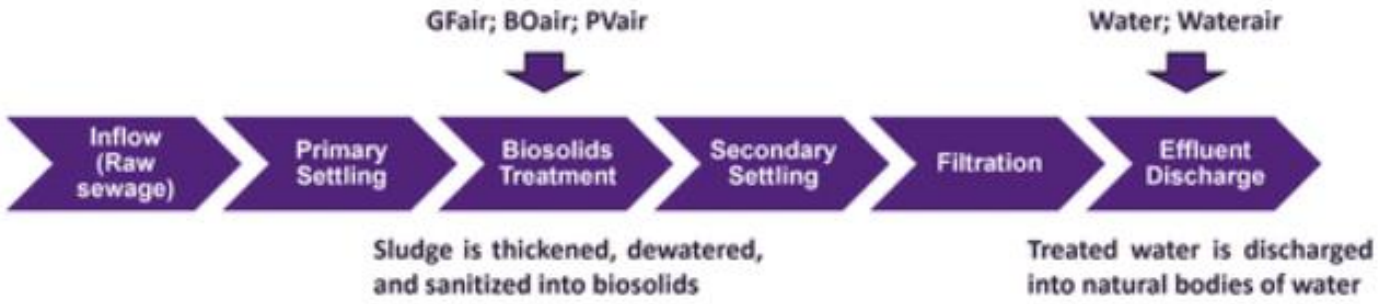


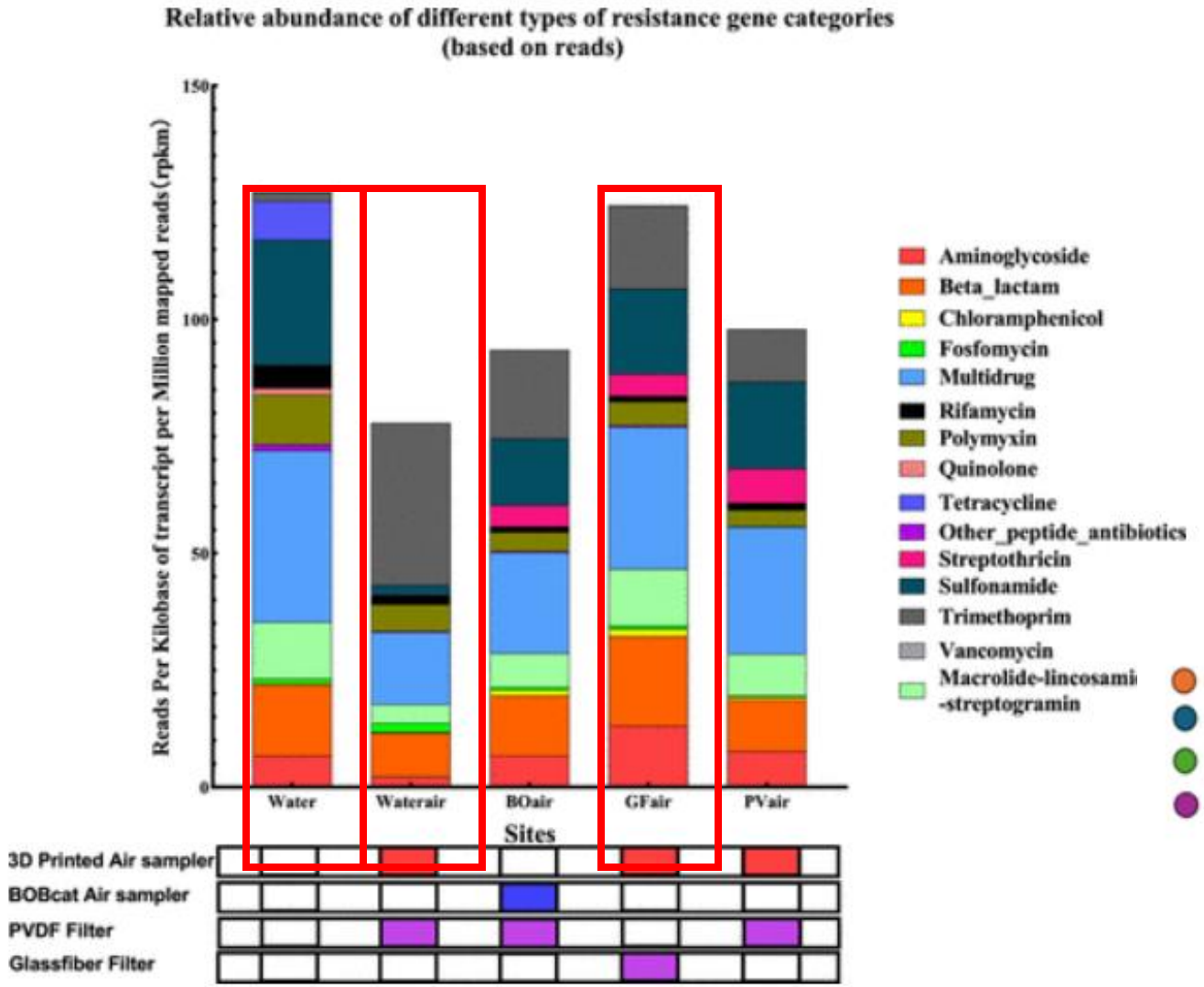


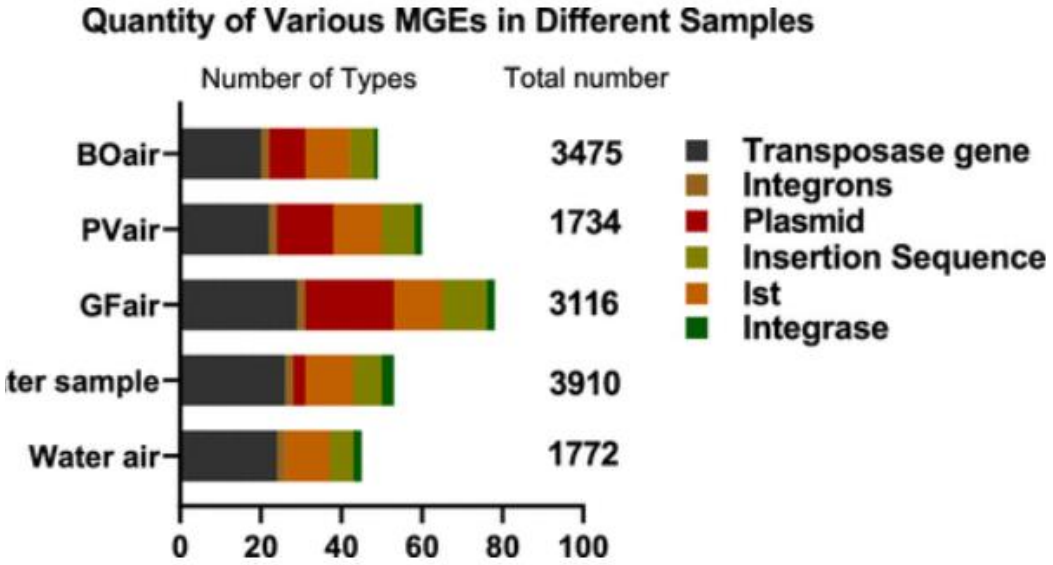
Approximately 0.46 m/s and a volumetric flow of 0.8 L/s.

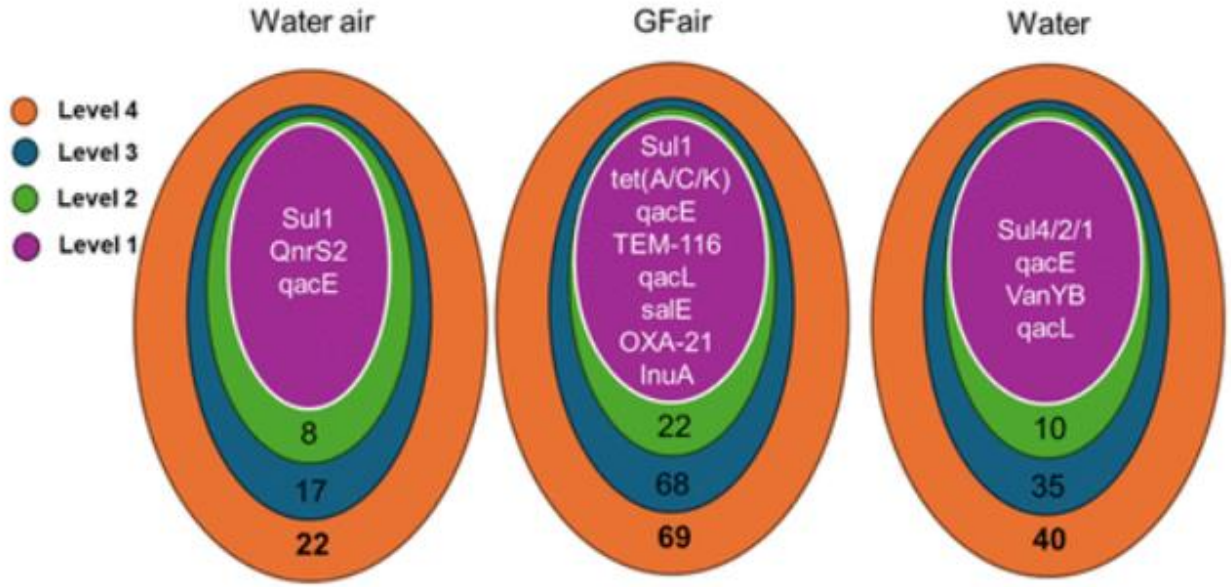
Applications in Wastewater Treatment Plants



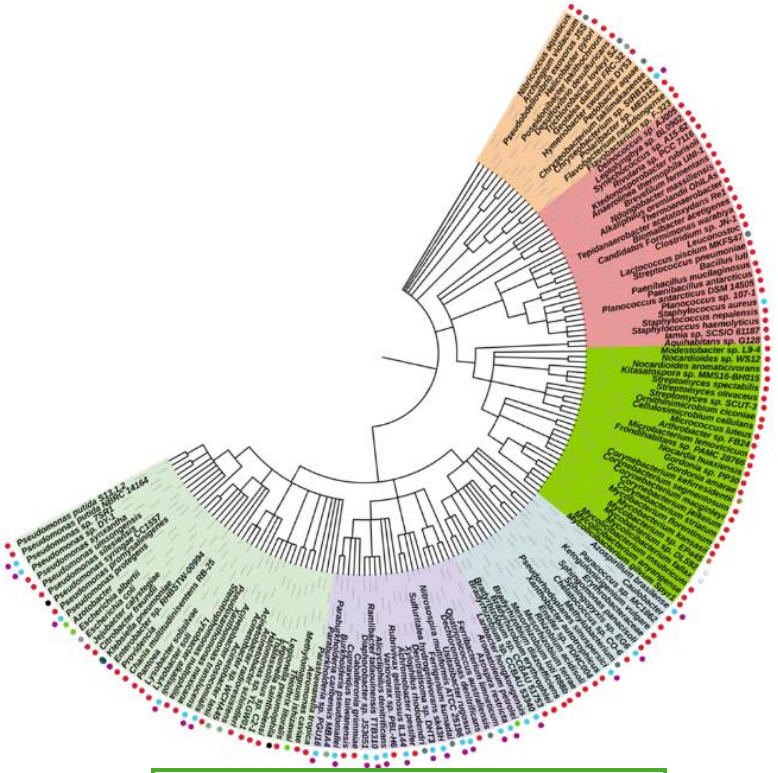



Relative abundance of different types of resistance gene categories
(based on reads)
Reads Per Kilobase of transcript per Million mapped reads (rpkm)
150
100
50
0
Water
Waterair
BOair
GFair
PVair
Sites
3D Printed Air sampler
BOBcat Air sampler
PVDF Filter
Glassfiber Filter
Aminoglycoside
Beta_lactam
Chloramphenicol
Fosfomycin
Multidrug
Rifamycin
Polymyxin
Quinolone
Tetracycline
Other_peptide_antibiotics
Streptothricin
Sulfonamide
Trimethoprim
Vancomycin
Macrolide-lincosami
-streptogramin

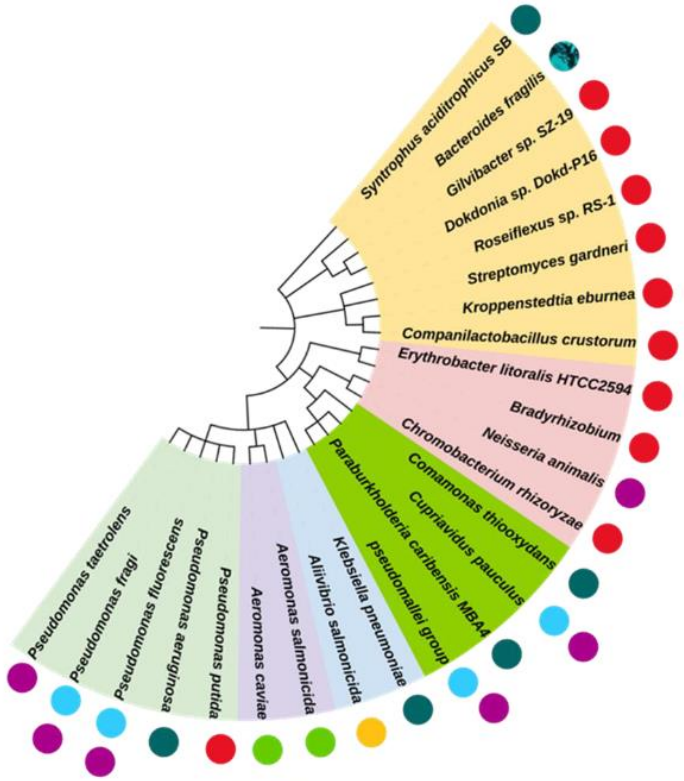

Quantity of Various MGEs in Different Samples
Number of Types
Total number
BOair
PVair
GFair
ter sample
Water air
3475
1734
3116
3910
1772
0
20
40
60
80
100
Transposase gene
Integrons
Plasmid
Insertion Sequence
Ist
Integrase


Water air
GFair
Water
Level 4
Level 3
Level 2
Level 1
Sul1
QnrS2
qacE
8
17
22
Sul1
tet(A/C/K)
qacE
TEM-116
qacL
salE
OXA-21
InuA
22
68
69
Sul4/2/1
qacE
VanYB
qacL
10
35
40

Eff water



Air around biosolids



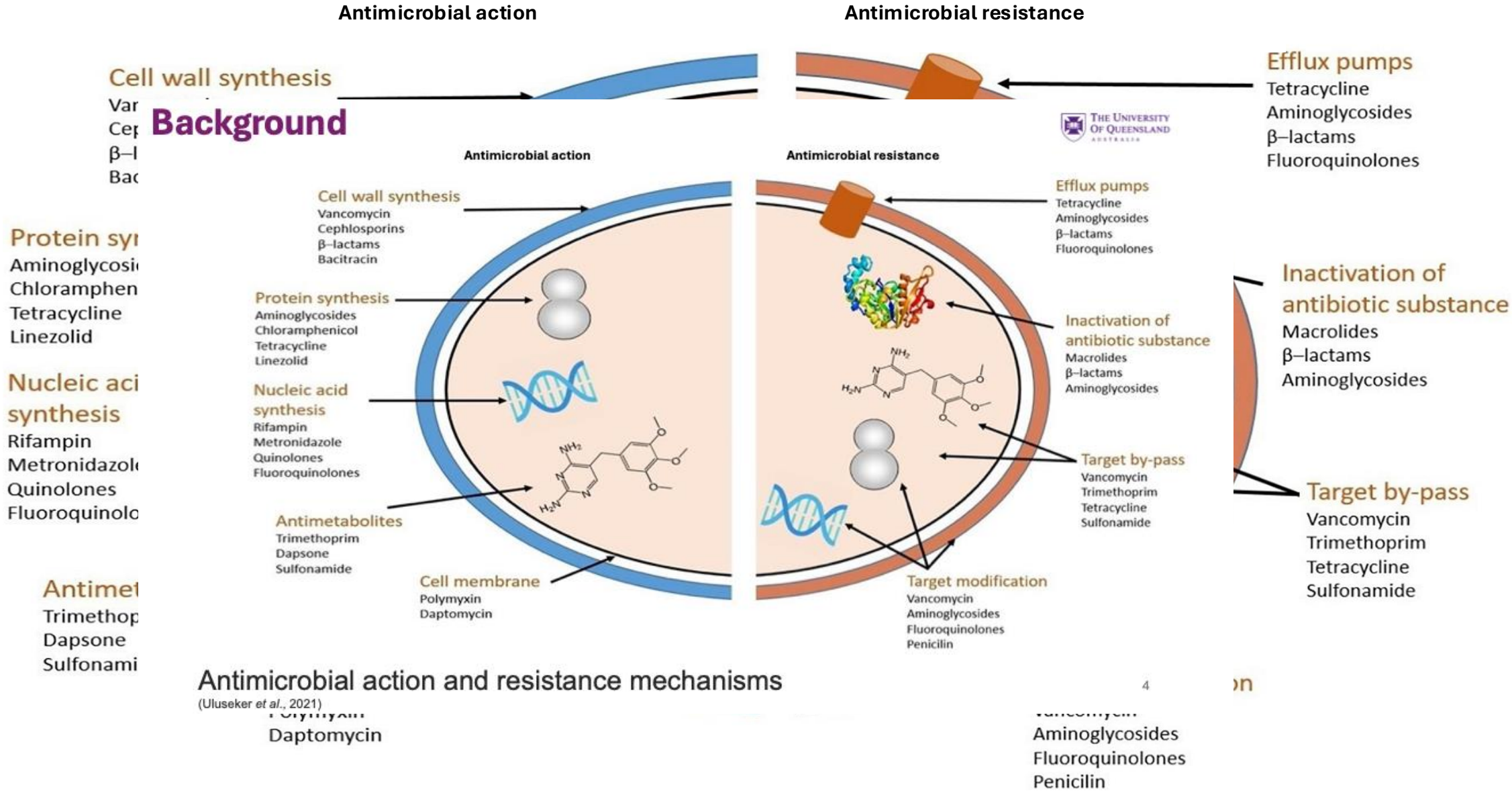
Air above water

Thanks for your attention !

ACKNOWLEDGEMENT

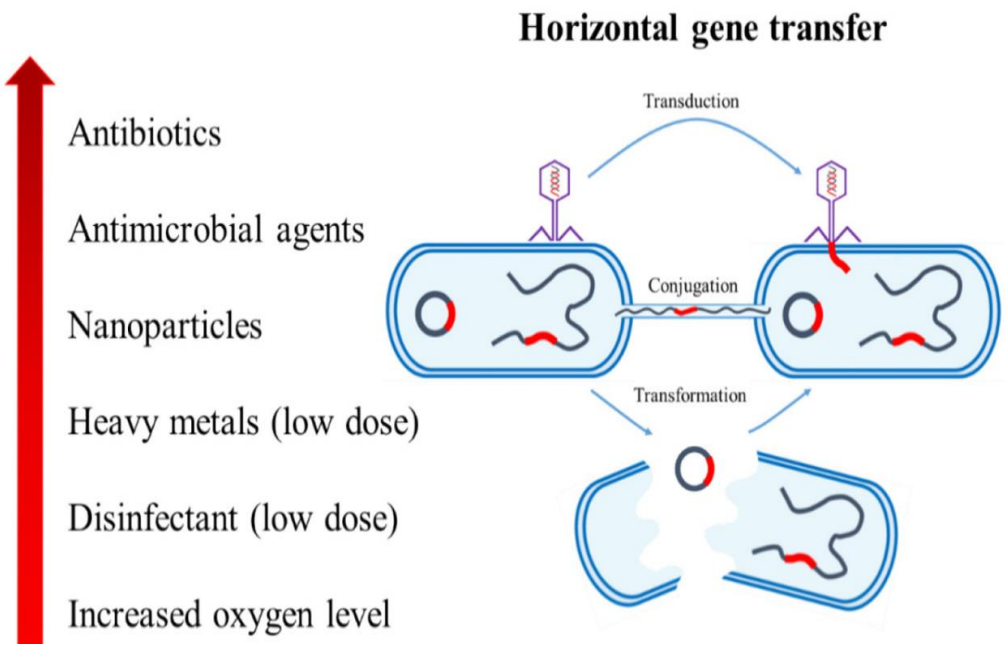
All staff and students from QAEHS
SCIEX: for the use of the 7500
Samples: WWTP operators
Queensland Health
UQ Ph.D. scholarship
QAEHS Top-up scholarship
Health & Environment 2025

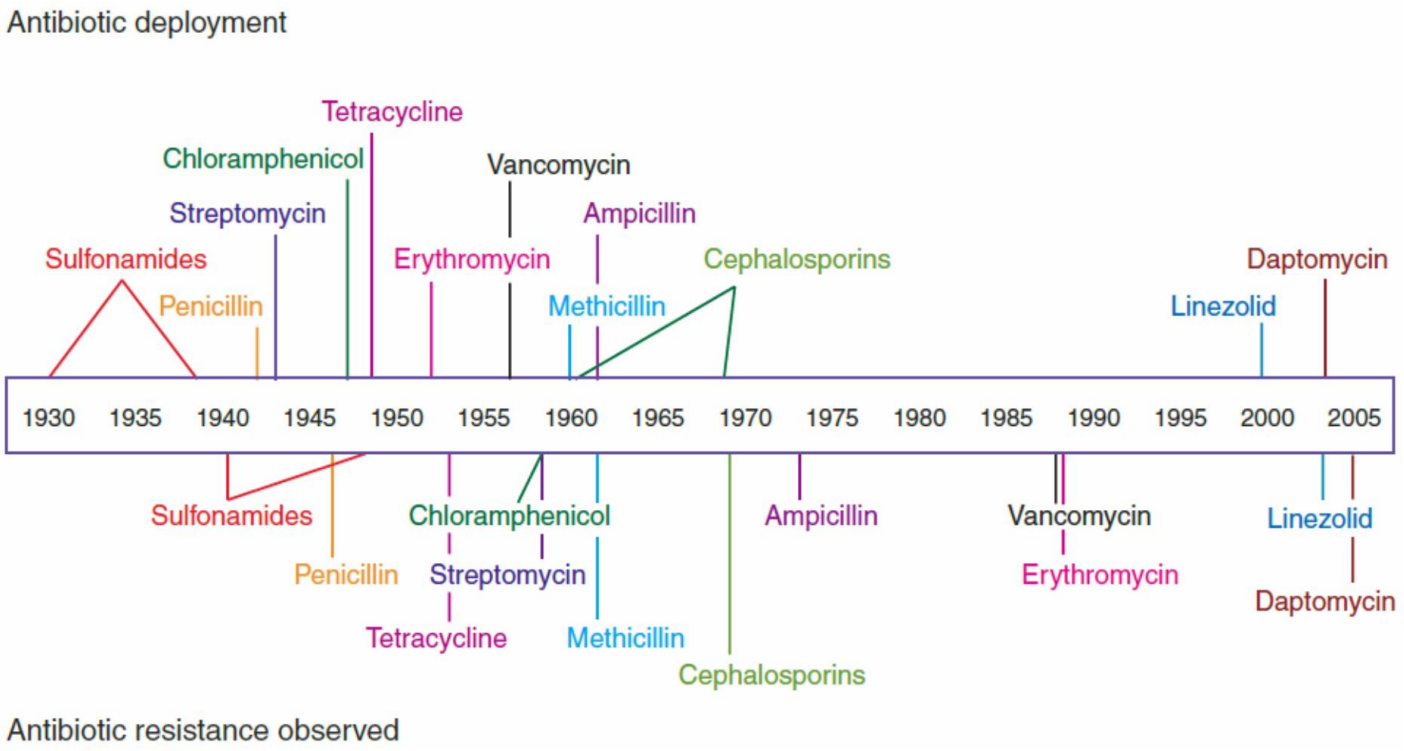
Background



Antimicrobial action and resistance mechanisms
(Uluseker *et al*., 2021)

Background

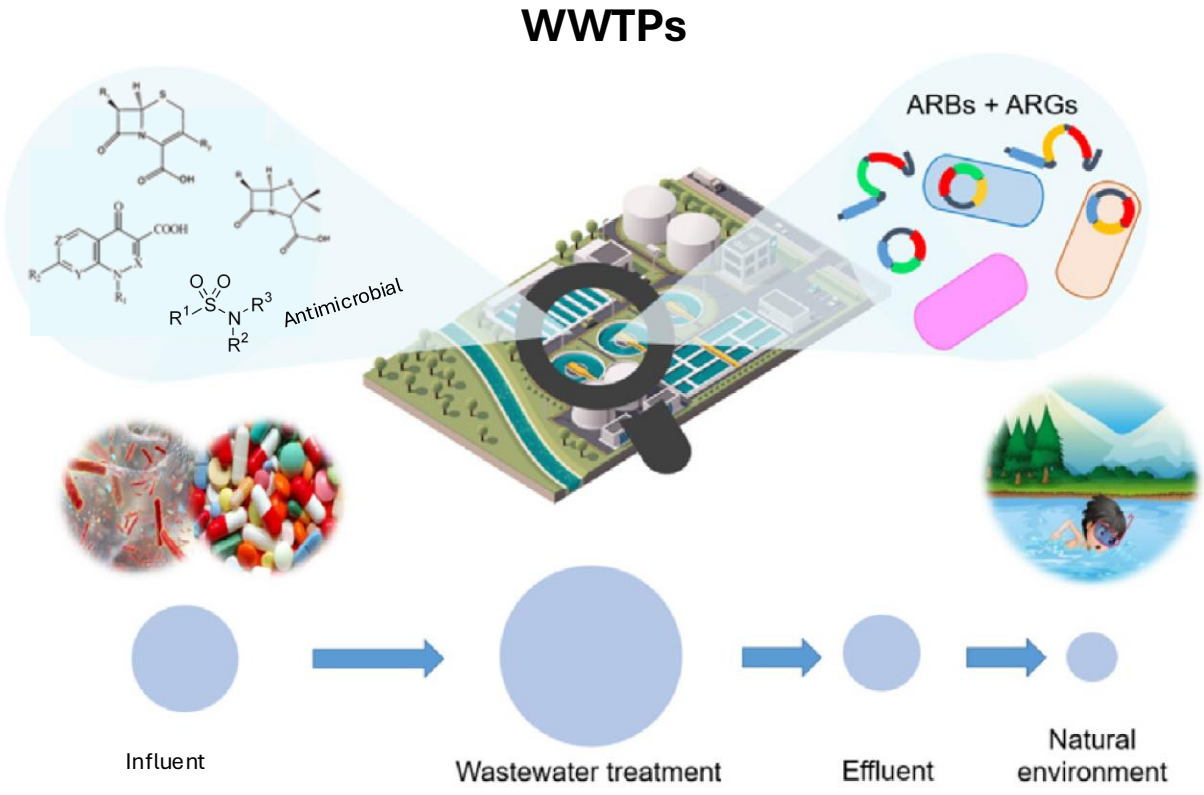




Selective pressure accelerates the evolution and spread of **Antimicrobial Resistance (AMR)**
(Nguyen *et al*., 2021)

AMR evolved rapidly (Clatworthy *et al*., 2007)

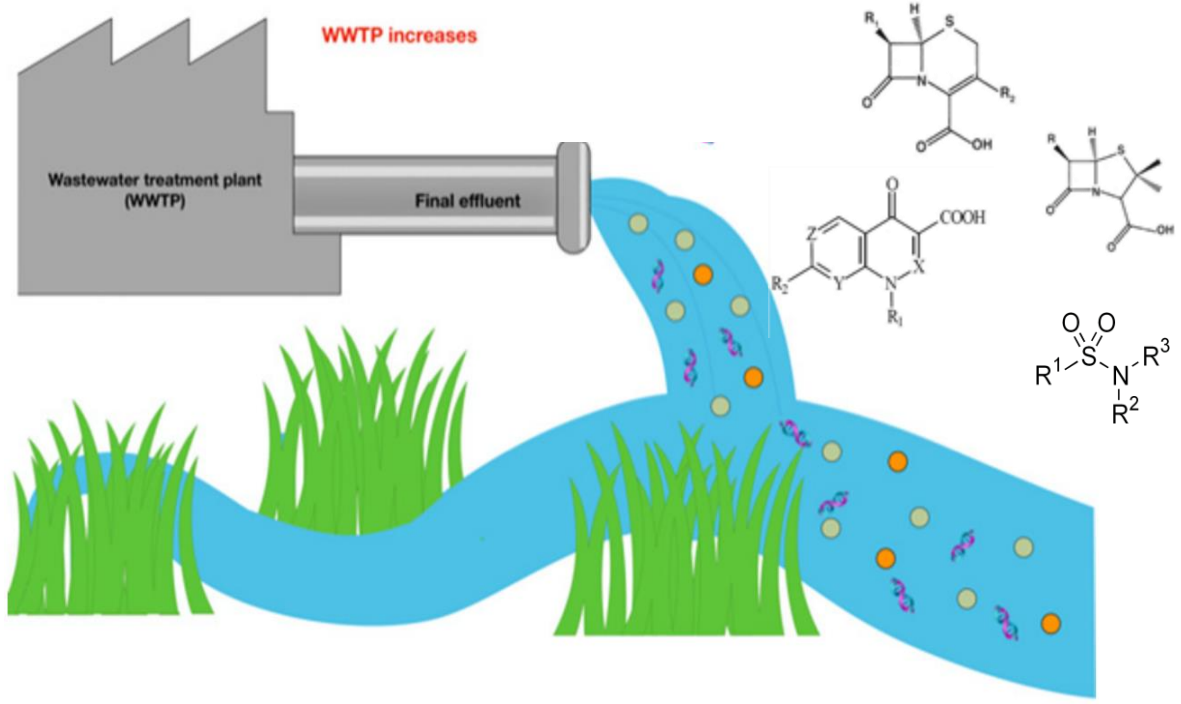
Wastewater-Based Epidemiology (WBE)



Adapted from Nguyen *et al.*, 2021

Wastewater-Based Epidemiology (WBE)

(O'Brien *et al.*, 2020)



AMR discharged to surface water

(Adapted from Makowska *et al.*, 2021)

Background: Antimicrobial Resistance and One Health

- 全球AMR负担（WHO预测2030或2050年死亡率）
- One Health框架（人-动物-环境）
- 水与空气在传播中的关键作用
- **视觉建议：**用一个三圆图（One Health）或WHO统计图。
  讲解时间：约1分钟。

Background: Antimicrobial Resistance and One Health

- 背景
- 全国初筛
- 然后一张图显示我们的不同支线
- 然后延伸到空气
- 然后介绍采样器
- 然后我们的workflow
- 然后我们的研究亮点

Background: Antimicrobial Resistance and One Health

- **Custom Air Sampler for WWTP Bioaerosols**
  Monitoring Airborne Pathogens and ARGs