Recent years have seen the debate over antimicrobial use in food-animal production increase, while the absence of sound data allows for the presentation of highly speculative, unsubstantiated arguments and innuendo. At the heart of the argument is the belief by some that use of these drugs in agriculture and aquaculture leads to the increase and spread of antibiotic resistance. In fact, the probability of resistance transfer from bacteria occurring in aquaculture systems to human pathogenic bacteria is believed to be low. The aquaculture environment and the industry’s practices present several barriers to such transfer, although these barriers also complicate quantitative risk analysis.

Many aquatic species are farmed, including vertebrate and invertebrate animals, as well as various plants. In the United States, for example, the United States Department of Agriculture recently conducted a survey indicating that at least 23 different species were farmed. Internationally, this number is far greater and continues to expand. Species are raised in freshwater and in brackish to marine waters, and in both cold and warm temperatures (5-30°C). Methods range from the relatively open-water net pen systems and flow-through raceways, to individual ponds and closed, recirculating systems. Each offers advantages and disadvantages, as well as the potential to transfer resistance.

S. pneumoniae is one of the leading bacterial pathogens causing community-acquired pneumonia, meningitis, sinusitis and acute otitis media. In recent years a significant increase in the incidence of penicillin-resistant, macrolide-resistant and multiply-resistant S. pneumoniae has been documented. The varying rates of nasopharyngeal carriage of penicillin-resistant S. pneumoniae (PRSP) in preschool children have been well-documented in publications from all over the world, and attendance in daycare centers has been proven to be an important risk factor for the rise of morbidity associated with penicillin-resistant strains.

Nasopharyngeal specimens were obtained from 152 children between the ages of 1 through 6 years, at three different daycare centers, located in Sofia, during October and November of 1999. These specimens were taken in an effort to better understand the rate of S. pneumoniae nasopharyngeal carriage, and to gauge the percentage of penicillin-resistant, macrolide-resistant and multiply-resistant strains in children. A questionnaire was given to parents to determine socio-economic status, preliminary antibiotic treatment, and perceptions about the prescription of these drugs (Table 1).

**Methods**

Swabs were inoculated onto blood agar plates and incubated at 37°C in 5 - 10% CO₂ for 18 - 24 hours. Identification of S. pneumoniae was by the routine criteria — haemolytic colonies with typical morphology that showed susceptibility to optochin disk (Becton Dickinson, BBL). Susceptibility testing was performed on Mueller-Hinton II agar (Becton Dickinson, BBL), supplemented with 5% sheep blood, according to the NCCLS, 1999. Susceptibility to penicillin was first screened by a disk of oxacillin, 1g. Strains with an inhibition zone <20 mm were tested with a penicillin E test, according to the instructions of the manufacturer. Sensitivity to other antimicrobial agents was determined by the Kirby-Bauer disk diffusion method and antimicrobial disks, obtained from the National Center for Infectious and Parasitic Diseases, in Sofia. The mechanism of macrolide resistance was detected by double disk method.

**Rates of Resistance**

Of the 152 children studied, 85 (55.6 %) showed S. pneumoniae nasopharyngeal carriage. A high level of penicillin resistance (>2 mg/L) was seen in 1.2% of strains, while 21.2% showed intermediate resistance (0.1 - 1 mg/L). This rate of PRSP is similar to percentages found in Italy, Canada and Portugal, but is relatively lower than that seen in Spain, France, and Hungary. It is higher than in some countries.
northern countries with restricted antibiotic policies, like Iceland and Sweden. A statistically significant percentage of children with PRSP (14 of 18; p<0.001) had received antibiotic therapy during the prior three months, mainly aminopenicillins. Similar findings have been documented by earlier investigators.5,11,12,14

Resistance to other antibiotics varied: 37.6% of the strains tested showed resistance to tetracycline, 10.6% to chloramphenicol, 21.2% to erythromycin, 9.4% to clindamycin and lincomycin, and 42.3% to trimethoprim-sulfamethoxazole. All of the strains tested were susceptible to cefotaxime, rifampicin, vancomycin and ciprofloxacin.

### Table 1. Parental perceptions about antimicrobials and their use, Bulgaria.

<table>
<thead>
<tr>
<th>Perception</th>
<th>Percentage</th>
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<tr>
<td>Number of respondents who consider antibiotics useful for the management of infection</td>
<td>43%</td>
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<tr>
<td>Number of respondents who think antibiotics lead to harmful consequences</td>
<td>46%</td>
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<tr>
<td>Number of respondents who consider antibiotics useful for the treatment of a viral infection</td>
<td>50%</td>
</tr>
<tr>
<td>Number of respondents who have insisted on an antibiotic from their doctor in the past</td>
<td>12%</td>
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</table>

Percentages are based on the distribution of 152 questionnaires, of which 108 were returned and answered anonymously.

### Mechanisms of Resistance

The rate of macrolide resistance was also demonstrated to be high (21.2%), although lower than in Italy,6 France and Spain.1 Analyzing the mechanisms of macrolide resistance has shown methylation of 23S rRNA, encoded byerm genes (MLS B phenotype) in 38.9% of strains, and an efflux pump mechanism, mediated bymeF genes (M phenotype) in 61.1%. This finding differs from the percentage found by other authors.13 Five of the erythromycin-resistant strains were penicillin-susceptible and presented M phenotype. All of the strains with MLS B phenotype were resistant to tetracycline.

While 35.3% of the strains tested were susceptible to all examined antibiotics, 20% were multiply-resistant (resistant to >3 different classes of antibiotics). It is interesting to note that 5 of the 17 multiply-resistant strains were susceptible to penicillin. As was expected, PRSP showed a higher rate of resistance to other antibiotics (Table 2).

This pilot project has demonstrated nasopharyngeal carriage of penicillin-resistant S. pneumoniae in the three daycare centers examined to be as much as 22.4%. This may represent a significant epidemiological risk. The

### References


Three New Chapters Added to APUA’s International Network

APUA officially welcomed new chapters from three different continents during the past quarter. One of these, APUA-Nepal, has already prepared a draft national antibiotic policy upon request by the Ministry of Health. This proposed policy was presented during a three-day workshop in late May, sponsored by the government, APUA and the Rational Pharmaceutical Management Project. Over 60 experts from various sectors attended the gathering. The chapter has been asked to translate the draft policy from English to Nepali, and it is currently being reviewed by the government for approval. With assistance from APUA-International, APUA-Nepal also published a Nepali-language educational brochure and poster (right) on antibiotic resistance and prudent drug use for distribution to clinics throughout the country. And chapter Board members were also recently interviewed during a live broadcast on Nepal’s Radio Sagarmatha, on the issues surrounding antibiotic use and misuse in that country.

In March, APUA welcomed Ecuador as the newest chapter in Latin America. Dr. Jeannette Zurita Salinas, a medical microbiologist, will serve as Chapter Coordinator. Dr. Zurita is Chief of the Microbiology Laboratory at the Vozandes Hospital in Quito, Professor of Microbiology in the School of Medicine at Catholic University of Ecuador, and Vice President of the Ecuadorian Society for Infectious Diseases. Other founding members include clinicians and academicians specializing in intensive care and emergency medicine, infectious diseases, and pediatrics. The chapter is sponsoring a symposium on antibiotic resistance at the First Ecuadorian Congress on Infectious Diseases, to be held in Quito in July.

Belarus is APUA’s latest chapter to be established in the former Soviet Union. Led by Professor Valery Semenov, the Head of the Department of Infectious Diseases at Vitebsk Medical University, the chapter is planning a national conference on the rational use of antibiotics and antibiotic resistance in October, 2000. This conference is endorsed by the Ministry of Health, with the participation of scientists from Russia and from throughout the Republic of Belarus.

Table 2. Percent of resistant nasopharyngeal *S. pneumoniae* strains in children in Bulgaria.

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<th>PEN</th>
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<tr>
<td>All <em>S. pneumoniae</em></td>
<td>22.4</td>
<td>37.6</td>
<td>10.6</td>
<td>9.4</td>
<td>21.2</td>
<td>9.4</td>
<td>42.3</td>
</tr>
<tr>
<td>PEN-resistant <em>S. pneumoniae</em></td>
<td>100</td>
<td>42.1</td>
<td>21</td>
<td>31.6</td>
<td>52.6</td>
<td>31.6</td>
<td>63.2</td>
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Note: This work was conducted and supported as part of APUA’s Small Grants Program for Chapter Affiliates. For more information on APUA’s Small Grant Program, contact Denise DeRoeck at dderoe01@emerald.tufts.edu.
differential impact the probability of resistance transfer.

Environmental Influences

The natural bacterial flora of aquatic ecosystems can be considerably different than that in terrestrial or human systems. Much depends on what bacteria flow into the water from terrestrial watersheds. Variation in salt and organic matter content, pH and turbidity can effect the population of flora. Temperature alone can have a profound effect on bacterial survival and abundance. Many of the human pathogens, especially those associated with food-borne illness, have temperature optima (37°C) far warmer than occurs in aquaculture operations. The difference is most striking for cold water salmonid aquaculture that occurs at 15°C or colder. Various Salmonella and Vibrio species can occur in warm water or marine aquatic systems. Both bacterial types have been recovered from aquaculture animals raised under these conditions. However, the pathogenicity or resistance status of these isolates has not been established. Meanwhile, a recent US Food and Drug Administration (FDA) survey found a very low prevalence of Salmonella on the processed fish sampled at supermarkets. The species with the most number of Salmonella was wild-captured shrimp from India.

While the causes of resistance in aquatic bacteria are not clear, certainly use of antibiotics can increase its probability in some species. However, organisms may be resistant to antibiotics in the absence of antibiotic use, so other factors appear to be important as well. Some resistance may occur because of elevated environmental nutrients. The type of water used for aquaculture also appears to be an important determinant. In many parts of the world, for example, animal and human excrement is added to fish ponds as an organic fertilizer; in Europe and Israel many wastewater treatment facilities have incorporated aquaculture for decades, although this practice does not occur in the U.S. Resistant bacteria have been recovered from wild fish exposed to sewage outfalls, and in India a relatively high prevalence of resistant bacteria from fish sampled at supermarkets was attributed to the rearing of fish in sewage-influenced waters. In spite of this, human disease associated with aquacultured animals is very rare.

Therapeutic Use

While antibiotics are not typically used for growth promotion in international aquaculture, and are outlawed in the US for this purpose, they are occasionally used in aquacultured animal husbandry to treat specific bacterial diseases. The drugs are primarily delivered in a medicated feed. This occurs because individual fish cannot be readily isolated for medication. In the U.S., antibiotics are incorporated into the feed instead of top-dressed (coated onto the feed after feed manu-

The occurrence of human disease associated with aquacultured animals is extremely rare...

facture). This precludes any leaking of the drugs into the environment during feed delivery. Additionally, medicated feed is only applied when most fish in a population are still actively feeding. By maintaining good husbandry practices with close scrutiny of fish, delivery of medicated feed can be timed to ensure its consumption within seconds. Furthermore, many types of aquaculture facilities have some means of capturing solid wastes prior to discharge. This allows for the removal from the water of antibiotics associated with fecal matter or waste feed.

Oxytetracycline and ormetoprim-sulfadimethoxine are the two antibiotics approved by the FDA for use in US aquaculture, and then only for the treatment of certain bacterial diseases in certain animal species (salmonids, catfish, and lobster). Other countries have approved several more antibiotics and higher dosages for use in aquaculture. In Japan, for example, 29 antibiotics or combinations may be used. Most countries do not exert the same level of approval or use stringency requirements as in the U.S.

It has been suggested that the use of antibiotics by the Ecuadorian shrimp farming industry caused the development of multidrug resistant V. cholera. There are little data to support this, and other plausible explanations have been suggested, including poor hygiene. The discharge of sewage containing antibiotics into coastal waters where shrimp farming occurs is another possibility.

How aquatic species are marketed or consumed can also influence the probability of exposure to aquatic bacteria, whether resistant or not. While the occurrence of human disease associated with aquacultured animals is extremely rare, there are two reports of human infection associated with the handling of live Tilapia. In both cases, people were punctured by the spiny rayed fins of the fish, resulting in direct inoculation of the bacteria (Streptococcus iniae or V. vulnificus). In Israel, where V. vulnificus exposure occurred, once the producer returned to his normal method of marketing his fish, human infection disappeared.

Consumption of raw fish and shellfish is a known risk factor for acquiring food-borne diseases. In Japan, infection with V. parahaemolyticus (a normal inhabitant of marine ecosystems) can occur as the result of consumption of raw, improperly cooked or recontaminated seafood, such as aquacultured finfish.

There is very limited actual evidence to indicate that the use of antibiotics in aquaculture is of any risk to public health. In fact, considerable historic evidence suggests various natural barriers, and aquaculture practices effectively prevent the transfer of any human pathogens. More credible data is needed for any quantitative risk analysis, and while existing information suggests the use of antibiotics in aquaculture is safe, the National
Aquaculture Association nevertheless supports the prudent use of these drugs and supports development of effective alternatives.

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**APUA News**

**APUA’s Small Grants Program in Developing and Transitional Countries**

During the past year, APUA’s small grants program has helped support innovative research and educational activities conducted by chapters in seven developing and transitional countries.

In the area of research, **APUA-Uruguay**, under the leadership of Dr. Maria Hortal, conducted a study that demonstrated the overprescription of antibiotics for children with pharyngitis. While 75% of the 212 children who presented with pharyngitis at an outpatient clinic were prescribed antibiotics, only 15% of the swabs taken from the children tested positive for Streptococcus pyogenes Group A, for which antibiotics are warranted. The goal of the study, which was co-funded by the Pan American Health Organization and the Uruguay Ministry of Health, is to help persuade pediatricians to modify their antibiotic prescribing habits.

**APUA-Russia**, headed by Leonid Stratchounski, is conducting a study to inventory antibiotics in home medicine cabinets. Investigators have visited 800 families in eight cities throughout the country, using a prepared questionnaire to learn more about antibiotic use in the population, where the drugs are being obtained, and the extent to which individuals are using expired medication. The study results will be used to develop educational materials for consumers in that country.

Other research activities include a study in India aimed at gauging the sensitivity patterns and cost-effectiveness of newer, more expensive antibiotics used in hospital settings, as compared to older, less expensive drugs. Additionally, **APUA-Bulgaria** recently finished an in-depth study of resistant *S. pneumoniae* in children at daycare centers (see page 1).

Educational activities conducted by APUA chapters have also received small grant funding. These include a week-long campaign in Moldova on Rational Antibiotic Use (see Vol. 18, No. 1), which targeted providers, pharmacists, policy makers, and the general public, and a portion of a year-long series of training courses on medicine awareness for school teachers and women’s groups, conducted by the new APUA chapter in Nepal.

APUA is currently reviewing a new round of research and educational proposals from several chapters.

**Project FAAIR: Facts about Antibiotics in Animals and their Impact on Resistance**

On June 6, APUA convened a committee of distinguished infectious disease experts, as part of the Alliance’s Project FAAIR, to consider the body of scientific evidence concerning the human health impact of antibiotic use in animals. Participating were Drs. Morton Swartz of Massachusetts General Hospital, Michael Barza of Carney Hospital, Thomas O’Brien of Brigham and Women’s Hospital, and Stuart B. Levy of the New England Medical Center. Dr. Paula Fedorka-Cray, of the USDA Department of Agriculture, presented data from its National Antimicrobial Resistance Monitoring System. The committee also considered staff-prepared information, including a literature review, and data on antibiotic use. Members of this core group are former participants of the 1989/1998 Institute of Medicine studies. They will be considering new studies and providing recommendations to link these data. The committee findings will be publicized and used to guide the current policy debate, and fill in gaps in knowledge concerning antibiotic use in animals. Members interested in this project should contact Kathy Young (kyoung02@tufts.edu) to be connected with the project team.

**APUA Hosts Conference**

A first of its kind conference, Antibiotic Resistant Infections A Global Problem with Local Solutions, was jointly sponsored by APUA, The American Association of World Health, The Massachusetts Department of Public Health and the Massachusetts Medical Society. Held on May 2, in Needham, Massachusetts, the conference attracted over 300 participants, with a target audience of community physicians and nurse practitioners. Topics included the surveillance, ecology and economics of resistance, and specific prescribing guidelines for hospital and community settings. The day’s events were generously funded through unrestricted grants from the Bayer and Ortho-McNeil Pharmaceutical Companies. Proceeds of the meeting will be posted on APUA’s website.

**Sales of Antibiotics in Chile Fall Fifty Percent**

According to a recent report published in the Chilean daily newspaper, La Tercera, the first 3 months of this year saw the sale of all types of antibiotics fall more than 50% in that country. This drop is thought largely to be the result of a strong campaign instituted by the Ministry of Health at the end of 1999, which, among other things, now requires a medical prescription to obtain these drugs in all cases. Broad-spectrum penicillin sales fell by 50.6% when compared to the first 3 months of 1999, and macrolides and other similar antibiotics saw a 17% decrease in sales. The APUA newsletter will publish a more comprehensive analysis of these findings in an upcoming issue. Those members interested in contributing to such a report should contact the editorial offices, or email Associate Editor Elizabeth Frame at emarf@mediaone.net.

**Study Section to Begin**

APUA is beginning a new study section concerning the ecology of antibiotic resistance. The group will produce several scientific reports to increase public understanding of resistance as an ecological problem. A new APUA website section on this topic will also be developed. APUA members interested in participating should contact Kathy Young at kyoung02@tufts.edu.
Highlights from the International Congress on Infectious Diseases, Argentina

In April, APUA-International and several APUA chapters and members participated in the International Congress on Infectious Diseases (ICID), held in Buenos Aires, Argentina. APUA, along with the Pan American Health Organization (PAHO) and the Pan American Association for Infectious Diseases (API), sponsored the symposium, “Prudent Use of Antibiotics in the Developing World.” Drs. Aníbal Sosa (APUA), Gabriel Schmunis (PAHO), Hugo Pezzarossi (API), Maria Hortal (APUA-Uruguay), Philip Walson (Ohio State University and Children’s Hospital) and Michael Bennish (Africa Centre for Health and Population Studies) spoke about partnerships to address antibiotic resistance, application of community research results to clinical practice, effective strategies for provider education, and the process of designing clinical guidelines for antibiotic use. The symposium and its reception attracted more than 200 professionals from a variety of disciplines.

In conjunction with ICID, APUA-Argentina, along with the Argentina Ministry of Health, PAHO/WHO, and APUA-International, presented the first regional training course on laboratory surveillance methods for detecting antimicrobial resistance. The course was dedicated to the memory of one of its designers, Dr. Alicia Rossi, and brought together 120 microbiologists from throughout Latin America. Participants had the opportunity to improve their knowledge of susceptibility testing techniques, specific organisms, interpreting results, quality control, and data management. In the coming year, APUA and PAHO will be sponsoring similar trainings in other Latin American countries.

Sixteen current and prospective APUA chapter leaders from Latin America met during ICID to share the latest developments in antibiotic resistance surveillance, policy and regulation of antibiotic prescription, professional training and local research agendas. The group stressed the importance of maximizing resources in the region by promoting cost-effective professional training, standardizing bacteriological diagnosis and antibiotic susceptibility testing, accessing existing surveillance tools and networks, and seeking international support for their efforts.

Sessions and Workshops

• First Ecuadorian Congress on Infectious Diseases, at the Four Points Sheraton Hotel, Quito, Ecuador, July 24 - 29, 2000 (sessions conducted in Spanish). On Friday, July 28 at the Congress, APUA will sponsor a symposium on antibiotic resistance, covering local and global perspectives, the effects of resistance on treatment of acute diarrheal and respiratory illnesses, and surveillance and collaborative efforts to contain resistance. Other topics will include occupational health, food-borne illnesses, the laboratory’s role in diagnosing infectious diseases, immunizations, and new antimicrobials. For more information contact Dr. Jeannette Zurita at jzurita@hcjb.org.ec.

• Regional Forum on Bacterial Resistance, Managua, Nicaragua, meeting venue to be determined, August 17-19, 2000 (Sessions to be conducted in Spanish). This conference is being planned and sponsored by the Pan American Association for Infectious Diseases. Featured topics will include, epidemiology of and strategies for controlling antibiotic resistance in the community and in the hospital, methods to detect resistance, the use and abuse of antibiotics in the community, establishing surveillance networks, WHOnet description and hands-on training, APUA’s role in Latin America, and a workshop to develop a proposal for a Latin American surveillance network. Country profiles from Costa Rica, Cuba, the Dominican Republic, El Salvador, Guatemala, Honduras, Nicaragua, and Panama will also be discussed. For more information, contact Dr. Carlos Mejia at carlosmejia@guate.net.

APUA to Inform WHO Strategy

APUA has been commissioned by the World Health Organization (WHO) to provide a synthesis of 25 years worth of major policy group studies and recommendations concerning antibiotic use and resistance. The intent of the report is to guide WHO global strategy aimed at curbing resistance. APUA chapters will provide grassroots input into the report.
If you are concerned about the public health threat of antibiotic resistance, become part of the solution. Make a tax deductible contribution and join our global network of citizens, clinicians, researchers and policy makers.

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Two-year memberships are charged the full rate the first year and will be granted a complimentary membership for the second year.

The Alliance for the Prudent Use of Antibiotics is a non-profit organization dedicated exclusively to curbing antibiotic resistance and improving the use of antibiotics throughout the world. Founded in 1981 as a global grassroots organization, APUA’s mission is to improve public health through education and research concerning antibiotic use and resistance. With members in over 100 countries and numerous foreign chapters, APUA provides a unique network to support country-based activities and facilitate international communication and planning.

APUA’s resources include an international scientific advisory board with members of national academies of medicine and science, and a professional staff with specialized expertise. APUA’s global network of affiliated chapters serves to tailor interventions to local customs and practices.

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