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Infection-causing bacteria are the bad guys of the health-care world, but dental detectives track them down

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"If we think we have cleaned 300 bacteria, but there are 1,000 still waiting for us—and we don't even know about them—that may put patients at risk," says Addy Alt-Holland. Credit: Iker Ayestaran

It began as a routine investigation. There were five disinfectants in town—ecofriendly types. Claimed they could wipe out bacteria in dental clinics. Tufts researchers had to figure out whether the cleaners were on

the up-and-up.

The case appeared straightforward enough. Addy Alt-Holland and Ronald Perry, scientists at Tufts School of Dental Medicine, rounded up bacteria from the protective plastic barriers used to cover surfaces in dental operatories, from chairs to instrument palettes to lamps, to evaluate the killing power of the green disinfectants. The barriers, which are discarded after each patient, should have been teeming with bacteria.

But the bacteria had gone missing. Something was fishy.

"It just didn't make any sense," says Alt-Holland, an assistant professor of endodontics and a co-principal investigator on the study. "You should find all kinds of bacteria on these barriers after a dental treatment."

In solving the mystery of the elusive bacteria, Alt-Holland and Perry, DG99, bagged an even bigger prize—a way to ferret out some of the most serious bacteria that infect patients in hospitals and other health-care settings. The discovery could have wide-ranging implications for the health and safety of all of us.

Scientists traditionally use a technique known as swabbing to collect and identify bacteria. They swipe a cotton swab across a surface and culture what's gathered on an agar plate in an incubator for 48 hours. In trying to hunt down the missing bacteria, Alt-Holland methodically eliminated all possible suspects along the testing chain: flaws in the agar plates used to grow the bacteria, the swabs, the swab solution, the incubation process. All came up clean.

The conclusion was troubling. Swabbing—the universally accepted method—must be the culprit. Had she overlooked something? And then it hit her: eliminate the middleman, the swab itself. She cut a piece off a discarded plastic barrier and pressed it directly on an agar plate. In 48

hours, multiple bacteria grew.

"It was such an easy idea, so simple, you wonder why no one thought of it earlier," says Alt-Holland, who named her bacteria-detection technique "stamping."

To confirm the reliability of stamping, Alt-Holland and Ramesh Thondapu and Kasun Rajapaksha, both D14, tested sets of barriers from five different operatories—half of each barrier was swabbed, the other half stamped. The results were nothing short of stunning: Swabbing turned up 294 bacteria colonies, while stamping identified 1,517 colonies—about five times as many.

In seven subsequent tests, the researchers used stamping to isolate and identify three harmless bugs—Staphylococcus haemolyticus, a common oral and skin bacteria; Pantoea calida, found in infant formula; and Bacillus infantis, a common ingredient in probiotics to aid digestion and boost immunity. "With swabbing, we likely would have detected none of them," Alt-Holland says.

"This is a very significant result," she adds. "If we think we have cleaned 300 bacteria, but there are 1,000 still waiting for us—and we don't even know about them—that may put patients at risk."

The implications are clearly unsettling. "What this means is that we cannot be 100 percent sure if the disinfectants we depend on to keep clinics, hospitals or public bathrooms clean and safe are working on all the bacteria," Perry says.

Game-Changer

The potential applications for stamping are enormous. It could be an important new weapon against antibiotic-resistant infections, which are

increasingly common in hospital patients. And the discovery could change infection-control protocols across the entire health-care industry.

"The stamping method is a breakthrough," says Toshihisa Kawai, chair of the immunology and infectious diseases department at the Forsyth Institute in Cambridge, Mass., who collaborated with Alt-Holland on the research. "The study clearly demonstrated that this method is remarkably more sensitive in detecting bacterial contamination than swabbing, although understanding why will take further study.

"We are always under threat of a potential pandemic infection, such as the E. coli strain that caused the deadliest outbreak on record, in 2011," Kawai says. "The stamping method allows us to detect sufficient bacteria to prevent the transmission of possible pandemic-causing germs in clinical settings." (The food-borne E. coli outbreak killed 53 people and sickened 3,950 others, primarily in Germany, but also in other countries, including the United States.)

Roughly one in 20 hospitalized patients acquires an infection, according to the Centers for Disease Control and Prevention (CDC). And approximately 1.7 million of these infections from bacteria and other microorganisms cause or contribute to 99,000 deaths in the United States each year.

Antibiotic-resistant bacteria, the so-called superbugs, are the most difficult to treat, and so can spread wildly. Among them is methicillin-resistant staphylococcus aureus (MRSA), which causes a life-threatening infection that accounts for 8 percent of all bugs patients pick up in the hospital, according to the CDC.

While more rigorous infection-control procedures have resulted in a decline in MRSA infections, another superbug, Clostridium difficile, which causes severe diarrhea, remains at historically high levels—linked

to 14,000 deaths each year in the United States alone. Another problem bacterium is vancomycin resistant enterococcus (VRE), which can result in urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis and meningitis. In one study, the VRE strain E. faecalis was found in seven out of 21 root canal-treated teeth involving an abscess at the root.

Bacteria flourish in all kinds of places. MRSA, for example, has been found on cabinets, counter tops, bed rails, bedside tables and other surfaces in most hospital patient rooms, according to the Committee to Reduce Infection Deaths (RID), a national advocacy and education group. Moreover, a nurse who has no direct patient contact, but touches objects in a hospital room, will have surgical gloves contaminated by bacteria 42 percent of the time, RID reports.

Clothing can also transmit bacteria. When doctors and nurses lean over a patient infected with MRSA, their white coats and uniforms pick up the bacteria 65 percent of the time—providing a free ride to another patient's room, according to RID. Perry and Alt-Holland suspect that the infant formula bacteria they found in their study probably hitched a ride to the dental clinic on a patient's jacket or shirt.

So far, Alt-Holland and Perry have used stamping only to evaluate the ability of environmentally friendly disinfectants to kill harmless bacteria, not the superbugs.

"With stamping, we may be able to identify solutions that can protect us in our cars, in our offices, where we eat," Perry says, "not to mention helping to lower risks for those in hospitals and clinics at greater peril due to surgical incisions, intravenous needles or just lowered immunity due to illness."

Environmental Practice

Stamping has allowed the Tufts researchers to return to their initial investigation of the dental operatory cleaners. They include the most common clinic disinfectant, isopropanol-based Cavicide, as well as some greener options containing ethanol, hydrogen peroxide and citric acid. After each patient visit in the Tufts clinics, all surfaces are sprayed with Cavicide, wiped down and then sprayed again, notes Shannon Balletto, the school's infection control administrator.

Perry, a clinical professor and director of the Gavel Center for Restorative Research at Tufts, was the other co-principal investigator on the eco-friendly cleaner research, which was funded by PureLife Dental, a dental supply distributor that is looking to add green cleaners to its product line. He's an advocate for green dentistry, a range of environmentally friendly practices now gaining national support among dental practitioners and professional organizations.

"I am looking for something to use in my private practice that kills the bacteria, but that I can use frequently and repeatedly over many years that will not corrode my equipment, be toxic to the environment or have fumes that endanger my health and that of my patients," he says.

Two of the green cleaners Perry and Alt-Holland evaluated proved as effective as isopropanol on all three of the harmless bacteria they tested using stamping. They presented their research during the annual meeting of the American Association for Dental Research earlier this year.

There has been very little research on disinfectants, says Perry, so most health-care providers choose one with isopropanol. While exposure to small amounts of isopropanol fumes is relatively harmless to humans, acute overexposure may affect the central nervous system, liver, kidneys, brain and cardiovascular system, according to the World Health Organization. Studies on long-term, low-level exposure are needed to determine if there are risks, Perry says. Stamping, he notes, will provide

a more accurate evaluation of the effectiveness of isopropanol as well as eco-friendly disinfectants.

"In terms of infection control and choosing the right kind of environmental solutions to work with, this research is super important," Alt-Holland says. "It will contribute greatly to protecting both our health and the environment."

Provided by Tufts University

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