

# **Antibiotic Resistant Bacteria and Genes in Wastewater Treatment Facilities**

**Presentation to the SCCWRP Commission  
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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 Pacific Ocean



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



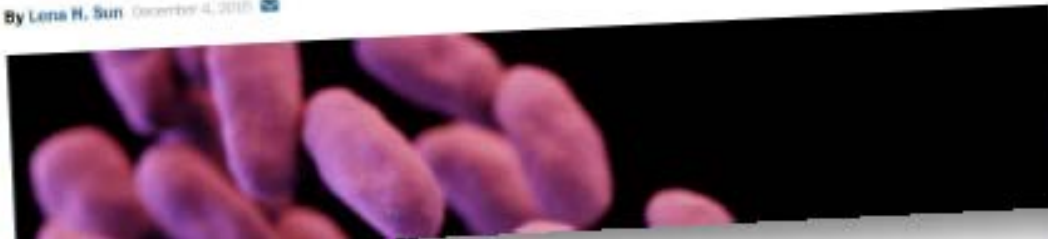
The Washington Times

The Washington Post

To Your Health

Superbug known as 'phantom menace' on the rise in U.S.

By Lena H. Sun December 4, 2010



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

May 2nd 2010 From the print edition

2 Images



# **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**

# WHAT DO WE KNOW?

- **Very little information about antibiotic resistant bacteria and antibiotic resistant genes in wastewater discharges**
- **WERF-funded study suggests antibiotic resistance in Gram-positive bacteria increases during the activated sludge process of wastewater treatment**
- **Methicillin Resistant *Staphylococcus aureus* (MRSA) is routinely found in beach water and sand**
- **Antibiotic resistance genes and resistance to antibiotics can be transferred**
  - Shared between bacteria
  - Transported by viruses
  - Taken up as naked DNA

# STUDY QUESTIONS

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

# APPROACH

- **Paired influent and final effluent samples to compare levels of antibiotic resistant bacteria before and after treatment**
  - Samples taken quarterly for one year to capture seasonality
- **Multiple plants to capture across-plant differences in treatment regimens**
- **Both culture and genetic methods to determine which resistance genes are prevalent**

# METHODS

- **Measure four types of antibiotic resistant bacteria by culture**
  - Fecal coliform and *Klebsiella* spp. for carbapenem resistance using imipenem
  - Vancomycin Resistant Enterococcus (VRE)
  - Multiple-drug Resistant *Staphylococcus aureus* (MRSA)
- **Verify resistance and test for multiple antibiotic resistant “Superbugs” by gene amplification**
- **Quantify of levels of antibiotic resistance genes using droplet digital Polymerase Chain Reaction**
- **Survey broad range of resistant genes in population using DNA sequencing**



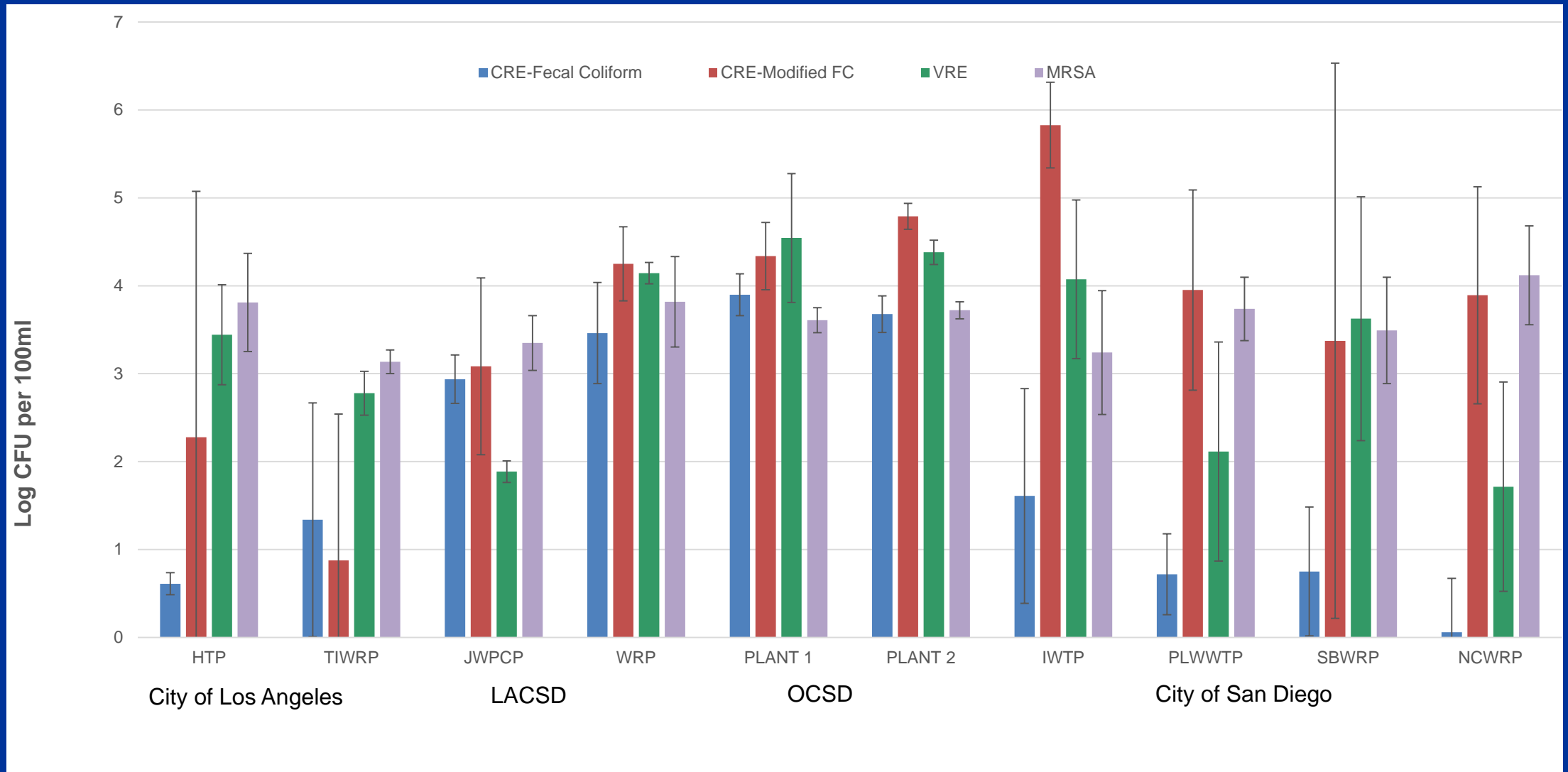
# PARTICIPATING AGENCIES AND PLANTS

| POTW   | Final Effluent Treatment                       | Agency                                 |
|--|--|--|
| Hyperion Water Reclamation Plant                   | Advanced Secondary Treatment (No Disinfection) | City of Los Angeles                    |
| Terminal Island Water Reclamation Plant            | Advanced Secondary Treatment (No Disinfection) | 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                               | City of San Diego                      |
| South Bay Water Reclamation Plant                  | Tertiary Treatment                             | City of San Diego                      |
| South Bay International Wastewater Treatment Plant | Secondary Treatment                            | City of San Diego/IBWC                 |
| Hampton Roads                                      | Advanced Secondary Treatment                   | HRSD                                   |

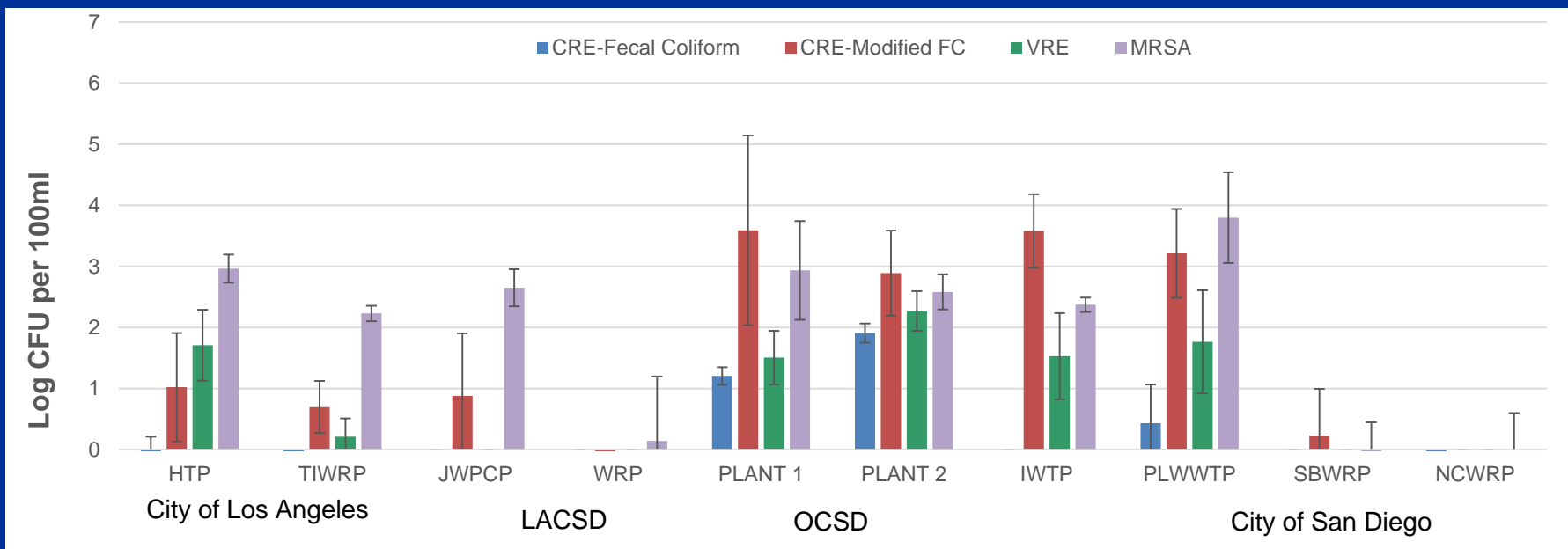
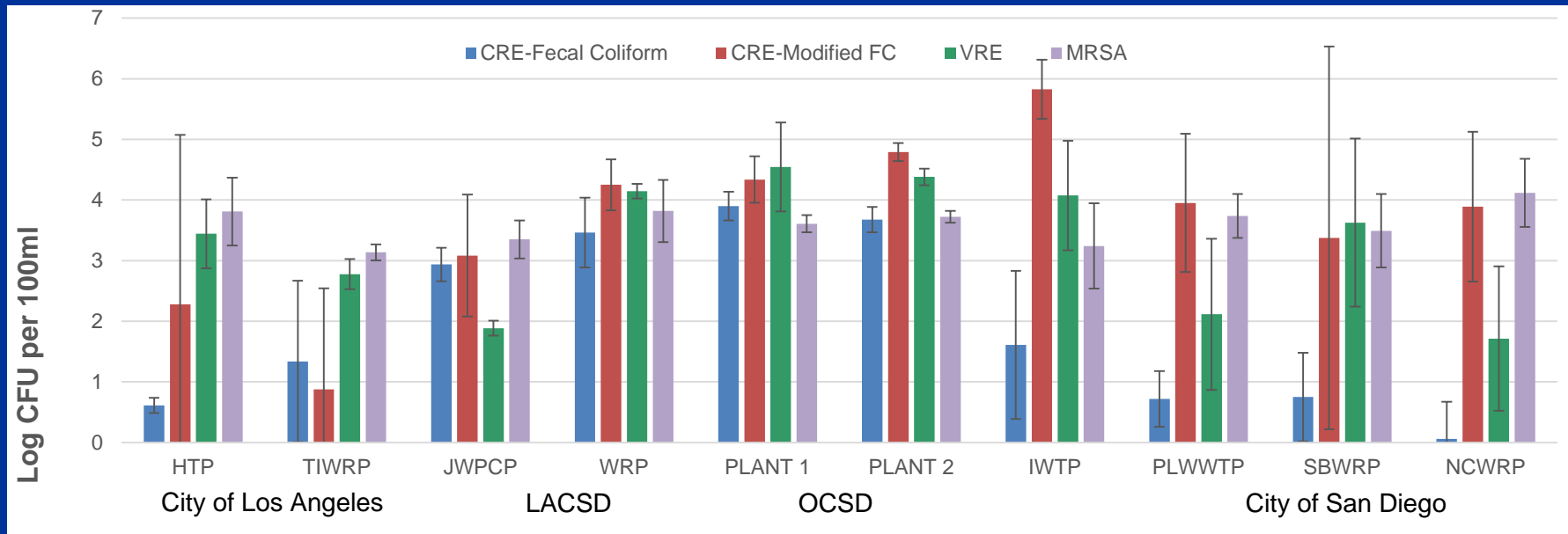
# PROGRESS TOWARD COMPLETION OF SAMPLING

| POTW   | Percent Sampling Completion |
|--|-----------------------------|
| Hyperion Water Reclamation Plant                   | 50%                         |
| Terminal Island Water Reclamation Plant            | 50%                         |
| Joint Water Pollution Control Plant                | 75%                         |
| Water Reclamation Plant                            | 75%                         |
| Plant No. 1  | 100%                        |
| Plant No. 2  | 100%                        |
| North City Water Reclamation Plant                 | 100%                        |
| Pt. Loma Wastewater Treatment Plant                | 100%                        |
| South Bay Water Reclamation Plant                  | 100%                        |
| South Bay International Wastewater Treatment Plant | 100%                        |
| Hampton Roads                                      | 0%                          |

# ANTIBIOTIC RESISTANT BACTERIA ENTERING TREATMENT PLANTS



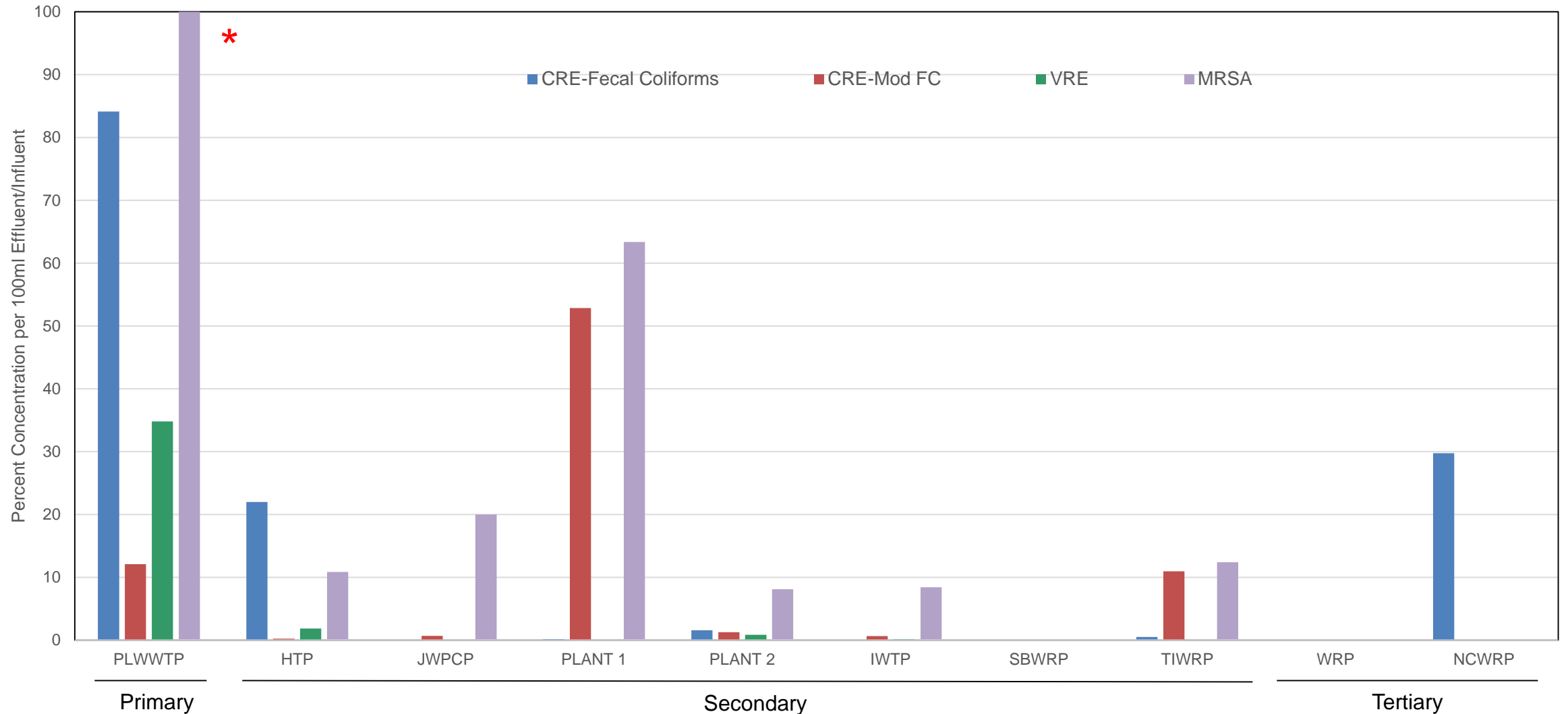
# TREATMENT PLANTS ARE NOT ENHANCING LEVELS OF RESISTANT BACTERIA



# INCREASING TREATMENT LEVEL IMPROVES ARB CONCENTRATION REDUCTIONS

\* >>100 % (275%)  
MRSA in Effluent

ARB Concentrations in Effluent  
as a percentage of Influent



# DO WASTEWATER TREATMENT FACILITIES IN SOUTHERN CALIFORNIA DISCHARGE ANTIBIOTIC RESISTANT BACTERIA?

- **Wastewater treatment facilities do discharge antibiotic resistant bacteria to the environment**
- **The antibiotic resistant bacterium that survived treatment in the greatest numbers across all facilities was methicillin resistant *Staphylococcus aureus***
  - Other groups also survived treatment in measurable numbers at certain facilities

# IS THE NUMBER OF ANTIBIOTIC RESISTANT BACTERIA IN WASTEWATER DISCHARGES HIGHER OR LOWER THAN IN THE INPUT?

- **Concentrations of antibiotic resistant bacteria were lower in treated wastewater discharges than in water coming into facilities**
  - The only exception was for methicillin resistant *Staphylococcus aureus*
  - Concentrations of methicillin resistant *Staphylococcus aureus* increased with primary treatment

# HOW DOES LEVEL OF TREATMENT AFFECT LEVELS OF ANTIBIOTIC RESISTANT BACTERIA?

- **Concentrations of all groups of antibiotic resistant bacteria we measured decreased with increasing treatment effort**
  - Primary treatment ineffective
  - Secondary treatment removed > 50%
  - Advanced tertiary treatment removed nearly all bacteria



# NEXT STEPS

- **Complete sampling**
- **Complete for multiple antibiotic resistant “Superbugs”**
  - Collaboration with Mt. Saint Mary’s University
- **Complete genetic quantification and survey of resistance genes**

# WE DON'T KNOW RISK ASSOCIATED WITH PASS-THROUGH OF ANTIBIOTIC RESISTANT BACTERIA

Estimated Daily Load of Antibiotic Resistant Bacteria in Discharges

