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A call for antibiotic alternatives research

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The persistence and spread of antibiotic resistance, in conjunction with decreased profitability of new antibiotics, have created the dangerous prospect of ineffective therapies against bacterial diseases. National strategies aimed at discovery, development, and definition of the mechanisms of effective antibiotic alternatives, especially for agricultural applications, should be encouraged.

The time has come for innovative and bold solutions to slow resistance to antibiotics and speed the development of new antibacterials [1]. Unlike the so-called golden age of antibiotics, when many antibiotic classes were discovered and commercialized, the discovery and release of new antibiotics have dramatically decreased in the past decades. For instance, there have been no new classes of antibiotics to treat Gram-negative bacteria, such as *Escherichia coli* and *Salmonella enterica*, in over 40 years [1]. At the same time, extensively drug-resistant and pan-resistant (resistant to all therapeutic antibiotics) strains of these bacteria have been identified in human and veterinary clinics.

Since the 1990s, both the decline in attractiveness of the antibiotic market and consolidations within the biopharmaceutical industry have resulted in a 75% decrease in the number of companies with large antibiotic R&D efforts [2,3]. In 2004, antibiotics represented fewer than 2% of drugs in clinical development by the 15 largest drug companies [4].

The pursuit of new antibiotics has decreased because of a decrease in profitability from the effort. As pointed out by Sharma and Towse [5], antibacterials are not an attractive option for investors seeking quick and substantial returns on their investments. The development of an antibiotic drug from discovery through approval is estimated to cost between $0.8 and $1.7 billion. Finding or creating new antibiotics in the laboratory has become more challenging [6]. Easily discovered antibiotic classes have already been found, often serendipitously and through traditional empirical assays of fermentation products. Current discovery approaches require specialized technologies and are aimed at specific molecular targets [1,5,6]. They have yielded chemical compounds that kill bacteria but lack the desirable biological and economic properties of the golden-age antibiotics, such as broad activity spectra [6]. In addition, government safeguards and regulatory requirements, especially for the approval of non-traditional antibiotics, prolong market release and add to development costs [1,3].

With rare exception, antibiotic therapy for humans is for short-term use. There is more profit and thus more incentive for companies to invest in drugs for frequent, long-term use, such as cancer, cardiovascular, psychoneurological, and lifestyle-targeted therapies. Despite legislative efforts to extend patent protection times for new antibiotics, they are insufficiently attractive as commercial products to be developed without guaranteed sales. In the absence of widespread life-threatening bacterial diseases, antibiotic products are currently not profitable and returns on R&D investments can no longer be assured.

Paradoxically, because the pursuit of new antibiotics has decreased there is an increasing need for the discovery of antibiotics to combat antibiotic-resistant bacteria. As discussed by Andersson and Hughes [7], it is very likely that the antibiotic resistance problem resulting from the extensive use and misuse of antibiotics for human, animal, and plant purposes will continue, at least for the foreseeable future.

At one time, antibiotic resistance was considered to be a biologically costly trait. In the absence of antibiotic selection, gene mutations or extra genes conferring resistance were predicted to be handicaps for resistant bacteria in competition with sensitive bacteria and were thus thought of as dispensable. Now, however, experimental data from both clinical and research laboratories have led to the conclusion that antibiotic resistance genes exist and stably persist in bacteria with or without antibiotic selection [7–9].

Host-associated microbial ecosystems are common sites where antibiotic resistance persists. One of the most diversely populated bacterial ecosystems is the animal (human) intestinal tract. The intestinal ecosystem contains approximately 100 trillion non-pathogenic bacteria representing upwards of 500–1000 different bacterial species. These microbes make essential contributions to the health and wellbeing of their host animal. They are also a reservoir for antibiotic resistance genes. A significant, and usually large, proportion of the bacterial species in that ecosystem is resistant to one or more antibiotics despite the fact that the individual animal (and its intestinal microbial denizens) has never been exposed to an antibiotic. In one study of antibiotic-free swine from organic farms, over 70% of *E. coli* and 16% of the total anaerobic bacteria populations were chlortetracycline-resistant [10]. Several explanations for the persistence of antibiotic resistance have been proposed or demonstrated (Box 1). By inference, antibiotic resistance will continue to exist at some level in microbial populations even with restrictions on antibiotic use. Antibiotic use selects for an increase in existing resistant bacteria, encourages the spread of resistance

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Box 1. Explanations for the persistence of antibiotic resistance*

- Genetic-free trade agreements: genes for antibiotic resistance flow freely and widely across bacterial groups within an ecosystem such as the intestinal tract. Non-pathogenic bacteria serve as reservoirs of antibiotic resistance for other non-pathogens and for pathogens.
- Multiple use: antibiotic resistance mechanisms have other uses in bacteria, for example, potassium transport or protection from intestinal bile acids.
- Co-selection of gene clusters: genes for resistance to one antibiotic physically link (on transferable genetic elements such as plasmids, transposons, integrons, and bacterial viruses) to other genes conferring fitness on bacteria or on the genetic element. Examples include resistance to mercury and resistance to copper. The benefits of a single gene can promote the survival of multiple genes when the genes are shared as a unit between bacteria.
- Constant exposure: antimicrobial resistance genes are continually recycled from the environment, carried via bacteria in food, water, air, soil, and animal-to-animal contact. Tetracycline resistance genes, for example, have been detected in feed and antibiotics given to animals. Resistance genes exist naturally in pristine soil and water environments [14].
- Compensation mechanisms: gene mutations resulting in antibiotic resistance are offset by other gene mutations or by duplication of genes and antibiotic resistance genes are switched off. As a result, the competitive fitness of the bacterium is not weakened when antibiotics are absent.
- Sub-MIC selection: ultra-low concentrations of antibiotics (contaminating residues or levels found in natural environments, such as soil) do not kill susceptible bacteria but reduce their growth rates, so resistant bacteria outgrow them.
- Subspecies diversity in complex ecosystems.

*Based on Stanton and Humphrey (http://www.feedinfo.com/console/PageViewer.aspx?page=185602) and Andersson and Hughes [7].

genes across species barriers through diverse bacterial communities, and drives the evolution of resistance mechanisms.

A reliable supply of effective antibacterials for food animals and humans is essential to the health and wellbeing of the citizens of a nation, as are a secure supply of wholesome food and a strong agricultural economy. Antibiotics in the USA are used for growth promotion and to prevent or treat diseases of livestock. Several hypotheses surround the growth promotion effects of antibiotics, including prevention of low-grade bacterial infections [11]. Antibiotic treatment of low-grade infections and antibiotic use to prevent animal disease induced by management stress, such as transport, have been considered prophylactic therapies. Annually, an estimated 1000 tons of macrolide and tetracycline antibiotics (alone) are added to swine diets for these purposes [12].

There is major debate over the effects of agricultural antibiotic use on human health. There is concern that the use of antibiotics in food animals can lead to dissemination of antibiotic resistance genes from animals to humans, especially through or to human bacterial pathogens.

Movement of bacteria occurs regularly, back and forth, between animal farms and human communities through air, water, direct physical contact, and, notably, via the food chain. In view of the animal origin of foodborne pathogens, such as *E. coli* O157:H7, *Salmonella enterica* serovar Typhimurium, and *Campylobacter* species, it is implausible that antibiotic use in animals does not select for antibiotic-resistant bacteria that can pass to humans through animal products. Detection of fecal contamination of meat products could be one mechanism to prevent dissemination of resistant bacteria through the food chain and to reduce antibiotic use (Box 2).

Box 2. A USDA invention to reduce animal-to-human transmission of antibiotic-resistant bacteria

In 2000, two ARS scientists, Tom Casey and Mark Rasmussen, and an Iowa State University colleague, Jacob Petrich, received a Superior Service Honor Award from the US Secretary of Agriculture and a National R&D 100 award for the invention of a fluorescence detector. The detector monitors, in real time, fecal contamination of animal carcasses in meat processing plants. Fecal contamination is a significant source of foodborne pathogens and spoilage bacteria in meat products [15].

This invention will reduce the transmission from meat to humans of antibiotic-resistant foodborne pathogens and non-pathogens. Ideally, the invention will be widely adopted if not mandated.

Any method that prevents the spread of fecally transmitted bacteria will curb the spread of antibiotic resistance genes. It will also reduce antibiotic use in farm animals and in humans, for example, by reducing the number of foodborne illnesses that must be treated with antibiotics.

Meat is a significant, often preferred, protein source in human diets. Large-scale efficient production of meat animals in the USA is an essential contributor to food biosecurity. Important unanswered questions surround the impact of antibiotic limitations on agricultural productivity. To what degree will restrictions on agricultural use reduce the incidence of human antibiotic resistant infections, given widespread antibiotic use in humans and the persistence of antibiotic resistance? To what degree will restrictions impact human health by reducing the affordability of an important protein source?

The continuous and substantial use of antibiotics for agriculture is prudent for those who have the goal of maintaining a healthy agricultural economy. Ironically, long-term substantial antibiotic use in agriculture leads to more difficult-to-treat animal diseases caused by multiply and highly resistant bacteria [13]. The continuous and large-scale use of antibiotics for agriculture is imprudent for those who support efficacious antibiotics for human health. Ironically, antibiotic use in agriculture ensures the profitability that supports R&D budgets for biopharmaceutical companies to discover new antibiotics. Both sides hold arguable, but incompatible, positions. An inability to find common ground has led to decades of debate and either legislation or no legislation, based on the political influence of either side. In the meantime, antibiotic resistance and the lack of new antibacterials loom as problems for both agriculture and human health.

It is time for a new prescription for antibacterials, one involving antibiotic alternatives. To sustain a healthy agricultural economy and preserve antibiotics for humans, we can no longer ignore any strategy to reduce antibiotic use and prevent the spread of antibiotic resistance (Box 2). The decades-long debates and impasse over agricultural antibiotic use and human health need to give way to a common-ground concurrence to evaluate existing products and discover or develop new products that are alternatives to traditional antibiotics for animal health [11].

New antibacterials are needed for human use. The discovery efforts of research laboratories are aimed at these products through the support of private venture capital. From a discovery perspective, these private laboratories should also recognize the potential profit and societal benefits of antibiotic alternatives for enhancing livestock performance and health, in other words, products fed daily to large numbers of animals over long periods of time. Furthermore, from a development perspective, antibiotic alternatives for livestock health, unlike products for humans, can be tested early and directly in the target animal species.

It is also time for government and public institutes to become more involved in the discovery of antibiotic alternatives. There is a need for nationally coordinated, interdisciplinary, multi-agency (human and animal health) efforts to encourage research on promising antibiotic alternatives. All physical, chemical, immunological, genetic, and biological approaches should be considered, with fast tracking of the most promising. Cooperative research should be encouraged between public institutes and private corporations to confirm, define, and improve the efficacy of existing non-antibiotic health products for animals.

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