From Pipes to Patterns: Tracking Antimicrobial Resistance in Hawaiian Wastewater

#IDLabCon

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DISCLOSURE

No conflict of interest to declare

Content Page

1. Introduction
2. Pilot Study: Experiment design and workflow
3. Results & Discussion
4. Summary & Conclusion

Introduction

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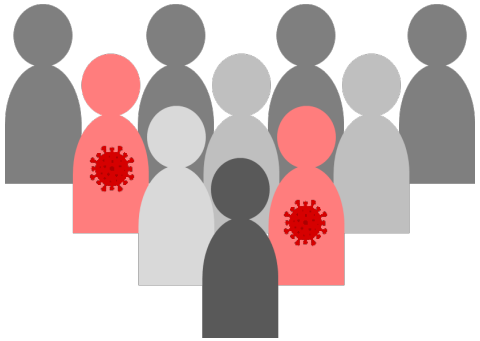
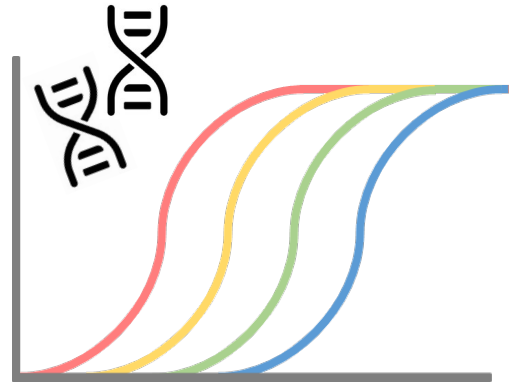
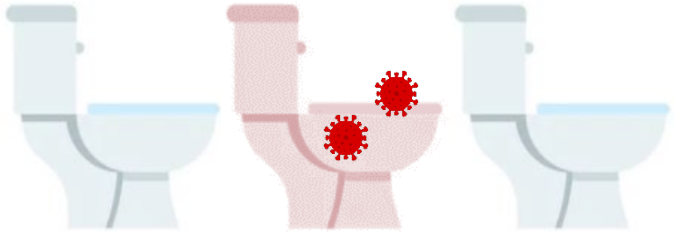
- What is wastewater surveillance?
- Wastewater Surveillance Program in Hawaii
- Why tracking Antimicrobial Resistance?

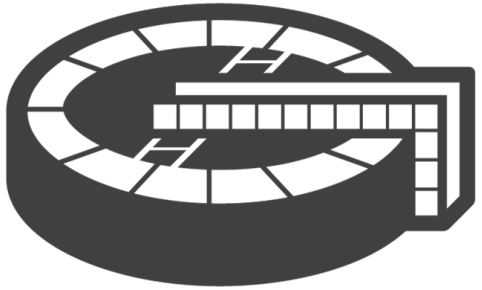
What is Wastewater Surveillance?

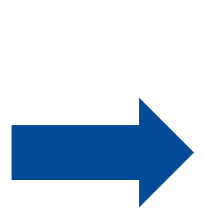


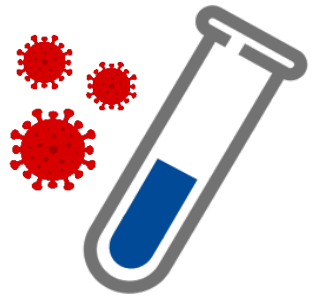




Poop Don't Lie







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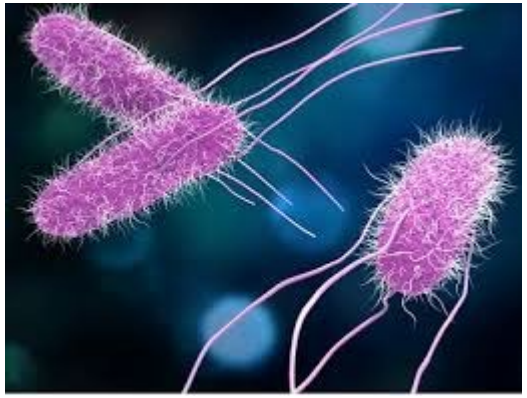
History of Wastewater Surveillance

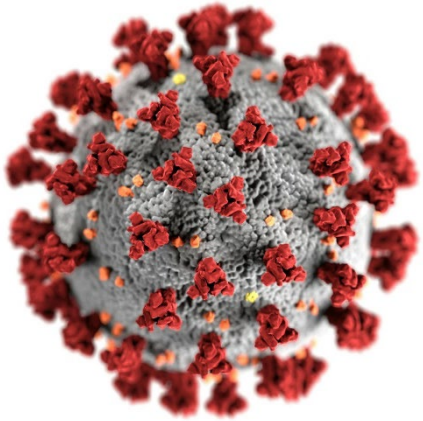
1950s



Salmonella

Enteric bacterial pathogen outbreaks

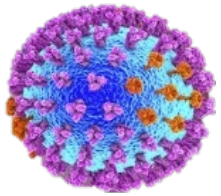
2020



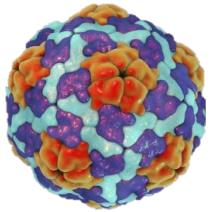
SARS-CoV-2

Pandemic respiratory virus

Today



SARS-CoV-2, Influenza, RSV

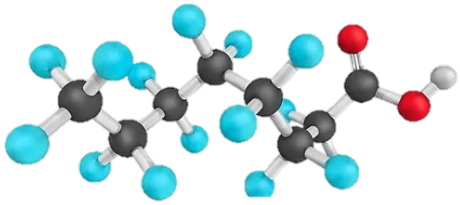


Hepatitis A

Candida auris

Norovirus, Rotavirus

Fentanyl, Heroin, Cocaine, methamphetamine



PFAS

Bacteria, Virus, Yeast, Illicit Drugs, Chemicals….

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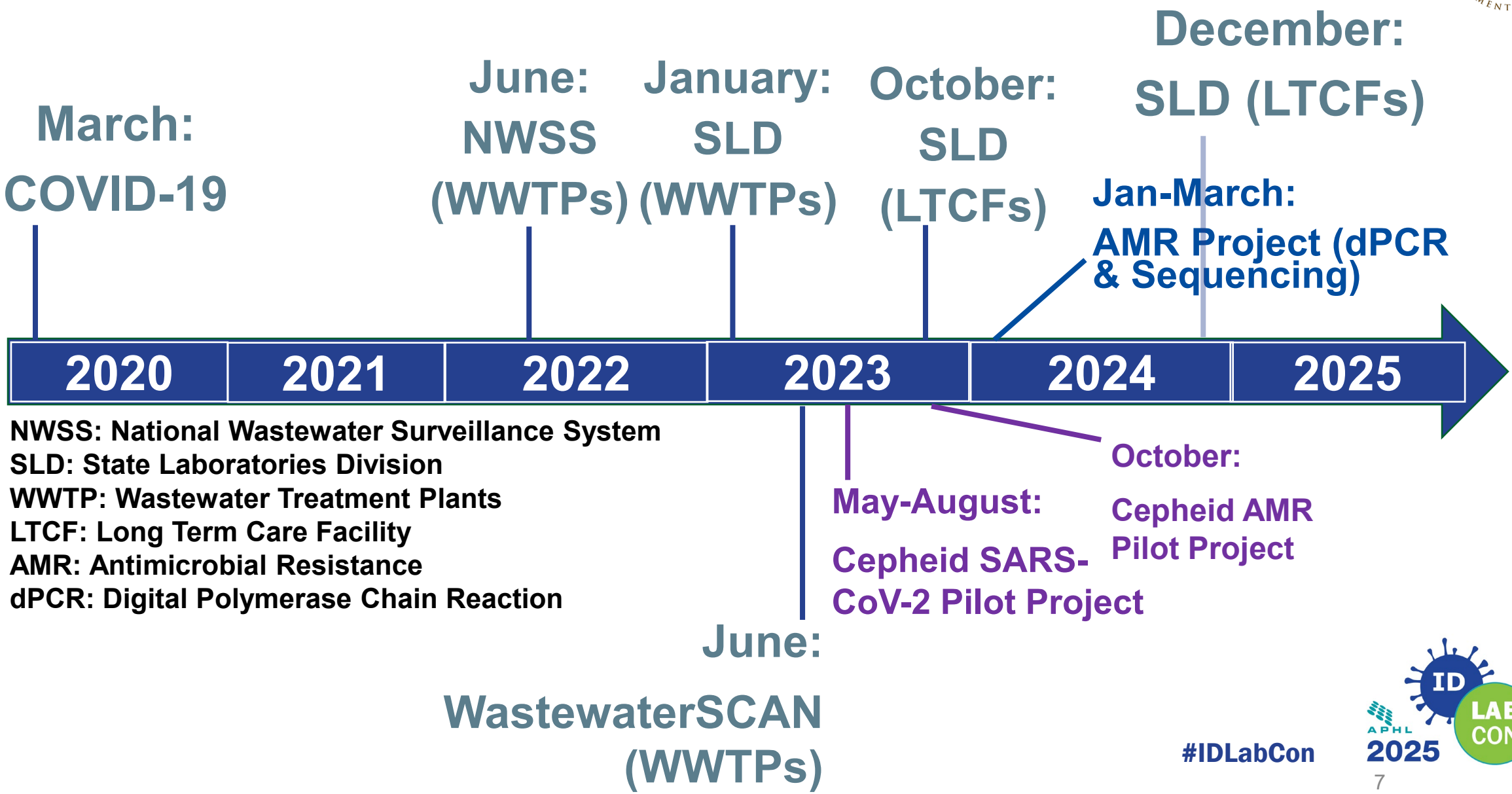
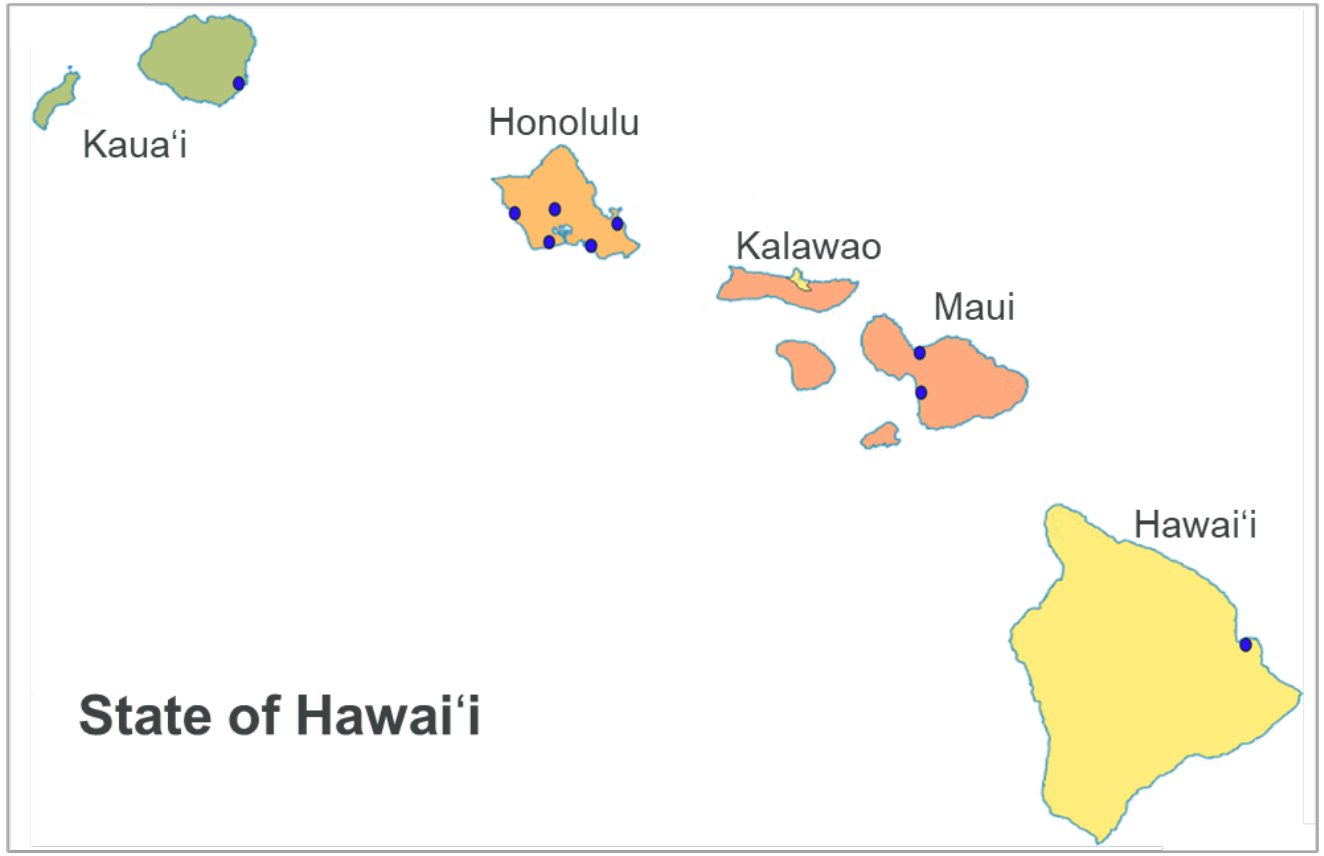
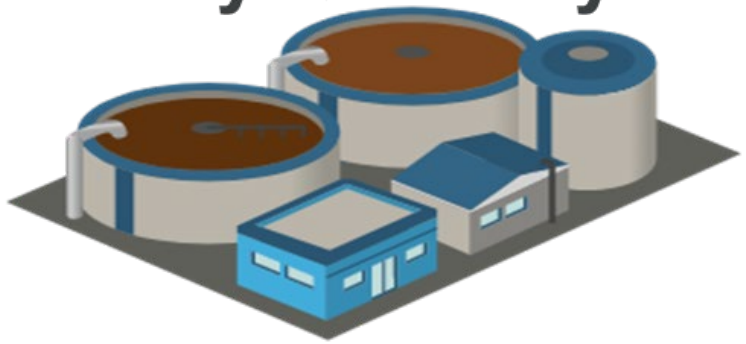
Wastewater Surveillance Program in Hawaii



Wastewater Surveillance Program in Hawaii



City & County



9 Wastewater Treatment Plants (serving 68.7% of the total population in the State of Hawaii)

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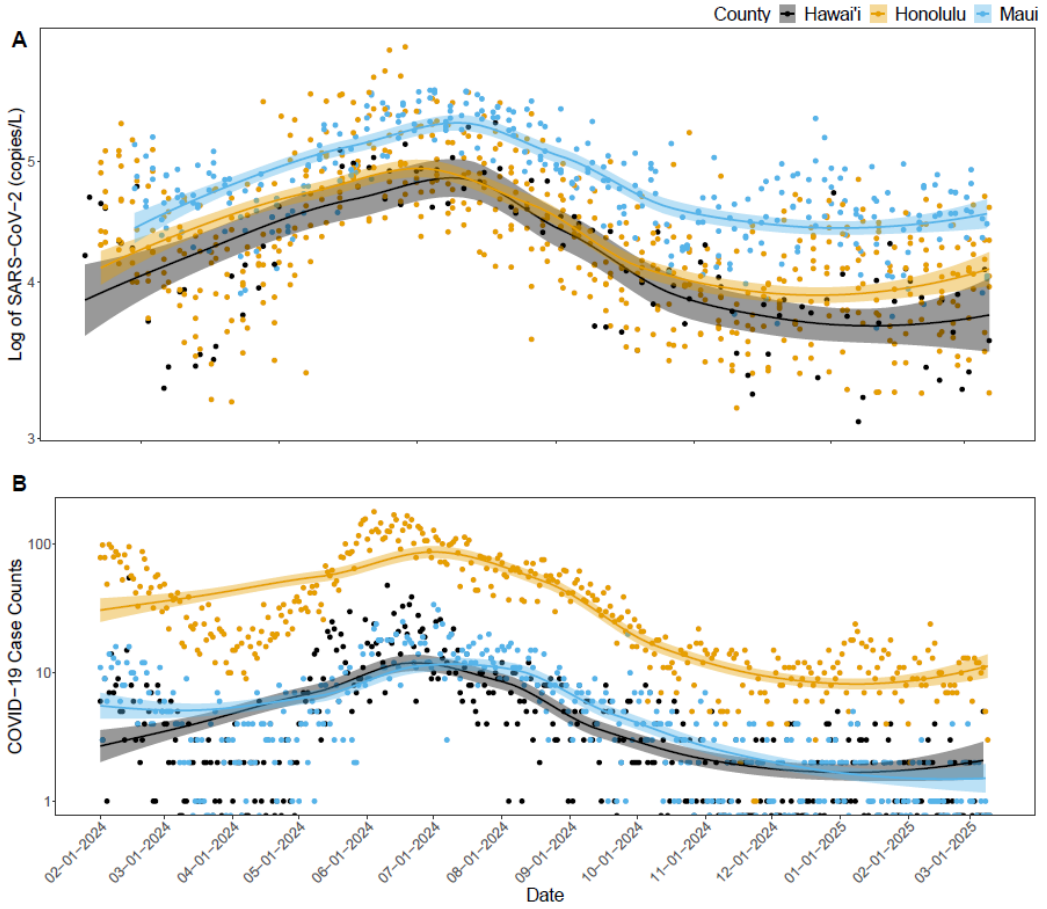
Wastewater Surveillance Data Report

Published on DOH Website Biweekly

Link: https://health.hawaii.gov/coronavirusdisease2019/what-you-should-know/covid-19-data-reports/



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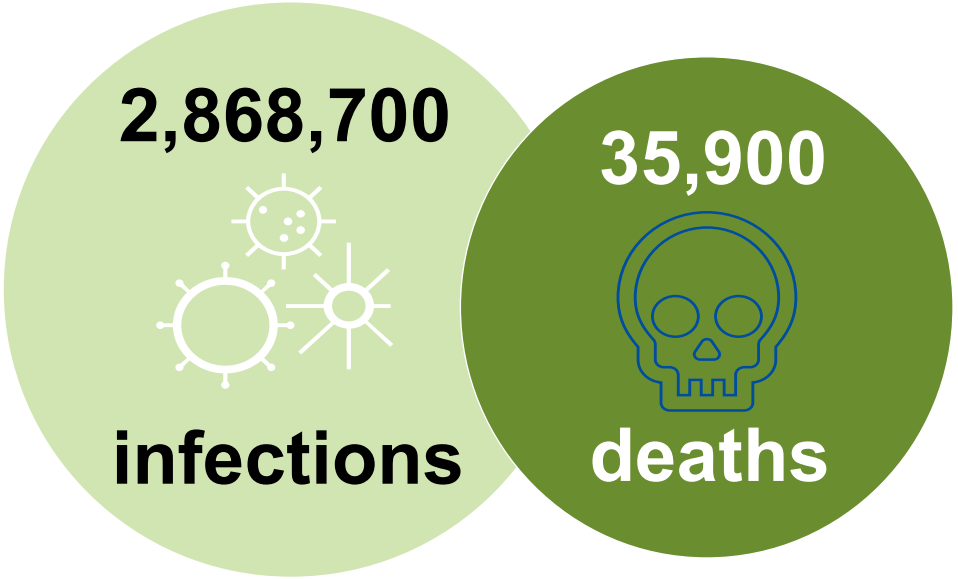
Why tracking Antimicrobial Resistance?

Antibiotic resistance threats in the US



~*50 000* deaths attributed to **Carbapenem-resistant** *Acinetobacter* and *Enterobacterales* globally

Antibiotic resistance threats list

Urgent threats

- **Carbapenem-resistant *Acinetobacter***
- *Clostridioides difficile*
- **Carbapenem-resistant *Enterobacterales***
- Drug-resistant *Neisseria gonorrhoeae*

Serious threats

- Drug-resistant *Campylobacter*
- Drug-resistant *Candida*
- ESBL-producing *Enterobacterales*
- Vancomycin-resistant *Enterococci* (VRE)
- Multidrug-resistant *Pseudomonas aeruginosa*
- Drug-resistant non-typhoidal *Salmonella*
- Drug-resistant *Salmonella serotype Typhi*
- Drug-resistant *Shigella*
- Methicillin-resistant *Staphylococcus aureus* (MRSA)
- Drug-resistant *Streptococcus pneumoniae*

Concerning threats

- Erythromycin-resistant group A *Streptococcus*
- Clindamycin-resistant group B *Streptococcus*

Watch-list

- Drug-resistant *Mycoplasma genitalium*
- Drug-resistant *Bordetella pertussis*

Source: CDC webpage www.cdc.gov/drugresistance/biggest-threats.html

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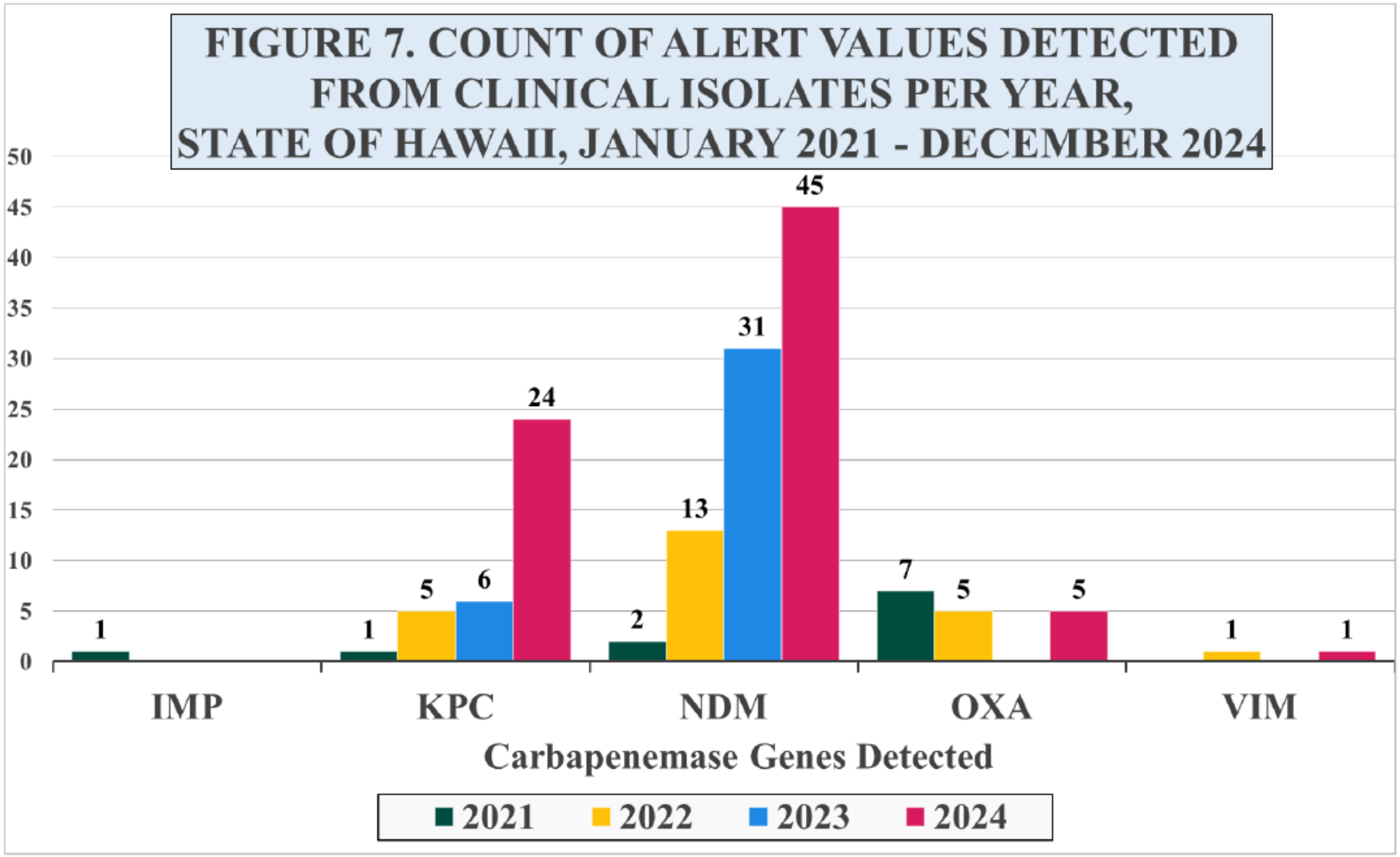
Why tracking Antimicrobial Resistance?



Source: Disease Outbreak Control Division (DOCD), HDOH

In Hawaii, clinical cases increased in recent years, especially KPC and NDM.

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Pilot Study: Experiment Design and Workflow

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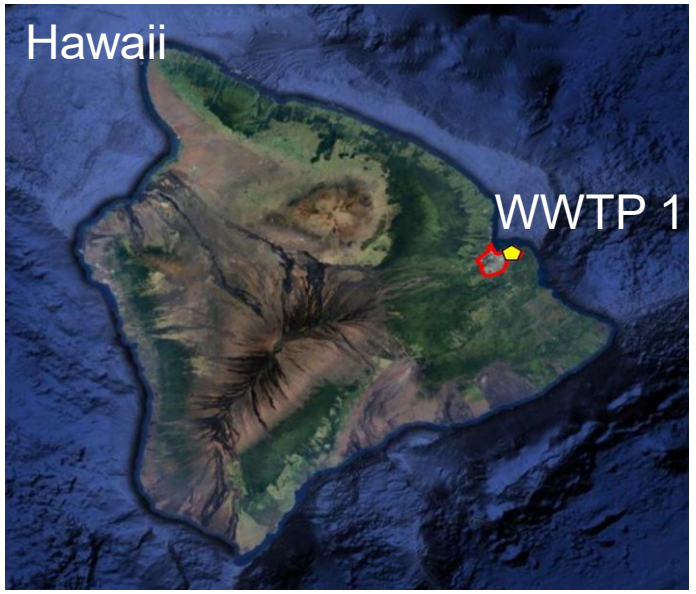
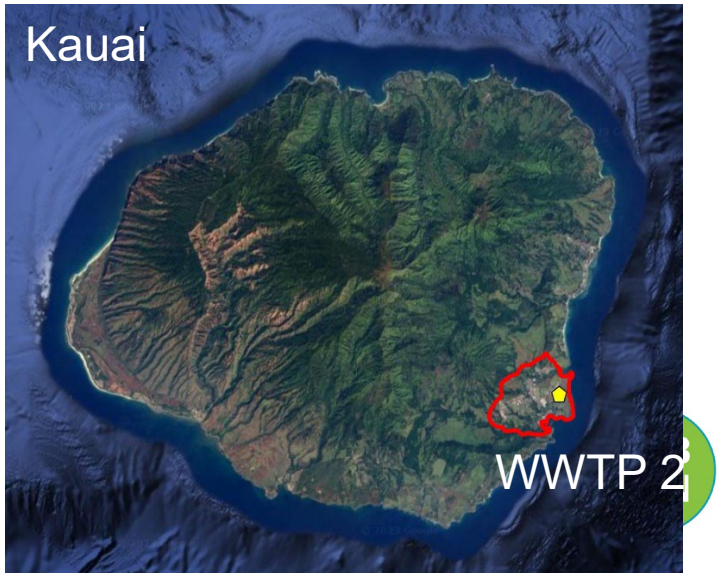
Study Sites and Sampling Time

- Pilot Location: **Hawaii Island and Kauai**
  - WWTP 1: population served ~32,000
  - WWTP 2: population served ~10,000

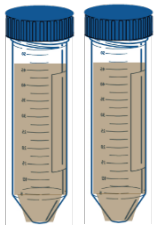
- Study Period:
  - Jan 1st to March 31st, 2024 (13 weeks)
  - 1 sample/week (duplicate)

- Objective:
  - To understand the spatial-temporal distribution and genetic diversity of AMR genes in the two Hawaiian communities.





Workflow and Methods



35 mL (×2)
Wastewater
Samples

Ceres Nanotrap®
Microbiome B Particles
+
Enhancement Reagent 3

QIAGEN AllPrep®
PowerViral®
DNA/RNA Kit

QIAcuity® Digital PCR 26k 24-well
&
dPCR Microbial DNA Detection Assays
(NDM, IMP, VIM, KPC, and OXA-48)

QIAseq® FX DNA Library Kit
QIAseq® xHYB AMR Panel (2,786 ARGs)
&
Illumina MiSeq™ System
MiSeq™ Reagent Kit v2
Illumina NextSeq 2000 P1 (300 cycles)

QIAGEN CLC Genomics Workbench

Results & Discussion

- Temporal and Spatial Trends
- AMR Metagenomic Diversity
- Bacterial Diversity

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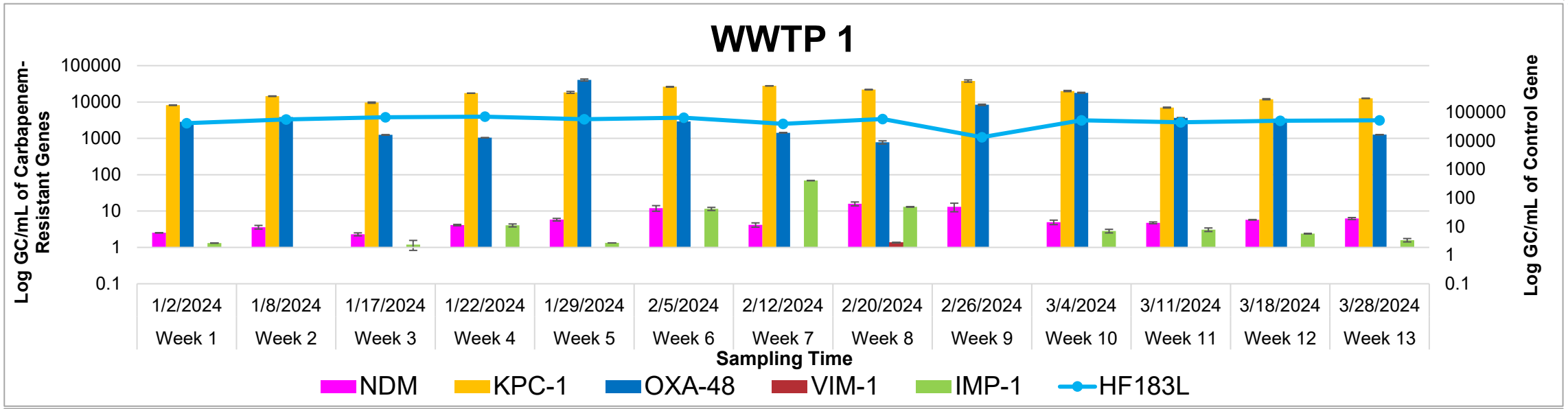
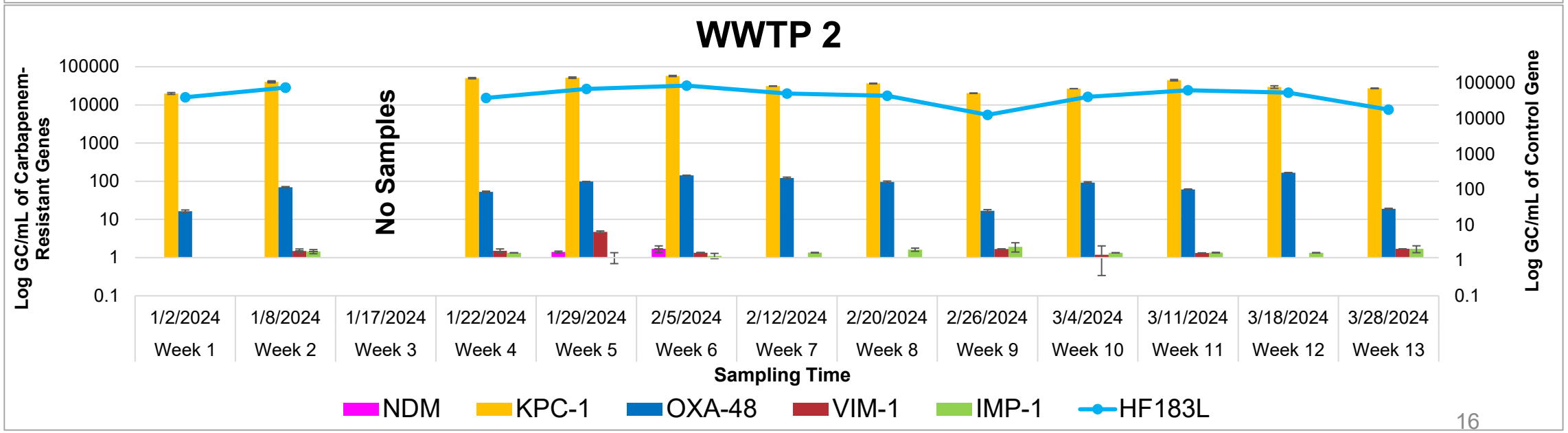
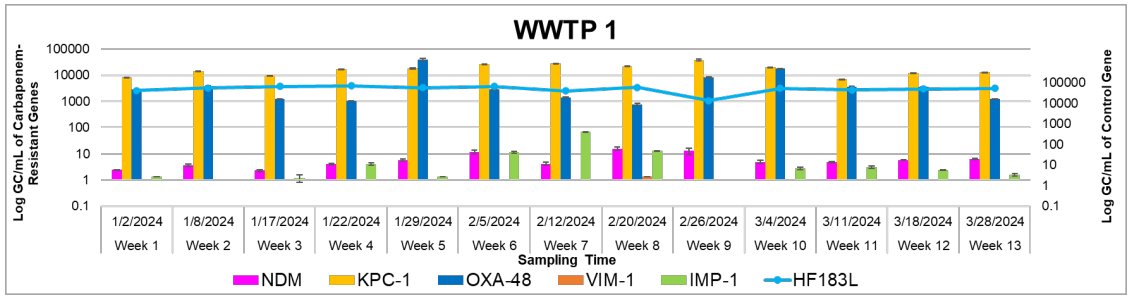
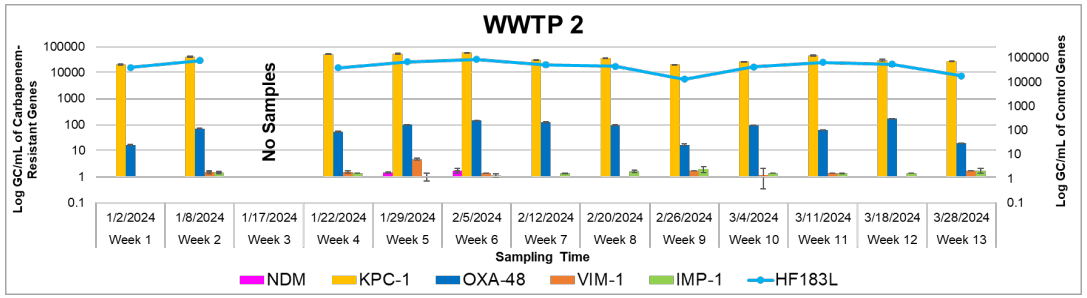
Results: Temporal and Spatial Trends





Results: Temporal and Spatial Trends





Over a 13-Week period, dPCR showed that

- **Both WWTPs** showed **high concentration** of **KPC** and **OXA-48** genes.
  - WWTP 1: KPC ($7.0 \times 10^3 – 3.8 \times 10^4$ GC/mL)
  - WWTP 1: OXA-48 ($7.8 \times 10^2 – 4.0 \times 10^4$ GC/mL)
  - WWTP 2: KPC ($1.9 \times 10^4 – 5.7 \times 10^4$ GC/mL)
  - WWTP 2: OXA-48 ($1.6 \times 10^1 – 1.4 \times 10^2$ GC/mL)
- **IMP** genes were **frequently being detected** in **both WWTPs** but at a **low concentration** (1.2 – 68.9 GC/mL and 1.0 – 1.9 GC/mL).
- **NDM** genes were **consistently being detected** in **WWTP 1** (2.3 – 15.8 GC/mL) but were only detected two times in WWTP 2 (1.4 & 1.7 GC/mL).
- **VIM** genes were **detected more frequently** in **WWTP 2** (1.2 – 4.7 GC/mL) than in WWTP 1 (1.4 GC/mL) at low concentration.

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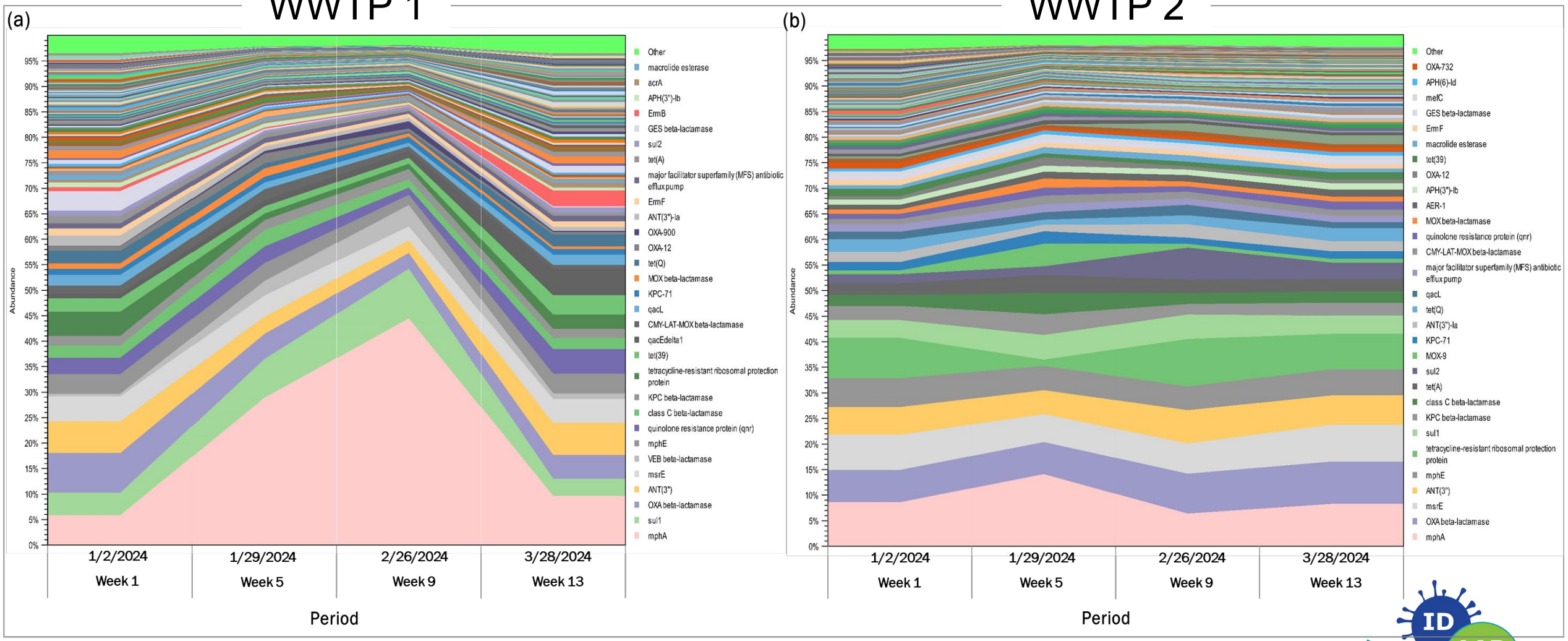
Results: AMR Metagenomic Diversity



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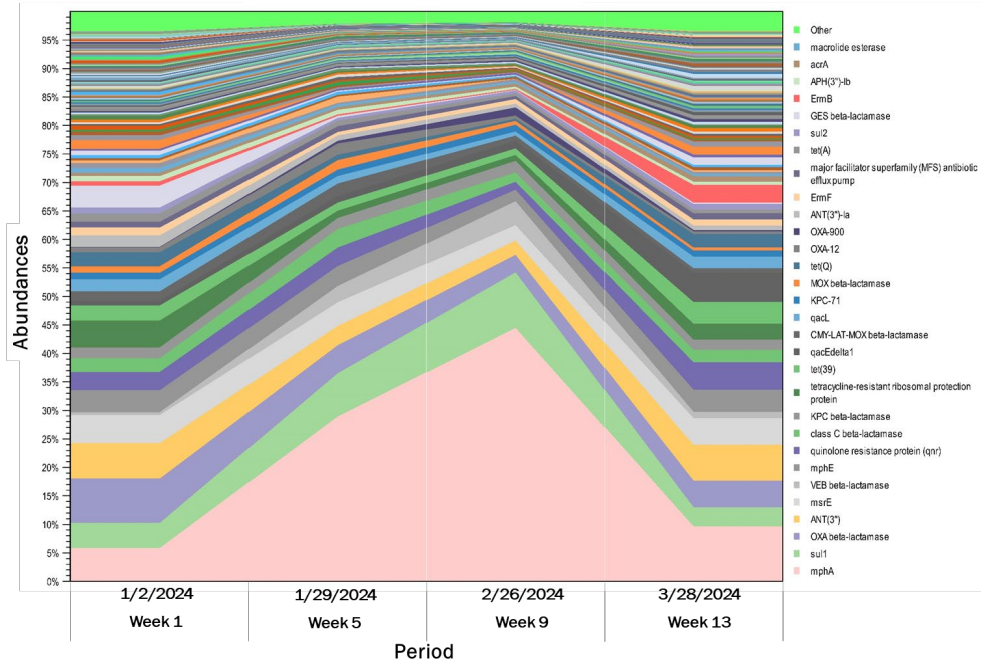
Results: AMR Metagenomic Diversity

WWTP 1



KPC ✓

OXA-48 ✓

IMP ✓

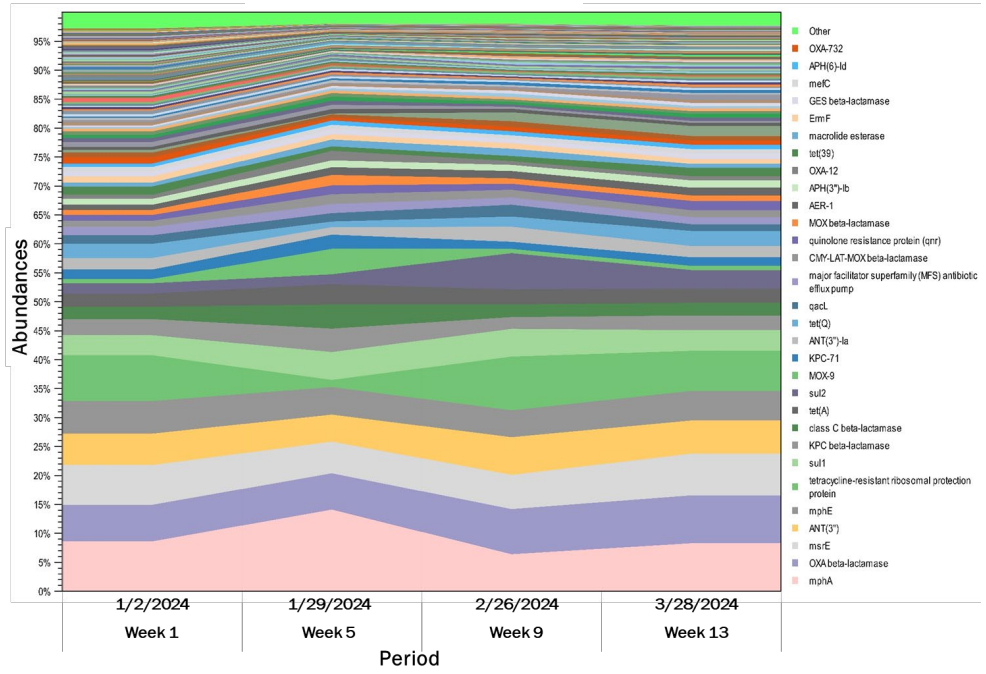
NDM ✓

VIM ×

WWTP 1							
Carbapenem Resistance Genes of Interest [Abundance (%) Rank]							
Week 1		Week 5		Week 9		Week 13	
OXA beta-lactamase	**7.78**	**OXA beta-lactamase**	**4.92**	**OXA beta-lactamase**	**3.07**	**OXA beta-lactamase**	**4.66**
KPC beta-lactamase	**1.91**	OXA-12	1.98	**KPC beta-lactamase**	**2.03**	**KPC beta-lactamase**	**1.78**
KPC-71	1.13	**KPC beta-lactamase**	**1.92**	OXA-900	1.44	KPC-71	1.05
OXA-12	0.88	KPC-71	1.13	KPC-71	1.19	OXA-12	0.48
OXA-732	0.66	OXA-780	0.62	OXA-12	0.57	OXA-732	0.41
OXA-46	0.55	OXA-900	0.47	OXA-732	0.20	OXA-780	0.25
OXA-296	0.32	OXA-732	0.30	OXA-780	0.17	OXA-900	0.25
OXA-780	0.32	OXA-46	0.14	OXA-46	0.10	OXA-347	0.20
OXA-347	0.24	OXA-164	0.11	OXA-347	0.09	OXA-46	0.13
OXA-164	0.14	OXA-347	0.11	OXA-164	0.06	OXA-209	0.06
OXA-900	0.08	OXA-372	0.04	OXA-372	0.03	OXA-372	0.03
OXA-209	0.07	OXA-209	0.03	OXA-209	0.02	OXA-164	0.03
OXA-372	0.03	OXA-308	0.01	OXA-308	0.01	OXA-296	0.02
OXA-308	0.01	OXA-464	0.01	OXA-299	0.01	OXA-308	0.01
OXA-664	0.00810	OXA-9	0.01	OXA-664	3.44E-03	OXA-664	0.01
OXA-9	0.00697	OXA-830	0.01	OXA-9	1.98E-03	OXA-9	0.01
OXA-37	0.00389	OXA-664	0.0045	IMP-35	1.94E-03	OXA-60	0.0044
IMP-35	0.00292	OXA-299	0.0043	OXA-830	1.76E-03	OXA-37	0.0034
OXA-18	0.00292	IMP-35	0.0027	**IMP beta-lactamase**	**1.35E-03**	OXA-299	0.0029
OXA-830	0.00130	OXA-18	0.0025	OXA-37	8.59E-04	OXA-830	0.0027
OXA-299	0.00097	**IMP beta-lactamase**	**0.0021**	OXA-464	7.85E-04	OXA-18	0.0022
OXA-464	0.00097	OXA-37	0.0013	**NDM beta-lactamase**	**5.60E-04**	OXA-464	0.0021
IMP beta-lactamase	**0.00065**	OXA-60	0.0011	OXA-60	5.60E-04	IMP-35	0.0009
OXA-443	0.00065	**NDM beta-lactamase**	**0.0003**	OXA-18	5.23E-04	OXA-198	0.0009
OXA-902	0.00065	OXA-198	0.0003	OXA-568	5.23E-04	OXA-568	0.0009
NDM beta-lactamase	**0.00049**	OXA-902	0.0003	IMP-27	4.86E-04	OXA-85	0.0007
OXA-198	0.00032	OXA-62	0.0003	OXA-198	2.99E-04	OXA-902	0.0005
OXA-154	0.00016	OXA-85	0.0003	OXA-443	2.24E-04	**NDM beta-lactamase**	**0.0003**
OXA-243	0.00016	OXA-296	0.0003	OXA-902	1.87E-04	**IMP beta-lactamase**	**0.0003**
OXA-60	0.00016	OXA-443	0.0002	OXA-153	1.49E-04	OXA-55	0.0003
		OXA-568	0.0002	OXA-727	1.49E-04	OXA-727	0.0003
		IMP-27	0.0001	OXA-154	1.12E-04		
		OXA-243	0.0001	OXA-243	7.47E-05		
				OXA-45	7.47E-05		
				OXA-258	3.74E-05		
				OXA-85	3.74E-05		

Results: AMR Metagenomic Diversity

WWTP 2



KPC ✓

OXA-48 ✓

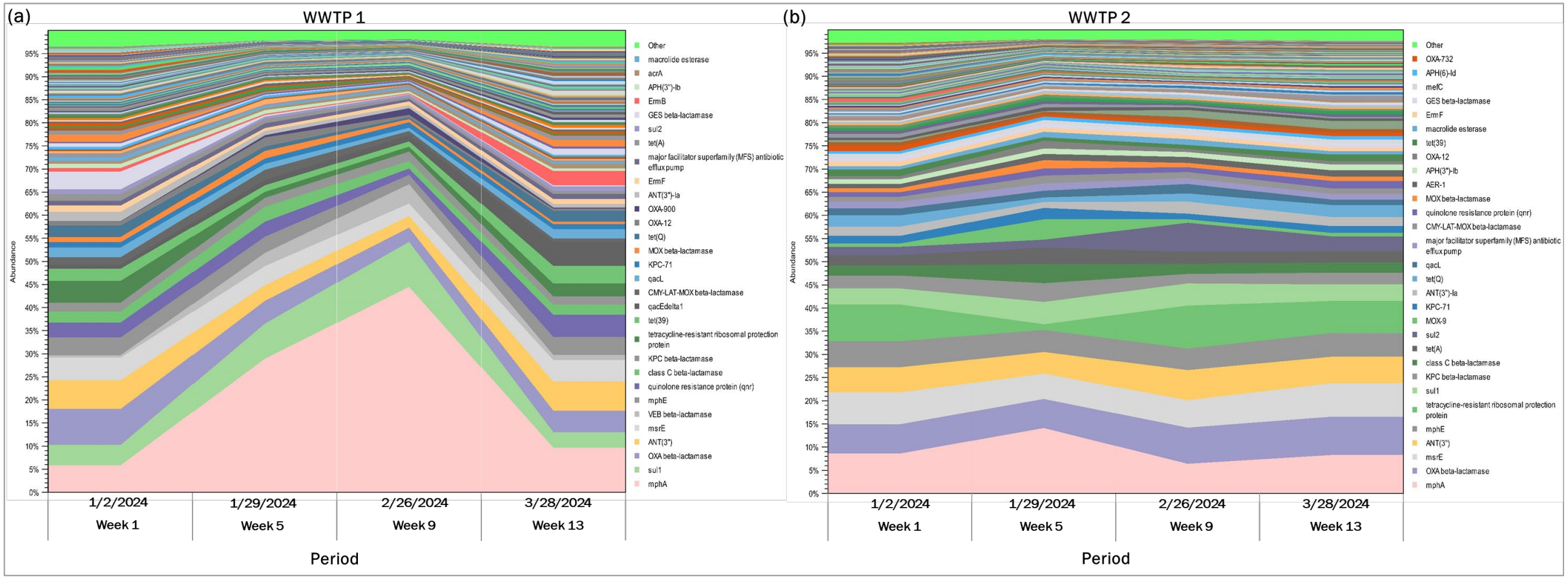
IMP ✓

NDM ×

VIM ✓

WWTP 2							
Carbapenem Resistance Genes of Interest [Abundance (%) Rank]							
Week 1		**Week 5**		**Week 9**		**Week 13**	
OXA beta-lactamase	**6.32**	**OXA beta-lactamase**	**6.27**	**OXA beta-lactamase**	**7.80**	**OXA beta-lactamase**	**8.27**
KPC beta-lactamase	**2.77**	**KPC beta-lactamase**	**4.05**	**KPC beta-lactamase**	**2.04**	**KPC beta-lactamase**	**2.54**
KPC-71	1.63	KPC-71	2.44	KPC-71	1.22	KPC-71	1.47
OXA-732	1.10	OXA-12	1.66	OXA-732	0.78	OXA-12	0.72
OXA-12	0.67	OXA-780	0.71	OXA-12	0.66	OXA-732	0.71
OXA-164	0.63	OXA-732	0.50	OXA-347	0.51	OXA-780	0.65
OXA-780	0.52	OXA-347	0.40	OXA-46	0.51	OXA-347	0.54
OXA-347	0.29	OXA-164	0.33	OXA-780	0.45	OXA-46	0.48
OXA-46	0.28	OXA-46	0.31	OXA-164	0.21	OXA-164	0.21
OXA-209	0.09	OXA-209	0.13	OXA-372	0.18	OXA-209	0.17
OXA-372	0.07	OXA-372	0.05	OXA-209	0.15	OXA-372	0.09
OXA-9	0.01	OXA-85	0.03	OXA-9	0.04	OXA-372	0.09
OXA-85	0.01	OXA-9	0.01	OXA-85	0.03	OXA-9	0.02
OXA-664	0.01	OXA-830	0.01	OXA-664	0.01	OXA-85	0.01
OXA-464	0.0043	OXA-902	0.01	OXA-464	0.0034	OXA-308	0.01
OXA-308	0.0037	OXA-464	0.0043	OXA-18	0.0027	OXA-664	0.01
OXA-60	0.0036	OXA-664	0.0024	OXA-902	0.0025	OXA-60	0.01
OXA-443	0.0033	OXA-37	0.0023	OXA-60	0.0022	OXA-443	0.0028
OXA-902	0.0030	OXA-153	0.0011	OXA-308	0.0018	OXA-18	0.0020
OXA-830	0.0027	IMP-35	0.0011	OXA-153	0.0016	OXA-830	0.0019
OXA-37	0.0017	OXA-60	0.0011	OXA-830	0.0015	**IMP beta-lactamase**	**0.0017**
IMP-35	0.0009	OXA-443	0.0010	OXA-299	0.0009	OXA-902	0.0017
IMP beta-lactamase	**0.0006**	**IMP beta-lactamase**	**0.0006**	OXA-62	0.0009	OXA-464	0.0016
OXA-153	0.0004	OXA-18	0.0006	IMP-35	0.0007	IMP-35	0.0015
OXA-299	0.0004	OXA-154	0.0005	**IMP beta-lactamase**	**0.0007**	OXA-299	0.0015
VIM-7	0.0003	OXA-308	0.0005	OXA-443	0.0007	OXA-62	0.0012
OXA-243	0.0003	OXA-62	0.0005	OXA-45	0.0006	OXA-37	0.0011
OXA-62	0.0003	OXA-299	0.0004	**VIM beta-lactamase**	**0.0003**	OXA-568	0.0009
OXA-18	0.0002	OXA-45	0.0003	OXA-900	0.0003	OXA-45	0.0009
VIM beta-lactamase	**0.0001**	IMP-27	0.0002	OXA-198	0.0003	**VIM beta-lactamase**	**0.0005**
OXA-727	0.0001	OXA-215	0.0002	OXA-37	0.0001	OXA-900	0.0003
		OXA-258	0.0002	OXA-243	0.0001	OXA-258	0.0003
		OXA-727	0.0002			OXA-153	0.0001
		VIM-7	0.0001			OXA-243	0.0001
		VIM beta-lactamase	**0.0001**			OXA-154	0.0001
		OXA-568	0.0001			OXA-198	0.0001
		OXA-243	0.0001				

Results: AMR Metagenomic Diversity



- Both WWTPs showed **very similar AMR genetic diversity** despite being located on **two different islands**.

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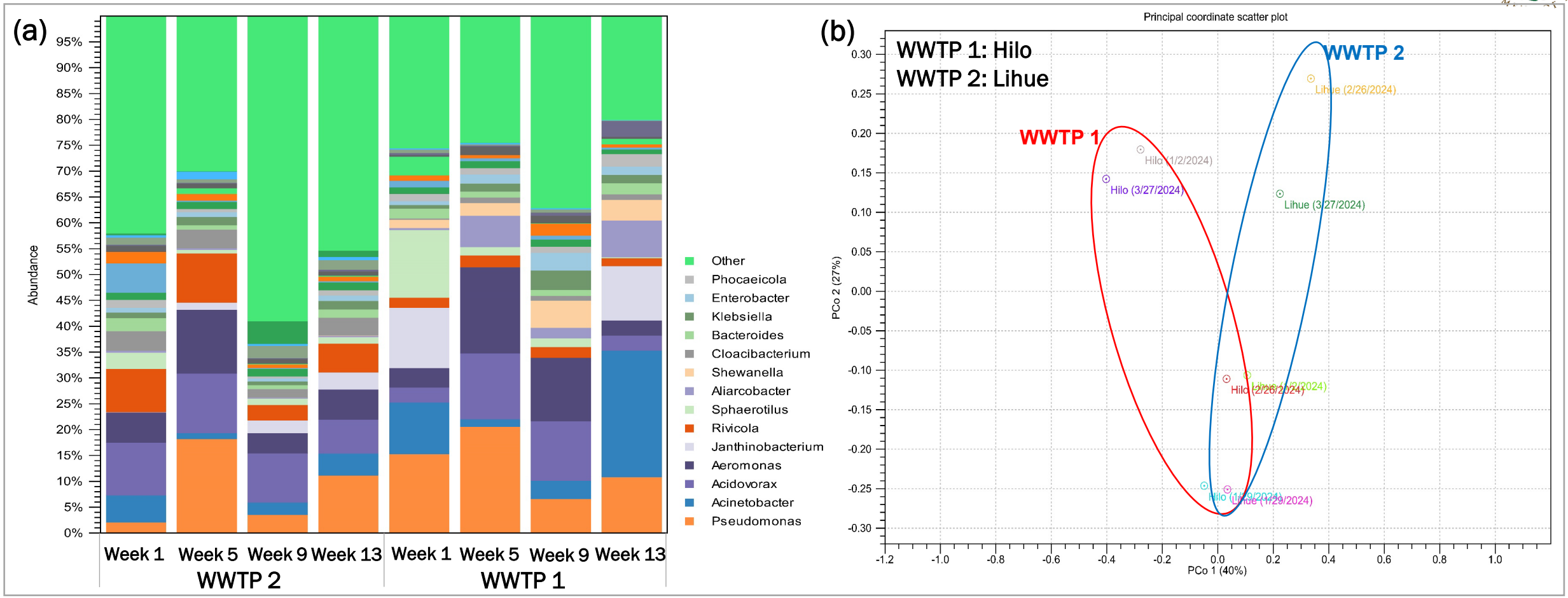
Results: Bacterial Diversity



- Both WWTPs showed that **highly comparable bacterial diversity** despite being located on **two different islands**.
- PCA analysis showed that, **by WWTPs**, bacterial diversity were not distinctly different.

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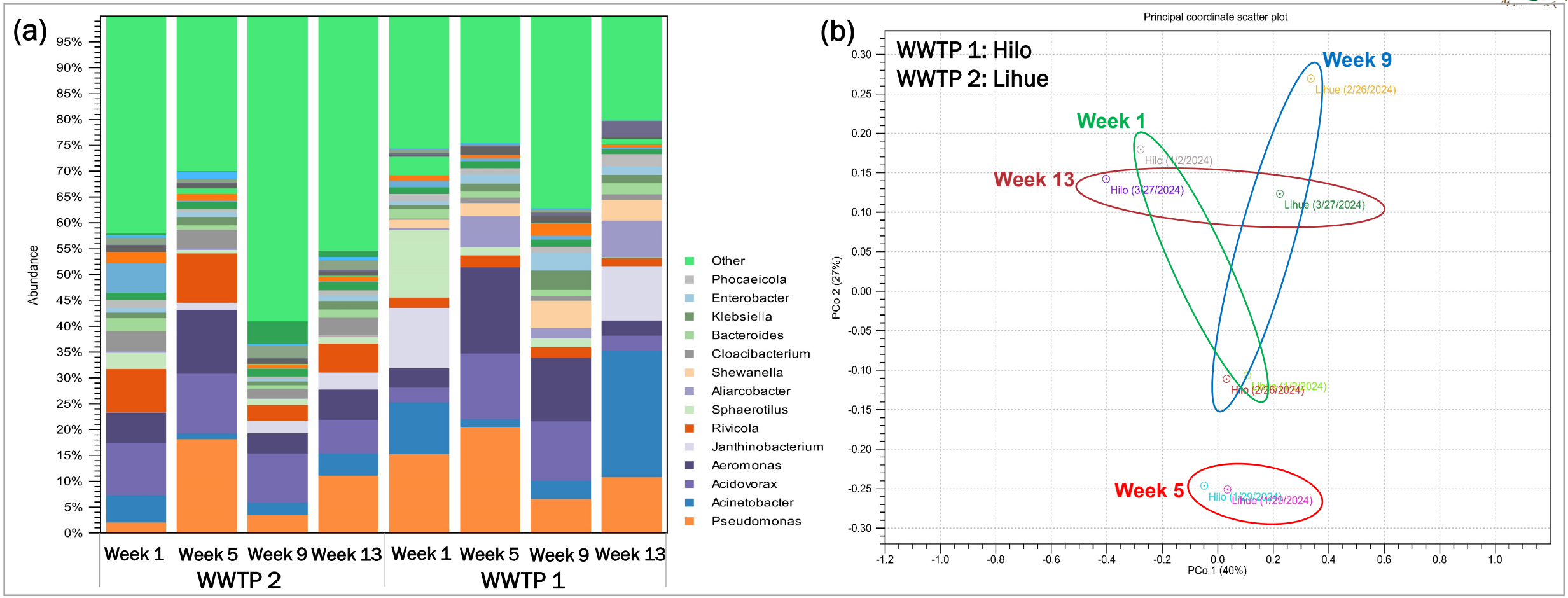
Results: Bacterial Diversity



- PCA analysis showed that, **by Weeks**, bacterial diversity were not distinctly different too.
- Especially Week 5

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Summary & Conclusion

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Summary

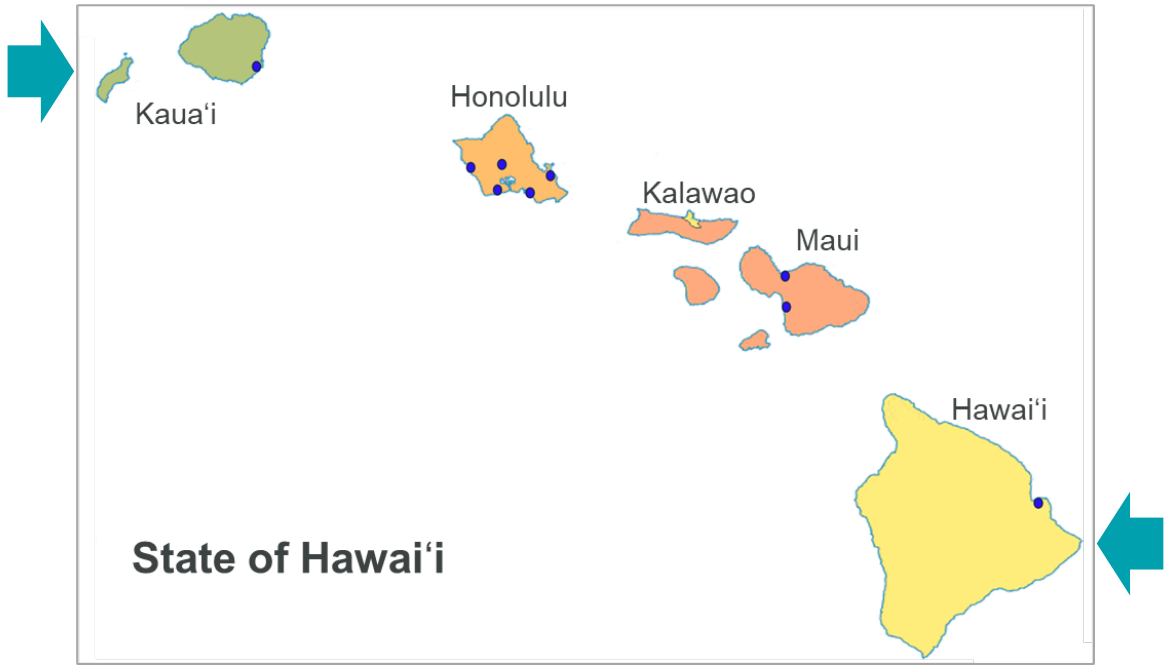
- In both WWTPs across the State of Hawaii, **KPC** and **OXA-48** were **always detected at a high levels**; whereas **NDM**, **IMP**, and **VIM** were **detected at a low levels or not detected at all**.

- AMR sequencing data were **correlated** with the dPCR data, where the more frequent detected carbapenem resistance genes by dPCR were also showed higher abundances in the sequencing data.

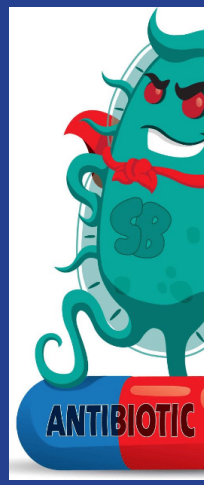
- Bacterial diversity were **not distinctly different** in both WWTPs.

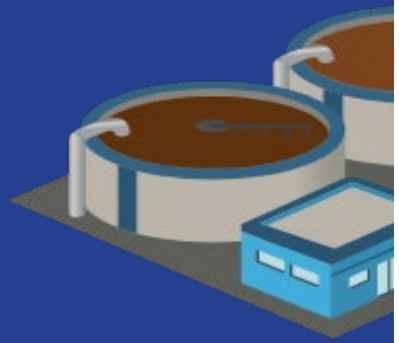
Conclusion

- Both WWTPs showed surprisingly **very similar AMR spatial & temporal distribution**, **AMR genetic diversity**, **and bacterial components** despite being located on two different islands.



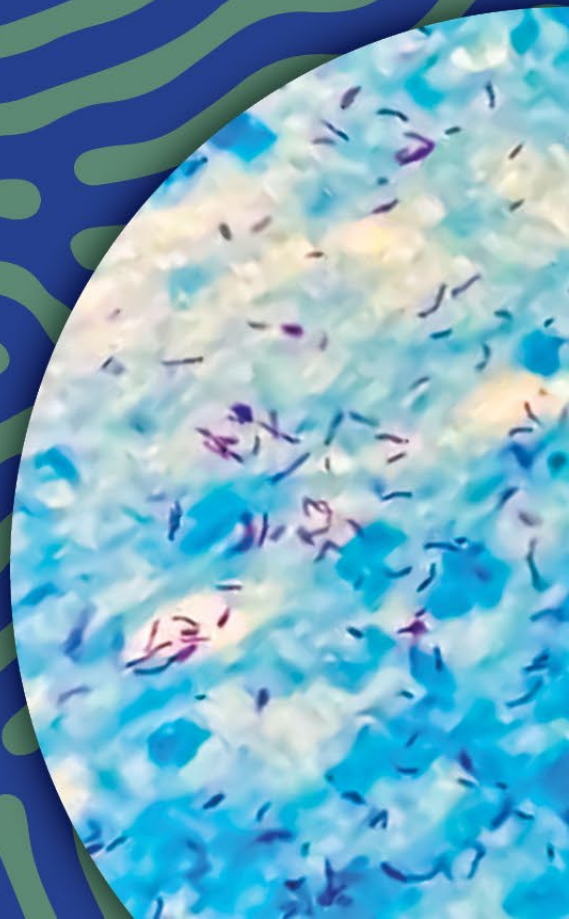
Takeaways





Human

Environment



Biofilm

Acknowledgement

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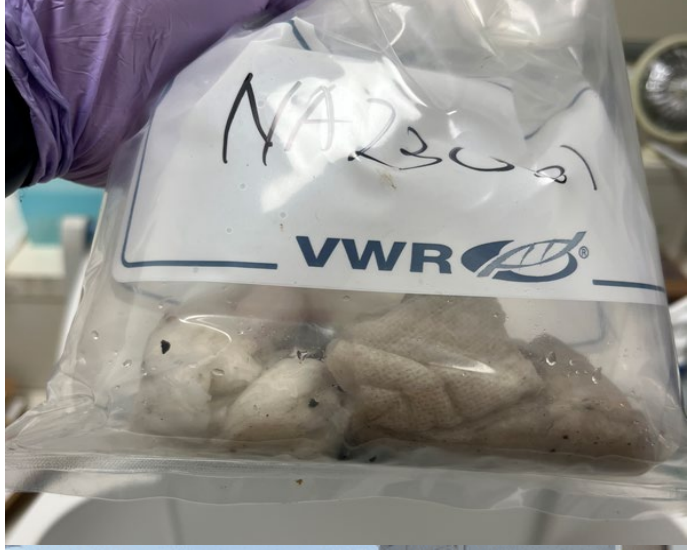

THANK YOU

Questions? Please contact

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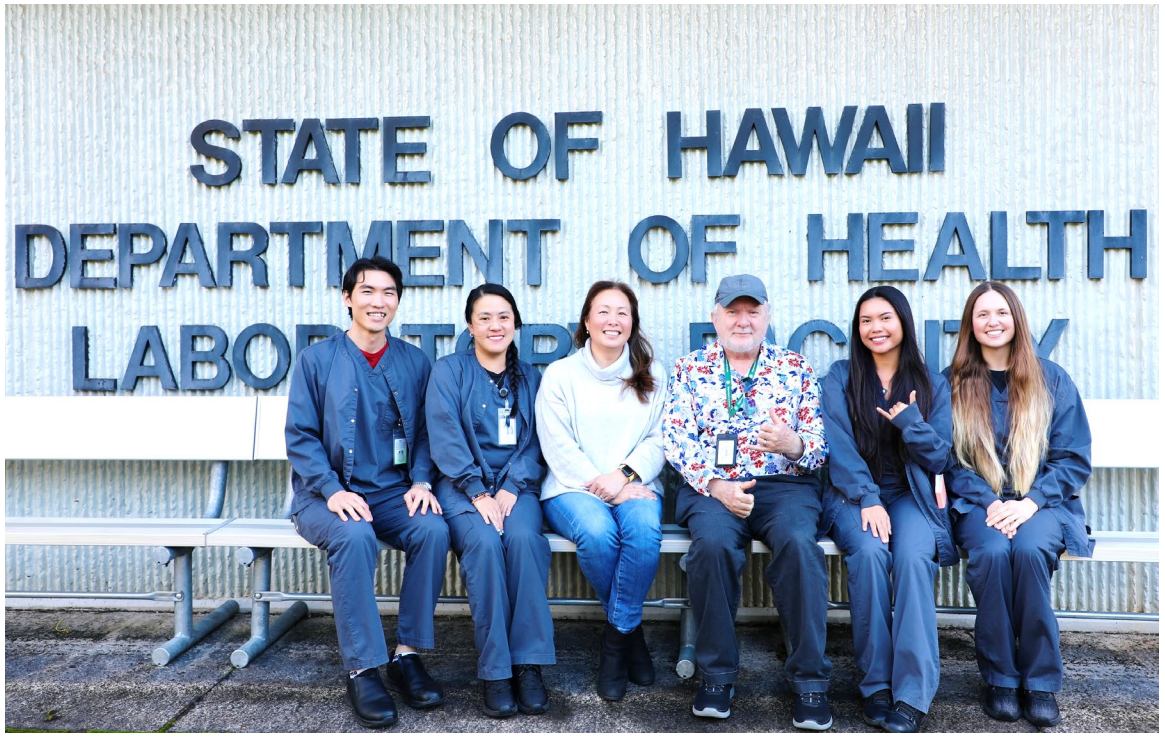

Wastewater Team in HI