AMROAR SCIENTIFIC MEETING REPORT
ON

COMMENSALS AS RESERVOIRS OF ANTIBIOTIC RESISTANCE

Coordinated by APUA- Sponsored by NBACC
Monday, June 2, 2008, 10:00am - 2:00 pm
Tufts University Health Sciences Campus
Room: Sackler 826
145 Harrison Avenue,
Boston, MA, 02111

Opinions, interpretations, conclusions, and recommendations are those of the author and are not necessarily endorsed by the National Biodefense Analysis and Countermeasures Center (NBACC), Department of Homeland Security (DHS), or Battelle National Biodefense Institute (BNBI).

I. Executive summary

On June 2nd, 2008, Alliance for the Prudent Use of Antibiotics (APUA) hosted an AMROAR scientific meeting to evaluate the state of research on antibiotic resistance in commensal microbes and to prioritize a research agenda. For the purposes of this report, commensal is defined as " bacterial strains deemed *not actively responsible for a pathogenic process* and derived from either humans, animals or plants, or recovered from environmental sources such as air, water, soil, sludge etc." Participants included project sponsor and CORE project director Dr. Kenneth Tucker, seven prominent scientists with significant expertise in antibiotic resistance in commensal organisms, and members of APUA staff who are coordinating the project.

The meeting began with an introduction of AMROAR and AMRIID projects which APUA is coordinating for NBAAC. The APUA AMRIID project is an international surveillance program that collects and verifies common environmental commensal organisms from foreign countries and evaluates how they may contribute to emergence of new resistance threats. The meeting documented the need for documenting and analyzing trends in antibiotic resistance among commensal organisms, the importance of synthesizing data from different, as well as previously uncharted, sources and suggested steps that can be taken to combat antibiotic resistance as a bioterrorism threat. Each scientist provided presentations on antibiotic resistance as it relates to their current research interests in commensal bacteria (see titles p.4 and power point attachments).

The discussion focused on the following major areas:

- Evaluation of the state of science in commensal antibiotic resistance
- Goals, responsibilities and stewardship of global surveillance on antibiotic use
- Ways to promote better understanding of the problem of antibiotic resistance
- Gaps in understanding the problem of antibiotic resistance
- Major interventions that can help to prevent the emergence and spread of antibiotic resistance
- Criteria for evaluation of antibiotic resistance
- Prioritization of a scientific research agenda

Dr. Kenneth Tucker emphasized that he is looking to predict where antibiotic resistance is coming from, whether there are ways to prevent this rapidly developing problem, and how NBACC can contribute to that.

The team of scientific experts noted that major gaps in studying antibiotic resistance stem from a lack of sharing and co-ordination of research findings between the pharmaceutical industry and the scientific community. They noted that much potentially useful information is still available in the hands of the pharmaceutical companies and needs to be disseminated in order to gain a fuller understanding of the problem. Bridging this gap would be an important aid in helping the scientific community set an agenda for addressing antibiotic resistance issues. It was also suggested that the ecological ("systems biology") aspects of antibiotic resistance are fundamental and need to be examined by assembling the diverse perspectives of ecologists, microbiologists, veterinary biologists and bioinformaticists.

Five priority questions were fielded for discussion. It was agreed that, with the current data gaps, it is difficult to predict when and where the next antibiotic resistance genes will emerge, but that resistance development to new antibiotics is inevitable—it is just a matter of time. Resistance emergence is directly related to the amount of antibiotics being used in a specific environment. Meeting participants agreed that it is important to conduct studies explicit to genome sequencing of environmental bacteria to enable us to identify and track with greater definition the emergence and proliferation of specific resistance genes within and between microbial environments. A major consensus point was that aquaculture and other water environments are ideal for studying the flow of resistance genes and should be funded as research priorities.

List of attendees and affiliations

Name	**Title**	**Department**	**Institution**
Julian Davies	Professor	Microbiology and Immunology	University of British Colombia
James Tiedje	Professor and Director	Center for Microbial Ecology	Michigan State University
Marilyn Roberts	Professor	Environmental & Occupational Health Sciences	University of Washington
Anne O. Summers	Professor	Microbiology	University of Georgia
David Skurnik	Post Doctoral Student	Laboratoire de Bacteriologie	Xavier Bichat Medical School, Paris 7 Univ.
Thomas O'Brien	Director	Microbiology Lab	Brigham and Women's Hospital
Lisa Nolan	Professor and Dean	Vet. Microbiology	Iowa State University
Kathleen Young	Executive Director		APUA
Bonnie Marshall	Research Scientist and Editorial Consultant	Molecular Biology and Microbiology	Tufts University; School of Medicine APUA
Morten Sommer	Research Fellow	Genetics	Harvard Medical School
Kenneth Tucker	Director	CORE Support	Battelle National Biodefense Institute
Wendy Lu	Special Projects Coordinator and Executive Assistant		APUA
Keely MacMillan	Intern		APUA
Dorothy Ochieng	Program Manager		APUA

Meeting Agenda

10:00 am	Welcoming remarks, project background and meeting objectives	**Kathleen Young**
10:10 am	Background on CORE and project Interests	**Ken Tucker**
10:20 am	*Omnipresent integrons*	**Julian Davies**
10:30 am	*Exploring antibiotic resistance in soils, manures and ocean sediments not impacted by humans*	**James Tiedje**
10:40 am	*Drug Resistant Environmental Bacteria Associated with Aquaculture*	**Marilyn Roberts**
10:50 am	**Q&A and discussion**	**Kathleen Young** (moderator)
11:30 am	**Break**	
11:40 am	*Perspectives on the Problem of Antibiotic Resistance*	**Anne Summers**
12:00 am	Individual presentation	**Lisa Nolan**
12:10	*Bacteria subsisting on antibiotics*	**Morten Sommer**
12.20	**Lunch and discussion**	**Thomas O'Brien** (moderator)

II. Highlights of the meeting *(supporting references were inserted subsequent to the meeting)*

The AMROAR meeting discussions focused on the problem of antibiotic resistance, its global spread, gaps in the current knowledge base and research needs for the future. Discussions emphasized the need to document and follow resistance trends in commensal flora as a barometer for resistance emergence in pathogens. For the purposes of this report, commensal is defined as " bacterial strains deemed *not actively responsible for a pathogenic process* and derived from either humans, animals or plants, or recovered from environmental sources such as air, water, soil, sludge etc." Measures were outlined that can be taken to combat the global spread of antibiotic resistance. The following are highlights of important issues raised:

A. General overview of commensal antibiotic research

Participants agreed that research in this field has been largely neglected and is still in its infancy. In part this is due to the difficulties of getting published without alluding to pathogens despite the fact that commensals are an enormous source of antibiotic resistance genes (Picard, Garcia et al. 1999; Alekshun and Levy 2006). There is a tendency to only support and recognize research that is related to infectious disease i.e., pathogenic strains of *E. coli, Staphylococcus or Enterococcus.* Researchers working primarily with commensals must identify relevancy to environmental applications in order to validate their work and avoid rejection of funding applications and publishing of their data. As we move forward, there is a significant need to raise global awareness of the role of that commensal microbes have in spreading resistance and to develop novel management strategies, utilizing our knowledge of commensals as reservoirs of antibiotic resistance genes.

To emphasize the importance of the commensal population, it was suggested that it would be worthwhile to estimate the ratio of known pathogens to the total commensal population, thereby erasing the erroneous concept that these are of equal numbers or significance. Commensals outnumber pathogens numerically by many orders of magnitude and are likewise more widely distributed throughout all ecosystems. Initiation of systems for early detection and tracking would aid in combating the problem of emergence of new resistance genes. While we cannot visualize every emergence, we can seek to identify insipient examples that would be extremely valuable in clarifying the source of the problem—enough such that one could surmise, for example, that agriculture is inevitably the source of resistance. The problem is an unbridged data gap surrounding a critical and growing public health threat.

B. Concepts regarding the origin and emergence of antibiotic resistance

- Resistance genes found in pathogens are the very same as those found in the environmental flora (Sunde and Sorum 1999; Chopra and Roberts 2001; Snyder and Saunders 2006). Many of these genes are associated with mobile elements which allow for rapid spread through bacterial populations and ecosystems. Consequently, there exists a very large potential reservoir of available mobile antibiotic resistance genes (Lima-Bittencourt, Cursino et al. 2007; Dib, Motok et al. 2008; Knezevic and Petrovic 2008; Sjolund, Bonnedahl et al. 2008). The strategy for using antibiotics operates with the knowledge that there is a similarity between the two (pathogens and commensals). While the enormous reservoirs of circulating resistance genes suggest an inevitable emergence in pathogens, this progression should be able to be contained or slowed.

- The concept that the microbial commensal populations are a main source of resistance genes and resistance elements for the world's miniscule pathogen population has emerged relatively recently. This is an idea that still requires intensive detailed investigation.

- Two paradigms, which are not mutually exclusive, were offered for the origins of resistance: 1) Conceptually, every resistance gene pre-exists somewhere in a

commensal and it is merely a matter of time until it emerges and manifests in a clinical pathogen; and 2) Resistance genes are not *all* pre-existing—there is a process of development in which a few mutational or recombinatorial steps are required for them to become resistance genes (Courvalin 2005). In recent years conspicuous new resistances have emerged with this mutational resistance exclusively (e.g., the modification of aminoglycosides by acetyltransferases [which are ubiquitous] – resulting in quinolone resistance) (Dever and Dermody 1991; Li 2005).

- The term "emergence" of antibiotic resistance has historically been restricted to the first time a resistance gene appears in a clinical isolate; but a better description for this stage of emergence may be needed, since it is possible that an unknown portion of resistance genes that are going to appear clinically already pre-exist or develop ("emerge") first in commensals.

C. Concepts related to the spread and containment of antibiotic resistance

- Many genes are shared across the entire spectrum of microbial environments, irrespective of whether they are specific to certain organisms or certain ecosystems.

- Globally, the current scenario for antibiotic resistance can be compared to a large collection of "rooming houses" shared by bacteria, humans, animals and plants, etc. On a certain subset of these, a very stringent [antibiotic] selection has been imposed over the past 50 years, which has changed the "baggage" that is being carried by all the inhabiting "clientele." We have, in essence, been "farming" antibiotic resistance. (Witte, Klare et al. 1999; Hawkey 2008). By recruiting the transfer and recombinatorial functions of very ancient bacterial structures, antibiotic selective pressures have propelled strains to the level of "swat team" bacteria that are well equipped to deal with the newest available antibiotic.

- Widely used agents other than antibiotics can select for antibiotic multi-resistance through linkage and/or direct functional overlap. Examples of the former are mercury selection for linked resistances (integrons in gram negatives and a newly defined Tet transposon in gram positive bacteria) and for the latter – salicylate (aspirin) selection for MDR efflux pumps, many of which are on conjugative plasmids as well as the chromosome. Antibiotic resistance systems have evolved such that agents other than antibiotics will perpetuate them in the commensal/pathogen gene pool.

- The unabated advancement of our current resistance problem stems from a total lack of futuristic thinking in prior decades. The realization of a possible "end" to the era of antibiotic efficacy should spur action that could protect the utility of antibiotics. Such action could have prevented the deficit of new antibiotics entering the pipeline. For example, in 2000 it was known that a new resistance (CTX-M beta-lactamase) was on the horizon. (Bonnet, Sampaio et al. 2000; Bonnet 2004; Munday, Boyd et al. 2004; Eisner, Fagan et al. 2006; Govinden, Mocktar et al. 2006) . A survey of 2007 hospital records indicates that CTX-M is now endemic. CTX-resistant strains have emerged with a total of 5 resistances. (Soge, Adeniyi et al. 2006; Karisik, Ellington et

al. 2008). The question remains – why were we powerless to do anything about this at the time it was foreseen?

- The opinion was expressed that resistance in nature and that found in hospital infections exist functionally for two different, unrelated reasons. Thus the prediction of resistance gene emergence is not straightforward. Prior events may pose little predictability of what can be expected (Courvalin 2005); for example, it would never have been predicted that aminoglycoside acetyltransferase could give rise to quinolone resistance, but now we know that this enzyme can be modified such that it will acylate an amino group on another antibiotic (Li 2005). Knowledge of this fact does lend a certain level of predictability that such an event could happen again.

- Since both commensal flora and agricultural practices vary greatly from region to region, the interaction between them that may yield any particular new resistance gene may be far more likely to happen in some regions than in others. This underscores the necessity for surveillance in a range of geographic sites.

- Given the ubiquity of resistance elements, and the mobilizing of resistance genes, we can anticipate arrival of resistance to new antibiotics on the market over time, but the time frames are varied and difficult to predict. Attempts at delaying this could prolong the lifespan of these lifesaving drugs.

- There is an enormous bias to where antibiotic resistance came from to begin with because of pharmaceutical industry's initial approach, i.e., few pathogens were screened initially (primarily gram-positives) and drugs were kept or discarded according to the industry's perspective of "usefulness." It was suggested that it could prove useful to re-examine the older discarded chemical data with a contemporary perspective and retest discarded drugs for possible utility against modern pathogens.

D. Summaries of recent research in antibiotic resistance in commensals

Each attending scientist shared a review of his/her respective research efforts in commensal organisms. The means of transmitting antibiotic resistant genes from the environment and its commensal reservoirs to human populations was conceptualized, followed by a discussion of relationships between commensal flora and the environment and how understanding this could help in prediction of emergence of resistant pathogenic species. Following are the key features of these scientific presentations:

- While awareness of the antibiotic resistance problem has certainly been elevated in the public health community, solutions to the problem still need to be identified and implemented. This will require resources.

- Our current antibiotic resistance problem is more accurately defined as a "multidrug resistance problem", which stems from, and depends upon, horizontal gene transfer, i.e., the cell infrastructure (Levy 2002; Summers 2006). The ancient cellular infrastructure of antibiotic resistance transport is continuously supported and promoted by sequential

exposure to new drugs. (Gillings, Boucher et al. 2008). These activities encourage transmissibility between commensals and multidrug-resistant pathogens.

- Integrons are major carriers of antibiotic resistance in gram-negative bacteria (Ploy, Lambert et al. 2000; Rowe-Magnus and Mazel 2002; Gillings, Boucher et al. 2008; Gillings, Krishnan et al. 2008). Although they have been found in a limited number of gram-positive bacteria (Ploy, Lambert et al. 2000; Ploy, Lambert et al. 2000; Nandi, Maurer et al. 2004), the extent to which they transmit resistance is not well known (Fluit and Schmitz 1999).

- Wastewater treatment facilities present an enormous "mixing pot" in which diverse bacteria and gene types co-exist. They are ideal sites for the creation of novel virulence functions and antibiotic resistance genes (Schluter, Szczepanowski et al. 2007). Wastewater contaminates the community environment when there are heavy rains, as well as the major events such as hurricanes or earthquakes. Partially processed wastewater may also be released into large bodies of water which are often used for recreation and thus the risk of exposure to the public from wastewater is of concern.

- Antibiotic resistance has been found in soils, manures, air and ocean sediments not impacted by human populations (Dantas, Sommer et al. 2008) This provides evidence that resistance occurred naturally and was subsequently dispersed to the other environments.

- Bacterial genetic exchange is ubiquitous, particularly for resistance genes. (Salyers, Gupta et al. 2004; Salyers and Shoemaker 2006). Few barriers appear to exist and the parameters surrounding gene exchange are ill-defined.

- "Bad bugs" (pathogens) and "good bugs" (commensals) are closely related. They are connected genetically by mobile genetic elements and ecologically by the commensal microbiota.

- Evolution of acquired antibiotic resistance genes and associated elements is an on-going process which varies in different ecosystems and over time.

- Bacteria that metabolize natural antibiotics and utilize them as a sole carbon source are widely distributed in the environment (Dantas, Sommer et al. 2008). They are resistant to virtually all antibiotics and are closely related to pathogens. They constitute an unrecognized environmental reservoir of antibiotic resistance determinants—hence a great source of information on antimicrobial resistance. Phylogenetic distribution suggests that resistance gene transfer from "antibiotic eater" to pathogens could occur (Dantas, Sommer et al. 2008).

- Aquaculture is an expanding industry which is poorly understood. This industry exposes the environment and its bacterial community to antibiotic residues, resistance genes, and other resistant strains. It is potentially very active in the spread of antibiotic resistance to plants, human and animal bacteria (Cabello 2004).

E. Discussions on priority research questions and possible research foci

The following questions Q.1 – Q.5 were posed as relevant to understanding some key issues surrounding antibiotic resistance in commensals:

Q.1. Is agriculture or aquaculture a richer source of resistance genes that impact resistance found in human infectious disease agents?

Because of the paucity of accessible data and studies, the general consensus was that it is currently difficult to tell whether either of these industries predominates as a major source of resistance genes, though both certainly contribute. However, aquaculture is suspected as a significant contributor, but definitely has not been adequately studied. There exists a large data gap here, but this question might be addressed with an expansion of aquacultural research. The problem is a complex one because of globally diverse cultures impacting antibiotic use In Mekong Delta, Vietnam for example, antibiotics and pesticides are sold together. Resistance is never reported as a problem, merely because it is never investigated. In contrast, Japan monitors antibiotic levels carefully due to huge monetary losses from rejection of antibiotic-laden fish by the more regulated European markets, yet antibiotic use is extensive in aquaculture here and fish food is often purchased from the developing world were antibiotic use is not regulated or recorded. The Japanese pharmaceutical industry is fueled by the high levels of bacterial resistance in fish pathogens, and the need to find new effective drugs for this huge industry. Resistance has yet to be examined in aquaculture workers and industry regulation in Europe and North America is spread over several agencies, while in other parts of the world there are no regulations or guidelines. There was a general consensus that resistance in aquaculture presents a serious growing problem, which directly affects the public, and would therefore warrant priority focus in a research agenda. While there are abundant data from Japan and Norway, few data are reported from the developing world. In order to compare the aquaculture and agriculture impacts of antibiotic use, studies are needed that examine resistance gene transmission to fish farmers and to agriculture farmers and to the consumers of these products.

Other points relevant to this question:

- Currently the only way to limit antibiotic resistance is to limit the use of antibiotics in these industries. There are studies showing that limiting antibiotic use in agriculture leads to a decrease in resistance problems (Aarestrup, Seyfarth et al. 2001).

- In Europe, antibiotic resistance has declined as a result of reducing antibiotic use in animals, specifically as food additives for growth promotion (van den Bogaard, Bruinsma et al. 2000; Aarestrup, Seyfarth et al. 2001; Heuer, Pedersen et al. 2002; Bengtsson and Wierup 2006). This is undeniable. Antibiotic use is more restrained there than elsewhere.(Wierup 2001).

- In order to significantly achieve a reduction in the amount of antibiotic resistance released into environment, antimicrobial use in agricultural animals must be reduced. Exact quantities being used are very hard to obtain but the amounts being released from all sources is great.

- Given the differences in culture, regulatory standards, policy, etc., it is difficult, but essential, to introduce standards of prudent antibiotic use worldwide in the agriculture and aquaculture industries.

- Veterinarian and medical communities are recognizing the need to track antibiotic resistance, but few systems for collection or analysis are in place outside of the EU. Now is the time to promote concrete examples of successful programs that show significant reductions in antibiotic resistance or likewise, to publicize those efforts which did not yield significant improvements. It was expressed that the European Union is definitely making progress in curbing the resistance problem (van den Bogaard, Bruinsma et al. 2000; Wierup 2001) in this regard and the US should follow suit.

Q.2. Do certain commensal species harbor higher frequencies or more diverse types of resistance genes?

- Aquaculture environments, harboring *Aeromonas* and *Vibrio*, were suggested to be the prime targets.

- Commensals that constitute the human flora are also key; but, it is possible to clarify this answer with data emanating from the Microbiome sequencing project.

- A closer examination is needed of the biology of commensals and mechanisms that are involved in the transfer of resistance genes to certain organisms. Species such as *Enterococcus* are more promiscuous in terms of gene transfer.

Q.3. Do domestic pets or farm/food animals pose a greater risk of human exposure to resistance genes?

What is known on this topic was summarized as follows:

- There is a large impact from agriculture because it is known that one can get bacterial resistance genes not only from water or farm, but also from air, soil and food products (van den Bogaard, Willems et al. 2002; Lewis, Molbak et al. 2008) Hummel and Tschape et al (1986) (Giraffa 2002; Johnson, Delavari et al. 2005; Perreten, Vorlet-Fawer et al. 2005).

- The use of agricultural (and urban) waste as fertilizer poses an enormous risk of developing resistance to both heavy metals and antimicrobials (Martins da Costa, Vaz-Pires et al. 2006)

- Pets are being treated with the same antibiotics classes that humans receive.

- Being a veterinarian has a higher risk of carriage of diseases transferred from animals to humans. Vets often have, for example, a higher rate of carriage of MRSA (Hanselman, Kruth et al. 2006; Lewis, Molbak et al. 2008). Studies done in Denmark (where MRSA rates are lower) showed that vets had higher levels of

carriage of MRSA than the public, and the MRSA was related to the animals being treated (Moodley, Nightingale et al. 2008; Wulf, Sorum et al. 2008).

Q4. How do *E. coli* compare with enterococci as major reservoirs of resistance genes?

There was a general agreement that enterococci might be the more important reservoir for the following reasons:

- There are ten times as many enterococci in the human gut as *E. coli*.
- They contain abundant conjugative transposons. (Salyers, Shoemaker et al 1995).
- While not an optimal species for study, *E. coli* is ubiquitous and therefore well-studied. Streptococcus and enterococcus are under-investigated and need more attention with respect to conjugative elements.

Q.5. What are the best examples of antibiotic resistance moving from agriculture/environment and emerging as pathogens in human infections?

- Beta-lactamase genes that were originated specifically in animal pathogens and ended up in *Hemophilus influenzae* - a specific human pathogen (Medeiros, Levesque et al. 1986; Brinas, Moreno et al. 2003; Bonnet 2004; Li, Mehrotra et al. 2007)
- VRE in Europe [*The finding of two ribotyping patterns among isolates from animals, sewage and clinical sources suggests that animals may serve as a reservoir of vancomycin-resistant enterococci (VRE) which may enter the human food chain.*] (Bates, Jordens et al. 1994; Bonten, Willems et al. 2001; Agerso, Lester et al. 2008).

F. <u>Knowledge gaps in commensal antibiotic resistance research</u>

The following were identified as knowledge gaps that need to be addressed:

- There is a general lack of data collection, "mining" and synthesis. An ongoing coordinated system of collection and analysis is needed on a global basis.
- Most findings on the epidemiology of antibiotic resistance have not yet been published as these data are sequestered as confidential information by the pharmaceutical industry. Release of these data will allow for some sort of intellectual information sharing that will help identify gaps yet to be filled.
- There is too much reliance on phenotypic data to determine the quantity of antibiotic resistance – this stems from a lack of reliable, standardized genotyping methodologies with which to promote uniform, broad-scale surveillance and genotypic analysis.

- There is need for phylogenetic grouping of resistance gene clusters.

- There are still no good models for predicting the emergence of antibiotic resistance. Following persistence in commensals could help.

- Financial gap – there is a major problem that lies in financing of commensal antibiotic research and the general financing of applied environmental research.
- "Best policies and practices" on antibiotic use and antibiotic resistance control need to be identified and disseminated as models for reducing antibiotic resistance in all environments.

- A poor definition of "commensal" and classification of the different levels at which they operate impedes discussion. Stratification (proximal to distal) in terms of their impacts on human disease could aid in focusing discussions and directing surveillance efforts appropriately.

G. Policy-related interventions for preventing the emergence and spread of antibiotic resistance

- There is a need to understand that the problem of antibiotic resistance lies in the both the ubiquity of resistance elements and management of antibiotics themselves.

- There was general agreement on instituting a good early warning system such that prompt action could be taken to contain the emergence of new resistance. Despite the identification of numerous resistance genes (i.e. penicillinase genes in the 1940s, CTX-M, qnr, qep, AAC 6'-ib-cr, etc.), any efforts undertaken to counter the spread of these genes came too late. There was insufficient fear to motivate containment of the MRSA problem in the 70's or 80's. When SARS emerged, it captured immediate attention, yet MRSA has ultimately done far more damage than SARS.
  While global alerts and containment plans are in place for any potential outbreak of H5 influenza or bioterrorism agent, such systems are lacking for the emergence of new antibiotic resistance. What happens when a new hospital is opened? Plans are needed to modify an antibiotic or change therapy before resistant strains become established.

- There is no management of antibiotic resistance from a proper ecological perspective. Novel ecology-based approaches are needed that can attract funders.

- Screening for MRSA has proved to be very effective against the spread of resistance in microorganisms. In Holland for example, the screening and quarantining of patients drove the rate down (van Trijp, Melles et al. 2007). Europe and Australia have employed an early warning system for a long time (McDonald 1997), but this is just barely beginning in the United States. In

the US, high community rates of MRSA have discouraged patient screening programs. While patients in the US may be screened for MRSA carriage and isolated, hospital workers are not, though they have 2-3-fold higher carriage rate then the general public. Plans should be instituted for screening of both patients and health workers to avoid a higher incidence of resistance spread.

- Despite some changes made in antibiotic use practices, there is a lack of follow-up regarding the impacts, e.g., there appears to have been no follow-up to measure the consequences resulting from the McDonald's Corp having switched to antibiotic-free meat several years ago.

- A two-pronged intervention was suggested for resistance control: 1) research that focuses on tracking resistance genes and 2) mobilizing efforts to raise public awareness of the emerging resistance problem.

- Antibiotics have demonstrated a defined period of "viability", but there exists a potential for extending this lifetime through skillful antibiotic management. Concern was expressed over why this has not been pursued more aggressively by government and professional agencies.

- If lessons cannot be learned from the past mismanagement of antibiotic resistance issues, the current erratic emergence of resistance will continue. More attention should be paid to what can be done to prevent repeat occurrences. With the advent of each new antibiotic, we anticipate the arrival of antibiotic resistance. This is a complicated economic and political problem that cannot be solved until the worldwide view of antibiotic resistance is broadened and there exists global collaboration to combat it. Although there is no guarantee, it might be worth trying a preventive measure. This could be a plan in place to evaluate the problem of emerging resistance as new antibiotics come to market. There is an urgent need to mobilize the medical community to combat the antibiotic resistance problem.

- The field of antibiotic resistance is suffering from lack of research, surveillance and data mining; every federal agency should be made aware that this area needs to be addressed urgently and adequate resources provided. Surveillance of antibiotic resistant bacteria, in clinical, agricultural and environmental settings is fundamental for understanding the resistance problem and instituting interventions.

- There is much emphasis placed on standardizing and testing antibiotic residuals in the environment, but no attention is paid to measuring the widespread impacts of these residuals on the bacteria themselves.

- There is a need to obtain a better idea of the information held confidentially by the pharmaceutical companies. Benchmark records should be published for accreditation. This information should be reported in order to promote some sort of intellectual sharing process, freedom of information, and help

drive awareness of the issue of antibiotic resistance forward. It is still not very clear if there are promising antibiotic prospects that are buried because the company abandoned them for either good or bad reasons. Prior roadblocks to a useful product may have been overcome at this point in time.

- Without common international standards, localized antibiotic restrictions in agriculture are pointless. There is difficulty in imposing standards of prudent antibiotic use in agriculture and aquaculture industries worldwide, given the differences in culture, regulatory standards, policy, etc. These hurdles need to be creatively addressed.

- There is a need for communication across diverse disciplines that study antibiotic resistance: ecologists, microbiologists, veterinary biologists and bioinformaticists. Engagement of these disciplines would encourage cross-talk.

- A major effective means of limiting resistance would be to employ a worldwide policy to implement antibiotic-free food production. Policies and guidelines for addressing antibiotic resistance need to take a global approach. Anything being done only at the local level will have limited success. Antibiotic surveillance without subsequent action is useless.

H. <u>Suggestions for science-related interventions</u>

- The issue of antibiotic resistance is significantly both an ecological issue and a cellular infrastructure issue, and both perspectives should be considered in addressing scientific solutions to the problem. It was suggested that the scientific community should work concertedly to devise a compelling "ecological approach" that will attract funding from NIH or any other funding organization. As we seek possible approaches, molecular biology studies appear to be invaluable. It was suggested to procure an antimicrobial to which minimal resistance has been expressed and to test it against environmental bacteria – looking for a previously unknown resistance mechanism that can be studied and tracked globally, simultaneously looking for escalating resistance. This could facilitate predicting the emergence of antibiotic resistance. For example, the van operon is known, but there may be other vancomycin resistance elements in the environment that could be screened for on a large scale.

- Molecular studies to project or estimate the size of microbial genomes should be undertaken.

- Sequencing is becoming increasingly more cost effective and fast and surveillance should be supported using a gene-based approach. One run on a 454 pyrosequencer could provide the sequences of more than 1000 plasmids from hospital pathogens. How can this information be used intelligently to

screen for "real" and "cryptic" resistance genes in the most vulnerable hospital patients?

- Data gaps need to be addressed. Prediction of the emergence of new antimicrobial resistance genes without evaluating and bridging the current data gaps is not possible. The following were identified as areas needing focused research:
    - **a.** Aquaculture: active screening for antibiotic resistance genes and gene tracking of new resistances from fish to human pathogens, in a variety of geographical settings. In order to evaluate the impacts of antibiotic use from agriculture and aquaculture use, studies are needed that compare gene transmission in these two different environments.
    - **b.** Contaminated water environments: microbial environments are all connected through wastewater treatment plants; better disposal of wastewater is needed, even in the US.
    - **c.** Sludge and manure as fertilizer: impacts of this "mixing pot" of genes are unclear.
- Bioinformatics and algorithms need to be employed for predicting the patterns and trends in antibiotic resistance.

- Genotyping methodologies are currently diverse, but the gene-based literature is improving and can be relied upon to design a quantitative method to study the emergence of antibiotic resistance. The identification of resistance mechanisms is needed in order to support studies on antibiotic resistance.

- An optimal system for managing and analyzing cumulative surveillance data needs to be designed. (APUA's ROAR isolate database is a step in that direction).

- More information can be derived from gene sequencing. Phenotyping alone cannot track gene flow, but this can be accomplished with genotype profiling. Sequencing proved that genes were circulating and were well distributed. Without genotyping, the presence of a resistance gene cannot be identified until and unless it is expressed in an appropriate host.

- Funding support should be given to the analysis of all genomic, microbiomic, and other data, which should be screened for known and potential resistance genes. As huge numbers of genes have been sequenced already, theoretically, there are already sufficient data that could be screened for discovering the first appearance of a particular allele (e.g., a transferase). The human microbiome project will provide all the information on cryptic or potential resistance genes in commensals that are needed. Surveillance must be done before resistance becomes established. The same could be done for potential virulence genes; when transferred into new hosts they could lead to novel pathogens.

- Several organisms should be the focus for expanding studies beyond *E. coli*:
  1). *Pseudomonas* - "a wild card that moves everything"
  2). *Enterococcus* – should be added to the list of organisms being tested (*E. coli, Streptococcus, Salmonella, Staphylococcus*) through APUA's AMRIID Surveillance project.
  3). *Vibrio* and *Aeromonas* –good representatives of water environments that are also generally confined there.
  4). *Stenotrophomonas* –an emerging opportunistic pathogen

- Focus on integrons. A collaborating team of epidemiologists, ontologists, and bioinformaticists should assist in deriving some good scientific direction.

- Soil drilling: look for resistance genes in a variety of global sites; in particular, organisms that are common pathogens in the tropics, but may soon emerge as pathogens in more polar regions.

- In retrospect, it was suggested that there is a need for a definitive review evaluating all the experiments showing animal to human transfer, as well as antibiotic use leading to resistance, etc. The farm industry cites a lack of evidence for animal use in antibiotics causing resistance in humans. Strong, convincing arguments are needed on this question of transmission. In addition a compilation and critical evaluation of data from the Netherlands and other countries is needed.

III. <u>APUA-recommended next steps</u>

1. In accord with the expert group suggestion, APUA recommends a series of mulitidisciplinary meetings to set a detailed research agenda utilizing an ecological or "systems biology" approach to address the many data gaps and paucity of modeling systems for predicting resistance. Building on the ROAR project approach, a collaboration of a small team of experts from diverse specialties (bioinformatics, epidemiology, microbiology, etc) should be established to research and design an integrated approach towards predicting and containing emerging resistance.

2. APUA recommends a coordinated research project involving ROAR project RFPs and subgrants for the "mining" of the commensal genomes for resistance and virulence elements as these sequences emerge from the human microbiome and other sequencing projects.

3. APUA encourages support for the expanded collection and consolidation of genome-based data within the ROAR isolate database and for the annual updating of the ROAR literature database.

4. To provide the scientific evidence for better public policy, APUA recommends a definitive review article that would evaluate all experiments showing animal-to-human transfer and antibiotic use leading to resistance. This article could be drafted utilizing

APUA's FAIIR Report (Barza, M., Gorbach, SL. [eds] 2002) and the updated ROAR literature database, with the input of key experts.

5. A meeting of pharmaceutical industry and academic scientific experts working on resistance should be convened to consider how to overcome barriers for sharing findings from antibiotic research.

6. A coordinated surveillance group project involving industry and government surveillance systems should be established to jointly interpret new findings, act as an early warning system, and develop intervention plans similar to the warnings and plans in place for H5 influenza. The APUA GAARD project, a public/private surveillance partnership, provides a foundation for this work.

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