Innovative approaches to confront the antibiotic resistance crisis

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In March, the White House released a 60-page National Action Plan for Combating Antibiotic-Resistant Bacteria, which outlines an ambitious, broad-based plan for drastically reducing the rates of most deadly superbug infections over the next five years. Under this 5-point plan, the administration aims to lower Clostridium difficile and MRSA infections by at least 50% and reduce carbapenem-resistant Enterobacteriaceae (CRE) by 60%. See news item here.
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In 2006, the APUA Newsletter featured an article titled: Will antibiotics become useless by 2015?¹ The article summarized a UK-based debate that argued the statement: “This House Believes that by 2015 all Antibiotics will be Redundant.” In his opening address, Chairman Peter Davey stated: “The fuse is lit, and unlike most fuses that burn at a constant rate, this one is accelerating.” In the end, the motion failed: four in favor and five against—but the vote demonstrated a rather alarming, closely divided opinion on the future efficacy of antibiotics.

Nine years later, in 2015, we still depend on, and to a large extent, succeed in controlling the majority of common bacterial infections with our current battery of antimicrobials. However, there is no dearth of dire warnings with regard to the imminent demise of these drugs. It seems that we have indeed inched closer to “the cliff”— spawning reports with titles such as “Antibiotics have gone from wonder drugs to wonder-if-they’ll-work-drugs”;² “Are you ready for a world without antibiotics?”³ and “Is the world failing in fight against antibiotic resistance?”⁴ And to some, the cliff-hanging is over. The CDC’s Arjun Srinivasan removes the questionable aspect and declares, “We’ve reached the end of antibiotics, period.”⁵

In a growing number of countries (including our U.S. neighbor, Cuba), most cases of urinary tract infections are resistant to the typical short-course fluoroquinolone treatment for urinary tract infections. Standard antibiotics are ineffective for treating more than 33% of severe Klebsiella lung infections in China. And in some settings in the Americas, as many as 90% of Staphylococcus aureus wound infections are multidrug resistant (MRSA) and fail standard treatment.⁶

The CDC now reports that at least two million Americans are thought to suffer from antibiotic-resistant infections annually, resulting in some 23,000 deaths directly, with many succumbing indirectly from complications of other illnesses.² Deaths from drug-resistant bacteria are estimated at 5,000 per year in the UK, and at 25,000 in Europe.³ Doctors are increasingly turning to what were once considered treatments of last resort. Moreover, in the face of unchecked resistance, the number of deaths is expected to escalate to 10 million globally by the year 2050, with an estimated cost of $100 trillion (Figure 1).⁹

In an attempt to counter the arrival of doomsday predictions, the U.S. government has publically declared an intricate and...
extensive plan to intervene. Expanding upon current patient safety efforts and goals, the FY 2016 President's Budget requests funding for CDC to increase the detection of antibiotic-resistant infections and to improve efforts to protect patients from infections.\textsuperscript{10}

The march to outpace antibiotic-resistant bacteria has challenged scientists for decades, inspiring researchers to investigate both older discarded methodologies and to mine new horizons for novel approaches to drug development. In their race to stall the “post-antibiotic era”, scientists are attempting to circumvent the mechanisms that cause resistance. In its 2014 report titled, \textit{NAIAD's Antibacterial Resistance Program: Current Status and Future Direction}, NAIAD outlines its strategic plan for antimicrobial resistance research.\textsuperscript{11} Among its list of seven pursuits is, “Exploiting Natural Predators: the Specificity of Phage Therapy”—specifically, “using phage or phage-derived lysins to kill specific bacteria while preserving microbiota.” This issue of the \textit{Newsletter} takes a fresh look at this older approach. Once actively pursued as a promising anti-infective, bacteriophage therapy was subsequently largely abandoned with the advent of chemical antibiotics. Today, it is utilized for human therapy in only a small number of foreign countries. Nonetheless, bacteriophage technology has emerged as a novel, viable and safe alternative for the prevention, treatment and or eradication of foodborne pathogens in a variety of food processing environments.\textsuperscript{12}

In the article by Sulakvelidze and Morris, the current status of investigation into bacteriophage therapy is examined—looking into the history, pitfalls, current trials, and the process for reinvigorating this technology in human therapy.

In President Obama’s recently released \textit{National Strategy for Combating Antibiotic Resistant Bacteria}, (Sept 2014),\textsuperscript{13} Goal #4 outlines the need to “accelerate basic and applied research and development for new antibiotics, other therapeutics and vaccines.” The second feature in this \textit{Newsletter} focuses on some new candidates in the continuing search to refuel the antibiotic pipeline. The article by Kim Lewis explores some fledgling compounds in various stages of development – in particular some promising agents that contain low probabilities for resistance; compounds that can effectively kill off recalcitrant “persister cells”; and also, species-specific drugs that target the pathogen, but preserve the microbiome. Some of these promising agents are being mined through new technological advances that tap into the vast numbers of previously “unculturable” microflora.

The work of the authors described here provide some optimism that effective antimicrobials can be perpetuated into future generations.

\textbf{References:}

2. Rosenberg, M. Antibiotics have gone from wonder drugs to wonder-if-they’ll-work-drugs. Huffington Post Oct 1, 2013
According to the United States Centers for Disease Control and Prevention (CDC), each year at least 2 million people in the US become infected with antibiotic-resistant bacterial pathogens and at least 23,000 people die as a direct result of those infections.\(^1\) While a variety of sophisticated techniques are being used in the development of new drugs, their promise has not yet been realized, and it may require many years or even decades before effective drugs resulting from those technologies are developed. At the same time, optimal control of antibiotic resistant microorganisms will be dependent not just on the development of new drugs, but also on movement toward a “multiple hurdle” approach that includes efforts to minimize emergence of resistance, reduce the risk of transmission of resistant microorganisms, and utilize modalities other than small molecules to kill resistant bacteria. The reincarnation of an old antibacterial approach using naturally occurring bacteriophages or phages (viruses that kill bacteria) – called “bacteriophage therapy” or “phage therapy” – may provide, in the relatively near future, an effective component of such a multi-modality approach to management of antibiotic-resistant bacteria.

**Bacteriophages**

Bacteriophages are bacterial viruses that are the oldest (ca. 3-billion-years-old) and most ubiquitous organisms (ca. \(10^{30}-10^{31}\) phage particles) on Earth. They were first identified during the early part of the 20th century by Felix d’Herelle who called them *bacteriophages* (“bacteria-eaters,” from the Greek *phago* meaning *to eat* or *devour*).\(^2\) This was the time when antibiotics were not yet available and infectious diseases of bacterial etiology were very difficult, if not impossible, to treat. Thus, the discovery of bacteriophages having potent antibacterial activity was met with great enthusiasm by the medical community, and bacteriophages started to be utilized therapeutically throughout the world almost immediately.\(^3\)

Using phages to treat humans was safe. However, phage therapy did not always work for various reasons, the main one being the specificity of phages. In this context, lytic phages are very specific: they lyse strains of their targeted host bacterial species, but not strains of other bacterial species. Thus, if the disease’s etiologic agent is not correctly identified and the appropriate phage is not selected to target it, phage therapy will be ineffective. This is very different when broad-spectrum antibiotics are used. The identity of the etiologic agent is not as critical because those drugs are bactericidal and/or bacteriostatic for many different bacterial species. Thus, phage specificity—and the associated uncertainty in the clinical outcome—was one of the key reasons for phage therapy gradually falling out-of-favor in the U.S. and Western Europe, particularly when antibiotics effective against a broad spectrum of pathogenic bacteria became widely available. Several additional factors contributed to the decline of interest in phage therapy in the West, as reviewed in more detail by several investigators.\(^3,4,5\)

However, the emergence of multi-antibiotic-resistant bacterial pathogens has rekindled interest in bacteriophages in the West, and the potential applications for lytic phages are now increasingly being examined for various purposes, ranging from improving food safety to preventing and treating bacterial diseases caused by multi-drug-resistant pathogens.

**How phages can help address the problem of antibiotic resistance**

Lytic phages have remarkable *bactericidal* activity against their specifically targeted bacterial strains. In addition, the
mechanisms by which phages kill bacteria, and the mechanisms
of bacterial resistance to phages, are different from those for
antibiotics (Table 1). Therefore, in theory, phages can help to
reduce the emergence of antibiotic resistance in a variety of
ways. Two major ones are: (1) using phages in agricultural
applications; e.g., to reduce contamination of foods with
specific foodborne bacteria (the approach commonly called
“phage biocontrol”), and (2) prophylactic or therapeutic
applications of bacteriophages for managing infectious diseases
of humans and domesticated, commercially important animals
(the approach commonly called “phage therapy”). Phage
biocontrol has the potential to reduce antibiotic usage by
significantly reducing the levels of specific pathogenic bacteria
in various foods – and, by association, reducing the pressure on
the industry to use antibiotics for food safety reasons. Such
post-harvest phage applications have been slowly but steadily

<table>
<thead>
<tr>
<th><strong>Table 1. Major similarities and differences between lytic phages and antibiotics</strong></th>
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</thead>
<tbody>
<tr>
<td><strong>Lytic bacteriophages</strong></td>
</tr>
<tr>
<td>Composition</td>
</tr>
</tbody>
</table>
| Mechanism of action | Lytic phages attach to their specific receptors on the bacterial membrane and inject their DNA into the host cells. Almost immediately afterwards, the host machinery is redirected to the synthesis of phage proteins and to phage assembly. The bacterial host cell is metabolically dead in ca. 60 seconds after injection. The lytic cycle will continue for another 30-60 min, at which time the host cell is ruptured. Released progeny phages (typically 40-200 per bacterial cell) can infect other bacterial cells with which they come into physical contact. | Antibiotics kill or inhibit growth of bacteria by various mechanisms, including by affecting their:
- Peptidoglycan synthesis (e.g., β-lactam antibiotics and glycopeptides),
- Protein synthesis (e.g., aminoglycosides),
- DNA gyrase (e.g., quinolones),
- RNA polymerase (e.g., rifampin),
- Production of tetrahydrofolic acid (e.g., trimethoprim) |
| Mode of action | All lytic phages are bactericidal. | Some antibiotics are bactericidal (e.g., vancomycin) and some antibiotics are bacteriostatic (e.g., chloramphenicol). |
| Specificity | Highly specific. Phages only lyse strains or subgroup of strains, usually within the same species or across closely related species. This requires that the etiologic agent is identified and that appropriate phages lytic for that agent are used for phage therapy to be successful. At the same time, this specificity permits the targeting of specific pathogens, without affecting desirable bacterial flora. | Broad specificity. They kill or inhibit the growth of the targeted pathogenic bacterium as well as many other bacterial species at the same time. This increases the chances of successful therapy even when the precise identity of the etiologic agent is not known. However, they may also deleteriously affect the host’s normal beneficial microflora. |
| Safety / side effects | Excellent safety profile. Only a few minor side effects (e.g., mild fever) have been reported for therapeutic phages, most of them likely due to using poorly purified phage preparations. | Multiple side effects, including intestinal disorders, ototoxicity, taste alteration, nausea, allergies, and secondary infections (e.g., yeast infections) have been reported. |
| Resistance mechanisms | Two fundamental mechanisms of resistance, caused by mutations in:
- Bacterial receptors that may prevent phage attachment, and/or
- Bacterial host restriction/modification system that degrades phage DNA after it has been injected into the bacterial cell. | Numerous mechanisms of resistance, and a single bacterium often possesses several of them simultaneously. Some examples:
- Mechanisms that inactivate the antibiotic (e.g., produce β-lactamase which inactivates β-lactam antibiotics by cleaving the β-lactam ring),
- Mechanisms that limit access of the antibiotic to its target (e.g., “efflux resistance” to tetracycline and macrolides),
- Mechanisms that alter the bacterium’s target for antibiotics (e.g., resistance to rifampin is often due to mutations in bacterial RNA polymerase). |
| Impact on overall resistance | Because of phages’ specificity, their use is not likely to select for phage resistance in other (i.e., not-targeted) bacterial species. | Because of their broad spectrum of activity, antibiotics may select for resistant mutants of many bacterial species, not just for resistant mutants of the targeted bacterium. |
| Developmental cycle | Natural co-evolution of bacteria and phages provides unprecedented flexibility in obtaining new lytic phages against phage-resistant bacteria emerging as a result of exposure to other phages or because of natural shifts in bacterial populations. Identifying a new lytic phage in the environment usually requires a few weeks to a few months. | Developing a new antibiotic (e.g., against antibiotic-resistant bacteria) is a time-consuming process and may take several years to accomplish. |
gaining recognition during the last few years, and several phage products for food safety applications have been approved by the U.S. FDA and by some other countries. Those products, when properly applied, may significantly reduce the incidence of foodborne illness; however, their impact on reducing antibiotic usage is likely to be fairly minor.

Arguably the biggest and most direct impact that lytic phages may have on the problem of antibiotic resistance is based on their direct use as therapeutic agents. As noted earlier, phage therapy was used to treat various infectious diseases until antibiotics became widely available. After that time, although phage therapy was all but forgotten in most developed countries (including the U.S.), it continued to be utilized in the former Soviet Union (FSU) and Eastern Europe (EE) – and, on a much smaller scale, in France, Switzerland and Egypt. Although the data from those countries are, for the most part, semi-anecdotal, they strongly suggest that phages, when carefully selected after determining that they are lytic for the infecting bacterial strain, were often more effective than antibiotics in treating many infections, and they were effective in managing infections refractory to treatment with available antibiotics. Significantly, even when the disease-causing bacteria were antibiotic-sensitive, administering phages together with antibiotics was reported to improve the clinical outcome.

Several hundred publications concerning various therapeutic applications of bacteriophages are available; however, the majority of them are in the non-English literature and are not widely available to the Western medical establishment. This situation has been a significant problem in conveying, to the Western scientific community, the FSU’s and EE’s experiences in using phages therapeutically. The situation is now gradually improving, with several English reviews recently published. However, the recent increase in English-language publications has not yet transitioned into a significant increase in the number of phage therapy clinical trials. So far, only a few clinical trials with phages have been performed in the West. The first FDA-approved human phage therapy application in the U.S. was for a physician-initiated Phase I clinical trial conducted in Lubbock, Texas during 2006-2008. The trial evaluated the safety of a phage cocktail (containing eight distinct phages against Staphylococcus aureus, P. aeruginosa and E. coli) used to treat infected venous leg ulcers.

Table 3 summarizes some of the recently completed and ongoing phage therapy trials. More phage-related studies and clinical trials must be conducted in order for phage therapy to become widely accepted in the U.S., and for designing optimally effective phage preparations and treatment regimens. It is encouraging that the NIH, U.S. Army, and USDA are increasingly soliciting projects for phage characterization and phage-based interventions. For example, recent Army SBIR topic A15-054 specifically solicited proposals for high-throughput bacteriophage isolation and characterization, particularly against the ES-KAPE* pathogens, and the NIH “Non-traditional therapeutics that limit antibacterial resistance (R21/R33)” (RFA-AI-14-066) program specifically listed bacteriophages as one of the non-traditional approaches of high interest.

Phage-based therapeutic preparations offer unprecedented flexibility for keeping up with the emergence of phage-resistance in bacterial populations. Phages have been co-evolving with their host bacteria for more than 3 billion years; therefore, when they are needed, it is relatively easy to isolate

*ESKAPE pathogens: Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp.
new environmental phages that can kill phage-resistant bacterial mutants. From a practical standpoint, that approach will require that the phage sensitivity of the targeted bacteria be continuously monitored, and that the phage preparations are updated as needed. The first approach is not novel or particularly difficult because bacterial isolation and antibiotic-sensitivity testing is a routine practice in all major hospitals and similar testing could be implemented for bacteriophages without too much difficulty. However, phage substitutions may be more challenging from a regulatory standpoint. Updating phage preparations by replacing old phages with new, more effective phages has been commonly and successfully used in the FSU and EE. However, this practice may be novel for Western regulatory agencies accustomed to approving defined chemicals and requiring that each change in a preparation must be the subject of a new regulatory application. Having similar requirements for

### Table 2. Approvals for bacteriophage preparations used for food safety applications

<table>
<thead>
<tr>
<th>Date</th>
<th>Agency</th>
<th>Phage preparation</th>
<th>Company</th>
<th>Target/food application</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006, August</td>
<td>FDA, 21 CFR 172.785</td>
<td>ListShield™</td>
<td>Intralytix, Inc.</td>
<td>RTE meats</td>
</tr>
<tr>
<td>2006, August</td>
<td>USDA, FSIS Directive 7120.1</td>
<td>ListShield™</td>
<td>Intralytix, Inc.</td>
<td>RTE meats</td>
</tr>
<tr>
<td>2006, October</td>
<td>FDA, GRN 198</td>
<td>Listex™</td>
<td>Micreos</td>
<td>Cheese</td>
</tr>
<tr>
<td>2007, January</td>
<td>USDA, FSIS Directive 7120.1</td>
<td>Finalyse™</td>
<td>Elanco, Inc.</td>
<td>Hides of livestock</td>
</tr>
<tr>
<td>2007, March</td>
<td>USDA, FSIS Directive 7120.1</td>
<td>BacWash™</td>
<td>OmniLytics, Inc.</td>
<td>Hides of livestock</td>
</tr>
<tr>
<td>2007, June</td>
<td>FDA, GRN 218</td>
<td>Listex™</td>
<td>Micreos</td>
<td>Foods, generally</td>
</tr>
<tr>
<td>2008, July</td>
<td>USDA, FSIS Directive 7120.1</td>
<td>Salmonella-targeting</td>
<td>OmniLytics, Inc.</td>
<td>Feathers of live poultry</td>
</tr>
<tr>
<td>2010, September</td>
<td>Health Canada</td>
<td>Listex™</td>
<td>Micreos</td>
<td>RTE meat, dairy, fish</td>
</tr>
<tr>
<td>2011, February</td>
<td>FDA, FCN 1018</td>
<td>EcoShield™</td>
<td>Intralytix, Inc.</td>
<td>Ground beef</td>
</tr>
<tr>
<td>2012, August</td>
<td>FSANZ</td>
<td>Listex™</td>
<td>Micreos</td>
<td>Meat, seafood, cheese, RTE foods</td>
</tr>
<tr>
<td>2013, February</td>
<td>FDA, GRN 435</td>
<td>SalmoFresh™</td>
<td>Intralytix, Inc.</td>
<td>Poultry, fish, fruits, vegetables</td>
</tr>
<tr>
<td>2013, February</td>
<td>USDA, FSIS Directive 7120.1</td>
<td>SalmoFresh™</td>
<td>Intralytix, Inc.</td>
<td>Poultry</td>
</tr>
<tr>
<td>2013, December</td>
<td>FDA, GRN 468</td>
<td>Salmonelex™</td>
<td>Micreos</td>
<td>Pork and poultry</td>
</tr>
<tr>
<td>2014, August</td>
<td>Health Canada</td>
<td>SalmoFresh™</td>
<td>Intralytix, Inc.</td>
<td>Poultry, fish, fruits, vegetables</td>
</tr>
<tr>
<td>2014, August</td>
<td>Israel Ministry of Health</td>
<td>SalmoFresh™</td>
<td>Intralytix, Inc.</td>
<td>Poultry, fish, fruits, vegetables</td>
</tr>
<tr>
<td>2014, August</td>
<td>Israel Ministry of Health</td>
<td>ListShield™</td>
<td>Intralytix, Inc.</td>
<td>RTE meats</td>
</tr>
<tr>
<td>2014, August</td>
<td>Israel Ministry of Health</td>
<td>EcoShield™</td>
<td>Intralytix, Inc.</td>
<td>Ground beef</td>
</tr>
<tr>
<td>2014, December</td>
<td>FDA, GRN 528</td>
<td>ListShield™</td>
<td>Intralytix, Inc.</td>
<td>Fruits, vegetables, dairy, fish</td>
</tr>
</tbody>
</table>

Source: (Woolston and Sulakvelidze, 2015)”

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Preparations containing naturally occurring phages that target a single or only a handful of bacterial pathogens will inhibit the development of new phage-based therapeutics.

Another unknown is how Western regulatory agencies might regulate phages for the “Pharmacy Approach” when phages are custom-selected for each individual patient. Since lytic phages are highly specific, mainstream commercial phage products may

### Table 3. List of recent clinical trials of bacteriophages, based on clinicaltrials.gov

<table>
<thead>
<tr>
<th>Clinical trials.gov identifier</th>
<th>Study title</th>
<th>Condition</th>
<th>Intervention</th>
<th>Sponsor</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCT00945087</td>
<td>Experimental phage therapy of drug-resistant bacterial infections, including MRSA infections</td>
<td>Bacterial infections</td>
<td>Bacteriophage preparation</td>
<td>Institute of Immunology &amp; Experimental Therapy of the Polish Academy of Sciences</td>
<td>Recruiting patients</td>
</tr>
<tr>
<td>NCT02116010</td>
<td>Evaluation of phage therapy for the treatment of <em>Escherichia coli</em> and <em>Pseudomonas aeruginosa</em> wound infections in burn patients (PHAGOBOURN)</td>
<td>Wound infections</td>
<td><em>E. coli</em> and <em>P. aeruginosa</em> phage cocktails; Standard of care: Silver sulfadiazine</td>
<td>Pherecydes Pharma</td>
<td>Not yet recruiting patients</td>
</tr>
<tr>
<td>NCT01818206</td>
<td>Bacteriophages effects on <em>Pseudomonas aeruginosa</em> present in sputum of cystic fibrosis (CF) patients</td>
<td>CF</td>
<td>Cocktail of 10 phages</td>
<td>University Hospital, Montpellier</td>
<td>Started in February 2012. Completed in April 2012</td>
</tr>
<tr>
<td>NCT00937274</td>
<td>Randomized, double-blind, placebo-controlled studies to evaluate the effect of an orally-fed <em>Escherichia coli</em> phage preparation for the management of ETEC- and EPEC-induced diarrhea in children</td>
<td>Diarrhea</td>
<td>T4 phage cocktail; standard oral rehydration solution</td>
<td>Nestlé</td>
<td>Started in August 2009. Completed in January 2013</td>
</tr>
</tbody>
</table>

The table only includes clinical trials in which phages were/are evaluated for therapeutic purposes. Other clinical studies with bacteriophages; e.g., the clinicaltrials.gov identifier NCT01617122 study in which phage øX174 is used to evaluate the immune response in humans, are excluded from the table. Also, some clinical trials have been reported in the literature but are not listed on www.clinicaltrials.gov; e.g., the trial to evaluate the efficacy and safety of a therapeutic bacteriophage preparation (Biophage-PA) targeting antibiotic-resistant *P. aeruginosa* in chronic otitis.17
not be as effective against one or more strains of the targeted species that happen to predominate in a particular hospital or clinical center. One approach for addressing this potential problem is to have all targeted bacterial strains examined for their sensitivity to various phages, and to select and use only the phages exhibiting strong lytic potency against the infecting bacterial strain. This approach is much like how antibiotics are used today; i.e., bacterial strains are screened for their sensitivity to various antibiotics before prescribing the most effective antibiotic for a given patient. For phage therapy to reach its full potential, such custom-designing and regular updates must be implemented. A positive development in that regard was the FDA’s flexibility regarding its approval of various phage preparations for food safety applications (Table 2): the agency allowed future updates of most approved phage preparations in response to the emergence of new, phage-resistant strains.

Concluding remarks

As discussed in more detail elsewhere,13,18 various technical challenges (e.g., the emergence of phage-resistant bacteria) and nontechnical challenges (e.g., current barriers against implementing a fundamentally different approach into clinical practice by a traditionally conservative medical establishment) will need to be addressed before phage therapy becomes widely available in the U.S. However, given the somber threat of antibiotic resistance, and the potential of bacteriophages to provide a safe and effective complementary therapeutic approach in the relatively near future, strong efforts to develop and evaluate this natural therapeutic approach seem long overdue.

References


For Further Reading


For a more in-depth look at Salmonella as a public health threat, see APUA Newsletter Vol.32 No.3 here.
There is a broad agreement among experts that the main bottleneck in solving the antibiotic crisis is the lack of novel leads that could be developed into therapies to fight drug-resistant pathogens.\textsuperscript{1,2} This in turn suggests a focus not only on \textit{ad hoc} discovery of single compounds, but more importantly, on the development of platforms that can reliably churn out leads, most of which will inevitably fail in development.\textsuperscript{3} In this feature, we will summarize new compounds at different stages of development that act against new targets and that illustrate the emerging platforms for discovery.

The oxaborole AN3365 (Anacor/GSK) (Fig. 1A) is an effective inhibitor of the leu-tRNA synthase of gram-negative bacteria.\textsuperscript{4} The compound is small and fairly polar, and penetrates well into gram-positive bacteria. It failed in Phase II clinical trials; resistance readily developed by mutations in the target. However, AN3365 is an excellent example of a well-penetrating molecule, and it is a promising compound for combination therapy. For most single-target compounds, resistance will be an inevitable problem. Combination therapy is already the standard for treating tuberculosis, \textit{H. pylori} infections, and in general, difficult-to-treat chronic infections. Combination therapy would open a large number of targets to antimicrobial discovery.

POL7080 (University of Zurich/Polyphor/Roche) was discovered by a lucky accident in a program aimed at producing mimetics of the membrane-acting antimicrobial peptide (AMP) protegrin.\textsuperscript{5} Studies of the mechanism of action revealed that the target is not the cytoplasmic membrane, but LptD, a β-barrel protein of the outer membrane of \textit{P. aeruginosa} (Fig. 1B). LptD participates in inserting lipopolysaccharide into the outer membrane. The target is on the surface of the cell, which obviates permeability issues. Even though it is a single target, frequency of mutations in LptD are very low, probably because POL7080 is a large compound with multiple binding interactions, and the target is not an enzyme and cannot be easily modified. POL7080 is specific against \textit{P. aeruginosa}, but other antimicrobials targeting the conserved LptD protein are likely to follow. Species selectivity has its distinct advantages—for one, the compound will not harm our microbiome. POL7080 is being developed as an anti-pseudomonas drug and is in Phase II clinical trials.

Lassomycin (Northeastern/NovoBiotic) was identified in a specific screen of a collection of uncultured bacteria against \textit{M. tuberculosis} and is produced by \textit{Lentsea}, an actinomycete.\textsuperscript{6} Extracts were counter-screened against \textit{S. aureus}, and only specific hits against \textit{M. tuberculosis} were followed. Since virtually no natural products are specific against mycobacteria, this simple screen allows one to determine extracts containing novel compounds before doing any chemistry. This resolves the bottleneck of known and toxic compounds that slows down natural product discovery. Lassomycin is a lasso-shaped peptide which inhibits the essential ClpP1P2C1 protease of \textit{M. tuberculosis} while increasing its ATPase activity. This dual mode of action kills growing and dormant cells of the pathogen. The compound also serves as proof-of-concept for species-selective screening as a new antimicrobial discovery platform.
M64, abenzamide-benzimidazole, is an inhibitor of MvfR, the transcriptional activator of the *P. aeruginosa* quinolone quorum sensing (QS) regulon (Fig. 1C) (MGH/Spero Therapeutics/Roche). M64 is an optimized analog of a compound identified in a high-throughput screen of commercial libraries (the Harvard ICCB collection). QS controls virulence in this pathogen. M64 decreased persister (dormant variants tolerant to antibiotics) formation in an exponentially growing culture of *P. aeruginosa* and improved survival of mice with a lung and wound infection. The in vivo efficacy of M64 was especially pronounced in combination with ciprofloxacin. The fact that it was discovered in a high-throughput screen suggests that commercial libraries, which failed to produce compounds with a broad spectrum, may harbor useful molecules acting against a single target. The promise of anti-virulence factors lies in the expectation that they will have a low probability of resistance development, though this needs to be verified. Discovery of M64 bodes well for two emerging platforms – species-specific compounds, and anti-virulence factors.

Acyldepsipeptide (ADEP4), developed by Bayer, but dropped due to a high frequency of resistance, activates the ClpP protease, which normally uses ATP-dependent chaperones to degrade misfolded proteins. Importantly, ADEP keeps the entrance channel of ClpP open, and chaperones are no longer needed. Since ADEP/ClpP does not require energy to cleave proteins, this seemed like a good compound to kill energy-depleted persisters. We found that ADEP4 kills *S. aureus* persisters by forcing cells to self-digest. ClpP is non-essential, and null mutants resistant
to ADEP4 arise with high probability. Combining ADEP4 with another antibiotic such as rifampicin solves this problem, and leads to eradication of a biofilm population in vitro and in a mouse model of infection. Advanced analogs of ADEP4 are in development (Northeastern/St Jude/Arietis). ADEP is the first example of an antibiotic capable of effectively killing persisters.

Teixobactin, a novel inhibitor of cell wall biosynthesis, was discovered in a screen of uncultured bacteria (Northeastern/NovoBiotic). It is produced by *Eleftheria terrae*, a β-proteobacterium belonging to a new genus. Teixobactin binds the lipid-pyrophosphate-sugar moiety of lipid II, (precursor of peptidoglycan), and lipid III, (precursor of wall teichoic acid) in gram-positive bacteria. There is no resistance development to teixobactin since its targets do not mutate. Sophisticated resistance mechanisms such as destruction of the antimicrobial usually originate in the producing organisms, but *E. terrae* is a gram-negative bacterium which protects itself by exporting teixobactin across the outer membrane, and there is nothing for target gram-positive organisms to borrow. It appears that teixobactin evolved to be essentially free of resistance. The discovery of teixobactin challenges the dogma of inevitable resistance.

References

APUA Headquarters in Action

APUA attends BAARN conference
The Boston Area Antibiotic Resistance Network was founded as a response to the antibiotic crisis and is guided by the philosophy of synthesizing solutions based on rational, scientific information. As a result BAARN organizes yearly symposia where interdisciplinary teams of scientists, academics, industry experts, and government agencies can come together to exchange knowledge.

The 2015 Boston Area Antibiotic Resistance Network (BAARN) Symposium took place on March 10 at the Broad Institute in Cambridge, MA. The 2015 symposium focused on novel approaches to rapid diagnostics and therapeutics. Barbara Lapinskas and Jane Kramer represented APUA at the event.

APUA President Dr. Levy presents at Boston conference
The 6th conference hosted by Boston University and the Consortium of Universities for Global Health (CUGH) was titled “Mobilizing Research for Global Health” and held in March in Boston, MA. With panel members Ramanan Laxminarayan (chair), Lance Price, Maryn McKenna, and Kevin Outerson, Dr. Stuart presented on the topic *The Challenge of Declining Antibiotic Resistance*. APUA intern, Casi Kadangs, was also in attendance at the conference and shared program updates on APUA’s social media platforms.

This year’s conference boasted a long list of experts in the field from prestigious institutions including Boston University, MIT, Harvard University, WHO, London School of Hygiene & Tropical Medicine, and the Bill and Melinda Gates Foundation among others. Conference themes included Ebola, technology in genetics, vaccines for the 21st century, disaster preparedness and response and much more. More information about the conference can be found [here](#).

APUA board meeting
On March 17th, 2015 members of the APUA Board of Directors gathered at the headquarters office for the first of their tri-annual meetings. The agenda of the meeting included an organizational overview presented by Dr. Levy, updates on current projects and organizational involvements, and considerations for future development and collaboration. A highlight of the meeting was the need for greater collaboration with the international chapters through small grants and other initiatives. The finer details of implementing these initiatives are being worked out and will be communicated in due time.

Upcoming Events

May 30–June 2, 2015: 115th General Meeting of the American Society for Microbiology (ASM), New Orleans, LA, USA
June 2-3, 2015: Uppsala Health Summit, Uppsala, Sweden
June 16-19, 2015: International on Prevention and Infection Control (ICPIC), Geneva, Switzerland
June 27-29, 2015: Association for Professionals in Infection Control and Epidemiology (APIC) Annual Conference, Nashville, TN, USA
September 18-21, 2015: The American Society for Microbiology and the International Society of Chemotherapy for Infection and Cancer (ISC) host ASM’s Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC) and ISC’s International Congress of Chemotherapy (ICC), San Diego, CA, USA
October 15-16, 2015: IACMAC Volga Region Conference on Antimicrobial Therapy, Saratov, Russia
April 9-12, 2016: 26th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID), Istanbul, Turkey

See more [events](#)
APUA-Lebanon partnerships and collaborations established in 2014:

- The chapter became officially represented in the Lebanese Society for Infectious Diseases and Clinical Microbiology (Lebanese Order of Physicians).
- The chapter has signed a MOU with the Ministry of Agriculture to collaborate in research activities on the spread of antimicrobial resistant organisms in Lebanese farm animals.

APUA-Lebanon chapter activities in 2014:

- Organization of a 5-day course on “Epidemiology of Resistance and Measures of Control” held September 26-30, 2014 in Beirut. Dr. John Stelling from the WHO Collaborating Center for Antimicrobial Resistance, Boston, USA gave three presentations and a hands-on training of WHONET software. Forty-five infectious diseases specialists, microbiologists, and infection control officers attended. Dr. Ziad Daoud, Dr. Eid Azar, and Dr. Claude Afif addressed the issue and concerns of antimicrobial resistance in the country.
- In partnership with national and international institutions, APUA-Lebanon has participated in the organization of two events:
  1) A one-day course on “Standardization of antimicrobial susceptibility course” (Beirut, December 2013), APUA-Lebanon coordinated part two of the course “Correlating antibiotic resistance to antibiotic consumption”
  2) A “WHONET workshop for 65 microbiology specialists in the PULSENET/AMR laboratory network on Foodborne diseases (Beirut, September 27, 2014), jointly presented with the ASM, the Ministry of Public Health (Lebanon), and the WHO.

Participation in local scientific meetings

The chapter team working on resistance in gram-negative bacilli presented “Resistance in gram-negative bacilli: patterns, consumption, and synergistic combinations” at the 16th annual congress of the Lebanese Society for Infectious Diseases and Clinical Microbiology.
APUA-Lebanon research collaborations:
The APUA-Lebanon has established research collaborations with 2 academic Lebanese institutions: The Faculty of Medicine at the University of Balamand and the Faculty of Medicine at the American University of Beirut. In this context, the APUA team was active and successful in the production of several abstracts presented at major conferences (See box).

APUA-Lebanon ongoing activities:
- The production of a “National Antibiotic Report” summarizing the antimicrobial resistance data of 10 hospitals countrywide is expected to be finalized in April 2015.
- A pilot study, initiated in Dec 2014, is examining the occurrence and spread of MDRO in livestock. The study, performed in collaboration with the University of Balamand, Faculty of Medicine, is funded by the Lebanese Center for Scientific Research (CNRS) and supported by the Ministry of agriculture in Lebanon.

APUA-Nepal
The APUA-Nepal chapter remains active and steadfast in promoting antibiotic stewardship. The following main activities were accomplished in the first quarter of this year:

- A program discussing problems of antimicrobial resistance was held by APUA-Nepal in February 2015. It consisted primarily of two presentations,
Resistance in the News

Medical Xpress interviews APUA President Levy

APUA President, Dr. Stuart Levy, was recently interviewed by the online journal Medical Xpress regarding the current state of antibiotics, lessons from the past, and prospects for the future. Three decades ago, few scientists or physicians gave much heed to the possibility that antibiotic misuse would become a global issue, even though it was already a problem in the developing world. Dr. Levy was one of the first to warn against antibiotic dependence in agribusiness and how it contributes to resistance in humans. In late 2014, he contributed to a report to President Obama on combating antibiotic resistance. Subsequently, the Obama Administration published an action plan to address this problem in the United States and beyond. The current spotlight on antibiotic resistance has generated renewed interest and investment in the development of new drugs and spurred the recent discovery of a novel antibiotic that shows considerable promise for circumventing the development of resistance. Although this is good news in many ways, Dr. Levy is cautious and says “when you give an antibiotic, you will select resistance. It may take some time, but you will get resistance to that antibiotic”.

Big pharma cuts costs, employees

In a wave of cutbacks to “narrow its research focus”, pharmaceuticals giant AstraZeneca announced in March the discharging of 95 employees from its Infection iMed unit in Waltham, MA. Similarly, Merck made over 100 job cuts after taking over Cubist Pharmaceuticals Inc. Some scientists have expressed concern over this trend, especially in light of the rise in antibiotic resistance globally. John S. Gilmore, professor at Harvard Medical School, states “This is highly disruptive. There are few places focused on this [anti-infective] problem in a significant way. As companies have given up the fight or have been taken over, you break that continuity” Despite the dismal news, others in the industry see a silver lining. As larger companies merge and take over smaller ones, more start-ups are entering the field, which is ultimately viewed as a good development. Read more here.

Global consumption of antibiotics in livestock on the rise

It is estimated that in the next 20 years, global antibiotic use in animals will increase by two-thirds in emerging economies such as Brazil, Russia, India, and China. Experts expect that antibiotic use will double—fueled by growing consumption of meat, milk and eggs by wealthier and increasingly urbanized populations. The data derive from a report in which researchers from Princeton University, the National Institutes for Health, and the International Livestock Research Institute measured global antibiotic consumption in livestock and how it drives the rise of super-bugs in humans.

Online treatment for STIs seen as a menace to public health

In 2003, the UK National Chlamydia Screening Programme launched an online platform where users can access diagnostic tests questionnaires for Sexually Transmitted Infections. The rationale was to encourage people to seek answers and help for illnesses that might be perceived as embarrassing in physical contact with a health care professional. However, a recent investigation into the web service has revealed incorrect treatments for STIs. For example, both an injection and pills are required to treat gonorrhea effectively, but the online portal only prescribes pills. In addition, there are no actual microbiological tests performed and thus treatments are not specific. Likewise, the issue of sexual partners at risk is not being addressed. Antibiotic resistance also becomes a factor when pills are prescribed indiscriminately. It is hoped that a better way to provide access and quality to patients will be found. Find the article here.

Rapid diagnostic research projects receive NIH funds

In early April, the National Institute of Allergy and Infectious Diseases (NIAID) announced that it had awarded over $11
Fake drugs drive up fatalities and increase resistance

The battle against antibiotic-resistant bacteria as well as a host of other infectious agents is made even more difficult by the barrage of fake pharmaceuticals that are in circulation globally. Unfortunately, the issue of fake drugs is not new to the world of medicine. In a special issue of the American Journal of Tropical Medicine and Hygiene, experts showed that in a sample of 16,800 drugs an estimate of 9-41% did not meet quality standards. This means that the drugs contained sub-therapeutic levels of the active ingredient or in some cases, no active ingredient at all. The sample included anti-malarial, anti-tuberculosis, antibiotics, and anti-leishmaniasis drugs. These bad drugs are not only ineffective in treating illnesses, they also help to make superbugs more resistant. The NIH’s Joel Breman states that what is needed in combatting the fake drugs market

million in funding for research projects around the country. These projects support enhanced diagnostics for rapidly diagnosing antimicrobial-resistant bacteria, with a focus on bacteria that cause infections in healthcare settings. NIAID Director Dr. Anthony S. Fauci states “one way we can combat drug resistance is by developing enhanced diagnostic tests that rapidly identify the bacteria causing an infection and their susceptibility to various antimicrobials.” The awardee institutions will focus their research on the following bacteria: Klebsiella pneumoniae, Acinetobacter baumannii, Psuedomonas aeruginosa, Enterobacter species, and Escherichia coli.

These awards for the advancement of research on rapid diagnostics are in line with the recently-released National Action Plan for Combating Antibiotic-Resistant Bacteria. For the list of awardees and more information, visit the NIH website here.
is an international body that decides the best and most cost-effective ways of detecting these drugs and training people on how to enforce the regulations.

Global response to antimicrobial resistance crisis not adequate, says WHO

A report titled “Worldwide country situation analysis: Response to antimicrobial resistance” was recently published by the WHO to determine the plans of action being implemented by countries in response to the current antibiotic crisis. The WHO assistant director-general for Health Security, Dr. Keiji Fukuda, calls it “the single greatest challenge in infectious diseases today”. The report reveals that out of 133 countries that participated in the survey, only 34 have a comprehensive national plan to fight resistance to antibiotics and other antimicrobial agents. This dismal response is amplified by the fact that in 2013, there were an estimated 480,000 new cases of multidrug-resistant tuberculosis. There has also been an increase in resistance to the first-line drugs used in HIV treatment since 2012. In order to draw more attention to the seriousness of this issue, the 68th World Health Assembly has asked governments to declare their commitment to address growing antimicrobial resistance by approving a global plan drafted by the WHO. The plan follows five strategic objectives namely:

♦ Improve awareness and understanding of resistance
♦ Increase knowledge through tracking and research
♦ Reduce the incidence of infection
♦ Optimize the use of antimicrobial medicines
♦ Ensure sustainable investment in countering antimicrobial resistance

The full report can be found here.

APUA-Georgia

The monitoring of antimicrobial resistance data continues through the laboratory network established by the Service of Antimicrobial Chemotherapies. The chapter regularly analyzes these data and updates its recommendations for empirical antimicrobial therapy. The Chapter also participates in the Study for Monitoring Antimicrobial Resistance Trends (SMART) conducted by the International Health Management Associates, Inc. (IHMA). Gram-negative clinical isolates of intra-abdominal and urinary tract origins are forwarded to the laboratory in Epalinges, Switzerland for identification and MIC tests. At the 2014 ECCMID in Barcelona, APUA-Georgia presented the poster: Prevalence of ESBL-producing E.coli and K. Pneumoniae in Georgia 2011-2012. Lastly, in October 2014 APUA-Georgia Chapter participated in a pilot Global Point Prevalence Survey of Antimicrobial Consumption and Resistance (GLOBAL-PPS).
President Obama’s National Action Plan for Combating Antibiotic-Resistant Bacteria

The discovery of penicillin in 1928 marked a turning point in human medicine, resulting in millions of lives saved from previously deadly infections. With the rise and spread of antibiotic-resistant bacteria, the security that antibiotics have given us is threatened. In the US alone, an estimated 23,000 deaths annually are caused by antibiotic-resistant bacteria. There are many factors that contribute to the surge in antimicrobial resistance including improper use of antibiotics by humans, overuse of antibiotics in food livestock, environmental pollution, and lack of new drugs. As a result, President Obama issued an Executive Order for the creation of the National Action Plan for Combating Antibiotic-Resistant Bacteria. This document will serve as a ‘roadmap to guide the nation in rising to this challenge’. It includes five main goals which are:

♦ Slow the emergence of resistant bacteria and prevent the spread of resistant infections.
♦ Strengthen national one-health surveillance efforts to combat resistance.
♦ Advance development and use of rapid and innovative diagnostic tests for identification and characterization of resistant bacteria.
♦ Accelerate basic and applied research and development for new antibiotics, other therapeutics, and vaccines.
♦ Improve international collaboration and capacities for antibiotic-resistance prevention, surveillance, control, and antibiotic research and development.

Read the full document here.
Antimicrobial resistance is a global scourge, and although statistics are not available for every country, it claims the lives of about 50,000 people each year in the U.S. and across Europe. Even in sophisticated health care systems, as can be found in many European countries, more and more patients are presenting with pan-resistant infections—meaning effective treatment is not available. Economic growth in the developing world and the BRIC (Brazil, Russia, India, and China) nations have contributed to the surge in antibiotic use, accounting for about three quarters of the growth between 2000 and 2010. Other factors such as intercontinental travel also play a role.

Due to this imminent threat to the “health and wealth” of nations, the UK Prime Minister and the Wellcome Trust initiated a panel of experts to develop action steps to address this issue. This document is the panel’s first report, and it focuses on the following 5 themes:

♦ The impact of antimicrobial resistance on the world’s economy if the problem is not tackled.

♦ How we can change our use of antimicrobial drugs to reduce the rise of resistance, including the game-changing potential of advances in genetics, genomics, and computer science.

♦ How we can boost the development of new antimicrobial drugs.

♦ The potential for alternative therapies to disrupt the rise in resistance and how these new ideas can be boosted.

♦ The need for coherent international action that spans drugs regulation, and drugs use across humans, animals, and environment.

APUA article examines European algorithms for potential CRP point-of-care diagnostic

Promoting the development of new rapid diagnostic tests is one of the strategies of combating antibiotic resistance recommended by the “National Strategy for Combating Antibiotic Resistant Bacteria”. APUA hosted a summit in May 2014 of international experts to discuss the potential of a point-of-care diagnostic in improving antibiotic use in outpatient settings. The article, *Improving outpatient antibiotic prescribing for respiratory tract infections: Results of new algorithms used in European trials* (by R. Gaynes and S. Levy), describes the difficulty U.S. physicians face in differentiation between bacterial and viral respiratory tract infections, which are responsible for the greatest amount of antibiotic prescriptions. This is because routine laboratory tests tend to be inconclusive and there is no general consensus in the field on what tests to order in the first place.

The authors point to the success of point-of-care rapid diagnostics in the European Union as a possible model for the U.S. However, before it gains traction, any potential diagnostic will have to successfully go through clinical trials in outpatient locations on the aspects of “accuracy, time to result, simplicity, and cost”.

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About us

Antibiotics are humanity's key defense against disease-causing microbes. The growing prevalence of antibiotic resistance threatens a future where these drugs can no longer cure infections and killer epidemics run rampant. The Alliance for the Prudent Use of Antibiotics (APUA) has been the leading global non-governmental organization fighting to preserve the effectiveness of antimicrobial drugs since 1981. With affiliated chapters in more than 65 countries, including 33 in the developing world, we conduct research, education and advocacy programs to control antibiotic resistance and ensure access to effective antibiotics for current and future generations.

Our global network of infectious disease experts supports country-based activities to control and monitor antibiotic resistance tailored to local needs and customs. The APUA network facilitates the exchange of objective, up-to-date scientific and clinical information among scientists, health care providers, consumers and policy makers worldwide.

The APUA Newsletter has been published continuously three times per year since 1983.
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