Bacterial Resistance of Antibiotics- I

Bacterial resistance to antibiotics has been recognized since the first drugs were introduced for clinical use. The sulphonamides were introduced in 1935 and approximately 10 years later 20% of clinical isolates of *Neisseria gonorrhoeae* had become resistant.

Penicillin was first used in 1941, when less than 1 % of *Staphylococcus aureus* strains were resistant to its action. By 1947, 38% of hospital strains had acquired resistance and currently over 90% of *S. aureus* isolates are resistant to penicillin. Equally, it is well recognized that certain bacteria are unaffected by specific antibiotics. In other word, these bacteria have always been antibiotic-resistant.

Intrinsic and acquired resistance

Antibiotic resistance is classified into two broad types: intrinsic and acquired.

1- **Intrinsic resistance**. This suggests that inherent properties of the bacterium are responsible for preventing antibiotic action. This type of resistance is also termed **innate**. There are many antibiotics active against Gram-positive bacteria which have no effect on Gram-negative bacteria and vice versa. This intrinsic resistance is thought to be associated with the outer cell layers, such as the outer membrane, which are absent in Gram-positive cells. The Gram-negative cell envelope is effectively impermeable, preventing certain antibiotics from reaching their intracellular targets.

2- **Acquired resistance**. This occurs when bacteria which were previously susceptible become resistant, usually, but not always, after exposure to the antibiotic concerned.

Intrinsic resistance is always **chromosomally mediated**, whereas acquired resistance may occur by **mutations in the chromosome or by the acquisition of genes coding for resistance from an external source normally via a plasmid or transposon.**

Genetic basis of acquired resistance

Three genetic elements are responsible for acquired resistance: **chromosomes, plasmids and transposons**.

1- ***Chromosomal mutations***

Resistance to certain antibiotics can arise as a consequence of mutations to chromosomal genes because of changes in the DNA sequence. Mutations can occur due to single base pair changes. **Transitions** involve the substitution of one purine (A or G) for another and therefore one pyrimidine (C or T) for another. **Transversions** involve a change from a pyrimidine to a purine and vice versa. **Frameshift mutations** occur when one or two bases are inserted into the DNA sequence, resulting in an altered reading frame and therefore an altered gene product.

More extensive changes in the DNA sequence (often referred to as **macrolesions**) can also occur. **Deletions** result in the loss of part of the DNA sequence. **Insertions** add extra base pairs to a gene. **Transversions** occur when a segment of the DNA is reversed and duplications occur when a segment of the DNA is repeated. Some of these changes also result in frameshifts.

2- ***Plasmids***

The bacterial chromosome contains all the genes necessary for the growth and replication of cells. Many, if not most, bacteria also possess additional circular elements of DNA which are capable of replicating and transferring independently of the chromosome. These extrachromosomal

genetic elements are known as **plasmids** and *can code for a number of properties including antibiotic resistance.*

Plasmids have the ability to transfer within and between species and can therefore be acquired from other bacteria as well as a consequence of cell division. This property makes plasmid-acquired resistance much more threatening in terms of the spread of antibiotic resistance than resistance acquired due to chromosomal mutation. Plasmids also harbor transposons which enhances their ability to transfer antibiotic resistance genes.

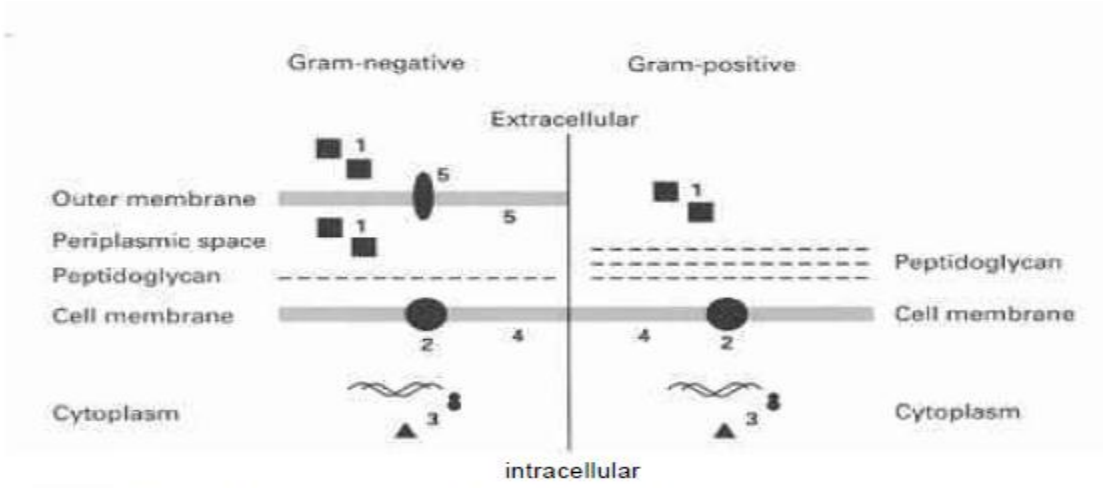
Transduction and transformation are generally limited to the same or related species and are therefore not effective as a means of antibiotic resistance transfer across species boundaries. However, our knowledge of transformation in nature is limited, and the significance of this mechanism of gene transfer is unknown.

3- ***Transposons***

Transposons are mobile genetic elements capable of transferring or transposing independently from one DNA molecule to another. The DNA molecules may be chromosomes or plasmids. Transposons are normally flanked by short regions of almost identical DNA sequence known as repeats. The central region of the transposon often codes for antibiotic resistance genes. The ability of transposons to mobilize from one DNA molecule to another has led to them being referred to as **jumping genes**. Transposons do not require homologous regions of DNA in order to integrate into a DNA molecule unlike the normal recombination process in bacterial cells and are **therefore a major cause of the transfer and spread of antibiotic resistance genes among different bacterial species**. Furthermore, it is possible for bacteria to acquire a series of transposons coding for different antibiotic resistances by insertion in existing plasmids or the chromosome.

Biochemical mechanisms of resistance

The following sections describe the biochemical mechanisms of resistance to different classes of antibiotics, with the antibiotics grouped according to their mechanism of action.



Schematic representation of possible mechanisms of resistance in Gram-negative and Gram-positive bacteria. 1, antibiotic-inactivating enzymes; 2, antibiotic efflux proteins; 3, alteration or duplication of intracellular targets; 4, alteration of the cell membrane reducing antibiotic uptake; 5, alterations in porins or lipopolysaccharide reducing antibiotic uptake or binding.

Inhibitors of nucleic acid synthesis

Antibiotics considered here can be divided into two mechanisms of action: those which **(1)inhibit nucleotide metabolism** and those which **(2)inhibit enzymes involved in nucleic acid synthesis**.

Sulphonamides

Two mechanisms of chromosomal resistance have been identified. A mutation of **dihydropteroate synthetase (DHPS)** in *Strp. pneumoniae* produces an altered enzyme with reduced affinity for sulphonamides. **Hyperproduction of *p*-amino benzoic acid (PABA)** overcomes the block imposed by inhibition of DHPS. The specific cause of PABA hyperproduction is unknown, though chromosomal mutation is the probable cause.

Duplication of DHPS, with the second version of the enzyme being resistant to the sulphonamides, is the cause of plasmid-acquired resistance. **Two different enzymes have been identified, both with lowered affinity for the antibiotic.**

Trimethoprim

Chromosomal mutations in *E. coli* result in overproduction of **dihydrofolate reductase (DHFR)**. Higher concentrations of trimethoprim, which may not be therapeutically achievable, are therefore required to **inhibit nucleotide metabolism**. Other mutations lower the affinity of DHFR for trimethoprim. These two mechanisms of resistance may coexist in a single strain, effectively increasing the level of resistance to the antibiotic.

Quinolones

The quinolones exert their action by binding to **DNA gyrase (bacterial topoisomerase II)** and inhibiting its functions. Acquired resistance to the quinolones arises due to chromosomal mutations in the genes coding for DNA gyrase. Levels of resistance can be increased by the presence of multiple mutations with a region of the *gyr*A gene known as the quinolone resistance determining region. The exact mechanism of resistance is unknown but is thought to involve a subtle conformational change in DNA gyrase which reduces binding of quinolones.

Other chromosomal mutations resulting in quinolone resistance have been found to **decrease permeability** of the antimicrobial agent.

Rifampicin

Resistance to rifampicin is primarily due to chromosomal mutations resulting in an **altered RNA polymerase** which is less well inhibited by the drug. The mutations tend to be clustered within short conserved regions of the β subunit gene of RNA polymerase.

Inhibitors of protein synthesis

Inhibition of protein synthesis is the antibacterial mechanism shared by most groups of antibiotics, though the exact action differs.

***Aminoglycoside-aminocyclitol group*(AGAC)**

Alteration of the antibiotic molecule is plasmid- or transposon-encoded. Three classes of enzyme can alter the AGAC molecule. **Aminoglycoside adenylyltransferases (AADs)** use adenosine triphosphate (ATP) as a cofactor in modifying certain hydroxyl groups in the antibiotic molecule by adenylylating them. **Aminoglycoside phosphotransferases (APH)** also use ATP to modify certain hydroxyl groups by phosphorylating them. **Aminoglycoside acetyltransferases (AACs)** use acetyl CoA as a cofactor and acetylate susceptible amino groups on the molecule. These three classes of enzyme have been further subdivided according to which site on the AGAC molecule is modified. For example, APH(6) phosphorylates the 6-hydroxyl group on the aminohexose group of streptomycin. Most AGAC antibiotics are susceptible to more than one modification reaction. Relatively small amounts of the antibiotic are modified, implying that resistance is determined by the relative rates of drug uptake and modification. A less efficient modifying enzyme will permit unmodified antibiotic to reach its ribosomal quantity. A more efficient enzyme, or greater quantities of the enzyme, will result in resistance.

AGAC-modifying enzymes are active outside the cytoplasmic membrane, in the periplasmic space in Gram-negative bacteria and extracellularly in Gram-positives.

A second mechanism of resistance to the AGACs involves an **alteration of the ribosomal target site**. Mutations in the gene coding for ribosomal protein S12 *(rpsL* in *E. coli)* prevent the antibiotics from binding to their target. In mycobacteria, which possess only one ribosomal RNA

operon, mutations in *rpoB*, coding for 16S rRNA, also inhibit binding of the drugs.

Tetracyclines

Plasmid- or transposon-encoded tetracycline efflux proteins. These efflux proteins are thought to span the cytoplasmic membrane and are dependent on the proton-motive force for their action. It is thought that **the efflux proteins bind tetracyclines and initiate proton transfer**, although no functional domains have been identified. Eight distinct tetracycline efflux proteins have been identified thus far.

Plasmid- or transposon-encoded ribosomal protection factors are a second mechanism of resistance to the tetracyclines. *These proteins are believed to alter the tetracycline binding site on the 30S ribosomal subunit, lowering the affinity for the drugs*.

Chloramphenicol

Plasmid- or transposon-encoded **chloramphenicol acetyltransferases (CATs)** are responsible for resistance by inactivating the antibiotic. **CATs convert chloramphenicol to an acetoxy derivative which fails to bind to the ribosomal target**.

Three other mechanisms of chloramphenicol resistance have been described. **First**, a transposon-encoded chloramphenicol efflux protein has been identified in *E. coli*. **Second**, some bacterial strains have been found to possess drug-resistant ribosomes, and **third**, low level resistance can arise by chromosomal mutations which reduce the amount of porins and therefore impair uptake. This last mechanism is essentially that described for the AGAC antibiotics.

Fusidic acid

Gram-negative bacteria are intrinsically resistant to low levels of fusidic acid (a steroid) **due to exclusion by the outer membrane**. Nevertheless, acquired resistance does occur which has the effect of increasing the level

of resistance to the antibiotic. Acquired resistance also occurs in Gram-positive bacteria normally susceptible to fusidic acid.

Plasmid-mediated resistance in Gram-positive bacteria is thought to **involve decreased uptake of the drug**, although the precise mechanism is unknown. Resistance to fusidic acid in Gram-negative bacteria is also poorly understood.

Chromosomal mutations have also been described which produce a modified translocation factor protein with lowered affinity for fusidic acid.

will become largely ineffective against bacterial infections