Bacteria boost antifungal drug resistance in severe childhood tooth decay

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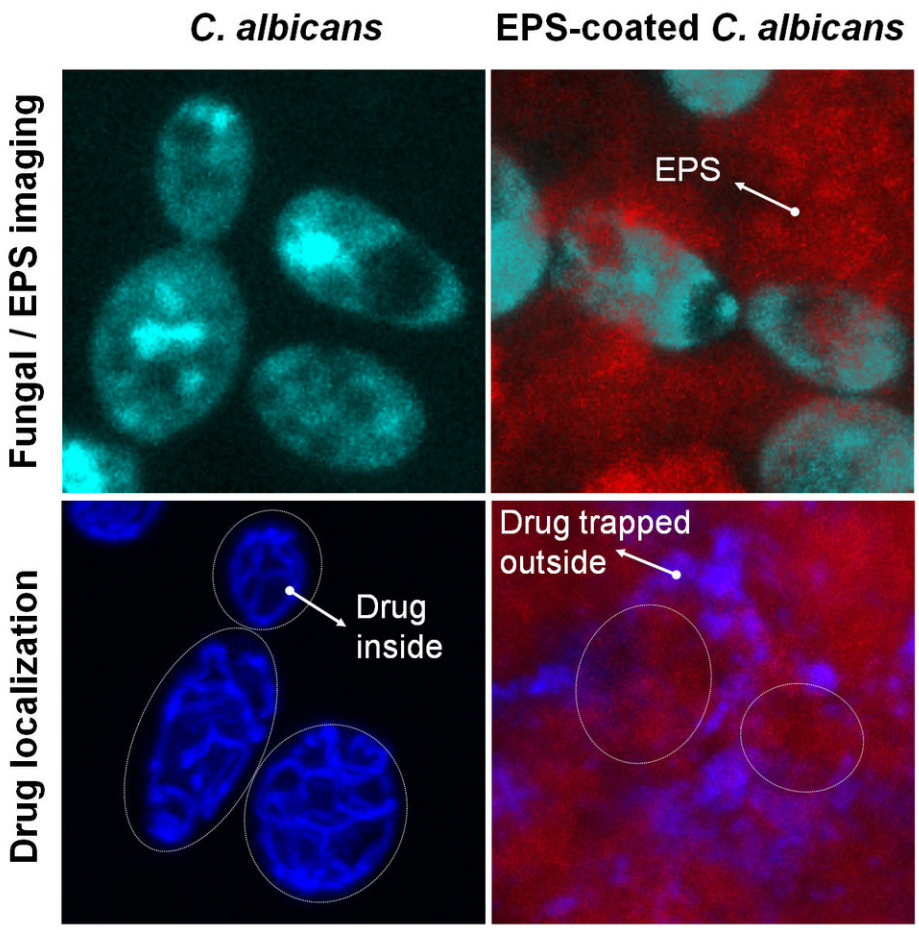

Credit: University of Pennsylvania

Early childhood caries, a form of severe tooth decay affecting toddlers

and preschoolers, can set children up for a lifetime of dental and health problems. The problem can be significant enough that surgery is the only effective way to treat it.

Recently researchers from the University of Pennsylvania School of Dental Medicine discovered that, in many cases, early childhood caries result from dental plaque that contains both bacteria and fungus working together to make the biofilm on the teeth more pathogenic and difficult to remove. Now they have shown that these two types of microorganisms synergize to enhance drug resistance, enabling the fungal cells to avoid being killed by antifungal therapies. Yet simultaneously targeting the matrix produced by the bacteria along with the fungus offers a way around this protection.

"The current antimicrobial modalities for treating early childhood caries have limited efficacy," says Hyun (Michel) Koo, a professor in the Department of Orthodontics and divisions of Pediatric Dentistry & Community Oral Health in Penn's School of Dental Medicine. "Available evidence shows that biofilm-associated diseases are polymicrobial in nature, including a mix of bacterial and fungal species; therefore a treatment aimed at just one type of microorganism may not be effective. I think this work gives us a glimpse into alternative ways to disrupt cross-kingdom biofilm, a combinatorial approach that considers the fungal and bacterial components."

Koo was the senior author on the work and Dongyeop Kim, a postdoctoral research fellow, was first author. They collaborated with teams from Tel Aviv University and the University of Wisconsin-Madison on the work, which was published in the *ISME Journal*.

During the last several years, researchers have observed that the dental plaque in children with early childhood caries often contained *Candida albicans*, a fungal species that normally colonizes mucosal surfaces, in

addition to Streptococcus mutans, the bacteria generally associated with tooth decay. Work in Koo's lab demonstrated that an enzyme produced by the bacteria, termed GtfB, can bind to *Candida* and when sugar is present (a dietary hallmark in childhood caries) a sticky polymeric matrix forms on its cell surface, enabling the fungus to bind to teeth and associate with bacterial counterparts. Once together, these organisms work in concert to increase severity of tooth decay in a rodent model.

Realizing this, Koo, Kim, and colleagues wanted to see whether a two-pronged approach might break apart the synergistic association and effectively treat the biofilm. "Initially, we decided to look into therapies that are clinically used in dentistry to attack or prevent either fungal or bacterial infections," Koo says.

They came up with fluconazole, which is used as an antifungal, and povidone iodide, which is an antiseptic agent with antibacterial properties. Used alone to treat biofilms grown on a tooth-like material in the lab, the drugs had only moderate effects, confirming that monotherapy doesn't work very well against polymicrobial biofilms. But in combination, the results were much more impressive.

"We completely eradicated the fungal infection, both in the lab-grown biofilms but also those formed in vivo using an animal model," Koo notes, yet this achievement came without enhancing antibacterial activity.

To understand why the combination approach was so effective against *C. albicans* even without killing many more bacteria, the researchers looked closely at high-resolution microscopic images of the biofilms with the various treatment combinations. They observed that, in untreated biofilms and those treated with solely fluconazole, the fungus was coated with abundant sticky matrix, which seemed to serve as a protective shield against the antifungal compound. But in biofilms treated with

povidone iodide as well, the matrix was substantially reduced, leaving the fungus exposed to the fluconazole.

"We thought, that's interesting," Koo says, and turned to the scientific literature to find out more. They discovered that iodide-containing drugs can inhibit the activity of GtfB. In a series of experiments, they found that povidone iodide acted as a powerful inhibitor of the sticky-matrix production. The agent was almost 100-fold more potent as an inhibitor of the matrix than it was as an antibacterial agent.

That led them to the hypothesis that the matrix was serving as a "drug-trapping shield," preventing the fluconazole from accessing and killing the fungal cells. To see whether disrupting the matrix could allow the fluconazole to penetrate and reach the fungus, they collaborated with Tel Aviv University scientists to track, in real time, fluorescently-labeled fluconazole as it moved through a biofilm.

Taking time-lapsed images, they found that the fluconazole were trapped in the matrix, largely failing to reach the fungal cells, which was further confirmed by directly measuring radiolabeled fluconazole absorbed in the matrix. In contrast, fluconazole readily moved inside the fungal cells when they were located in biofilms with the matrix disrupted by povidone iodine.

Using three different assays to disrupt the matrix, either by directly degrading the matrix or using bacteria defective in GtfB, the researchers found that the antifungal-killing ability of fluconazole could be completely restored, confirming the role of the bacteria-produced matrix in promoting antifungal drug resistance.

The fungus itself has its own mechanisms for avoiding being killed by antifungals, but this resistance is exacerbated by the shielding effect of the matrix, the researchers found.

Looking ahead, the Penn-led team hopes their findings lead to new strategies for treating bacterial-fungal infections associated with early childhood caries and possibly other polymicrobial diseases. For the researchers' part, they are making use of nanotechnology to develop targeted approaches that can precisely target the matrix and both the fungal and bacterial components of the oral biofilm.

More information: Dongyeop Kim et al, Bacterial-derived exopolysaccharides enhance antifungal drug tolerance in a cross-kingdom oral biofilm, *The ISME Journal* (2018). DOI: 10.1038/s41396-018-0113-1

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