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Factors impacting on the problem of antibiotic resistance

Stuart B. Levy*

Center for Adaptation Genetics and Drug Resistance, Tufts University School of Medicine, Boston, MA 02111, USA

Antibiotic resistance has become a major clinical and public health problem within the lifetime of most people living today. Confronted by increasing amounts of antibiotics over the past 60 years, bacteria have responded to the deluge with the propagation of progeny no longer susceptible to them. While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem. Selection of resistant forms can occur during or after antimicrobial treatment; antibiotic residues can be found in the environment for long periods of time after treatment. Besides antibiotics, there is the mounting use of other agents aimed at destroying bacteria, namely the surface antibacterials now available in many household products. These too enter the environment. The stage is thus set for an altered microbial ecology, not only in terms of resistant versus susceptible bacteria, but also in terms of the kinds of microorganisms surviving in the treated environment. We currently face multiresistant infectious disease organisms that are difficult and, sometimes, impossible to treat successfully. In order to curb the resistance problem, we must encourage the return of the susceptible commensal flora. They are our best allies in reversing antibiotic resistance.

Today we can list a number of organisms in both hospitals and the community that thwart treatment because they are resistant to not one, but to many different antibiotics (Table).[1] The term multidrug resistance (MDR), which initially described resistant mammalian tumour cells, and later strains of *Mycobacterium tuberculosis*, now describes multidrug resistance in *any* microorganism—bacterium, fungus or parasite. The emergence of MDR is clearly related to the quantity of antibiotics and how they are being used.[2,3] It may reflect acquisition of different resistance determinants on the same DNA molecule, or single determinants, such as multidrug pumps, that specify efflux activity against different antibacterials.[4] Besides the known pathogens, the relatively recent appearance of opportunistic organisms, intrinsically resistant to many drugs, is now complicating the advances that we have made in medical technologies. With a larger number of immunocompromised patients and longer time periods spent in an immunocompromised state, these organisms have become 'specialized' pathogens—typically attacking only the most vulnerable patients. Among these opportunistic pathogens are the enterococci, the coagulase-negative staphylococci, *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. Those physicians attending medical school 20–30 years ago probably did not even discuss these organisms as important pathogens, though today they cause prominent, even potentially lethal, problems in hospitals worldwide.

Importantly, organisms known since the early days of clinical microbiology are becoming critical health hazards because of the lack of therapeutic options. A good example, reported by the Public Health Laboratory Service in the UK, is multidrug-resistant *Salmonella typhi* with resistance to ciprofloxacin, a drug becoming essential in treating this organism.[5] The frequency of resistance to ciprofloxacin was found to be nearly 35%. Some may remember the problems in Central America when resistance to ampicillin and chloramphenicol in *S. typhi* led to deaths. In these same areas, strains of *S. typhi* now bear resistance to five or six different agents, including fluoroquinolones.

*Tel: +1-617-636-6764 ; Fax: +1-617-636-0458; E-mail: stuart.levy@tufts.edu

Table. Current problems of multidrug-resistant bacteria

Hospital	Community
Gram-negative	
Acinetobacter sp.	*E. coli*
Citrobacter sp.	*Neisseria gonorrhoeae*
Enterobacter sp.	*S. typhi*
Klebsiella sp.	*Salmonella typhimurium*
P. aeruginosa	
Serratia marcescens	
Gram-positive	
Enterococcus sp.: vancomycin-resistant enterococci (VRE)	*Enterococcus* sp.: VRE
coagulase-negative *Staphylococcus*	*Mycobacterium tuberculosis*
MRSA	MRSA
MRSA heterogeneously resistant to vancomycin	*Streptococcus pneumoniae*
	Streptococcus pyogenes

In simplifying the resistance phenomenon, we can focus on two factors: the antibiotic, which, acting as a selective agent, helps to propagate organisms that have the second factor: the resistance gene.[6] If either the antibiotic or the resistance gene were not present, we would not face a resistance problem. I refer to a clinical resistance problem, i.e. a patient with a MDR infection. The finding of resistance to a new agent in an organism is not unexpected, since antibiotics and other related organic molecules are, or resemble, natural products. Over their many millennia of existence, bacteria have continuously confronted organic structures that affect their growth; to survive, bacteria have acquired resistance genes. It is, however, the appearance of these resistance genes in a clinical isolate and in a clinical setting that is a warning to clinicians to control use of the new drug. In this regard, the discovery of resistance in a bacterial strain, for instance a commensal, portends future problems with resistance in clinical pathogens in that hospital or community. Attention should be focused on the use of the drug and the spread of resistance genes.[7]

An important feature contributing to the dissemination of antibiotic resistance is the ability of the resistance genes to move into other bacteria by a variety of genetic means. One transfer mechanism is by plasmids, extrachromosomal elements that can move genes between bacteria of vastly different evolutionary backgrounds, including transfer between Gram-positive and Gram-negative bacteria. There are bacteriophages that can deliver chromosomal- or plasmid-associated resistance genes to a new bacterial host. Finally, naked DNA, released from dead bacteria, can be picked up and incorporated into new strains. The last mechanism, called transformation, is documented in the emergence of resistance among pneumococci and *Haemophilus* spp. Not all organisms have all three mechanisms, but each one helps to amplify the resistance determinant within the microbial world.[2] In the cell, resistance genes can move from one DNA vehicle to another, e.g. from a plasmid to the chromosome, if they are part of a smaller piece of DNA, called a transposon. The microbial environment has carried these various gene distribution systems over evolutionary periods, using them to defend itself against threats to its existence, such as those posed by antibiotics.[2]

In the USA, an estimated 23×10^6 kg of antibiotics are currently used annually; about half are provided to people and the rest are manufactured for agriculture.[8] In hospitals, they are generally administered parenterally, while in the community they are delivered mostly as oral preparations. About 7×10^6 kg of antibiotics, chiefly penicillins and tetracyclines, are used as growth promotants for food animals. Some 45×10^3 kg of antibiotics, namely tetracyclines and streptomycin, are provided as pesticides for agriculture; these are sprayed on to fruit trees in the southern and western USA. While this last amount seems small compared with overall antibiotic use, the geographical spread can be considerable. Some strains of *Erwinia amylovora*, the bacterial target of these drugs, have become resistant to antibiotics. While the emergence of resistant bacteria in agriculture is a small part of the overall global microbial resistance pool, it is an example of widespread antibiotic use in which the environment of microorganisms is besieged with growth-inhibitory agents. The result is the survival of those organisms that bear transposons and other mechanisms for self-preservation, leaving an environment of microorganisms that are largely resistant.

How are antibiotics provided? In some parts of the world, antibiotics are available over the counter in pharmacies, like other commodities. In some countries, vendors sell antibiotics on the street. This means of distribution provides the worst scenario for emergence of resistance: the possibility of too little drug for treatment and provision of drugs when not necessary. Of course, in industrialized

areas like Europe and the USA, a prescription is required. But is that enough to assure prudent use? It appears not to be. When antibiotic resistance began to reach public awareness in the USA (largely because of drug resistance among pneumococci associated with otitis media) the news media took an interest. *Newsweek* featured a cover story in 1994. Fox television sent a 26-year-old assistant producer to four doctors' offices in Denver, Colorado, claiming that she didn't feel well. Four out of four doctors prescribed an antibiotic. The Canadian Broadcasting Company sent a well 'patient' into three doctors' offices in Toronto and obtained the same results, as did another individual from Dan Rather's *48 Hours* team in Park Avenue physicians' offices. I am reminded of a study performed in Iran where a medical student, feigning a cold, entered 40 doctors' offices and recorded physicians' diagnosis and treatment. Thirty-seven of the forty physicians diagnosed a viral infection but still prescribed antibiotics.[9]

A few years ago, the *New Yorker* magazine aptly satirized American attitudes toward antibiotics with a cartoon of a doctor's office sign stating, 'Don't forget to take a handful of our complimentary antibiotics on your way out'.[10]

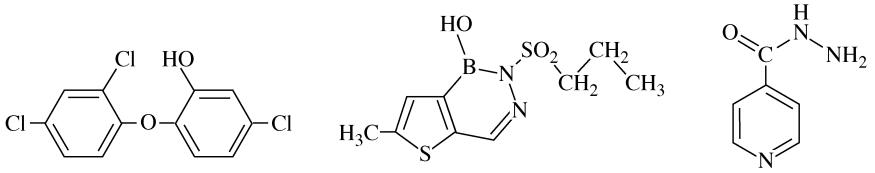
Antibiotics are also fed to animals. In the mid-1970s, we performed a study that involved raising 300 chickens on a small farm outside Boston. We provided 150 newly hatched chicks with oxytetracycline-laced feed and another 150 without.[11] We followed the effect of the antibiotic-laced feed on the animals and people on the farm. As we began the study, the control group had little or no resistant organisms. In the group receiving low levels (200 ppm) of oxytetracycline, tetracycline resistance began to emerge among the faecal *Escherichia coli*. What was surprising was that, within 12 weeks, we detected as much as 70% of all *E. coli* with resistance to more than two antibiotics, including ampicillin, sulphonamides and streptomycin.[11] The resistances were all on transferable plasmids that emerged following use of just tetracycline. This finding is reminiscent of faecal flora data obtained from long-term use of ampicillin to treat female urinary tract infections[12] and changes in skin and faecal flora after prolonged antibiotic treatment of acne.[13,14] All these studies demonstrate that chronic, single drug treatment leads to MDR.

A unique, previously ignored, feature of antibiotics is the effect of individual use on the people sharing that environment; what I call a 'societal effect'.[1] A dermatology group in the UK reported a statistically significant difference in the frequency of drug resistance among the skin flora of people living in the same home as patients taking antibiotics for acne, as compared with gender- and age-controlled groups in homes who were not.[15] There were 1000-fold differences in the frequency of resistance in skin flora to tetracycline and erythromycin, and of MDR.[15] Clearly, there is continual exchange of microbes among people, and an environmental impact from antibiotic usage in the home. This effect is generally linked to extended periods of drug use. It does not usually follow a short, 5–7 day period, but as one uses the antibiotic for longer periods, a broad effect on other bacteria in the environment appears.

There is a further disturbing feature of resistance, besides quantity of antibiotic use and the presence of resistance genes themselves. To counteract the selective forces exerted by antibiotics, it would help the environment if resistant organisms actually destroyed the antibiotic. Unfortunately, there are relatively few mechanisms that inactivate these drugs.[3] These include the β-lactamases, the acetylase for chloramphenicol, the esterases for macrolides and the aminoglyoside-inactivating enzymes. All other mechanisms deal with changing the target of the drug or exporting it out of the cell, leaving the drug active in the environment. Thus, antibiotics are excreted by individuals or by animals into the environment, e.g. on to crops or into municipal waters, where the antibiotic can continue to exert its selective pressure. The result is a 'post-therapy' environmental selection phase of the antibiotic.[16] At that time, the antibiotic is at less than therapeutic concentrations, which is ideal for selecting resistance. In considering this situation, it may not be the treatment period (when there are heavily concentrated amounts of antibiotics) that is responsible for the present resistance emergence. Instead, it may be the post-treatment period when the antibiotic is dispersed in diluted amounts into the environment and has ample time to select resistant organisms.

Antibiotics are only one inhibitory substance against which bacteria must defend themselves. There are other antibiotic-like substances called 'antibacterials' that have appeared in products of diverse forms ranging from floor cleaners to dishwashing detergents, and even chopsticks. The two major types of compound are triclosan and the quaternary ammonium compounds (QACs, e.g. benzalkonium chloride). My laboratory explored whether triclosan had an intracellular target or acted non-specifically like alcohols and peroxides. We isolated *E. coli* mutants resistant to triclosan, with which we identified the genetic target for triclosan, enoyl reductase, the product of the *fabI* gene.[17] Of interest, this protein is also one of the targets for isoniazid, used in the treatment of tuberculosis. Thus, triclosan joins a group of other drugs, including an experimental antibiotic, diazoborine, that has FabI as its target; a mutation in the enzyme leads to resistance to all three drugs (Figure 1).

Another mechanism for triclosan resistance occurs through multidrug efflux pumps, such as AcrAB in *E. coli*[18] and the Mex proteins in *P. aeruginosa*.[19] Do these antibacterial products impact on drug resistance? The potential is certainly there.[20] We examined clinical isolates of *E. coli* and found that overexpression of the AcrAB pump produced not only antibiotic resistance, but also cross-resistance to triclosan.[18] Similarly, efflux pumps in *Pseudomonas* provide resistance to both antibacterials and antibiotics.[19]



Triclosan Diazaborine Isoniazid

Figure 1. The enoyl reductase (product of *fabI* among Gram-positive and Gram-negative bacteria and *inhA* in *Mycobacterium*) involved in fatty acid biosynthesis is the target for a number of structurally unrelated drugs, including triclosan.

Today there are a growing number of antibacterial-containing products in the USA—from 23 in 1993 to over 700 today. In the last decade, a new kind of methicillin-resistant *Staphylococcus aureus* (MRSA), unlike those in hospitals, has emerged in the community. The so-called community-acquired MRSA (cMRSA) have been reported in the USA, Australia and Canada. The unique feature of this organism is that it is resistant principally to the β-lactam antibiotics and not, like hospital strains, to multiple other drugs. This fact, together with some studies from Japan,[21] suggests a link between cMRSA and antibacterials.[20] The Japanese investigators selected MRSA mutants with one-step higher resistance to benzalkonium chloride. The mutants showed much higher levels of resistance to methicillin and other β-lactam antibiotics, but not to other antibiotics. Recently, a group examined the phenotype of *Pseudomonas stutzeri* selected for resistance to the biocide chlorhexidine. As chlorhexidine resistance increased, so did resistance to many antibiotics, including erythromycin and ampicillin, and other antibacterials, namely triclosan and QACs.[22] These two laboratory studies show a link between resistance to antibacterials and resistance to antibiotics.

Antibacterials should be reserved for use to protect vulnerable patients from transmission of disease agents in the hospital and when they are completing recovery at home.[23] But if the home is already exposed casually to these same antibacterial products, (e.g. triclosan, the QACs) what is happening in that environment? If you were a bacterium, you would have a hard time finding a place of refuge because you are being bombarded by antibacterials in addition to antibiotics.

We need to change both our mentality and our course of action. First, we should implement shorter courses of antibiotics. Secondly, we should consider cycling antibiotics when new ones enter the market (or become available). We also need new drugs, including ones with new targets and those that block resistance mechanisms. For instance, work beginning in my laboratory at Tufts University and now continuing at Paratek Pharmaceuticals (Boston, MA, USA) aims to discover new tetracyclines that block or overcome the two tetracycline resistance mechanisms, active efflux and ribosomal protection. The work strives to restore the efficacy of tetracyclines; a renaissance for this family of antibiotics. In this regard, we are producing new tetracycline derivatives that either block resistance mechanisms or are not subject to resistance.[24] In continuing studies at Tufts, we have achieved purification of the Tet efflux protein as a histidine-tagged protein in one step on a nickel column.[25] Recently, we reported the two-dimensional structure of the Tet protein as a trimer in a lipid membrane.[26] This finding confirmed the genetic and biochemical data that the N-terminal domains of two different Tet proteins bind to each other.[27] These findings lead us closer to the goal of a three-dimensional structure of the protein, an achievement that will allow us better to devise antibiotics that are not subject to, or can block, the efflux function.

A non-chemical, non-classical approach to reversing the resistance problem would be the revival of the susceptible strains. By encouraging their regrowth and repopulation in areas where they have been severely reduced, they can replace resistant strains. One action is to re-introduce a susceptible, competitive flora. This approach has been exemplified by the use of probiotics. 'Preempt' is a commercial product consisting of different bacterial strains from adult hens, which, by exclusion colonization, prevents *Salmonella* colonization in chickens.[28] Likewise, the GG lactobacillus fed to adults has shown efficacy in gastrointestinal illnesses without the need for antibiotics.[29] In yet another biological approach, bacteriophages could substitute for antimicrobials in agriculture and, potentially, even in humans.

As we step back to look at the role of antibiotic use in producing resistance, we see many factors including human, antibiotic and bacterial, that encourage a forward impetus towards resistance and block the potential for a reverse reaction back toward susceptibility[30] (Figure 2). Much work is needed on education of the consumer and the prescriber. To try to affect each of these factors can be overwhelming. Jumping from one course of action to another merely magnifies the overwhelming nature of the problem and its solution. But, by focusing on one action at a time, individuals and groups can move towards reversing the resistance phenomenon and ultimately succeed in the full control of resistance. Instead of attacking the whole field of problems, one should adopt the action of cows—grazing—dealing with each patch of the problem consecu-

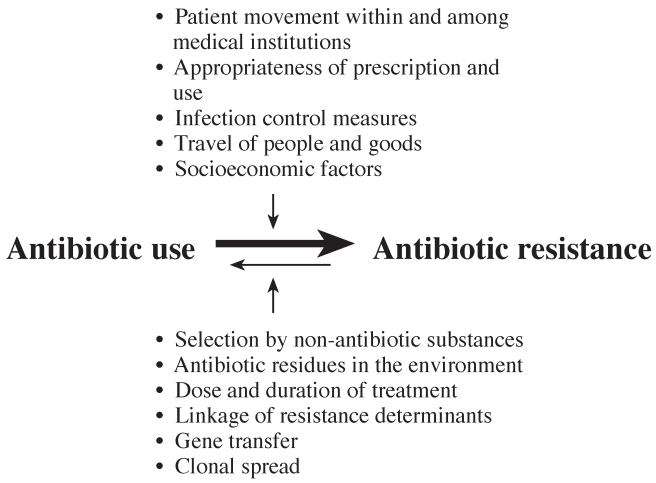


Figure 2. While the major factor driving the emergence of drug resistance is antibiotic use, a number of other factors influence the antibiotic resistance problem, including the spread and fate of bacteria, resistance genes and antibiotics, as well as the behavioural and medical activities of people. Each contributes to producing the problem and to the difficulty in reversing it (adapted from Barbosa & Levy[30]).

tively, clearing up one area before moving on to another (Figure 3). This philosophy is being practised by the Alliance for the Prudent Use of Antibiotics (APUA) (www.apua.org), an international organization established in 1981 that focuses efforts of individuals and its 28 country-based chapters on problems at the local level. APUA has also instituted two global actions to look at the frequency of antibiotic resistance. The first is the ROAR (Reservoirs of Antibacterial Resistance) project, which examines resistance among commensal organisms. This programme aims to encourage identification of commensals (non-pathogenic strains) as initial reservoirs of resistance traits and harbingers of the problem before it appears in clinical strains.

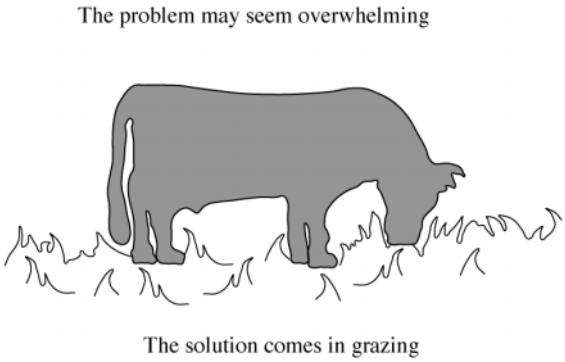


Figure 3. Taking on all the factors (see Figure 2) at one time is overwhelming. It is far better to focus efforts on specific areas within the expertise of the individual or group. We should 'adopt' the action of cows and 'graze', taking on one patch of the problem at a time, as we move together to achieve reversal of the resistance problem.

The second is an international cooperative effort dealing with global surveillance systems of clinical isolates. Called GAARD (Global Advisory Board on Antibiotic Resistance), it includes representatives from three global surveillance systems: SENTRY, the Alexander Project and TSN, with input from the CDC and WHO. It aims to establish an integrated view of antibiotic resistance in clinical strains worldwide. APUA acts as the GAARD coordinator.

We began the antibiotic era with a full-fledged attack on bacteria. It was a battle misconceived and one in which we cannot be the winner. We cannot destroy the microbial world in which we have evolved. The best solution now is to take a broader view of the microbial world. While focusing on the pathogens, our efforts should act in ways that impact fewer commensal flora. We need to forget 'overcome and conquer' and substitute 'peace' when regarding the microbial world. The commensal organisms are, in fact, our allies in reversing the resistance problem. As they rebuild their constituencies, they will control the levels of resistance by out-competing resistant strains. Then, when bacteria or other microbes cause infections, they will be drug susceptible, and we will have the armamentarium to treat them effectively and successfully.

Acknowledgements

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