THE EFFECT OF ELECTROMAGNETIC FIELDS ON THE INTERACTION BETWEEN ANTIBIOTICS AND BACTERIA

by

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ABSTRACT

THE EFFECT OF ELECTROMAGNETIC FIELDS ON THE INTERACTION BETWEEN ANTIBIOTICS AND BACTERIA

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The effect of electromagnetic fields on the interaction between the antibiotics erythromycin and bacterial species *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)* are determined by its influence on the growth rate of bacterium. Previous studies have shown the electromagnetic field effects were on effectivity of erythromycin on *E. coli*. Erythromycin has been studied in detail previously. Experimental samples of *E. coli* and *S. aureus* were prepared in varying environments of magnetic field. Measurements of optical density were done to determine the growth rates and behavior of bacteria after its exposure to the magnetic field. Experiment data sets were used to visualize the response of bacteria to antibiotics in different concentrations. It has been shown clearly that the

perturbative effect of the magnetic field reduce the growth rate significantly for lower concentrations of antibiotics. For higher concentration of 200 microgram/milliliter the low magnetic field effect is totally suppressed.

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CHAPTER 1: CELL MECHANICS

1.1 Introduction

Bacterial cells are the simplest example of life and their detailed study helps to understand the mechanisms involved in the growth process and life and death of eukaryotic cells. Bacteria plays a big role in several mechanism in human body and the excessive growth of unwanted bacteria is a cause of infectious diseases and may lead to the deadly damages in human being. Therefore, a detailed study of growth rate, impact of ecological changes and the mechanism to control or enhance the growth of different bacterial species is a topic of great interest. A better understanding of the mechanism of bacterial growth and the control of growth rate by ecological factors or chemical composition by antibiotics is related to human survival and safety directly.

Mechanical forces are normal physiological functions which are generated and sustained by biological cells. Cells are always active and are capable of detecting mechanical stimuli via mechanosensitive signaling pathways which are activated and in turn respond physical signals by reorganizing the cytoskeleton and generating the appropriate forces. Various properties of cells such as viscosity, elasticity and adhesiveness are susceptible to change as a result of disruption in pathogen-induced cytoskeleton and mutation. Conversely, cell behavior can be influenced by perturbations in the ability to sense and respond to various external forces. Theoretical models have been used to characterize the mechanical properties of cells, which are based on the basic concepts from soft matter physics and mechanical engineering. Current knowledge of cell mechanics and its associated roles in the disease prevention and control, physiology of multicellular

organisms and genetic development has been indebted to interdisciplinary studies which amalgamate research in contemporary molecular biology and advanced techniques for cellular mechanics characterization.

Modern biomechanical research is at an intriguing stage whereby more is being discovered about the influence of mechanical cellular functions and characteristics both as a regulating factor and as a direct result of cellular architecture. Contemporary biomechanical research is aimed at combining the experimental, theoretical and computational approaches in order to develop an elaborate description of the mechanical behaviors of cells. This information can then be used to develop new perspectives on the correlation of cellular mechanics and disease, and the procedures which are used to pursue such research avenues are becoming increasingly complex. One of the greatest challenges facing such endeavors is the requirement for the reconciliation of physical and biological research, which requires a lot of expertise in both fields.

1.2 Physiological Forces

All living organisms need to interact with the physical forces that exist within their environments. This is one of the fundamental requirements for life and survival, and it is one of the main influencing factors of biological design. Various body parts in human beings are designed structurally to serve specific physiological functions in order to mitigate natural physical forces, such as the skeleton which poses a barrier to gravitational force. Even the most minor body functions are based on the generation of force, such as in breathing and exhaling for respiratory purposes. In recent times, biomechanical research has been focused on understanding this phenomenon at more basic organism and organ

levels. New experimental techniques have made it possible to evaluate the effects of physical interactions in disease development and physiology via advanced techniques in surface and cell culture sciences. The sustenance, detection, and generation of physical forces at unicellular levels is an important step which is necessary for understanding mechanical sensitivity, and organ and tissue-level physiology.

1.3 Mechanical Properties of Cells

The mechanical properties of a material are the traits which dictate a material's response to mechanical stimuli. Essentially, the cellular mechanical properties define a cell's deformation reaction to applied stress, and the manner in which this deformation changes over time. The unit of measurement for a solid material's strain and stress is known as Young's modulus. This is a basic property of solids since it relates their ability to resist stress by retaining their shapes under mechanical pressure. Unlike elastic solids, fluids flow do not store elastic energy, and instead flow when they are exposed to mechanical stress. The viscosity of a fluid is the measure of its rate of flow under a specific stress load. Still, many materials are viscoelastic; they exhibit both elasticity and viscosity when exposed to mechanical stress. Such materials concurrently undergo deformation while storing and dissipating mechanical energy. Over time, mechanical stress in viscoelastic materials relaxes while deformation continues increasing over time. Fluid mechanics to describe the cellular motion in fluids such as bacterial motion in nutrient broth [1].

One of the biggest challenges in cell mechanics lies in explaining the origins of the structures of measured mechanical properties in cells. It is generally understood that cells are complicated and heterogeneous structures which contain a variety of proteins, sub-

cellular structures, filaments and organelles, all of which have different physical properties and thus contribute variedly towards the viscosity and elasticity of the cell. The nucleus in particular is much stiffer than the other cytoplasmic constituents of the cell. In cell compression procedures, the cortex and cell membrane have always been observed to undergo deformation within the first 200 nm of force. Increasing the force applied in the cell causes a larger contribution from the nucleus, which implies that there is no simple, linear relationship between the stress applied and the strain on the cell. Still, mechanical models can be used to compare the characteristics of cell mechanics under pharmacological and genetic perturbations, which can reveal a lot of interesting information about the interpretations of the cell mechanics.

1.4 Cell Mechanics

Cells have interior fluid and are packed with organelles, structures and macromolecules which have specific functions. The cytoskeleton, a network of sub-cellular filaments, makes up higher order meshes which enable cells to invariably sustain mechanical stress. With regards to cell mechanics, there are three particular cellular cytoskeletal filaments which draw interest: the actin microfilaments, microtubules, and intermediate filaments. Actin forms polarized filaments which in turn interact with a variety of ancillary proteins. Actin is also among the most abundant eukaryotic proteins. The mechanical properties of actin include semi-flexibility, persistence of length, and dynamicity. Actin filaments can quickly reorganize themselves and migrate in order to change their shapes. Individually, actin filaments may not necessarily have a lot of influence on the mechanics of a cell. However, by forming complex structures and

interacting with polymerizing and crosslinking factors, actin filaments contribute a great deal towards the overall mechanical properties of a cell.

Cells are active materials which exhibit essential pre-stress characteristics that are formed by myosin motors. When myosin proteins crosslink and process along filaments, they create stress between the neighboring filaments which are not aligned in parallel. The best illustration of pre-stress is in contractile actin formations, which includes structures like intercellular junctions and stress fibers. The transaction of chemical signals between cells spans a wide range of scales, from inter-organ hormonal traverses to paracrine signals transfer among groups of cells, and even intracellular signals cascading. Research conducted in recent times in the field of cell biology has indicated that cells have ways of sensing mechanical impetuses within their immediate environment.

The vitality of sustaining, sensing and generating cellular mechanical forces is evident in the analysis of cytoskeletal diseases. Some genetic disorders have been known to interrupt cellular actin cytoskeletal formations and binding of actin- erythrocyte. This causes red blood cells to develop abnormal shapes and compromises their function, as is evident in cases of sickle-cell anemia and malaria. Establishing links between cellular mechanosensitivity and other factors such as underlying molecular mechanisms in cells is one of the most exciting prospects for future cell mechanics.

1.5 Cellular Structure

Cells are fundamental building blocks of life. Eukaryotes such as humans contains trillions of cells in their body while prokaryotes such as bacteria are single cell creatures compared to eukaryotes, lack the nuclear membrane and do not possess membrane–bound

organelles such as mitochondrion. The cell is made up of many parts with distinct functions; these organelles are highly specialized structures that can perform specific tasks in vivo (within the cell).

The fundamental organelles in eukaryotic cell include: mitochondrion that is the powerhouse of metabolism, generating energy by process of respiration in the form of Adenosine Triphosphate (ATP). It has its own stranded DNA hence able to self-replicate itself and multiply to replace the worn out cells during process of respiration. The mitochondrion is sac-like in its structure. The cell membrane is an organelle with the ability to select permeable substances into the cell hence able to transport desired material into the cell. In prokaryotic cells, the cell membrane is made up of

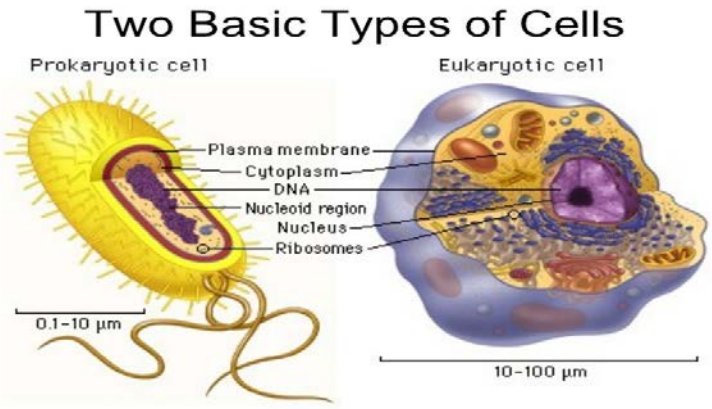

Figure 1.1: Type of cells [16]

glycolipid and glycoprotein thus providing mechanical resistance to the cell by being the base of attachment for cytoskeleton in some organisms. The proteins in cell membrane provide chemical climate required by the cell while the lipids can offer flexibility. Cell membrane is a lipid bilayer in its composition. Cytoplasm is made up of all the contents

outside the nucleus and enclosed by the cell membrane as shown in Figure [1.1]. These contents includes the cytosol and in eukaryotic cells; mitochondria and ribosome are contained; cytoskeleton fibers are also in the cytoplasm. The cytoplasm is gel-like and is clear in color, and the main constituents are enzymes, water, organelles and organic salts. The role of cytoplasm is to move materials along the cell and dissolve the cellular materials within the cell. The endoplasmic reticulum is found near the nucleus and has flat sacs called cisternae which is a flattened disk of an endoplasmic reticulum which has a lot of ribosome w continues to make nuclear envelope. However, smooth endoplasmic reticulum lacks ribosome. Rough endoplasmic reticulum transports proteins synthesized by the ribosome while smooth synthesizes lipids [17].

A eukaryotic cell is also made up of other organelles with sacs. Golgi apparatus contains flattened sacs with a protein and handles modifying the proteins received from the endoplasmic reticulum. The vesicle then transports these proteins to the desired region of the body. Lysosome has a spherical sac containing the enzymes necessary for breaking down cellular materials in the cell such as microorganisms that are engulfed by the cell. The nucleus of the cell carries genetic information, it contains DNA and RNA, which are important in transcription and translation during genetic decoding. DNA sequence is transcribed into the messenger RNA controlling the manufacture of proteins by the ribosome during growth and reproduction. The nucleus control every cellular activity of the cell since it handles sending a blueprint of proteins required to be synthesized by the ribosome. Contained within the nucleus is a dense, membrane-less structure composed of RNA and proteins called the nucleolus. The **nucleolus** contains nucleolar organizers, which are parts of chromosomes with the genes for ribosome synthesis on them. The nucleolus helps to synthesize ribosomes by transcribing and assembling ribosomal RNA subunits. These subunits join together to form a ribosome during protein synthesis [18].

1.6 Bacterial Cells

Bacteria, singular bacterium, are large and important form of life that live in enormous numbers in almost every environment on Earth. They are single celled lacking nucleus reproducing by spore formation or fission. They can live in other organisms and normally cause diseases (harmful) but there are other bacteria beneficial to humans, these include bacteria located in the stomach and those aiding in digestion. There are many kinds of bacteria classified according to the physical characteristics. They can also be classified

if they are beneficial or harmful for life. Bacteria are differentiated by morphology, nutritional requirements or the chemical composition of cells. Biochemical pathways and the source of energy also classify bacteria. There are three basic shapes of bacteria; coccus (spherical), bacillus (rod-shaped) and spiral (twisted) as shown in Figure [1.2].

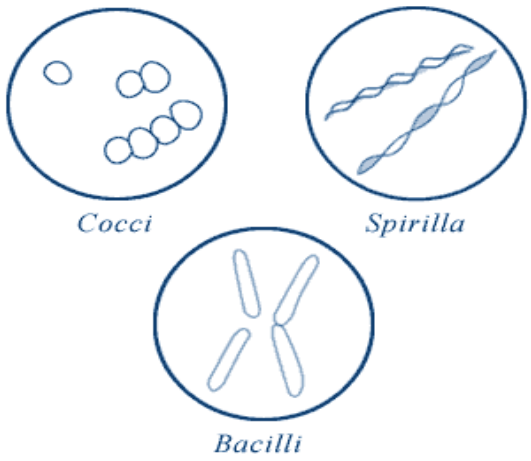

Figure 1.2: Bacteria come in many different shapes. Some of the most common shapes are bacilli (rods), cocci (spheres), and spirilli (spirals). Bacteria can be identified and classified by their shape[19].

However, there exist a pleomorphic bacterium that assumes no definite shape. Most bacteria are about 0.2 µm in diameter and about 2-8 µm in length. The arrangement of cocci is oval, elongated of flattened on one of its side. However, they remain attached after cell division. Diplococci remain in pairs after dividing, streptococci remain in chains after division, tetrads are cocci that divide in two planes and remain in-group. Sarcinae are divided into three planes and form cubes with eight groups and staphylococci that divide into multiple planes and form grape-like structures.

Bacilli are bacteria, which ivied across their axis and most appear as single rods. Diplobacilli appears in pairs after division, streptobacillus appear in chains after dividing while coccobacilli are short and flat hence look like cocci. Spiral bacteria are twisted in nature Vibrio as spiral has curved rods while spirillums have a rigid body that is helical in shape. Spirochetes have helical and flexible bodies; they move by using axial filaments. Bacteria also has other shapes including star-shaped Stella with about 0.5 μm in diameter, rectangular shaped Holoarcula with a diameter of about 0.5 μm and is from genus archaea.

1.7 Gram-Staining Classification

There are different types of bacteria grouped according to how useful or harmful to man. They are classified into gram-positive and gram-negative bacteria depending on their differences in their cell wall structure and staining. When crystal violet dye is used and is stained then the type of bacteria is gram-positive bacteria. However, gram-negative bacteria do not stain crystal violet but pink or red color appears as shown in Figure [1.3]. Comparing the resistance to antibiotics of both bacteria, gram-positive have impenetrable cell wall hence are more resistance to antibiotics.

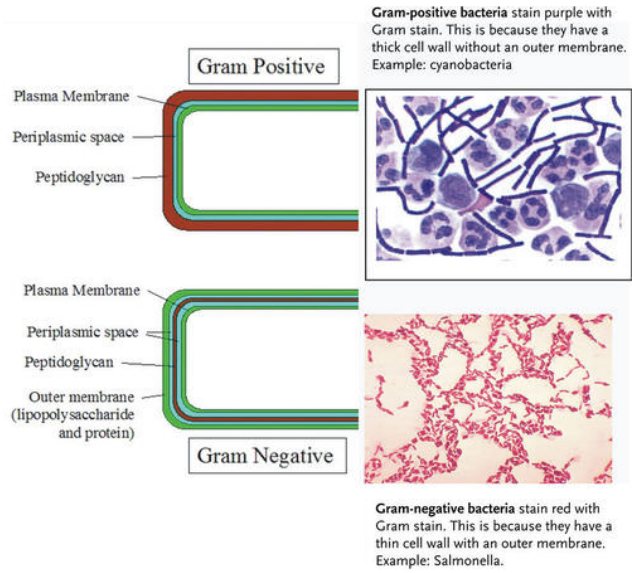

Figure1.3: The structure of gram staining classification. Gram positive cells stain bluish-purple, just as these Bacillus anthracis bacteria (the purple rods) have done. Gram negative cells are stained pinkish-red, like these Bacillus coagulanscells[20].

Gram-negative bacteria have thin or single layered peptidoglycan while gram-positive bacteria have thick or multilayered peptidoglycan. Teichoic acids are also absent in gram-negative bacteria but are present in most of gram-positive bacteria. Teichoic acid are polysaccharides of glycerol phosphates or ribitol phosphates found in bacteria and are linked by phosphodiester bonds.

Gram-negative bacteria have high lipid and lipoprotein content due to the high presence of the outer membrane; gram-positive bacteria have virtually no lipid and lipoprotein. Gram-negative bacteria have four rings in the basal body on the flagella while gram-positive bacteria have two rings of the basal body in the flagella. Gram-negative bacteria are endotoxins while the gram positive are exotoxins, toxigenesis is the process by

which pathogenic bacteria produce toxins. Endotoxin is a polysaccharide found in gram-negative bacteria such as *E. coli*; however, exotoxin is soluble proteins that act as an enzyme and catalyze biochemical reactions. The cell wall of gram-negative bacteria has 70-120 Angstrom thick two layers, the lipid content is very high at about 30% while murein content is very low at less than 10 percentage. In gram-positive bacteria, it has a single layer of 100-120 Angstrom with very low lipid content and very high murein content at about 90%. There economic uses of bacteria depending on their benefits and harm it causes to man. Lactobacilli are Gram-positive bacteria located mostly in the human intestine, vagina and the mouth. It secretes lactic acid hence prevents overgrowth of harmful bacteria. These bacteria are also found in the fermented milk products such as yogurt and are taken to maintain the amount of the probiotics in the human body. Bifidobacteria are branched rod shape gram-positive bacteria that are important in humans since they maintain the concentration of probiotics in the human body. It also prevents yeast infection and diarrhea; probiotics are the organism that improve health conditions when consumed. *E. coli* is a gram negative bacteria which is rod-shaped; it is important because it breaks down undigested sugars in the intestine thus aid in the digestion. They also provide vitamin k and biotin that are crucial in cellular activities in the body. Streptomyces and cyanobacteria are other helpful bacteria found in the environment; they prevent the proliferation of harmful bacteria. There are also some of the harmful bacteria, which cause disease or adversely affect the health. Mycobacteria are rod-shaped and are neither gram-negative nor gram positive, they cause infection of primary organs of the body such as skin and lungs. Leprosy and tuberculosis are common diseases caused by mycobacteria. Other bacteria that cause

harm are *Clostridium tetani* a Gram-positive bacteria that infect the gastrointestinal and skin; as a result cause tetanus that can result in death. *Yersinia pesitis* is gram-positive bacteria infecting skin and lungs leading to bubonic and pneumonic plague. The terrorists can use these bacteria as a dangerous biological weapon. *Helicobacter pylori* is a common bacteria associated with ulcers of the gastric and peptic glands. *Bacillus anthracis* are gram-positive rods occurring in animals, such as goats, cattle and sheep and can cause abnormal problems such as diarrhea.

Bacteria are organisms that are found everywhere and therefore, there is a need for humans to develop methods of getting rid of the bacterial infections. Depending on the type of bacteria, different methods are available. The standard method is boiling water prior to drinking or any other commercial use, and heat can minimize the bacterial infections. Prescription drugs are used in the synthetizing of antibiotics such as methicillin and penicillin has effects on different species of bacteria. Doctors and pharmacists study the interaction of drugs to different strains of microbes. The potential side effects of these medicinal products in the body is also analyzed. Chlorination is also efficient method of eliminating bacteria in water; it is the most used method in public places but debate by scientists on the efficiency of the method is still on because of the safety concerns.

1.8 Cell Membrane and its Function

A membrane is basically a layer around the cell that permits some materials to go through it and not the others [21]. This layer covers protoplasm inside a cell except the nucleus. The main purpose of this membrane is to safeguard cohesion of inside of a cell by permitting the passage of certain materials and blocking the supplementary bodies

[22]. In certain living things cytoskeleton is attached through membrane and in others it is linked through cell wall. In this way, a membrane of the cell assists in preserving its structure as well as act as a pillar for the cell [21].

This outer layer of the cell enhances the development of a cell by achieving an equilibrium between the processes of attaining and releasing substances through a vesicle that is present in the cytoplasm. The process through which substances are taken in and proteins and lipids are extracted out of the cell is called as endocytosis [22]. While the process that involves integration of the vesicles with the membrane of a cell is named as exocytosis. Through this process the size of a cell is enlarged. The particular bodies inside a cell are also covered through these membranes.

1.9 Cell Membrane Structure

A cell membrane is made up of both the lipids and the proteins. Lipids constitute twenty to eighty percent of a cell layer but this percentage varies with respect to function and position of the membrane. The rest of it consists of the proteins. These membranes achieve elasticity with the help of lipids [22]. The molecules are transported in and out of the cell through proteins. Additionally, the substance atmosphere of the cell is examined through proteins.

This cell membrane is comprised of four kinds of phospholipids. The major section of the lipid is constituted by these phospholipids. Phospholipids are dispersed in an irregular way between bilayer of the membrane. The exterior sheet of the membrane is formed by sphingomyelin and phosphatidycholine. While the inner sheet of the cell membrane is made up of phosphatidylserine and phosphatidylethanolamine. There is

another phospholipid that is placed in the interior of the membrane. This lipid is named as phosphatidylinositol. Although, this lipid constitutes smaller part but it performs a significant role in motion of the cell and transferring of information. The heads of Phosphatidylinositol and phosphatidylserine have a negative charge. So their prevalence in inner sheet leads towards development of an overall negative charge in the solution of membrane. In addition to these phospholipids, cholesterol and glycolipids are also present in most of the membranes in case of animals. These glycolipids are present on the outer sheet of the membrane. In animals, cholesterol is the main element of the cell membrane. The characteristics of bilayer of phospholipids are very important for functioning of the membrane. For example, the membrane can perform its main task due to the shape of bilayer of phospholipid because they act as a fence between both sections that carry water. Phospholipids consist of a head and a tail. Molecules of proteins are very crucial for the lives of living organisms.

1.10 Protein Structure

Proteins are actually organic substances that are made up of different molecules particularly amino acids. These amino acids are connected with each other through a specific bond. Polypeptide series are formulated through these amino acids that further crumple to develop a protein. These proteins are obtained as a result of a decryption procedure that is done inside the DNA of a cell. The components that cause motion and trigger organization in a cell are made up by proteins. Also proteins act as a substance that trigger the speed of chemical processes in organisms [22].

Proteins in combination with RNA trigger biotic actions in the cell. Chemistry of the enzymes is regulated by proteins. Proteins are highly distinctive in nature. The functioning of proteins is regulated by the peptide strings that are arranged in an organized manner. This arrangement of peptide strings enhances connection between hydrogen bods of the polar sets.

Amino acids, the main components of proteins are made up of a central carbon molecule that is further linked with a hydrogen molecule, amino group, an inconsistent element and a carboxyl group. The specific bonds through which these amino acids are connected to each other are named as peptide. These bonds are developed in reaction to a biochemical procedure. This biochemical process results in production of a water atom. In this process, amino group of one amino acid is linked to carboxyl group of another amino acid.

Each protein is created through a deposit of amino acids that contains 20 amino acids. Every amino acid has its own side string. It is important to understand the nature of the side string because these strings can join with one another to control protein length in a structures. The amino acid chain of proteins is responsible for directing connection between molecules and overlapping of the chain. It eventually regulates the 3D structure of proteins [22].

1.11 Types of Protein Structures

Different types of proteins structures have been identified on the basis of convolution in polypeptide arrangement. These structures are discussed as in Figure [1.4].

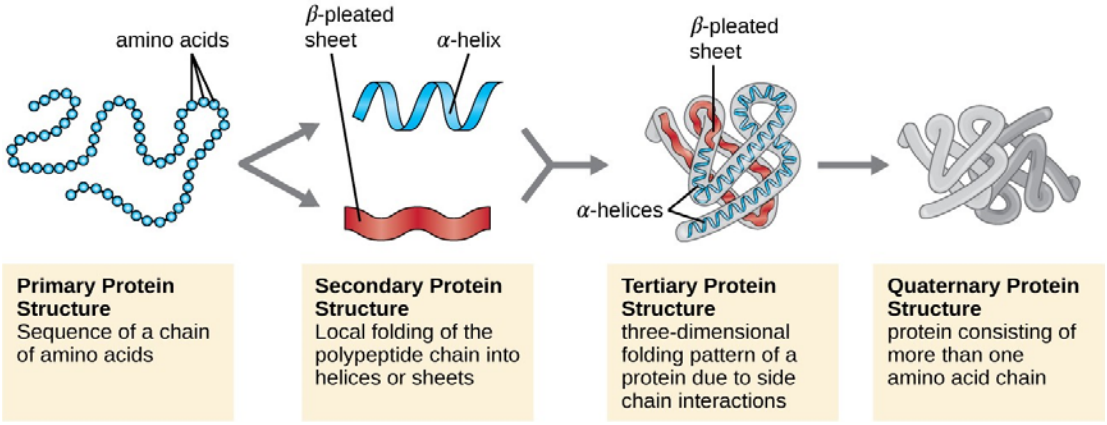

Figure1.4: Four levels of protein structure. Modification of work by National Human Genome Research institute [23].

- ***Primary structure:*** primary structure of proteins is established on the foundation of twenty amino acids. In each of these amino acids, a central carbon is attached to a carboxyl group, a hydrogen molecule, an amino group and an unsteady mass [21]. This unsteady mass is different for each amino acid and responsible for finding out the distinction between protein molecules that can be linked to similar molecules to create a polymer. The series of amino acids for each protein is decided through the data that is present in genetic key. A specific protein possess a specific arrangement of amino acids in its chain and if one amino acid is replaced then it can result in modification of genes. It further causes inappropriate working of protein [21].
- ***Secondary structure:*** in secondary structure, polypeptide chain is overlapped and produces a 3 dimensional form of proteins. Secondary structure is distributed into two forms i.e. alpha helix and beta pleated. In the first kind of structure, hydrogen

bond safeguards the structure and it is in spiral form. While beat pleated structure is in folded form.

- ***Tertiary structure:*** in this structure different kinds of drives and bods are involved. For example, overlapping and forming of proteins is done through particular type of corresponding actions. The inconsistent component in each amino acid either tends to mix with water or repels to combine with water. The amino acids which have such variable components that are attracted by water will try to find watery surroundings while the amino acids that have variable components which repel water will settle themselves in the middle of proteins.
- ***Quaternary structure:*** This type of structure originates as a result of corresponding influence between strings of polypeptides. These polypeptide strings are called as subunits. Proteins that have this kind of structure may composed of similar subunits.

1.12 Lipid Structure

The significant component of food are lipids. Lipids offer vitality to a great extent. The extreme significance is linked to Lipids because of presence of naturally occurring fatty acids in organic fat. Lipids perform the function of coating for some parts and they also provide a sheath for those tissues that are present under the skin [20]. Most of the membrane structure is made up of lipids. Lipids are produced in a natural way in the form of fats and oils. Lipids are produced by all living organisms. Lipids also perform their role in various organic procedures [19].

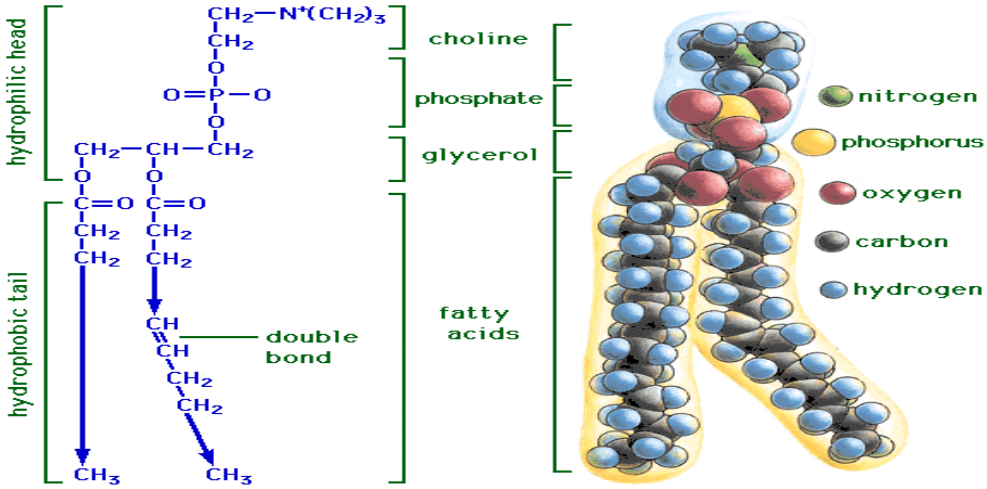

Figure1.5: Lipids are fat soluble molecules; a property conveyed by its structure. The most commonly occurring lipids are triglycerides which consist of a glycerol backbone bonded to three fatty acids [24].

There is no habitual structure for Lipids and an example is shown in Figure [1.5]. The two types of Lipids found frequently are phospholipids and triglycerides [25]. The cell membranes of plants and animals indicate the presence of phospholipids. Structure of a phospholipid contains fatty acids, alcohol, glycerol and phosphoric acid (H_3PO_4). Phospholipids are organized in the form of a collateral layer i.e. bilayer. This bilayer of the phospholipid is very important for smooth functioning of the cell. Also the membrane of a cell is constructed by this bilayer of phospholipids. The two fatty acids in phospholipids are connected to a glycerol that is the head. The hydrophilic section of phospholipids is formed when a phosphate group and a glycerol get attached to each other. In the bilayer of phospholipids, edges of tails point towards interior while heads point towards exterior. This organization of heads and tails is crucial because most of the portion of the cell is made up of water. A layer that encloses a nucleus is developed through phospholipids. These lipids are utilized as a source of energy because they can

be smashed inside a cell. Phospholipids are distributed in molecular form. These molecules of phospholipids are named as chemokines. Chemokines are responsible for controlling different actions inside a cell. For example, synthesis of proteins is controlled by these chemokines and transferring of cells from one region of the body to another region of the same body is monitored by these tiny molecules. In addition to these functions, chemokines provide lubrication to the cells particularly in the joint and lung areas. Phospholipids are essential for healing of the wounds and body parts. Because they enhance the impact of pain killers and transmit the drug to that region of the body which is affected by pain or injury. The body of the human can engross phospholipids in a successful and easy way.

Lipids that are present in oils and fats are called as triglycerides. In triglyceride, 1 glycerol is connected to 3 fatty acids. Sometimes these fatty acids are identical to each other and sometimes they differ from each other. Triglycerides are the lipids that move through the blood. Glycerol is the main component of triglycerides. This glycerol contains three carbons. Each of these three carbons has its own hydroxyl group. Fatty acids react with these hydroxyl groups. The category of triglycerides is determined by the formation of the fatty acids. Hydrogen and carbon are the key elements of fatty acids. There exist two kinds of fatty acids that are saturated fatty acids and un-saturated fatty acids. These triglycerides mainly execute the function of provision of energy.

1. 13 Escherichia Coli

E. coli are the kind of bacteria that is present in food and intestines of the living organisms that include animals and human beings. The majority of these bacteria are

beneficial because they maintain the health of digestive area. But some of these bacteria can prove harmful for the health [26]. For example, if a polluted water of food is ingested that it leads towards developing of an ailment. These bacteria are responsible for generating diseases of urinary area. 75 percent to 95 percent of these ailments are caused by Escherichia coli [26]. *E. coli* bacteria is discharged into the surroundings through fecal substance. These bacteria develop in fresh faces for a duration of three days. But the quantity of *E. coli* decreases as the fecal substance becomes old. *E. coli* are capable of shifting DNA by changing it into another form. In this way, genetic substance is expanded in a horizontal manner in a populace. They are present in the guts of the human beings. They indicate short term variations in frequency as well as long term dormitory forces which alter due to the use of antibiotics and changes in food. The protein which is found in serum and cell of the immune system is produced by the gut of the individuals that further ease the production of biofilms of *E. coli* on mucous membrane of the intestines. *E. coli* offers various advantages to the host. The main benefits of *E. coli* are the production of vitamin B12 and vitamin K that are necessary for the mammals. Moreover, it facilitates those organs that require oxygen by preserving constructive surroundings through the absorption of the oxygen. In other words, *E. coli* keeps those harmful bacteria out of the gut that are responsible for various ailments.

The connection of *E. coli* with the host is very strong and it initiates from the birth of the host. For example, newly born children are introduced to *E. coli* through the fecal substance of the mother at the time of childbirth. This exposure to *E. coli* is very significant. Actually, the amounts of *E. coli* increases in microorganisms of the female

throughout the pregnancy. This enhances the probability of exposure of the newly born children to *E. coli* . These populating *E. coli* have hair like attachments and discharging methodology that enable *E. coli* to get connected to epithelium of the baby's gut. This quickly developing and recently built populace of *E. coli* alters the working and shape of the cells of the epithelium. These changes are important for better growth of microbiome. However, this population of *E. coli* in the human babies is decreasing on continuous basis because of the increased number of cesareans and better cleanliness at the hospitals. This reduction in population of *E. coli* results in larger population of *S. aureus*. Increased population of *S. aureus* causes various ailments in the human beings that include asthma, diabetes and obesity etc. the life of *E. coli* in the interior of the host is smooth and steady. But it should be capable of adapting outside the host to combat the extreme surroundings. The main reason behind the survival of *E. coli* in harsh surroundings is the constant adaptation of *E. coli* .

E. coli is categorized as gram negative bacterium because of presence of a cell wall and a slender membrane. The structure of *E. coli* bacteria changes with respect to surroundings or genetics. However during rapid development, it continues to possess a cylindrical structure. The mutant forms of *E. coli* which are deficient in molecular mass usually expand in order to look like spherical bacteria or cells. When they are motionless then adapt into a rounded form. In the initial stages of development, these bacteria adjust into different shapes but after formation of a new cell their shape remains constant. The membrane lipid structure of *E. coli* has 3 types of phospholipids. Zwitterion is the main phospholipid of its lipid membrane. The exterior membrane of *E. coli* contains nearly

one hundred lipoproteins. However, the role of these proteins is still undiscovered. These proteins have a beta barrel shape. These proteins are enclosed in chambers. This exterior membrane protects *E. coli* from harmful antibiotics for example penicillin by acting as an obstacle. The interior membrane is gram negative bacteria and it is a bilayer of phospholipids. In case of *Escherichia coli*, the major phospholipids of the bilayer are phosphatidyl glycerol and phosphatidyl ethanolamine. But cadiolipin and phosphatidyl are also present in the bilayer in small quantities. *E. coli* can live in various types of matter.

1.14 *Staphylococcus Aureus*

A bacteria that is found in the nose is named as *S. aureus*. For majority of the time, it does not present any health risk. Due to *S. aureus*, various infections are generated on periodical basis. People of all ages are prone to this infection but there are some particular age groups that have more chances of acquiring this infection. For example, the individuals who are suffering from cancer, diabetes, lung ailments, eczema and vascular ailments have more probability of acquiring Staphylococcus infection [27]. These individuals have weak system of fighting with diseases so they easily develop staphylococcus infection.

In United States of America, nearly 500,000 individuals are suffered from various diseases due to Staphylococcus on yearly basis. It influences different groups of organisms on a wider scale and it easily shifts from one group to another group [27]. This infection is transferred from one person to another person through the air. When an individual is suffering from transfer of staphylococcus into the air through cough or

sneeze which are inhaled by other healthy individuals. Moreover, touching those objects directly that are polluted by the bacteria can also develop this infection.

It is vulnerable to curcumin reticence to a considerable level. Basically, it is a bacterium that leads towards origination of different diseases like osteoarticular, bacteremia, IE and lungs and pleura infections. Staphylococcus has developed with the passage of time to stay away from the immune structure of the human beings and antibiotics therapy. As a result, Methicillin-resistant *S. aureus* has progressed. This a kind of infection that is caused by *S. aureus* and is difficult to cure through antibiotics. Because MRSA show resistance towards those antibiotics. It is a type of bacteria that take quick advantage of the opportunities and produces a large number of diseases. These ailments range from minor infections of the skin to lethal offensive ones. However, this pervasive bacteria is very significant because of the presence of the aggressiveness, severity of the poisonous impact and refusal towards antibiotics. It has a huge contribution in the infections whether they are obtained from the hospitals or the community. It does not produce cells that are capable of multiplying but creates staining of the food items during handling and composition. This bacterium develops in different temperature ranges like it can grow in a temperature of 7 degree to 48.5 degree centigrade. It can survive in the conditions of severe dryness and tension. For example, it can survive on human skin and inside of the nose including inorganic exteriors like clothing etc. because of these features, it is capable of developing in different food items. *S. aureus* is able to germinate for a long period of time on different surfaces and hands.

Almost 30 percent of total population is affected by population of *S. aureus* and MRSA in the US. Mostly these bacteria reside in the nostrils of the host. Although, population of staphylococcus is not dangerous itself for the individual but it leads towards development of multiple contaminations. The hosts are classified as constant and occasional bearers of *S. aureus* who are able to transmit these bacteria to other individuals. Most of the bowel disruptions are caused by *S. aureus*. *Staphylococci enterotoxins* are developed by *S. aureus* in the absence of cold ties. They can be removed through heat therapy but still they have the ability to develop due to their tolerance towards extremities of temperature. It must be taken into consideration while formulating actions for the health of the individuals.

S. aureus is categorized as a gram positive bacteria because of thickness of the cell wall and purple stain. This bacterium is circular in form. The thick cell wall of *S. aureus* is formulated by murein, proteins and teichoic acids. *S. aureus* cell is also surrounded by a succulus. Glycan structures in murein are inter connected through peptide linkages. This is the unique characteristic of *S. aureus*. *S. aureus* have profuse wall for its cell. The thickness of cell wall of *S. aureus* range from 20 to 40mm. And it is very difficult to find the exact direction of glycan structures in the cell wall of *S. aureus*. This bacteria is found in the form of groups and they do not possess any thread-like figure. This murein is further made up of thin rope like glycan. These glycan are further connected in an oblique manner. This enhances organizational unification of sacculus. As they are gram positive so they do not contain lipoprotein and lipids. The murein and peptide constitution of the cell is complicated to a great extent. Thus the

design of wall of *S. aureus* is convoluted. It presents higher degree of cross connections as compared to other kinds of gram positive bacterium that possess simple cell wall. The oligomeric strings have not been found in the cell walls of other bacteria that are gram positive. Moreover, peptide feelers are prolonged in the case of *S. aureus* in comparison with other bacteria of the same class.

In binary fission of *S. aureus*, the daughter cells do not segregate from each other. They are only partially separated. As a result various clusters are developed. *S. aureus* grows in asexual manner. The plasma membrane is extended and segregates the molecules of DNA. A vacant space is formulated by the cells that further distributes in more cells. The contemporary cell wall is connected to the previous cell wall and results in formation of groups [28].

1.15 Binary Fission

Binary fission is the process for growth of the bacteria. In binary fission, a cell grows in its volume and then it divides into two cells. However, it is essential that this division should occur at an appropriate time. Each daughter cell should contain a duplicate of the DNA. Before the process of binary fission, DNA is duplicated and disseminated to other parts of the cell. Different kinds of proteins gather at that place in the cell which is prone to division in the future. The main element in binary fission is the proteins. These proteins gathers at central location in the cell in a circular form. Other elements also stick to this circular structure of proteins afterwards. It is placed in such a manner in the cell that upon division it does not harm DNA.

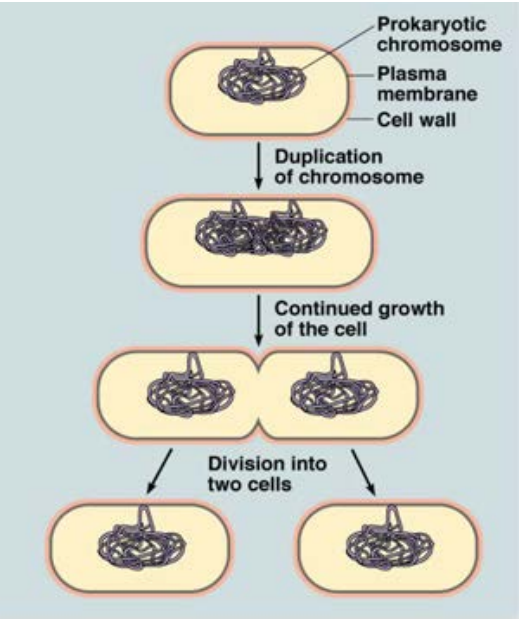

Figure 1.6: Binary fission [29]

This duplication of the genetic matter persists until the whole chromosome is duplicated and reproducing enzymes reaches at the other side of the cell as shown if figure [1.7]. The cytoplasm distributes upon completion of transmission of chromosome to other area of the cell [30].

When cell divides, cytoplasm is divided into two cells. In most of the bacteria, cell wall is also produced as a result of division of a cell. However, the duration of the division processes like replication and separation of DNA is strictly monitored.

CHAPTER 2: QUANTUM EFFECTS ON BIOLOGY

2.1 Introduction

Atoms are electrically neutral fundamental building blocks of matter with negatively charged electrons orbiting around a positively charged nucleus in the center. By the early twentieth century, it had already been established that electrons can transfer in between atoms and are responsible for chemical bonding. A large group of scientists including Ernest Rutherford, Niels Bohr, Max Planck, Albert Einstein and Schrodinger could then succeed to develop an early model of atoms and describe the distribution of electrons around the nucleus using the uncertainty principle and spin along with the basic principles of quantum mechanics.

Electrons are only able to jump from one orbit to another by either emitting or absorbing a particular amount of energy due to the quantization of angular momentum in quantum mechanics, and each transition has a specific wavelength and frequency associated with it and is referred to as a quantum jump.

In nature and science, quantum mechanics may offer an alternative interpretation of some events. Many effects and occurrences that cannot be explained using intuition are often ascribed to quantum mechanics. Quantum biology considers the prospects of such unusual occurrences happening in biological systems, as well as how such occurrences may actually be fundamentally important to the functioning of said systems. Whereas it is relatively easy to reconcile and examine the effects of quantum mechanics in physics, replicating the same procedures for biological systems is an extremely difficult feat which

requires specialized expertise and machinery. The field of quantum biology is still in its infancy, although more research is being directed towards the field presently than before.

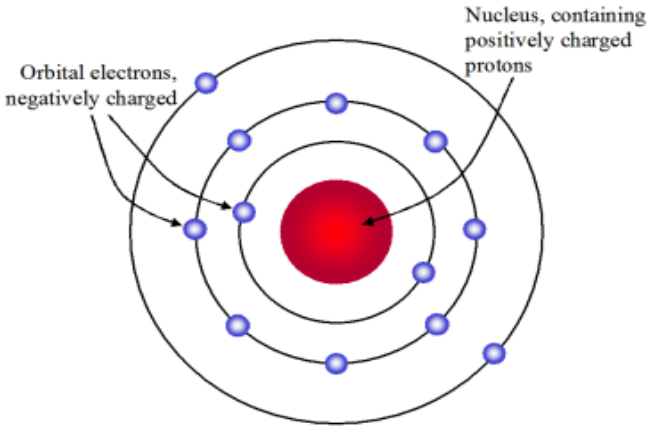

Figure 2.1: The Bohr's Atom and an Illustration of Quantum Jumps [31].

Important complicated processes in the interior of cells including enzymatic activity, DNA replication and gene mutation are governed by the electron transfer in chemical bonds which is facilitated by the energy production along with the specific ions production and transport in cells. Most of these chemical processes are described in detail using quantum mechanics. Quantum mechanical tunneling of electrons and protons is used to explain some of the DNA mechanisms and may be held responsible for mutation as well [40]. Enzymatic activity in cells including bacterial cells could be well understood using the quantum mechanical approach [33].

2.2 **Quantum Mechanical Description of Bacterial Growth**

Quantum biology can help explain processes by which the growth of bacteria in deep sites can be inhibited. For bacterial infections, tunneling and barrier potential are the two fundamental aspects that can help in the understanding of the time required to completely treat bacterial infection-related ailments. Tunneling works particularly

effectively in situations whereby bacterial infections are hidden deep within a broth. Using tunneling approaches, researchers can successfully develop means of accessing bacterial locations that are heavily layered in various locations within the body. Bacterial growth is most commonly inhibited through the use of antibiotics. Antibiotics form virtual barriers which prevent bacteria from utilizing the nutrients they need to multiply. As such, ensuring that antibiotics are able to penetrate the broth is essential to their operations.

Despite the fact that there is limited evidence-based research in the field, it is imprudent to disregard the findings that have been established by the little research in quantum biology with respect to health sciences. Quantum biology is understood to have immense potential for application in various contexts. This is primarily why research in this field has often taken exceedingly long periods of time in order to first establish whether the results are legitimately verifiable. The results of such research will be the basis of future measures aimed at improving antibiotics performance. The calculation of barrier potential provides insight into the factors that impede the optimal functioning of antibiotics. The principle of tunneling is generally understood to be based on the assumption that particles set in resonance with barriers. The particles will hence penetrate the barriers in order to ensure that the antibiotics are also able to pass through the protective membranes in order to reach the cell interior to stop its growth.

2.3 The Significance of Quantum Biology

Researchers use models based on quantum biological principles to better understand the means by which bacteria develops resistance to antibiotics. This is one of the major problems in drug administration in the world today, since drug resistance would

pose a dire threat to many people all over the world who rely on antibiotic treatment to treat their bacteria-related ailments. This also justifies the immense and frenzied efforts that are being directed towards understanding the process of combating bacteria as they develop more and more resistance to conventional antibiotic medication.

Energy of bacteria $\boldsymbol{E}$ is written in terms of the energy provided by enzymatic reaction by E_0 and from magnetic field will provide extra energy $\boldsymbol{eB}$ to the particles such that total energy E can be expressed as:

$$E_0 = E + \boldsymbol{eB} \tag{1}$$

In this setup, the addition of an antibiotic into an environment that contains bacteria is aided by the use of a virtual membrane in areas of the body which exhibit bacterial activity. A chemical is thus introduced to ensure that the bacteria does not multiply and continue affecting the environment in which it resides.

Tunneling refers to the ability of electrons to get transferred by enzymes, regardless of the preexisting energy barriers, hence moving in a way that is reminiscent of a tunnel. Research in this area utilizes knowledge of cell activities which feature the movement of electrons that is enhanced using natural light, especially in the case of photosynthesis [34]. In human-related health control and research into the inhibition of microbial activity, researchers use laser light, which raises a lot of skeptic concerns and possible health hazards.

Enzymatic activity is understood to be largely influenced by the phenomenon of quantum tunneling. In photosynthesis and cellular respiration, long distance electron transfers traversing various redox centers are dictated by quantum tunneling. Long range

electron tunneling plays a major role in cellular respiratory enzymatic redox reactions. Despite the fact that there are relatively large distance separations between redox sites that exist within enzymes, there is a consistent, efficient, temperature independent, and distance dependent traversing of electrons that occurs. This implies that electrons are capable of tunneling in physiological environments. However, there is little research that conclusively illustrates that this manner of tunneling follows a coherent procedure.

The bacteria-related diseases that are extremely difficult to treat are known as the multivariate bacterial ailments because the resistance that the bacteria involved develop towards antibiotics leads the body to become more susceptible to unique infections. Tunneling is used in the treatment of such bacterial ailments to ensure that the antibiotics do not access other strains of bacteria. The means of operation for antibiotics is selective. In essence, the antibiotics eliminate the bacterial strains that are responsive to medicine, but leave behind the unresponsive, resistant strains. Some strains of *Salmonella typhi* have been reported to be responsible for thirty-five percent of antibiotic drug resistance in the U.K. This does not necessarily imply that the medication that is used for such cases is poor, rather, that there are some modifications that are necessary in order for the starting points of the bacteria to be eliminated in the future.

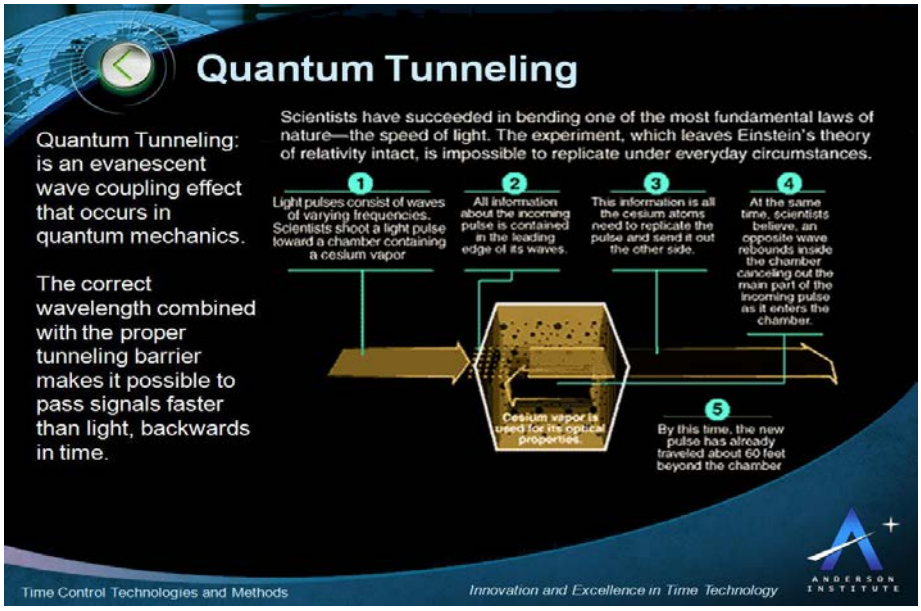

Figure 2.2: Quantum Tunneling [34]

While addressing quantum biology, it is necessary to understand the concepts which can demonstrate the progression of resistance, and the development of antibiotics which can successfully traverse the barriers. Various strains of bacteria undergo a diversity of mutations which ultimately result in their resistance to antibiotics. As more antibiotics invade the ecology of the bacteria, it actually makes it more favorable for the bacteria to survive since it enhances the adaptability of the bacteria. Initial efforts aimed at evaluating the potential applications of the principles of tunneling and barrier potential were directed towards existing radiological methods. The efforts successfully illustrated that the fission of radioactive elements and radioactivity successfully bore through the barriers, regardless of their thickness. Likewise, adapting the same principles for quantum biological applications can aid the development of treatments which can overcome potential barriers and access layered bacteria.

In many systems it is observed that the basic particle is the most influential one, but in quantum biology the case is quite the opposite. As quantum biology follows the bottoms up model, the basic point or the starting part is the higher level. This makes it essential to understand the transitional changes or properties which occur between different levels and sublevels of a living organism [36]. In order to understand the concept in detail, mathematical models and equations are used as they provide a detailed picture of the process [36]. It is expected that by carrying out more research in the field, medicines will be developed using the principles of quantum biology, which will be able to heal people by normalizing the information patterns.

One of the important factors which are involved in quantum biology is the Hamiltonian matrix. The Hamiltonian matrix is an operator that represents the total or overall energy of the living system under discussion. It is related to the environment the living being is placed in. Therefore, this factor is kept under consideration in most of the calculations of the systems that come under the category of quantum biology. One significant equation of quantum biology which explains the role of the environment on the overall quantum systems in living things is denoted as follows:

$$H = H_S + H_E + H_I \tag{2}$$

In the equation (2), H represents the overall Hamiltonian matrix, H_S is the Hamiltonian for the system, H_I represents the interaction Hamiltonian between the system and the environment, and H_E denotes the free evolution of the environment. From the equation it can be clearly deduced that the overall Hamiltonian matrix depends on the all

the three factors [35]. The sum of these factors is known as the total Hamiltonian effect. This means that by increasing any one of the three factors we can increase the value of Hamiltonian matrix, and by reducing any of the factors the value of the Hamiltonian matrix will also be reduced. It has also been observed that during experiments the environmental conditions are kept controlled to a huge extent, hence the Hamiltonian effect`s significance is reduced. But when these systems are observed outside of the laboratories under natural conditions, all these factors play their role [35]. The example of a freely moving protein particle can be taken here in order to explain the concept. Protein is one of the simplest or micro particles of a living organism, and it can be observed that the environment the protein moves in affects its total energy. If the environment is defined as wet, noisy and warm it particle is expected to have greater Hamiltonian matrix. Hence it can be concluded that the environment does influence the total Hamiltonian matrix of a biological system. This indicates that the amount of energy in a system can be altered easily by altering the environment in which the system is operating in [35].

2.4 The Density Matrix

The density matrix is a matrix that serves to describe quantum systems in mixed states. In contrast, a single state vector serves to describe quantum systems in their pure states. A mixed state occurs when the particular state that is under manipulation in an experiment is not fully understood. This may include situations such as those whereby the experiment involves a quantum system with two or more entangled subsystems. In such a case, each subsystem must be treated as a mixed state regardless of the pureness of the complete system.

Many biological systems are capable of remaining in quantum coherent states at room temperature for extended periods of time. By maintaining the optimal balance between chaos and regularity, systems can successfully increase their time spent in coherent states by orders of magnitude.

2.5 The Hartree-Fock Computational Model

Hartree-Fock is among the most important and efficient quantum biological techniques since it recovers nearly 99% of the aggregate electronic energy. The method has seen more widespread application in physics and chemistry compared to biological adaptations. Still, the Hartree-Fock model is understood to be quite unreliable and offers a weak approximation in many cases. However, the model has been refined and customized into more sophisticated post-Hartree-Fock models which are more accurate in their depiction of the Hartree-Fock wave function. In addition, the Hartree-Fock computational model offers mathematical structures upon which molecular orbits can be studied and interpreted in detail [37].

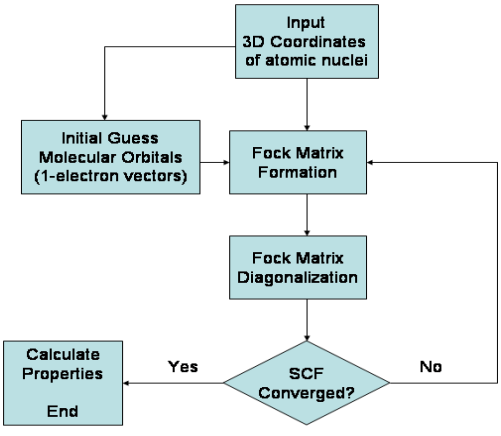

Figure 2.3: Algorithmic Illustration of the Hartree-Fock Method [37]

The basis of the Hartree-Fock computational model is the evaluation and manipulation of $O(M^4)$ integrals with two electrons. The algorithm then scales in the interval between $O(M^2)$ and $O(M^3)$ for all instances in which the computation dominates the runtime. However, given that the Hartree-Fock method is based on the solution of nonlinear Eigenvalue sequences of equations via iteration, there are some key hindrances which prevent worst-case scaling from forming polynomials. The main hindrance is the convergence of implementations which are self-consistent.

2.6 Interpretations of Quantum Biology

Quantum biology can be interpreted using sets of statements which are aimed at describing the role of quantum mechanics on human understanding of natural processes. Even though quantum mechanics largely helps to rigorous testing and experimentation, there are various interpretations that can be deduced from quantum mechanical phenomena. Among the sources of contention and divisive interpretations of quantum mechanics is the lack of clarity over which facets of quantum mechanics can be interpreted as deterministic, and which ones can be considered real. This likewise affects the applications of quantum mechanics in biology, since this is a field which is heavily dependent on the influence of non-trivial quantum phenomena applied to many-body systems.

Quantum biology is a very intriguing subject because it presents the answers to some of the most fundamental concerns and questions regarding basic operations in nature. At a more specific and practical level, quantum biology presents immense opportunities

for resolution of impending problems related to human health, and the development of new solutions to issues which have bugged mankind for a very long time. In antibiotics, for instance, the development of medication that is able to combat resistant strains of bacteria depends greatly on the prospects of quantum biology theory to come up with ways of handling such issues. Currently, mankind is facing a serious problem in the near future which may result from the development of super resistant strains of *Salmonella typhi* and other bacteria. Quantum biology presents people with opportunities to better understand the fundamental functioning of cell-level structures, which in turn informs us of the best procedures to utilize for the solution of problems such as hard antibiotic resistance. In the case of antibiotic resistance, the solution is through tunneling. With the wide array of applications of quantum biology currently in various stages of research, one can only ponder what the future holds for humanity as a result of such research.

2.7 Tunneling in Quantum Biology

The application of theoretical chemistry and quantum mechanics to biological problems and objects is referred to as quantum biology. Biological processes involve various transformations that are chemical in nature and apply the nature of quantum mechanics [36]. Tunneling in quantum biology is defined as the ability of particles with masses that are small in nature to travel and pass through various energy barriers. It is therefore the metaphorical name given to the process, possible in quantum mechanics, but not in classical mechanics, whereby a particle can disappear from one side of a potential-energy barrier and appears on the other side without having enough kinetic energy to

overcome the obstacle. Quantum tunneling in biology is an important factor that plays a major role in processes that involve enzymatic reactions, such as in the case of redox reactions relating to photosynthesis and cellular respiration.

The following figure [2.4] is a demonstration of quantum tunneling through a barrier. The energy of the tunneled particle is the same, but the probability amplitude is decreased.

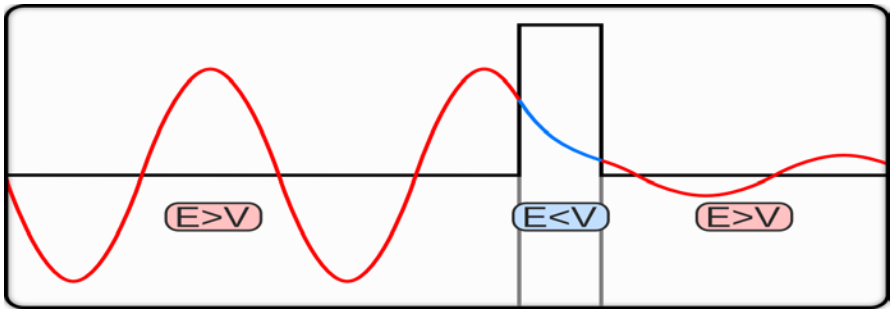

Figure2.4 The energy of a particle that has tunneled through a barrier remains unchanged but its wave function amplitude goes down [38].

2.8 Schrödinger Equation for Quantum Tunneling

Schrödinger Equation can be used in order to explain the phenomenon of quantum tunneling and the example of how the energy distributed in the barrier remains the same throughout. These particles move in the form of a wave and the Schrödinger equation (also known as the "wave equation") shows how these particles exhibit such a behavior [39]. In the equation below, *H* is the Hamiltonian operator and the behavior of the proton in the tunnel is regulated by this time-dependent equation.

$$H\Psi = \frac{-h}{2\pi i}\frac{\partial \Psi}{\partial t} \quad (3)$$

There are three states in the whole process, the first is the initial state at t = 0 when the particle is stationary and E >V, and the second is the tunneling level when the value of the function changes at some time t. The final is the new state where E >V, which shows that the energy remains same at both the initial and final points. This phenomenon is known as quantum tunneling [39].

2.9 Tunneling and Probabilities

$$\Psi(t) = e^{-\left(\frac{2\pi i}{h}\right)Ht}\ \Psi o \tag{4}$$

The Schrödinger equation at the stationary state t=0 will then become a time dependent function again as it will move into its next state and could be written as following:

$$\Psi(t) = \sum e^{-2\pi i/hE_n t}\ \Phi n\ <\Phi n|\Psi o> \tag{5}$$

Equation 4 can be made into an orthonormal equation when $H\Phi_n = E_n\Phi_n$, hence it becomes a set in the tunneling process which can be made up into an identity to solve the equation hence in this way $1 = \sum_n |\Phi_n><\Phi_n|$ which again becomes a time dependent function proving that value remains same at all intervals.

$$a = [1/2\,(a + b)] + [1/2\,(a - b)] \tag{6}$$

The above equation can be used to make equation 3 to be in linear form and solve the function as well. This will help in corresponding to the 50-50 distribution of the proton's energy in the tunneling stage and also denote the proton orbitals that are

associated with the two different positions as well at $t = 0$ $(i.e. \Psi o = a \; or \; \Psi o = b)$ [39]. Hence, equation 3 will be deduced as below [39]:

$$\Psi(t) = 1/2\,(a + b)e^{-2\pi i h E_g t} + 1/2\,(a - b)e^{-2\pi i/h\, E_u t} \quad (7)$$

For,$\nu = (Eu - Eg)/h$ the time will become 1/v, so Equation 7 will be as following [39]:

$$|\Psi(t)|^2 = |\,1/2\,(a + b) + 1/2\,(a - b)e^{-2\pi i t/T}\,|^2 \quad (8)$$

2.10 Proton Tunneling in DNA

Proton tunneling is an example of quantum tunneling that involves disappearance of a proton instantaneously and its appearance in an adjacent site that is separated by a potential barrier. It is a fundamental factor in spontaneous mutation of DNA which occurs when a normal DNA replicates after an important proton has taken advantage of quantum tunneling. Hydrogen bonds are associated with proton tunneling, and as such DNA strands which are made up of hydrogen bonds are subject to proton tunneling [39]. The arrangement of the hydrogen bond is unique in a DNA strand, and it defines the genetic code. Basically, there exists a double well potential along the hydrogen bond separated by a potential barrier. The potential well is usually asymmetric and one of the wells is deeper than the other. The proton usually lies in the deeper well. During the replication of a DNA strand, proton tunneling takes place that is responsible for the changes and the configuration of the hydrogen bond. At times the tunneling will depend on the height and form of barrier in the DNA. The form of the double-well potentials regulating the hydrogen

bonds depends on both base and neighboring pairs involved, their net charges and the entire electric environment. Proton tunneling is of great importance since it controls the occurrences of tumors. The growth of a person is a highly refined balance between the factors that enhance the cell duplication and that limit the duplication so that an organism takes a desired shape.

In a quantum mechanical system, the proton can be represented by a wave packet which allows the proton to penetrate into hindered areas in the classical system. This enables the proton to move from one equilibrium state to another by means of tunneling through the potential barrier. The figure [2.5] shows how the quantum tunneling allows a quantized wave packet to penetrate the barrier and move from one potential well to another.

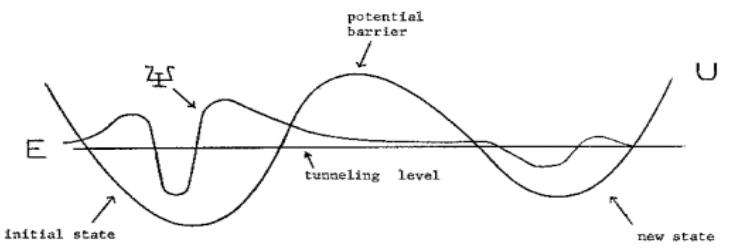

Figure: 2.5 Quantum tunneling effect allows a quantized wave packet to penetrate through the barrier and move from one potential well to another [39].

2.11 Electron Transfer

Electron transfer is defined as the relocation of electrons from a molecule or an atom to another chemical entity. It involves the movement of an electron from one molecular species known as the donor to another known as the acceptor. It is an important and essential step in biological photosynthesis where the electron transfer initiates a downstream process such as the proton pumping through the membrane and the production

of glucose. The chemical reactions such as the oxidation or redox involve the movement of electrons from one atom to another [40]. Numerous biological and chemical processes in nature are carried out by electron transfer reactions. These include respiration, oxygen binding and detoxification.

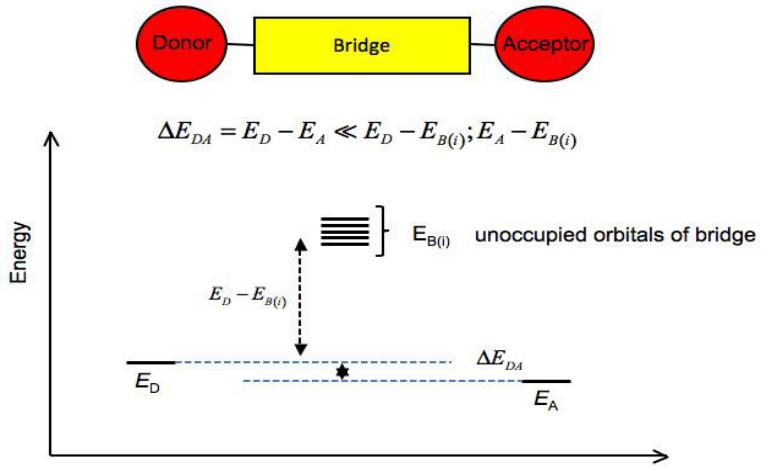

Figure.2.6: The two state model of electron transfer [41]

Electron transfer in quantum biology can be considered and viewed in terms of quantum mechanical and semi-classical models as shown in the figure [2.6]. In biological systems we base on a specific example of plant cryptochrome, where electrons are essential for functioning. In this case, proteins and electrostatics play a significant role in the transfer of electrons. The polarization forces are also crucial in propelling the electrons through the cryptochrome. Consequently, the quantum mechanical description of electron transfer is applicable in variety of biological systems, including DNA photolyase.

CHAPTER 3: ANTIBIOTICS INTERACTION WITH BACTERIA

3.1 Antibiotics Use common methods

Antibiotics are antimicrobial type of drug used in the treatment and prevention of bacterial infections. Antibiotics kill or stop bacterial growth; however they are not effective against viruses such as influenza or common cold. Antibiotics use has sometimes led to the eradication of deadly diseases like tuberculosis. At times, the term antibiotic is mostly used to refer to any substance used against microbes. The revolutionary of antibiotic medicine occurred in the 20^{th} century. Conversely, their easy access and effectiveness have led to their overuse stimulating bacteria to gain resistance [42]. Alexander Fleming, a Scottish researcher and botanist began the use of modern antibiotics and -discovered penicillin in 1928.

Antibiotics can be classified based on the system they affect or their cellular component. Antibiotics successfully work by bacterial microorganisms' elimination, referred to as bactericidal or by hindering the microorganisms' development known as bacteriostatic. Since the penicillin discovery, many more effective antimicrobials have been developed and discovered by elucidation of drug molecule modification and by drug target interactions. The increasing drug resistant bacteria prevalence, resistance gaining means, has significantly made it clear of the multilayered mechanisms through which available antibiotics eliminate bacteria and also find alternative therapies of antibacterial drugs. Antibiotics induced cell death has been linked with formation of breaks of double-stranded DNA following DNA inhibitor treatment. Most current bacterial antimicrobials

inhibit RNA synthesis, DNA synthesis, protein synthesis, metabolic pathway, cell membrane disorganizing and cell wall synthesis [43].

3.2 Method of Action

The method of action or mechanism of action for antimicrobial drugs is the means by which they kill or inhibit bacterial colonies. There should be three conditions for an anti-microbial to powerfully be against microscopic organisms. These include; the antibiotic need to reach the objective in adequate amount, the anti-infection must not be altered / in-activated and a vulnerable target of anti-microbes should exist in the cell. They fall generally into the following categories: inhibition of protein synthesis, inhibition of nucleic acid, inhibition of cell wall synthesis, inhibition of metabolic activity and alteration of cell membranes.

Cell wall synthesis interference; the bacterial cell is enclosed by murrain or, peptidoglycan layers which is polymer matrix covalently cross-linked. The mechanical strength managed by this layer of the cell wall is crucial to the ability of bacteria to survive conditions of the environment that may alter osmotic pressure prevailing. Glycopeptides and ß-lactams are among the antibiotic classes that interfere with specific steps in homeostatic biosynthesis of cell wall [44]. Effective treatment with an inhibitor of cell wall synthesis can results in cell changes of size and shape, culminate in cell lysis and induce cellular stress response. In *Staphylococcus aureus* (*S.aureus*), an extra holing-like system was discovered in *S.aureus* and found to activate autolysins making *S. aureus* more prone to β-lactam facilitated killing [44].

Protein synthesis inhibition; Cellular structures and enzymes are built of proteins. Protein synthesis is a critical process necessary for the survival and multiplication of all bacterial cells. The process of mRNA (messenger Ribonucleic acid) translation into protein occurs over three phases that is initiation, elongation and termination involving the ribosome. Ribosome organelle contains two ribonucleoprotein subunits the 30S and 50S [44]. Erythromycin, a 50S ribosome inhibitors, works by physically blocking either exit of growing protein or peptidyl tRNA (transfer Ribonucleic acid) translocation. The 30S ribosome inhibitors that include aminocyclitol and tetracycline families of antibiotics work by blocking the access of aminoacyl-tRNAs to the ribosome. This activity then leads to disruption of normal bacterial cellular metabolism and subsequently leads to the inhibition of multiplication or death of the organism [44].

Interference with nucleic acid synthesis; DNA and RNA are keys to the replication of all living forms, including bacteria. Some antibiotics work by binding to components involved in the process of DNA or RNA synthesis, which causes interference of the normal cellular processes which will ultimately compromise bacterial multiplication and survival. Examples: quinolones, metronidazole, and rifampin [44].

The inhibition of synthesis of RNA by rifamycin has a disastrous effect on prokaryotic metabolism of nucleic acid and it is an effective way of inducing death of bacterial cells. The antimicrobial class of quinolone interferes with the chromosomal topology maintenance targeting DNA topoisomerase and gyrase preventing strand rejoining.

Inhibition of a metabolic pathway; some antibiotics act on selected processes of cells essentially for the bacterial pathogens survival. An example is a case whereby trimethoprim

and sulfonamides disrupt the pathway of folate that is important step for production of precursors by bacteria for DNA synthesis. Sulfonamides bind to dihydropteroate synthase while trimethoprim inhibits enzyme dihydrofolate reductase [44].

Cell membrane disorganizing; cell membranes are significant barriers that regulate and separate the extracellular and intracellular flow of substances. Damage or leakage to this structure may result in important solutes crucial for survival of cells leaking. Polymyxins apply inhibitory impacts by expanding the penetration power of bacterial layer hence causing bacterial substance spillage. Most of the clinical use is limited to topical applications. Also, daptomycin indicates fast action of bacteria to the cytoplasmic film thus encouraging efflux of potassium from the cells of bacteria [44].

3.3 Erythromycin

In this investigation, the antibiotic utilized is erythromycin. Erythromycin was isolated from *Saccharopolyspora erythraea*. The drug has been listed by the World Health Organization as a crucial medicine and has been given title as one of the safest medicine. Erythromycin is relatively inexpensive range of antimicrobial drug indicating that it is effective for an extensive variety of disease causing microorganism. Erythromycin can be used to treat skin contaminations, tract infections, inflammation of pelvic, syphilis and contaminations by chlamydia [45]. Due to its slight adverse reactions, the drug is generally prescribed to the pregnant or nursing women.

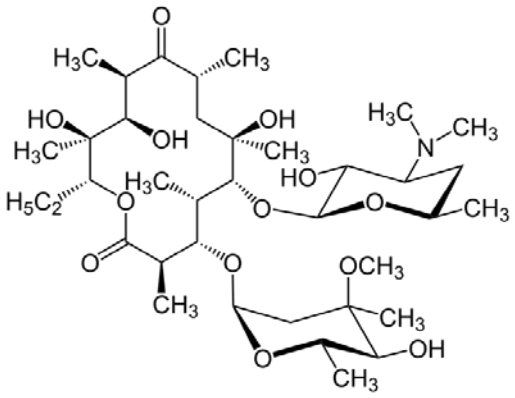

Figure 3.1: Erythromycin structure [46]

Erythromycin method of activity is restraint of protein synthesis by binding to the 23S location of rRNA molecule of the bacterial ribosome therefore blocking the sensitive microorganism growing peptide chain exit. This produces bacteriostatic cell response which stops replication. Certain resistant microorganisms with changes in mutation in components of the subunit of the ribosome fail to fix the drug. The ribosome and erythromycin association is reversible and takes place only when the 50S subunit is free from tRNA molecules bearing emerging peptide chains. Erythromycin interferes with translocation of aminocyl, keeping the tRNA exchange bound at the A-site of the rRNA complex to the P-site of the complex rRNA.

A time that the translocation cannot take place due to the A-site as yet being used, at that event protein synthesis ends. Any approaching tRNA and its attached amino corrosive to the incipient polypeptide chain are restrained from moving further along the chain of mRNA. This prevents the creation of practical proteins used as a part of replication of cells [47]. Erythromycin is assimilated immediately through layers of tissues and equally diffuses between phagocytes. For this, the high phagocytes number show in circulatory

system takes into account the irritable erythromycin dissemination to the diseased area. The larger erythromycin part is utilized by the liver using hepatic catalysts. Erythromycin contains mycrocyclic center, which is a ring made out of an extensive moderate molecule number. The rRNA chain assumes a critical part in the blend of protein. On the erythromycin account, of which ties to 50S site of ribosomes, the anti-toxin viability

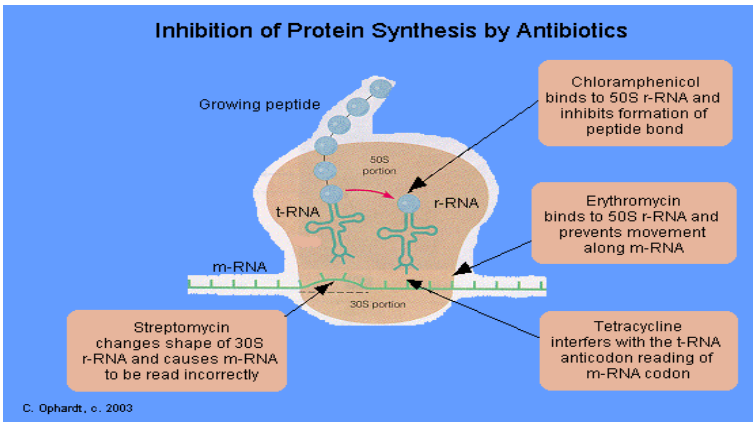

Figure 3.2: Action method of erythromycin [47]

is controlled by the anti-infection ties amount. Antibiotic resistance in the case of erythromycin can come from many changes in the cellular behavior and its structure. Antibiotic resistance can evolve through the inactivation of rRNA in the cell. Methylation enzymes would decrease the effectiveness of the drug due to other rRNA sequences being bound to the 50s site. This allows the bacterial cell to produce critical proteins needed for replication by effectively passing the 50s site. Without any place for the drug to bind to the drug would lose its effectiveness [47].

Macrolides are molecules that have a macrocyclic lactone ring in their structure. It is believed that antimicrobial and antifungal properties of substances stem from macrocyclic ring structures. Examples of these types of molecules that exhibit

antimicrobial properties are erythromycin, clarithromycin, and azithromycin. Macrolides specifically inhibit protein synthesis. This is most commonly done by prohibiting the tRNA from binding to activation sites on the rRNA. By preventing peptidyl-transferase from adding the growing peptide attached to tRNA to the next amino acid, erythromycin stops the amino acid from changing into a functional protein complex [47]. This in turn hinders translation of ribosomes along the sequence of rRNA.

3.4 Magnetic fields

Magnetic fields are characterized by attraction impact delivered by magnetic materials or electrical currents. A wide variety of magnetic fields are vectors, this means they have both direction and magnitude with them. Magnetic field cooperation changes when separation occurs. Magnetic field strength has a $1/r^2$ dependence on its size indicating that the further one moves from the magnetic field source, the field drops away with the square of the distance. The distribution of magnetic fields is similar to that of electric fields. The bar magnet fields resembles scattered lines that on top of one other get stack. To find out the magnetic fields effect on the biological system behavioral field uniform is applied to the system. If, on the system a gradient of field is applied, then it would be difficult to find out if the observed effect is solely due to the fields' gradient or magnetic field.

Figure 3.3: Magnetic field lines [48]

S. aureus is a major pathogen that produces super antigens and toxins causing skin and soft tissue infections in communities or hospitals. Antibiotic therapy is not greatly proficient because the intensive and routine usage has led to emergence of both community and hospital linked methicillin resistant *S.aureus.* A study conducted on the effect of paused intensity of magnetic field and the pulse number on bactericidal property of pulsed magnetic field in sterilization of fresh juice of watermelon. The results showed that the overall effect of the bactericidal was reinforced as the pulse number and magnetic field intensity was 21 and 2.52 respectively. Effects of electromagnetic field on bacteria study are important not only for environmental stress influences investigating on biological systems but also to discover the possibility of controlling the sensitivity of bacteria toward environmental antibiotics.

Magnetic field effect on growth and antibiotic susceptibility of bacteria *S. aureus* was tested. This was aimed to examine the exposure effect of different magnetic fields that is; 400, 800, 1200 and 1600 Gauss for 2 to 24 hours on the rate of growth and antibiotic sensitivity of *S. aureus.* The bacteria were isolated from the medical case and identified

using a system known as API STAPH. The vulnerability of the antibiotic of *S. aureus* measured according to the technique of diffusion. The results exhibited an important logarithm reduction in the number *of S. aureus* treated with high frequency magnetic field as shown in Figure [3.4]. The sensitivity of *S. aureus* to antibiotic increases during a short period of 4 to 6 hours and increase its resistance to the same antibiotic at a long term exposure of 18-24 hours [50].

Some biochemical tests results indicated positive effects of magnetic fields on the biochemical properties. The enzymes bacterial lactose, trehalose, sucrose, mannitol, acetyl-glucosamine and maltose were affected by magnetic field at 24 hours of incubation. From the research, it is concluded that the cellular membrane of the microorganism had been affected by the fields of magnet [49]. Furthermore, the response amplified when the intensity of the magnetic fields increased. According to this, the effects of magnetic field on bacteria are considered bactericidal and thus a change in the number of cells or the change measured in the sensitivity of membrane to antibiotic demonstrated the change in the structure of the cells internally.

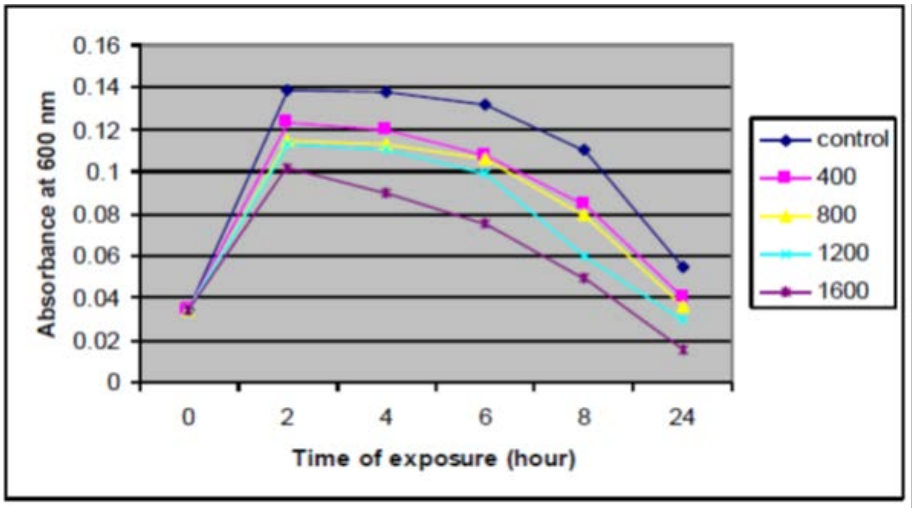

Figure 3.4: Absorbance at 600 nm of S. aureus cells with different exposure periods [50]

It was found that magnetic fields increased the phase of logarithm of *S. aureus* growth within 4 hours of treatment but reduced growth curve after 8 hours period.

There was a change which was considerable in the rate of growth of *S. aureus*. A decrease in the colony forming units started instantly after the magnetic field effect on the bacteria could be deliberated as bactericidal. These results are concurring with others

Time of exposure to magnetic field in hour	*Optical Density (O.D.) at 600 nm and bacterial cells count(McFarland)*									
	Control		*400G*		*800G*		*1200G*		*1600G*	
	O.D.	*Bac. count x 10^6*	*O.D.*	*Bac. count x 10^6*	*O.D.*	*Bac. count x1 0^6*	*O.D.*	*Bac. count x10^6*	*O.D.*	*Bac. count x 10^6*
0	0.034	10.2	0.034	10.2	0.034	10.2	0.034	10.2	0.034	10.2
2	0.139	41.7	0.123	36.9	0.115	34.5	0.113	16.95	0.102	30.6
4	0.138	41.4	0.120	36	0.113	33.9	0.110	33	0.090	27
6	0.110	33	0.108	32.4	0.106	31.8	0.099	29.7	0.075	22.5
8	0.098	29.4	0.085	25.5	0.080	24	0.060	18	0.050	15
20	0.055	16.5	0.040	12	0.036	10.8	0.030	9	0.015	4.5

Table 3.1: Growth rate of S. aureus for each group [48]

who reported the exposure of *salmonella typhi*, *E. coli* and *S. aureus* to the magnetic field has the same effects [51].

Antibiotics	*Mode of action*	*Inhibition antibiotics zone diameter in mm*							
		Un exposed to M.F	*M.F exposure (G) time*						
			2 hour				*20 hour*		
			400	*800*	*1200*	*1600*	*400*	*800*	*1200*	*1600*
Gentamycin	Inhibition of protein synthesis (30 S-R)	25	35	25	30	30	22	17	16	16
Tetracycline	Inhibition of protein synthesis (30 S-R)	25	36	38	35	30	17	16	16	16
Chloramph-enicol	Inhibition of protein synthesis (50S-R)	18	12	10	10	10	10	10	R	R
Ceftriaxone	Inhibition of cell wall	25	30	R	R	R	R	R	R	R
Ceftazidium	Inhibition of cell wall	17	30	25	23	23	16	15	13	10
Rifampin	Inhibition of nucleic acid	32	18	R	R	R	R	R	R	R
Metronidazole	Inhibition of nucleic acid	R	R	R	R	R	R	R	R	R

Table.3.2 Antibiotic test of exposed and unexposed S. aureus to magnetic field. R: Resistance, M.F: magnetic field, S-R: Subunit-Ribosome [50]

The previous table [3.2] showed the antibiotics susceptibility test at various exposure periods 2,4,6,8 and 24 hours which estimated according to the action mode. The results indicated that *S. aureus* were sensitive for ceftazidime, gentamycin, rifampin, chloramphenicol, ceftriaxone and tetracycline where else resistant to metronidazole [52]. The diameters of the stimulation or inhibition zone of the different forces of magnet were measured after 24 hours from the process of exposure compared with samples unexposed. These results concurred with a study which found that moderate intensity static fields were able to lead to a reduction in resistance of *E. coli* and sensitivity. In addition, found that the possibility of magnetic field interfering with the charge on the antibiotic molecule or surface charges of the membrane altering the antibiotic penetration rate may exist.

Also it was exhibited that magnetic field can affect functions of membrane however, the magnetic field could relate with other specific process that help the bacteria

adapt to the new environment [53]. Due to this, the bacteria are able to retort to stresses of environment by initiating suitable inducible systems like DNA repair system and in turn destroy processes which increase the variability of genes.

3.5 Bacterial growth curve

Bacterial growth curve shows the time that it takes to populate. This time changes with different types of bacteria. Some of the bacteria requires more time for development while other bacteria requires less time for growth. When these bacteria are provided with a suitable surroundings then they show a particular design [54]. It is represented by a curve that is called as growth curve. The growth of bacteria or the growth of other microorganisms can be modeled with different phase as shown in figure below [3.6].

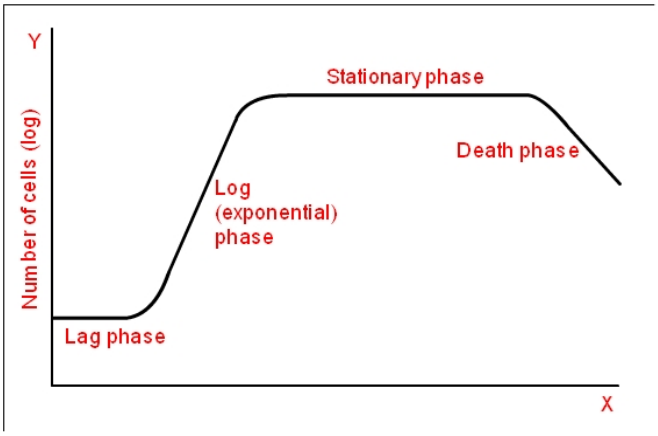

Figure 3.5: Show the growth phases where Y-axis is absorbance and X-axis is the time [54].

These phases are (A) lag phase (B) exponential phase (C) stationary phase (D) death phase. In the Lag phase, bacteria are maturing independently and therefore not able to divide. When cells are transferred to the fresh media they required time to adapt the environment, express specific genes, and synthesize components. In this phase bacteria adapt themselves to growth condition. During this, lag phase cells changes very less because the cells do not quickly reproduced in new medium [54].

The time which cell takes to adapt the environment is called incubation time and bacteria is said to be in the lag phase. After passing by the lag phase bacteria enters into the exponential phase, in which cells grow exponentially. In addition, this is the time of reproduction characterized by cell doubling. Binary fission occurs at a maximum rate, and the cells divide as rapidly as possible. For this type of exponential growth, plotting the natural logarithm of cell number against time produced a straight line. In this phase if the growth is not limited, doubling will continue at a constant rate so both the number of cells and population increase with each consecutive period of time [10]. At stationery phase, the growth stops and there is no net increase or decrease in the number of cells. Bacteria use new forms of metabolisms to survive and in some cases produce secondary metabolites, which are often useful to humans such as antibiotics. Spores are also produced during this phase. The result is a “smooth “horizontal linear part of the curve during the stationary phase. But mutation can occurs during stationary phase. The death phase is also called as decline phase. In this phase bacterial life ends and it dies. This could cause changes in environmental temperature that are above and below the

tolerance band for the species or some injuries. More cells are dying as compared to production.

CHAPTER 4: EXPERIMENT AND DISCUSSION

4.1 The Experiment and Results

The purpose of this experiment is to testify the interaction between antibiotics and bacterial cells inside two types of magnetic fields. *E. coli* has been previously studied because it gives a comparison of a good prokaryotic Gram-positive bacteria which exhibit both aerobic and anaerobic properties and has a well-understood structure. In this experiment, *S. aureus* was studied as an example of Gram positive prokaryotic cell and a common bacteria which can attack human beings. We used erythromycin as it is a relatively effective broad-spectrum antibiotic. Erythromycin is a common and well-studied antibiotic and is known to affect *S. aureus* and *E. coli* growth. We investigate the effect of weak magnetic field on the interaction of erythromycin with bacteria. The effect of weak magnetic field on the interaction of erythromycin with bacteria gives enough justification of the impact of weak field on the enzymatic activity of the drug with bacteria, which includes the activity of ribosomes.

4.2 Experiment Setup

This experiment was designed in a natural and clean laboratory environment with minimum resources. We used Bausch and Lomb Spectrometer 20 as shown in Figure [4.1] Colorimeter to measure the optical density to observe the bacterial growth. By taking the transmittance and absorbance of the bacterial culture in nutrient broth, we were able to plot a growth curve. Same bacterial growth was studied while bacteria was treated with the magnetic field and a comparative behavior between treated and untreated bacteria was

done. The impact of magnetic field on the interaction between bacteria and antibiotics is studied in detail.

4.3 EXPERIMENTAL SETUP: Materials and Design

The bacterial growth was studied in a constant magnetic field and time varying magnetic field and the results were compared with the one with no field exposure and used as control (B=0). Magnetic fields were very low (~ 5G) to study the perturbative effect of the field. Strong magnetic field effect are totally different and should not be mixed with the weak field effects. Following the instructions on the package the original bacterial culture was grown and then through dilution and segregation over agar plates. The original culture was diluted three times and a colony was isolated in a standard concentration of LB Broth. The common culture of this bacteria was then used to study the growth rate over the magnets and compared with the growth rate without magnets in the same nutrient broth under exactly the same laboratory environment.

The growth rate of the bacteria was measured be measuring the optical density using Spec.20 as shown in Figure [4.1]. The field strength was variable depending on the location. However the maximum strength of the magnetic field was 5 Gauss in magnitude.

Figure 4.1: Picture of the spectroscope. This is used for measuring the optical density at 650 nm.

The process of preparing samples began with streaking a sample of isolated *S. aureus* and a sample of *E.coli* into Petri dish with a mixture of agar and peptone. The samples were grown in an incubator at a 37 °C for 24 hours. Bacterial colony was selected and showed visible growth. Peptone broth samples were prepared then the selected colony was introduced into 10 ml of liquid peptone broth. Antibiotics were recommended and prepared by instructions from Sigma Aldrich. 10 Capsule from erythromycin, 100gm/ml was prepared by powdering the antibiotics and took out 10 grams. The powder was introduced to 100 ml of peptone broth. This led to a final concentration of 100 mg/ml. This was used to treat 100 microliter of bacteria stock.

Magnetic Fields Environments	Field Strength
Time Varying Magnetic Field	5,3,1 for 10 minute intervals
Static Magnetic Field	4 Gauss
No Magnetic Field	0 Gauss

Table 4.1: Table of magnetic environments.

In order to search for varying effects, different magnetic fields were set up in relatively simple configurations. Bacterial tubes were placed in a time-varying electromagnet and static electromagnets and results were compared with the samples of unexposed magnetic field. The magnetic field strength and types are described in table [4.1].

The static magnetic field is produced using a series of Helmholtz coil which are connected together and act as a solenoid, shown in Figure [4.2]. A static magnetic field is generated passing a steady current in Helmholtz coils that produced 4 guess in the middle of the coils. The tubes also were placed inside the coils to study the effect of the static magnet on the bacteria growth.

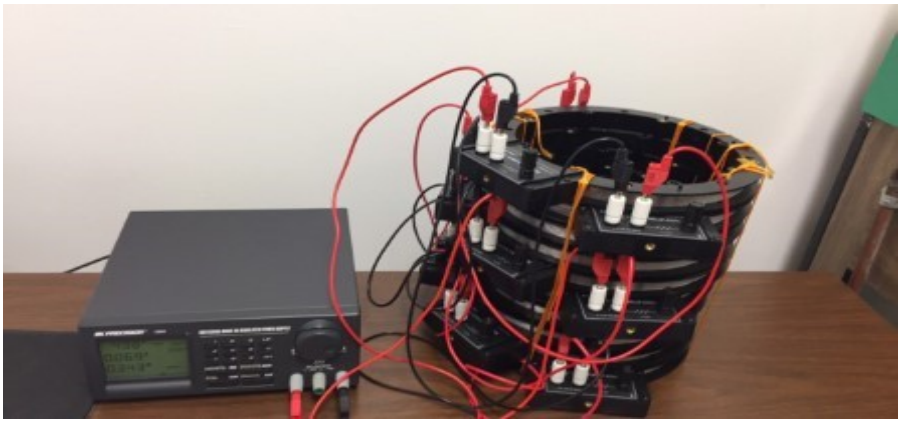

Figure 4.2: Picture of Helmholtz coil. This is used to generate a uniform magnetic field of 4 gauss inside the coils.

The time-varying magnetic field was produced using a similar arrangement as for the production of uniform fields and it was turned on and off every 10 minutes to produce oscillation of very low frequency. The current is running from 1 amps, 3 amps, and 5 amps every 10 minutes and it starts over. The tubes were placed inside the coil after the bacteria was inoculated to the nutrient broth.

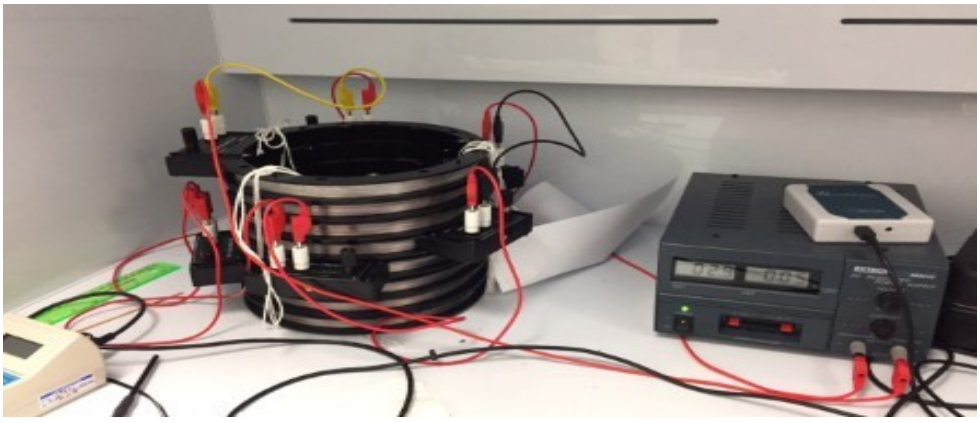

Figure 4.3: Picture of Helmholtz coil with power supply. This is used to generate a time varying magnetic field. The current running every 10 minute from1 amps, 3amps and 5 amps.

Spec. 20 was used for measuring the optical density at 650 nm. The tubes were placed inside the Helmholtz coils to be exposed with the magnetic field. The measurement of absorbance and transmittance was taken. Two blank tubes were used in the experiment, for calibration of the spec. 20. One containing only nutrient broth and another one containing nutrient broth and antibiotics. The replication time for *E.coli* is between 20 to 40 minutes and *S. aureus* is between 27 to 40 minutes. For this purpose, the tubes were checked periodically and the measurement was taken every 2 hours. After 12 hours, samples marked to receive antibiotics at 100μg/m. From the data was taken and graphed as a function of time.

4.4 Result and Discussion:

Run1

Starting with the regular lab environment with no magnets and a blank tube of nutrient broth in order to calibrate the spec. 20 to zero and remove any interference. For the samples which received antibiotics, the blank contained antibiotics and nutrient broth as described previously used to calibrate the spec. 20 to zero as well.

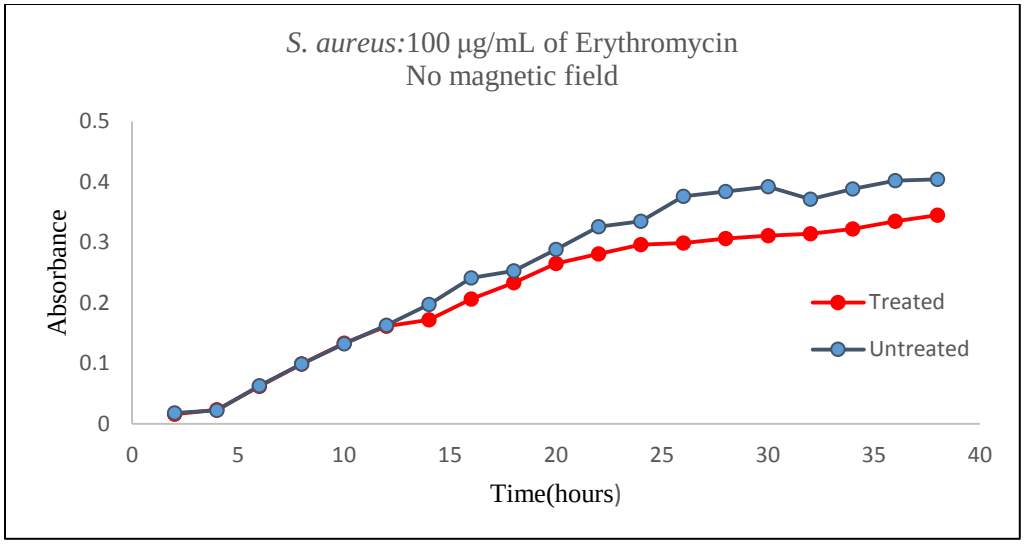

Figure 4.4: Comparison of growth curves between treated and untreated bacteria in the absence of magnetic field.

The data from the first run shows similar behavior from the initial growth for both samples until 12 hours. And two obviously different growth curves were generated after treating the bacteria with antibiotics. The antibiotics were introduced to half of the samples after 12 hours. The results of two fairly close initial concentrations are compared determine the effect of magnetic field on the bacterial interaction with antibiotics. After the antibiotics were given, the curve moved to stationary phase for an hour and regrow slowly. Unlike untreated samples, the growth curve continuously growing until 28 hours then fluctuated. As is indicated from the Figure 4.4, the growth curve of both sets of samples shows steady growth after 25 hours and end up with .05 different absorbance.

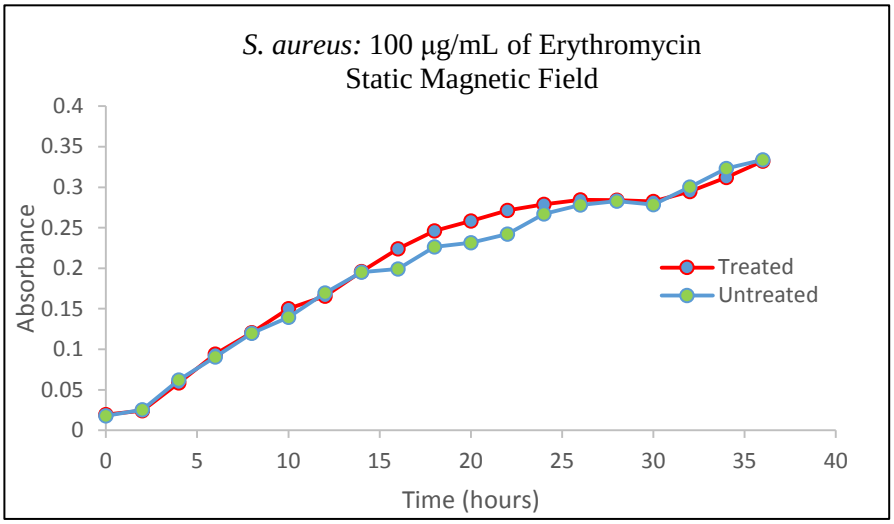

Figure 4.5: *Comparison of growth curves between treated and untreated bacteria In the effect of static magnetic field.*

The static magnetic field samples showed relatively different behavior to what we observed in the controlled vails. The static magnetic field shows the steady growth curve for both samples. Untreated samples show a lower growth curve than the treated samples after 14 hours. Due to a low concentration of bacteria at the time when antibiotics were introduced, the magnetic interaction between antibiotics with cells may be causing bacteria to accelerate the absorbance of ions from nutrient broth.

Lastly of run 1 is time varying magnetic fields. Uniform magnetic fields were applied to these samples every 10 minutes from 1 amps, 3 amps and 5 amps. After the antibiotics were introduced, the growth curve increased slowly due to bacteria resistance. However, the unexposed sample increased more than exposed sample with 0.1 absorbance. Since these samples are not interacting with the magnetic field, the only explanation for this difference growth for static and time varying field could be the interaction of antibiotics with the magnetic fields.

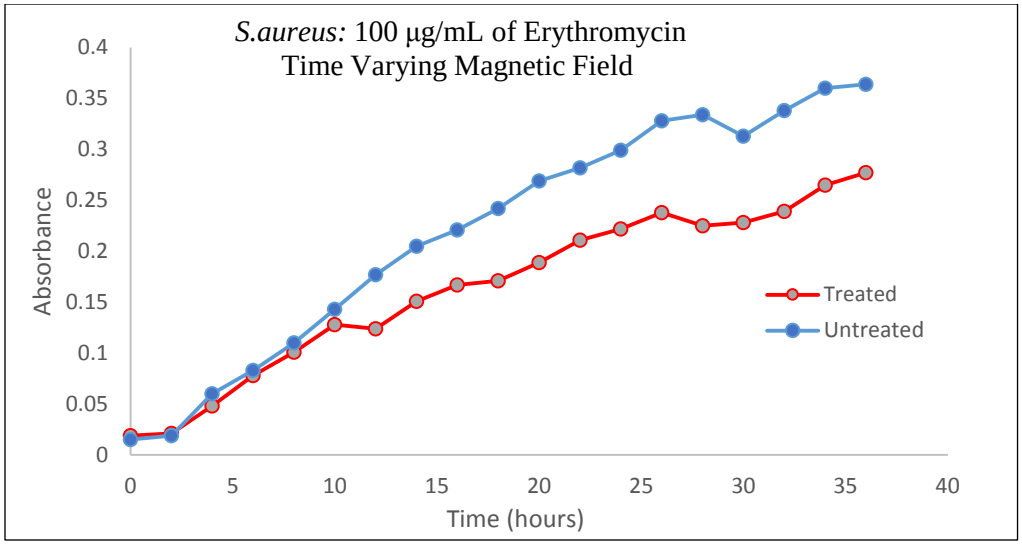

Figure 4.6: Comparison of growth curves between treated and untreated bacteria in the time varying magnetic field with a concentration of 100 µg/mL.

When comparing the graph for time variable and static magnetic environments, we observed the growth curve samples have a small difference. A comparison of antibiotic treated samples which were exposed to the magnets were compared with the unexposed samples and static magnets have lower growth curve after antibiotics were given. Static magnetic environments cause a change to measurements taken as well as in the fluid. To understand this, a higher concentration of 150 µg/mL was used to look at the effect of magnetic fields on the interaction between the antibiotics and bacteria.

Run 2

As mentioned previously, this run has higher concentration to be 150µg/ml. Preparations are similar as in run 1. The same number of tubes were prepared and antibiotics dosage as well. The antibiotics were given after 12 hours for treatment. The first

run shows a difference between the samples applied to magnetic environment and no magnetic.

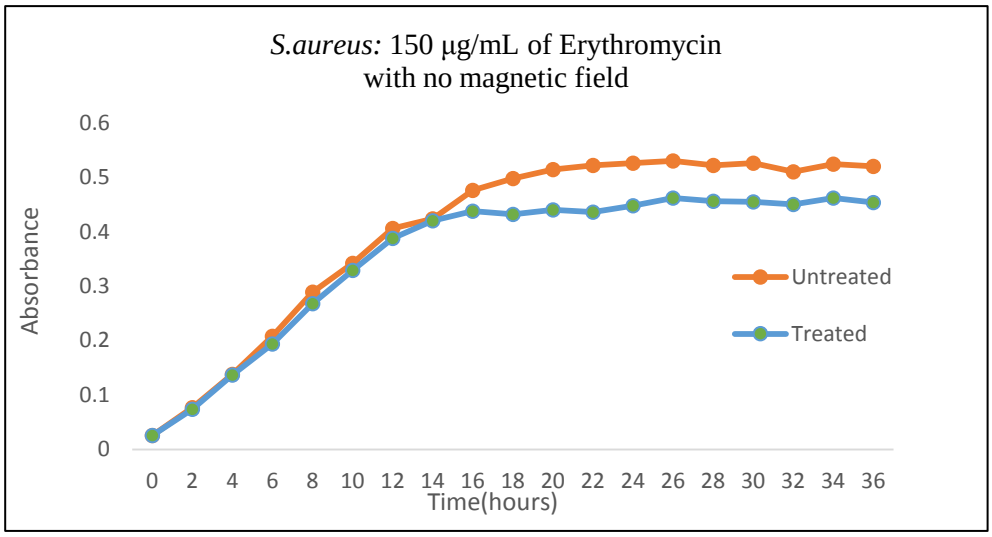

Figure 4.7: Comparison of growth curves between treated and untreated bacteria in the absence of magnetic field with a concentration of 150 µg/mL

The control samples for run 2 shows a slight increased to the growth curve for both samples until the dosages were given, the curves increased for 12 hours for both samples. After 12 hours, samples treated with antibiotics have moved to stationary phase. However, the untreated sample curve increased until 20 hours then showed a steady growth. Both samples end up with 0.07 different absorbance.

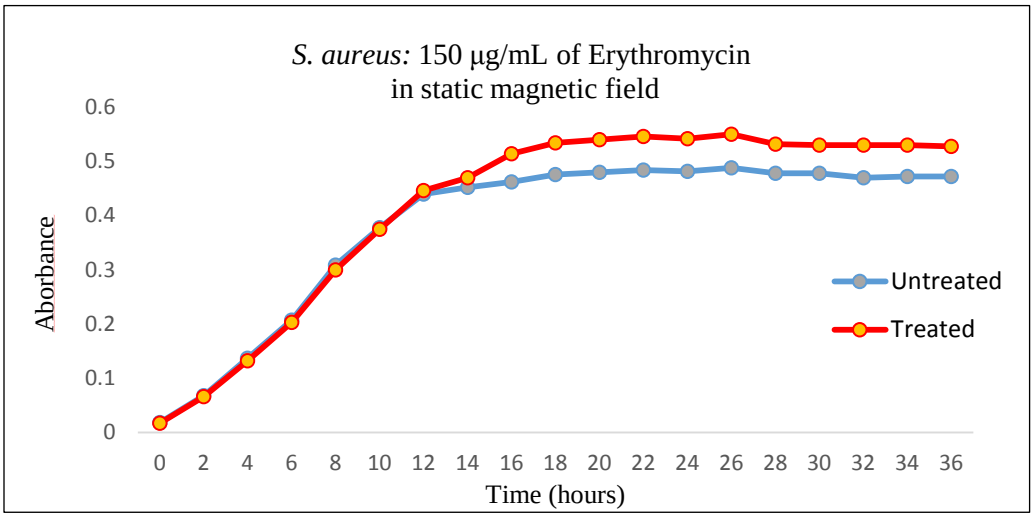

Figure 4.8: Comparison of growth curves between treated and untreated bacteria in the static magnetic field with a concentration of 150 µg/mL

The static field growth curve initially started out with similar curves as shown in Figure [4.8]. After 12 hours of growth, the untreated samples move to stationary phase. On the other hand, treated samples after 18 hours moved to stationary phase when the antibiotics were introduced. Treated samples continued to grow after untreated samples moved to stationary phase although the antibiotic were given. After 20 hours, both samples show insignificant decreased in the growth curve. In this run, the constant force applied and shows different behavior of the curves similar to previous run with 150µg/ml concentration.

The time-varying magnetic field's growth curve started out with a similar curve as if it is not exposed to magnetic fields as shown in Figure [4.9]. After 12 hours the treated samples show steady growth curve. In contrast, untreated samples continued to grow until 22 hours. The difference between the untreated and treated samples is comparable throughout and has a difference in absorbance almost close to 0.05.

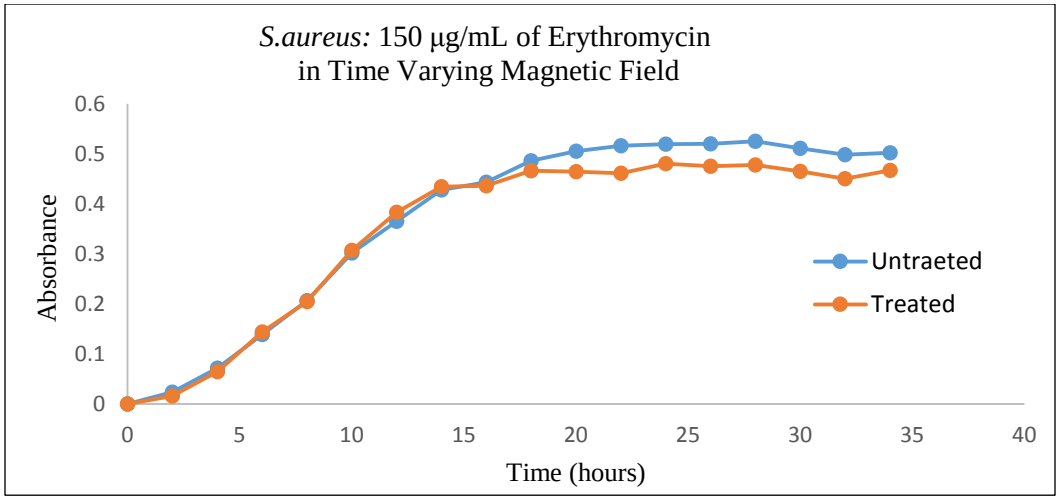

Figure 4.9: Comparison of growth curves between treated and untreated bacteria in the time varying magnetic field with a concentration of 150 µg/mL.

In this run, it was expected to have a similar result for time varying magnetic field and the constant current producing static magnetic field. However, the result was different from the static magnet fields. The reason behind the difference in magnetic field effect could be explained considering that the bacteria acquire a state of dynamic equilibrium in the constant field after some time. Whereas the regular change in the time varying field does not let this happen. However, the constant magnetic field effect will also depend on temperature and the small variation in temperature will affect the bacterial response to the magnetic field. Moreover, magnetic field effect is related to the concentration of bacteria as well as temperature because the terminal velocity of bacteria is related to the terminal velocity and the parameters of the medium as well.

Run 3

Since the magnetic field effect with the initial concentration of 100µg/ml and 150µg/ml, was small, it was natural to check with the higher concentration that if the effect

becomes more visible with higher concentrations such as 200µg/ml to check if the higher concentration can subside the magnetic field effects. In this run we just changed the concentration of antibiotics and compare the effect of antibiotic concentration on the growth pattern. So we determine the effect of higher starting concentration and compare the bacterial response to antibiotics or not.

Figure [4.10] shows a comparative study of the effect of magnetic field on the growth rate for the addition of higher concentration of antibiotic after 10 hours on two samples with similar initial growth. After the antibiotics were given, the samples obviously dropped then regrew again. Both samples after 14 hours moved to stationary phase until 18 hours. Untreated samples started dropping slowly and continue for 6 hours. Unlike the treated samples, continued on the stationary phase for two more hours after the untreated samples started dropping slowly. However, the growth curve shows lower growth of treated sample as compared to untreated samples. This happened in the absence of magnetic fields.

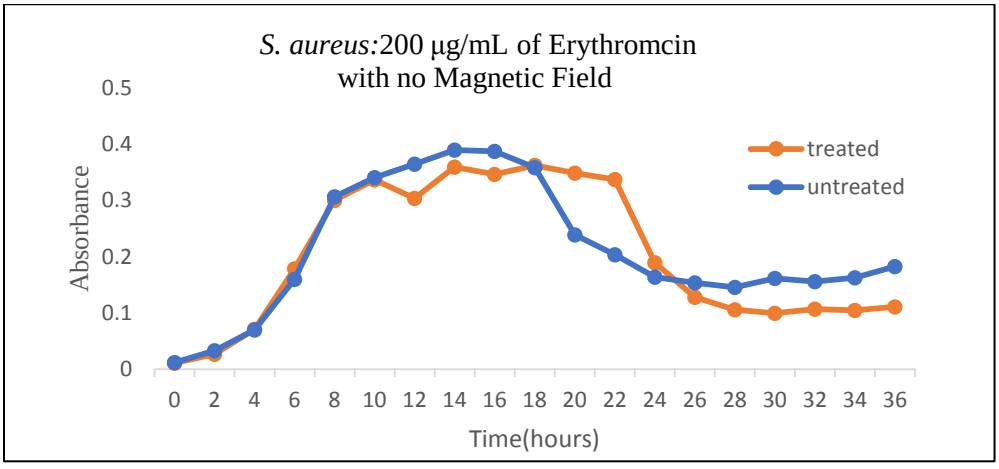

Figure 4.10: Comparison of growth curves between treated and untreated bacteria in the absence of magnetic field with a concentration of 200 µg/mL.

Figure [4.10] clearly shows that the growth curve steeper for both samples. The antibiotics were introduced after 12 hours. After the addition of antibiotics, the treated sample dropped quickly and almost maintained the growth rate whereas untreated sample had a different behavior.

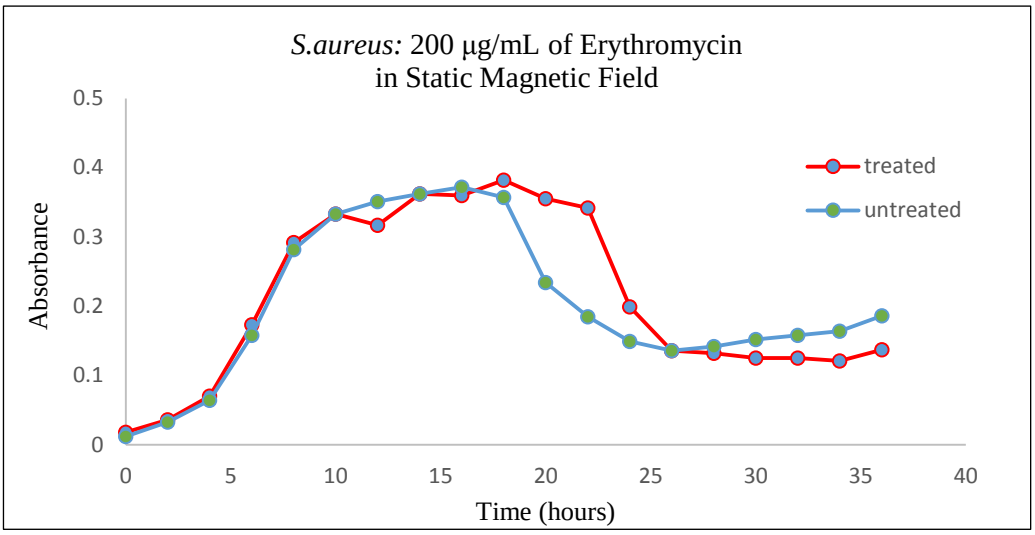

Figure 4.11: Comparison of growth curves between treated and untreated bacteria in the static magnetic field with a concentration of 200 µg/mL

The data plotted in Figure [4.11] was plotted for the same concentration of antibiotics and with the same initial concentration of bacteria with exposed to the static magnetic field. The growth curve for both samples is grown for 36 hours. Treated samples dropped after the antibiotics introduced then regrew again after 2 hours and moved to stationary phase for 6 hours. Treated samples moved to death phase after 22 hours. Unlike the untreated samples dropped after 18 hours. Similarly to the previous run, the treated samples end up with a lower curve.

From the Figure [4.12] is clearly the curve growth increased for both samples for 12 hours. The antibiotics were introduced after 12 hours similarly to the previous run. After the antibiotics were given, the treated sample dropped quickly within an hour and increased.

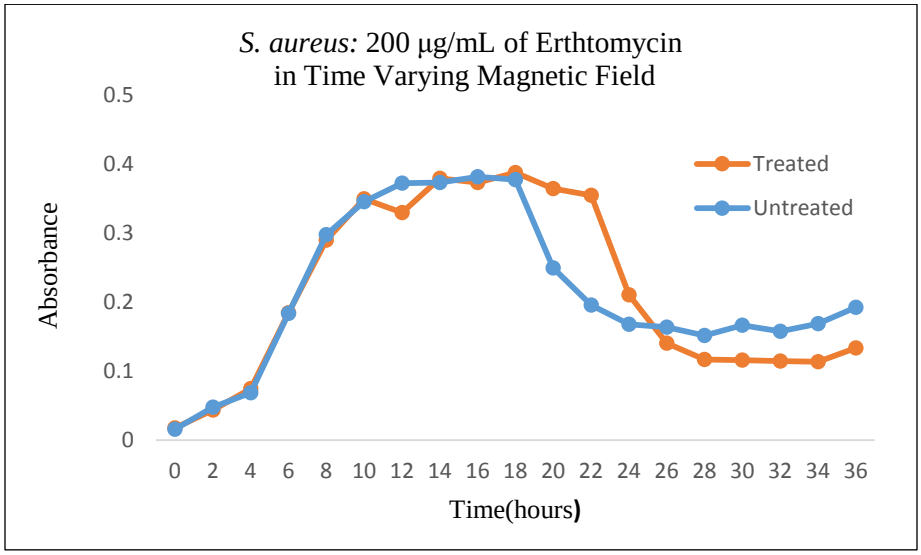

Figure 4.12: Comparison of growth curves between treated and untreated bacteria in the time varying magnetic field with a concentration of 200 µg/mL.

Similar to the previous section, the data from the same concentration was applied to time varying magnetic field as well. The growth curve for both samples grown for 10 hours. Treated samples dropped after the antibiotics introduced then grew again after 2 hours and moved to stationary phase for 6 hours. Treated samples moved to death phase after 22 hours. Unlike the untreated samples dropped after 18 hours. Similarly to the previous run, the treated samples end up with a lower curve.

In this run, it was expected to have a similar result for time variable magnet and no magnet. However, the result was different from previous concentrations of 100 µg/mL and 150 µg/mL. Static magnetic field sets have shown increased growth rate for treated

samples for 100 μg/mL and 150 μg/mL. On the other hand, static magnets for 200 μg/mL shown no effect on growth curve. One reason could be that force by uniform magnets changed the behavior of the bacteria growth for low concentration. This difference in behavior is possibly due to the change in concentrations in each fixed magnetic flux. Using different bacteria was suggested to better understand with high concentration.

Run 4

Previous results of the effect of magnetic field on the *S. aureus* were compared with the corresponding *E.coli* Similar preparations were made as in trial 3, and the time spent in the fields before the treatment of antibiotics was given was 12 hours. The results from the first run show a similarity in the behavior of no magnets, the static field, and the time-varying field. As you can see, from the figure 4.13 the drop for treated sample was after 12 hours for the control samples.

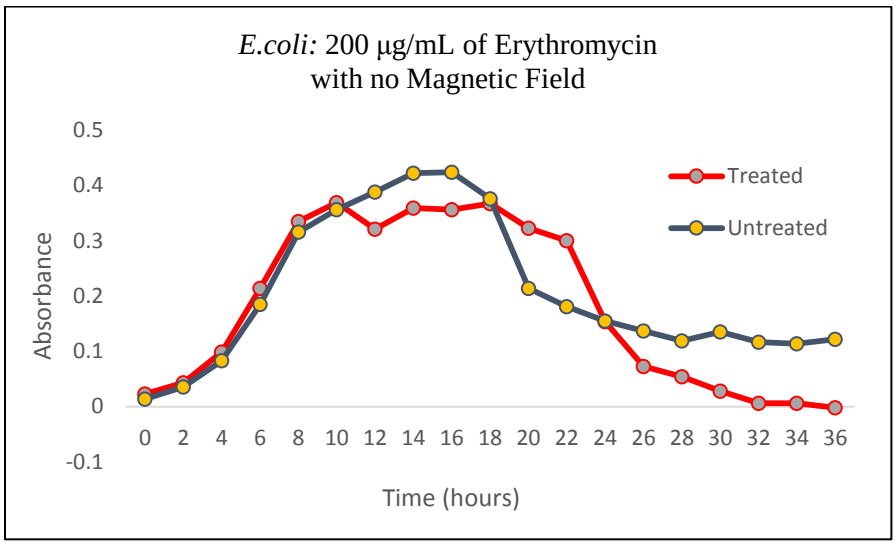

Figure 4.13: Comparison of growth curves between treated and untreated bacteria in the absence of magnetic field with a concentration of 200 μg/mL.

The growth curves of both sets of samples in the control go quickly into the log phase of growth. After 12 hours, the antibiotics were introduced and the growth rate dropped for treated samples almost immediately. Untreated samples declined after 18 hours however the treated samples declined after 22 hours. Treated samples growth curve end up with almost zero absorbance.

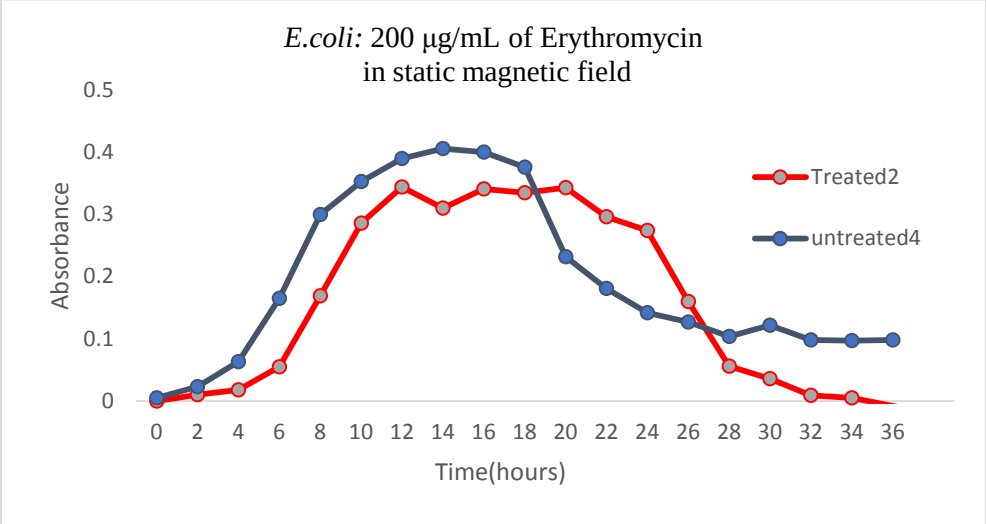

Figure 4.14: Comparison of growth curves between treated and untreated bacteria in the static magnetic field with a concentration of 200 µg/mL.

In the static magnetic run, it was expecting to have identical curve growth without magnetic field and static magnetic field. After the dosages were introduced, the treated samples dropped and fluctuated for 8 hours then moved to death phase. Unexposed samples dropped after 18 hours however treated samples have shown sharp drop after 24 hours to zero absorbance.

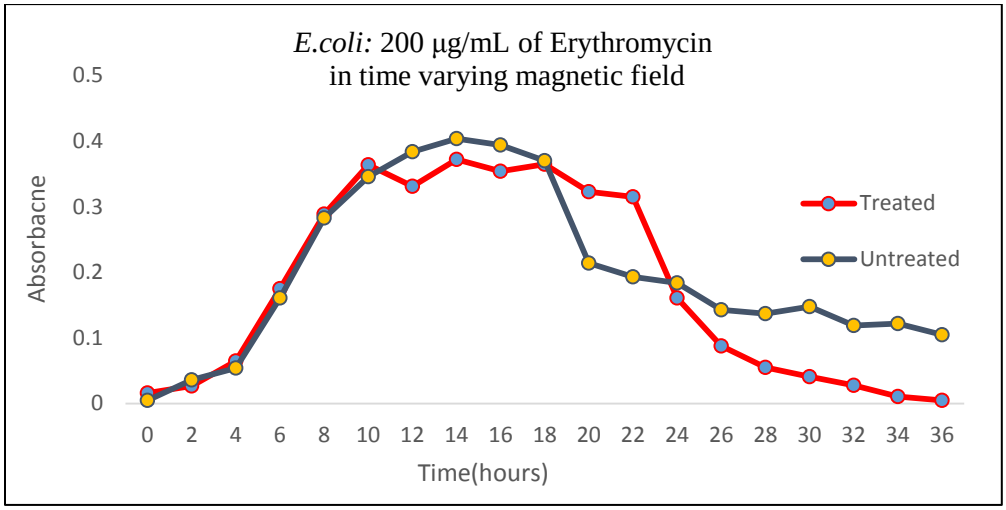

Figure 4.15: Comparison of growth curves between treated and untreated bacteria in the time varying magnetic field with a concentration of 200 μg/mL.

Last run with *E.coli* was applied to time variable magnetic fields as shown in Figure [4.15]. It was obvious for both bacteria with high concentration the examining of the entry into log phase of the graph is showing similar behavior. However, there is a slight dip at 12 hours for treated samples. Untreated samples declined after 16 hours but treated samples show more bacterial death at 36 hours. As expected, with high concentration is too similar to the pattern seen in the control field data, implying that same effect may take place.

4.5 Comparison of the Results

To better understand, data from both magnetic fields and no magnetic field were plotted with the same concentration for comparison. To summarize the effect of different magnetic fields on the interaction between *S. aureus and* erythromycin, we compared those

different environments at the same concentration. From Figure [4.16] it is obvious that the treated samples which applied to magnetic fields have lower curves than the control samples. Appling magnetic fields on *S. aureus* reduced the growth curve. Due to low concentration, erythromycin interacts with bacteria and gives effective results on weak electromagnet fields. Higher concentration shows different result as discussed in run 2.

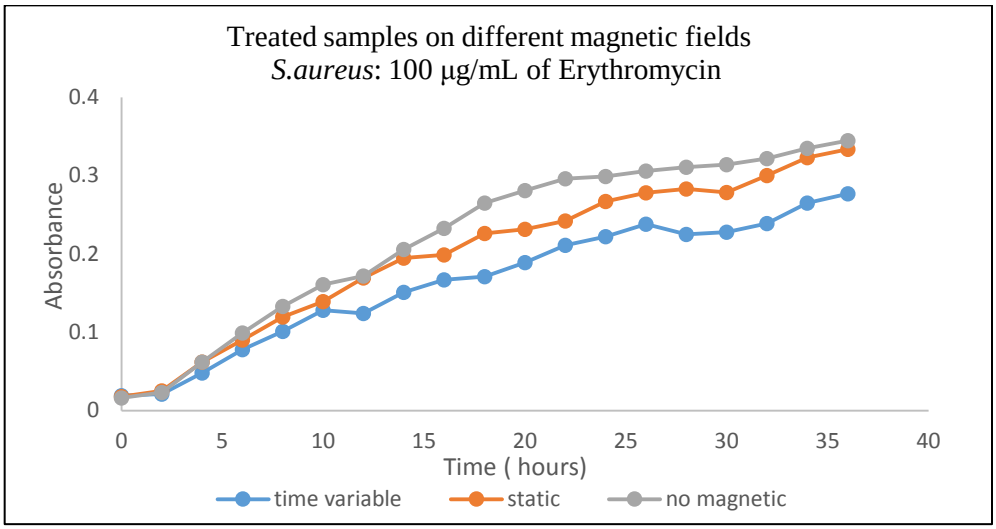

Figure 4.16: Comparison of treated samples on time variable magnetic fields, static magnetic fields and no magnetic fields at 100 µg/mL concentration.

When comparing the behavior of the treated samples on different magnetic fields at 150 µg/mL, it can be seen that the growth curves for time variable and static magnetic fields match up very closely until the dose of antibiotics is introduced. As seen in Figure [4.17], at 12 hours after the antibiotics are supplied to the samples, and a decrease of the growth curves is seen. Once the antibiotic dose is introduced to the samples, the growth curve drops off and moved to stationary phase.

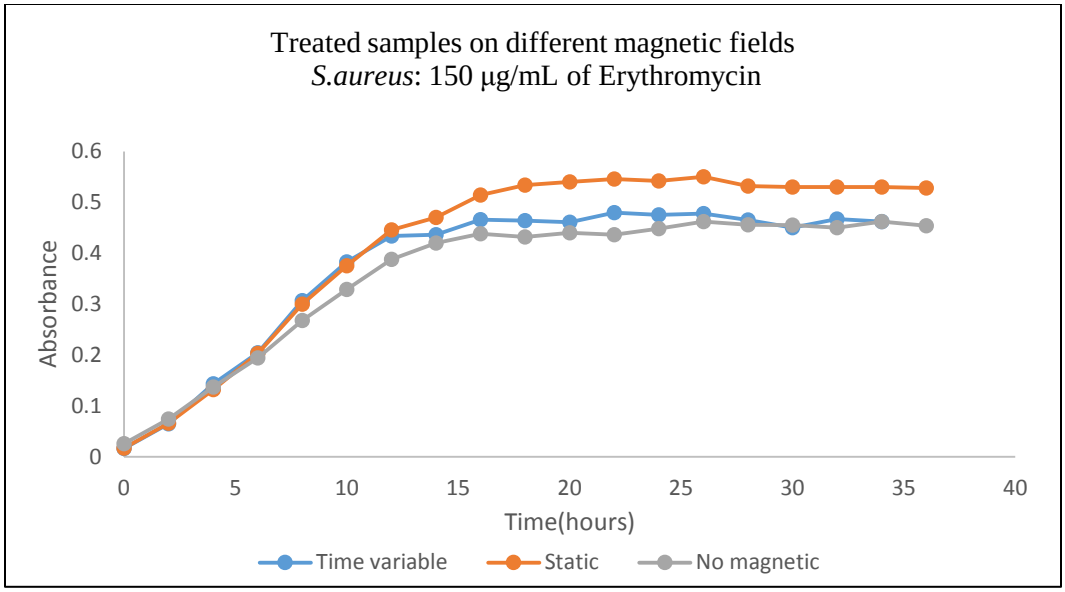

Figure 4.17: Comparison of treated samples on time variable magnetic fields, static magnetic fields and no magnetic fields at 150 µg/mL concentration.

Interesting results with 150 µg/mL concentration was the control set. The unexposed samples to magnetic fields have lower curve than static magnetic field. Static magnetic field produces a constant flux of 4 gauss and this makes a constant perturbative effect in the fluid. Due to the continued force, the free particles contuse the acceleration. However, in time varying magnetic field the bacterial samples, the system changed due to force applied and this may cause to the particles acceleration and deceleration in the low frequency environment.

When observing the Figure [4.18], it can be seen the high concentration has shown to have no effect on the growth curve for both bacteria *S. aureus* and *E. coli*. The behavior of the both magnetic field samples are similar to the control set.

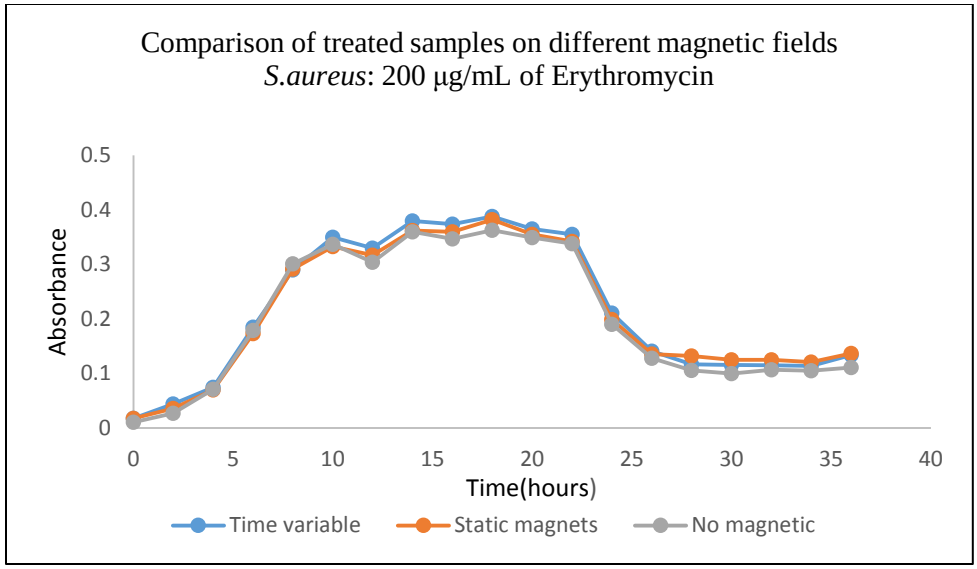

Figure 4.18: Comparison of treated samples on time variable magnetic fields, static magnetic fields and no magnetic fields at 200 µg/mL concentration.

The first drop was for all samples at 12 hours when the antibiotics introduced to the samples. After long exposure such as 22 hours for both magnetic field sets, the curve drop off similarly to the control set. All samples end up with insignificant different absorbance.

4.6 Conclusion

In this experiment we looked at the interaction between the antibiotics and bacteria on different magnetic fields. The concentration of the antibiotics has shown effect on growth curve of the bacteria. At low concentrations of the antibiotics, the bacterial growth is increased on static magnetic fields.

On the other hand, at high concentrations of antibiotics has shown similar effect on growth curve for time varying magnetic fields, static magnetic fields as compared to the control. Uniform magnetic fields have shown effects on the growth curve.

Bacterial growth is affected by weak magnetic field. Antibiotics effectivity is influenced by the extremely weak fields. Static magnetic field has an effect on the interaction of antibiotics also. Relative motion between magnets and cells has an impact the growth of bacteria.

Quantum mechanical description of important processes in DNA replication and a detailed study of processes like proton transfer may help to understand how the perturbative effect can change the morphology and behavior of bacteria. Different bacterial shapes and gram staining properties may be affected differently.

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