# Antibiotics, present and future

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## ABSTRACT

The problem of antibiotic resistance in bacterial pathogens is a problem in genetic ecology. For the past half century, microbes have been exposed to enormous quantities of toxic agents (antibiotics) and they have survived. The principal survival strategy employed was the functional acquisition and horizontal transfer of antibiotic resistance genes. It is apparent that the genetic mechanisms involved in these processes were novel and that they were likely to have been involved in the broader aspect of bacterial genome evolution for a long period of time.

## Introduction

Prior to the late 1930s no antibiotics, as we know them, were available. At that time metal-based disinfectants containing mercury and detergents made from phenolic compounds were employed and their use may have contributed to present-day resistance problems. The antibiotics introduced in the 1940s and thereafter were not new biological agents, but rather natural products whose useful antibiotic activity had been discovered; they were naturally present in the environment in small amounts, and genes encoding resistance to them would have been present in the producing organisms and possibly in the microbial community. The roles of these low-molecular-weight biologically active components in microbial ecology were poorly understood then, as now [8]. What *was* new and different was the nature and magnitude of their use as a result of their commercialisation for the treatment of infectious diseases in humans, animals, and plants. Enormous quantities of natural products with demonstrated therapeutic activities (antibiotics) were manufactured for human use by a new generation of pharmaceutical companies and released into the environment, creating a catastrophic situation for terrestrial bacteria. (It has been estimated that more than a million metric tons of antibiotics have been released into the biosphere during the last 50 years.) What happened biologically is a matter of speculation, since microbial ecology was a fledgling science at the time and retrospective analyses are often difficult to interpret; components of certain localised microbial populations may have been wiped out, with significant effects on the microbial ecology in many different eco-systems, such as changes in species distribution and extensive genetic modification. The use of antibiotics induced an intensive flux of genetic information in the microbial world. Essentially a large, uncontrolled microbiological 'experiment' took place world-wide in the second half of the 20$^{th}$ century, with only the survivors available for investigation. Retrospective analyses of this 'experiment', properly executed and interpreted, give important information on subjects such as horizontal gene transfer and genome evolution.

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## Development of antibiotic resistance

With few exceptions antibiotic resistance in bacterial pathogens was identified very quickly after the introduction of antibiotics into clinical practice. While mutations leading to resistance occurred extensively (for example, streptomycin resistance in *M. tuberculosis*), it is likely that mutations leading to low levels of resistance were only a 'first' step in the development of the refractory strains found today [9]. However, the majority of bacteria acquired the genes encoding resistance to antibiotics from exogenous and still largely unidentified sources. Obviously only infection-related strains (the tip of the iceberg) were identified and studied; it is unfortunate that changes in antibiotic susceptibility patterns of microbial populations could not have been analysed at earlier times and even more disappointing that the demographics of microbial populations under antibiotic stress have been little studied since! It would have been of great value to trace the course of genetic traffic on the introduction of antibiotics. Did resistance determinants develop in many species or in only a limited number, before being mobilised and transferred to the pathogens identified as antibiotic-resistant? This may never be known. However, it is to be hoped that the next time a completely novel antibiotic (chemical entity) is introduced into human clinical practice, steps will be taken to ensure analysis and elucidation of the route(s) by which antibiotic resistance can be established in microbial communities.

Prior to the introduction and use of antibiotics, antibiotic-resistant microbes were absent from human or animal flora [7, 11]. With antibiotic use, rapid increases in bacterial resistance to tetracycline and other antibiotics was documented in previously susceptible strains. Recent examples come from studies of antibiotic-resistance development in *E. coli* 0157:H7 in the State of Washington: in 1984 all strains were antibiotic-sensitive, in 1989 7% of the isolates carried antibiotic-resistance determinants, and since that time the number and incidence of resistance characters has expanded. The dramatic appearance of antibiotic resistance in pathogenic *Shigella* spp. during an epidemic of intestinal infections in the 1950s in Japan is yet another example of the rapid response of bacterial pathogens to the threat of antibiotic use [17] and signalled the appearance of resistance plasmids.

At the present time many different resistance genes (often termed 'cassettes') exist in accessible and mobilisable form in the microbial gene pool. Upwards of a dozen biochemical mechanisms of resistance have been characterized (Table 1): these are encoded by hundreds of different genes, many of them identified as classes of related allelic determinants. The bacterial population has essentially stockpiled a considerable armamentarium of genetic defences. Not surprisingly the majority of resistance genes are carried by plasmids, transposons, and other elements capable of inter-generic and inter-specific mobility. While information on the origins and acquisition of these genes is hard to come by (where did they come from and how did they move to where they are?), significant advances have been made.

## Origins of antibiotic-resistance genes

As with the pathogenicity islands found in clinical isolates, resistance genes are often clustered on their resident genomes and differ in base composition from their immediate sequence environment. No single source of resistance genes can be identified but a number have been proposed, including housekeeping genes (alternate substrates, mutation to new substrate recognition, etc.), antibiotic-producing microbes (self-protection), and

**Table 1**: Biochemical mechanisms of antibiotic resistance and their genetic determinants

| | | Genetic determinants | |
|---|---|---|---|
| **Mechanism** | **Examples** | **Mutation** | **Gene acquisition** |
| Reduced permeability | aminoglycosides | + | + |
| Pro-drug not activated | isoniazid | + | |
| Active efflux | tetracycline | | + |
| | fluoroquinolones | + | |
| Alteration of drug target | erythromycin | | + |
| | fluoroquinolones | + | |
| | rifampicin | + | |
| | tetracycline | | + |
| Inactivation of drug | aminoglycosides | | + |
| | chloramphenicol | | + |
| | ß-lactams | + | + |
| By-pass inhibited step | sulphonamides | | + |
| | trimethoprim | | + |
| Immunity protein | bleomycin | | + |
| Amplification of target | trimethoprim | + | |
| | sulphonamides | + | |
| Sequestration of drug | ß-lactams | + | + |

**Table 2**: Resistance determinants with biochemical homologues in antibiotic-producing organisms

| **Antibiotic** | **Resistance mechanisms*** |
|---|---|
| Penicillins | ß-lactamases |
| Cephalosporins | penicillin-binding proteins |
| Aminoglycosides | acetyltransferases, phosphotransferases, adenyltransferases |
| Chloramphenicol | acetyltransferases |
| Tetracyclines | efflux, ribosomal protection |
| Macrolides | ribosomal RNA methylation |
| Streptogramins | esterases, acetyltransferases |
| Lincosamines | phosphotransferases, acetyltransferases |
| Phosphonates | phosphorylation, glutathionylation (?) |
| Bleomycin | acetyltransferase, immunity protein |
| Vancomycin | D-Ala-D-Lac ligase |
| Rifamycin | ribosylation |

*Multiple drug resistance (MDR) efflux systems are common to producing organisms and clinical isolates

'natural' resistance genes in soil communities. Supporting evidence is largely circumstantial and comes from a variety of studies. In no case can a direct (single transfer) relationship be established between the resistance gene(s) in clinical isolates and the putative source organisms. For example, methicillin resistance (the *mec* cluster) found in *Staphylococcus aureus* is closely related to that of *Staphylococcus sciuri* [24], but transfer *in vivo* has not been shown. The enterobacterial aminoglycoside acetyltransferase (*aac*) genes, some of which are derived from chromosomal genes in gram-negative bacteria [13], are another example; interestingly the *aac* genes have been shown to be members of a large protein N-acyltransferase family [18].

That resistance to aminoglycosides, macrolides, tetracycline, chloramphenicol, and other antibiotics may have originated in their producing organisms is indicated by biochemical and nucleic acid sequence similarities of the genes and their products (Table 2). An example of an antibiotic-resistance gene of unusual parenthood is provided by mupirocin (pseudomonic acid), an antibiotic employed in the treatment of topical infections by gram-positive bacteria. Resistance to mupirocin is due to the presence of an altered isoleucyl tRNA synthase that may have originated in eukaryotes and was only recently acquired by *S. aureus* strains [5]. The molecular details of this unusual acquisition remain to be ascertained.

## Mutation

Extensive 'tailoring' and adaptation of the different genes must occur during transfer between the 'source' organisms and the ultimate clinical isolates, which are usually characterised by a different G + C composition, codon usage pattern, and regulatory elements. How do these modification occur? It is probable that uncharacterised intermediate transfer hosts play a role in the tailoring of the determinants currently identified. Hyper-mutable strains and mutator genes must have played roles, and recent studies have shown that many natural bacterial isolates, particularly pathogenic strains, are hyper-mutagenic and carry mutator genes (*mut*S, *mut*L, *mut*T) [14, 21]. In some cases the mutator genes involved, such as *mut*T, favour the reduction of G/C-rich sequences, contributing to adaptation of codon usage patterns to new cellular environments [20]. The natural incidence of mutator strains in the environment and their potential roles in the evolution of antibiotic resistance genes (or any horizontally transferred determinants, such as biodegradation clusters) can only be hypothesized. The critical juxtaposition of mutational and gene transfer events is not understood, apart from examples such as the evolution of extended ß-lactamases by natural protein engineering [6]. In addition, the genetic ecology of antibiotic resistance is inextricably linked to the ecology of microbial populations in humans, animals, and plants.

## Mechanisms of acquisition (resistance-gene capture)

The tandem assembling of multiple drug-resistance genes in a single, mobile genetic element has many implications:

(a) A single plasmid replicon or transposon may carry resistance to a number of clinically significant antibiotics at the same time.

(b) A single gene may encode a biochemical mechanism that engenders resistance to an entire class of antimicrobials, e.g. all available macrolides (*erm*), all ß-lactams (*bla*), or several different aminoglycosides (*aac*, *aph*, *ant*).

(c) A single resistance gene may encode resistance to at least two structurally unrelated antibiotics, e.g. streptomycin and spectinomycin (*aad*).
(d) Use of a non-antibiotic substance, such as a disinfectant or metal salt, may select for linked antibiotic-resistance genes carried on a plasmid or transposon.
The most common mechanism by which tandem arrays of antibiotic-resistance genes are assembled in the Enterobacteriaceae is gene-capture by integrons. This process has been dissected genetically and biochemically by Roy, Sundstrom, Hall and Stokes, and their co-workers[4, 10, 12]. All known integrons are composed of three essential elements: an integrase gene, a primary recombination site and a strong promoter. The integron-associated integrases belong to the site-specific recombinase family and are able to recombine with discrete units of DNA, the gene cassettes, providing them with a promoter for their expression. The resistance cassettes contain a single resistance gene associated with a specific recombination sequence, known as the 59-base elements. An outline of the integron-integrase acquisition process is illustrated in Fig. 1. Many important details of this process need to be elucidated. For example, what is the origin of the integrases and the 59-base-pair elements which are essential for the recognition and integration of the captured gene cassettes? Are they derived from bacteriophages? What is the origin of the gene cassettes and how do the 59-base-pair elements become attached?

Recent work by Mazel *et al*. on the antibiotic-resistance integrons and the *Vibrio cholerae* repeated sequences (VCRs) [16] has demonstrated striking similarities, which suggests important functional relationships between these compound structures. Both the VCR and antibiotic-resistance integrons possess specific integrases that insert coding sequences (ORFs) into a unique chromosomal attachment site, leading to the formation of tandem arrays of genes. In the case of *V. cholerae* the cluster of VCR-associated ORFs represents 60-100 genes and occupies 4% of the 2.5Mb genome [3]. Preliminary studies indicate that such integrase-linked structures are found in a number of different *Vibrio* spp. The VCR integrases so far identified form a class related to, but distinct from, IntI2 (of class II integrons).

The VCR structures differ from the integrons in that (1) a defined integration site is lacking, (2) a certain number of the ORFs possess their own promoter, (3) an identical repeat (VCR) is associated with each ORF, (4) the ORFs encode largely unknown functions; those so far identified appear to be related to virulence, and no well-defined antibiotic-resistance genes are in the clusters, although it should be noted that the *bla*P and *dfr*VI cassettes of integrons are VCR-associated. Thus resistance and virulence are linked, which implies that the selective pressure of antibiotic use played a part in the dissemination of pathogenicity determinants in the development of ‘new’ pathogens. Many *Vibrio* spp. possess similar clusters, and detailed analysis of the open reading frames (in process) confirms the diversity of sequences present. If each *Vibrio* species possesses VCR-like clusters of hundreds of unidentified genes, a veritable treasure trove will be available for functional genomic studies. The number of different bacterial integrons may be very large, each classified according to its associated site-specific integrase. Integron-driven gene capture is likely to play an important role in the evolution of bacterial genomes beyond the functions of antibiotic resistance and pathogenicity. While such tandem arrays have been identified only in enteric bacteria, incomplete integrons have been found in the gram-positive *M. tuberculosis* and *Corynebacterium glutamicum*, and clusters of resistance genes have been characterised in *Staphylococcus aureus* [1].

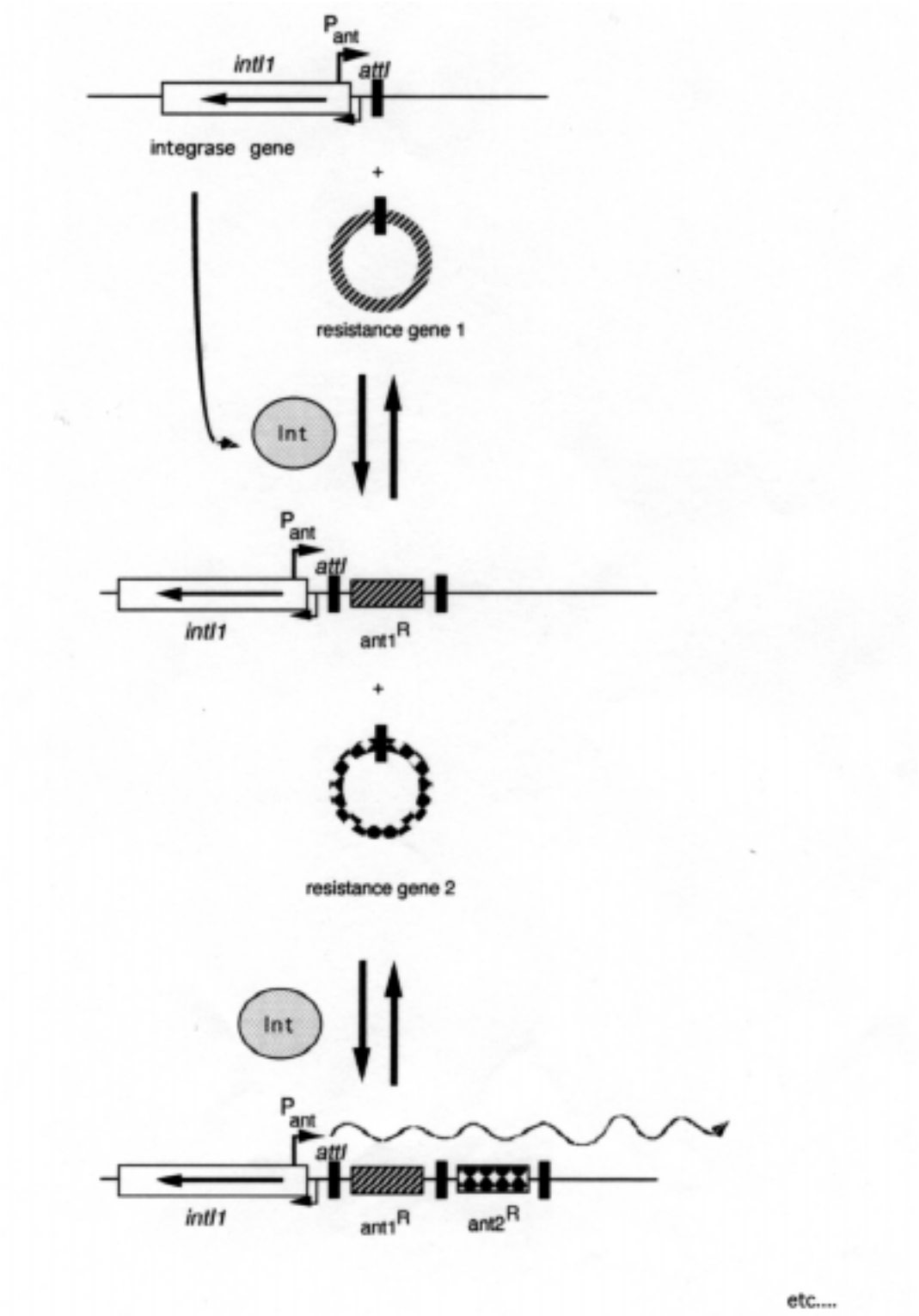


**Fig. 1**: The mechanism by which resistance-gene cassettes are incorporated into capture elements such as integrons or VCRs (see text). The gene *intI1* encodes the integrase (Int) and the promoter ($P_{ant}$) necessary for transcription of inserted downstream genes. The direction of transcription of the *int* gene is shown by the lower arrow. The resistance gene cassettes ($ant1^R$, $2^R$) are integrated at the attachment site (attI) which interacts with the 59-base-pair elements (solid bar) in each resistance-gene cassette.

## The big picture – the ecology of antibiotic-resistant bacteria

We have focused on the acquisition, dissemination and organisation of genes within the bacterial kingdom: the genetic ecology of antibiotic resistance. On the other hand, the wide distribution of antibiotic-resistant bacteria in many different ecological niches defines the macro-ecology of infectious disease; the spread of antibiotic-resistant organisms as well as the horizontal transfer of resistance genes must be considered. Antibiotic resistance is associated with many different sources, and the following are important factors:

a) health care institutions and the maintenance and transmission of resistant strains within the community (food, domestic animals, etc.)

b) the dissemination of antibiotic-resistant bacteria in animals and their different environments

c) global transfer of antibiotic-resistant strains (pathogens and non-pathogens) due to increased international travel and the food 'business', including traffic in live animals and associated products.

The components of the food chain are inextricably linked and interactions between bacterial populations of humans, animals, plants and environment disseminate antibiotic-resistant pathogens within the food chain. The threat of serious infection from food-borne pathogens has become a significant risk to the consumer [15]. The implications for human health, especially among disease-susceptible groups such as the very young and the elderly, must be addressed as a priority. Typically, antibiotic usage in agriculture at first led to transfer of resistance genes from animal isolates (such as *Salmonella typhimurium* 29] to human pathogens. However, the increasing incidence of multidrug-resistant strains of *S. typhimurium* DT104 and *E. coli* 0157 H7 [2, 22, 23], which are pathogenic to *both* animals and humans, is a different matter and is cause for concern [19].

## Conclusions

A general understanding of the origins, mechanisms of acquisition and dissemination of antibiotic-resistance genes within microbial populations exists currently. However, there are many missing links in this picture. Studies of antibiotic-resistant bacteria have focused on the properties of the antibiotic-resistant pathogens isolated in human clinical situations, with little effort expended in trying to establish their genesis. Such information will be critical in providing the means to control the problem of antibiotic resistance in microbes. Efforts to reduce the development and incidence of antibiotic resistance in hospitals and the community must be increased on a world-wide basis. The introduction of novel antibiotics and effective vaccines is a priority, as is improved technology in the agricultural and food supply industries that will reduce dependence on antibiotic use and the exclusion of resistant organisms from the food chain. Nationwide and international surveillance will be needed to identify outbreaks and to institute effective measures in containment.

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