The impact of antibiotic use on resistance development and persistence

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Abstract The intense use and misuse of antibiotics are undoubtedly the major forces associated with the high numbers of resistant pathogenic and commensal bacteria worldwide. Both the volume and the way antibiotics are applied contributes to the selection of resistant strains. Still, other social, ecological and genetic factors affect a direct relationship between use and frequency of resistance. Resistant bacteria, following their emergence and evolution in the presence of antibiotics, appear to acquire a 'life of their own'. They proliferate and maintain the resistance traits even in the absence of antibiotics, thus jeopardizing the reversal of bacterial resistance by simple reduction in antibiotic use. Reversing resistance requires restoration of the former susceptible flora in people and in the environment.

INTRODUCTION

Following the introduction of penicillin into human therapeutics in the 1940s and throughout the past 60 years, antibiotics have been used and misused. Developed originally to treat human infectious diseases, their properties in veterinary, animal and plant agriculture and aquaculture were applied soon thereafter. Broad use has created a strong selective pressure, which consistently has resulted in the survival and spread of resistant bacteria, providing an excellent example of Darwinian evolution. The emergence of resistance has revealed multiple and complex mechanisms by which resistance genes spread across the bacterial kingdom, with apparent disregard for species barriers. But the bacterial evolutionary response has not been limited to the acquisition of resistance genes. Bacteria have also developed means for stabilizing the resistance phenotype, thus dashing initial hopes of reversing resistance by simply reducing antibiotic use.

Clearly we were unaware of the implications associated with the indiscriminate use of these therapeutic entities and underestimated the genetic flexibility of the microorganisms that were targeted. Presently we face a global public health crisis, as infectious diseases top the list for causes of death worldwide (www.who.ch/whr/1998/reports.html). While it is likely that antibiotic resistance contributes significantly to this problem, data on consumption and resistance to antibiotics are limited for most countries[1,2] and the relationship of resistance to morbidity and mortality is quantitatively unclear.

SELECTION OF RESISTANCE: THE FORWARD REACTION

Resistance determinants were present prior to the introduction of antibiotics, but were mostly found in natural antibiotic-producing microorganisms.[3] Other neighboring species possibly had already acquired those genes or developed new mechanisms to protect themselves from the inhibitory effects of the antibiotics to which they were exposed. Consistent with this theory is the homology between resistance determinants (e.g. VanA and VanB[4]) found in antibiotic-producing bacteria and in unrelated bacteria. Moreover, a collection of bacteria isolated prior to the therapeutic use of antibiotics showed little if any resistance, although many had conjugable plasmids without resistance determinants.[5]

Bacterial resistance has evolved with the increased number, volume and diversity of antimicrobial applications. As new drugs were introduced clinically, resistant strains were identified relatively soon after. Many of these resistant bacteria are not obligate pathogens, being part of the indigenous microflora. However given the right associations, such as immunocompromised patients and the use of antibiotics, these organisms have the potential to cause life-threatening disease.[6]

A direct relationship between the quantity of antibiotic used and the development of resistance has not been easy to determine. Data on total antibiotic utilization in particular areas are unfortunately limited, unreliable, or many times non-existent. An attempt to estimate worldwide antibiotic usage taking antibiotic availability as an indicator of human use has been reported.[1] Antibiotic availability varied considerably in different countries, but differences were also present within a country and throughout time. The authors state that while many countries do not have data on the antibiotic availability, those that have them use different systems of data collection, making comparisons difficult or impossible to establish. The situation gets even harder to analyze in many developing countries where antibiotics are available without prescription. Unfortunately, with the exception of a few attempts at quantification,[7] data in this area are still limited 13 years since this report. More importantly, data are presented for whole cities or countries, while resistance reflects local practices.[3,8] Therefore, what clearly matters is the antibiotic consumption in designated areas, whether home, hospital or community as these data will more closely reflect the incidence and patterns of resistance observed in those smaller environments. For example, Ridley et al.[9] described that, although chloramphenicol was infrequently used in a hospital, it was routinely prescribed in a particular ward where more than half of the chloramphenicol-resistant hospital staphylococcal isolates originated.

In 1994 a 'threshold' hypothesis proposed that resistance could be curtailed if total antibiotic use in a particular environment stayed below a critical quantitative level.[10] The proposal was founded on the natural competition among bacteria and the potential for the return of susceptible flora after antibiotic treatment – a possibility that decreased as antibiotic consumption in a particular environment increased. Definition of the threshold values for different antibiotics would be important in controlling bacterial

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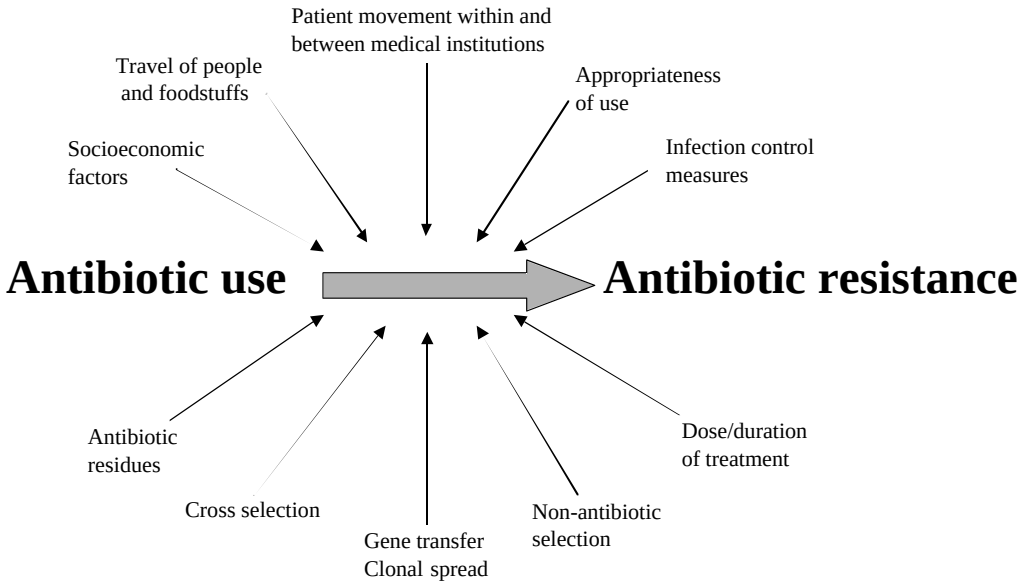

Fig. 1 **Relationship between antibiotic use and development of resistance. Antibiotic use is the main factor in the forward process, i.e. selection of resistance, but other factors can influence that relationship. Factors dependent on humans, and their management of antibiotics, are represented above the horizontal arrow, while factors related to the antibiotic itself and the genetic basis of resistance are represented below the horizontal arrow.**

resistance. In a further elaboration of this idea, it was suggested that resistance followed a 'selection density'. Here again, ecology was the basis of the suggestion, i.e. the more antibiotic used for individual persons, animals or plants in a particular geographic unit, the fewer susceptible bacteria would survive to repopulate.[8]

Using population genetics theory, another group analyzed the relationship between the amount of antimicrobials used in the community and the frequency of resistance. They described a 'sigmoidal rise in resistance over time in the presence of a constant rate of antibiotic consumption' and a threshold level of antibiotic usage needed to 'trigger the emergence of resistance to significant levels'.[11] The study provided mathematical modeling to known data which further supported the threshold concept.

Both the amount of antibiotics used and *how* they are used contribute to the development of resistance. The use of broad-spectrum antibiotics rather than narrow-spectrum drugs is known to favor the emergence of resistance by broadly eliminating competing susceptible flora. For example, the empiric use of amoxicillin-cefotaxime combination for suspected neonatal sepsis in a neonatal ICU was associated with the emergence of resistant gram-negative bacilli.[12] The risk of colonization with bacteria resistant to the empirical treatment was 18 times higher for the broad-spectrum therapy than for an alternative regimen of narrower-spectrum antibiotics.[12] Some antibiotics cause unpredictable ecological consequences because strains bear intrinsic resistance to them. For example, cephalosporins select for enterococci, and broad-spectrum antibiotics select for drug-resistant *Acinetobacter* and *Xanthomonas*.

Antibiotics are frequently prescribed in the treatment of viral infections or at wrong doses for incorrect periods of time. Guillemot et al.[13] described how antibiotic prescription practices can be more closely related to the emergence of resistance than the volume used. They demonstrated an association between the use of long-term, less-than-recommended daily doses of oral β-lactams with increased risk for pharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* as compared to shorter courses with higher doses of the drug.

Other factors, difficult to quantify, impact the relationship between use and resistance (Fig. 1). Education, poverty, hygiene, and communal facilities (child care centers and nursing homes), affect the adequacy of treatment provided, the compliance of the patients, and the development and spread of resistance. A significant relationship between long-term, full-time day-care attendance of young children (<3 years) and the carriage of respiratory pathogens has been described.[14]

The trade of foods and goods and the movement of people encourage the establishment and dissemination of resistant bacteria, making it difficult to determine direct correlations between the use of antibiotics locally and the emergence of resistance. Penicillin-resistant *S. pneumoniae* serotype 23F, originating in Spain, has been isolated in North and South America, Asia, and Europe.[15]

Genetics of bacterial resistance

When addressing the impact of antibiotic use on the development of resistance, we need to understand not only the selection process, but also the complex evolution and ecology of the resistance determinants involved. Over time, in response to antibiotics, bacteria have evolved and optimized their genetic arsenal to deal with the action of antibiotics. Resistance mechanisms include inactivation of the compound by detoxifying enzymes (e.g. TEM β-lactamases, chloramphenicol acetyltransferase), reduced cell permeability or expulsion of the drug by specific or non-specific pumps

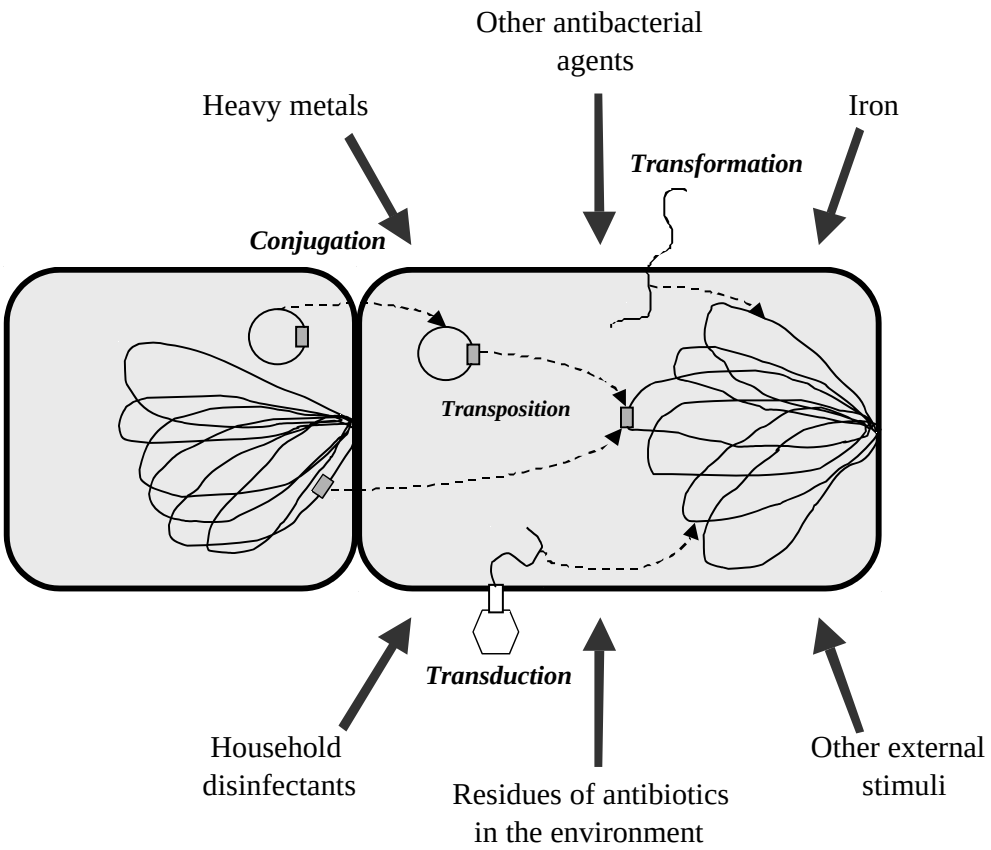

Fig. 2 **Acquisition, transfer and stability of antibiotic resistance. Bacteria can acquire exogenous DNA bearing resistance genes via transduction mediated by bacteriophage, transformation of free DNA and conjugation of plasmids and conjugative transposons (small grey boxes). Transposons can move within the cell by transposition from one DNA molecule to another. Following selection many factors can contribute to the maintenance of the resistance developed. While some are represented in this figure, others, such as the development of compensatory mutations, can also be involved in the stabilization of resistance traits.**

(e.g. Tet, AcrAB/TolC), and modification of the antibiotic targets (e.g. altered penicillin-binding proteins). These mechanisms can be constitutive or inducible by the antibiotic itself or by environmental signals. Bacteria can also acquire a resistance phenotype via global or specific transcriptional regulators.[16]

Bacterial resistance to an antibiotic may be mediated by multiple mechanisms and/or resistance determinants in the same bacterial cell. For example, Tet K and Tet L determinants have been described in single gram-positive isolates with Tet M and/or Tet O, which encode a different mechanism of tetracycline resistance.[17]

Bacteria can achieve resistance by many routes.[16] These include intrinsic resistance to particular antibiotics, such as anaerobes for aminoglycosides; mutations in the chromosomal genes encoding drug targets, such as *gyrAB/parCE* and *rpoB* mutations causing resistance to fluoroquinolones and rifampicin respectively; and activation of intrinsic low-level resistance loci, such as the broad-spectrum *mar* locus[16] and the β-lactamase *ampC* gene.

Different mechanisms of gene transfer can mediate acquisition of resistance determinants[3,16] (Fig. 2). Transduction transfer is mediated by bacteriophages, while transformation involves the uptake of naked DNA. The latter has proven to be important in the evolution of *S. pneumoniae* β-lactam resistance via mosaic penicillin-binding-protein gene structures. Conjugation involves direct cell-to-cell contact for transfer of extra-chromosomal or chromosomal DNA and is believed to be the most important transfer mechanism in the acquisition and spread of antibiotic resistance.

Conjugative transposons, frequently located on the chromosome of a number of bacterial species (e.g. Tn*916*-like structures in *Streptococcus spp.*) also play an important role in broad-host-range gene transfer. In addition to promoting their own transfer, self-mobile plasmids and conjugative transposons are able to mobilize other genetic elements, which may also carry resistance determinants. Bacteria have other mobile or mobilizable elements, e.g. classic transposons, insertion sequences and integrons bearing gene cassettes, which help spread antibiotic resistance.[16,18] Additionally, deleted, associated and rearranged versions of these elements are frequently found, which may reflect a cellular evolution and adaptation to the external stress.

Locales of antibiotic use

Human therapy accounts for approximately half of the total consumption of antibiotics in the European Union and the USA.[19,20] Until 10–20 years ago, resistance was concentrated chiefly in the hospitals due to the intensive use of antibiotics there compared to the community. This difference is less sharp today, as the numbers of treated patients in community settings have increased and resistance has become widespread among community-acquired pathogens and commensal bacteria.[3,6,20]

The emergence of resistance in nosocomial pathogens has been shown to be associated with antibiotic misuse (overuse plus inappropriate use) in therapy and prophylaxis.[21] Similar associations can be found in the community.[22] In Finland, a clear relationship was established between the increased consumption of erythromycin and the increase in erythromycin resistance among group A streptococcal isolates in the early 1990s.[23] In Iceland, a strong association between antimicrobial use in the community and nasopharyngeal carriage of penicillin-resistant pneumococci in children was described.[24] In The Netherlands, increased resistance to norfloxacin in *E. coli* correlated with increased prescription of fluoroquinolones for urinary tract infections.[25] Importantly, Calva et al.[26] showed a high frequency of resistance to antimicrobial agents in fecal isolates from healthy Mexican children and similar observations have been reported in other countries.[27] These findings provide strong evidence that the community environment is being overwhelmed by antibiotics and that commensal bacteria have become reservoirs of antibiotic resistance determinants.

Current major problems of antibiotic resistance in the community are methicillin-resistant *S. aureus* (MRSA), penicillin-resistant *S. pneumoniae* (PRSP), multidrug-resistant *M. tuberculosis* and vancomycin-resistant enterococci (VRE), although they do not have an equal incidence across the globe. While *M. tuberculosis* is a major problem in the USA and developing countries, PRSP is a major problem in Europe, especially in Spain and France, countries renown for their intensive use of antibiotics, including penicillin. In the UK and Germany, the low incidence of resistance among pneumococci seems to reflect decreased utilization of antibiotics and close adherence to recommendations on their use.[28]

While human consumption of antibiotics bears primary responsibility for the development of resistance in human pathogens, one can no longer dismiss the contribution of antibiotic use in animals and plant agriculture to the development of resistance. Nevertheless, this subject remains controversial after almost three decades of intense debate. Although a number of studies have correlated the animal use of antibiotics with subsequent development of resistance in animal isolates,[29] the quantitation of the risk of this use to resistant isolates in humans has not been established.

Antibiotics are used in animals for therapy, prophylaxis and growth promotion. According to FEDESA,[19] 48% of the total antibiotic consumption in the EU and Switzerland in 1997 was for animals: 33% in veterinary therapy and 15% as feed additives for growth promotion. In the USA, nearly half of the total consumption of antibiotics is in agriculture, mainly for animals, where ~80% is used in prophylaxis/growth promotion.[20]

In 1998 the EU banned the growth-promotion use of those antibiotics which are currently designated for human therapy, or that are known to select for cross-resistance to antibiotics presently used in human medicine. However, clinically important drugs such as tetracyclines, penicillins, macrolides, aminoglycosides and fluoroquinolones are part of the drug arsenal used in veterinary therapy.[19]

In the USA, similar legislation has not been proposed and antibiotics allowed as growth promoters, such as penicillins and tetracyclines, are also used in human therapy. Many argue that resistance to some of these antibiotics, such as tetracyclines, is so widespread that their use as feed additives will not compromise their limited use in human therapy. However, this argument disregards co-selection of resistance, as tetracycline resistance is frequently associated with determinants of resistance to other antibiotics on the same genetic elements. One particularly disturbing aspect of growth promotion is the delivery of large quantities of antibiotics at sub-therapeutic concentrations in food and water for long periods of time. This practice creates special conditions for selection, spread and evolution of resistant strains and establishment of stable resistance traits.[3,30]

Resistant animal *Salmonella*, *Campylobacter* and *E. coli* isolates can be transferred to people through the food chain with subsequent colonization and proliferation, and development of difficult-to-treat or even untreatable disease.[29] Multidrug-resistant *S. enterica* serotype *typhimurium* DT104 is becoming endemic in several countries among humans and animals, and is also frequently isolated from foodstuffs.[29,31] A significant increase in fluoroquinolone resistance among animal and human *Campylobacter* spp. was observed after veterinary application of this drug.[32] Between 1982 and 1989, resistance in *Campylobacter* isolates increased from 0–14% in poultry products and 0–11% in humans.[32] A study performed during 1997–1998 in Spain showed a very high level of ciprofloxacin resistance among *Campylobacter* strains: 99% in broilers and pigs and ~72% in foods and humans.[33]

Unfortunately much of the research involves pathogenic bacteria and is, in most cases, restricted to facultative bacteria. Very little is known about the impact of the use of antibiotics on the commensal obligate anaerobes, which are the predominant bacteria among the gut microflora of humans and most other animals, and can therefore constitute large pools of resistance determinants potentially transferable to human pathogens. A striking example is the more than 99.9% nucleotide sequence homology between the tetracycline resistance gene *tet*(W), of the rumen anaerobe *Butyrivibrio fibrisolvens* and the *tet*(W) of the human gut anaerobes *Fusobacterium prausnitzii* and *Bifidobacterium longum*.[34] These observations provide strong evidence for the occurrence in nature of recent horizontal gene transfer events between obligately anaerobic bacterial populations from animal and humans.

The emergence of VRE provides an illustrative example of how antibiotic use in the hospitals and community affects the resistance levels in both environments and how antibiotic resistance in a particular host may impact resistance in isolates from a different host. In the last 15 years we have been witnessing a steady and worrying increase of VRE among clinical and commensal isolates. In the USA, a high

frequency of VRE is found among hospital isolates with little evidence for their presence in animal and environmental sources.[35] On the other hand, in Europe, VRE is rarely found in clinical hospital isolates, but is widespread among farm and pet animals, foodstuffs, and other environmental sources.[36–38] These are thought to be major reservoirs for the large numbers of VRE found in fecal flora of healthy subjects in European countries.

In several studies an association between the use of the glycopeptide avoparcin, the incidence of VRE, and the role of the food chain in the spread of resistance is proposed.[38,39] Additionally, evidence for horizontal transfer of *vanA* elements between animals and human enterococcal isolates has been described.[40] In contrast, VRE have not been detected in the food chain, or the fecal flora of healthy people in Sweden, where the use of avoparcin as a growth promoter was never approved.[29]

The differences in VRE incidence found in both geographical areas appear to be associated with different glycopeptide applications in the two continents: the overuse of vancomycin in USA hospitals and the European use of avoparcin for growth promotion, which causes cross-resistance to vancomycin.

Other examples demonstrate the development of reservoirs of resistance genes to human clinically important drugs due to the use of analogues in animal veterinary or growth promotion. For example the use in the past of the streptogramin Virginiamycin as a growth promoter meant that before the introduction of the streptogramin combination *Synercid* into human therapy, resistance mechanisms had already been developed.[41]

Antibiotics have also been extensively used in the treatment and prevention of bacterial diseases in crops, fruit trees and ornamental plants.[42] In the USA, the volume of antibiotics used in plant disease control is less than 0.1% of the total usage. The risk of the use of antibiotics in plant agriculture is closely linked with the methods of application, e.g. spraying of large areas, which contaminates the surrounding environment and leads to accumulation of considerable levels of residues. Streptomycin and oxytetracycline are used in the treatment and prevention of several bacterial infections in plants and fruit trees in the USA.[42] As occurred with human and animal bacteria resistance has developed among plant pathogens,[3] e.g. streptomycin resistance in *Erwinia amylovora* and other plant pathogens. Even if we do not face the risk of zoonoses from plant pathogens, the possibility of gene transfer from these bacteria to animal and human flora certainly exists.

'Post-utilization' effects of antibiotics

While the flow of resistant bacteria and resistance genes in nature has been described, little or nothing is known about the flow of antibiotics. What is the impact of antibiotic residues left in the environment following treatment, such as antibiotics excreted in the feces of animals and humans, or agricultural utilization? What plays the major role in the development of resistance: the antibiotic during the course of utilization or its continued presence in the environment after use? Moreover, although bacteria have multiple ways of dealing with antimicrobials, most do not involve structural inactivation of the drug (SB Levy, Infect Dis Clin Practice, in press). Consequently, active antibiotics accumulate and appear to be relatively stable in the environment where their selective effects are perpetuated. Some antibiotics are quite stable in liquid media (such as milk) and some are tolerant to heat treatment. For example, neomycin residues in eggs were considerably tolerant to the cooking procedures normally used.[43] Tetracyclines in human and rodent feces were still active after several months of storage at room temperature.[44]

Antibiotic residues excreted in the feces in one particular area can impact on geographically distant ecosystems, as they can easily be dispersed in soil and waters via manure, sewage, etc. Importantly, antibiotics have already been found in natural waters.[45] In entering the environment, antibiotics will be diluted compared to their therapeutic concentrations. It is widely recognized that sub-therapeutic concentrations over long periods of time are ideal conditions for selection of resistance. This post-usage presence may contribute to the level of resistance found in the community and environmental pathogens and commensals, although the quantification of that contribution has been overlooked. The continuous exposure of bacteria to antibiotics accumulated in the environment will likely translate into a considerable decrease in the susceptible organisms and the selection and stabilization of the resistant ones.

Antibiotic use can cause considerable alterations in the gut and skin ecology of human and animals. The fluoroquinolone ciprofloxacin excreted in sweat was able to select for intermediate-level and high-level resistant *S. epidermidis* in the axilla and nostrils of volunteers.[46] One can only expect that continuous exposure to antibiotic residues will cause similar changes in the different ecosystems.

REVERSAL OF RESISTANCE: THE BACKWARD REACTION

Successful decrease in antibiotic resistance following implementation of antibiotic reduction policies in local environments, such as hospitals, has been described[47] (Table 1), but examples are limited. Antibiotic control measures not only deal with specific problems of antibiotic resistance but also help to control nosocomial infections resulting from antibiotic use. The extensive use of broad-spectrum antibiotics (e.g. cephalosporins), especially in ICUs, is linked to outbreaks caused by resistant nosocomial organisms, such as *C. difficile* and *Acinetobacter sp*.[51] Those outbreaks were controlled by introducing changes in antibiotic use, namely switching to narrow-spectrum antibiotics and/or reducing the use of broad-spectrum antibiotics.[51,53]

The incidence of resistance in *S. aureus* hospital isolates was affected by different antibiotic policies, e.g. restriction in the use of erythromycin resulted in significant decreases in erythromycin resistance among *S. aureus* isolates.[9] A multidrug resistant *Klebsiella aerogenes* outbreak in a neurosurgical ICU was only controlled when the use of all antibiotics was suspended.[49] An impressive decrease in antibiotic consumption, paralleled by a significant reduction in bacterial resistance, followed implementation of an antibiotic restriction policy program in a Greek hospital.[50]

Table 1 **Illustrative examples of decrease in antibiotic resistance frequency following implementation of antibiotic control measures**

Location	Organism	Reference
Hospitals		
London, UK	Staphylococci	Barber et al., 1960[48]
Glasgow, UK	*Klebsiella aerogenes*	Price and Sleigh, 1970[49]
London, UK	*Staphylococcus aureus*	Ridley et al., 1970[9]
Athens, Greece	Multiple organisms	Giamarellou and Antoniadou, 1997[50]
Texas, USA	Multiple organisms	White et al., 1997[51]
Virginia, USA	*Clostridium difficile*	Climo et al., 1998[52]
Indiana, USA	Multiple organisms	Smith DW, 1999[53]
Community*		
Japan	Group A streptococci	Fujita et al., 1994[54]
Iceland	Penicillin-resistant pneumococci	Kristinsson et al., 1995[55]
Finland	Group A streptococci	Seppälä et al., 1997[56]
Germany	Vancomycin-resistant enterococci	Klare et al., 1999[57]
The Netherlands	Vancomycin-resistant enterococci	van den Bogaard et al., 2000[58]

*Humans and animals.

In Texas, following an outbreak of multidrug resistant *Acinetobacter* infection in the surgical ICU, a control program requiring pre-approval for selected parenteral antimicrobial agents resulted in an increase in susceptibility to all β-lactams and quinolones, especially in those bacteria isolated in ICUs.[51] The changes were observed not only in *Acinetobacter* but also among the *Enterobacteriaceae*.

An association between a *C. difficile* diarrheal outbreak and the use of clindamycin was reported.[52] Control measures which reduced use of clindamycin resulted in a decrease in *C. difficile*-associated diarrhea and a decrease in clindamycin resistance among *C. difficile* isolates (from 91% 6 months before restriction to 39% 20 months after restriction). A significant reduction in the incidence of resistant gram-negative bacteria, VRE and MRSA in a hospital was reported following a decrease in cephalosporin utilization.[53]

Similar successes have also been described in the community (Table 1). In Japan a significant decrease in erythromycin resistance in group A streptococcal isolates was observed (from 22.2% in 1981 to nearly 0 in 1990), following a decrease in macrolide consumption.[54] In Finland, in response to the considerable increase in erythromycin resistance among group A streptococcal isolates (from ~5% to 16.5% between 1988 and 1992), a number of recommendations in the community were implemented.[56] These resulted in a considerable decrease in the use of macrolides and a statistically significant decrease (to 8.6% in 1996) in the incidence of erythromycin resistance among group A streptococcal isolates from throat-swab and pus samples.

Recent studies performed in several European countries following the ban in the use of avoparcin report an encouraging and sometimes dramatic decrease in the frequency of VRE not only in animals and food products of animal origin, but also in the intestinal flora of healthy subjects.[57,58]

Although a substantial drop in the frequencies of resistance may indeed be observed, the values do not retreat to those observed before the use of antibiotics.[3] This means that resistance will probably increase very quickly if the same drug or analogues are reintroduced to therapy. Moreover the fall in resistance rates does not mirror the rise;[11] i.e. the backward reaction is much slower than the forward one. This point is well demonstrated in an individual whose rise in resistant flora following tetracycline use was rapid (48 h), but whose return to normal susceptible flora was slow (10–14 days).[8]

The cause of these successful decreases is not totally clear since many of these studies do not consider other factors which may contribute to the decrease in resistance, e.g. infection control measures. Furthermore, the decrease in the use of one antibiotic is normally accompanied by an increase in another, but rarely are the resistance levels of the alternative antibiotic reported. Of even greater impact to the rate of the backward reaction are factors which influence the persistence of resistance in the absence of the drug.

Stability of resistance

The idea that resistance genes and/or resistance elements would be an unnecessary burden to the bacterial cell in antibiotic-free environments led to the belief that elimination of the selection pressure would encourage the return of susceptible strains. This has not stood the test of time. A persistent and high frequency of resistance can be found in commensal bacteria of people and animals without a recent history of antibiotic intake.[26,59] The relative stability of resistance in the absence of selection pressure underscores the problem of trying to correlate resistance frequency with antibiotic use at any one time or place.

We cannot eliminate contamination with resistant bacteria or antibiotic residues from other environments or sources as possible causes. As described earlier, resistant bacteria are routinely isolated from foodstuffs. Corpet et al.[60] have shown that after feeding volunteers a near sterile diet, the numbers of resistant bacilli in their fecal flora decreased almost 1000-fold. Resistance traits among lactic acid bacteria isolated from several food products have been frequently described.[61] The innocuous bacteria present in food, although transient in normal circumstances, may colonize the gut of people, especially when the indigenous flora has been wiped out, e.g. by antibiotics.

Many factors could contribute to the stability of resistance in the absence of antimicrobial use (Fig. 2). Bouma and Lenski[30] describe the co-evolution of host and plasmid over time in the presence of the drug, that results in a symbiotic relationship being established between the cell, resistance determinant and the DNA carrier. The development of second-site mutations often compensates for the cost of the original mutation or the presence of new resistance genes, and possibly the associated genetic element.[62–65]

Hypothetically, bacteria can also adapt to new determinants, for example, to membrane-embedded antibiotic efflux pumps, by rearranging membrane structures in such a way that they will be compatible and interact with the new

acquisition. Or a number of other genes may need to change in order to effect full expression of a new gene, e.g. *mecA* in Staphylococci.[66] Alternatively, genes and mechanisms involved in antibiotic resistance may perform additional physiological functions in the cell that are not related to antibiotic resistance, but which nevertheless confer advantages to the cell even when antibiotics are not present, e.g. the tetracycline efflux pump TetA(L) has been shown to perform multiple functions including tetracycline, Na^+, and alkali resistance and K^+ acquisition.[67]

Selection and spread of resistance of a certain antibiotic by other antibiotics, i.e. the co-selection process, can also account for the maintenance of resistance to a particular antibiotic, even if its use has been discontinued. Multiple resistance genes are frequently found on the same plasmid or transposon and therefore the use of any of the antibiotics would select for resistance to all the others.

Resistance can also be maintained by non-antibiotic selective pressures, but the extent to which these environmental factors impact resistance is not fully known. A multitude of transposons and plasmids code not only for resistance to multiple antibiotics, but also for genes mediating heavy metal tolerance, virulence and metabolic functions. For example Tn*1691* specifies resistance to gentamicin, streptomycin, sulfonamides, chloramphenicol and mercury. A number of studies have shown a strong association between resistance to Hg and resistance to multiple antibiotics.[68]

Compounds such as household disinfectants and other antibacterial agents can also select for antibiotic resistance. Triclosan and pine oil, which are widely used in home cleaning products are able to select for multidrug-resistant mutants, either by mutation in the target genes[69] or in the regulatory *mar* system, providing a pleiotropic resistance to disinfectants, multiple structurally unrelated antibiotics, organic solvents and oxidative stress agents.[70] Constitutive expression of an MDR efflux pump which confers resistance to triclosan is also reported in *P. aeruginosa*.[71] Given the increased use of these agents in households, one can imagine dramatic changes in the environmental flora that impact antibiotic resistance.

Multidrug resistance transporters such as AcrAB and LmrA, with a broad range of substrate specificities (e.g. antibiotics, amino acids and sugars) can also impact stability of resistance traits. Some of these pumps, and other regulatory resistance mechanisms, can be regulated by multiple environmental stress conditions and compounds besides antibiotics,[72] for example iron starvation and presence of bile salts. Additionally resistance can be activated by multiple transcriptional regulators, some of which are primarily engaged in response to stresses other than antibiotics, e.g. SoxS, the activator of the oxidative stress response system, also activates *mar*. Obviously the larger the number of signals that can maintain a phenotype, the more difficult will be loss of that phenotype, especially when the energy cost is reduced by a mechanism which is regulated rather than constitutive.

Within this framework it is not difficult to understand that reduction or elimination of a particular antibiotic may not directly eliminate resistance. Strategies like those described in Finland are nevertheless important in prolonging the efficacy of the present antimicrobial repertoire until new antibiotics are developed. Development of vaccines and phage therapy may constitute important alternatives in the future.

While antibiotics appear to be the central element in selecting resistant bacteria, they are not wholly responsible for the persistence and spread of them once selected. The selection event (forward reaction) is clearly the most important one, which fosters resistant bacteria of different genotypes and phenotypes in the environment. But once selected, the resistant strains can 'take on a life of their own' in response to other selection and maintenance factors in the absence of antibiotics. This makes the reversal of resistance (backward reaction) more difficult to achieve.

There is a need to create more ingenious ways of re-establishing the susceptible flora. This goal may theoretically be accomplished by using bacteria themselves in the form of probiotic formulations. However the potential presence of antibiotic resistance genes in probiotic organisms also needs to be evaluated. Understanding the impact of antibiotics on the evolutionary and ecological flexibility and versatility of the bacterial world is essential for strategically responding to the global problem of antibiotic resistance.

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References

1. Col NF, O'Connor RW. Estimating worldwide current antibiotic usage: report of Task Force 1. Rev Infect Dis 1987; 9: S232–243.
2. Report of the ASM task force on antibiotic resistance. Antimicrob Agents Chemother 1995; Suppl: 1–23.
3. Levy SB. The antibiotic paradox. New York: Plenum Press, 1992.
4. Marshall CG, Broadhead G, Leskiw BK, Wright GD. D-Ala-D- Ala ligases from glycopeptide antibiotic-producing organisms are highly homologous to the enterococcal vancomycin-resistance ligases VanA and VanB. Proc Natl Acad Sci USA 1997; 94: 6480–6483.
5. Hughes VM, Datta N. Conjugative plasmids in bacteria of the 'pre-antibiotic' era. Nature 1983; 302: 725–726.

6. Levy SB. The challenge of antibiotic resistance. Sci Am 1998; 278: 46–53.
7. Carrie AG, Zhanel GG. Antibacterial use in community practice: assessing quantity, indications and appropriateness, and relationship to the development of antibacterial resistance. Drugs 1999; 57: 871–881.
8. Levy SB. Antibiotic resistance: an ecological imbalance. Ciba Found Symp 1997; 207: 1–9; discussion 9–14.
9. Ridley M, Lynn R, Barrie D, Stead KC. Antibiotic-resistant *Staphylococcus aureus* and hospital antibiotic policies. Lancet 1970; 1: 230–233.
10. Levy SB. Balancing the drug-resistance equation. Trends Microbiol 1994; 2: 341–342.
11. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci USA 1999; 96: 1152–1156.
12. de Man P, Verhoeven BA, Verbrugh HA, Vos MC, van den Anker JN. An antibiotic policy to prevent emergence of resistant bacilli. Lancet 2000; 355: 973–978.
13. Guillemot D, Carbon C, Balkau B, et al. Low dosage and long treatment duration of β-lactam: risk factors for carriage of penicillin-resistant *Streptococcus pneumoniae*. JAMA 1998; 279: 365–370.
14. Principi N, Marchisio P, Schito GC, Mannelli S. Risk factors for carriage of respiratory pathogens in the nasopharynx of healthy children. Ascanius Project Collaborative Group. Pediatr Infect Dis J 1999; 18: 517–523.
15. McGee L. Emergence and spread of antibiotic-resistant *S. pneumoniae*. Southern African J Epidemiol Infect 1999; 14: 117–120.
16. Alekshun MN, Levy SB. Bacterial drug resistance: Response to survival threats. In: Storz G, Hengge-Aronis R, eds. Bacterial Stress Responses. Washington: ASM Press, 2000: 323–366.
17. Roberts MC. Genetic mobility and distribution of tetracycline resistance determinants. Ciba Found Symp 1997; 207: 206–218; discussion 219–222.
18. Hall RM, Collis CM. Antibiotic resistance in gram-negative bacteria: the role of gene cassetes and integrons. Drug Resist Updates 1998; 1:109–119.
19. FEDESA. Press release. September 6 1998 (www.fedesa.be/eng/PublicSite/PressRelease).
20. Harrison PF, Lederberg J. eds. Antimicrobial resistance: Issues and options (workshop report). Washington: National Academy Press, 1998.
21. McGowan JE, Jr. Antimicrobial resistance in hospital organisms and its relation to antibiotic use. Rev Infect Dis 1983; 5: 1033–1048.
22. Baquero F. Antibiotic resistance in Spain: What can be done? Task Force of the General Direction for Health Planning of the Spanish Ministry of Health. Clin Infect Dis 1996; 23: 819–823.
23. Seppälä H, Klaukka T, Lehtonen R, Nenonen E, Huovinen P. Outpatient use of erythromycin: link to increased erythromycin resistance in group A streptococci. Clin Infect Dis 1995; 21: 1378–1385.
24. Arason VA, Kristinsson KG, Sigurdsson JA, Stefansdottir G, Molstad S, Gudmundsson S. Do antimicrobials increase the carriage rate of penicillin resistant pneumococci in children? Cross sectional prevalence study. Brit Med J 1996; 313: 387–391.
25. Goettsch W, van Pelt W, Nagelkerke N, et al. Increasing resistance to fluroquinolones in *Escherichia coli* from urinary tract infections in The Netherlands. J Antimicrob Chemother 2000; 46: 223–228.
26. Calva JJ, Sifuentes-Osornio J, Cerón C. Antimicrobial resistance in fecal flora: longitudinal community-based surveillance of children from urban Mexico. Antimicrob Agents Chemother 1996; 40: 1699–1702.
27. Levy SB, Marshall B, Schluederberg S, Rowse D, Davis J. High frequency of antimicrobial resistance in human fecal flora. Antimicrob Agents Chemother 1988; 32: 1801–1806.
28. Pradier C, Dunais B, Carsenti-Etesse H, Dellamonica P. Pneumococcal resistance patterns in Europe. Eur J Clin Microbiol Infect Dis 1997; 16: 644–647.
29. van den Bogaard AE, Stobberingh EE. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs 1999; 58: 589–607.
30. Bouma JE, Lenski RE. Evolution of a bacteria/plasmid association. Nature 1988; 335: 351–352.
31. Threlfall EJ, Frost JA, Ward LR, Rowe B. Epidemic in cattle and humans of *Salmonella typhimurium* DT104 with chromosomally integrated multiple drug resistance. Vet Rec 1994; 134: 577.
32. Endtz HP, Ruijs GJ, van Klingeren B, Jansen WH, van der Reyden T, Mouton RP. Quinolone resistance in campylobacter isolated from man and poultry following the introduction of fluoroquinolones in veterinary medicine. J Antimicrob Chemother 1991; 27: 199–208.
33. Sáenz Y, Zarazaga M, Lantero M, Gastañares MJ, Baquero F, Torres C. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and humans in Spain in 1997–1998. Antimicrob Agents Chemother 2000; 44: 267–271.
34. Scott KP, Melville CM, Barbosa TM, Flint HJ. Occurrence of the new tetracycline resistance gene *tet*(W) in bacteria from the human gut. Antimicrob Agents Chemother 2000; 44: 775–777.
35. Coque TM, Tomayko JF, Ricke SC, Okhyusen PC, Murray BE. Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. Antimicrob Agents Chemother 1996; 40: 2605–2609.
36. Torres C, Reguera JA, Sanmartin MJ, Pérez-Díaz JC, Baquero F. *vanA*-mediated vancomycin-resistant *Enterococcus* spp. in sewage. J Antimicrob Chemother 1994; 33: 553–561.
37. Bates J, Jordens JZ, Griffiths DT. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J Antimicrob Chemother 1994; 34: 507–514.
38. Klare I, Heier H, Claus H, et al. *Enterococcus faecium* strains with *vanA*-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. Microb Drug Resist 1995; 1: 265–272.
39. Kruse H, Johansen BK, Rørvik LM, Schaller G. The use of avoparcin as a growth promoter and the occurrence of vancomycin-resistant *Enterococcus* species in Norwegian poultry and swine production. Microb Drug Resist 1999; 5: 135–139.
40. Simonsen GS, Haaheim H, Dahl KH, et al. Transmission of VanA-type vancomycin-resistant enterococci and *vanA* resistance elements between chicken and humans at avoparcin-exposed farms. Microb Drug Resist 1998; 4: 313–318.
41. Marwick C. Animal feed antibiotic use raises drug resistance fear. JAMA 1999; 282: 120–122.
42. McManus PS. Antibiotic use in plant disease control. APUA Newsletter 1999; 17: 1–3.
43. Katz SE, Levine PR. Determination of neomycin residues in eggs and stability of residues after cooking. J Assoc Off Anal Chem 1978; 61: 1103–1106.
44. Levy SB. Microbial resistance to antibiotics. An evolving and persistent problem. Lancet 1982; 2: 83–88.

45. Raloff J. Drugged waters: Does it matter that pharmaceuticals are turning up in water supplies? Science News 1998; 153: 187: (21 March 1998).
46. Høiby N, Jarløv JO, Kemp M, et al. Excretion of ciprofloxacin in sweat and multiresistant *Staphylococcus epidermidis*. Lancet 1997; 349: 167–169.
47. Gould IM. A review of the role of antibiotic policies in the control of antibiotic resistance. J Antimicrob Chemother 1999; 43: 459–465.
48. Barber M, Dutton AAC, Beard MA, Elmes PC, Williams R. Reversal of antibiotic resistance in hospital Staphylococcal infection. Brit Med J 1960: 11–17.
49. Price DJ, Sleigh JD. Control of infection due to *Klebsiella aerogenes* in a neurosurgical unit by withdrawal of all antibiotics. Lancet 1970; 2: 1213–1215.
50. Giamarellou H, Antoniadou A. The effect of monitoring of antibiotic use on decreasing antibiotic resistance in the hospital. Ciba Found Symp 1997; 207: 76–86; discussion 86–92.
51. White AC, Jr., Atmar RL, Wilson J, Cate TR, Stager CE, Greenberg SB. Effects of requiring prior authorization for selected antimicrobials: expenditures, susceptibilities, and clinical outcomes. Clin Infect Dis 1997; 25: 230–239.
52. Climo MW, Israel DS, Wong ES, Williams D, Coudron P, Markowitz SM. Hospital-wide restriction of clindamycin: effect on the incidence of *Clostridium difficile*-associated diarrhea and cost. Ann Intern Med 1998; 128: 989–995.
53. Smith DW. Decreased antimicrobial resistance after changes in antibiotic use. Pharmacotherapy 1999; 19: 129S–132S.
54. Fujita K, Murono K, Yoshikawa M, Murai T. Decline of erythromycin resistance of group A streptococci in Japan. Pediatr Infect Dis J 1994; 13: 1075–1078.
55. Kristinsson KG, Hjalmarsdottir MÁ, Gudnason Th. Epidemiology of penicillin resistant pneumococci in Iceland-Hope for the future? 35th Intersci Conf Antimicrob Agents and Chemother 1995: 42-Abstract C9.
56. Seppälä H, Klaukka T, Vuopio-Varkila J, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. Finnish Study Group for Antimicrobial Resistance. N Engl J Med 1997; 337: 441–446.
57. Klare I, Badstubner D, Konstabel C, Bohme G, Claus H, Witte W. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb Drug Resist 1999; 5: 45–52.
58. van den Bogaard AE, Bruinsma N, Stobberingh EE. The effect of banning avoparcin on VRE carriage in The Netherlands. J Antimicrob Chemother 2000; 46: 146–147.
59. Gilliver MA, Bennett M, Begon M, Hazel SM, Hart CA. Antibiotic resistance found in wild rodents. Nature 1999; 401: 233–234.
60. Corpet DE. Antibiotic resistance from food. N Engl J Med 1988; 318: 1206–1207.
61. Teuber M, Meile L, Schwarz F. Acquired antibiotic resistance in lactic acid bacteria from food. Antonie Van Leeuwenhoek 1999; 76: 115–137.
62. Schrag SJ, Perrot V, Levin BR. Adaptation to the fitness costs of antibiotic resistance in *Escherichia coli*. Proc R Soc Lond B Biol Sci 1997; 264: 1287–1291.
63. Björkman J, Nagaev I, Berg OG, Hughes D, Andersson DI. Effects of environment on compensatory mutations to ameliorate costs of antibiotic resistance. Science 2000; 287: 1479–1482.
64. Lenski RE, Simpson SC, Nguyen TT. Genetic analysis of a plasmid-encoded, host genotype-specific enhancement of bacterial fitness. J Bacteriol 1994; 176: 3140–3147.
65. Björkman J, Andersson DI. The cost of antibiotic resistance from a bacterial prespective. Drug Resist Updates 2000; 3: 237–245.
66. Berger-Bächi B, Tschierske M. Role of Fem factors in methicillin resistance. Drug Resist Updates 1998; 1: 325–335.
67. Wang W, Guffanti AA, Wei Y, Ito M, Krulwich TA. Two types of *Bacillus subtilis tetA*(L) deletion strains reveal the physiological importance of TetA(L) in K(+) acquisition as well as in Na(+), alkali, and tetracycline resistance. J Bacteriol 2000; 182: 2088–2095.
68. Summers AO, Wireman J, Vimy MJ, et al. Mercury released from dental "silver" fillings provokes an increase in mercury- and antibiotic-resistant bacteria in oral and intestinal floras of primates. Antimicrob Agents Chemother 1993; 37: 825–834.
69. McMurry LM, McDermott PF, Levy SB. Genetic evidence that InhA of *Mycobacterium smegmatis* is a target for triclosan. Antimicrob Agents Chemother 1999; 43: 711–713.
70. Moken MC, McMurry LM, Levy SB. Selection of multiple-antibiotic-resistant (*mar*) mutants of *Escherichia coli* by using the disinfectant pine oil: roles of the *mar* and *acrAB* loci. Antimicrob Agents Chemother 1997; 41: 2770–2772.
71. Schweizer HP. Intrinsic resistance to inhibitors of fatty acid biosynthesis in *Pseudomonas aeruginosa* is due to efflux: application of a novel technique for generation of unmarked chromosomal mutations for the study of efflux systems. Antimicrob Agents Chemother 1998; 42: 394–398.
72. Ma D, Cook DN, Alberti M, Pon NG, Nikaido H, Hearst JE. Genes *acrA* and *acrB* encode a stress-induced efflux system of *Escherichia coli*. Mol Microbiol 1995; 16: 45–55.