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Super Drugs for Super Bugs

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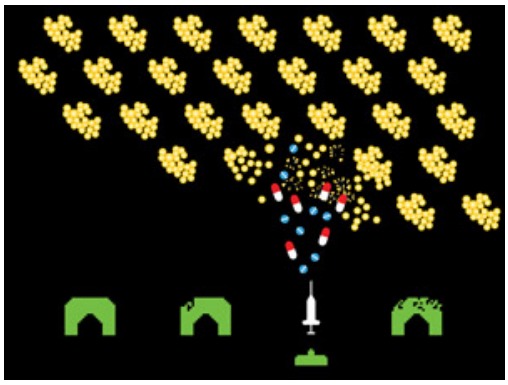


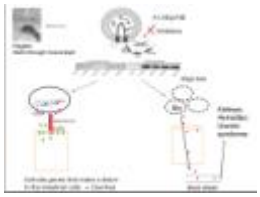
It's a typical workday in 1929, and in Oxford, UK, Dr. Alexander Fleming has just entered his lab. He sips his morning coffee, then one of the researchers in his lab brings a strange observation to his attention. It seems a mold has grown on one of the bacterial culture plates. Thinking that the mold is just contamination, Fleming orders the researcher to discard the plate. But destiny wouldn't let Fleming leave the plate in the trash. Later that day, he examines the plate again, this time a bit more closely. He observes that the mold has inhibited the growth of bacteria in the area around it. Fleming didn't realize it then, but he had just made one of the most important discoveries, albeit accidental, in human history.

What Fleming discovered that day was the first antibiotic: penicillin. A natural product of the *Penicillium* species of mold, penicillin would not be analyzed in pure form for another 11 years. And it would not be until 1943 before penicillin would be used to treat bacterial infections in humans. Viewing penicillin as a panacea, microbiologists and clinicians alike agreed that no more microbiological research needed to be done. However, due to its widespread use in World War II, bacteria quickly became resistant to penicillin, rendering the antibiotic useless. It was discovered soon thereafter that the resistant bacteria were producing enzymes that could destroy the structure of penicillin. To combat this, drug developers produced semi-synthetic versions of penicillin in 1960; a year later, bacteria became resistant to this, too.

According to Kunyan Zhang, MD, MSc, PhD, assistant professor, **University of Calgary**, Calgary, Alberta, Canada, *Staphylococcus aureus* became resistant to methicillin (a derivative of penicillin) in 1961, soon after the introduction of methicillin in 1959 to overcome the problem of penicillin-resistant S. aureus due to ß-lactamase (penicillinase) production. The source of resistance for methicillin-resistant Staphylococcus aureus (MRSA) is the methicillin-resistant gene (*mecA*) carried on a mobile genetic element, termed the staphylococcal cassette chromosome *mec* (SCC*mec*).

The danger of this cassette is that it does not just carry the methicillin-resistant gene but also resistance genes for other antimicrobials as well. This cassette can be transferred to susceptible bacteria found in the same environment as resistant bacteria, rendering them resistant. "It used to be that MRSA was limited only to the hospital and confined to vulnerable hospital patients," says Zhang. "But starting early 1990, MRSA started to be found in the community. This newly emerging, community-associated MRSA is now causing serious community-acquired infections/outbreaks in otherwise healthy children, athletes, and other individuals lacking typical risk factors for nosocomial MRSA acquisition." The community MRSA appears to be more virulent. And what's worse is that now community-associated MRSA has gone back to the hospital in a multi-drug-resistant form.

Bala Hota MD, MPH, assistant professor, Section of Infectious Diseases, **Stroger Hospital of Cook County/Rush University Medical Center**, Chicago, Ill., knows both forms of MRSA all too well. Hota's interest in antimicrobial resistance is both community- and hospital-acquired MRSA. In his most recent study, published in *Clinical Infectious Diseases*, he looked at cases of hospital-associated MRSA over a seven year period. "In that study, we found that while our overall rate of bloodstream infections was stable and quite low compared to some other studies we had looked at, our strain types were being replaced," says Hota. In other words, the hospital strain was being replaced by a community-acquired strain called USA300, an event which, according to Hota, has occurred in half of all US hospitals. Another study Hota did, published in May 2007 in Archives of Internal Medicine, showed a seven-fold increase in the incidence of community-associated MRSA over a seven year period. In this study, those who had served jail time or had lived in public housing were the greatest sources of community-acquired MRSA. "So, for example, some people might be housed in the jail or correctional facility where outbreaks of MRSA have been well reported. … And when they get discharged, they might enter public housing … and then that becomes a new epicenter of a MRSA epidemic," says Hota, who speculates that this is why there is now a higher incidence of MRSA in the community.



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The novel antimicrobial inhibitors prevent QseC from recognizing its signals (autoinducer-3, epinephrine and norepinephrine), thereby inhibiting the signaling cascade that leads to the activation of the bacterium's virulence genes. (Source: Vanessa Sperandio, PhD)

All adults are at risk for infection with the community-associated MRSA strain, but efforts to reduce the incidence of MRSA are occurring nationwide. For example, Hota says that in an effort to reduce spreading of MRSA in the hospital facility,

Crossing disciplines

Another researcher studying the resistance problem in Gram-negative bacteria is Vincent H. Tam, PharmD, BCPS (Infectious Diseases), assistant professor, Department of Clinical Sciences & Administration, **University of Houston College of Pharmacy**, Houston, Texas. Tam is studying the relationship between antibiotic exposure and the emergence of bacterial resistance, particularly in the Gram-negative bacterium Pseudomonas sp. And the ultimate goal of the study is to cross the disciplines of pharmacology and microbiology. The studies employ the traditional microbiological surveillance approach as well as a population pharmacokinetic approach.

In the microbiologic approach, Tam and his collaborators at the University of Texas Medical Center periodically harvest bloodstream isolates of *Pseudomonas sp*. bacteria from hospitals and analyze these isolates for prevalence and mechanism of resistance to antimicrobial agents. Tam accesses the antibiotic susceptibility profile for these isolates through the hospital's database and uses this information to calculate the prevalence of resistance and track the prevalence over time. Tam is currently studying mechanisms of carbepenem and multi-drug resistance. To determine the molecular mechanism of resistance, he uses a wide variety of tools ranging from spectrophotometry to standard molecular techniques such as PCR.

The population pharmacokinetics approach involves optimizing the dose of an antimicrobial agent at the site of infection. In this study, clinicians expose different patient groups to different antimicrobial agents and then collect serum samples or other body fluids, prospectively. "Since we know how long it has been since they received the drug, we are able to do pharmacokinetic modeling to describe the disposition of the drug over time as a form of population kinetics model," says Tam.

A third kind of study performed by Tam involves experimental therapeutics. "Essentially, we use an infection model to expose different types of bacteria to different concentration-time profiles in order to understand what is a good exposure to eradicate the bacterial population and what doses facilitate the emergence of resistance," says Tam.

Tam collaborates with many pharmaceutical companies including AstraZeneca, Merck, and Schering-Plough. These companies sponsor projects to study antibiotic resistance in *Escherichia coli*, *Acinetobacter*, and *Pseudomonas sp*. bacteria**.**

hospitals in the state of Illinois require that, prior to admission, all patients be screened for MRSA colonization. "The common site that *Staphylococcus aureus* colonizes individuals is in the nose. And what hospitals are doing is taking a cotton swab, rubbing it in the nose and then setting that up for culture. So that will either show MRSA or not," says Hota.

Hota is also interested in better characterizing the community-associated strains of MRSA. For all of the clinical isolates his group has collected, they first identify the strain type (i.e., USA300 or USA400) using pulsed-field electrophoresis; determine by PCR whether or not the strain contains the SCC*mec4*; and, determine by PCR whether or not the strain carries specific toxins such as Panton-Valentine leukocidin (PVL), which is associated with boils produced by MRSA. "So we are finding that SCC*mec4* and PVL are very strongly associated with the community-associated MRSA strains," says Hota. The results of these tests are then compared to strains from national outbreaks. And Hota's strains are identical to those found in the national outbreaks.

In addition to Hota's work, Zhang has developed and published a multiplex PCR assay that distinguishes MRSA from methicillin-susceptible S. aureus and other bacteria and, more importantly, can be used to determine whether the isolate carries the PVL gene and whether it is the USA300 or USA400 strain. This test is for research purposes only.

Down, but not out

The issue of antimicrobial resistance has caused a significant decrease in the number of available antibiotics for treatment. "I think, especially in children, we are running out of therapeutic options," says Margaret R. Hammerschlag, MD, professor of pediatrics and medicine, and director, Division of Pediatric Infectious Diseases, **SUNY Downstate Medical Center**, Brooklyn, N.Y., who adds that there are many antimicrobials coming out for resistant Gram-positive bacteria like MRSA, but there is nothing for Gram-negative bacteria like *Klebsiella sp.* and *Acinetobacter sp.* Hammerschlag faces bugs that are resistant to every antibiotic and for which there is no foreseeable antibiotic development. "We have got a real crisis. We'll be looking down a hole to the pre-antibiotic era and antibiotics might end up as orphan drugs."

But researchers like Vanessa Sperandio, PhD, associate professor of microbiology, **University of Texas Southwestern Medical Center**, Dallas, Texas, are designing antibiotics to combat the resistance problem and Gram-negative bacteria. Design of novel antimicrobials to combat resistance requires a higher degree of understanding of the biology of superbugs. In her work, Sperandio started by primarily targeting *Escherichia coli* 0157:H7, a cause of *E. coli*-related, food-borne illness. She studies a hormone-based signaling system that allows for communication between bacterial cells and between the bacterial cell and the host, which in this case is human. This system, which is more complex than quorum sensing, is activated when an aromatic compound called autoinducer-3 is sensed by a kinase called QseC found in the *E. coli* 0157 plasma membrane. Autoinducer-3 is produced by many species of bacteria including *E. coli* and by the normal intestinal flora of humans. A second signal recognized by QseC is produced by the two stress hormones normally present at significant levels in the human intestine: epinephrine and norepinephrine. "So basically by recognizing these cues, *E. coli* 0157 notices that it is inside the colon, the right site for infection," says Sperandio. Upon sensing any of these signals, QseC autophosphorylates, consequently initiating a signaling cascade that leads to regulation of virtually all of the bacterial virulence genes. "So because we noted that this system was important *in vitro* and *in vivo* for this pathogen—which basically has no treatment whatsoever at this point—we wanted to target it for antimicrobial development," says Sperandio.

And so Sperandio *et al.* have developed a QseC inhibitor as an antimicrobial agent. Currently, this small molecule inhibitor is being tested in mice. The preliminary toxicology work performed on this drug has shown it to be nontoxic. Preliminary efficacy data show that the compound does not inhibit bacterial growth and it does not kill the bacteria. And this is a good thing because treating E. coli 0157 with an antibiotic that is bacteriocidal causes these bacteria to release a potent toxin that can cause immediate kidney failure, which in some cases can lead to death. "So the line of thought in the field is that if you're going

to try to develop something that is going to prevent pathogenesis, but is not at the same time going to kill the bacteria, you're engendering less evolutionary pressure for development of resistance," says Sperandio.

In addition to *E. coli* 0157, Sperandio has also observed QseC homologues in at least 25 other medically-important pathogens including *Salmonella sp.*, *Shigella sp*., *Yersinia sp*., and *Francisella tularensis*. And in fact, her QseC inhibitor has been shown to protect mice from being infected by salmonella and Francisella tularensis. For example, Sperandio says that a single dose of this inhibitor will protect mice from *Francisella* infection. "So basically what this inhibitor does is compete with the signals to bind the kinase. So the signal cannot activate in the animal. And if the kinase does not activate, the virulence genes do not activate. And in this way the bacteria just passes through."

All of the preliminary work on this compound thus far has been performed in animals, but Sperandio hopes that the drug will make it into clinical trials. She says "all of the work has been done academically and we have a small start-up company as a partner. But big pharma is going to have to take over if this [compound] is to go somewhere."

In summary, antimicrobial resistance is a monumental public health problem. It affects everyone from infected patients and physicians to researchers and drug developers. Increased research efforts toward understanding mechanisms of resistance, as well as identifying new antimicrobial targets, is greatly needed. Large drug companies are well aware of the problem, but have been fighting an uphill battle to develop antibiotics that exist long enough on the market to turn a profit. Consequently, smaller drug companies and academic institutions have taken it upon themselves to develop new antimicrobials. However, these drug developers require large drug companies to put their drug discoveries on the market. Hopefully, big pharma will heed the call.

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http://www.dddmag.com/Article-Researcher-Fight-Antimicrobial-Resistance.aspx