Rich Display of Novel Antimicrobial Agents at 47th ICAAC

Measured in terms of sheer numbers, the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) was noteworthy in terms of the novel antimicrobial structures being presented, according to Karen Bush of Johnson & Johnson Pharmaceutical (J&J) in Raritan, N.J., a co-convenor of the leadoff poster summary session, “All New Antimicrobial Agents.” More than 100 novel antimicrobial structures were unveiled at that and other sessions—the “biggest number in many years,” she says—referring to the most recent ICAAC, held in Chicago, Ill., last September. These novel agents, still in preclinical development, include diphenyl urea compounds, a hybrid version of established antibiotics, lipopeptides that block cell-wall synthesis, antisense compounds, phage-carried proteins that block bacterial DNA, inhibitors of transfer-RNA (t-RNA) synthesis, and new types of β-lactamase inhibitors, as well as antifungal agents belonging to a new class.

The compound designated AR-2474 and several other members in a novel class of 1,3-diphenyl urea agents are bactericidal against a broad range of gram-positive as well as a narrower set of gram-negative pathogens, according to Stephen Hawser of Arpida AG in Reinach, Switzerland. AR-2474 is active topically, and is being developed for treatment of skin infections caused by methicillin-resistant Staphylococcus aureus (MRSA), he says. AR-2474 and other members of this class are also active against many additional drug-resistant gram-positive clinical isolates, and the “propensity of mutants to develop resistance to the [diphenyl urea compounds] is very low.” Although he calls the mechanism of action “distinctly different from anything to date,” Hawser declined to describe that mechanism or how the new agent is being formulated.

CBR-2092 is a “hybrid” antimicrobial, consisting of a quinolone linked to rifamycin, that is active against gram-positive bacteria, according to Simon Lynch of Cumbre Pharmaceuticals Inc., in Dallas, Tex., and his collaborators. Not only is it superior in potency when compared to the parent antibiotics from which it is formed, but also “retains many of their key attributes,” he says. Moreover, the hybrid is effective against bacteria in biofilms, including those cells called “Persisters” that typically prove resistant to many other types of antibiotics. This hybrid does not appear to break down through metabolism when administered to animals, and it also does not act as a “self-antagonist,” even though rifamycin can antagonize quinolones when the two are administered separately.

Fruilimicin, a cyclic lipopeptide antimicrobial compound, blocks cell-wall synthesis in gram-positive bacteria through a “novel mechanism,” according to Stefan Pelzer of Combi-nature Biopharm AG of Berlin, Germany, and his collaborators there, at AB Biodisk in Solna, Sweden, Anti-infectives Intelligence GmbH in Rheinbach, and at several universities in Germany. Thus, this compound, which was derived from soil-dwelling microorganisms, blocks synthesis of two components of gram-positive cell walls, namely peptidoglycan and teichoic acid, and it also induces proteins that are “markers... for cell envelope stress.” Although its spectrum of activity is like that of daptomycin, another cell-wall inhibitor, their modes of action differ, and there is no cross-resistance of fruulimicin with daptomycin-resistant isolates. However, mutants that develop thicker-than-usual cell walls are “less susceptible” to the new agent, he notes.

Another approach to developing novel antimicrobial compounds involves oligomers, in this case acting through an antisense mechanism in which phosphorodiamidate morpholino oligomers (PMOs) block translation by binding to messenger RNA molecules in bacterial cells, according to Bruce Geller of Oregon State University and AVI BioPharma, both in Corvallis, Ore., and collaborators there and at the National Institutes of Health (NIH) in Bethesda, Md. The PMOs being tested contain 11 bases that are coupled to peptides. So far these compounds are being targeted to specific bacterial pathogens, including Escherichia coli and, separately, the Burkholderia cepacia complex (BCC).

A set of small acid-soluble spore proteins (SASP) can tie up the DNA of bacteria, halting replication and transcription while leading to loss of viability, according to Heather Fairhead of Phico Therapeutics Ltd., in Cambridge, U.K., and her collaborators there and at the Health Protection Agency, also in Cambridge. Although these proteins cannot enter cells on their own, they prove to be potently antimicrobial when transported into bacterial cells via phage, she says. The first candidate product being devel-
A bacterial methionyl tRNA synthetase in *Clostridium difficile* is the target of Rep3123, a diaryldiamine with a narrow activity spectrum that is being evaluated for its activity against this pathogen, according to Nebojsa Janjic of Replidyne in Louisville, Colo., and his collaborators. Rep3123 inhibits the charging of this particular tRNA molecule and is bacteriostatic against this and various other gram-positive bacteria, he says. The compound, which has a low propensity for disrupting gut bacteria, can block sporulation by *C. difficile* and production of its toxins. Moreover, it appears to be more effective than vancomycin against *C. difficile* when tested in animals, he adds.

Meanwhile, several novel bridged bicyclic monobactams prove to be potent inhibitors of class C β-lactamase enzymes, whose catalytic activity can undermine the effectiveness of penicillins and other β-lactam antibiotics against gram-negative pathogens, including multidrug-resistant *Acinetobacter* spp., according to Malcolm Page of Basilea Pharmaceutica AG in Basel, Switzerland. Some of these compounds, particularly a siderophore designated BAL0019764, when mixed with other β-lactamase inhibitors such as clavulanic acid, prove highly active against gram-negative pathogens that produce multiple types of β-lactamase, he says. However, the activity against strains that produce extended-spectrum β-lactamase inhibitors (ESBLs) is “significantly weakened” when clavulanic acid is not added along with the novel inhibitor.

In a similar vein, novel derivatives of maleic acid make up a new class of metallo-β-lactamase inhibitors (MBLs) with “potent” activity, according to Munee Hikida and his collaborators at Meiji Seika Kaisha, Ltd., in Yokohama, Japan. One such derivative, designated CP3242, “remarkably enhanced activity of β-lactams against resistant strains,” including *Pseudomonas aeruginosa*, leading to 100% survival of infected mice that were treated with this compound combined with carbapenem, he says. CP3242, which has “no antibacterial activity by itself,” is a “specific inhibitor of plasmid-encoded MBLs.”

Moving from bacterial to fungal pathogens, a bis-arylamine, designated T-2307, is “potently active” against *Candida albicans*, showing more activity than either amphotericin B or fluconazole when tested in vitro, according to Jun-ichi Mitsuyama of Toyama Chemical Co. Ltd., in Toyama, Japan, and his collaborators. T-2307 also shows “superior efficacy compared to reference drugs” when used to treat various fungal infections in mice, he says. Fungi but not mammalian cells “selectively accumulate” the compound, which acts on targeted fungal cells by causing a “collapse of mitochondria” through a loss of membrane potential.

The development of these novel agents comes mainly from researchers in industry who are nearly evenly scattered among Japan, Europe, and the United States, according to Bush
of J&J. Although researchers “are responding to the challenge to find new antimicrobials agents with new mechanisms of action...we’re not seeing a payoff from biodefense programs,” she adds. “We are not seeing the returns we’d like, and still need more resources for antibiotic research.”

Jeffrey L. Fox
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**Phage Sometimes Remodel Host Genomes**

When cyanophage infect *Prochlorococcus*, they kill some host bacteria but remodel others in ways that makes the survivors harder, according to Sallie Chisholm of Massachusetts Institute of Technology (MIT) in Cambridge, Mass., Debbie Lindell of the Technion Israel Institute of Technology (TIIT) in Technion City, Haifa, Israel, and their collaborators. Their analysis of the seesaw genetic relationship between this phage and its ocean-dwelling photosynthetic bacterial host, which lies at the base of the sea food chain and produces much of the oxygen we breathe, appears in the September 6 issue of *Nature*.

“We know little about the role of phage in [regulating *Prochlorococcus*], other than that they are abundant in the oceans, can be ecotype specific, and appear to play a role in ‘shuttling’ genes around, including photosynthesis genes,” Chisholm says. “We thought it would be informative to get a close look at gene expression over the course of infection in both the host and phage to better understand this dynamic.”

What they found runs counter to dogma. Instead of the virus merely shutting down host function and control, cyanophage P-SSP7 upregulates some 41 of the 1,717 host genes, based on changes in levels of messenger RNA molecules (mRNAs). Many of those upregulated genes are located in “genomic islands,” regions of the genome that can be hot spots for exchange of DNA between a bacterium and an infecting virus. Indeed, the P-SSP7 genome contains several “bacterial-like” genes that likely were acquired from *Prochlorococcus*, according to Chisholm and Lindell.

Some of those genes encode proteins that aid cells in adapting to environmental changes, including food deprivation and lack of sunlight. The viral genome also harbors bacterial-like genes that function during photosynthesis and when DNA is being replicated. Intriguingly, even though those genes are scattered along the viral genome, they are transcribed at the same time during an infection rather than in the left-to-right pattern that ordinarily leads to early and late activation of viral genes.

“The most parsimonious evolutionary scenario” is that *Prochlorococcus* is activating stress response genes in response to cyanophage, according to the MIT and TIIT researchers. In a subsequent infection, phage that incorporated such genes upregulate them and thus help to drive the infection and shuttle those genes back into the bacterial genome. Some infected bacteria survive, in part, because of these transplanted and virally modified genes.

The findings may have more profound evolutionary implications, according to Chisholm and Lindell. “We cannot understand life by studying single organisms,” they argue. “We have to also study the system in which they are embedded. . . . Phages move genes around, and if the host receives them and they turn out to be useful then it is evolving as a result of the interaction. As far as the bacterium is concerned, it would attempt to fight off the infection and doesn’t welcome it, but a positive side product of it is the gene shuffling that goes on, which increases diversity and can lead to evolutionary benefits.”

“Microbiologists used to think that the microorganisms could be basically understood simply by knowing enough about the componentry of individual
bacteria in pure culture,” says Carl Woese of the University of Illinois, Champaign-Urbana. “We are now finding that this kind of understanding, detailed as it can be, is not sufficient to understand the microbial world; it does not capture the essence of microorganisms. There is a new microbiology, a new way of looking at microorganisms, emerging and it is represented and being developed in the research done by the Chisholm lab (and certain other like-minded groups).”

Brian Hoyle
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Fluorinated Antimicrobial Peptide Resists Proteases, Has Elevated Potency

Fluorinating antimicrobial peptides (AMPs) renders them dramatically more resistant to proteolytic enzymes and, in some cases, elevates their antimicrobial potency, according to Neil Marsh and his collaborators at the University of Michigan, Ann Arbor. They presented their findings during the 234th national meeting of the American Chemical Society, held last August in Boston, Mass.

Although AMPs disrupt negatively charged bacterial membranes, they are not particularly effective against neutral phospholipid cell membranes of humans or other eukaryotes. Moreover, when administered in very high doses to overcome proteolytic degradation, they begin to exert toxic side effects, such as rupturing red blood cells. “We knew AMPs have potential to be therapeutic molecules, but they need some tweaking to become beneficial therapeutics,” Marsh says. Typically, such AMPs contain 15 to 30 amino acids, and are highly susceptible to proteases.

Marsh and his collaborators chose to modify a synthetic AMP, called MSI-78 or pexiganan, that is an analog of magainin-2, which can be isolated from frog skin. The researchers replaced two leucine and two isoleucine residues in MSI-78 with hexafluoroleucine, and named this modified AMP fluorogainin-1. When MSI-78 is added to liposomes to mimic interactions with bacterial membranes, the proteases trypsin and chymotrypsin degrade it within 30 minutes, whereas fluorogainin-1 remains stable for up to 10 hours.

MSI-78 and fluorogainin-1 are both active when tested against both gram-negative and gram-positive bacterial species, including human pathogens. For example, the two peptides show similar minimum inhibitory concentrations (MIC) in vitro against Bacillus subtilis, Salmonella enteritis, and Shigella sonnei. “That was not a guarantee after introducing fluorinated atoms,” notes Marsh. More surprisingly, fluorogainin-1 kills Klebsiella pneumoniae, whereas MSI-78 is inactive against this pathogen. Furthermore, fluorogainin-1 kills Staphylococcus aureus at a dose four times lower than does MSI-78, but fluorogainin-1 is less active than MSI-78 against Streptococcus pyogenes.

Fluorinated AMPs “seem to be at least as good at killing bacteria as their nonfluorinated counterparts, and for some bacteria they may be significantly better,” Marsh says. The improved resistance to proteolysis in vitro could mean that fluorinated AMPs will have prolonged bioavailability in vivo. Although some researchers are investigating AMPs in clinical trials, protease destruction and bioavailability problems constrict their therapeutic range.

Marsh, a chemist, had no particular application in mind when he began exploring how fluorine-containing amino acids change proteins and pep-

Several Developments on the Antimicrobial Front

• Officials of the Food and Drug Administration (FDA) in October approved the antiviral drug Isentress™ (raltegravir), for use in combination with other antiretoviral agents in HIV-infected patients; raltegravir, which was developed at Merck of Whitehouse Station, N.J., is the first drug to be approved that works by inhibiting integrase, the enzyme that inserts HIV DNA into human DNA.

• Also in October, FDA officials approved the antibiotic Doribax (doripenem), an injectable drug for treating serious gram-positive and gram-negative bacterial infections, including complicated intra-abdominal and urinary tract infections; it was developed by OrthoMcNeil, Inc., of Raritan, N.J.

• Administering the antibiotic minocycline within 24 hours after an individual experiences a stroke reduces brain damage and associated physical impairments, according to Yair Lampel of Tel Aviv University in Tel Aviv, Israel, and his collaborators, whose small-scale clinical study is published in the October issue of Neurology.

• The lipid-lowering drug Simvastatin, a Merck product, shows moderate antibacterial activity in vitro against several gram-positive pathogens, and this activity might help to explain epidemiological findings linking statin usage with a decreased risk for severe infections, according to Jon Cohen of Royal Sussex County Hospital in Brighton, United Kingdom, and his collaborators, who presented their findings during the 2007 Interscience Conference on Antimicrobial Agents and Chemotherapy, held in Chicago, Ill., last September.

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tides. However, he was approached by his office neighbor, Ayyalusamy Ramamoorthy, who was interested in using fluorine as a solid-state nuclear magnetic resonance (NMR) probe for studying the interactions of AMPs with cell membranes. Working together, they went beyond those narrower goals to address the stability of AMPs and advance them as therapeutic agents.

“Adding peptide modifications that enhance stability of antimicrobial peptides in vivo could represent an advance,” says Paul McCray, a pediatric pulmonologist at the University of Iowa, Iowa City. However, animal experiments of effectiveness and safety are needed before embracing this preliminary approach as a therapeutic advance, he cautions.

Chemist Beate Koksch of Free University Berlin, Germany, emphasizes the chemical advantages in making and evaluating fluorinated AMPs.

“Given the ominously increasing resistance against antimicrobial drugs, Marsh’s investigations of fluorinated antimicrobial peptides as potential drug candidates against bacterial infections is important,” she says. Applying fluorine chemistry “is an impressive step towards the creation of new peptide-based drugs.” Further, she notes, fluorine dramatically affects the stability and interactions among peptides into which it is incorporated, and also provides a useful label for spectroscopic analyses.

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AAM Report Cites Progress in Assessing Drinking Water Microbial Risks

Despite its limitations, microbial risk assessment can be extremely useful for safeguarding drinking water quality, according to a new report from the American Academy of Microbiology (AAM), “Clean Water: What is Acceptable Microbial Risk?” The report, based on a colloquium convened during November 2006 in Tucson, Ariz., recommends a series of improvements for assessing the risks associated with waterborne microorganisms, emphasizing the need to develop better indicator organisms, credible models for evaluating waterborne diseases, and an international database of waterborne pathogen incidence. It also says that an iterative approach to assessing risks can lead toward better water quality standards and help to correct inappropriate approaches of the past.

With U.S. and European regulatory officials seeking better ways to protect public health, there is heightened interest in refining microbial risk assessment tools and applying them to water quality issues, according to Mark LeChevallier of the American Water Works Service Company in Vorhees, N.J., who chaired the AAM colloquium. Such assessments provide regulatory agencies with the means for developing regulations to improve the safety of drinking water, he says. Also, because of recent advances on the scientific side of assessing microbial risks, it is an opportune time to bring such experts together with regulators to take advantage of those advances.

Thimerosal in Vaccines Deemed Unlikely To Account for Autism in Children

Exposure to thimerosal, a mercury-containing preservative used in vaccines since the 1930s, does not appear to account for autism or other neuropsychological deficits in children, according to William Thompson and his collaborators at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. They examined 1,047 children between the ages of 7 and 10 years using standardized tests to assess 42 neuropsychological outcomes, and then analyzed those outcomes against various measures of the children’s early exposure to mercury. The study by the CDC investigators appears in the September 27, 2007, issue of the New England Journal of Medicine along with articles reviewing recent legal disputes involving thimerosal use in vaccines.
Although risk assessments were long used in developing water quality standards, misunderstandings sometimes led to faulty regulations, particularly when regulators accepted risk assessments as unassailable, according to LeChevallier. Instead, they need to take uncertainties into account.

The report emphasizes another important point: the need to repeat and refine risk assessments. “It’s always an iterative process,” says LeChevallier. “Each time you go through the risk assessment process, you really learn more about the situation.” Organizing available information, determining uncertainties, and anticipating research needs are also important, according to the report.

In the past, much of the supporting data necessary for making meaningful assessments of risk, including information on pathogens, exposure, and dose-response relationships, was not available, according to colloquium steering committee member Joan Rose of Michigan State University in East Lansing. Now research is beginning to fill these gaps in our understanding, she says, so microbial risk assessment is becoming increasingly more accurate, particularly with respect to characterizing the quality of drinking water sources, such as reservoirs and ground water.

The public is largely unaware of the risk of waterborne illness, says Rose. “People presume our water is safe without us having to think about it much or put in any kind of strategy that protects it,” she says. Paradoxically, the risks have become greater in some respects. “We have hundreds of different types of microbes that can be spread through water,” she continues. “In the past we’ve thought of the traditional types, like cholera and typhoid, but those are really easy to control compared to some of the emerging pathogens, such as Cryptosporidium, norovirus, and Legionella.” Microbial risk assessment can help to gauge and manage these risks.

The AAM report is available electronically on the Academy website at http://www.asm.org/Academy/index.asp?bid=2093 and also can be ordered via e-mail (colloquia@asmusa.org).

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Serial Growth in Microchambers Adapts Balky Microbes to Culture

Domesticate them serially, and they will grow—even otherwise balky bacteria, including strains from the phylum Verrucomicrobia, whose members are notoriously difficult to raise in the lab, according to Slava Epstein and colleagues at Northeastern University in Boston, Mass. The key development entails growing specimens first in special diffusion chambers and then “domesticating” each species of interest by growing it serially in those chambers until it adapts to more conventional equipment that better suits the convenience of investigators. Details appear in the October issue of Applied and Environmental Microbiology (73:6386–6390).

Unlike the natural environments in which these balky microorganisms are found, “the in vitro environment lacks critical elements necessary for [them] to grow,” Epstein says. More than five years ago, he and his collaborators developed a miniaturized diffusion chamber in which such recalcitrant bacteria can be placed on one side of a

Disturbing News on the Antibiotic Resistance Front

Several recent disturbing developments on the antibiotic resistance front include:

• Methicillin-resistant Staphylococcus aureus (MRSA) caused more than 94,000 life-threatening infections and nearly 19,000 deaths in the United States in 2005, with 85% of them associated with health care settings, according to Denise Cardo and her collaborators at the Centers for Disease Control and Prevention (CDC) in Atlanta, Ga. These estimated figures are based on extrapolations of invasive MRSA cases at nine U.S. sentinel sites. Details of the CDC report appear in the October 17 issue of the Journal of the American Medical Association.

• A strain of Streptococcus pneumoniae, which is circulating in upstate New York and causes acute otitis media (ear infections) in children, appears to be resistant to all available antibiotics, according to Michael Pichichero of the University of Rochester in Rochester, N.Y., and his collaborators, who presented their findings during the 2007 Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), held in Chicago, Ill., last September. They point out that this strain emerged following introduction and ongoing use of a conjugate vaccine that protects against seven widely circulating strains of this bacterial pathogen.

• The KPC genes encoding resistance to carbapenem antibiotics are showing up globally, including in the Midwest of the United States, Puerto Rico, Brazil, Colombia, and Israel, according to several sets of investigators who also presented their findings during ICAAC.
semipermeable membrane while samples of its particular niche in nature, presumably containing nutrients, growth-specific factors, and other species-specific necessities, are placed on the other side.

While the chambers allowed difficult-to-grow microorganisms to be cultivated, those chambers are “a pain” for conducting experiments, and lead to a whole assortment of logistical problems, according to Epstein. One of the big problems is that each microorganism’s “natural” environment must be maintained outside its diffusion chamber, contained in the form of specific aquaria or terraria that stack up in the lab. Other challenges include maintenance of purity of cultures when chambers are opened and inherent difficulties in keeping radioactive tracer compounds from becoming diluted as they leak through the membranes. “Standard physiology and biochemistry experiments have really been developed for the lab cultures, and are mostly useless for the diffusion chamber-reared cells,” he says.

The trick to overcoming these drawbacks is to “domesticate” the microbes to grow under standard laboratory conditions. This takes place as they are serially cultivated in one diffusion chamber after another, according to Epstein. Perhaps some “uncultivable” microbes “require signals from neighboring synergistic species,” he speculates, to let the cell know that the biological environment is good to grow in; then, over several generations that dependence on signals presumably is lost.

Another hypothesis is that the diffusion chamber environment “represents an intermediate between nature and a Petri dish, and growth there may select for variants more capable of coping with the shock of standard lab conditions,” Epstein continues. In any case, he adds, “I think the arrival of a ‘domesticated’ variant is a probabilistic event.” The more cells you have, the higher the probability that variants will appear that are capable of growing readily in vitro.

During experiments to test this approach, species from 10 phyla were cultivated and then isolated from diffusion chambers, including representatives from the rarely cultivated Acidobacteria and Verrucomicrobia. The probability of cultivating five previously ungrowable species of Verrucomicrobia in one series of experiments is very low, about $1.6 \times 10^{-4}$, Epstein notes. Furthermore, this single effort boosts the number of cultivable Verrucomicrobia species by nearly $5\%$, a sizable jump over other efforts that date back more than 150 years.

Limited success using this serial approach to adapt diffident microorganisms to grow in culture does not mean that “we can cultivate any formerly unculturable microorganisms,” Epstein cautions. The immediate goals are “less ambitious,” he adds. “Our approach may be very selective and biased, but the biases are dramatically different from all the other approaches used to date.” Nonetheless, he and his collaborator Kim Lewis, also at Northeastern University, founded a company, Novobiotic Pharmaceuticals in nearby Cambridge, whose approach to discovering drugs is based, in part, on exploiting this approach to adapting microorganisms from environmental specimens to grow in culture.

Their results are noteworthy in themselves and also because of the message that, molecular approaches notwithstanding, cultivation remains “fundamentally important in generating cells from which to study growth-dependent physiology,” says David A. Relman of Stanford University in Stanford, Calif. “As the authors point out, what is now needed is an analysis of the mechanisms and reasons why this system revealed life forms that Petri dishes alone do not.”

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### Potential Water Quality Problems

Increased use of corn to produce ethanol for fuel could lead to deterioration of water quality in the United States, according to a report, “Water Implications of Biofuels Production in the United States,” made public in October by a committee of the National Research Council, which is part of the National Academies in Washington, D.C. Not only does the additional irrigation that would be required to grow crops for biofuel pose “a major concern,” but refineries for producing such ethanol could also “substantially affect local water supplies,” the report notes. Meanwhile, pollens and toxins—specifically, insecticidal proteins derived from Bacillus thuringiensis—that are released from widely used, genetically engineered varieties of corn could be harming U.S. aquatic ecosystems by killing off waterborne caddisflies, which are an important food source there, according to Emma Rosi-Marshall of Loyola University in Chicago, Ill., and her collaborators. Details appear in the October 9, 2007 issue of the Proceedings of the National Academy of Sciences.