

A shifting threat

Development of antibiotic resistance and spread outside of hospitals weighs on researchers' efforts to tame *Staphylococcus*

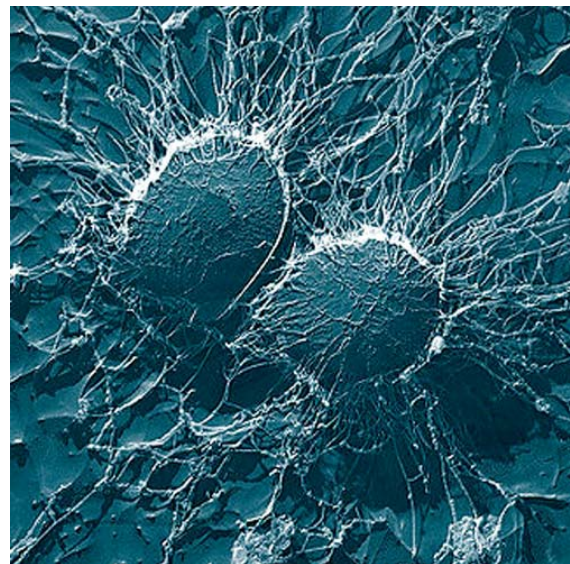
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Otherwise-healthy children die of unusual infections. Antibiotic-resistant bacteria spread through sports teams. And the last line drug loses effectiveness. The threat of *Staphylococcus* to human health continues, and presents new challenges for doctors and scientists. Researchers are delving into developing new antibiotics, understanding why staph infections aren't restricted to hospitals anymore, and figuring out how the bug evades the host immune system.

Staphylococci are gram-positive bacteria that commonly inhabit humans; approximately one-third of the population harbors the bacteria in the nose or on skin. *Staphylococcus*, or staph, is normally benign, and occasionally causes relatively harmless infections such as skin boils and acne. But the bug also causes ear infections, and even more harmful diseases such as endocarditis, pneumonia, and toxic shock syndrome. The bug is also responsible for infections around medical devices such as replacement joints, catheters, and breathing tubes. Dialysis patients are also at risk for staph infections. *Staphylococcus aureus* is typically the first bacterial species that infects cystic fibrosis patients.

Staphylococci cause these varied problems because they can produce numerous types of toxins. The bacteria can produce hemolysin and leukocidin, molecules that destroy blood cells. They can make an enterotoxin that can cause food poisoning. They can make proteins that trigger massive immune responses, tissue-degrading proteins, and other proteins that interfere with host defenses. Several species of staphylococci can spur infections in humans, but *Staphylococcus aureus* is the most common cause.

Up until recently, staph infections occurred almost exclusively in hospitals, playing on vulnerable patients, such as those with compromised immune systems or who had just undergone surgery. The introduction of penicillin in the 1940s stemmed staph infections in hospitals. However, staph strains quickly developed resistance to penicillin. In response, scientists developed methicillin, a synthetic penicillin, but staph quickly learned to evade it, as well. Methicillin was introduced in Europe in 1959 and in the United States in 1961; methicillin-resistant strains appeared in Europe by 1961 and in the United States by 1968.



Bacterial cells of *Staphylococcus aureus*
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In part, resistance has developed because bacteria are built to adapt. They divide quickly, mutate readily, and can swap genetic material with other bacteria. But humans have helped them along. Overuse of antibiotics, both in medicine and in agriculture, creates more opportunities for resistant bacteria to emerge. In addition, people who are prescribed antibiotics often don't complete their course of treatment, increasing the selection for resistance. Furthermore, poor hygiene practices in hospitals can help resistant bacteria spread to vulnerable patients.

Now, 60% or more of hospital *S. aureus* strains resist all forms of penicillin; these strains are termed methicillin resistant *S. aureus*, or MRSA. Perhaps half of all hospital infections are caused by *Staphylococcus aureus*, so the development of antibiotic resistance is a major health threat.

Hunting for Antibiotics

An antibiotic called vancomycin is currently the drug of last resort against *Staphylococcus*. It has drawbacks: it is toxic, and must be given intravenously. But it kills MRSA. However, in the late 1990s, strains appeared that required larger than normal doses of vancomycin to kill. And in 2002, the first cases of vancomycin resistance were reported. Once treatable infections are now more likely to kill, as doctors lose one antibacterial weapon after another.

At the same time, pharmaceutical companies no longer devote the same effort to discovering new antibiotics as they once did. New versions of existing antibiotics might work for a short time, but they would likely quickly lose effectiveness as bacteria inevitably develop resistance. As a result, companies could only sell a new product for a short time and would face difficulty in earning back the cost of developing a drug. Instead, more and more drug companies are turning to drugs that treat chronic disease, where profits are more reliable.

Devising antibiotics with completely new modes of action is arduous. In the last 40 years, only three new antibiotics with truly new modes of action have made it to market: daptomycin, tigecycline, and linezolid. Daptomycin, a so-called cyclic lipopeptide, is sold by Cubist Pharmaceuticals as Cubicin. It was approved in the United States in 2003 to fight *S. aureus* skin infections, and some studies hint that it might be effective against blood and heart infections caused by the bug.

Tigecycline was approved in 2005 for *S. aureus* skin infection. Wyeth markets it as Tygacil. Linezolid is the first antibiotic in the oxazolidinone class, and is used to treat pneumonia and skin infections caused by staph. In addition, Synercid was approved in 1999 for staph infections. This is the first member of the streptogramin class of drugs, which combines two existing antibiotics that together kill bacteria. While these new drugs may restock the arsenal against bacterial infections for a short time, doctors will constantly be playing catch up, as bacteria develop resistance to the newest antibiotic.

Other molecules are also showing promise as future antibiotics. Osterhelt and colleagues showed in October 2005 in *Nature Medicine* that molecules called acyldepsipeptides prevent the growth on Petri plates of antibiotic resistant bacteria including *S. aureus*. The molecules also helped 80% of mice infected with staph survive.

Further studies revealed that the molecules hone in on a bacterial protease, a new target for antibiotics. The molecules unhinder the protease, and the unrestrained protease activity somehow halts cell division. It's unclear whether molecules such as these will develop into full-fledged antibiotics, but discovering entirely new kinds of antibiotics will be crucial to keep up with the spread of antibiotic resistant bacteria.

Most antibiotics are derived from naturally occurring anti-bacterial compounds. Scientists have thoroughly searched the soil bacteria that produce most of the known antibiotics, but they are still finding new leads. For instance, Wang and colleagues in *Nature* (2006) and *PNAS* (2007) have described related natural products that inhibit fatty acid production and block growth in bacteria, including antibiotic-resistant strains.

Still other groups are gaining an improved understanding of how existing antibiotics work to get clues about how to improve them. In the March 9, 2007 issue of *Science*, Lovering and colleagues used x-ray crystallography to get a 3-dimensional picture of a penicillin-related antibiotic called moenomycin clinging to a staph protein called PBP2. This protein helps the bacterium make its cell wall; moenomycin and related antibiotics block this protein from making normal cell walls. Moenomycin is used in animal feed to help livestock grow and has thus far bacteria seem to have not developed resistance. Understanding this structure could help researchers develop new antibiotics for humans that similarly evade the development of resistance.

Other groups are probing new ways of treating infections once they develop. Buonpane and colleagues, reporting in the June 2007 issue of *Nature Medicine*, are developing an antidote to one *S. aureus* toxin, Staphylococcus enterotoxin B (SEB). SEB causes toxic shock syndrome by binding to T cells through T cell receptors and over activating the immune system throughout the body. The researchers are developing small, free-floating versions of the T cell receptor; in this strategy, these receptor mimics soak up the toxin and prevent it from binding to the receptors on T cells, thereby preventing an immune response. They've developed one such molecule and have shown that it protects rabbits exposed to toxin.

To be successful, the molecule would need to be combined with one or two other molecules that bind to other toxins. Once the team has identified other molecules, they intend to test animals against infections by whole bacteria. Because it interferes with the host immune response rather than killing the bacteria, this strategy would be most useful for treating patients that have already gone into sepsis. It might complement human intravenous immunoglobulin, the last resort for patients in septic shock.

Researchers are also delving into ways that *S. aureus* evades the immune system. For instance, *Staphylococcus* forms biofilms, organized communities of bacteria growing together in a molecular matrix. This organization is especially important for the growth of bacteria on medical devices, such as catheters or breathing tubes, or in the lungs of cystic fibrosis patients. In the April 23, 2007 issue of *PNAS*, Rice and colleagues show that staph do not form biofilms when they have a mutation in a gene called *cid*, which is involved in a bacterial cell death pathway. They further showed that cells that die release DNA, which forms an important component of the biofilm's matrix. Normal biofilms disintegrated when treated with a DNA-dissolving enzyme, supporting the idea.

As the prevalence of antibiotic resistance grows, so does interest in vaccines that prevent infections from taking hold. Despite some promising findings in animals, however, human testing of staph vaccines has foundered. Testing is risky, because researchers must show that vaccines protect healthy people, rather than treat sick ones. That means that trials need more people enrolled to be able to show statistically significant protection.

And two trials of staph vaccines have failed recently. In 2005, clinical trial data showed that StaphVAX, a vaccine developed by Nabi Biopharmaceutical, failed to protect kidney dialysis patients from staph infections. The vaccine contained fragments of sugars found on the surface of bacteria. The company has since stopped work on the vaccine. Veronate, under testing by Inhibitex, Inc., appeared to protect low-birthweight babies from staph infections in Phase II trials, but that initial finding didn't emerge from Phase III trials, the company announced in 2006. Veronate contains antibodies that bind to adhesion molecules carried on the surface of bacteria.

Despite these failures, additional vaccine targets are in the offing. In the October 30, 2006 issue of PNAS, Schneewind and colleagues immunized mice with four staph surface proteins that they found were similar among many staph strains. Each of the proteins provide some degree of protection to mice from kidney infections, but when combined, the proteins protected against kidney disease to an even greater extent. In addition, they saved mice exposed to a lethal dose of *S. aureus*. Mice vaccinated with only one of the four proteins did not survive the lethal dose.

Other promising targets for vaccines include a molecule called RAP. This protein activates a regulatory RNA molecule called RNAIII, which in turn turns on genes that help staph attach to and invade host tissue. Another possible candidate is a surface sugar called PNAG, which apparently encourages *S. aureus* to form organized biofilms. Vaccinating mice with PNAG protects them from *S. aureus* infection. But despite numerous leads and tantalizing findings in animals, the road to an effective, approved vaccine is long.

Yet efforts to find new antibiotics, treatments for infection, and vaccines are only becoming more important. Once restricted to vulnerable patients in hospitals, staph infections are becoming increasingly common outside of the hospital. Numbers of infections by so-called community-acquired MRSA (CA-MRSA) are on a rapid rise: perhaps 12% of staph infections are due to CA-MRSA, which infects healthy people, rather than people with compromised immune systems, or those who have just had surgery. They have hit football and wrestling teams, and have spread through prison populations and populations of gay men. They typically cause skin infections, but can be much more pernicious, triggering a form of pneumonia that kills lung tissue, and causing Waterhouse-Friedreichsen syndrome, a disease in which skin, joints, and internal organs hemorrhage and can be deadly.

CA-MRSA strains don't seem to be derived from hospital strains, but instead are new versions of the bacteria. For instance, they carry a different version of the DNA element containing the methicillin-resistance gene than do hospital MRSA strains. In addition, although resistant to methicillin and other penicillin-related antibiotics, they are sensitive to other classes of antibiotics—at least some good news for treating the rising infections..

In addition, they are clonal: most infections are caused by the same strain, rather than different strains being responsible for different outbreaks. In 2006, researchers sequenced the complete

genome of the most common clone of CA-MRSA, called USA300; their findings were reported in the March 4, 2006 issue of *The Lancet*. They identified genetic elements unique to USA300 that didn't appear in related *S. aureus* strains. Understanding the function of these elements could lead to better knowledge about why USA300 is so virulent, and could point to new drugs that prevent the spread of CA-MRSA.

CA-MRSA seem to be more virulent than hospital versions, up to now the reason for that is not understood. Some teams are now investigating whether particular molecules make community-associated staph so deadly—and are making apparently conflicting findings. Infections in the community frequently are caused by staph strains that produce a toxin called Panton-Valentine Leukocidin, or PVL. But it has been unclear how this toxin influences disease.

In 2006, researchers tested the effect of PVL by infecting mice with strains that produced PVL; other mice received strains that did not produce PVL. Voyich and colleagues reported in the *Journal of Infectious Disease* that strains without PVL were just as virulent as those that made the toxin: just as many mice died when treated with PVL-lacking strains. In addition, the different strains caused similar extents of skin abscesses, and were similarly able to kill human immune cells.

In 2007, another team investigated the role of PVL in pneumonia. Reporting in the 23 February 2007 issue of *Science*, Labandeira-Rey and colleagues tested strains with and without PVL, and found that only when a strain contained PVL did it cause necrotizing pneumonia in a mouse. This severe form of pneumonia kills lung tissue. In addition, they found that PVL boosts quantities of a molecule called protein A by the bacteria. This molecule prevents bacteria-eating immune cells from working. The team went on to show that bacteria lacking protein A weren't as able to cause disease and death in mice.

These seemingly conflicting studies have raised an apparent controversy about whether PVL is or is not responsible for disease, and further studies are needed to resolve the discrepancy. Researchers may find ultimately that PVL is a crucial trigger for some types of diseases but not for others. Or the combination of PVL with other proteins expressed by a particular strain might play a large role in determining the severity or type of disease caused by *S. aureus*.

Overall, the current picture of *S. aureus* is a dire one. Drugs to treat staph infections are diminishing, the bug is spreading outside of hospitals, and the antibacterial drug pipeline has slowed to a trickle. Staph biology is receiving considerable attention from basic researchers, but translating those findings into real clinical solutions is an urgent, daunting challenge.