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Introduction to this issue

With the current spotlight focused nervously on the Ebola virus outbreak, the whole matter of vector transmission in the environment has advanced to the forefront of attention. In our ever more closely entwined global society, the issue of environmental vectors and transport can no longer be ignored. The relatively recent formation of the “One Health Initiative” (http://onehealthinitiative.com) as a more holistic approach for addressing health and disease issues in our tightly knit global community, has highlighted the need for attention to the intricacies of animal, human, insect, water and soil interconnectedness and the vectors that overtly or subtly interface with pathogens and non-pathogens in these environments.

This issue of the APUA Newsletter explores the concept of resistance genes as emerging contaminants or pollutants in our environment and examines a small sampling of the environmental niches that are impacted by antibiotics. The total tonnage of antibiotics applied in humans and animal husbandry is disputed, but up to 95% of the antibiotics consumed are excreted in an unaltered state. This antibiotic “spillage” from excretion and discarded drugs ends up in the environment – rivers, streams, farmlands and even waters processed and reclaimed through water treatment facilities.

The increasing reports of antibiotics in our environment indicate wide dispersion through our ecosystem via multiple and complex pathways, many of which are largely unexplored in terms of resistance gene transmission. As “societal” drugs, antibiotics have profound ramifications—far beyond the boundaries of the original consumer. Persisting at levels well below the original therapeutic doses, these contaminants are capable of inducing, selecting and generating antibiotic resistance genes (ARGs) that have now also been identified in a wide variety of environmental niches (Fig. 1). This propagation of ARGs in the environment makes them unique among contaminants. Through low-level selection pressure and the process of horizontal gene transfer, ARGs may be multiplied through pathogens and non-pathogens alike, including distantly related species. Moreover, they may persist with, or without, a viable host. ARGs are known to coalesce into movable “cassettes” of resistance genes (integrons) and even “superintegrons,” which reportedly contain over 100 cassettes. The co-existence of these linked genes indicates the potential for cross-resistance, i.e., the ability to resist multiple antibiotics when confronted with only a single drug.

Wastewater processing breeds resistance genes

This Newsletter issue focuses on some of the often overlooked elements of the environment that play varying, but potentially significant roles in the dissemination of ARGs. Water is an element that interfaces all our environmental niches. Recent efforts in water conservation have forced the issue of water reclamation, accompanied by an upsurge in interest in how these processes affect the spread of pathogens and antibiotic resistance.

The article by McGowan takes a closer look at the structure, age and function of our wastewater treatment plants (WWTPs) and examines their shortcomings in dealing with the problem of propagating and spreading resistance genes. WWTPs are interfaces between multiple different environments (domestic, industrial, healthcare) and have become a reservoir for the convergence of pathogens, opportunistic pathogens and environmental bacteria. Even the most high-tech plants may be

fertile breeding grounds for antibiotic-resistant superbugs, and some multi-resistant strains reportedly can resist as many as 7 or 8 antibiotics. Of interest in this regard is the recent collaborative study between Rice University (Texas, US) and Nankai and Tianjin Universities (China) which garnered the Grand Prize in University Research for their work on the proliferation of NDM-1 positive superbugs in Chinese activated sludge treatment plants. Four to five-fold more highly resistant NDM-1 positive strains were exiting the wastewater treatment plant than entered it, and the resistance genes remained transferable to other benign bacteria.

Of recent concern are the outputs of WWTPs—the sludge and treated wastewater (multi-stage treated, plus UV and bleach), the latter of which is circulated through so-called “purple pipes” and subsequently dispensed for irrigation of golf courses, grounds of city schools, sports fields and municipal parks. Biosolids (dry sewage) application is a common practice that disperses unknown amounts of both antibiotics and resistance genes. While this practice has been banned in Switzerland, the Netherlands and parts of Canada, in the US alone, millions of tons of biosolids are generated and about one-half is processed for use as fertilizer.

The effects of individual wastewater treatment processes are largely unknown and study is needed on both the fate of the antimicrobials and the resistant bacteria themselves. Antibiotics may be reduced as much as 85%, (e.g., norfloxacin and ciprofloxacin), remain unchanged (e.g., enrofloxacin), or actually increase in these processes. (e.g., nalidixic acid). Although seemingly eliminated from effluent, antibiotics are actually adsorbed to sludge, rather than undergoing biodegradation, and can persist in sludge for long periods. Because ARGs are now so widely dispersed in the environment and sometimes display high background levels, it can be challenging to determine what constitutes elevated levels of greater concern. While evidence points to a proliferation of resistance genes within the WWTP transport pipes themselves, the implications of finding resistance genes at sludge application sites are as yet unknown. The lack of sufficient data prevents an assessment of quantitative health risks at present.

Establishing causal links

While an abundance of ARGs in the greater environment is not disputed, the greater challenge has been demonstrating the causal links between antibiotic use in one environment and disease emergence in another – particularly with regard to the substantial use of antibiotic growth-promoters in animals. Nonetheless, with recent rapid advances in the newer molecular technologies (i.e., sequence-based metagenomics), microbial hosts from different communities can be compared and the links between these environments are becoming more evident.

In a recent issue of the APUA Newsletter (volume 25 issue 2), the significance of extended-spectrum beta-lactamase (ESBL)-producing bacteria was discussed in depth. Here, the article by Hachler and Stephan demonstrates how tracking of ESBL genes can be an ideal model for examining the many environments that interface with humans. Their studies of humans, wildlife, pets, farm animals, food and water illustrate the scope of spread of ESBL genes and highlight in particular the role of WWTP in disseminating and concentrating ESBL types that are shared with humans.

A third area addressed in this Newsletter is the less well-recognized role of insects, which harbor considerable potential for dispersing bacterial pathogens and facilitating the spread of resistance genes to and from pathogens in our environment. The article by Ghosh and Zurek looks more closely at these vectors, examining the exposure of insects to antibiotics used in food-animal husbandry and the habitats of excreted manure that provide ideal breeding grounds for these insects. Not surprisingly these authors show evidence for the sharing of multidrug-resistant clonal lineages between insects and the manure of commercial swine operations. Their tracking then extends to the environs of restaurants, restaurant food, and wastewater treatment facilities, following in particular, Enterococcus spp.

As highlighted in the recent WHO report, there is a crisis in antibiotic resistance that demands urgent action for this complex problem. In addition to the dire need for new antimicrobials, a reduction in antibiotic overuse is mandated. Actions to mitigate contamination through environmental pathways are also warranted, but these are not yet addressed by WHO.

Noting that formal risk assessments are necessary—but will only postpone the crucial action needed—Pruden et al in their recent critical review, outline multiple options that can be implemented immediately, but often at minimal cost. They identify simple strategies, such as nutrient management, runoff control, and infrastructure upgrades that work synergistically with current policies. Clearly new capture technologies are needed. One such novel removal method would engineer the native bacterial efflux pump (Acr-B) by coupling it with a solar-driven proton motive force (Delta rhodopsin) in a membrane-bound vesicle. In the presence of direct sunlight, the vesicle system can selectively capture twice the volume of antibiotics.
Transfer of extended-spectrum $\beta$-lactamase (ESBL)-producers at the human-food chain-environment-wildlife interface in Switzerland

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Extended-spectrum $\beta$-lactamases (ESBL) are the resistance determinants most appropriate to serve as a model to show the dissemination of antibiotic resistance from the human to the non-human environment. This, for a number of reasons: (i) $\beta$-lactams are the earliest antibiotics, and are in clinical use since the 1940s, (ii) because of their perfect selective toxicity, they are the most popular antibiotics being administered in nearly two thirds of all human antimicrobial treatments worldwide, (iii) a vast number of chemical derivatives of $\beta$-lactams, including five generations of cephalosporins have been developed (iv) thus, enormous selective pressure has been exerted by $\beta$-lactams, prompting bacteria to adapt through evolutionary diversification and fine tuning of resistance factors, and (v) extensive research on $\beta$-lactamases over decades has facilitated the development of tools for rapid detection, as well as easy and precise molecular characterization.

The first $\beta$-lactamase was discovered in 1940, almost simultaneously with the introduction of the first penicillin into clinical practice. The first plasmid-mediated and horizontally transferable $\beta$-lactamase in Escherichia coli - TEM-1 - was described in 1962 and hydrolyzed penicillins and first-generation cephalosporins (1G-Ceph) soon followed by SHV-1 with a similar substrate spectrum. During the 1970s and early 1980s, 2nd and 3rd-generation cephalosporins (2G-Ceph and 3G-Ceph) came into market, followed by the 4G-Cephs in the 1990s. These new formulations were to become indispensable for clinicians, and were accordingly over-used. Consequently, bacteria became exposed to heavy selection pressure and reacted by optimising their $\beta$-lactamase structures. Starting from the broad-spectrum $\beta$-lactamases TEM-1 and SHV-1, two whole families, TEM- and SHV-ESBLs, arose through microevolutionary processes that led to few amino acid substitutions, thus extending the substrate

![Figure 1. Prevalence in Switzerland of bla\textsubscript{ESBL} genes in Enterobacteriaceae of farm-animal or healthy-human origin.](image)

A: chickens; B: sheep, C: cattle; D: pigs; E: healthy humans. (Graphics taken from Ref. 7 with written permission from the original publisher).
spectra to also include 2G-, 3G- and 4G-Ceps and monobactams. The first such ESBL, SHV-2, was described in Germany in 1982, followed by TEM-3 in 1987. Not only did these two ESBL families grow to encompass further types, but at least two more families conferring an ESBL phenotype were discovered, the most important being the cefotaximases of the CTX-M type. Bush et al. provided a classification system, and Jacoby and Bush set up an internet platform for all authors reporting new β-lactamases. This platform, when accessed in August 2014, had 219 TEM-, 188 SHV-, and 159 CTX-M β-lactamases on display: truly a formidable evolutionary record by the bacterial pathogens within just three decades.

Bacterial isolates producing ESBLs also underwent a remarkable development, and, with time, became an issue of much concern. Although ESBL-producers were at first sporadic, opportunistic pathogens—mainly E. coli isolated from long-term hospitalized patients—they were soon found responsible for nosocomial outbreaks. Moreover, ESBL-encoding plasmids were soon transferred horizontally into many other species of Enterobacteriaceae, including obligate pathogens such as Salmonella sp., and even non-fermenters, including Pseudomonas aeruginosa. By the end of the century, ESBL-producers had disseminated around the world, and, interestingly, CTX-M group enzymes had replaced TEM- and SHV types as the dominating ESBL family for as yet unknown reasons. By around 2005, ESBL-producers made up an ever-increasing proportion of isolates from patients of private practitioners, heralding a shift from the hospital to the general public. Thus, with some delay, this paralleled a similar tendency of the methicillin-resistant Staphylococcus aureus (MRSA) from hospital- towards community-acquired MRSA. This tendency, together with an illustrative flow chart showing the putative pathways of dissemination of ESBL-producers into the environment has been outlined in an excellent recent review.

Switzerland is a country with highly developed industrial and food technological standards, as well as a sophisticated medical system. By installing nationwide infectious disease experts and hospital hygienists etc., it imposes strict prescription guidelines for the prudent use of antibiotics in human and veterinary clinical practice as well as in agriculture. Consequently, we decided to search for producers of ESBLs along the food chain, in patients from primary care, in healthy humans, in wild animals, and in the environment. The aim was to (i) collect sets of strains from the mentioned origins, (ii) characterize all strains in much detail using molecular methods, and (iii) compare the sets in order to gain insights into possible routes of dissemination.

ESBL-producers are most prominent among Enterobacteriaceae, and are by far most frequent in E. coli. Considering our knowledge gained from foreign studies on the shift of prevalence of such organisms from hospitals to the general public, food was primarily suspected as a vehicle, and in particular meat and animal products, because of the well-known therapeutic application of antimicrobials in animal husbandry. Consequently, faecal carriage of ESBL-producers in various farm animals and corresponding food products such as meat and milk were assayed. Faecal swabbing of cattle, pigs, sheep, and chickens at slaughter yielded ESBL-positive isolates in 13.7%, 15.3%, 8.6%, and 63.4% of samples, respectively. In contrast, no ESBL-producers were found in minced beef or pork from retailers, and neither were any of 100 bulk raw milk samples contaminated, although one sample (withheld from market because of mastitis) grew a producer of CTX-M-14 ESBL. However, 78% of poultry samples from retailers yielded E. coli expressing CTX-M-1, and 15% of cutting boards from a hospital kitchen grew ESBL-producers after processing of poultry meat. Again, CTX-M-1 was predominant. Concurrent studies on humans in Switzerland revealed that 5.8% of healthy subjects and 5.2% of primary care patients carried ESBL-producers in their stool. Sequencing of the blaESBL genes from the strain sets of these studies provided an astonishing multiplicity of expressed ESBLs (Fig. 1), and even a novel type, CTX-M-117. However, it became obvious that CTX-M-1 was predominant in food animals and poultry meat, while CTX-M-15 (42%) was most frequent in humans (Fig. 1). Interestingly, 8/107 (7.5%) isolates from cats and dogs with urinary tract infections also yielded CTX-M-15. Considering these frequencies and the Swiss consumer habits, animal food products, particularly poultry, offer a plausible explanation for the 29% of CTX-M-1 producers found among the isolates from human ESBL carriers. However, the main proportion—the 42% of human carriers of CTX-M-15 producers—could not be explained by these studies.

Prompted by the relatively high ESBL prevalences encountered within the realm of humans and farm animals, the scope of the investigation was extended to cover wild animals and the environment. Among a total of 235 hunted ibex, chamois, red deer, and roe deer, a single roe deer was identified as a carrier of E. coli expressing CTX-M-1. It had been shot in a rural area.
of the central lowlands of Switzerland, where nocturnal grazing on a cow paddock could not be excluded. Among 298 street pigeons from the City of Zürich, one carried a producer of CTX-M-15. Of 30 great cormorants one each was a carrier of E. coli expressing CTX-M-15 or CTX-M-27, respectively. Sampling 139 fish (8 species) caught in two Swiss lakes (Lake Zürich and Lake Thun) we identified 26 (18.7%) as ESBL carriers. Some even carried multiple strains, yielding a total of 32 ESBL-producers and one producer of plasmid-mediated AmpC β-lactamase. Interestingly, the most frequent ESBL type was CTX-M-15 (13/32 [40.6%]).

Eventually, a systematic investigation into surface waters covering the German part of Switzerland was performed, whereby 40 rivers and 18 lakes from urban and rural areas, including low and high altitudes, were surveyed by means of filtering 500mL per sample for examination. The results are depicted in Fig. 2. Alarmingly, 21 of the 58 samples from the water bodies (36.2%) yielded a total of 74 Enterobacteriaceae producing ESBLs. A variety of ESBL types were found. However, as found in healthy humans, CTX-M-15 was the dominating type (62%). Moreover, ESBL-producers were clearly confined to the urban areas, while samples from altitudes above 1000m remained negative even though sampling had been executed during the alpine summer farming season (Fig. 2, red circles and blue squares). Very worrisome was the detection of a Klebsiella pneumoniae strain expressing VIM carbapenemase (Fig. 2, red triangle). VIM belongs to a relatively recently discovered family of metallo β-lactamases that, unlike the ESBLs, compromises the last remaining effective treatment option among the β-lactam antibiotics—the carbapenems.

In conclusion, ESBL-producers are extremely widely disseminated in humans, in food animals and pets, in various wild animals, and even in the urban low altitude surface waters in Switzerland. Careful determination of ESBL types has yielded convincing evidence that outlines four major findings: (i) food animals, particularly poultry, are an important reservoir of E. coli producing CTX-M-1 ESBL and may be
responsible for a part of the ESBL-producing *E. coli* that colonize humans; (ii) although the reservoir of CTX-M-15-producers has not so far been discovered, CTX-M-15 is the most frequently found ESBL (41%) among the 5.8% of healthy humans excreting ESBL-producers; (iii) humans and pets largely share the same ESBL type, CTX-M-15; and (iv) surface waters and humans share the most frequent ESBL type, again CTX-M-15. The latter finding strongly suggests that CTX-M-15-producers may be disseminated by human sewage via waste water treatment plants (WWTP) into the environment. This view is convincingly supported by a very recent French study showing that ESBL-producing *E. coli* are less efficiently eliminated by WWTPs than are susceptible *E. coli* of the normal flora, and are thus relatively enriched.  

Finally, owing to the fact that ESBLs are almost exclusively encoded on conjugative plasmids, they are currently so evenly disseminated over a plethora of different clones of *Enterobacteriaceae* that any endeavour to trace particularly promiscuous clones—e.g., by genetic typing of chromosomal backgrounds with pulsed field gel electrophoresis—must fail (e.g., Figure 1 in Ref. 6). In order to generate even more precise data on the routes of dissemination than is shown in this review, there will therefore be no way around laborious genome sequencing of whole series of the involved conjugative plasmids, as has been attempted in a recent pilot study.  

**Acknowledgements**

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**References**

Antibiotic resistance genes in wastewater treatment and reclamation: hazards and challenges

Edo McGowan, PhD (retired/Emeritus): Dr. McGowan has over 40 years’ experience in the development and direction of local, regional, and international programs and policy relating to health aspects of water quality, vector control, the analyses and disposal of hazardous materials, and the use of water as a vehicle for bioterrorism.

The undisputed current reality is that we are losing our antimicrobials to resistance, hence, a potential loss of an effective defense against increasingly serious pathogens. This is not news. Nonetheless, I’d like to broaden the context of this discussion beyond addressing prudent use of antimicrobials and consider controllable “things” that are generating resistant bacteria and why those “things” are ignored.

We have seen the basis of antibiotic-resistant infections expand from narrowly confined classic sites, such as hospital ICUs, to the community at large. Through the ease of modern transport, this community disbursement has broadened to all corners of the globe. Because this expansion has been a relatively recent event, questions of community sources remain essentially unanswered and largely under investigated. What do we find in the community at large that might be a reservoir for resistant pathogens? Are these pathogens circulating back into hospitals and again into the community? How might we demonstrate such? If we could identify a common causative factor, would that factor be controllable? Would there be the political will to control? What kinds of technologies, policies and expenditures would be needed?

In fact, there is such a source embedded in our communities. It is directly connected to hospitals, is currently inadequately controlled (but potentially controllable), and generally ignored at the industry and regulatory levels. This source is wastewater—but wastewater viewed within a broader context than is typically considered.

Wastewater is derived from potable water that has been utilized and subsequently discharged to sewers. It generally undergoes some type of processing in a wastewater treatment plant (WWTP) and is then released back into the environment—usually into a river or lake where that water will again be used for supplying drinking water and irrigation water for food crops (Fig. 1).

Propagation of antimicrobial resistance

The close juxtaposition of sub therapeutic levels of discarded and excreted antimicrobials, together with microbes in a WWTP, fosters gene exchange, thereby enhancing resistance. Several major studies have demonstrated this. In one study, the authors followed fecal coliforms, tracing the movement and frequencies of resistant bacteria through a WWTP at various locations along the treatment process, i.e., the inlet, primary sedimentation tank, activated sludge digestion tank, final settling tank, outlet and return activated sludge drain. Both resistant and susceptible bacteria were tracked and examined for the presence of drug resistance plasmids. From 900 individual isolates tested for resistance to tetracycline, kanamycin, chloramphenicol, streptomycin, ampicillin, nalidixic acid, rifampicin, and sulfisoxazole, more than half contained plasmids encoding multi-drug resistance. While this was interesting, another finding raised even greater concern. The further along that the wastewater progressed through the treatment process, the greater the tendency was to encounter strains that had developed multi-resistance and simultaneously carried transferable drug-resistance plasmids. Thus, the development of drug resistance and the transfer of multi-drug resistance are enhanced in WWTPs.

Dispersal of resistance genes into the environment

Current academic studies on some of the nation’s most sophisticated sewer plants document that they are discharging resistant microbes and antibiotic resistant genes (ARGs) in
impressive amounts directly into US rivers and lakes, from which other cities subsequently draw drinking water.\textsuperscript{13} However, because of current antiquated standards and non-action by regulators, this is perfectly legal. Researchers are also documenting the passage of ARG's into drinking water supplies.\textsuperscript{29, 30} These resistant organisms are also present in reclaimed (recycled) sewer water which is already legally allowed to be used on vegetables consumed raw and to recharge ground water basins used for drinking water.\textsuperscript{31}

Chlorine and ultra-violet light are the two main forms of disinfection used by the water industry. The emergence of organisms resistant to chlorine used in water treatment have now been reported.\textsuperscript{32, 33} Chang, et al, (2007) noted that exposure of \textit{Staphylococcus aureus} to chlorine causes shifts in the genes that enhance virulence factors.\textsuperscript{33} Resistance genes and genes involved with virulence are essentially unaffected by both chlorine and UV when used at the levels found in water treatment.\textsuperscript{13, 35, 36} Additionally, the filters typically used by industry do not stop through-put of these genes.

Some of these same authors have also examined the reclaimed (recycled) water which is used ubiquitously for irrigation of farmlands as well as municipal parks and recreation fields. Antibiotics escape capture by the current non-specific activated carbon filtration methodology, due to their relative low abundance. While reverse osmosis will accomplish this goal, it is still relatively costly.

Resistant bacteria can transfer their ARGs to the intestinal microbes of humans and animals. That information may persist for years — contributing to increased resistance in higher grade pathogens through interspecies transfer.\textsuperscript{41}

**Outdated and failing systems**

Although water processing is regulated, controls of

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**Figure 1. Conceptualized modern wastewater treatment plant (WWTP)**

The above diagram conceptualizes the many processes that may be involved in the treatment of sewage and wastewater derived from a diverse set of community services (domestic, industrial, health care, etc.). The resulting products are 1) sludge, which may be applied to agricultural farmlands; and 2) recycled water products, both potable and non-potable. The latter is increasingly utilized for irrigation of farmlands as well as municipal parks and recreation fields. Antibiotics escape capture by the current non-specific activated carbon filtration methodology, due to their relative low abundance. While reverse osmosis will accomplish this goal, it is still relatively costly.

Source: "Tropical Connections: South Florida's marine environment" (pg. 101)  http://ian.umces.edu/imagelibrary/displayimage-lastup-0-7574.html
such systems (WWTPs and drinking water plants) are based on engineering and operational standards/concepts that predate the antibiotic era. Wastewater treatment plants (in the U.S. and globally) were never designed to fully eliminate pathogens and their resistance genes. Additionally, the standards under which these plants operate (e.g., water quality standards of the US, as well as those of the World Health Organization [WHO]), do not effectively consider the realities of numerous pathogens as well as resistance nor the complexities caused by modern industrial waste discharged to sewers. Overwhelmed and unequal to the task, our current wastewater treatment plants are failing.

**Regulatory shortcomings**

Typically, the water industry has been in control of this failing process. The challenge has outstripped the cumulative control capacity of those in charge, including the regulatory community. Such loss of control encompasses the generation of antimicrobial resistance and other critical contaminants that are presumably removed in the waste water treatment process. In addition, other constituents of concern interact with both sewage and its byproducts with little effective oversight or investigation by the regulatory community.

The subject of wastewater plant-generated resistance was extensively studied and confirmed in the late 1970s by the US EPA, through a series of studies at its Wastewater Research Division, Municipal Environmental Research Laboratory in Cincinnati. That series of studies noted that “Several researchers have pointed out that wastewater, treated or untreated, is the primary contributor of bacteria to the aquatic ecosystem.”

Citing data sources that reach back into the 1950’s, the report from this study continues: “Waters contaminated by bacteria capable of transferring drug resistance are of great concern since there is the potential for transfer of antibiotic resistance to a pathogenic species.”

Unfortunately, rather than build upon these studies to propose new plant designs, the report and any data from the study were subsequently removed from the entirety of the US EPA data base. It was as if the topic never came up. Once deleted from circulation, the subject seems to have been promptly forgotten. In fact, the Agency and its upper management seemed reluctant to even open any discussion of the topic. Freedom of Information Act requests for such information were met with non-action. The topic seemed to be taboo. Fortunately, a 1982 peer-reviewed journal article preserved the essence of the study. Absent that journal article, the topic would have disappeared and with it, discussion of the issue. This journal article had been published following an extensive internal US EPA review by a scientific panel that vetted the information for accuracy (hence, for external release). The whole of the study was based originally on the author's doctoral dissertation.

The question that must be asked is, why did US EPA remove the report and all evidence of the study? That question is especially germane today because the Obama Administration is discussing large expenditures to correct deferred maintenance in US infrastructure. If we, as taxpayers, are expected to refurbish infrastructure, we should be assured that the best interests of the nation are being considered and that the best designs are presented so that generation of resistant organisms and their discharge into the environment will be finally terminated. Repouring concrete into the same old systems and forms may not only waste money, but also exacerbate the current issues regarding discharge of resistant organisms and contaminants of emerging concern.

**Antiquated diagnostics**

The typical water quality test used by industry is the Most Probable Number (MPN), using coliforms as the indicator. That test is known to have serious flaws. The point within the system where these tests are conducted also plays a critical part in how that water is viewed. Typically, industry and regulators choose to test bacteria at the point of release (POR) from the processing plant, but almost never at the point of use (POU), which can be miles down the pipe. Investigators who test at both the POR and POU are finding demonstrably higher indicator bacteria (coliform counts) at the POU (Fig. 2).

Susceptibility testing (by Kirby-Bauer disk diffusion) at both the POR and POU typically finds multi-drug resistant bacteria. However, industry does not employ this test. Using the state standard MPN test at the POR often finds low (or non-detectable) counts, and again, this is only for coliforms. Retesting at the POU will often detect coliform counts that are completely off the chart. Thus, something is evolving as the water travels down the pipes. We hypothesized that it is resuscitation of the indicator bacteria from a viable but non-culturabe (VBNC) state, or the sloughing of biofilms, or both. Also, these high counts at POU were not a simple momentary blip in the system, but rather found to be a constant state. Using the state standard tests, (i.e., MPN on coliforms) a positive reaction would not be expected from bacteria in the VBNC state. It is thus easy to obtain a false negative. The regulatory community is aware of this—but seems disinclined to correct it.
Addressing shortfalls

In summary, obtaining an effective grip on the increasing levels of multi-drug resistant pathogens and their antibiotic resistance genes will involve far more than a mere call for prudent use from within medicine. A coordinated, multi-disciplinary approach will be needed. To give the reader some perspective regarding the bureaucratic challenges, the following is offered:

At the 2006 Environmental Law Conference in Yosemite an interesting insight exposed how the effects of dysfunctional regulators can impact Public Health. The topic was presented vis-a-vis analyses of "non-action". One paper addressed the inadequacies of WWTPs in the removal of pharmaceuticals. Current WWTP designs accelerate the shift from solids into solutions and this is why we now have pharmaceuticals in our drinking water. Other contaminants—such as carcinogens, mutagens, endocrine disrupters, and fire retardants, etc., pass through without being effectively removed or filtered out.\textsuperscript{44, 45} Consequently, recharge of aquifers with reclaimed water carries serious risks.

Of particular interest at the Environmental Law Conference was the analysis of the Safe Drinking Water Act (SDWA) by one of the conference US/EPA drinking water toxicologists. Keep in mind that he was discussing drinking water, which must now include the "toilet to tap" conversion of reclaimed sewage into drinking water that is being proposed across the nation. The toxicologist concluded with the following: “Bottom line on almost all of the ‘emerging’ contaminants that have attracted attention: It will be a long time, if ever, before they are regulated under the SDWA.” But, industry is bound by what is in statute, regulation, and standards. Under existing law and standards, industry cannot just move to correct many of these issues. We are on the horns of a dilemma. It would be illegal to do so. Nonetheless, should we allow them to fall back on the adage—“but we meet state standards”?  

The treatment and discharge of wastewater, under existing standards, creates the perfect storm for the production and dispersal of resistant pathogens back into the commons. Discharged sewage effluent is, however, a ubiquitous but currently legal carrier for spreading multi-drug resistant pathogens. Getting a grip on the increasing levels of multi-drug resistant pathogens (MDRP) and their antibiotic resistance genes (ARGs) will involve far more than a response from within medicine for prudent usage. Needed will be a coordinated multidisciplinary

\begin{figure}
\centering
\includegraphics{Figure_2.png}
\caption{Recovery of selected antibiotic resistance genes from treated wastewater transit pipes}
\end{figure}

The above graphs highlight the importance of considering the bacterial activity that transpires as treated water flows through WWTP pipes from point of exit (POE, clear bars) to the point of use (POU, solid bars). The presence of \textit{vanA} (detectable throughout) is noteworthy because vancomycin is a drug of last resort for MRSA, a common community infection.

POE samples represent 2 WWTPs (A, B) that emit water to a co-mingled distribution system. \textbf{WWTP A:} 1 site tested; \textbf{WWTP B:} 2 sites tested; POU= 8 different sites tested randomly.

*Shows significant differences in ARG concentrations between POE and POU samples (p<0.001).

Source: Adapted from Fahrenfeld et al. Ref.\textsuperscript{43}. 

\begin{thebibliography}{9}
\item Fahrenfeld et al. Ref.\textsuperscript{43}
\end{thebibliography}
approach that in-stitches issues arising from political economy and acknowledges the fact that there is widespread clientele capture by industry of its regulatory community. To understand this, and hence gain the necessary control, will include incorporating several non-medical disciplines from various other sciences. This interdisciplinary interaction will also require broadly based generalists to act as coordinators and interpreters for discussions amongst and between the various and generally disparate and highly technical disciplines. The end result then needs to be distilled into carefully crafted transparent policies, new standards, and development of clearly directed law. This may heighten emphasis and focus on the changing areas of public health and public health law generally that seem to have been neglected or sacrificed to the political calculus.

The author and staff greatly appreciate the valuable assistance of Amy Pruden, PhD.

References


Genes in Wastewater • The APUA Newsletter Vol. 32. No. 2 • © 2014 APUA • 13


Insects are a numerous and diverse group found in many environments; however, their potential to play a role in the ecology of antibiotic resistance traits has not been well recognized.1 With continuing urban expansion into agriculturally zoned areas, the concern in the public health community about insect pests, such as flies and cockroaches associated with animal productions and waste treatment facilities, has increased because of the capacity of these insects to spread zoonotic food-borne pathogens. Flies and roaches have a great potential to dissemi nate fecal bacteria because of their developmental habitat, unrestricted movement, mode of feeding, strong attraction to human food, and synanthropic nature.2,3

Bacteria proliferate and share antibiotic resistance genes in the insect gut

Bacterial proliferation and transfer during insect feeding has been demonstrated previously in house flies for Escherichia coli.4,5 We used a GFP-labeled Enterococcus faecalis OG1RF:pMV158 to track the fate of this bacterium in the digestive tract of house flies and to assess the vector potential of this insect for E. faecalis.6 Analysis of viable fluorescing cells within various gut components over several time points revealed the highest bacterial count in the midgut in the first few hours (1-4h) after feeding, followed by a subsequent gradual decline; while the CFU peaked in the fly foregut (crop) after 48h and remained high until the end (96h) of the experiment. This suggested that E. faecalis was digested in the midgut, but proliferated in the crop.6 Bacterial proliferation in the house fly crop and digestion in the midgut have also been reported for Aeromonas hydrophila and Pseudomonas aeruginosa.7,8 This is important because the content of the crop, including associated bacteria, is typically released on a food source by house fly regurgitation during feeding.2,9 Furthermore, we also directly assessed the ability of house flies to contaminate ready-to-eat food with enterococci under laboratory conditions.10 Within 30 minutes, exposure of as few as five flies collected from a cattle feedlot resulted in an average of ~10³ enterococcal CFU/g of crop deposit on the food (beef patty from a hamburger).10 These studies further support the notion that house flies can act not only as a mechanical, but also as a bioenhanced vector for bacteria, and have great potential to contaminate substrates by microbes during feeding and by defeacation.

In addition, the potential for horizontal transfer of genes coding for toxins and antibiotic resistance among bacteria within the digestive tract of house flies was also evaluated. Petridis et al.11 observed relatively frequent (10⁻³ to 10⁻² transconjugants per donor) transfer of genes for chloramphenicol resistance and the Shiga toxin among strains of E. coli in both the midgut and crop of house flies 1h post-feeding. Our study showed that the tetracycline resistance gene (tetM) on a pheromone-responsive plasmid pCF10 was frequently (10⁻⁵ to 10¹ transconjugants/donor) transferred between E. faecalis strains in the house fly mouthparts and digestive tract within 24h after exposure.12 The implications of these studies are significant to public and animal health as they point to the ability of bacteria to actively share toxins and antibiotic resistance genes within the house fly gut beyond what is consumed initially by the fly and beyond simple bacterial proliferation.

Insects on animal farms carry antibiotic-resistant bacteria

Extensive use of antibiotics, especially as growth promoters, in the animal industry has resulted in great pressure for evolution and selection of antibiotic-resistant bacteria in the food-animal environment.13,14 Many antibiotics used as growth promoters are poorly absorbed in the animal digestive tract and are therefore released to the environment in animal feces.15,16,17 At the same time, organic waste in and around animal productions provides an excellent habitat for the development of insects such as house flies and stable flies. In addition, some animal facilities (e.g. confined swine productions) provide a new and ideal habitat for insects that are typically considered urban
pests, particularly German cockroaches. As a consequence, the likelihood that the livestock insect pests acquire and carry bacteria with antibiotic resistance traits is high.

The first report on the potential of flies to acquire antibiotic-resistant E. coli from food animals (swine and cattle) was published in 1990 by Marshall et al. The Australian bush fly was reported as a carrier of multi-drug resistant Salmonella sp. and Shigella sp. on a cattle farm and in urban areas in Australia. Literak et al. found that house flies from two swine operations in the Czech Republic carried E. coli with the same antibiotic resistance patterns and genotypic profiles as those from swine manure. The same group isolated E. coli with identical antibiotic resistance phenotypes and genetic backgrounds from both flies and manure on a dairy farm. Usui et al. sampled flies (house flies and false stable flies) and cattle feces from a cattle farm in Japan and found 14.3% (13/91) of house flies, 10.3% (7/68) of false stable flies and 7.5% (7/93) of cattle feces were positive for a third-generation cephalosporin-resistant strain of E. coli that contained transferrable plasmids encoding the blacTXM-15 gene. Pulsed-field gel electrophoresis (PFGE)-based genotypic analysis indicated that the flies carried the same E. coli clones that were detected in cattle feces. Extended-spectrum beta-lactamase (ESBL)-producing E. coli were also isolated from house flies and blowflies from two poultry farms in the Netherlands, and the genetic background of these isolates was identical to that of ESBL-producing E. coli isolates from the chicken manure. In a study from poultry farms in the U.S., house flies collected at and near confined chicken operations carried antibiotic-resistant enterococci that matched genotypically and phenotypically those from poultry litter. Our research team compared enterococci from house flies, German cockroaches, and pig feces from two commercial swine operations in Kansas and North Carolina. Enterococci were detected in the majority (>89%) of all samples and multi-drug (mainly tetracycline and erythromycin) resistant enterococci were common from all three sources. Genotypic PFGE analysis of selected E. faecalis and E. faecium isolates demonstrated that cockroaches and house flies shared the same enterococcal clones that were detected in the swine manure, indicating that insects acquired enterococci from swine manure. The above studies demonstrate that insects on farms commonly carry the same clonal lineages of multidrug-resistant bacteria that are found in animal feces.

Table 1. Antibiotic resistance profiles of Enterococcus faecalis from sludge and house flies (HF) onsite and nearby (offsite) of a WWTF.

<table>
<thead>
<tr>
<th>Resistance profile</th>
<th>Sludge (n=88/24) *</th>
<th>HF onsite (n=120/44) *</th>
<th>HF offsite (n=98/31) *</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. of resistant isolates (%)</td>
<td>no. of resistant isolates (%)</td>
<td>no. of resistant isolates (%)</td>
</tr>
<tr>
<td>TET</td>
<td>6 (6.8)</td>
<td>11 (9.2)</td>
<td>7 (7.1)</td>
</tr>
<tr>
<td>ERY</td>
<td>3 (3.4)</td>
<td>2 (1.6)</td>
<td>5 (5.1)</td>
</tr>
<tr>
<td>GM</td>
<td>D</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 (1.1)</td>
<td>11 (12.5)</td>
<td>11 (9.2)</td>
</tr>
<tr>
<td>TET, D</td>
<td>2 (2.3)</td>
<td>2 (1.6)</td>
<td>5 (4.2)</td>
</tr>
<tr>
<td>TET, S</td>
<td>14 (15.9)</td>
<td>15 (12.5)</td>
<td>6 (6.1)</td>
</tr>
<tr>
<td>TET, ERY</td>
<td>6 (6.8)</td>
<td>6 (5.0)</td>
<td>4 (4.1)</td>
</tr>
<tr>
<td>TET, ERY, S</td>
<td>3 (3.4)</td>
<td>2 (2.0)</td>
<td>1 (0.8)</td>
</tr>
<tr>
<td>TET, ERY, GM</td>
<td>1 (1.1)</td>
<td>1 (1.0)</td>
<td></td>
</tr>
<tr>
<td>TET, ERY, S, GM</td>
<td>8 (9.1)</td>
<td>6 (5.0)</td>
<td></td>
</tr>
<tr>
<td>TET, ERY, S, GM</td>
<td>2 (2.3)</td>
<td>1 (0.8)</td>
<td></td>
</tr>
<tr>
<td>TET, ERY, S, GM</td>
<td>2 (2.3)</td>
<td>1 (0.8)</td>
<td></td>
</tr>
<tr>
<td>TET, ERY, S, GM</td>
<td>22 (25.0)</td>
<td>24 (20.0)</td>
<td>1 (1.0)</td>
</tr>
</tbody>
</table>

Pan-susceptible: 8 (9.1) 32 (26.7) 71 (72.5)

TET-tetracycline, D-doxycycline, ERY-erythromycin, S-streptomycin, GM-gentamicin, NIT- nitrofuratoin.

* number of E. faecalis/number of samples
Antibiotic-resistant bacteria in insects from restaurants, apartments, and wastewater treatment facilities

Previous studies using fly traps and multi-locus DNA fingerprinting reported random dispersal (up to 125 km) of house flies from poultry and cattle farms.\textsuperscript{27,28} We screened the digestive tract of house flies collected at five fast-food restaurants and found that antibiotic-resistant enterococci were common.\textsuperscript{29} Enterococcus faecalis was found as the most abundant species (88.2%)—harboring resistance to tetracycline (66.3\% of isolates), erythromycin (23.8\%), streptomycin (11.6\%), ciprofloxacin (9.9\%), and kanamycin (8.3\%). Our subsequent study showed that ready-to-eat food from the same restaurants was commonly contaminated with antibiotic-resistant enterococci.\textsuperscript{30} Overall concentration of enterococci throughout the year averaged $\sim10^6$ CFU/g, with greater prevalence during the summer than the winter. The higher prevalence of enterococcal contamination among food samples in summer correlated with house fly activity. These studies implied that food served in restaurants is commonly contaminated with antibiotic-resistant enterococci and that house flies may play a role in this contamination.

Most recently, we assessed the prevalence of enterococci in house flies collected from four municipal wastewater treatment facilities (WWTF) as these sites are another potential source of antibiotic-resistant strains. Interestingly, the highest prevalence of multidrug-resistant enterococci was detected from a WWTF (sludge and associated house flies) that processed the waste from a nearby sausage factory, pointing again to animal agriculture as a source of these bacteria (Table 1).\textsuperscript{31} Genotypic analysis (PFGE) revealed the same clones of E. faecalis present in the waste and in the house fly digestive tract. Doud et al.\textsuperscript{31} also collected house flies from the residential environment (restaurant, apartment complex, mobile homes) close (0.7-2.0 km) to one of the WWTF and found similar antibiotic resistance profiles in E. faecalis and E. faecium, although in lower prevalence, and with no clonal matches to enterococci isolated directly from the WWTF environment (Table 1).

We propose that integrated pest management should be incorporated into pre- and post-harvest food safety programs to minimize spread of antibiotic-resistant bacterial strains. In addition, the insect link between agricultural and urban environments presents another reason for implementation of prudent use of antibiotics in the food-animal industry.

References

In an effort to update the US taxpayers and the public on the implications of antimicrobial resistance, this paper provides an overview of the Antimicrobial Resistance Monitoring and Research (ARMoR) Program. Since its initiation in 2009, the government-funded ARMoR program has collected and archived >20,000 AMR isolates for further support of outbreak investigations. Healthcare providers collaborate to collect relevant AMR data, conduct centralized molecular characterization, and use AMR characterization feedback to implement appropriate infection prevention and control measures. Since its initiation in 2009, the government-funded ARMoR program has collected and archived >20,000 isolates for further support of outbreak investigations. In an effort to update the US taxpayers and the stakeholders, this paper provides an overview of the program, its policy development and collection methods, program costs and communications, and challenges and mitigations of future outcomes.

**Recommended Reading**

**Antimicrobial Resistance Monitoring and Research Program**

In response to the increasing antimicrobial resistance, the US Department of Defense founded the Antimicrobial Resistance Monitoring and Research (ARMoR) Program to aid in infection prevention and control. This network of epidemiologists, bioinformaticists, microbiology researchers, policy makers, hospital-based infection preventions, and healthcare providers collaborate to collect relevant AMR data, conduct centralized molecular characterization, and use AMR characterization feedback to implement appropriate infection prevention and control measures and influence policy.

**Upcoming Events**

- **September 5-9, 2014:** Interscience Conference on Antimicrobial Agents and Chemotherapy (ICAAC), Washington, DC, USA
- **September 10-12, 2014:** TEDMED2014: Unlocking Imagination; CDDEP Director Ramanan Laxminarayan has been scheduled to speak at this year’s TEDMED conference (Session 4), where he will discuss an unusual, yet practical, approach to conserving antibiotics. Washington DC and San Francisco, CA, USA
- **September 23, 2014:** Accelerating the engineering of life for human health applications, Cambridge, MA, USA
- **September 24, 2014:** Roundtable on Improving knowledge and understanding of antimicrobial resistance (BSAC), London, England
- **September 27-30, 2014:** 5th ASM Conference on Beneficial Microbes, Washington DC, USA
- **October 8-12, 2014:** Infectious Diseases Society of America (IDSA), the HIV Medicine Association (HIVMA), and the Pediatric Infectious Diseases Society (PIDS)’s ID Week 2014, Philadelphia, PA, USA
- **October 14-16, 2014:** The Cuban Society for Microbiology and Parasitology hosts the 8th Cuban Congress on Microbiology and Parasitology/5th National Congress on Tropical Medicine/3rd International Symposium on HIV/AIDS Infection in Cuba, Havana, Cuba
- **October 22-24, 2014:** ESCMID hosts Conference on Reviving Old Antibiotics, Vienna, Austria.
- **October 27-29, 2014:** Re-entering Anti-Bacterial Drug Development Summit, Boston, MA, USA
- **October 31-November 3, 2014:** 5th International Meeting on Emerging Diseases and Surveillance (IMED 2014), Vienna, Austria.
- **November 12-14, 2014:** National Institute for Animal Agriculture (NIAA) Antibiotics Symposium, Atlanta, GA, USA
- **November 26-29, 2014:** 15th Asia Pacific Congress of Clinical Microbiology and Infection (APCCMI), Kuala Lumpur
- **December 9, 2014:** Roundtable on Safeguarding the effectiveness of existing antimicrobial treatments for serious infections (BSAC), London, England

See [more](#) events
foodborne bacteria. Samples of *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcus* were collected from humans, food animals, and retail meat sources to determine if these bacteria showed resistance to multiple human antibiotics. NARMS, established in 1996, monitors antimicrobial resistance in foodborne bacteria and assists the FDA in making evidence-based decisions regarding effective antimicrobials for use in animals.

Key findings from the 2011 Executive Report include:

- In people, the five-drug resistance pattern “ACSSuT” (resistance to ampicillin, chloramphenicol, streptomycin, sulfonamide, and tetracycline) in *Salmonella* has declined to 19.5% in 2011 from its peak in 1997 at 35.1%.
- Multi-drug resistance in *Salmonella* from humans, slaughtered chickens and slaughtered swine was the lowest since 1996. However, multi-drug resistance in *Salmonella* from retail poultry meats generally increased.
- Erythromycin resistance in *Campylobacter jejuni* (C. *jejuni*) has remained at less than 4% in isolates obtained from humans, retail chicken and slaughtered chicken since testing began. The antibiotic erythromycin is the drug of choice for treating *Campylobacter* infections, more than 90% of which are caused by C. *jejuni*.
- *Campylobacter* resistance to the fluoroquinolone ciprofloxacin has increased slightly in isolates from humans since 2005. Ciprofloxacin is not approved for use in poultry, and the FDA withdrew approval for the use of enrofloxacin in poultry in 2005. Ciprofloxacin and enrofloxacin are both in the same class of drugs (fluoroquinolone).
- Resistance to third-generation cephalosporins, another important drug class for the treatment of *Salmonella* infections, rose among isolates from retail ground turkey between 2008 and 2011 and among certain *Salmonella* serotypes in cattle between 2009 and 2011. In April 2012, FDA prohibited certain uses of cephalosporin drugs in cattle, swine, chickens, and turkeys. NARMS will continue to monitor these trends over time.

**Antibiotics found in Minnesota groundwater**

A recent study of groundwater contaminants (2009 – mid 2012) by the US Geological Survey and the Minnesota Pollution Control Agency has found measureable levels of antibiotics—most commonly, sulfamethoxazole (in >10% of samples), azithromycin and lincomycin (an animal antibiotic). Previously identified in surface waters, these and other consumer and industrial contaminants are thought to have leached into the ground water from landfills, septic systems, and sewage treatment plants. While none were found in excess of drinking water quality standards, the report does raise awareness for the 75% of Minnesota residents who drink groundwater.

**Mixed results in FDA’s annual antibiotic resistance survey**

The US FDA released its National Antimicrobial Resistance Monitoring System (NARMS) [2011 Executive Report](#) in mid-August, showing increasing and decreasing trends in antimicrobial resistance. This report summarizes data from the FDA, the CDC, and the USDA which tracks antibiotic resistance in foodborne bacteria. Samples of *Salmonella*, *Campylobacter*, *E. coli*, and *Enterococcus* were collected from humans, food animals, and retail meat sources to determine if these bacteria showed resistance to multiple human antibiotics. NARMS, established in 1996, monitors antimicrobial resistance in foodborne bacteria and assists the FDA in making evidence-based decisions regarding effective antimicrobials for use in animals.

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APUA names MedImmune a new corporate sponsor

APUA signed a contract with MedImmune for a $25,000 corporate sponsorship to run through August 2015. MedImmune will support APUA’s presence at ICAAC this September. MedImmune shares APUA’s goals of promoting innovative antibiotic development and ensuring prudent antibiotic use as both a patient safety and cost containment strategy.

APUA to host Resistance documentary showing

On September 25, APUA will host a showing and panel discussion of the documentary Resistance. The showing is free and open to the public and will take place at the Coolidge Corner theatre in Brookline, MA from 7-9pm. The 72-minute documentary explores the bacteria that give rise to antibiotic resistance infections through microscopic footage and personal stories. The Uji film traces the mass production of antibiotics through the rise of the superbugs in the 21st century. Following the film, APUA Vice President Tom O’Brien, filmmaker Michael Graziano, and a Cubist pharmaceuticals representative will answer audience questions about antibiotic resistance.

APUA hosts Boston summit on point-of-care biomarkers

In May, APUA hosted an international meeting of key opinion leaders concerning the utility of biomarkers to guide antibiotic therapy and reduce antibiotic overuse. The 12 key opinion leaders had relevant health care expertise from Europe and diverse US geographic locations, including two US CDC leaders in antibiotic stewardship and Get Smart About Antibiotics. Also in attendance were staff from APUA and Alere Inc. APUA planned and coordinated the summit meeting with the help of an unrestricted educational grant from Alere, Inc.

The Boston-based May 21 summit meeting, titled Improving antimicrobial stewardship in outpatients: Potential for CRP and other biomarkers, explored how biomarkers such as C-Reactive Protein (CRP) can be used at the point-of-care (POC) from finger-stick blood samples, thereby providing additional information to guide the physician in antibiotic decision-making and reducing diagnostic uncertainty in community healthcare settings. This practice is now common in some northern European countries, which are well known for prudent antibiotic use and low levels of resistance. A rapid, point-of-care CRP test is not currently approved in the US, and there are multiple barriers to its implementation, including physician and patient uptake, cost issues and federal approval.

A consensus evolved among attendees that US-based studies to evaluate and clarify the utility and outcomes of CRP are needed in order to augment the dearth of sometimes conflicting data—most of which are based in Europe at present. A summary manuscript of the meeting outcomes, submitted to a primary health care journal in July, is under review, pending publication.
APUA-Russia Chapter Activities

Submitted by: Dr. Roman Kozlov, APUA-Russia chapter leader

APUA Russia was established in 1997 in affiliation with the Interregional Association for Clinical Microbiology and Antimicrobial Chemotherapy (IACMAC). IACMAC members (over 1,500 in Russia) frequently participate in national and international conferences and symposiums organized jointly by IACMAC with APUA.

IACMAC activities includes several annual meetings (one international congress in Moscow and two international conferences in different parts of Russia), antibiotic resistance monitoring, educational workshops and meetings with online and offline schooling of bacteriologists, clinicians and clinical pharmacologists and publishing activities (official international -peer-review quarterly publication “Clinical Microbiology and Antimicrobial Chemotherapy”, practical guidelines on anti-infective chemotherapy etc.)

Specifically, there are three annual meetings scheduled for 2014 in Siberian region (Krasnoyarsk), Moscow and Far-east region (Vladivostok), two of which have been held:

- IV Siberian conference on antimicrobial therapy, Krasnoyarsk, 3-4 April 2014: 1579 participants from 19 regions of Russia
- XVI International IACMAC Congress on antimicrobial therapy, Moscow, 21-23 May 2014: 1328 participants from 64 regions of Russia and 17 countries
- Vladivostok, regional conference, Far East region - will be on October 16-17.

APUA-Mexico coordinates AMIMC workshops

Submitted by: Dr. Miguel Angel Peredo, APUA-Mexico chapter leader

The 39th Annual Congress of Infectious Diseases and Clinical Microbiology Mexican Association (AMIMC) was held on May 28-31 in Acapulco, Mexico. This Congress is the main scientific forum on infectious diseases in Mexico. As very high rates of resistance have been observed in bacteria that cause common healthcare-associated and community-acquired infections, the APUA-Mexico chapter and the AMIMC coordinated the workshop of rational antibiotic use to discuss the treatment of the following therapeutic guidelines: urinary tract infections, gastrointestinal infections, upper and lower respiratory infections and multi-resistant bacterial infections. The workshop was held to exchange and improve best practices and discussion about solutions, such as improved data collection and surveillance, eliminating the overuse of antimicrobials and reducing the use of critically important antibiotics.

Chapter leader, Dr. Miguel A. Peredo, and APUA-Mexico continue their educational activities with medical students and physicians through lectures and workshops to promote the prudent use of antibiotics.
APUA-Nepal: Government approves Antibiotic Treatment Guidelines

Submitted by: Dr. Kumud K. Kafle, APUA-Nepal chapter leader

APUA-Nepal has recruited experts from different disciplines for the drafting of new Antibiotic Treatment Guidelines. Following review and approval by program managers and experts from the Ministry of Health and Populations (MOHP) of Nepal, the Guidelines have been adopted and implemented. The new treatment Guidelines were developed for common infective health problems related to general medicine, surgery, pediatrics, obstetrics and gynecology, sexually transmitted diseases, ophthalmology, ear, nose/oropharynx and dental infections. Guideline topics cover the relative safety of antimicrobial agents in pregnancy, lactation and breast feeding, antibiotic prophylaxis in surgery, topical antibiotics, antimicrobial combinations and also a section on requirements for considering antibiotic prescriptions. The new Guidelines have classified antimicrobials into non-restricted prescriptives, nonrestricted prescriptives for TB and leprosy, restricted prescriptives (prescribed by a medical officer or higher qualification) and those highly restricted for prescription by faculty, a specialist or consultant only.

References


Recommended Resource

Antibacterial Drug Development: Challenges, Recent Developments, and Future Considerations

The Nature Publishing Group recently released a commentary by Nambiar et al. regarding the challenges of antibiotic development. The authors discuss appropriate clinical trial designs in order to continually generate effective therapies to sustain patient needs. The authors critique the use of non-inferiority trials during antibiotic development and recognize the FDA-drafted guidance for streamlined pathways to expedite antibacterial production. While the GAIN Act has made significant progress, Nambiar et al. advocate further research and policy developments to make the new therapies available.
German livestock producers required to report antibiotic usage

Under a new German regulation, livestock farmers are now required to report every 6 months on what antibiotics they have administered to which animals in order to survey and track antibiotic usage and resistance. The amendment to the German Drug Act will help minimize the use of antibiotics in food animal production. If a livestock farm’s antibiotic usage index is greater than the federal average, then producers and veterinarians must identify the causes and take steps to reduce use. If livestock producers fail to report, they may be fined or farm operations may be suspended. In 2008, a federal German antibiotics resistance strategy required drug makers and wholesalers to report how many antibiotics were distributed to veterinarians. This regulation has contributed to a 170-ton reduction in sales from 2012 to 2013. Similarly, The Netherlands are preparing a benchmarking system to curb antibiotic use on poultry farms. Already the Dutch have reduced antibiotic use by 40-50%, but are aiming for a 70% reduction in antibiotic usage across all sectors by 2015.

FDA reverses ruling on animal antibiotic use

At the end of July, the US Court of Appeals ruled that the US FDA is not required to hold hearings concerning the safety of sub-therapeutic antibiotic use in food animals. The Natural Resources Defense Council, the Center for Science in the Public Interest, Food Animal Concerns Trust, Public Citizen, and the Union of Concerned Scientists argued that the FDA is required to hold hearings to withdraw approval for the use of penicillin and tetracyclines in animal feed. The 1970s report and recent research shows that the low-dose routine use of antibiotics in food animals contributes to the development of resistant bacteria. The 2013 CDC Report on antibiotic resistance states, “Because of the link between antibiotic use in food-producing animals and the occurrence of antibiotic-resistant infections in humans, antibiotics should be used in food-producing animals only under veterinary oversight and only to manage and treat infectious diseases, not to promote growth.” Not requiring hearings means that the FDA does not have to consider banning the routine feeding of low-dose antibiotics to healthy animals as growth promotion.

Pew Charitable Trusts proposes new pathway for drug development

The rise in drug resistance and the dry antibiotic pipeline are contributing to the post-antibiotic era and the rise of superbugs in the 21st century. The healthcare community is searching for innovative new drugs for patients whose treatment options are nonexistent. Last January, Pew hosted a conference to explore drug development for limited populations and proposed to bring urgently needed medicines by reducing economic and regulatory barriers to antibiotic innovation. The government regulators, infectious disease physicians, public health specialists, pharmacists, pharmaceutical representatives, and payers in attendance concluded that proper management and data-driven use of the limited-population antibiotics would be important for the success of this new development pathway.
“Care follows the dollar” is an established health policy maxim, meaning that money is a powerful motivator of health care provider behavior. Pharmaceutical development is one of the country’s more successful enterprises whose profits derive from aggressive marketing deemed necessary to gain adequate return on investments. The antibiotic development business faces unique challenges however, in that aggressive marketing over time can result in antibiotic overuse and antibiotic resistance, leading to the premature loss of the drug’s effectiveness. To slow the emergence of antibiotic resistance, public health authorities are instituting tighter antibiotic stewardship and infection control requirements which, if successful, could constrain antibiotic sales even further.

Faced with limited sales projections and ROI for antibiotics, many pharmaceutical companies have abandoned the antibiotic development business in favor of the more lucrative drugs for chronic conditions. To address this dilemma, APUA recently signed on with several initiatives to explore radical solutions such as that proposed by APUA President, Dr. Stuart Levy in his 2002 publication of *The Antibiotic Paradox*. Antibiotics are uniquely societal drugs …” he stated, “By establishing a special regulatory category, we can improve how they are used, marketed, and developed through incentives to industry.” While that proposal seemed unorthodox at the time, similar untraditional economic models are now being seriously explored to address the rising rates of untreatable resistant infections.

APUA is participating in several pioneering programs to promote innovative business models aimed at reconciling the seemingly incompatible goals of antibiotic development and stewardship. As a consortium member in the original proposal, APUA is now serving as a member of the antibiotic stewardship study section of The EU DRIVE-AB project (Driving Re-Investment in R&D and Responsible Antibiotic Use) recently funded by the EU Innovative Medicines Initiative (IMI)—a three-year project which aims to develop innovative public/private collaborations to promote development of novel antibiotics while ensuring rational antibiotic use and enhancing competitiveness of the EU biopharmaceutical sector. Having joined in the consortium on the original proposal, APUA will now serve on the stewardship study section.

The Chatham House Working Group on Antibiotic De-linkage is another promising initiative which was recently established by the UK-based international think-tank. It engages representatives from academia, industry, NGOs and government to develop breakthrough solutions to major global policy issues. APUA will contribute as a reviewer to this study group, which is exploring alternative economic models to ensure antibiotic company profits, while eliminating perverse incentives to maximizing antibiotic sales or use. The group will statistically evaluate feasibility of various incentives to simultaneously spur antibiotic development and conservation. The final report will serve as a framework for the DRIVE-AB project and include recommendations regarding payment models; geographic scope; financing credits; IP ownership and marketing utilization incentives. Professor Kevin Outterson, of Boston University School of Law, who has published extensively on the topic of de-linkage, is coordinating the working group. Their report is due out in the fall of 2014.

As APUA’s representative on these projects, I look forward to contributing APUA’s antibiotic stewardship expertise and an understanding of the public health and industry interests I gained as an FDA Anti-infective Drug Development Advisory Committee member. Development of novel antibiotics is urgently needed to treat resistant infections now. At the same time, stronger incentives for antibiotic stewardship are essential in order to extend their lifespan and ensure a sustainable supply of these lifesaving agents for all.

**US developments to drive antibiotic stewardship and development**

The Infectious Diseases Society of America (IDSA) and pharmaceutical executives criticize the US regulatory disincentives as a major barrier to US antibiotic development.
The aforementioned European regulatory environment, in contrast, implements strategies to fund antibiotic development and address drug resistance as a public health priority.

In July 2012, President Obama signed the FDA’s Generating Antibiotics Incentives Now (GAIN) Act to incentivize the research and development of novel antibiotics. Due to the dry antibiotic pipeline, antibiotic-resistant infections are a severe public health risk—according to the CDC antibiotic resistant infections cause over 2 million illnesses and 23,000 deaths in the US, costing over $20 billion annually. Incorporated into the FDA’s Safety and Innovation Act, the GAIN Act created a pathogen-focused antibacterial drug development pathway and identifies antibiotics for priority review. The Act also removes some financial developmental barriers to expedite antibiotic production, provided the compounds fulfill minimum efficacy data. To review the progress of GAIN to date, read here.

Following the President’s Council of Advisors on Science and Technology (PCAST) this summer, IDSA wrote white papers exploring the PCAST recommendations to incentivize federal action regarding the increasing antibiotic resistance and dry antibiotic pipeline. IDSA encourages PCAST to: consider Europe’s successful antimicrobial surveillance and tracking system; stimulate antibiotic R&D with collaborative work through public-private-partnerships; and increase federal funding. The White House responded by releasing the 2016 Budget for Combating Antibiotic Resistant Bacteria Resource Priorities. The budget proposal allocates funds to minimize the development of resistant bacteria, to strengthen national one-health surveillance, to develop rapid diagnostic technology, to accelerate and develop new antibiotics, therapeutics, and vaccines, and to improve international collaboration.

APUA has joined the new U.S. Stakeholder Forum on Antimicrobial Resistance (S-FAR), convened by IDSA, to help coordinate efforts to inform and advise the U.S. government on matters relating to antibiotic resistance (AR). S-FAR already includes 60+ organizational partners. Members will also occasionally be notified of opportunities to engage in AR advocacy, such as sign-on letters, legislative activities, and public events. S-FAR went live on September 4 and has opened resources to the general public.

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**Funding Opportunity**

**ASMCUE® Leadership Grant for International Educators**

The Leadership Grant for International Educators enables a select group of microbiology, biology, and health science undergraduate educators from resource-limited countries to attend the ASM Conference for Undergraduate Educators (ASMCUE) and a pre-conference workshop in order to provide these future leaders with the resources to develop and pilot innovative pedagogy and learning modules that engage students and lead to enduring understandings in microbiology, biology, and health science.

**Program Description:** The goals of this program are to provide educational leaders from resource-limited countries with training in the latest developments in microbiology education in order to improve microbiology and STEM education in their home country and to build capacity for disseminating change within their local and national STEM educational communities.

**Application Deadline:** October 1, 2014

**Funding:** ASM will provide up to $3,000 US dollars towards round trip economy airfare to the US & ground transportation to the conference. Complimentary registration and conference housing is provided to recipients.

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**Recommended Resource**

**URTI Stewardship Guidelines**

The Global Respiratory Infection Partnership (GRIP) has prepared a continuing professional development module to meet the needs of patients with upper respiratory tract infections (URTIs). After completing this module, physicians should:

- Understand antibiotic resistance as a result of antibiotic overuse and/or misuse
- Acknowledge the importance of communicating with patients on appropriate antibiotic use in URTIs
- Recognize the importance of meeting patients’ symptomatic treatment needs in URTIs
- Be aware of when antibiotic use is appropriate for patients with sore throat
- Have a knowledge of the 1,2,3 approach to sore throat management
MASSPIRG's campaign to stop the abuse of antibiotics in factory farms

MASSPIRG, a Massachusetts consumer advocacy group and coalition of Boston medical professionals released a white paper on August 5 asking the Obama administration to immediately restrict the use of antibiotics on factory farms. Campaign associate, Amirah Mitchell urged, “The medicine chest may be empty soon. We need to end the abuse of antibiotics on factory farms right now to preserve their ability to treat infections.” More than 70 percent of antibiotics used in human medicine are sold for use in food animals to promote weight gain. This use fuels the creation of resistant bacteria that can spread from farms via food, animal to human contact, and animal waste that enters the environment. Last December, the U.S. FDA issued guidelines for antibiotic use on farms. Critics argue that the guidelines were voluntary and narrow in scope, and are unlikely to lead to significant reductions in antibiotic misuse on farms. In July, it was ruled in a 2-1 decision that the FDA is not required to hold hearings concerning the safety of feeding antibiotics to food animals at sub-therapeutic levels.

CDC: Antibiotic resistance could be 'next pandemic'

At a recent National Press Club luncheon, CDC director Dr. Tom Frieden addressed the growing threat of antibiotic-resistant bacteria. Over-prescribing of broad-spectrum antibiotics in hospitals has caused bacteria to mutate and develop drug resistance with the potential to “kill anyone in the country, undermine modern medicine, to devastate our economy, and to make our health care system less stable”. He cited the highly resistant CRE (carbapenemase-resistant Enterobacteriaceae) as the most problematic of these infectious agents. The cost of antibiotic resistance amounts to $20 billion and 23,000 US deaths per year. Frieden lists improved detection, control, prevention, and innovation as the four keys to stopping antibiotic resistance. There is a need for national surveillance and tracking of prescribed antibiotics, infection control, implementation of antibiotic stewardship programs, and new incentives to develop antibiotics and diagnostics.

Global antibiotic consumption on the rise

A recent Princeton University study published in The Lancet Infectious Disease concluded that antibiotic use has risen by 36% from 2000-2010. The BRICS countries (Brazil, Russia, India, China, and South Africa) account for 75% of the increase in global antibiotic use. Researchers Van Boeckel and colleagues examined the IMS Health MIDAS database for retail and hospital pharmacy antibiotic sales data in 71 countries in order to examine global trends in antibiotic consumption. Cephalosporins, broad-spectrum penicillins, and fluoroquinolones account for half of the increased antibiotic consumption, in addition to significant increases in carbapenems and polymixins—two last-resort antibiotic classes. Peak antibiotic use correlated with the end of the winter and monsoon seasons, which in the US corresponds with flu season. The authors recommend educational programs to improve prescriber habits and to reduce antibiotic distribution to patients with viral respiratory tract infections.

Five-fold increase in CRE superbug infections prompts warning of epidemic

Community hospitals in the southeastern US are urged to prepare for the oncoming carbapenem-resistant Enterobacteriaceae (CRE) epidemic. As classified by the WHO, CRE are “one of the three greatest threats to human health” as they are a class of highly antibiotic-resistant bacteria that cause lung, blood, and urinary tract infections. Over a 5-year study period, CRE infections increased five-fold. Researchers speculate that increased and overuse of broad-spectrum antibiotics, poor infection control, staff shortages, and financial constraints are reasons for the increased infection rate.

Antibiotic resistance prompts reinvestigation of phage therapy

With the looming threat of a post-antibiotic era, Western researchers and governments have revitalized the study of bacteriophages for treating infections. Phage therapy uses viruses to kill bacteria and is commonly used in Russia, Georgia, and Poland today. The US National Institutes of Allergy and Infectious Disease (USAID) has listed phage therapy among its plans to combat antibiotic resistance, and Swiss researcher
Gregory Resch has developed plans for the first multi-center clinical trial of phage therapy for human infections (the Phagoburn study). In contrast to broad-spectrum antibiotics, phages kill one species or strain of bacteria. Resch and the Phagoburn researchers hope that phage therapy will be regulated similarly to the seasonal influenza vaccine—continually updated as new bacterial strains emerge. With the EU contributing $5.2 million to the Phagoburn study, Resch and his team will recruit burn victims infected with *E. coli* or *Pseudomonas aeruginosa* and treat them with a variety of phage cocktails. While phage therapy is not expected to replace antibiotics, it can provide alternative treatment options for those patients in whom drug treatments have failed.

**Asymptomatic bacteriuria: resisting the urge to treat**

Infectious disease physicians are aware that “asymptomatic bacteriuria” patients do not require antibiotics. However, many physicians feel the urge to prescribe antibiotics when diagnostics reveal bacteria in the urine. Up to 80% of asymptomatic bacteriuria patients receive antibiotics despite the IDSA’s 2005 guidelines, which recommend treatment only for pregnant women or those with a genitourinary procedure with anticipated bleeding. Professor Leis and his University of Toronto colleagues published a pilot study in *Clinical Infectious Disease* which required physicians to call the microbiology lab to get test results. Of the 415 subjects, 2% were diagnosed with asymptomatic bacteriuria; antibiotic prescribing rates declined from 48% to 12% when physicians had to call the lab for the test results. (Leis JA, Rebick GW, Daneman N, et al. Reducing antimicrobial therapy for asymptomatic bacteriuria among noncatheterized inpatients: a proof-of-concept study. Clin Infect Dis. 2014;58:980-983.)

**New route identifies drugs to fight bacterial infections**

Daniel M. Czyz and colleagues have identified a novel approach for treating infections caused by the intracellular bacterial pathogens *Coxiella burnetii*, *Legionella pneumophila*, *Brucella abortus*, and *Rickettsia conorii*. By screening a library of FDA-approved compounds, they found numerous non-antibiotic candidates that had limited toxicity to the infected host cell, but simultaneously, effectively inhibited intracellular bacterial growth by targeting 3 probable host cell functions: (i) G protein-coupled receptors, (ii) intracellular calcium signals, and (iii) membrane cholesterol distribution. The findings suggest that drugs that disrupt intracellular pathogen growth pathways can act as therapeutic candidates to decrease the emergence of drug resistance.
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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