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The origins and molecular basis of antibiotic resistance

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We frequently refer to bacteria as being resistant to antibiotics, but rarely do we consider what that means. Even the most resistant bacterium can be inhibited or killed by a sufficiently high concentration of antibiotic; patients, however, would not be able to tolerate the high concentration required in some cases. Bacterial species vary tremendously in their susceptibility to an antibiotic—for example, most strains of *Streptococcus pneumoniae* in Britain are inhibited by 0.01 mg/l of benzylpenicillin (the minimum inhibitory concentration), whereas for *Escherichia coli* 32-64 mg/l are required to inhibit growth, a level which cannot be achieved in the human body. This introduces the concept of clinical resistance, which is dependent on outcome and is all too often ignored. Clinical resistance is a complex concept in which the type of infecting bacterium, its location in the body, the distribution of the antibiotic in the body and its concentration at the site of infection, and the immune status of the patient all interact.

Summary points

- Antibiotic resistance should be defined in terms of clinical outcomes, not laboratory methods
- Resistance occurs by means of four main mechanisms—more than one may be present in a single bacterium
- Resistance mechanisms have probably evolved from genes present in organisms producing antibiotics
- Resistance genes occur not only in bacteria that carry disease but also in commensal bacteria, to which we are continuously exposed and which are found in food, the environment, and animals
- The plethora of genetic mechanisms for evolution and reassortment of antibiotic resistance genes ensures that useful genes will be disseminated rapidly
- Action must be taken to slow the rate of evolution and spread of antibiotic resistance genes, in which the biggest single factor is the amount of antibiotics used in human medicine and agriculture

Mechanisms of antibiotic resistance in bacteria

The many mechanisms that bacteria exhibit to protect themselves from antibiotics can be classified into four basic types (fig 1). Antibiotic modification is the best known: the resistant bacteria retain the same sensitive target as antibiotic sensitive strains, but the antibiotic is prevented from reaching it. This happens, for example, with β lactamases—the β lactamase enzymatically

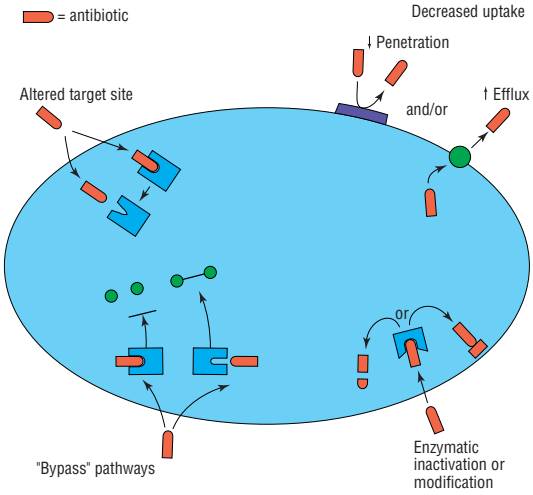

Fig 1 Four major biochemical mechanisms of antibiotic resistance

cleaves the four membered β lactam ring, rendering the antibiotic inactive. Over 200 types of β lactamase have been described (table). Most β lactamases act to some degree against both penicillins and cephalosporins; others are more specific—namely, cephalosporinases (for example, AmpC enzyme found in *Enterobacter* spp) or penicillinases (for example, *Staphylococcus aureus* penicillinase). β Lactamases are widespread among many bacterial species (both Gram positive and Gram negative) and exhibit varying degrees of inhibition by β lactamase inhibitors, such as clavulanic acid.[1]

Some antibiotic resistant bacteria protect the target of antibiotic action by preventing the antibiotic from entering the cell or pumping it out faster than it can flow in (rather like a bilge pump in a boat). β Lactam antibiotics in Gram negative bacteria gain access to the cell that depends on the antibiotic, through a water filled hollow membrane protein known as a porin (fig 2). In the case of imipenem resistant *Pseudomonas aeruginosa*, lack of the specific D2 porin confers resistance, as imipenem cannot penetrate the cell. This mechanism is also seen with low level resistance to fluoroquinolones and aminoglycosides. Increased efflux via an energy-requiring transport pump is a well recognised mechanism for resistance to tetracyclines and is encoded by a wide range of related genes, such as tet(A), that have become distributed in the enterobacteriaceae.[2]

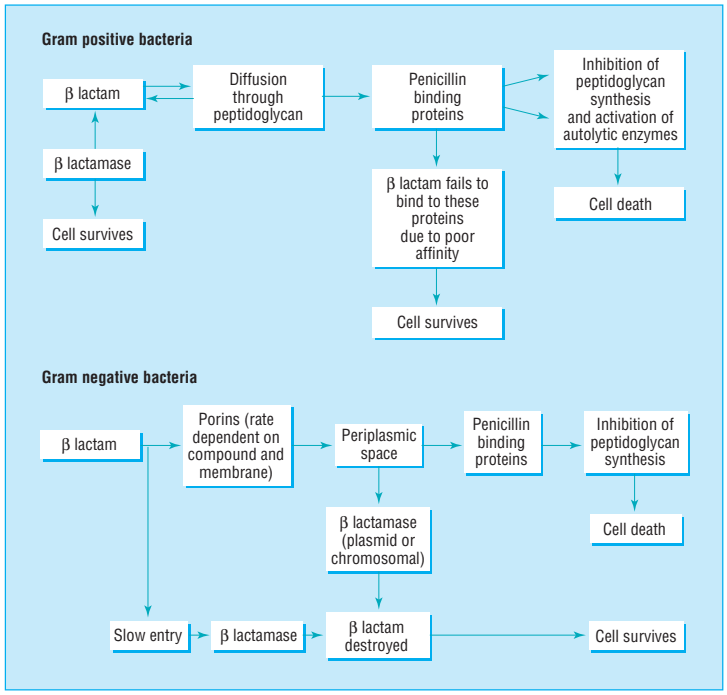

Fig 2 Interplay of β lactam antibiotics with Gram positive and Gram negative bacteria

Alterations in the primary site of action may mean that the antibiotic penetrates the cell and reaches the target site but is unable to inhibit the activity of the target because of structural changes in the molecule. Enterococci are regarded as being inherently resistant to cephalosporins because the enzymes responsible for cell wall synthesis (production of the polymer peptidoglycan)—known as penicillin binding proteins—have a low affinity for them and therefore are not inhibited. Most strains of *Streptococcus pneumoniae* are highly susceptible to both penicillins and cephalosporins but can acquire DNA from other bacteria, which changes the enzyme so that they develop a low affinity for penicillins and hence become resistant to inhibition by penicillins.[3] The altered enzyme still synthesises peptidoglycan but it now has a different structure.[4] Mutants of *Streptococcus pyogenes* that are resistant to penicillin and express altered penicillin binding proteins can be selected in the laboratory, but they have not been seen in patients, possibly because the cell wall can no longer bind the anti-phagocytic M protein.

The final mechanism by which bacteria may protect themselves from antibiotics is the production of an alternative target (usually an enzyme) that is resistant to inhibition by the antibiotic while continuing to produce the original sensitive target. This allows bacteria to survive in the face of selection: the alternative enzyme "bypasses" the effect of the antibiotic. The best known example of this mechanism is probably the alternative penicillin binding protein (PBP2a), which is produced in addition to the "normal" penicillin binding proteins by methicillin resistant *Staphylococcus aureus* (MRSA). The protein is encoded by the mecA gene, and because PBP2a is not inhibited by antibiotics such as flucloxacillin the cell continues to synthesise peptidoglycan and hence has a structurally sound cell wall.[5] The appearance in 1987 of vancomycin resistant enterococci has aroused much interest because the genes involved can be transferred to *S aureus*, and this can thus theoretically result in a vancomycin resistant MRSA. The mechanism also represents a variant of the alternative target mechanism of resistance.[6] In enterococci sensitive to vancomycin the normal target of vancomycin is a cell wall precursor that contains a pentapeptide that has a D-alanine-D-alanine terminus, to which the vancomycin binds, preventing further cell wall synthesis. If an enterococcus acquires the vanA gene cluster, however, it can now make an alternative cell wall precursor ending in D-alanine-D-lactate, to which vancomycin does not bind.

Molecular epidemiology of resistance genes

Resistance in bacteria can be intrinsic or acquired. Intrinsic resistance is a naturally occurring trait arising from the biology of the organism—for example, vancomycin resistance in *Escherichia coli*. Acquired resistance occurs when a bacterium that has been sensitive to antibiotics develops resistance—this may happen by mutation or by acquisition of new DNA.

Mutation is a spontaneous event that occurs regardless of whether antibiotic is present. A bacterium carrying such a mutation is at a huge advantage as the susceptible cells are rapidly killed by the antibiotic, leaving a resistant subpopulation. Transferable resistance was recognised in 1959, when resistance genes found in shigella transferred to *E coli* via plasmids. Plasmids are self replicating circular pieces of DNA, smaller than the bacterial genome, which encode their transfer by replication into another bacterial strain or species. They can carry and transfer multiple resistance genes, which may be located on a section of DNA capable of transfer from one plasmid to another or to the genome—a transposon (or "jumping gene"). Because the range of bacteria to which plasmids can spread is often limited, transposons are important in spreading resistance genes across such boundaries. The mecA gene found in MRSA may well have been acquired by transposition.[7] Plasmid evolution can be complex, but modern molecular techniques can give an understanding (as is the case with the plasmids that contain the tetM gene and are found throughout the world in *Neisseria gonorrhoeae*).[8]

Bacteriophages (viruses that infect bacteria) can also transfer resistance, and this is frequently seen in staphylococci. When bacteria die they release DNA, which can be taken up by competent bacteria—a process known as transformation. This process is increasingly recognised as important in the environment and is probably the main route for the spread of penicillin resistance in *Streptococcus pneumoniae*, by creation of "mosaic penicillin binding protein genes."[3]

Origins of resistance genes

The origins of antibiotic resistance genes are obscure because at the time that antibiotics were introduced the biochemical and molecular basis of resistance was yet to be discovered. Bacteria collected between 1914 and 1950 (the Murray collection) were later found to be completely sensitive to antibiotics. They did, however, contain a range of plasmids capable of conjugative transfer.[9] None of the Murray strains was resistant to sulphonamides, although these had been introduced in the mid-1930s; resistance was reported in the early 1940s in streptococci and gonococci.[10] The introduction of streptomycin for treating tuberculosis was thwarted by the rapid development of resistance by mutation of the target genes. Mutation is now recognised as the commonest mechanism of resistance development in *Mycobacterium tuberculosis*, and the molecular nature of the mutations conferring resistance to most antituberculosis drugs is now known.[11] Favourable mutations that arise in bacteria can be mobilised via insertion sequences and transposons on to plasmids and then transferred to different bacterial species.

Simplified functional classification of main groups of β lactamases, as proposed by Bush et al[12]

Group	Molecular type	Preferred substrate	Representative enzyme (bacterium)
1	C	ceph	AmpC (*Enterobacter* spp etc)
2a	A	pen	Penicillinase (*Staphylococcus aureus*)
2b	A	pen, ceph	TEM-1 (*Escherichia coli*), SHV-1 (*Klebsiella* spp)
2be/r	A	pen, ceph*	TEM 364, SHV 212 (enterobacteriaceae)
2c	A	pen	PSE-1 (pseudomonas)
2d	D	pen	OXA-111 (pseudomonas/enterobacteriaceae)
2e†	A	ceph	Inducible chromosomal enzymes from proteus
2f	A	pen, ceph, and carbapenems	SME-I (serratia)
3	B‡	pen, ceph, carbapenems	IMP-I (pseudomonas)
4	—	pen	Pencillinase (*Pseudomonas cepacia*)

*Includes third generation cephalosporins, such as ceftazidime; some are not inhibited by clavulanic acid.
†Inhibited by clavulanic acid.
‡Metallo-β lactamase, which requires zinc for activity.

In considering the evolution and dissemination of antibiotic resistance genes it is important to appreciate the rapidity of bacterial multiplication and the continual exchange of bacteria among animal, human, and agricultural hosts throughout the world. There is support for the notion that determinants of antibiotic resistance were not derived from the currently observed bacterial host in which the resistance plasmid is seen. DNA sequencing studies of β lactamases and aminoglycoside inactivating enzymes show that despite similarities within the protein studies of the two families, there are substantial sequence differences.[12 13] As the evolutionary time frame has to be less than 50 years it is not possible to derive a model in which evolution could have occurred by mutation alone from common ancestral genes. They must have been derived from a large and diverse gene pool presumably already occurring in environmental bacteria. Many bacteria and fungi that produce antibiotics possess resistance determinants that are similar to those found in clinical bacteria.[10] Gene exchange might occur in soil or, more likely, in the gut of humans or animals. It has been discovered that commercial antibiotic preparations contain DNA from the producing organism, and antibiotic resistance gene sequences can be identified by the polymerase chain reaction.[14]

Genes either exist in nature already or can emerge by mutation rapidly. Rapid mutation has been seen with *(a)* the TEM β lactamase, resulting in an extension of the substrate profile to include third generation cephalosporins (first reported in Athens in 1963, one year after the introduction of ampicillin) and *(b)* the IMI-1 β lactamase (reported from a Californian hospital before imipenem was approved for use in the United States).[15] The selection pressure is heavy, and injudicious use of antibiotics, largely in medical practice, is probably responsible—although agricultural and veterinary use contributes to resistance in human pathogens. The addition of antibiotics to animal feed or water, either for growth promotion or, more significantly, for mass treatment or prophylaxis (or both treatment and prophylaxis) in factory farmed animals, is having an unquantified effect on resistance levels.[16] Bacteria clearly have a wondrous array of biochemical and genetic systems for ensuring the evolution and dissemination of antibiotic resistance.

Competing interests: None declared.

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Antiviral drug resistance

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The development of effective antiviral drugs is an important biomedical scientific achievement of the late 20th century. Highly potent drugs are now available against herpes viruses, HIV, hepatitis B virus, and influenza virus. This list will extend to papillomaviruses, respiratory viruses, enteroviruses, and hepatitis C virus over the next 5-10 years. Viruses that maintain latency (the herpes viruses) or persistence (HIV and hepatitis B virus) are not specifically cleared from the body by these drugs, but their replication can be effectively suppressed. Currently, 18 specific antiviral drugs (excluding interferons) are licensed in the United Kingdom, with many more in phase 3 clinical trials or available on expanded access. For the common viral infections, prescribing will shift into primary care, as has already occurred for shingles and herpes simplex infections.

Against this exciting background comes the news of drug resistance. Virally encoded drug resistance has been documented against nearly all compounds with antiviral activity, and the genetic basis of resistance is now known.

Summary points

- Resistance has developed to nearly all specific and effective antiviral agents
- Resistance has developed to all drugs against HIV, and treating hepatitis B with nucleoside analogue monotherapy gives rise to drug resistant variants
- Resistance develops rapidly when viral replication is not maximally suppressed
- Drug resistant viruses may be transmitted
- Assays to measure drug resistance are available in specialised laboratories

Biological basis of resistance

Drug resistance is defined as a reduced susceptibility to a drug in a laboratory culture system and is expressed as an altered IC_{50} or IC_{90} (drug concentration required to inhibit viral growth by 50% or 90% respectively). This is termed the phenotype. This phenotype is determined by specific mutations in the viral genome (the genotype), which leads to alterations in the viral target protein (for example, HIV reverse transcriptase) or the viral drug activator (for example, herpes simplex thymidine kinase). The high rate of replication of some viruses determines that many of these genetic variants will already exist in untreated infected people. This is consequent on an inherent error rate of viral polymerases, especially for RNA viruses such as HIV[1] and influenza, which replicate the viral genome. A wide range of viral variants, including those with mutations associated with drug resistance, will therefore be present. This collection of variants in one person is termed the viral quasispecies, with the "fittest" virus representing the majority population. The use of an antiviral drug will provide a selective pressure for the preferential growth of variants with a reduced susceptibility to drugs in accordance with Darwinian evolutionary principles. The emergent drug resistant virus will be the fittest in the presence of drug. Some drug resistant viruses, however, seem not to replicate as well as wild type virus (in the absence of drug).[2] In some cases, multiple mutations are required for the development of high level resistance, and insufficient suppression of viral replication by antiviral drugs will predispose to their sequential acquisition.

Laboratory tests for resistant virus comprise phenotypic or genotypic assays.[3] Phenotypic assays are generally regarded as the standard but are time consuming and depend on the ability to propagate the virus—for example, hepatitis B and C viruses cannot routinely be grown in the laboratory. Genotypic assays are easier to undertake, but they are unable to detect mutations associated with drug resistance that occur in a small proportion of the viral population. Furthermore, the relation between results obtained by genotypic and phenotypic assays may be variable. Cur-