ORIGIN OF RESISTANCE

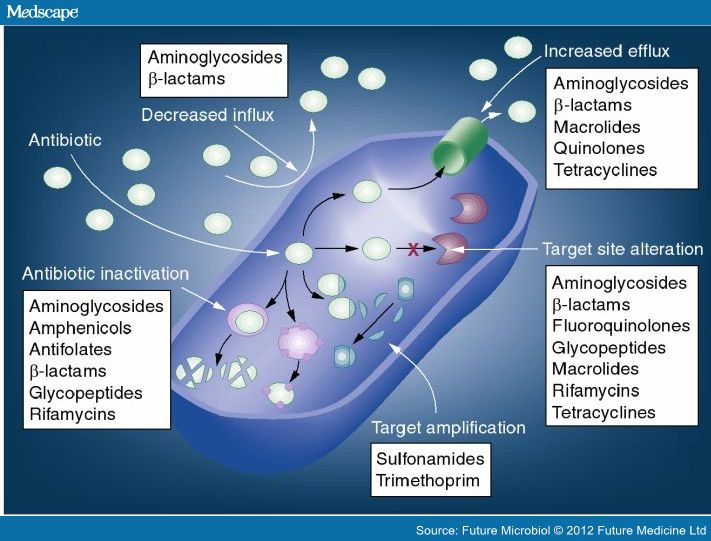
Some bacteria are said to have innate resistance against antibiotics and this typically reﬂects variations in the structure of their cell envelope. These will be identiﬁed in subsequent sections on resistance mechanisms. Resistance or reduced susceptibility may also be phenotypic, resulting from adaptation to growth within a speciﬁc environment. A characteristic of such phenotypic resistance is reversion to antibiotic susceptibility upon subculture in conventional laboratory media and failure to isolate genotypic resistant mutants. The origins of antibiotic resistance genes are unclear; however, studies using clinical isolates collected before the introduction of antibiotics demonstrated susceptibility, although conjugative plasmids were present. Resistance can be achieved by horizontal acquisition of resistance genes, mobilized via insertion sequences, transposons and conjugative plasmids, by recombination of foreign DNA into the chromosome, or by mutations in different chromosomal loci. Given that it is only 60 years since the introduction of antibiotics, mutation of common ancestral genes could not be the only resistance mechanism. Many resistance genes will have derived from the diverse gene pool present in environmental microorganisms, most likely produced as protective mechanisms by antibiotic - producing organisms. Genetic exchange is likely to arise in soil and the general environment as well as in the gut of humans and animals. Rapid mutation can occur and there is clearly a heavy selective pressure resulting from the overuse of antibiotics in medical practice. Agricultural and veterinary use of antibiotics also makes an important and unhelpful contribution. The mutation process is not a static event and a complex network of factors inﬂuences the rate and type of mutants that can be selected under antibiotic selective pressure. Antibiotic concentration, physiological conditions such as nutrient availability and stress can each regulate mutation rates. The structure of a gene is relevant to mutability. Size is not the main factor, as not every mutation in a gene that encodes an antibiotic target leads to resistance. Resistance only occurs by mutations which are both permissive (i.e. not lethal or leading to an unacceptable reduction in ‘ﬁtness’ or ability to cause infection) and able to produce a resistance phenotype. The probability that such a mutation arises will be proportional to the number of target sites within the gene. In *Escherichia coli*, mutations in the gyrA gene, encoding the GyrA subunit of topoisomerase II and leading to ﬂuoroquinolone resistance have been identiﬁed in at least seven locations, whereas mutational changes in only three positions in the parC gene, encoding a subunit of topoisomerase IV, have been observed. As a consequence, the prediction that the mutation rate would be higher in gyrA than parC is correct. Such observations and predictions cannot be extrapolated to other organisms. Indeed, the opposite is true for ﬂuoroquinolone resistance in *Strep. pneumoniae*.

Mechanisms of resistance

Resistance to antimicrobial agents typically occurs by one or more of the following mechanisms:

• Inactivation of the drug • Alteration of the target • Reduced cellular uptake

• Increased efﬂux.



**Microorganisms Demonstrate Resistance in Several Ways (From Olowe O. Adekunle, 2012)**

**Intrinsic Resistance** to an antimicrobial agents characterizes resistance that is an inherent attribute of a particular species; these organisms may lack the appropriate drug susceptibility targets or poses natural barriers that prevent the agents from reaching the target; examples are the natural resistance of gram- negative bacteria to vancomycin because of the drug’s inability to penetrate the gram-negative outer membrane, or the intrinsic resistance of the penicillin.

**Circumstantial Resistance** is the difference between the in vitro and in vivo effects of an antimicrobial agent. Agents that appear to be active in the laboratory may be ineffective in vivo because of failure to reach the site of infection, such as the inability of first generation cephalosporins to cross the blood-brain barrier. Drugs such as aminoglycosides may be inactivated; in vivo antagonist of trimethoprim-sulfamethoxazole can be overcome by enterococci via their inability to take up and internalize corporate environmental folate.

**Acquired Resistance**, which is the primary focus of this work, reflects a true change in the genetic composition of a bacterium so that a drug that once was effective in vivo no longer is effective. The major mechanism that bacteria employ to avoid the actions of antimicrobial agents include limiting the intracellular concentration of the antimicrobial agent by decreased influx or increased efflux, neutralization of the antimicrobial agent by enzymes that reversibly or irreversibly inactivate the drug, alteration of the target so that the agents no longer will interfere with it, and elimination of the target altogether by the creation of new metabolic pathways. Bacteria may employ or combine multiple mechanisms against a single agent or class of agents or a single change may result in development of resistance to several different agents.

**MECHANISM OF DISSEMINATION OF RESISTANCE GENES**

Bacteria avail themselves of a variety of efficient mechanisms for the transfer of resistance genes to other organisms and other species. The bacterial genome consists of chromosomal DNA, which encodes for general cellular characteristics and metabolic repair pathways, and smaller circular DNA elements known as plasmids that encode for supplemental bacterial activities such as virulence factors and resistance genes. The vast majority of resistance genes are plasmid- mediated, but plasmid-mediated traits can interchange with chromosomal elements. Transfer of genetic material from a plasmid to the chromosome can occur by simple recombination events, but the process is greatly facilitated by means of transposons. Transposons are small, mobile DNA elements capable of mediating transfer of DNA by removing and inserting themselves into host chromosomal and plasmid DNA and include Insertion Sequences, Transposons and integrons. If these elements become associated with either transmissible or mobile plasmids, chances are increased that they will be transferred to other organisms. Many resistance genes, such as plasmid-mediated β-lactamase, tetracycline-resistance genes and aminoglycosides-modifying enzymes are organized on transposons, which can vary greatly in size and complexity. Transposons may have a broader host range than their parent plasmids and may be important in the dissemination of resistance genes among species. Resistance determinants carried on the chromosome are transmitted by clonal dissemination. Resistance determinants on plasmids also are transferred vertically, although plasmids may be lost from the bacterial population if they no longer contain particular selective advantage. In bacteria, gene transfer that can lead to recombination which may occur in any of three different easy: *transformation, transduction and conjugation.*

**Transformation** is the simplest type of gene transfer. A recipient cell acquires genes from ‘free floating’ DNA molecules in the surrounding medium. In nature, the DNA may come from dead cells that lyse and release their DNA. In the laboratory, however, the DNA is extracted by chemical methods from a suspension of donor bacteria and then added to a culture of recipient bacteria. In nature or in the laboratory, a recipient bacterium can acquire one or more inheritable characteristics from a donor bacterium and become what is called transformed. Only certain species of bacteria are known to undergo transformation, and even these must be in a state of growth receptive to the incorporation of donor DNA; that is they must be competent. This condition usually occurs when the recipient bacteria are in the late logarithm phase of their growth. Competent bacteria cells produce a special protein that binds donor DNA fragments at specific sites on the cell surface. Although chromosomal DNA can be readily transferred to competent recipient bacteria, plasmid DNA is not easily transferred by ordinary transformation procedure that simply add DNA to recipient cells. However, special procedures widely used in genetic engineering can be used to accomplish transformation with plasmid DNA.

**Transduction** is gene transfer in which a virus serves as the vehicle for carrying DNA from a donor bacterium to a recipient bacterium. A phage consists of a nucleic acid, usually DNA surrounded by a protein coat to form a head. A tail –like appendage serves to attach the phage to the surface of a susceptible host bacterium. After the phage injects its DNA into the host cell, the phage DNA is replicated rapidly while the bacterial DNA is degraded. The phage DNA then directs the synthesis of new phage proteins by the host cell. Within a short time the new phage, DNA molecules combine the new phage proteins to form numerous whole phages, which are released as the host cell disintegrates. During assembly of the phage progeny within the infected host cell, any fragment of the host bacterium’s DNA that is approximately the same size as the phage DNA may be accidentally incorporated into a new phage head instead of the phage DNA. A phage carrying such a fragment is called a transducting phage because if it affects another bacterium, it injects the bacterial DNA fragments into the new host. Because the transducting phages do not contain the entire viral DNA, they do not kill the new host). The fragment can then undergo recombination with the corresponding part of the new host’s chromosome and become a permanent part of that chromosome. Thus, the second bacterial host acquires one or more genes.

**Conjugation** is a process of gene transfer that requires cell to cell contact. Plasmids also are capable of horizontal transfer via conjugation, although the efficiency of plasmid transfer both within and between species can vary tremendously. DNA may be transferred directly from one bacterium to another. Bacterial conjugation differs from sexual mating in eukaryotes in that it does not involve the fusion of two gametes to form a single cell. In some types of conjugation, only a plasmid may be transferred from the donor bacterium to the recipient bacterium. In other types, large segments of the donor cell’s chromosome or even the entire chromosome may be transferred to a recipient’s cell. This differs from transformation and transduction, in which only relatively small chromosomal fragments may be, transferred. Studies of conjugation in *E. coli* have revealed that this bacterium has two different mating types: a donor and a recipient. The ‘donor’ cells contain a plasmid called the F plasmid (‘F’ stands for fertility). Like most plasmids, this F plasmid is a small, circular piece of double-stranded DNA that is not part of the bacterial chromosome and can replicate independently. It contains about 40 genes that control the plasmid’s replication and the synthesis but the host cell of a filamentous appendage called the sex pilus. Cells containing the F plasmid are referred to as ‘F’ cells and are donors in mating. Recipient cells lacking the ‘F’ plasmid are called F- cells. When F+ and F- cells are mixed together in what is termed an F+ (x) F- cross, the end of the F+ sex pilus binds to a nearby F- cell and then retracts, pulling the F+ and F- cells into close contact. A channel is formed between the two cells, through which transferred one is DNA strand from the donor’s F plasmid to the F- cell. Once inside the recipient’s cell, the DNA strand acts as a template for the synthesis of a second, complimentary DNA strand. The end of the double stranded DNA molecule then joins to form a circular F plasmid and the recipient cell has become an F+ cell capable of donating DNA. In this way, the conjugation process can continue until all the F-cells in the culture are converted. Whilst DNA transport readily occurs at the conjugational junction, there is no general mixing of the cytoplasmic contents of conjugating bacteria. Only a single strand of DNA is transferred. The single strand is produced when the plasmid is nicked at the specific origin of transfer (oriT) site. Unwinding of the duplex by one or more DNA helicases following this, a single strand of DNA is then progressively displayed 5’ to 3’ and transported into the recipient. When transfer is complete, the F factor is re-circularized in the recipient and a complementary strand synthesized. Transfer can proceed until cell contact is interrupted or until a break in the DNA or the 3’ end of oriT is reached. As transfer of the single strand proceeds 5’ to 3’, the F genes are transferred, the recipient will not become F+. The features of F factor transfer appear to be characteristics of other conjugative transfer systems in Gram-negative bacteria. Chromosomal genes can be transferred along with the F plasmid, but this is a rare event, occurring only 1 in 10 million mating.

**β-Lactamases**

A number of different β-lactamases have been described, but all share the feature of catalysing the ring - opening of the β-lactam moiety. Thus, the structural homology with the terminal d-Ala-d-Ala of maturing peptidoglycan, shared by all β-lactam antibiotics, is lost. β-lactamases may be chromosomal or plasmid-borne, inducible or constitutive, and for this reason their terminology can be confusing. A number of classiﬁcation systems have been proposed, including classes A– D based on peptide sequence. Classes A, C and D have a serine at the active site, whereas class B enzymes have four zinc atoms at their active site and these are also called metallo-β-lactamases. Class A enzymes are highly active against benzylpenicillin; class B β-lactamases are effective against penicillins and cephalosporins. Class C enzymes are usually inducible, but mutation can lead to overexpression. Class D consists of the OXA-type enzymes, which can hydrolyse oxacillin. Increasing resistance to β-lactam agents, mainly by β-lactamase, prompted the discovery and introduction of agents with greater β-lactam stability such as cephalosporins, carbapenems and monobactams. Resistance ﬁrst appeared in organisms such as *Enterobacter cloacae* and *Pseudomonas aeruginosa*, due to mutations causing overproduction of the class C chromosomal AmpC β-lactamase. Subsequently, in the late 1980s, resistance occurred in organisms such as *Klebsiella pneumoniae* and *E. coli* that lack an inducible AmpC enzyme. Resistance was found to be mediated by plasmids encoding extended-spectrum β-lactamases (ESBLs). These arose from mutational development of more limited-spectrum β-lactamases such as TEM and SHV that either increased the size of the active-site pocket or altered its binding characteristics to allow the larger cephalosporins to enter and be broken down. TEM derivatives predominate, possibly favored by the use of ceftazidime and other slowly penetrating cephalosporins. These mutations also increase the binding of clavulanic acid and so these ESBLs remain susceptible to inhibition by this and other β-lactamase inhibitors such as sulbactam and tazobactam, which are generally ineffective against class C β-lactamases.

In December 2009, the ﬁrst report of a carbapenemase β-lactamase, referred to as New Delhi metallo-β-lactamase (NDM - 1), was recorded. It was discovered in a carbapenem-resistant *K. pneumoniae* strain isolated in Sweden from a Swedish national who acquired the infection in India. The enzyme is one of class of B metallo-β-lactamases and is conferred by the gene *bla* NDM-1. This is considered a serious threat to the carbapenem family of antibiotics.

**β-Lactamase inhibitors** In addition to introducing agents with increased stability to β-lactamase inhibition, β-lactamase inhibitors including clavulanic acid, sulbactam and tazobactam have been developed. Clavulanic acid is produced by a streptomyces and is a suicide inhibitor of β-lactamases from a number of Gram-negative and Gram-positive organisms. These β-lactamase inhibitors do not have any signiﬁcant antimicrobial activity against bacterial transpeptidases, but their combination with a β-lactam antibiotic has extended the clinical usefulness of the latter.



**Multiple drug resistance (R-factors)**

Of equal concern are instances where isolates can become resistant to multiple, chemically distinct agents in a single biological event. One of the earliest examples was in Japan in 1959. Previously sensitive *E. coli* became resistant to multiple antibiotics through acquisition of a conjugative plasmid (R - factor) from resistant *Salmonella* and *Shigella* isolates. A number of R – factors have now been characterized including RP4, encoding resistance to ampicillin, kanamycin, tetracycline and neomycin, found in *Ps. aeruginosa* and other Gram – negative bacteria; R1, encoding resistance to ampicillin, kanamycin, sulphonamides, chloramphenicol and streptomycin, found in Gram - negative bacteria and pSH6, encoding resistance to gentamicin, trimethoprim and kanamycin, found in *Staph. aureus*.