EDO UNIVERSITY IYAMHO  
Department of Biochemistry  

BCH 415: Clinical Biochemistry and Pharmacology  

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General overview of the Course  

This course covers the theoretical basis of laboratory tests of clinical significance in diagnosis and management of diseases. Routine tests of general well being - renal function tests and liver function tests, Introduction to pharmacological concepts, Drug administration, absorption, distribution and receptors. Xenobiochemistry, Drug metabolism, Drug metabolizing enzymes and drug interactions. Role of Cytochrome P450. It also covers drug design, Theories of the mechanism of drug action. The physiological and biochemical actions of some selected drugs. Biochemical mechanisms of drug action against parasites. Metabolic factors affecting chemotherapeutic agents. Drug resistances and other factors affecting drug efficacy. Biochemical basis of drug resistance.  

Intended Learning Outcomes  

At the end of this aspect of the course, students should be able to  

1. Discuss the significance of biochemical tests; liver function test, kidney function test and other routine tests  
2. Explain the metabolism of drugs (xenobiotics)  
3. Understand the basics of drug design  
4. Know basic principles on the mechanism of drug action  
5. Understand the concept of drug resistance.  
6. Understand the role of traditional medicinal plants in the management of some common ailments.
Assignments: We expect to have 5 individual homework assignments throughout the course in addition to a Mid-Term Test and a Final Exam. Home works are due at the beginning of the class on the due date. Home works are organized and structured as preparation for the midterm and final exam, and are meant to be a studying material for both exams. There will also be 2 term papers are expected to be written by individuals taking this course. The is aimed at broadening students; knowledge of the course.

Grading: We will assign 10% of this class grade to home works, 10% for the term papers, 10% for the mid-term test and 70% for the final exam. The Final exam is comprehensive.

Textbooks: The recommended textbooks for this class are as stated:
Title: Lehninger Principles of Biochemistry
Authors: David L. Nelson, Michael M. Cox
Publisher: Addison-Wesley 2nd edition

Title: Textbook of Biochemistry for Medical Students (6th ed.).
Author: Vasudevan DM, Sreekumari S, Vaidyanathan K.
Publisher: Jaypee Brothers Medical Publishers (p) Plc

Title: A Textbook of Modern Toxicology.
Author: Edited by Ernest Hodgson (3rd ed.)
Publisher: Wiley Interscience

Title: Textbook of Medical Biochemistry
Author(s): MN Chatterjea and RanaShinde.
Publisher: Jaypee Brothers Medical Publishers Ltd, Eighth Edition
ISBN: 978-93-5025-484-4
Year: 2012
MAIN LECTURE

Biochemical Tests

Biochemical tests are conducted to assess the functionality of organs in the body. They are usually conducted on body fluids and tissues particularly, blood, urine. These tests include; liver function tests, kidney function tests and test of electrolyte balance. Liver function tests are of immense value in diagnosis and monitoring of liver diseases. LFTs are the most widely performed biochemical tests in the laboratory.

Liver Function Tests

These liver function tests are broadly classified as:
1. Tests to detect hepatic injury:
   a. To detect the disease, whether mild or severe; whether acute or chronic.
   b. To assess the nature of liver injury; hepatocellular or cholestasis.
2. Tests to assess hepatic function.

Necessity for liver function tests
1. Liver diseases
2. Any chronic diseases
3. Coagulation diseases
4. Therapies that have potential for hepatotoxicity

Classification of liver function tests

Broadly speaking, liver function tests can be classified into three groups
1. Test of excretory function of the liver
   i. Serum Ï Bilirubin; total, conjugated and unconjugated.
   ii. Urine Ï Bile pigments, bile salts and urobilinogen.
2. Test of liver enzyme activities. They are markers of liver injury/cholestasis
   i. Alanine amino transferase (ALT)
   ii. Aspartate amino transferase (AST)
   iii. Alkaline phosphatase (ALP)
   iv. Gamma glutamyl transferase (GGT)
3. Tests for synthetic function of liver
i. Total proteins
ii. Serum albumin, globulins, albumin: globulin (A:G) ratio
iii. Prothrombin time

Renal Function Tests
The kidneys are key in maintaining health. The kidneys perform important functions such as; excretion of metabolic waste products, maintenance of electrolyte balance, reabsorption of glucose, amino acids etc and hormone production. The kidneys perform these functions by its functional unit called nephron.

Classification of Renal Function Tests

I. To screen for kidney disease; complete urine analysis, plasma urea and creatinine, plasma electrolytes
II. To assess renal function
a. To assess glomerular function; glomerular filtration rate, clearance tests, glomerular permeability
b. To assess tubular function; secretion and reabsorption tests, concentration and dilution tests renal acidification

**Glomerular Filtration Rate**
Measurement of glomerular filtration rate (GFR) is an index for the assessment of the severity of renal damage. A decrease in the renal function is due to the loss of functional nephrons, rather than a decrease in the function of individual nephron. It is the product of filtration rate in single nephrons and the number of nephrons in both kidneys. Substantial kidney damage occurs before GFR is decreased.
It is also affected by age, sex, body size, protein intake and pregnancy. Normal GFR for young adults is 120-130 ml/minute/1.73 m2. GFR is constant in a normal individual, but may vary even with normal kidney function. A decline with age is significant and more than 25% of people older than 70 years may have a GFR less than 60 ml/minute. This may be due to decline with age
or any systemic disease that may be coexisting. Glomerular filtration rate cannot be measured directly, it is estimated from the clearance of a filtration marker.

**Renal Clearance**
Renal clearance is defined as the volume of blood or plasma completely cleared of a substance per unit time and is expressed as milliliter per minute. Clearance estimates the amount of plasma that must have passed through the glomeruli per minute with complete removal of that substance to account for the substance actually appearing in the urine.

Clearance= mg of substance excreted per minute/mg of substance per ml of plasma

It is calculated by using the formula:

\[ C = \frac{U \times V}{P} \]

where \( U \) = concentration of the substance in urine; \( P \) = concentration of the substance in plasma or serum and \( V \) = the ml of urine excreted per minute. It is expressed as ml/minute.

If the substance is freely filtered across the capillary wall, and neither secreted nor reabsorbed, then its clearance is equal to glomerular filtration rate. A substance which meets these requirements is an ideal filtration marker. If the substance is also secreted by the tubules, the clearance exceeds GFR. For those which are reabsorbed by tubules, clearance is less than GFR. Exogenous markers are inulin, 51Cr-labelled EDTA, 99Tec-labelled EDTA, etc. These are not used in clinical practice, since it involves administration of extraneous compounds. Endogenous markers are urea and creatinine. None of these markers are ideal, but creatinine is the best out of all of them

**Creatinine Clearance Test**
Creatinine is a waste product, formed from creatine phosphate. It is a spontaneous and non enzymatic process that depends on body muscle mass. Creatinine is not affected by diet, age or exercise. Men excrete more creatinine than women and children because of their smaller muscle mass. About 98% of creatine pool is in muscle. About 1.6% of creatine is converted to creatinine per day which is rapidly excreted. Its blood level does not significantly fluctuate in spite of its continuous production. This makes it an ideal substance for clearance test. Creatinine excretion is relatively constant.

Advantages of Creatinine Clearance Test
1. Less interference by extra-renal factors.
2. The blood level does not fluctuate in spite of continuous production. This allows collection of blood at any time.
3. Plasma creatinine is not affected by diet or exercise.

Disadvantages of Creatinine Clearance Test
1. Creatinine is filtered by glomeruli, and actively excreted by the tubules. When the GFR is reduced, the secretion component is increased, and will interfere with the results. The creatinine clearance is said to overestimate GFR by about 10-20 ml/mt.
2. Extra-renal excretion increases when GFR is significantly reduced.
3. Early stages of decrease in GFR may not be identified by creatinine clearance.
4. Other pre-renal, renal and post-renal causes can influence creatinine clearance

**Urea Clearance Test**
Urea clearance is the number of ml of blood which contains the urea excreted in a minute by kidneys.

The urea clearance is calculated by the formula $U \times V/P$
where $U = \text{mg of urea per ml of urine;}$ $P = \text{mg of urea per ml of plasma}$ and $V = \text{ml of urine excreted per minute.}$ This is called maximum urea clearance and the normal value is found to be 75 ml/minute.

The clearance value may not be normal even though the plasma urea values are within normal limits. The plasma urea values will start to rise only when the clearance value falls below 50% of the normal. Urea is normally reabsorbed from renal tubules and therefore tubular function also affects urea clearance. This makes creatinine clearance over urea clearance.

**Inulin clearance**
Inulin is a polysaccharide of fructose. It is neither absorbed nor secreted by the renal tubules. This makes it ideal for determining GFR. The value of GFR as measured by inulin clearance is 125 ml/minute. About 100 ml of sterile 10% solution of inulin is given as slow intravenous drip within 2 hours. Urine specimen formed during this period is collected totally. Blood sample is taken in the course of the test. Inulin is estimated by resorcinol giving a red color. The test needs continuous infusion of inulin so as to keep the plasma level adequate. Since it involves administration of an extraneous compound, this procedure is not used routinely.
Introduction to Pharmacological Concepts

Pharmacology is the study of the systemic effect of drugs. Two concepts are important; pharmacodynamics and pharmacokinetics.
Pharmacodynamics of a drug refers to the processes of interaction of pharmacologically active substances with the target sites, the biochemical and physiological effects leading to either therapeutic or adverse outcomes. Pharmacokinetics refers to the processes of drug absorption, distribution, metabolism and elimination.

Xenobiotics are compounds that are foreign to the body. They include drugs, insecticides, pesticides, heavy metals etc. Drugs are introduced into the body via several routes, each route is specific for a purpose. Some of the routes are; orally (via mouth), intramuscularly (via muscle), intravenously (via vein), sublingually (placed under the tongue), cutaneously (via the skin) etc

Upon absorption, drugs are distributed to different body organs. Drug distribution to organs is dependent on; permeability of tissue membranes, vascularity of organs, size of the organ etc. Drug absorption in the gastrointestinal tract depends; blood flow to the site of absorption, surface area, the transit time of GI, co-administration of other drugs etc. The pharmacological effect of a drug is enhanced by interaction of the drug with a receptor controlling a specific function.

Drug Metabolism

Drug metabolism occurs in two phases; Phase 1 and Phase 2 reactions

Phase 1 reactions

Phase 1 reactions the xenobiotic, usually by adding a functional group which can lead to other metabolic processes in phase 2. These reactions include hydroxylation, hydrolysis, oxidation, reduction, dealkylation etc

Hydroxylation involves the addition of hydroxyl group.
Hydrolysis: These are chemical reactions whereby the addition of water splits the toxicants into smaller molecules. Example: Acetyl salicylic acid (aspirin) is hydrolysed to salicylic acid and acetic acid
Oxidation: This includes suloxidation, N-oxidation, epoxidation, hydroxylation. The oxidation of some compounds may lead to the production of more toxic substances. Example: hydroxylation of toluene to benzyl alcohol.

Dealkylation: This involves the removal of alkyl group from a xenobiotic.

Reduction: Example: the reduction of aldehydes and ketones to alcohol.

Phase 2 Reactions.

Phase 2 reactions are basically conjugation reactions in which a molecule normally present in the body is added to the reactive site of the Phase one metabolite. This often makes the compounds non-toxic and easily excretable. Examples of conjugating agents are: glucuronic acid, cysteine, sulphate and acetic acid. Moreover, glutamine and glycine can also act as conjugating agents.

Glucuronide Conjugation

This type of conjugation involves glucuronic acid. It is the most common Phase two reactions. It generally results in the formation of products that are less biologically and chemically reactive.

Glucuronic acid is added to xenobiotics by UDP-glucuronyl transferases that is present in the endoplastic reticulum. Glucuronic acid can conjugate with hydroxyl, carbonyl, sulphhydryl and amino compounds. A good example of compound conjugated and excreted as its glucuronide is the Bilirubin.

Sulphate Conjugation

Sulphate conjugation reduces the toxicities of xenobiotics by ensuring that the highly polar sulphate conjugates are easily excreted in urine.

Phenolic and alcoholic compounds are conjugated with sulphate involving sulfo-transferase. Examples of compounds excreted as their sulphates include; steroids and indole compounds.

Methylation

A large number of both endogenous and exogenous compounds can be methylated by several N-, O-, and S-methyl transferases. The most common methyl donor is S-adenosyl methionine (SAM), which is formed from methionine and ATP. Even though these reactions may involve a decrease
in water solubility, they are generally detoxification reactions. There are three types of methylation reaction; N-methylation, O-methylation and S-methylation reactions

Cysteine conjugation
Cysteine is derived from glutathione. Epoxides, alkenes, alkyl or aryl halides are detoxified by this type of conjugation

Conjugation with Glycine
Benzoic acid is conjugated with glycine to form benzoyl glycine (hippuric acid) which is excreted in urine.

Acetylation
This is conjugation involving acetic acid. Drugs; isoniazid, sulfanilamide and para amino salicylic acid are conjugated by acetylation.

The Cytochrome P 450 System
This is a system of monooxygenases that are involved in the biotransformation of xenobiotics. They absorb light at the wavelength of 450 nm upon exposure to carbon monoxide.

**Drug Design**
A drug is a key molecule involved in a particular metabolic or signaling pathway that is specific to a disease condition or pathology.

Action of activation (agonist) or inhibition (antagonist) to a biological target (protein, receptor, enzymes, cells… ) which in turn results in a therapeutic benefit to the patient.

Drug design is the approach of finding drugs by design, based on their biological targets.

It is the inventive process of finding new medications based on the knowledge of the biological target.

In the most basic sense, drug design involves design of small molecules that are complementary in shape and charge to the bio-molecular target to which they interact and therefore will bind to it.
**Drug target**

A biomolecular target (most commonly a protein or nucleic acid) is a key molecule involved in a particular metabolic or signaling pathway that is associated with a specific disease condition or pathology or to the infectivity or survival of a microbial pathogen.

Potential drug targets are not necessarily disease causing but must by definition be disease modifying.

**Traditional drug design**

Traditional drug design (is also known as forward pharmacology) involves the origin of drug that emanated from natural sources in the form of accidental discovery.

It was not target based and not much systemised as today.

Improvements and advancements in pharmaceutical science and technology has made traditional drug design evolve to become a more systemized modern drug discovery.

Traditional methods of drug discovery rely on trial-and-error testing of chemical substances on cultured cells or animals, and matching the apparent effects to treatments. These methods include:

- Random screening
- Trial and error method
- Ethno pharmacology approach
- Chemical structure based drug discovery
- Serendipity method
- Classical pharmacology

**Random screening**

Random screening involves the screening of synthetic compounds or chemicals or natural products by bioassay procedures and it involves two approaches:

- Screening for selected class of compounds like alkaloids, flavonoids, etc
- Screening of randomly selected plants for selected bioassays

Random screening led to the discovery of the first truly effective sulphonamide antibacterial Prontosil in 1935.

It also resulted in proving two success stories, which were those of paclitaxel and camptothecin
**Trial and error method**

Trial and error method includes the use of berries, roots, leaves and barks for medicinal purposes to alleviate symptoms of illness. Examples include the use of:

- Willow bark: contains salicin - fever reducing in general
- Cinchona bark: contains quinine - fever associated with malaria
- Chinese herbal remedies: used to treat many illness.

**Ethno pharmacology approach**

Depends on empirical experiences related to the use of botanical drugs for the discovery of biologically active new chemical entity.

This process involves the observation, description, and experimental investigation of indigenous drugs and is based on botany, chemistry, biochemistry, pharmacology, and many other disciplines like anthropology, archaeology, history, and linguistics.

Examples of drugs discovered via this method include:

- Morphine from Papaversomniferum,
- Artemisinin for malaria from Artemesiaalba
- Guggulsterones from Commiphoramukul (for hyperlipidemia)

**Chemical structure based drug discovery**

In 1891, Paul Ehrlich coined the term chemotherapy, used synthetic chemicals to try and cure disease.

**Serendipity**

"Serendipity" refers to an accidental discovery; i.e., finding one thing while looking for something else.

However, no scientific discovery has ever been made by pure luck. All happy accidents in science have one point in common: each was recognized, evaluated and acted upon in the light of the discoverer's total intellectual experience.

The serendipitous discovery of penicillin in 1928 by Alexander Fleming is a good example.

**Classical pharmacology**

Also known as function based approach.

Anciently, drug discovery programmes were often based-successfully-on measuring a complex response in vivo.
Such as prevention of experimentally induced seizures, lowering of blood sugar, or suppression of an inflammatory response, without the prior identification of a drug target.

**Rational drug design**

Rational drug design refers to the development of medications by studying the structures and functions of target molecules.

Rational drug design uses a methodological approach towards the invention of a new drug, as opposed to the traditional drug design where it is hoped that some stroke of luck would help design a new drug, or instead of randomly testing hundreds of drug molecules with hopes that one of them binds to a receptor and exerts a therapeutic effect.

In rational drug design, three general steps are used to create a new drug:

Step 1. Identify a receptor or enzyme that is relevant to a disease they are going to design a drug for.

Step 2. Elucidate the structure and function of this receptor or enzyme.

Step 3. Use the information from step two in order to design a drug molecule that interacts with the receptor or enzyme in a therapeutically beneficial way.

Rational drug design can be broadly divided into two categories:

- **Structure based drug design**
- **Ligand based drug design**

**Structure based drug design**

This relies on finding new medication based on the knowledge of the target. Also known as DIRECT DRUG DESIGN.

Structure based drug design (direct drug design) relies on knowledge of the three dimensional structure of biological target obtained through methods such as X-crystallography or NMR Spectroscopy.

If an experimental structure of a target is not available, it may be possible to create a homology model of the target based on the experimental structure of a related protein.

Using the structure of the biological target, candidate drugs that are predicted to bind with affinity and selectivity to the target may be designed using interactive graphics and the intuition of a medicinal chemist.

**Ligand based drug design**

Ligand based drug design relies on knowledge of other molecules that bind to the biological target of interest. Also known as INDIRECT DRUG DESIGN.
Ligand-based drug design (or indirect drug design) relies on knowledge of other molecules that bind to the biological target of interest.

These other molecules may be used to derive a pharmacophore model which defines the minimum necessary structural characteristics a molecule must possess in order to bind to the target.

In other words, a model of the biological target may be built based on the knowledge of what binds to it and this model in turn may be used to design new molecular entities that interact with the target.

Alternatively, a quantitative structure-activity relationship (QSAR) in which a correlation between calculated properties of molecules and their experimentally determined biological activity may be derived.

These QSAR relationships in turn may be used to predict the activity of new analogs.

**Principles of drug action**

Drugs alter the pace of ongoing activity but do not impart new function.

They act based on the following principles:

- Stimulation
- Depression
- Irritation
- Replacement
- Cytotoxic action
- Antimicrobial action
- Modification of immune status

**Stimulation:**

In stimulation, there is selective enhancement of the level of activity of specialized cells. For example:

- Adrenaline - heart
- Pilocarpine - salivary glands

Also, there could be excessive stimulation, followed by depression e.g.

- Picrotoxin: convulsions, coma & respiratory depression

**Depression:**

There could be selective diminution of activity of specialized cells. E.g.

- Barbiturates - CNS, Quinidine - heart
However, certain drugs stimulate one type of cells & depress the other. Eg:

Acetyl choline stimulates intestinal smooth muscle and depresses SA node in the heart

**Irritation**

Some drugs act by irritating cells. E.g.

Liniments to relieve pain.

Mild irritation may stimulate associated function e.g. Bitters increase saliva secretion but strong irritation results in inflammation, necrosis and morphological damage resulting in loss of function e.g. acids.

**Replacement:**

Use of natural metabolites, hormones or congeners in their deficiency. E.g:

Levodopa- parkinsonism, Insulin- diabetes mellitus, Iron-anemia

**Cytotoxic:**

Selective cytotoxic action for invading parasites or cancer cells. Eg:

Antibiotics, antivirals, anticancer drugs

**Antimicrobial action:**

Prevention, arrest & eradication of infections. Act specifically on causative organisms. Eg:

antibiotics

Modification of immune status:

Enhancing or depressing the immune status. Eg:

Vaccines, sera, levamisole, corticosteroids

**Drug target sites**

Drugs can interact with the following target sites in a cell

Receptors

Ion channels which incorporate a receptor and act as target sites

Enzymes

Carrier molecules

Based on the drug target sites, the mechanisms of drug action can be classified broadly as,
Receptor mediated mechanisms
Non-receptor mediated mechanisms

**Receptor mediated mechanism**

A Receptor is a membrane bound or intracellular macromolecular protein which is capable of binding the specific functional groups of the drug or endogenous substance.

Binding of a drug with its receptor results in the formation of drug receptor complex (DR) which is responsible for triggering the biological response.

\[ D + R = (D R) \xrightarrow{\text{Response}} \]

**Definition of important concepts**

Affinity: This refers to the ability of a drug to form the complex with its receptor (Drug-Receptor complex) e.g., the affinity a key has for the levers of the lock.

Intrinsic activity (or) Efficacy: The capacity of a drug to elicit the pharmacological response after forming the drug-receptor complex.

Ligand: Any molecule that can attach selectively to particular receptors or sites

Agonists: An agonist is any agent that possesses the ability to activate a receptor and produce an effect similar to that of the physiologic signal molecule. An agonist possesses both high affinity and high intrinsic activity and consequently can trigger the maximal biological response.

Antagonists: These are agents which prevents the action of agonists on a receptor but have no effect of its own. Antagonists bind to the receptor and prevents the binding of endogenous agonist. Antagonists have only affinity but no intrinsic activity.

Partial agonists: A partial agonist activates a receptor, causing it to produce a sub maximal effect and antagonizes the actions of full agonist. A partial agonist has high affinity but low intrinsic activity and hence is only partly as effective as agonists.

Inverse agonists: This is also known as a negative agonist and it is an agent that activates a receptor to produce an effect in a contrary direction to that of the agonist. Inverse or negative agonists have high affinity and very low to no intrinsic activity.

**Mechanism of action of selected drugs**

1. **ASPIRIN**

Aspirin is a member of the family of drugs known as Non-steroidal anti-inflammatory drugs (NSAIDS). Aspirin has an anti-inflammatory, analgesic and anti-clotting properties.

**Mechanism of Action**

Most of the properties of Aspirin is as a result of its ability to cause decreased production of prostaglandins and Thromboxane A2.
Prostaglandins are paracrine hormones that subserves various functions in the body such as transmission of pain impulses to the brain, modulation of the hypothalamic thermostat and inflammation.

Aspirin irreversibly inactivates the enzyme cyclooxygenase (COX). COX is required for the synthesis of prostaglandin and thromboxane.

Aspirin is different from other NSAIDS in the fact that it is an irreversible inhibitor of COX while others are reversible inhibitors.

2. QUININE

Quinine is an antimalarial that is effective against different strains of the malaria-causing parasite plasmodium including P. ovale, P. falciparum, P. vivax etc.

Mechanism of Action

Quinine acts primarily on the erythrocytic stage of human malarias with little parasiticidal effect on sporozoites, hepatic stages, or gametocytes of P. falciparum.

Quinine acts by interfering with the parasite’s ability to digest haemoglobin. Quinine also inhibit the spontaneous formation of beta-haematin (haemozoin or malaria pigment) which is a toxic product of the digestion of haemoglobin by parasites.

Drug resistance

Drug resistance could be defined as the ability of microbes, such as bacteria, viruses, parasites, or fungi, to grow in the presence of a chemical (drug) that would normally kill it or limit its growth.

It can also be defined as the reduction in the effectiveness of a drug in curing a disease or condition

Mechanisms of drug resistance

The following mechanisms have been postulated to be responsible for drug resistance:

Production of enzymes that destroy/modify the active drug.

Synthesis of an altered target site against which the drug has no effect.

Reducing drug accumulation through:

Decreasing the permeability of cell membrane.

Actively exporting drugs through Multi Drug Resistant pump (MDR OR EFFLUX pump).

Altering the metabolic pathway so that the reaction inhibited by the drug can be bypassed.
Developing an altered enzyme that is less inhibited by the drug but can still perform its metabolic function.

**Types of resistance**

**PRIMARY/NATURAL/NON GENETIC ORIGIN OF RESISTANCE**

In this type of resistance, bacteria possess an innate property to resist drug, which could be as a result of its mode of binding to the host or certain characteristics it possesses.

For example:

The bacteria may infect host at sites where drugs are inaccessible or not active as seen in Salmonellae.

The cell wall may be covered with an outer membrane that establishes a permeability barrier against the antibiotic as seen in Gram negative bacteria.

Bacteria may remain in dormant resting state without multiplying and become phenotypically resistant to drugs as seen in M. tuberculosis.

Micro-organisms may lose the specific target structure for a drug for several generations and become resistant.

In such cases, drug resistance would occur.

**ACQUIRED/GENETIC ORIGIN OF DRUG RESISTANCE**

Bacteria acquire/develop resistance to antibiotics either through the modification of existing genetic material (mutation) or the acquisition of new genetic material from another source (plasmid/gene transfer).

Further classified into:

1. **CHROMOSOME MEDIATED RESISTANCE:**

   Resistance acquired due to spontaneous mutation of gene that controls the susceptibility to a given antimicrobial drug.

   Structurally alters the target of the drug or the transport system that controls the uptake of the drug. This could occur in two forms:

   Stepwise mutation: As occurs with Penicillin

   One step mutation: As occurs with Streptomycin

   Vertical gene transfer of resistant genes to progeny.

   Growth of resistant mutants.

   Seen in M. tuberculosis and streptomycin.
2. TRANSFERABLE DRUG RESISTANCE

A. PLASMID MEDIATED RESISTANCE:

Resistance acquired through the transfer of extrachromosomal resistance plasmids (R factors)

R factor = RTF (Resistance Transfer Factor) + r determinant

Main features:
Frequency of resistance transfer is high.
Resistance transfer can occur to cells of different species.
Plasmids can mediate resistance to multiple drugs.
R factors provide resistance to metal ions and bacterial viruses/bacteriophages.
R factors code for enzymes causing inactivation of drug.

B. TRANSPOSON MEDIATED RESISTANCE:

Transposons are genes/segments of DNA that are transferred within themselves or between chromosomes and extrachromosomal plasmids.

They are also known as jumping genes and this mode of genetic transfer as transposition.

Transposons attach themselves to chromosomal, plasmid or phage DNA molecule and confer resistance to drugs under suitable environmental conditions.

Transposons are not self replicating.

R determinant segments of R Factors are said to be collections of Transposons.

Methods of transfer of resistance

Horizontal gene transfer (HGT) is a process whereby genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species.

Conjugation

Transduction

Transformation

Transposition

Summary and Conclusion

This course has informed students on the biochemical basis and clinical applications of some routine laboratory tests of wellbeing. It has also introduced students to pharmacology that deals
with the systemic effects of drugs. It also covered the physiological and biochemical actions of some selected drugs. Biochemical mechanisms of drug action against parasites. Metabolic factors affecting chemotherapeutic agents. Drug resistances and other factors affecting drug efficacy. Biochemical basis of drug resistance.

**Interaction and Question**

1. State three indications for Liver function test
2. What is clearance test? What are the advantages of creatinine clearance test over other clearance tests?
3. Discuss phase 1 and phase 2 reactions

**Bibliography/ Further Reading**