Antibiotic Resistant Bacteria and Genes in Primary to Tertiary Treated Wastewater

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Background

- "Superbugs", multi-antibiotic resistant bacteria, have been recognized as an emerging public health threat
- Historically, the focus has been on hospital-acquired infections
- Attention has shifted to include concern over antibiotic resistant bacteria released into the environment

Los Angeles Times

Deadly superbugs from hospitals get stronger in the sewers and could end up in the Pa Ocean

The Washington Times

'Nightmare bacteria' resistant to antibiotic of last resort found in U.S.

The Washington Post

To Your Health

Superbug known as 'phantom menace' on th in U.S.

By Lena H. Sun December 4, 2015

The Economist

World politics Business & finance Economics Science & technology Culture

Antibiotic resistance

The grim prospect

The evolution of pathogens is making many medical problems worse. Time to take drug resistance seriously

Wastewater treatment may enhance levels of Antibiotic Resistant Bacteria

- Processes such as aerobic digestion encourage bacterial growth and may serve as an incubator for antibiotic resistant bacteria

- Residence time and rapid growth during treatment may promote transfer of antibiotic resistance genes

- Little work has been done to quantify the effect of treatment on levels of antibiotic resistant bacteria in wastewater

Study Questions

- Do wastewater treatment facilities in Southern California discharge antibiotic resistant bacteria or genes?
    - If so, what kind?
- Are the number of antibiotic resistant bacteria or genes in wastewater discharges higher or lower than in the input?
- How does level of treatment affect levels of antibiotic resistant bacteria or genes?

Approach

- Paired influent and final effluent samples to compare levels of antibiotic resistant bacteria before and after treatment
- Multiple plants to capture across-plant differences in treatment regimens
- Four sampling events (once per quarter) to capture seasonal variability
- Genetic methods to identify and quantify resistance genes

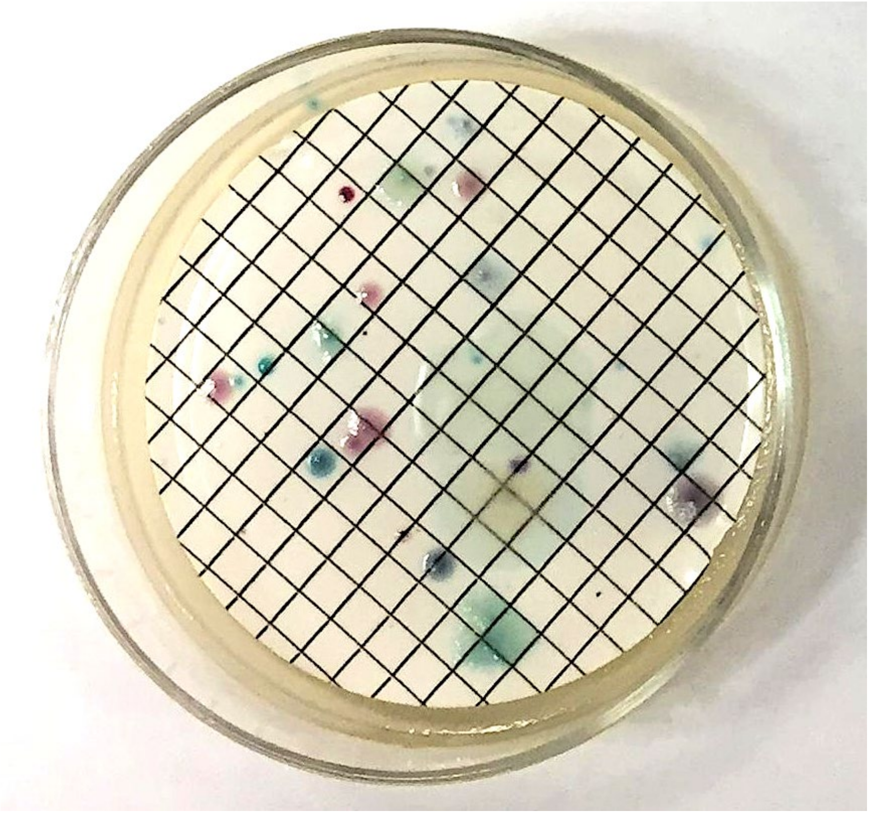
POTW	Final Effluent Treatment	Agency
Hyperion Water Reclamation Plant	Advanced Secondary Treatment (No Disinfection)	City of Los Angeles
Terminal Island Water Reclamation Plant	Tertiary Treatment	City of Los Angeles
Joint Water Pollution Control Plant	Advanced Secondary Treatment (Disinfection)	Los Angeles County Sanitation District
Water Reclamation Plant	Tertiary Treatment	Los Angeles County Sanitation District
Plant No. 1	Advanced Secondary Treatment	Orange County Sanitation District
Plant No. 2	Advanced Secondary Treatment	Orange County Sanitation District
North City Water Reclamation Plant	Tertiary Treatment	City of San Diego
Pt. Loma Wastewater Treatment Plant	Advanced Primary Treatment	City of San Diego
South Bay Water Reclamation Plant	Secondary Treatment	City of San Diego
South Bay International Wastewater Treatment Plant	Secondary Treatment	City of San Diego/IBWC

High Priority Antibiotic Resistant Bacteria

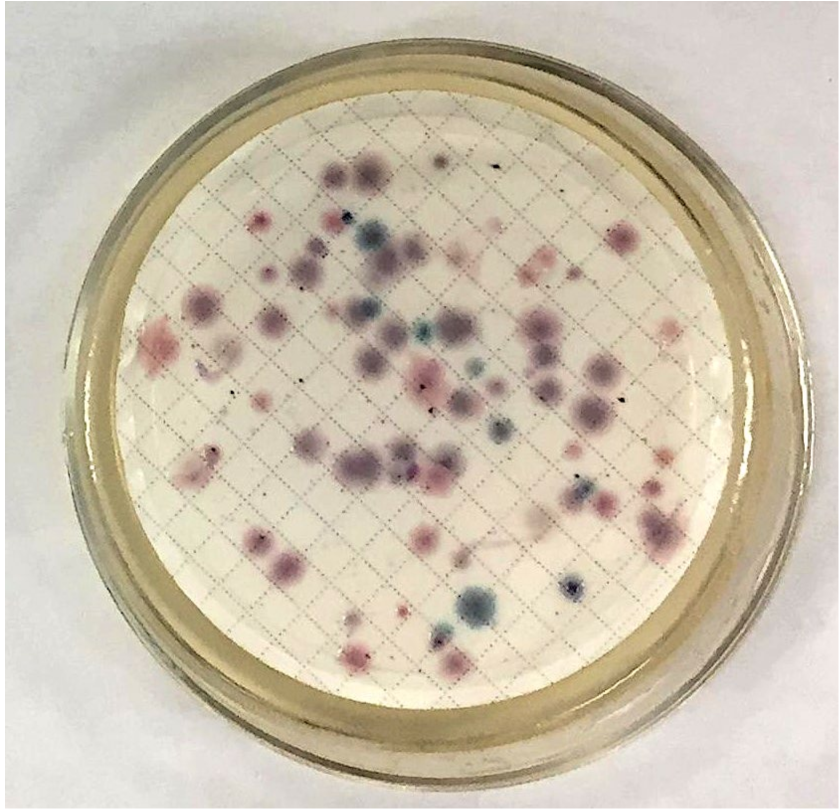
Antibiotic Resistant Bacteria	Bacterial Groups	Selective Media	Antibiotic Family	Motivation
Carbapenem Resistant Enterobacteriaceae (CRE)	Fecal coliforms, Klebsiella	mFC; mod mFC	Beta-lactam	EPA study found CRE in wastewater; last-line antibiotic
Vancomycin Resistant Enterococci (VRE)	Enterococcus spp	mEI	Glycopeptide	Sewage spills at FL beaches found persistent VRE; last-line antibiotic for MRSA
Methicillin Resistant Staphylococcus aureus (MRSA)	Staphylococcus aureus; Staphylococcus spp.	CHROMAgar SA	Beta-lactam	Studies have found it in California ambient water

Quantification Methods: Viable ARB

- Quantification and isolation of high priority antibiotic resistant bacteria by culture
  - CRE-FC, CRE-Kleb, VRE, MRSA



1 ml Effluent
no antibiotics



10 ml Effluent
with antibiotics

Antibiotic Resistance Genes

Specific

Type of analysis	Antibiotic Resistance Gene	Gene Names	Method	Sample Type	Outcome
Targeted	carbapenemase	KPC, OXA	Quantitative or Digital PCR	Isolates; Wastewater samples	Precise detection and quantification of a specific gene
Screening panel	List of ARGs	Multiple	Quantitative PCR	Isolates; Wastewater samples	Detection and quantification of multiple genes
Survey	Resistome	Multiple	Sequencing	Wastewater samples	Detection of ARGs in a sample

Broad

Results: Antibiotic Resistant Bacteria

Are there antibiotic resistant bacteria in discharge?

Yes

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Is the number higher or lower than in the influent?

Lower (mostly)

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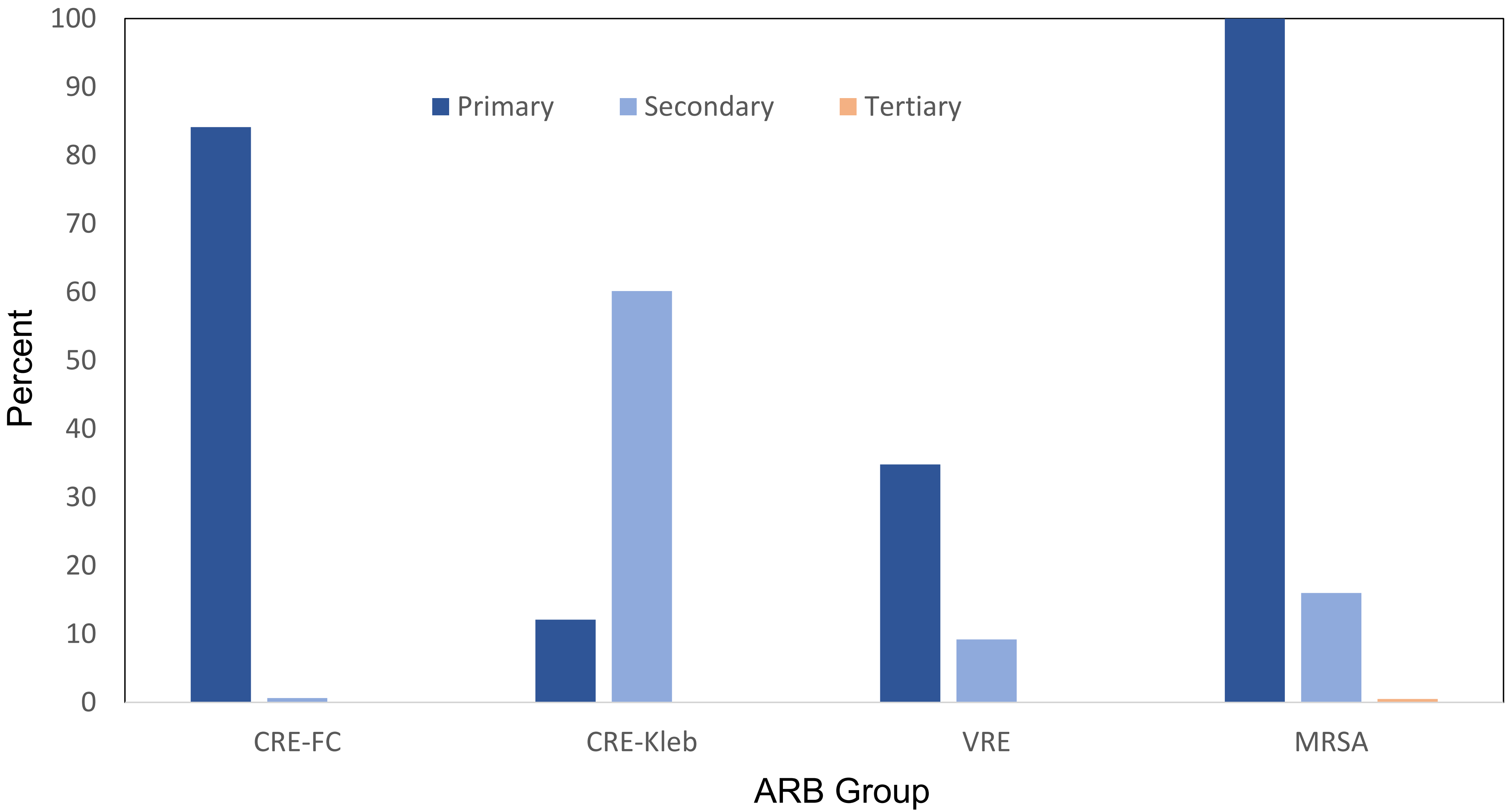
What is the effect of treatment?

As treatment increases, ARB numbers decrease

Percent ARB by High Priority Group



Results: Antibiotic Resistance Genes

Are there antibiotic resistance genes in discharge?

Yes

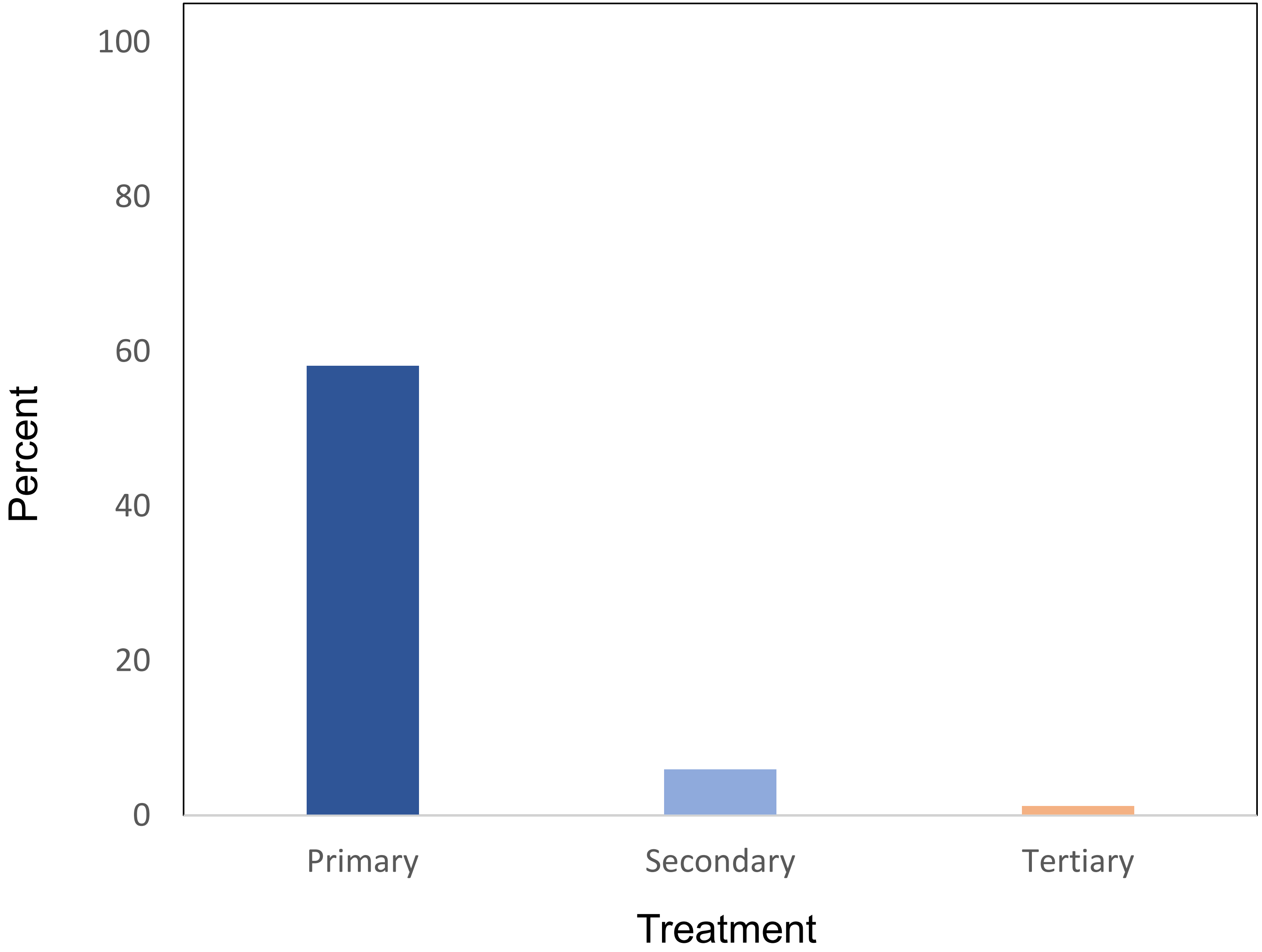
Is the number of genes higher or lower than in the influent?

Lower

What is the effect of treatment?

As treatment increases, ARGs decrease

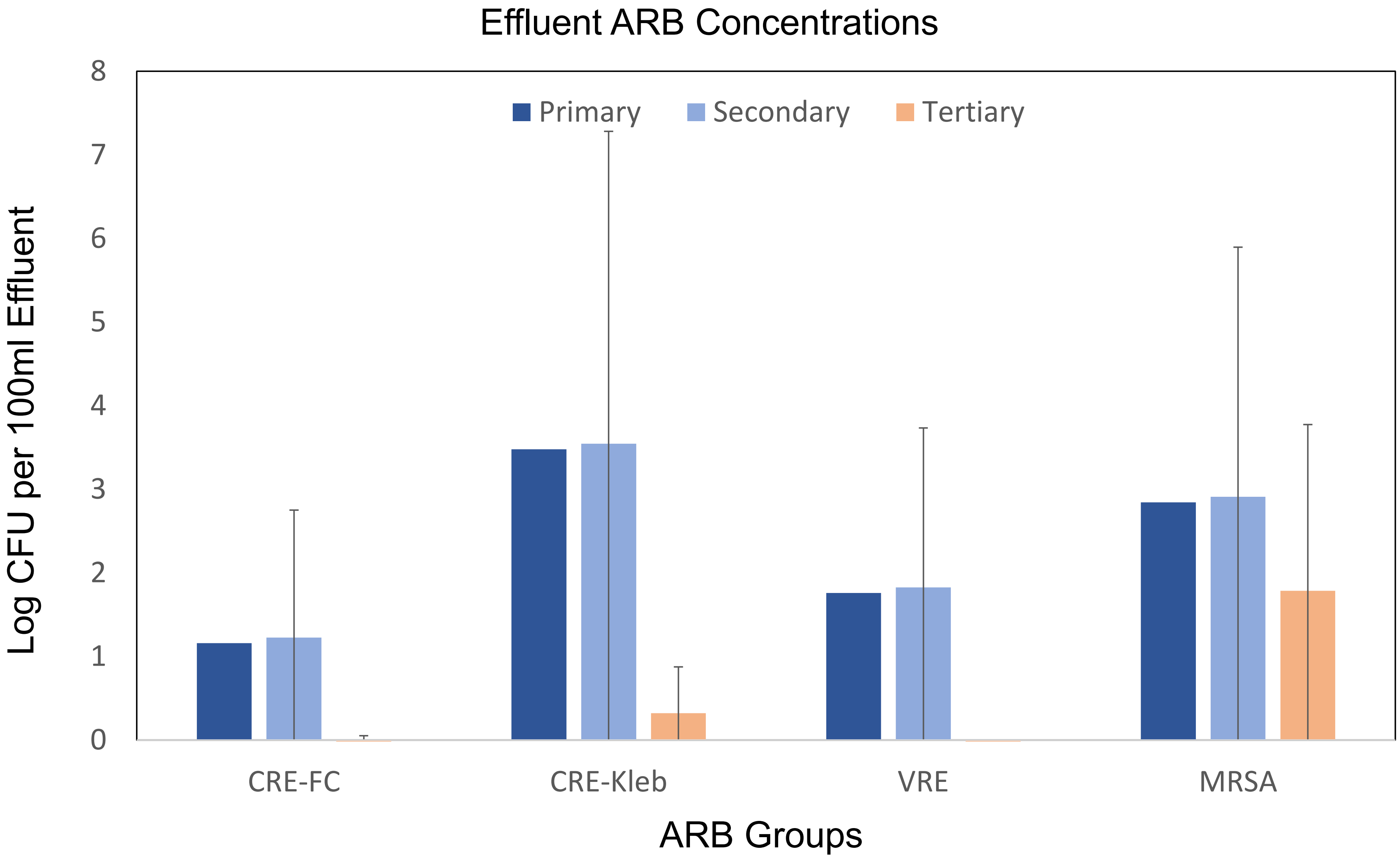
Percent Carbapenemase Genes in Effluent



The Good, The Bad, The Unknown

- Higher treatment levels reduce the number of ARB and ARG in effluent
- ARB and ARG are being discharged
- What happens to the ARB and the ARG?
  - In environmental waters?
  - Health risk?

Effluent ARB concentrations: What do these concentrations mean?



Next Steps

- Determine extent of ARB/ARGs influence in environmental waters
  - Investigate waters around an ocean and an inland outfall
  - Compare concentrations and measure extent of plume

- Measure risk of colonization and human illness in recreational waters
  - Epidemiology studies: expensive and time-consuming
  - Study team in place and pursuing funding