# Proof-of-Concept: Antibiotic Resistance in Coastal Wetland Sediments of Urban and Agricultural Watersheds

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*Black, anoxic sediments prevail just below the surface in typical wetlands. The Sea Grant-funded study is focused on the uppermost sediment layer.*


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*Sea Grant biologist David Cummings of Point Loma Nazarene University (PLNU) collects sediments from a tidal channel of the Tijuana River Estuary.*


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*Professor David Cummings of PLNU collects channel sediments from the Ballona Creek Estuary in Los Angeles County.*

## BACKGROUND

After years of antibiotic use, the normal flora in the human intestinal track can develop antibiotic resistance. If this happens, antibiotic-resistant (AR) bacteria, as well as AR genes, can be shed in feces. To the extent that raw sewage flows to the coast, and to the extent that sewage treatment processes do not destroy DNA, estuaries and other coastal wetlands are potentially the receiving waters for AR bacteria and genes. Because livestock are also fed antibiotics, animal waste is another potential contributor.

## PROJECT

This project set out to compare concentrations of AR bacteria and genes in sediments of urban and rural watersheds in California. Of particular interest was to begin to understand the significance of AR genes in the environment, how they accumulate and attenuate and whether they are components of plasmids – small circular pieces of DNA that can transfer themselves from one host species to another – or chromosomes.

"Most people might not think of genes as a pollutant," said biology professor David Cummings of Point Loma Nazarene University in San Diego, "But, the genes that confer antibiotic resistance may in fact pose a more serious long-term public and ecosystem health threat than many chemical pollutants in urban storm water."

"Unlike chemical pollutants, DNA pollutants have a natural mechanism for their own amplification," he said. "They can actually increase in concentration on their own, under the right conditions."

## STUDY SITES

The rural sites sampled for the project were Stone Lagoon and Dry Lagoon, both in Northern California, and Pine Valley Creek in the undeveloped mountains east of San Diego. The urban sites were Ballona Creek Estuary in Los Angeles County, Famosa Slough in San Diego and the Tijuana River Estuary in Imperial Beach.

## METHOD

Two basic techniques were employed for measuring antibiotic resistance. In one, bacteria were cultured in the presence or absence of different antibiotics to estimate the frequency of AR strains at each site. The other used PCR techniques to search for three genes (tetA, tetC and qnrA) associated with antibiotic resistance.

The tetA and tetC genes confer resistance to tetracycline by encoding for an efflux pump mechanism that, as the name suggests, pumps antibiotics out of the cell before the drugs can destroy the bacteria. The qnrA gene, in contrast, confers resistance to fluoroquinolone drugs by protecting the target of the antibiotic, DNA gyrase.


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*Professor David Cummings of PLNU and students extrude a core of channel sediments from the Ballona Creek Estuary.*

## CULTURING RESULTS

Urban estuaries contained a greater abundance of AR bacteria than rural ones. The one watershed receiving untreated human sewage (the Tijuana River Estuary) harbored the highest fraction of AR bacteria and these bacteria were resistant to the broadest range of antibiotics.

Some numbers: More than 10 percent of the cultivable bacteria at one Tijuana River Estuary site were resistant to ampicillin. More than 80 percent of bacteria from Famosa Slough were resistant to tetracycline. Ballona Creek showed a relatively high concentration of bacteria resistant to nalidixic acid and kanamycin. AR bacteria were uncommon at Pine Valley Creek.

## PCR RESULTS

The most interesting results came from studying AR genes in sediment samples taken from two sites in the Tijuana River Estuary during the wet and dry seasons. This work suggests that clinically relevant AR genes are introduced to the coast during heavy rains. These genes attenuate markedly during the dry season (summer).

In particular, the tetA, tetC and qnrA genes were detected in samples collected at the end of a February 2007 storm from both sites; test results were mixed for samples collected 10 weeks later in April. By June, both sites tested negative for the genes. The enrichment study described below shows that the AR genes were not absent in June but were below the PCR-detection limit.

## ENRICHED SEDIMENT STUDY

To amplify the signal in the summer samples, the scientist and his students enriched samples in a nutrient broth mixed with tetracycline. DNA extracted from this enriched culture was then subjected to another round of PCR probing. This experiment showed that the tetracycline-amended enrichment culture contained all three genes. DNA sequencing revealed that these AR genes were nearly identical to those found on plasmids in clinical isolates.

It is important to emphasize that the addition of tetracycline magnified the signal of the qnrA gene, which is unrelated to tetracycline resistance. Cummings said this strongly suggests that multiple AR genes are genetically linked on the same element, likely a plasmid. If true, this means that selecting for resistance to one antibiotic results in resistance to another, or multiple, antibiotics.

## NEXT STEP

California Sea Grant recently awarded Cummings support to quantify background levels of AR genes in the Tijuana River Estuary and to document the processes that attenuate them during the dry season. He and students would like to determine whether AR genes are components of plasmids or chromosomes and whether AR genes are spreading to other bacterial species beyond their original host organism.

"We anticipate that, despite the obvious attenuation, urban wetlands like the Tijuana Estuary, have higher background levels of AR genes due to their chronic introduction and accumulation," Cummings said.

## COLLABORATING ORGANIZATIONS

NOAA's Tijuana River National Estuarine Research Reserve
Moss Landing Marine Laboratories
Loyola Marymount University
California Department of Parks and Recreation
U.S. Fish and Wildlife Service
City of San Diego

## PRESENTATIONS

D. Arriola, Ryan, C., Singh, N., Unruh, K. 2008. "Investigation of antibiotic-resistance genes in polluted coastal environments." West Coast Biological Sciences Undergraduate Research Conference, San Diego, Calif.

D. E. Cummings. 2007. "Bacteria, the wetland's most underappreciated members." Tijuana Estuary Summer Speaker Series. San Diego, Calif. (Invited)

D. E. Cummings. 2007. "Microbiology at the Tijuana River Estuary." Loyola Marymount University, Los Angeles, Calif. (Invited)

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*David Cummings, professor of biology, PLNU.*

*PLNU undergraduates (from left) Kelsey Unruh, Cody Ryan, Nathan Singh and David Arriola. A primary objective of the project is to prepare undergraduate biology students for post-graduate studies.*

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