10. Mechanism of action of antibiotics

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Introduction

Antibiotics and similar drugs, together called antimicrobial agents, have been used for the last 70 years to treat patients who have infectious diseases (CDC, 2017). Antimicrobials are probably one of the most successful forms of chemotherapy in the history of medicine. Antibiotics are drugs of natural or synthetic origin that have the capacity to kill or to inhibit the growth of micro-organisms (FAO, 2005). Such chemical agents have been present in the environment for a long time, and have played a role in the battle between man and microbes. In the last century, the discovery of new antibiotics revolutionized the treatment of infectious diseases, leading to a dramatic reduction in morbidity and mortality, and contributing significantly to improvements in the health of the general population (FAO, 2005). Antibiotics can be classified based on the cellular component or system they affect, in addition to whether they induce cell death (bactericidal drugs) or merely inhibit cell growth (bacteriostatic drugs) (Kohanski *et al* 2010). Antibiotics that inhibit growth with no loss of viability are called bacteriostatic drug. Where as antibiotics that inhibit growth and kill bacteria upon exposure are called bacteriocidal drugs. Since the discovery of penicillin was reported in 1929 (Fleming A, 1929) other, more effective antimicrobials have been discovered and developed by elucidation of drug-target interactions, and by drug molecule modification.

Antibiotics as growth promoters have got succeeded widely in all the fields of science. The feeding of antibiotics is associated with decreases in animal gut mass, increased intestinal absorption of nutrients and energy sparing. This results in a reduction in the nutrient cost for maintenance, so that a larger portion of consumed nutrients can be used for growth and production, thereby improving the efficiency of nutrient use (FAO, 2005). Antibiotics act by eliminating the subclinical population of pathogenic microorganisms. Eradicating this metabolic drain allows more efficient use of nutrients for food production. Antibiotics alter the non-pathogenic intestinal flora, producing beneficial effects on digestive processes and more efficient utilization of nutrients in feeds. Antibiotics may prevent irritation of the intestinal lining and may enhance the uptake of nutrients from the intestine by thinning of the mucosal layer. Intestinal

bacteria inactivate pancreatic enzymes and metabolize dietary protein with the production of ammonia and biogenic amines. Antibiotics inhibit these activities and increase the digestibility of dietary protein. Bacteriostatic antibiotics include Tetracyclines, Spectinomycin, Sulphonamides, Macrolides, Chloramphenicol, Trimethoprim. Bactericidal antibiotics are Penicillins, Cephalosporins, Fluoroquinolones (Ciprofloxacin), Glycopeptides (Vancomycin), Monobactams, Carbapenems.

But no matter what class or kind of antibiotics exists or is discovered, all of them operate by one of the following mechanisms:

1). Inhibition of cell wall synthesis
2). Inhibition of protein synthesis
3). Inhibition of membrane function
4). Disruption of Metabolism
5). Inhibition of nucleic acid synthesis

1. The Cell Wall Synthesis Inhibitors

It includes those antibiotics which inhibit the synthesis of microbial cell wall (mostly bacteria, which possess cell walls). There are three mechanisms of inhibition of cell wall, and hence three classes of antibiotics in this regard:

a. Inhibition of peptidoglycan synthesis:

Beta-Lactams is the class of antibiotics that act by this mechanism. Examples of antibiotics in this class are Penicillins (Ampicillin, Amoxicillin, Methicillin etc), Cephalosporins, Monobactams, Carbapenems etc. The bacterial cell is encased by layers of peptidoglycan (PG, or murein), a covalently crosslinked polymer matrix composed of peptide-linked β-(1–4)-N-acetyl hexosamine (Bugg et al,1992). The mechanical strength afforded by this layer of the cell wall is critical to a bacterium's ability to survive environmental conditions that may alter prevailing osmotic pressures. In the other way, the degree of PG cross-linking can be correlated with the structural integrity of the cell (Holtje et al.,1993). β-lactams and glycopeptides are among the classes of antibiotics that interfere with specific steps in homeostatic cell wall biosynthesis. Successful treatment with a cell wall synthesis inhibitor can result in changes to cell shape and

size, induce cellular stress responses, and culminate in cell lysis (Tomasz A,1999). For example, β-lactams (including penicillins, carbapenems and cephalosporins) block the cross-linking of PG units by inhibiting the peptide bond formation reaction catalyzed by transpeptidases, which are also known as penicillinbinding proteins (PBP) (Holtje JV,1998; Wise EM,1965; Tipper DJ,1965).

b. Inhibitors/Disruptors of peptidoglycan cross-linkage:

Glycopeptide class of antibiotics act by this mechanism. Most common example includes Vancomycin. Other are teicoplanin, telavancin, bleomycin, ramoplanin etc. Most actinobacteria-derived glycopeptide antibiotics (e.g., vancomycin) inhibit PG synthesis through binding with PG units (at the D-alanyl-D-alanine dipeptide) and by blocking transglycosylase and transpeptidase activity (Kahne *et al,*2005). In this manner, glycopeptides (whether free in the periplasm like vancomycin or membrane-anchored like teicoplanin (Cooper *et al*,1999) generally act as steric inhibitors of PG maturation and reduce cellular mechanical strength, although some chemically-modified glycopeptides have been shown to directly interact with the transglycosylase enzyme (Ge M *et al* 1999). It is worth noting that β-lactams can be used to treat Grampositive and Gram-negative bacteria, whereas glycopeptides are effective only against Grampositive bacteria due to low permeability.

c. Disruptors of Precursor Movement:

This class of antibiotics blocks the movement of precursors required for peptidoglycan. Cyclic polypeptides like Bacitracin include such antibiotics. They are mostly used as ointments (topical use) because of their toxicity and poor bioavailability when taken through oral route.

2. **Inhibition of protein synthesis:**

The process of mRNA translation occurs over three sequential phases (initiation, elongation and termination) involving the ribosome and a host of cytoplasmic accessory factors .

(Garrett, 2000). The ribosome organelle is composed of two ribonucleoprotein subunits, the 50S and 30S, which organize (initiation phase) on the formation of a complex between an mRNA transcript, f-Met charged aminoacyl-tRNA, several initiation factors and a free 30S subunit (Nissen *et al*,2000). Drugs that inhibit protein synthesis are among the broadest classes of antibiotics and can be divided into two subclasses: the 50S inhibitors and 30S inhibitors.

50S ribosome inhibitors include the macrolide (e.g., erythromycin), lincosamide (e.g., clindamycin), streptogramin (e.g., dalfopristin/quinupristin), amphenicol (e.g., chloramphenicol) and oxazolidinone (e.g., linezolid) classes of antibiotics (Katz and Ashley, 2005). In general terms, 50S ribosome inhibitors work by physically blocking either initiation of protein translation (as is the case for oxazolidinones), or translocation of peptidyl-tRNAs, which serves to inhibit the peptidyltransferase reaction that elongates the nascent peptide chain. Studies on macrolide, lincosamide and streptogramin drugs have provided for a mode-of-action model that involves blocking the access of peptidyl-tRNAs to the ribosome (to varying degrees), subsequent blockage of the peptidyltransferase elongation reaction by steric inhibition, and eventually triggering dissociation of the peptidyl-tRNA. 30S ribosome inhibitors include the tetracycline and aminocyclitol families of antibiotics. Tetracyclines work by blocking the access of aminoacyl-tRNAs to the ribosome(Chopra and Roberts,2001). The aminocyclitol class is comprised of spectinomycin and the aminoglycoside family of antibiotics (streptomycin, kanamycin and gentamicin), which bind the 16S rRNA component of the 30S ribosome subunit.

In a overview, 30S Binders include Aminoglycosides like Gentamicin, Amikacin, Tobramycin etc come into this category which bind irreversibly to 30S subunit of ribosomes, Whereas 50S Binders can bind to 50S subunit in following ways:

a. Binding to peptidyl transferase

- Some antibiotics bind to the peptidyl transferase component of 50S ribosome therby blocking peptide elongation. Ex: Chloramphenicol

b. Inhibitors of amino acid-acyl-tRNA Complex binding

It includes those antibiotics which bind to 50S subunit in such a way that they block the binding of amino acid-acyl-tRNA complex and hence inhibit peptidyl transferase action and hence peptide elongation. Ex: Clindamycin.

c. Reversible Binders

These bind to 50S subunit in a reversible manner to temporarily block peptide elongation (hence these are bacteriostatic). Example: Macrolides like Azithromycin, Erythromycin, Roxithromycin, Clarithromycin etc

d. t-RNA binding blockers

This class of antibiotics block the binding of tRNA to 30S ribosome-mRNA complex. Tetracyclines like doxycycline, minocycline, plain tetracycline etc.

3. The Disruptors of Membrane Function

There are the class of antibiotics that render the microbial cell membranes disfunctional by inducing random pores by detergent like activity. This leads to the disruption of osmotic balance causing leakage of cellular molecules, inhibition of respiration and increased water uptake leading to cell death. Gram-positive bacteria possessing a thick cell wall are naturally resistant to such antibiotics.

Example: Lipopeptides like Polymyxins belong to this call of antibiotics.

4. The Disruptors of Metabolism (Folate Pathway Inhibitors)

This class of antibiotics inhibit the pathway responsible for the synthesis of folic acid which is essential for the synthesis of adenine and thymine (important nucleic acids for DNA and RNA synthesis; thymine is not required for RNA though, but required for DNA). And, since humans don't synthesize folic acid, so these antibiotics donot have an inhibitory toxic effect on humans.

The folic acid synthesis inhibition can take place by:a. Inhibition of the enzyme dihydrofolate reductase required for folic acid synthesis.

Example: Trimethoprim/Sulfamethoxazole acts by inhibiting dihydrofolatereductase.

b. Substrate competition with p-aminobenzoic acid (PABA) thereby preventing synthesis of folic acid. Example: Sulfonamides & Dapsone.

5. Inhibition of nucleic acid synthesis

a. Inhibition of DNA replication by quinolones:

Modulation of chromosomal supercoiling through topoisomerase-catalyzed strand breakage and rejoining reactions is required for DNA synthesis, mRNA transcription and cell division (Espeli and Marians, 2004). These reactions are exploited by the synthetic quinolone class of antimicrobials, including the clinically-relevant fluoroquinolones, which target DNA-topoisomerase complexes. (Sugino *et al* 1977, Gellert M *et al,*1977). Quinolones are derivatives of nalidixic acid, which was discovered as a byproduct of chloroquine (quinine) synthesis and introduced in the 1960s to treat urinary tract infections. Nalidixic acid and other first generation quinolones (i.e., oxolinic acid) are rarely used today owing to their toxicity. Second (i.e., ciprofloxacin), third (i.e., levofloxacin) and fourth (i.e., gemifloxacin) generation quinolone antibiotics can be classified based on their chemical structure along with qualitative differences in how these drugs kill bacteria (Hooper, 2003). The quinolone class of antimicrobials interferes with the maintenance of chromosomal topology by targeting DNA gyrase (topoisomerase II) and topoisomerase IV (topoIV), trapping these enzymes at the DNA cleavage stage and preventing strand rejoining (Drlica and Zhao, 1997). DNA gyrases (Type II Topoisomerases) are responsible for relieving the positive supercoils in the DNA (or introducing negative supercoils) ahead of the moving DNA polymerase, thereby enabling the availability of relaxed DNA strands for continuation of replication, as well as the compaction (negative supercoiling) of the large strands of newly synthesized DNA to pack them in the bacterial cell. Some antibiotics form a stable complex with these DNA gyrases, thereby inhibiting the DNA replication. Example: Quinolones like Cinoxacin, Ciprofloxacin, Levofloxacin, Norfloxacin, Ofloxacin act by this way.

Table 1.Typical modes of action of antibiotics
Source: Pharmacology, 1997

Mechanism	Examples
Damage cell membrane, allowing contents to leak out. Bactericidal	Polymixins.
Inhibitors of bacterial cell wall synthesis.	Penicillins; aminopenicillins; cephalosporins (cephalexin); bacitracin (topical); vancomycin.
Inhibitors of folic acid synthesis. Folic acid is needed to make RNA and DNA for growth and multiplication, and bacteria must synthesize folic acid. Bacteriostatic.	Sulphonamides; sulfasalazine; trimethoprim; co-trimoxazole.
Inhibitors of DNA function. DNA is needed for cell growth and division. Most are bactericidal.	Nalidixic acid; ofloxacin; metronidazole; rifampin; enrofloxacin; sarafloxacin
Inhibitors of protein synthesis. Proteins are synthesized on cell structures called ribosomes. Bactericidal or bacteriostatic.	Tetracyclines;aminoglycosides; chloramphenicol; florfenicol; macrolides; spectinomycin; lincosamides.

b. **RNA inhibitors**:

This class of antibiotics block the initiation and thus the synthesis of RNA in microbial cells. Example: Rifampin and Rifabutin which bind to DNA-dependent RNA polymerase, thereby inhibiting the initiation of transcription. Rifamycins were first isolated from the Gram-positive bacterium, *Amycolatopsis mediterranei* (originally *Streptomyces mediterranei*) in the 1950's, (Sensi *et al* 1959) and mutagenesis of this organism has led to the isolation and characterization of more potent rifamycin forms, including the clinically relevant rifamycin SV and rifampicin. In general, rifamycins are considered bactericidal against Gram-positive bacteria and bacteriostatic against Gramnegative bacteria. The rifamycin group includes the "classic" rifamycin drugs as well as the rifamycin derivatives rifampicin (or rifampin), rifabutin, rifapentine, rifalazil and rifaximin. Rifamycin drugs inhibit DNA-dependent transcription by stable binding, with high affinity, to the subunit (encoded by the *rpoB* gene) of a DNA-bound and actively-transcribing RNA polymerase enzyme (Hartmann, 1967)

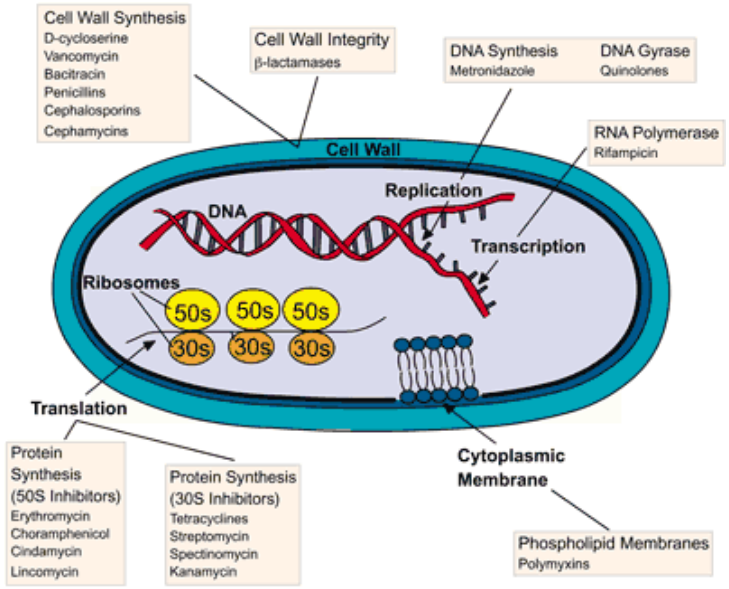

Fig a. Pictorial representation of mode of action of antibiotics on target

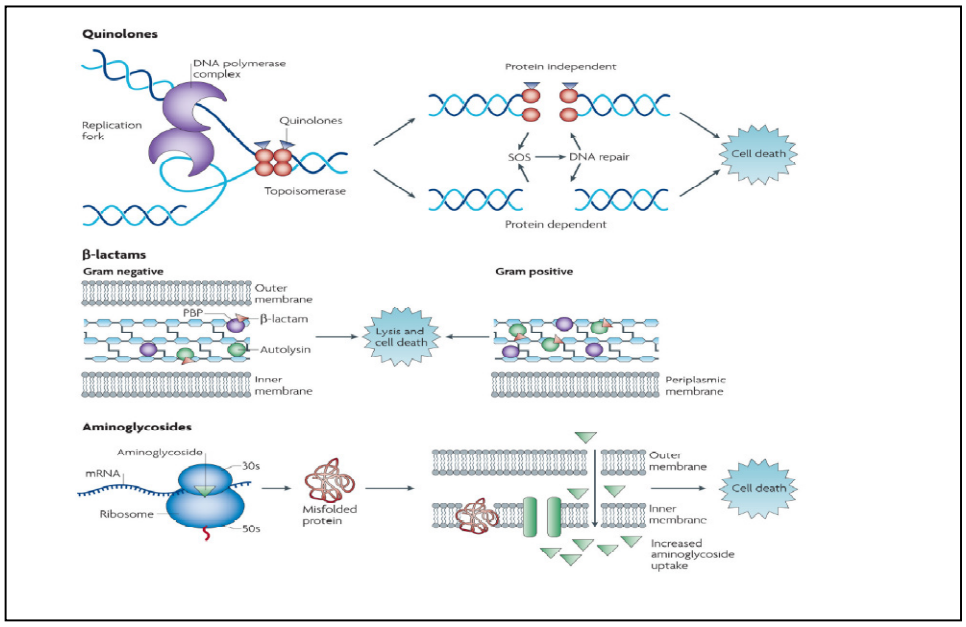

Fig b.Drug-target interactions and associated cell death mechanisms

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