Antibiotics and antibiotic resistance

Antibiotics are antibacterial biological substances produced by other microorganisms (actinomycetes/fungi) whereas chemotherapeutic agent is synthetically produced. Antibiotics such as chloramphenicol and erythromycin are derived from other bacteria or fungi. Some of the antibiotics are chemically modified to improve the therapeutic range or pharmacodynamic properties. Originally purified from the cultures of bacteria or fungi, antibiotics are now synthetically manufactured. The term antimicrobial agent is now preferred to include both modified biological products and chemically synthesized biological products.

Antimicrobial drugs are either bactericidal or bacteriostatic; the former kills the bacteria whereas the latter only inhibits its multiplication. Bactericidal drugs are preferred over bacteriostatic drugs as the cessation of therapy with the latter may lead to renewed multiplication and relapse of infection. Bacteriostatic drugs often rely on individual's immune response to eradicate bacteria. These drugs should not be used where effective immune response does not occur (endocardium or meninges). Examples of bactericidal drugs include penicillin, ceftriaxone, and gentamicin. Examples of bacteriostatic drugs include chloramphenicol and tetracycline.

Some antimicrobial agents have narrow range of activity or active on specific bacterium, others have broad-spectrum range of activity. Examples of the former include erythromycin, azithromycin, clindamycin, and vancomycin whereas; the examples of the latter include cefotaxime, ciprofloxacin, and amikacin.

While most antimicrobial agents are effective when administered alone, sometimes a combination of antimicrobial agents provide a synergistic effect. Penicillin with gentamicin is used to treat enterococcal endocarditis. A combination of bacteriostatic and a bactericidal drug may not be beneficial and instead my antagonize each other. Sometimes, another compound may be tagged to the primary antimicrobial agent to nullify antibiotic degrading enzymes. For example, clavulanic acid is used to protect amoxicillin from degradation by beta-lactamase enzyme produced by bacteria. Quinupristin and dalfopristin are bacteriostatic by themselves but the combination of the two is bactericidal.

The term cross resistance implies that a single mechanism confers resistance to multiple antimicrobial agents. This is usually seen with closely related antimicrobial drugs or that have a similar mode of binding or action. In case of multi-drug resistant isolates, resistance to multiple antibiotics are often due to different mechanisms. A single plasmid may harbour resistant genes conferring resistance to 7-8 different antibiotics.

Minimum inhibitory concentration (MIC) of an antibiotic refers to the least amount of that drug required to inhibit visible microbial growth. Whereas, minimum bactericidal concentration (MBC) is the least amount of drug required to kill 99.9% of bacteria. MBC values are usually 1-2 dilutions more than MIC values. The drug will be effective only if the MIC value obtained in vitro is achievable in vivo at the site of infection.

Mechanism of action:

Since bacterial metabolism is different in many ways from the human metabolism, their metabolic pathways are chosen as targets for inhibition by antimicrobial agents. This way, the antimicrobial agents offer selective toxicity. Penicillins and related compounds inhibit peptidoglycan synthesis, but since such a metabolic pathway is non-existent in humans, the antibiotic agent poses no toxicity (excluding hypersensitivity and other drug reactions). Antibiotics can be functionally classified on the basis of their activity or site of action in the following way:

A. Action on cell wall /cell wall synthesis
B. Action on cytoplasmic membrane/ membrane lysis
C. Inhibition of protein synthesis
D. Inhibition of nucleic acid synthesis

A. Action on cell wall

Bacterial cell wall is made of peptidoglycan, which has to be constantly built and rebuilt. Enzymes involved in this process are good target for antimicrobial agents. Various classes of antibiotics involved in inhibition of cell wall synthesis include:

a. Penicllins and cephalosporins

The transpeptidation enzymes of bacteria are involved in cross linking of peptidoglycan molecules during cell wall synthesis. Penicillin and related antibiotics have molecular structure that is analogous with d-alanyl-d-alanine of the peptide side chain. These enzymes, which are also known as penicillin binding proteins (PBP), mistakenly bind to antibiotics resulting in inhibition of the enzyme. Inhibitor of autolysis too is inactivated, leading to cell lysis.

b. Bacitracin

During the peptidoglycan synthesis, sugar-peptide units are transported to the ends of peptidoglycan chain by a lipid carrier molecule. After the transfer, the carrier molecule is dephosphorylated so that it can participate in the transportation again. But, bacitracin prevents this dephosphorylation reaction and interrupts peptidoglycan synthesis.

c. Glycopeptides

Vancomycin and teicoplanin have a cleft into which d-alanyl-d-alanine of peptide side chain bind stably. This prevents formation of cross-linkages between two peptidoglycan chains and ultimately weakens the cell.

d. Monobactams

Aztreonam has very high affinity for penicillin-binding protein 3 (PBP-3) and mild affinity for PBP-1a. It inhibits peptidoglycan synthesis in the bacterial cell wall, thereby blocking peptidoglycan cross-linking.

B. Action on cytoplasmic membrane

Integrity of cytoplasmic membrane is vital for bacterial survival. By binding to Lipid A moiety of the gram negative outer cell membrane's lipopolysaccharide, polymyxin B disrupts the membrane's function. Polymyxin B is detergent-like cyclic peptide. In gram positive cell, the cytoplasmic membrane is not accessible; hence this drug is effective against gram negative cells only. However, it is also able to disrupt mammalian cell membranes, making it slightly toxic. Other antimicrobial agent such as daptomycin, binds to cell membrane in a calcium dependent manner and causes depolarization of bacterial membrane potential resulting in release of potassium ions.

C. Inhibition of protein synthesis

Ribosomes are the sites of protein synthesis; while mammalian ribosomes are 80S, bacterial ribosomes are 70S. This makes selective toxicity easier to achieve.

a. Aminoglycosides

Antibiotics such as streptomycin, kanamycin, gentamicin, tobramycin, amikacin, neomycin etc bind to 16S rRNA of the 30S subunit thereby blocking the 30S initiation complex (30S-mRNA-tRNA). Besides, they also they slow down protein synthesis that has already initiated and induce misreading of mRNA.

b. Macrolides

All macrolides such as erythromycins, azithromycin, clarithromycin, and roxithromycin inhibit bacterial protein synthesis by binding to the 50S ribosomal subunit with a specific target in the 23S ribosomal RNA molecule. By doing so, they may interfere with formation of initiation complexes for peptide chain synthesis or may interfere with aminoacyl translocation reactions.

c. **Lincosamides** (e.g. lincomycin, clindamycin) bind to the 23S portion of the 50S subunit of bacterial ribosomes and cause premature dissociation of the peptidyl-tRNA from the ribosome.

d. **Tetracycline** binds to 30S ribosome and inhibit binding of aminoacyl-t-RNA to the acceptor site on the 70S ribosome thereby prevent the introduction of new amino acids to the nascent peptide chain.

e. **Chloramphenicol** binds to the 50S subunit of the ribosome and inhibits peptidyl transferase, thereby it interferes with the binding of new amino acids to the nascent peptide chain.

f. Streptogramin

Dalfopristin binds to 23S portion of 50S ribosomal subunit and changes its conformation, thereby allowing the binding of quinupristin. Quinupristin and dalfopristin act synergistically to inhibit peptide chain elongation.

D. Inhibition of nucleic acid synthesis

These antimicrobials exploit the differences in RNA/DNA synthesis mechanism to exert selective toxicity.

a. **Quinolones** Antimicrobials such as nalidixic acid, ciprofloxacin, ofloxacin, pefloxacin etc bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA replication and transcription.

b. **Rifampin** inhibits bacterial growth by binding strongly to the DNA-dependent RNA polymerase of bacteria thereby inhibiting bacterial RNA synthesis.

c. For several bacteria, p-aminobenzoic acid (PABA) is an essential metabolite for the synthesis of folic acid, which is an important precursor to the synthesis of nucleic acids. Sulphonamide, being similar in structure to PABA, binds to dihydropteroate synthetase (DHPS) and inhibits it. DHPS catalyses the conversion of PABA to dihydropteroate, a key step in folate synthesis. Trimethoprim inhibits dihydrofolate reductase and prevents reduction of dihydrofolic to tetrahydrofolic acid; thereby block further production of purines. A combination of sulfonamide (five parts) plus trimethoprim (one part) is often used (co-trimoxazole).

Mechanism of antimicrobial drug resistance

Some bacteria are naturally resistant to certain antimicrobial agents and their resistance is irrespective of the introduction of antimicrobial agent. Such a resistance is said to be intrinsic. Examples include ampicillin/cephalosporin resistance in Enterococcus and aminoglycoside resistance in non-sporing anerobes. Most other bacteria develop resistance due to mutation following exposure to antimicrobial agents or receive resistant property passively from resistant organisms. Such resistance is called acquired resistance. It is the latter that is more worrisome and antimicrobial agents previously known to be effective are turning out to be ineffective. Mutations in bacteria occur during DNA replication all the time. Spontaneous mutations occur in bacteria with a frequency of 10^{-12} to 10^{-7}. Antimicrobial agents don't induce mutations but are known to exert pressure on bacteria to acquire resistance. Those mutations that allow the bacterial cell to survive the antimicrobial onslaught are positively selected and then get propagated in the population. Once acquired, these mutant genes can be transferred to plasmids or transposons and eventually get disseminated. Mutations usually confer resistance to antimicrobials by way of loss of, altered or novel target proteins, which bind to the antimicrobials with lesser affinity. Mutations can affect genes present on chromosomes or plasmids.

Self transferable plasmids that have genes coding for antimicrobial resistance are called R factor. Plasmids may have resistance determinants to one or several different antimicrobials. Possession of such plasmids renders the bacterium multi-drug resistant. These are shared among bacteria by conjugation. Besides conjugation, genes coding for antibiotic resistance can be transferred to other bacteria by way of transduction and transformation. Transposons help the genes to move from chromosome to plasmid, from where they can be transferred to other cells by conjugation.

Bacteria have evolved in various ways to counter the toxic properties of antimicrobial agents. These are the following:

i. Inactivation of the antimicrobial drug

Resistance to penicillin and cephalosporin antibiotics in many bacteria is due to production of an enzyme beta-lactamase, which inactivates the antimicrobial agent. Genes encoding beta-lactamases may be present on chromosome or plasmid. These genes may be constitutively expressed or induced by a beta-lactam antibiotic. Such form of resistance is seen in *S. aureus, H. influenzae, E. coli, K. pneumoniae* etc. Aminoglycoside modifying enzymes destroy the drug by adenylylating, phosphorylating, or acetylating them. The genes encoding for aminoglycoside modifying enzymes are usually found on plasmids and transposons. This type of resistance is seen in members of Enterobacteriaceae, *Acinetobacter sps, Pseudomonas aeruginosa, S. aureus, Campylobacter jejuni* etc.

ii. Alteration of the antimicrobial target

As a result of mutation, the targets of the antimicrobial agents get lost or altered. Sometimes, the existing target may be replaced by an entirely novel protein. Resistances to penicillins/cephalosporins in MRSA, *S. pneumoniae* or enterococci are often to due to production of altered/novel penicillin binding proteins.

Methylation of 23S ribosomal RNA renders the receptor on 50S subunit altered, thereby preventing the binding of erythromycin. Mutations in the 30S subunit of the ribosome interfere with ribosomal binding of streptomycin.

Methylation of a single adenine in the bacterial 50s ribosome can lead to resistance against macrolides, lincosamides, and streptogramin B in *S. aureus* and *S. pneumoniae.*

iii. Adaptation of alternative metabolic pathway

Some sulfonamide-resistant bacteria do not require extracellular PABA but, like mammalian cells, can utilize preformed folic acid. A mutational loss in bacteria make them dependent on an external supply of thymine, which contributes to trimethoprim resistance. A mutational change

in *H. influenzae* results in overproduction of dihydrofolate reductases, leading to trimethoprim resistance.

iv. Active efflux pumping out of the drug

Mutations in certain bacteria permit the over-expression of the efflux-pump protein. Sometimes, an amino acid substitution in the efflux-pump protein makes it more efficient at export of the drug. In either case, the intracellular antibiotic concentration is decreased and the bacterium becomes less susceptible to that antibiotic. This kind of resistance is seen to chloramphenicol (*P. aeruginosa, K. pneumoniae, E. coli, S. typhimurium, V. cholerae*), macrolides (*Streptococcus pneumoniae, Enterococcus sps, Bacteroides sps, Pseudomonas sps* and Enterobacteriaceae members), tetracyclines (*S. aureus, E. coli, A. baumannii, S. typhimurium*), aminoglycosides (*E. coli, P. aeruginosa, A. baumannii*) and beta-lactams (*H. influenzae, P. aeruginosa, A. baumannii*).

v. Decreased permeability of the drug

Some strains of *P. aeruginosa* and other gram-negative bacilli exhibit aminoglycoside resistance due to a transport defect or membrane impermeabilization. Resistance to cefoxitin in *E. coli* and *K. pneumoniae* is due to mutations leading to narrowed outer membrane proteins.

Transfer of antimicrobial drug resistance

Spontaneous mutations that are selected favouring antimicrobial resistance are stably inherited by daughter cells following cell division. Such genes can escape from the chromosome into plasmids, transposons, or integrons. Once present in mobile genetic elements, they can be disseminated into similar or dissimilar species. Conjugation, transduction and transformation are the mechanisms by which resistant genes are shared among bacteria.

Characteristics of different elements involved in resistance gene spread*

Element	Characteristic	Role in spread of resistance genes
Self-transmissible plasmid	Circular, autonomously replicating element; carries genes needed for conjugal DNA transfer	Transfer of resistance genes; mobilization of other elements that carry resistance genes
Conjugative transposon	Integrated elements that can excise to form a non-replicating circular transfer intermediate; carries genes needed for conjugal DNA transfer	Same as self-transmissible plasmid
Mobilizable plasmid	Circular, autonomously replicating element; carries gene that allows it to use conjugal apparatus provided by a self-transmissible plasmid	Transfer of resistance genes
Transposon	Can move from one DNA segment to another within the same cell	Can carry resistance genes from chromosome to plasmid or vice versa
Gene cassette	Circular, nonreplicating DNA segments containing only open reading frames; integrates into integrons	Carry resistance genes
Integron	Integrated DNA segment that contains an integrase, a promoter, and an integration site for gene cassettes	Forms clusters of resistance genes, all under the control of the integron promoter

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Detection of antimicrobial drug resistance

It is vital to test for antimicrobial resistance in pathogenic isolates obtained from infected patients where the organism is known to harbour resistance or susceptibility pattern is unpredictable. There are several ways of testing antimicrobial drug resistance; these include the following:

A. Phenotypic

Phenotypic methods consist of detection or estimation of phenotypic expression of resistance to one or several antimicrobial drugs. These include agar screen, disk diffusion, MIC determination and automated methods.

B. Genotypic

Genotypic methods rely on detection of genes or nucleotide sequences responsible for coding antimicrobial resistance. Nucleotide sequences specific to resistance genes can be detected by DNA hybridization, nucleic acid amplification (PCR) technique or by microarray techniques.