# Current Topics

## Experts Formulate Seasonal and Prototype Avian Flu Vaccines on the Fly

Early each calendar year, experts assemble to reformulate the trivalent vaccine that protects people against influenza viruses anticipated to circulate in the northern hemisphere during the next season, designating appropriate antigens based on what seems to be circulating locally and in the opposite hemisphere. This past February, members of the Food and Drug Administration (FDA) advisory committee who undertake this task opted for a vaccine containing three new antigenic ingredients to ward off the seasonal influenza expected late this year—something of a departure from customary practices, but sensible in the face of flu viruses now circulating. The advisory committee also grappled with a more abstract and perplexing set of issues of how to formulate and use vaccines against what could become the next pandemic flu.

In terms of seasonal flu, although influenza A (H1N1) viruses predominated through mid-January of 2008, (H3N2) viruses were on the rise through late February, making them "the most common identified subtype for the season overall," according to officials of the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. Based on those findings as well as reports from elsewhere in the Northern Hemisphere, advisory group members concurred with the World Health Organization (WHO) and recommended formulating the 2008–2009 seasonal flu vaccine to contain three new antigens: A/Brisbane/10/2007-like H3N2, A/Brisbane/59/2007-like H1N1, and B/Florida/4/2006.

(In a related development involving a CDC advisory panel whose members met late in February, experts recommended that children from 6 months through 18 years of age be vaccinated yearly against influenza. This advice marks a shift from the previous recommendation to vaccinate only those children aged from 6 to 59 months against flu. The revised recommendation is to take effect no later than the 2009–2010 influenza season.)

Once finished with their annual duty of reformulating the seasonal flu vaccine, members of the FDA vaccine advisory panel wrestled with a related but grander challenge: How to formulate and then deploy vaccines to protect humans against avian flu—not only the H5N1 strain that continues to be more than 60% deadly for those it infects, but also its anticipated mutated successors that could prove much more efficient at moving from one human to the next.

Avian flu is continuing to circulate among humans, albeit causing small numbers of cases. In 2007, for example, it caused 86 human cases with 59 fatalities, mainly in Indonesia and the Nile Valley in Egypt, with additional cases in Vietnam, China, and other countries, according to WHO. In the majority of those cases, infected individuals had been exposed to sick or dead poultry. As of 11 March this year, WHO officials recorded another 23 human cases of H5N1 flu, 18 of them fatal. Thus, avian flu is "off to a rapid start" in 2008, says Nancy Cox of CDC, who advises the FDA committee.

Still relatively rare but extraordinarily deadly in humans, H5N1 influenza is circulating abundantly in domestic poultry and wild bird populations in many regions, particularly Asia and the Middle East, providing plenty of opportunities for genetic variants to emerge. By now, those variants fall into 9 clades (10 if those from the 1997 Hong Kong outbreak are counted), making the nomenclature to describe and track them increasingly "arcane," Cox says. Although viruses from any of those clades could lead to what instigates a pandemic, "only some" are being used to develop experimental vaccines, leaving "gaps where antisera don't inhibit well."

All those H5N1 variants and plenty of other uncertainties raise practical and philosophical questions about vaccine readiness plans. Experts now talk of developing both "pre-pandemic" and pandemic vaccines to protect against such infections—aiming first to prevent deaths and, next, to block severe infections. For example, officials of the FDA-like European Medicines Agency in February endorsed Prepandrix, a vaccine developed by GlaxoSmithKline Biologicals to trigger protective immune responses against H5N1 influenza, as acceptable for use in humans. It is one of several such vaccines intended to help European member states "prepare for pandemic influenza." Other "mock-up vaccines" in this group include Daronrix, Focetria, and Pandemrix.

None of these vaccines is considered ideal, and exactly what populations are to receive them is uncertain. For instance, the antigens in several of these vaccines prove poorly immunogenic on their own but are enhanced when adjuvants are added. The big questions are when and how to use them. "Look how long we've lived—5 years—with the risk of a pandemic

[with H5N1]," says advisory committee member Robert Couch of Baylor College of Medicine in Houston, Texas. In terms of using these experimental anti-H5N1 vaccines in humans, he adds, "if you can't assess the risk, how do you assess the benefit?"

For now at least, U.S. and European populations likely face a greater risk from being vaccinated with these products than they do of contracting H5N1 infections. Not only is there considerable uncertainty over whether any of those vaccines will adequately protect against whichever highly infectious-to-humans variants of H5N1 eventually break out. FDA officials also say that new adjuvants used with those vaccines will need to meet "high safety" standards. That safety requirement will need to be balanced against the goal of inducing high host-immune responses, figuring that higher levels of antibodies just might broaden the response to protect against the particular H5N1 variants that arise and threaten to wreak public health havoc.

**Jeffrey L. Fox**
Jeffrey L. Fox is the *Microbe* Current Topics and Features Editor.

### Several Developments on the Vaccine Front

Recent developments involving vaccine development and use include:

- An experimental vaccine, which was produced in insect cells with genetically engineered baculoviruses and uses virus-like particles, fully protected monkeys against the Ebola and Marburg viruses, according to Kelly Warfield of the U.S. Army Medical Research Institute of Infectious Diseases and her colleagues, who reported their findings during the 5th ASM Biodefense & Emerging Diseases Research Meeting, held in Baltimore, Md., last February.
- Noting that mitochondrial disorders are "associated with" degeneration of nervous and muscle tissue and with autism, officials of the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga., and several other federal agencies in March told parents that these conditions are not among "the risks associated with vaccines for normal children," and thus they urged parents to continue having their children immunized.

## In Biodefense Setting, Other Experts Outline Efforts to Confront Pandemic Flu

"We are definitely not prepared for a flu pandemic," says virologist and veterinarian Albert Osterhaus of Erasmus MC at University of Rotterdam in Rotterdam, the Netherlands, who was keynote speaker at the 5th ASM Biodefense & Emerging Diseases Research Meeting, held in Baltimore, Md., last February. In sharing that gloomy forecast, however, he and other participants reviewed some among the wide array of efforts under way to confront the pandemic potential of H5N1—a flu variant that is proving tenacious and deadly, but also elusive and remote.

Experts consider H5N1 influenza, also called avian flu, strains that are circulating throughout much of the globe a likely source of the next pandemic. More than 60% deadly in humans that they infect, those avian flu viruses have caused fewer than 400 human cases during the past 5 years—mainly in Asia, especially Indonesia, and also lately in the Middle East, particularly Egypt. Recent analyses indicate that H5N1 is not a well-adapted human pathogen. Thus, despite its deadliness, it only rarely infects humans, and there are not many asymptomatic cases or mild cases being overlooked, according to Kanta Subbarao of the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health in Bethesda, Md.

However, true to its name, avian flu is extensively distributed among bird species, including wild migratory birds throughout Europe and, less often, among domestic poultry flocks, mainly in Asia, according to Osterhaus. H5N1 infections among many types of wild birds are asymptomatic, abetting the spread of the virus, whereas the same viruses typically are "highly lethal" for farm-raised chickens and turkeys, he says. An additional worry is that commercial poultry flocks being raised on a free-range basis are even more often exposed than are their confined commercial counterparts to wild birds, including swans, ducks, geese, and gulls, carrying H5N1 avian flu viruses.

Osterhaus and his collaborators enlisted amateur bird watchers to help in tracking the avian flu virus by catching and releasing birds after taking cloacal swab samples with which to measure flu viral types and levels. One problem with this monitoring scheme is that investigators recently realized that the avian flu virus might be more commonly found in the throats and respiratory systems than in the cloacae of those wild birds, he says. "Maybe we're sampling the wrong end of the birds."

Some infected birds are likely delivering avian flu viruses to other species, including feline species such as domestic cats and tigers, lions, or similar species in zoos. In feline species, H5N1 infections are not limited to the respiratory tract, but tend to become systemic, according to Osterhaus. Something similar may occur in humans, perhaps helping to explain in part why H5N1 infections, although relatively rare, are so often deadly.

Subbarao of NIAID and her collaborators are evaluating a series of live, attenuated H5N1-based viral vaccines that can infect animals without causing serious illness. In both mice and ferrets, for example, these vaccines cause only mild infections, and the attenuated viruses are confined to the respiratory tracts of the mildly infected animals, she says. Although single doses produce little or no detectable neutralizing antibodies in treated mice, those animals are protected against otherwise lethal challenges by unattenuated H5N1 viruses, which are still found at high levels in the lungs of such animals. However, treating animals with two instead of one dose of vaccine blocks that nonlethal replication of the virus.

In separate experiments, monoclonal antibodies that are directed against hemagluttinin of H5N1 also can protect mice against the lethal effect of the avian virus, even when those antibodies are administered to mice 72 hours after they are challenged with the pathogenic H5N1 virus.

**Jeffrey L. Fox**

## Specific Cholesterol-Lowering Agents Blanch and Undercut *S. aureus*

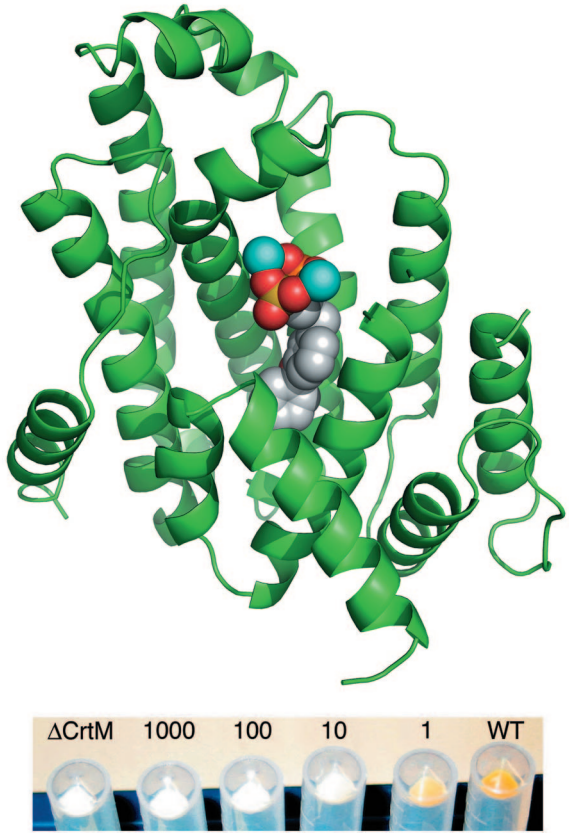
An experimental drug that interferes with the first step in cholesterol biosynthesis in humans also interferes with key pigment-forming steps in *Staphylococcus aureus*, rendering this bacterial pathogen more vulnerable to the innate immune system, according to chemist Eric Oldfield of the University of Illinois, Urbana-Champaign, Victor Nizet of the University of California, San Diego, and their collaborators. Not only might this approach lead to a new means for treating staph infections, but it may also prove applicable for treating diseases caused by protozoan parasites.

The collaboration between the Illinois- and California-based research groups traces to Oldfield's browsing through the October 2005 issue of *ASM News*, predecessor to *Microbe* (p. 450). A photo depicting carotenoid pigments in colonies of *S. aureus* caught his attention, as did the accompanying news story describing research by Nizet and his collaborators that linked those golden pigments with virulence. Knocking out a gene encoding an enzyme in the carotenoid pathway not only turned those bacteria white, but also makes them more susceptible to killing by the innate immune system.

"I noticed that the metabolic pathway involved had similarities to the one for the production of cholesterol in humans," Oldfield says. That led him to wonder whether cholesterol-lowering drugs could also render *S. aureus* and perhaps other pathogens more vulnerable to innate immunity. After speaking to Nizet, the two agreed to collaborate on testing drugs that block squalene synthase, the enzyme that controls the first step in cholesterol biosynthesis. In planning these efforts, they took advantage of drug development efforts by companies working on agents to target that enzyme. Commercial interest in that approach subsided after statins, which target other metabolic steps in the cholesterol pathway, captured this market segment.

Before being shelved, one candidate among those squalene synthase blockers, a phosphonosulfonate called BPH-652, made its way with a good safety record through two clinical trials as a cholesterol-lowering drug. BPH-652 binds not only human squalene synthase, according to X-ray crystallographic analysis, but also dehydrosqualene synthase, the bacterial enzyme that catalyzes the first step in making carotenoid pigments.

When low doses of BPH-652 are added to cultures of *S. aureus* cells, they lose their pigmentation. In other in vitro experiments, BPH-652 renders *S. aureus* 15 times more susceptible to killing by hydrogen peroxide, which is a key antimicrobial agent of the innate immune system, and 4 times less likely to survive in human whole blood than are cells of *S. aureus* that were not treated with BPH-652. Additionally, when *S. aureus*-injected mice are treated with BPH-652, there is a 50-fold decrease in bacteria accumulating in their kidneys, compared




Structure of the *Staphylococcus aureus* dehydrosqualene synthase enzyme, CrtM, involved in staphyloxanthin biosynthesis showing bound inhibitor BPH-652 (top), together with (bottom) effects of BPH-652 on pigment formation in *S. aureus* (concentrations shown are micromolar). (Images courtesy of Eric Oldfield, University of Illinois, Urbana-Champaign.)

## New Developments Involving Tuberculosis

Recent developments involving tuberculosis and *Mycobacterium tuberculosis* include:

- Multidrug-resistant tuberculosis (MDR-TB) soared to the highest rates ever recorded, based on surveys conducted between 2002 and 2006 in 81 countries, and extensively resistant (XDR-TB) is being found in at least 45 countries, according to a February report from officials of the World Health Organization (WHO). They estimate nearly half a million new cases of MDR-TB arise per year, amounting to 5% of 9 million new TB cases of all types. The highest rates of TB are in Baku, the capital of Azerbaijan, where nearly a quarter of all new TB cases (22.3%) are multidrug resistant.
- WHO officials estimate 9.2 million new cases of TB in 2006, including 700,000 among people with HIV and 500,000 cases of MDR-TB, according to a report released in March. An estimated 1.5 million people died from TB in 2006, while another 200,000 died from HIV-associated TB. Meanwhile, efforts to diagnose TB cannot keep pace with the expanding epidemic.

to mice treated with saline. Although other, newer inhibitors based on BPH-652 show even higher activity, their development "is years ahead," Oldfield says. Details appear in the online version of *Science*, February 14, 2008.

Cholesterol-lowering drugs such as BPH-652 or others like it could be combined with antibiotics to "deliver a one-two punch to really knock out the pathogen," says Julio Urbina, chairman of the Scientific Advisory Committee for the Drugs for Neglected Diseases Initiative in Geneva, Switzerland. Non-life-threatening staph infections of the skin and kidneys often turn into chronic infections, causing significant morbidity. This persistence of *S. aureus* might be due, in part, to limits of the innate immune system. Agents such as pigment-blockers that render pathogens more susceptible to that system "may finally clear these debilitating chronic staph infections," he says.

Meanwhile, some cholesterol-lowering drugs also show activity against protozoan parasites, including *Trypanosoma cruzi*, the agent of Chagas' disease, according to Urbina and his colleagues. Squalene synthase is essential for making ergosterol for its cell membrane, and depriving *T. cruzi* of these sterols leads to cell death in preclinical studies, he says.

**Carol Potera**
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## Plasmid Family Contains Stable but Genetically Supple Backbone

All five members of the IncW family plasmids share a remarkably conserved genetic backbone, according to Fernando de la Cruz of the University of Cantabria–CSIC-IDICAN, Santander, Spain, and his collaborators. Those five family members diversified only recently from a common ancestral genome, apparently responding to selective pressure from widespread use of antibiotics, the researchers report in the April *Antimicrobial Agents and Chemotherapy* (**52:**1472–1480).

Plasmids are nothing but extrachromosomal DNA molecules that replicate more or less autonomously within host cells. They commonly occur in bacteria, but are sometimes found in eukaryotic cells. Plasmids, which may be symbiotic or pathogenic, often carry genes that confer antibiotic resistance on their bacterial hosts. Little is known about their natural history.

"Our work suggests that plasmids are much more stable than bacteriophages," says de la Cruz. "Bacteriophages are highly modular, and their different modules (often single genes) evolve at high recombination frequencies. Plasmids preserve backbones for a much longer evolutionary time. We do not know why." Moreover, he continues, "Plasmids are relatively stable genetic entities, [which] suggests that many of the highly conserved backbone genes . . . are important in the plasmid's survival." The functions of those backbone genes remain "largely unknown," he adds, speculating that are "probably used in housekeeping [functions], such as in replication and conjugation."

These efforts to understand plasmid backbone genes in the IncW plasmids came after de la Cruz and his collaborators spent more than 15 years studying conjugation mechanisms, mainly in R388, which is one member within this larger family of plasmids. Once they knew how R388 behaved, they realized that many genes within this plasmid are not involved in either conjugation, replication, or stability. Nonetheless, those genes are highly conserved, he says. "We wanted to find out to what extent they were conserved in this plasmid family. Now we are certain that these genes are important for survival, and we want to find functions for them."

Some but not all the IncW plasmids, which are found in *Proteobacteria* species, acquired "integron platforms," and this acquisition happened relatively recently for those plasmids, according to de la Cruz and his collaborators, who first identified these gene-

capturing devices almost two decades ago. In addition to being found on plasmids, integron platforms also are sometimes found in transposons and some chromosomes.

Among their other attributes, integron platforms are particularly adept at acquiring genes encoding antibiotic resistance. Three of the IncW family members contain the class I integron platform. Because it is located at the same place along these three otherwise varied plasmids, it probably was acquired only one time. The platform itself remains identical within these three family members, further reinforcing the notion that it is a recent acquisition. Meanwhile, however, other IncW plasmids lack integron platforms and have another means, namely an $Ab^R$ transposon, for acquiring antibiotic resistance genes.

"I think these researchers provide two very important results," says Didier Mazel of the Pasteur Institute in Paris, France, referring to de la Cruz and his collaborators. "First, from a single backbone, you can develop very different resistance phenotypes," Mazel says, especially when it contains an integron. And second, he adds, "Selection for . . . the transfer region is very strong, and this is a very strong argument in favor of the selfish and parasitic behavior of such plasmids."

The project undertaken by de la Cruz and his collaborators is "ambitious" for seeking ways to "understand general principles of genome evolution," adds Ellen Zechner of the Institut für Mikrobiologie in Graz, Austria. By looking at this family of plasmids, the researchers are also providing valuable mechanistic insights into the conservative forces that preserve some plasmid gene modules, while allowing other genes to move more or less freely in and out of these established structures, she points out. On a more practical, public-health level, "understanding how antibiotic use drives genome dynamics and, specifically, the persistence and spread of resistance genes in bacterial populations is . . . [vital] for prolonging the efficacy of [those] antibiotics."

**David Holzman**
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## Promising Antimicrobial, Biodegradable Food Packaging Material

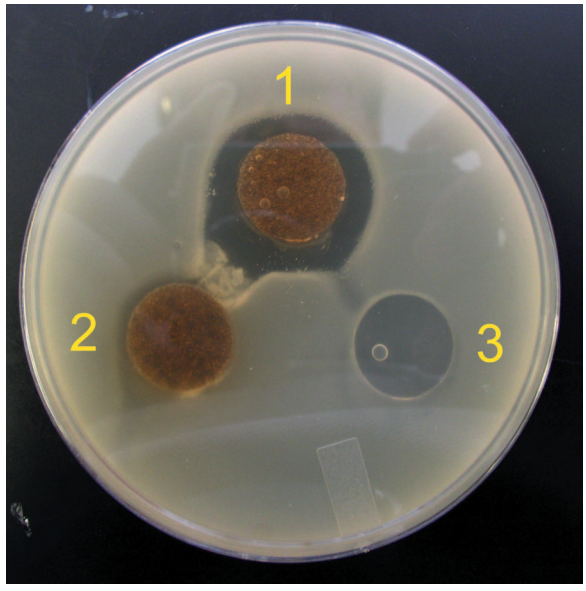
Biodegradable plastics embedded with antimicrobial bacteriocins may provide a new means for protecting perishable foods and curbing foodborne illnesses, according to chemist Tony Jin and his colleagues at the U.S. Department of Agriculture (USDA) Eastern Regional Research Center in Wyndmoor, Pa. The biodegradable material that they are developing not only targets foodborne pathogens, but also makes use of low-value agricultural wastes.

The USDA scientists are embedding bacteriocins into plastic films made from polylactic acid (PLA) and pectin. Nisin, a heat-stable antibacterial peptide, targets gram-positive bacteria, disrupting their membranes and also interfering with peptidoglycan synthesis. Nisin is uniformly distributed throughout the PLA film and is slowly released from it, according to Jin and his collaborators.

Produced by strains of *Lactococcus lactis* bacteria, nisin has been used as a food preservative for more than 50 years and was added to the Food and Drug Administration (FDA) list of generally recognized as safe (GRAS) food additives about 20 years ago. Because of its GRAS status, nisin can be added directly to foods such as packaged meats, cheese, eggs, and canned vegetables. Although nisin can kill sensitive bacteria on contact, it does not prevent survivors from recovering or subsequent contaminant bacteria from growing. "If you add nisin directly into food, it combines with other proteins, and its antimicrobial activity is less," Jin says. However, by loading nisin into food packaging materials, its antimicrobial activity can be extended, prolonging shelf-life for packaged foods and improving their safety.

During this testing phase, nisin is incorporated into PLA films, which then are juxtaposed with liquid foods such as orange juice and liquid egg whites that are "spiked" with familiar foodborne pathogens, including *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella enteritidis*. The nisin-containing PLA film reduces pathogen growth by 1,000-fold for up to 72 hours, compared to films lacking nisin. Details are reported in the April 2008 issue of the *Journal of Food Science* (**73**:M127–M134).

Adding pectin to the PLA enhances bacterial killing without detracting from the strength or flexibility of the



Antilisterial activity of films as determined by the agar diffusion method: 1, Pectin/PLA film with nisin; 2, Pectin/PLA film without nisin; 3, PLA film with nisin. (Image courtesy of Tony Jin, Eastern Regional Research Center, USDA-ARS, Wyndmoor, Pa.)

films, according to Jin. Moreover, pectin roughens the film surfaces, allowing higher amounts of nisin to adhere to them. In general, incorporating pectin into such nisin-containing films enhances their activity against bacteria such as *L. monocytogenes* by at least several orders of magnitude compared to comparable films lacking pectin. This work will be described in a forthcoming issue of the *International Journal of Food Science and Technology*.

Some food companies already use biodegradable PLA containers to package consumer products such as water, juice, and yogurt. PLA derives from renewable corn and wood residues, making this packaging material a "green" alternative to petrochemical-based plastics. Similarly, pectin, which is used as a thickening agent in jellies and jams, is made from renewable sugar beet and fruit pulp residues. "Food companies are very interested," Jin says, referring to the experimental, nisin-based packaging materials. "But we're still testing this [material] on a small scale, and commercial testing is at least two years off."

**Carol Potera**

## Odds, Ends, and Gems of Microbiological Developments

Here are some highlights amid recent microbiological developments:

- During conjugation, DNA molecules travel through pili from donors to recipient cells of *Escherichia coli*, according to Miroslav Radman of INSERM and Universite Paris Descarte Faculte de Medecine in Paris, France, and his collaborators, who report their findings in the March 14, 2008 *Science*.
- When in a vegetative state, cells of the parasite *Giardia intestinalis* maintain a pair of identical nuclei, and those two nuclei carry identical copies of the genome—indicating that this organisms transfers DNA efficiently during encystation to maintain homozygosity, according to W. Zacheus Cande of the University of California, Berkeley, and collaborators, who provide details in the March 14, 2008 *Science*.
- From a set of 276 *Caenorhabditis elegans* genes that modulate aging, there is an overlapping set of 25 genes that regulate aging in both yeast and nematodes, and 15 of those 25 yeast genes are "highly similar" to human genes that also might regulate longevity, according to Erica Smith, Matt Kaeberlein, and Brian Kennedy of the University of Washington, Seattle, and their collaborators, whose report appeared online, March 13, 2008, in *Genome Research*.

## Modeling Resistance Outbreaks May Improve Infection Control Strategies

Modeling antibiotic-resistant infectious-disease outbreaks in hospital settings could help in developing more effective infection control strategies, according to several participants at the symposium, "Modeling the Dynamics of the Drug-Resistant Killers of the 21st Century," convened during the 2008 meeting of the American Association for the Advancement of Science (AAAS), held in Boston last February. Such modeling works, in part, by enabling investigators to "create virtual hospitals," says symposium participant Glenn Webb, a mathematician from Vanderbilt University in Nashville, Tenn.

By incorporating parameters such as types of antibiotic treatments, their durations, and clinical outcomes, hospital administrators may begin to use such models to design infection control strategies that address a variety of different scenarios and contingencies, according to Webb. In untreated patients, nonresistant bacteria prevail over resistant strains, keeping the latter at very low numbers, according to his model. Factor in antibiotics, and the resistant strains make the patient a potential source of infections by resistant bacteria for as long as the treatment lasts. After treatments are completed, however, the nonresistant bacteria rebound.

One finding from such modeling, which can reveal unexpected patterns, is that the timing of antibiotic treatment courses can have important effects on clinical outcomes, particularly efforts to curb second-round infections, Webb says. Thus, for example, in those simulated cases in which antibiotic treatments are begun three days after diagnosis and continued for 18 days, there are high numbers of cross infections. However, starting antibiotics immediately upon diagnosis and continuing treatment for more than one week can greatly reduce the rate of such cross-infections within a hospital.

Another key factor to antibiotic resistance in hospitals is the behavior and practices of health care workers, who sometimes ferry infections around the hospital "like mosquitoes in malaria; they are the vector of disease spread," Webb says. However, when health care workers improve hygiene, the frequency of such cross-infections can be sharply reduced.

Antibiotic cycling, prompt diagno-

sis, and efforts to isolate patients harboring dual-resistant bacterial pathogens—those that are resistant to two unrelated antibiotics—can be "quite effective" in damping down dual resistance in infections within a virtual hospital being analyzed through modeling efforts, according to Carlos Castillo-Chavez of Arizona State University, another modeler who spoke during the AAAS symposium last February.

Castillo-Chavez and his collaborators use their model to compare antimicrobial cycling treatments of infected patients—in which different-acting antibiotics are strategically alternated—with an approach in which individual patients are randomly and successively treated with different antibiotics, and thus are not really considered as belonging to the same hospital population.

"This seems to be the first time that models have been used to deal with the evaluation of two distinct methods of reducing the impact of dual resistance in hospitals," Castillo-Chavez says. However, he adds, "Focusing on reducing dual resistance results in increases in the levels of individuals experiencing single resistance. . . Drugs provide no silver bullet, and only policies that reward their judicious use have a shot at slowing down what appears to be a losing battle."

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