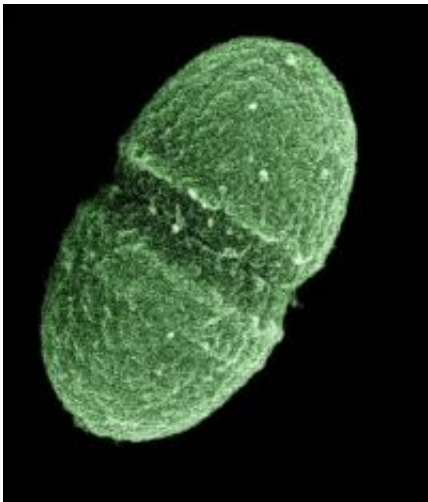


Genetically engineered plasmid can be used to fight antimicrobial resistance

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Enterococcus faecalis. Credit: United States Department of Agriculture

Researchers have engineered a plasmid to remove an antibiotic resistance gene from the *Enterococcus faecalis* bacterium, an accomplishment that could lead to new methods for combating antibiotic resistance. The research is published this week in *Antimicrobial Agents and Chemotherapy*, a journal of the American Society for Microbiology.

In vitro, and in mouse models, the engineered plasmid removed the [antibiotic resistance gene](#) from *E. faecalis*. In mouse models, it reduced the abundance of the resistance gene threefold..

"Our concern with organisms that cause hospital-acquired infections that are resistant to many clinical antibiotic therapies motivated the research," said co-senior author Breck A. Duerkop, Ph.D., Assistant Professor of Immunology and Microbiology, University of Colorado School of Medicine, Anschutz Medical Center, Aurora.

E. faecalis is part of the normal, benign intestinal flora, but when [antibiotics](#) kill off beneficial intestinal flora, *E. faecalis* can become pathogenic. As such, it can also acquire single or multidrug resistance. Antibiotic resistant *E. faecalis* infections are a major problem in hospitals.

The mechanism used to remove [antibiotic resistance](#) genes is the specialized protein, CRISPR-Cas9. It can make cuts just about anywhere in DNA.

Along with CRISPR-Cas9, RNA sequences homologous to DNA within the antibiotic resistance gene have been added to the engineered plasmid. These RNAs guide the CRISPR-Cas9 to make the cuts in the right places.

Previous work in animal models by co-senior investigator Kelli L. Palmer, Ph.D., found that CRISPR-Cas9 could prevent intestinal *E. faecalis* from acquiring resistance genes. Dr. Palmer is Fellow, Cecil H. and Ida Green Chair in Systems Biology Science, Associate Professor of Biological Sciences, University of Texas, Dallas.

The delivery vehicle for the engineered plasmid is a particular strain of *E. faecalis*, which conjugates with *E. faecalis* of various different strains. Conjugation is the process whereby bacteria come together to transfer genetic material from one to the other via direct cell to cell contact.

"*E. faecalis* strains used to deliver these plasmids to drug resistant strains

[of *E. faecalis*] are immune to acquiring drug resistant traits carried by the [target cells](#)," said Dr. Duerkop. "The engineered plasmid can significantly reduce the occurrence of antibiotic resistance in the target bacterial population rendering it more susceptible to antibiotics. We envision that this type of system could be used to re-sensitize antibiotic resistant *E. faecalis* to antibiotics," he said.

Nonetheless, Dr. Duerkop cautioned that it remained possible that *E. faecalis* could still circumvent the engineered plasmid. Some bacteria have anti-CRISPR systems that can block CRISPR-Cas9 function, and some others have systems that can degrade foreign DNA. "Future studies will need to be done to address such an issue as *E. faecalis* avoiding the targeting system and under what conditions this may happen," said Dr. Duerkop.

Provided by American Society for Microbiology

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