Chapter One

Introduction

1.1 Background of the Study

With an estimated 374 million new infections occurring each year, the prevalence and risk of sexually transmitted infections (STIs) among the general population continue to rise on a global scale[1]. For people between the ages of 15 and 49, more than one million infections are recorded worldwide every day, and this has made STIs one of the most common infectious diseases[2]. STI's are infections that are propagated by sexual activity, especially vaginal intercourse, anal, and oral sex[3]. Use of contaminated sharp instruments, vaginal birth with an infected mother during childbirth, or breastfeeding from an infected mother to her kid are the main ways that STIs are spread[4]. STIs constitute a major burden and a significant public health challenge, in terms of the impact they place on worldwide reproductive, sexual, and maternal-child health[5].

There are claims that sexually transmitted infections (STIs) are illnesses caused by a number of organisms that can spread through sexual contact, although they can also occasionally spread through other forms of intimate physical contact[6]. This is due to the fact that skin-to-skin contact can transmit some STDs, such as herpes and the human papillomavirus (HPV). STI-causing microbes can also proliferate through non-sexual channels like tissue and blood exchange. Direct sexual contact between infected individuals (with acute, chronic, or asymptomatic clinical manifestations) is the method of transmission. Many sexually transmitted infections (STIs) can also be passed from mother to child during pregnancy and childbirth, including chlamydia infection, gonorrhea, hepatitis B, HIV, human papilloma virus (HPV), herpes simplex virus 2

(HSV2), and syphilis. Some STIs can also be passed from mother to child through breastfeeding[7].

Sexually transmitted infections (STIs) are a major global public health concern that affects quality of life and causes serious morbidity and mortality. They are among the top five disease categories for which adults seek medical attention. It is a typical public health concern because it places a high burden on both the health care system and individual patient care. STIs have a direct impact on reproductive and child health through infertility, cancers, and pregnancy complications. They also have an indirect impact on sexual health through their role in facilitating HIV sexual transmission, which has an impact on both national and personal economies[8].

An estimated 50% of new HIV infections in women are thought to be related to STIs[9], and it has been demonstrated that STIs, such as syphilis, gonorrhea, and HSV, increase the risk of contracting HIV by a factor of three or higher[10]. STI transmission from mother to child can cause congenital abnormalities, low birth weight, preterm, sepsis, pneumonia, stillbirth, and congenital conjunctivitis. Women's pelvic inflammatory disease (PID) and infertility are primarily caused by sexually transmitted infections (STIs), such as gonorrhea and Chlamydia infections. Significant morbidities for pregnant women with untreated infections include gonorrhea, trichomoniasis, and Chlamydia infection[11]. Untreated gonorrhea and Chlamydia infections can lead to serious complications for women, such as infertility, ectopic pregnancies, pelvic inflammatory disease, and chronic pelvic pain. [12]. Additionally, there is a higher chance of unfavorable outcomes like stillbirth, premature amniotic sac rupture, spontaneous miscarriage, and preterm birth. Hyper acute conjunctivitis can cause blindness in newborns born to mothers who have

cervical gonorrhea if it is not promptly treated[13]. Pneumonia in babies can also result from the vertical transmission of gonorrhea and chlamydia infections[14].

With the exception of viral hepatitis, all STIs are generally contagious and past exposures do not provide immunity, meaning that the risk of reinfection endures before a new exposure and may even recur after the same partner if treatment is not received by both parties. Every day, an estimated one million people are estimated to contract a sexually transmitted infection[15]. According to WHO estimates, there are 374 million new cases of treatable STIs globally in 2020, with one of the four STIs being chlamydia (129 million cases), gonorrhea (82 million cases), syphilis (7.1 million cases), and trichomoniasis (156 million cases)[16]. Of the STIs that are incurable, 38 million people worldwide are estimated to be living with HIV as of 2019. In 2016, it was estimated that over 490 million people had genital herpes virus (HSV)[17], and the main cause of cervical cancer, HPV infections, affect an estimated 300 million women worldwide.

In 2018, there were 570,000 cases of cervical cancer linked to HPV infection; annually, there are over 311 000 cervical cancer deaths[18]. An estimated 296 million people worldwide have chronic hepatitis B, which is expected to cause 820,000 deaths in 2019, primarily from hepatocellular carcinoma (primary liver cancer) and cirrhosis[19]. Maternal syphilis prevalence has not changed in recent years. Syphilis was estimated to have affected almost a million pregnant women in 2016. This resulted in over 350,000 adverse birth outcomes, including 200,000 stillbirths and newborn deaths. Syphilis is now the second most common infectious cause of stillbirth globally[20]. There were 661,000 cases of congenital syphilis in 2016 despite the disease's decline since 2012[21]. The infection mainly affects the reproductive system, which can lead to cancer, infertility, and pregnancy difficulties that can ultimately harm a child's health. Furthermore, there were

604,000 new cases of cervical cancer overall, and 1.5 million new cases of hepatitis B infection.[22]. Symptoms may lead to chronic infections, congenital abnormalities, and stillbirth, thus stressing the urgent need to develop effective biomedical control[23].

Given that pathogenic bacteria can cause severe infections, protracted illness, high medical expenses, and increased morbidity, bacterial resistance is one of the most dangerous challenges associated with infections. Since the invention of antibiotics, there has been a persistent worry about bacterial resistance to them[24]. Controlling STIs is still difficult, especially in low- and middle-income nations where the infrastructure of the health system is less developed[25]. Most STIs have no symptoms, and this predisposes most patients to a higher risk if left untreated[26]. When they are discovered as a result of these setbacks, traditional medicines become the main source of medical care for their diverse health demands[27]. Due to their mostly unexplored chemical diversity, medicinal herbs have long been a desirable choice for the identification of new molecular entities[28]. Some plants have been utilized as a primary raw material for the creation of other traditional medicines, while others have been used in the synthesis of various pharmaceuticals, either singularly or in combination[29]. These plants' compounds may yield a new class of antibiotics with different target locations from those previously employed antibiotics that can be successful against drug-resistant infections.

In Nigeria, herbs have been used for their medicinal benefits from the beginning of time. Civilization has made people develop a keen interest in plant-based drugs and pharmaceutical products[30]. This was encouraged by the large quantity of diverse herb and plant species seen across the country. Herbs can be used to treat or prevent infections as well as conditions including diabetes, heart disease, and cancer[31].Over eighty percent of people around the world have benefited from medicinal herbs, and from the conventional

medicines they produce[32]. There are about 374,000 plants, yet humans use 28,187 different types of medicinal herbs[33]. The attraction attributed to herb use in promoting health and treating several diseases in different traditional healthcare settings since the earliest of times has been they are readily available and inexpensive[34]. In addition, they are also important in the pharmaceutical industry in the production of drugs, as half of the drugs in the world are derived from herbs [35]. Due to their antibacterial qualities, herbal medicines made from medicinal plants have been used to treat a variety of diseases since the dawn of time[36].

Medicinal herbs have many bioactive compounds that possess antimicrobial properties, making them suitable for use in different ways[37]. These medicinal plants can be used to treat human ailments since they contain phytochemical components[38]. Phytochemicals are a class of chemical compounds that are found in plants[39]. In comparison to macro- and micronutrients, phytochemicals are organic, actively functioning chemical compounds found in plants[40]. In order to protect themselves against infections, predators, and abiotic environmental factors, plants create complex phytochemicals including phenols, steroids, alkaloids, and other substances. They also give plants their odor, color, and flavor[41]. Phytochemicals have been demonstrated to contain anti-inflammatory, antiallergic, antioxidant, anticarcinogenic, antimutagenic, and antimicrobial properties[42]. Consequently, it is not unexpected that many herbs have been used historically and still are to cure a variety of ailments [43].

Over 30,000 plants have been found to have antimicrobial chemicals, and over 1340 have been examined and found to have antimicrobial action[44]. It has been reported that different plant phytochemicals can interact to give a synergistic, antagonistic, and/or non-interactive effect[45].The situation of the economy in Africa, which made imported

methods and drugs less accessible, geared people toward utilizing abandoned traditional medicine to improve their health. Herbal mixtures have been tagged by the lay public as 'natural, which means safe, having no side effects compared to conventional drugs[46]. The continued occurrence of health issues in communities with the experience gathered over time has pointed out that traditional healers do not use one species of plant in the preparation of herbal mixtures but combine different species of plant to increase their potency and absorption, consequently leading to an increase in demand for plant-based products, bringing about job opportunities in the community and hence a vital source of income for the herb trade sector. However, as a result of this advantage, there is now fierce competition, which could encourage product adulteration or the sale of low-quality goods along with unsupported claims[47].

1.2 Statement of the Problem

The increasing prevalence and resistance of STIs pose a serious threat to human health and well-being. There is a lack of effective and affordable antimicrobial drugs to treat these infections, especially in resource limited settings. Therefore. This study aims to investigate the phytochemical and in-vitro antimicrobial activity of selected herbal mixtures against clinical isolates causing STIs such as *Candida albicans, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumonia*.

Due to the various advertising techniques used by its marketers and producers, including television and radio programs, herbal goods are given legitimacy and credbility in order to improve consumers' awareness of their efficacy[48]. These study also aims to identify the bioactive compounds of these herbal mixture as alternative therapy for STIs.

1.3 Justification of the Study

STIs can cause serious complications such as infertility, pelvic inflammatory disease, ectopic pregnancy, cervical cancer, and increased risk of HIV transmission. Many of the common STIs are caused by bacteria and protozoa that can develop resistance to conventional antibiotics. Therefore, there is an urgent need to discover new sources of antimicrobial agents that can effectively treat these infections. One of the potential of such agents is medicinal plants, which have been used for centuries in traditional medicine for various ailments, including STIs.

The fact that a sizeable section of the global populace continues to use traditional medicine may speaks to its effectiveness and universality. In order for medical plants to be effective against STIs, specific chemical compounds must be present, and it has been found that medicinal plants contain bioactive compounds known as phytochemicals. They are crucial for a range of microbial illnesses and STIs since they can have an inhibitory effect on germs and infectious viruses. The purpose of the study is also to evaluate the phytochemical and in vitro antimicrobial activity of the selected herbal mixtures against clinical isolates of STIs. The results of this study will provide a scientific validation for the traditional use of these herbal mixtures as complementary therapy for STIs.

1.4 Aim & Objectives of the Study

The purpose of this study is to evaluate the phytochemical properties, in vitro antimicrobial activity and microbiological quality of the selected locally made herbal mixtures sold within Ibadan metropolis for treatment STIs.

The objectives of this study are:

1. To enumerate and determine each herbal mixture's microbiological composition.
2. To verify each herbal mixture's antibacterial properties.
3. To evaluate the effectiveness of different antibiotic discs that are readily accessible on the market against the chosen clinical isolates.
4. To do a qualitative and quantitative phytochemical screening on each chosen herbal blend.

1.5 Significance of the Study

The significance of this study is to confirm the microbiological quality of specific herbal mixes and to assess their in vitro antibacterial properties against a small number of clinical isolates causing STIs in Nigeria.

1.6 Scope of the Study

The study includes the evaluation of the antimicrobial activities of six herbal mixtures against four clinical isolates causing sexually transmitted infections, the activity of multiple antibiotic discs against some clinical isolates causing STIs, the microbial profile of the randomly selected herbal mixture marketed for STI treatment, and the screening of each herbal mixture for its phytochemical constituents, both quantitatively and qualitatively.

1.7 Limitation of the Study

The study includes the assessment of the antimicrobial activities of six herbal mixtures against five clinical isolates causing STIs, the effectiveness of various antibiotic discs against STI-causing clinical isolates, the microbial composition of the chosen herbal

remedy, and the quantitative and qualitative screening of each herbal mixture for its phytochemical constituents.

1.8 Operational Definition of Terms

Antimicrobial Activities: Antimicrobial activity is a phrase used collectively for all active agents slowing the growth of bacteria, preventing the formation of microbial colonies, and also eradicating microorganisms.

Herbal Mixtures: The World Health Organization defines traditional medicine as "medical knowledge systems that evolved over many generations in various societies before the advent of modern medicine, including the practices seen in health, belief, and knowledge incorporating plant- and mineral-based medicines used singly or in combination to prevent, diagnose, and treat illnesses or maintain well-being."

Clinical Isolates: This is the study that comprises a global collection of epidemiologically unrelated strains isolated at diverse geographical locations.

Sexually Transmitted Infections (STI): These are infectious diseases with a variety of viral etiologies, and sexual transmission is the key epidemiologic factor in their spread. However, they can also spread in other ways, such as from mother to child, by the transfer of blood products, or through tissue. Since asymptomatic forms may involve transmission-risk subclinical lesions, this term also covers them. This is why this term is preferably used instead of the term “sexually transmitted disease”, previously used.

Endnotes

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Chapter Two

Literature Review

2.1 Medicinal Plants

The term "medicinal plants" refers to the use of certain plant species in traditional medicine, which entails the investigation and application of plants as therapeutic agents[1]. Whether in conventional medicine or modern mepdicine, many times, medicinal plants are used solely for sustaining health and given for specific objectives[2]. Herbal medicines are characterized as industrially produced plant preparations that are still pure and natural, have not undergone chemical alterations, and have the active components that are solely responsible for the therapeutic benefits[3]. Plant-based medicines are among the most well-known sources of natural products[4]. Medicinal plants are used to treat a variety of illnesses because they are thought to be both safe and effective[5]. Because consumers believe plant-derived remedies are natural and safer than conventional medicines, they have become more popular[6].

Medicinal plants have been used to treat ailments since the dawn of mankind, and most nations still do today[7]. Up until the early nineteenth century, medicinal herbs served as the cornerstone of alternative medicine, serving as a primary production pathway for new drugs[8]. The UN's Food and Agriculture Organization estimated in 2002 that more than 50,000 medicinal plants were used globally[9]. The drugs derived from these plants in modern-day medicine have been associated with the utilization of materials derived from plants as a native cure in conventional systems of medicine[10]. Almost all the historic inventions in drug discovery originated from the natural and herbal fields[11].

80% of people worldwide, including millions of people living in rural parts of developing countries, utilize drugs made from plants for primary healthcare[12]. Because they are safe and effective, medicinal plants are frequently utilized to cure illnesses. They are also cheap and have fewer side effects[13]. Soft or dry medicinal plants can be used, and the raw materials can be used to create a variety of liquid and solid extracts[14]. In affluent countries, more than 25% of medications that are prescribed come either entirely or partially from plants notwithstanding the great advancements in synthetic organic medicinal goods of the 20th century[15]. With the advancement of civilization, the ongoing need for medicine, and the expansion of its applications, its significance only increases.

Chemically produced medications have a quick onset of action, but they also have long-term adverse effects that are harmful to the human body, whereas Medicinal plants have minimal to no negative effects on the body due to their comprehensive action[16]. However, in comparison to traditional medications, HMs contains many chemical components that can have a variety of pharmacological effects on the body, resulting in both minor and major side effects, such as renal failure, damage to the liver, a rise in blood pressure, and even death[17]. Many important medicinal plants exist, however some are poisonous plants since they are equally lethal and therapeutic. There is a discrepancy in the dosage offered since the plant may be beneficial in tiny amounts while being harmful in bigger doses. Therefore, when using these plants, precaution and accuracy must be taken in determining the exact dose[18]. HMs may also pose a safety risk if they are combined with conventional medications or infected with mold, bacteria, or yeast[19]. Chemical components found in plants that are medicinal have beneficial impact on the body, which indicates a significant opportunity for the development of novel medications[20]. These chemical substances are known as

phytochemicals, which are pharmacologically active compounds that are crucial in the treatment of various ailments. The sections of the plant where phytochemicals are deposited include the flowers, leaves, stem, roots and seeds and are mostly visible as colored molecules in the outer layer of plant tissue[21].

2.2 Phytochemicals

The pharmaceutical business has been gifted with new medicinally active chemicals from plants that have a long history of treating a number of ailments. The chemical components present in various plant parts that have a clear physiological impact on the human body are thought to be the source of these plants' therapeutic value[22]. Phytochemicals, a term derived from the Greek word for "plant," are secondary metabolites found in plants. These substances guard plant cells against environmental dangers including strain, exposure to ultraviolet radiation, a lack of water and infections[23]. Additionally, current research has demonstrated that when a person's nutritional consumption is significant, they play a part in preserving human health[24]. Numerous anticancer, anti-inflammatory, antioxidant, antimicrobial and immunostimulatory effects have been found in several plants[25]. The presence of antimicrobial compounds in the tissues of plants is a crucial factor that makes them active against pathogens that cause human diseases and can also serve as botanical pesticides or fungicidal and bactericidal agents[26]. Phytochemicals can be found in vegetables, fruits, whole grains, seeds, herbs, and spices in a variety of diets[27]. Different plant sections such as leaves, flowers, roots, fruits, leaves or seeds also accumulate phytochemicals[28]. In plants, levels vary according to variety, processing, cooking and growing circumstances[29].

Based on their chemical components, phytochemicals can be divided into different categories. An estimated 150 phytochemicals have been thoroughly investigated out of the over 4,000

phytochemicals that have been categorized and separated based on their chemical makeup, physical makeup, and protective role[30]. More than 200,000 naturally occurring compounds with diverse bioactive qualities are found in plants, highlighting the significance of natural products in the development of novel medications[31]. Primary and secondary metabolites include plants, which have pharmacological effects as a result of the accumulation of phytochemicals that are biologically active in plant tissue. Primary metabolites are unadulterated substances that include glucose, polysaccharides, starch, proteins, nucleic acids, and lipids. These substances are necessary for the formation and development of the body of humans. The remaining substances are the secondary metabolites produced by plants including lignans, alkaloids, steroids, terpenes, phenolic, saponins, glucosides and flavonoids[32].

2.3 Plant Constituents of Pharmacological Importance

Phytochemicals can be classified as main or secondary metabolites depending on how they function in a plant's metabolism. Common sugars, amino acids, chlorophyll, pyrimidines, proteins and purines from nucleic acids are examples of primary metabolites. Additionally, plants create metabolites that are secondary in nature, such as: (a) alkaloids, which have analgesic, antimalarial, antispasmodic, and osmotic actions (b) terpenoids, which are also anthelmintic, antiviral, antimicrobial, anti-inflammatory, cancer-fighting, and antimalarial, (c) antibacterial and antifungal activities are possessed by glycosides d) anti-allergic, antioxidant, and antibiotic effects of flavonoids and phenols are said to exist and (e) the antiviral, anti-inflammatory, and plant defense properties of saponins[33].

2.3.1 **Alkaloids**

The alkaloids, which are metabolic byproducts obtained from amino acids, are one of the most important and substantial substances produced by plants[34]. The first alkaloid, narcotine (also known as noscapine), was extracted from opium by Derosne in 1803. Today, there are more than 12,000 recognized alkaloids. Different creatures, including bacteria, fungi, plants, and mammals, create alkaloids. Alkaloids are natural nitrogenous bases that are formed by the cellular breakdown of amino acids. They tend to occur in plants and often have heterocyclically associated nitrogen. Alkaloids protect plants from disease attacks, deter herbivore grazing, and thwart competitors' growth, among other vital functions[35].

Some alkaloids comprise caffeine, nicotine, and morphine, which have stimulant properties and are used as analgesics, as well as quinine, an anti-malarial medication[36]. The molecule's structure, the existence of functional groups, and their placement all affect how basic a molecule is[37]. Without any water being produced, they combine with acids to create crystalline salts[38]. Alkaloids are used pharmacologically as CNS stimulants and anesthetics[39]. Strychnine, for example, is toxic, whereas cocaine and morphine are both addictive and used in medicine. Alkaloids can be found as salts of naturally occurring acids that include oxalic acid, tartaric acid and acetic acid in the cell sap of plants. They can be removed from the cell using acidified water or alcohol, or when the plant extract is made alkaline, they become soluble in organic solvents like chloroform.

2.3.2 **Flavonoids**

The family of natural products known as flavonoids is significant; in particular, they are secondary metabolic products of plants with a polyphenolic framework that are frequently

encountered in vegetables, fruits, and some drinks. Flavonoids are the most prevalent and extensively dispersed group of plant phenolics and rutin and quercetin are the flavonoids that are most frequently ingested[40]. Vegetables require flavonoids to support the development of their cells and protect themselves against plaques[41]. In the majority of angiosperm families, several flavonoids are easily identifiable as floral pigments. However, they are capable of being found in all plant sections and are not just present in flowers[42]. Flavonoids are known as dietary flavonoids because they are widely distributed in a variety of plant-based meals and beverages, including tea, vegetables, fruits, chocolate, and wine.

Flavonols, flavones, chalcones, flavanones, flavan-3-ols, flavanonols, and isoflavones are just a few of the subclasses of flavonoids[43]. Unique significant sources make up these groupings. For instance, prominent dietary sources of flavonols and flavones are onions and tea. The largest class of phytochemicals is flavonoids[44]. Typically, flavonoids can significantly reduce the risk of disease through a variety of physiologic pathways. Examples of these qualities include anti-inflammatory, cytotoxic, antiviral, antibacterial, and antioxidant properties[45]. Endothelial activity, cholesterol, blood pressure, and resistance to insulin as well as a reduction in blood pressure, are factors that reduce the risk of heart disease[46]. Long known to be produced at specific places in plants, flavonoids give flowers their color and perfume and assist fruits disperse so that pollinators can reach seeds and spores, which in turn promotes seed and spore germination and seedling growth[47]. Flavonoids operate as special UV filters and shield plants from a variety of stressors, both abiotic and biotic[48]. Flavonoids may be functional in plant heat acclimatization and freezing tolerance in addition to their involvement in frost hardiness, drought resistance, and other biological processes[49]. They have a variety of beneficial biochemical and antioxidant

properties linked to a number of illnesses, including atherosclerosis, Alzheimer's disease (AD), cancer etc[50]. Flavonoids are essential in a wide range of nutraceutical, pharmacological, medical, and cosmetic uses because they are linked to a wide range of health-promoting benefits. Additionally, they have been found to be potent blockers of a number of enzymes, including phosphoinositide 3-kinase, lipoxygenase and cyclo-oxygenase (COX)[51].

2.3.3 **Glycosides**

In the natural world glycosides are extensively dispersed. Various amounts of them can be found in the fruits, seeds, barks, roots and leaves. In certain situations, the same plant will have two or more glycosides. When hydrolyzed, glycosides release either one or several sugar molecules along with an organic hydroxide. The less specific general word "glycosides" was proposed because several plant components produced sugars other than glucose. A chemical may be referred to as a "glucoside" if glucose is the sugar that is generated. Additionally, more precise words like rhamnoside, fructoside and others can be used. The term "aglycone" or "genin" often refers to the glycoside's non-sugar part. Chemically, glycosides consist of a non-carbohydrate component (genin or aglycone) and a carbohydrate (glucose)[52].

There are claims that many medicinal formulations include plant extracts that contain cyanogenic glycosides as flavorings[53]. Chemically speaking, glycosides are acetals in which a non-sugar component's hydroxyl group is condensed with the sugar's hydroxyl group. To indicate that the bond is through oxygen, this form of glycoside is more specifically referred to as O-glycosides. Glycosides are acetal derivatives produced when a monosaccharide and an alcohol interact with an acid catalyst. The "oside" suffix is used in place of the "ose"

suffix in the names of glycosides, and the alcohol group name comes first. However, it is intriguing to note that only β shapes exist in plants. This is due to the enzyme emulsion's ability to hydrolyze naturally occurring α-glycosides while not being able to do so with synthesized α-glycosides. Glycosides are often hydrolyzed by acids and have a fair amount of stability in alkalis. Compared to other glycosides, some are significantly more resistant to hydrolysis. A vast range of chemical substances, such as cardiac, saponin glycosides, etc., represent the aglycone or non-sugar parts of glycosides. Because the solubility characteristics of the sugar residues have a significant impact, the sugar moiety promotes water solubility making many glycosides soluble in water or hydro alcoholic solutions. Enzymes that can synthesize or hydrolyze glycosides are frequently found with them. This occurrence causes complications with the isolation of glycosides because, in certain instances, partial or complete hydrolysis of the glycosides results from the disintegration of plant tissues without any safeguards to block enzymatic activity.

2.3.4 **Saponins**

In essence, saponins are phytochemicals that are present in the majority of vegetables, legumes, and herbs[54]. Plants frequently create saponins, which are structurally complicated amphiphatic glycosides of triterpenoids and steroids[55]. Some marine creatures, like sea cucumbers and starfish, also manufacture it[56]. Because of their surfactant qualities, which cause stable soap-like foam to form when shaken in aqueous solution, their name is derived from the Latin sapo, which means soap. Chemically, the name "saponin" refers to a class of high molecular weight glycosides that include an aglycon, also known as genin or sapogenin, connected to a glycosyl component[57]. Saponins may also be further divided into 12 primary classes based on the carbon skeleton of the aglycon. Additionally, saponins are frequently

found in intricate combinations, and their chemical makeup can change based on a plant's age, tissue type, background genetics, physiological state, and surrounding conditions. Because they hemolyze blood and are known to poison cattle, saponins are particularly dangerous[58]. It features immunostimulant, anti-inflammatory, anti-apoptotic, and anti-oxidant activities[59]. The linkage with the carbohydrate component, which includes one or more sugar moieties comprising the sugars rhamnose, galactose, glucose, glucuronic acid, arabinose, xylose, glucoronic acid glycosidically, is another crucial aspect of sapogenin (aglycone)[60]. Numerous saponins have been found to have antimicrobial properties, to limit the growth of mold, and to shield plants from damage caused by insects. Saponins are included in a wide class of defensive chemicals called phytoanticipins or phytoprotectants, which are compounds that are part of plants' defense mechanisms[61]. Because saponins are not required for the fundamental metabolic functions of the plant, they are classified as metabolites that are secondary and differ from the elements of primary metabolism. As phytoprotectants, such as the constitutively generated and inducible phytoalexins phytoanticipins, they also support the innate immune system[62]. Additionally, saponins have been investigated for a variety of qualities, including sweetness, bitterness, molluscicidal, fungicidal, pesticidal, insecticidal action, as well as additional industrial uses including foaming and surface-active chemicals.

2.3.5 **Tannins**

A complex, big biomolecule of the polyphenol type that has enough hydroxyls and other appropriate groups, like carboxyl, to form powerful complexes with a variety of macromolecules is commonly referred to as tannin[63]. Tannins are secondary polyphenolic compounds found in higher plants. Tannins are typically utilized in the tanning process and

as anti-gonorrhea, anti-pile, anti-burn and anti-inflammatory medications[64]. The terms "macromolecular phenolic compounds" and "condensed" tannins are used to describe the two main categories of tannins[65]. The first are broken down into two categories: (i) ellagitannins and (ii) gallotannins, which also include meta-depsids. Galloyl and hexahydroxydiphenoyl esters and their derivatives are the first of two major structural categories into which plant polyphenols are divided. Condensed proanthocyanidins are (ii). There are several main groups into which galloyl and hexahydroxydiphenoyl esters and their derivatives have been further divided: Simple esters are one. (2) Metabolites of depside (syngallotannins). (3) 'Open-chain' derivatives of glucose based on (a) the 4 C1 conformation of glucose, (b) the 1 C4 conformation of glucose, and (c) the dehydrohexahydroxyhexahydroxydiphenoyl and hexahydroxydiphenoyl esters (syn-ellagitannins). (4) "Dimers" and "higher oligomers," primarily those of class (3) above, created by the oxidative coupling of "monomers." Condensed tannins and complex tannins are the only two kinds of tannins that are fully discussed. The described tannin structures, however, demonstrate that the galloyl residues can also be linked to other residues or to one another by their aromatic carbon and/or phenolic oxygen atoms, in addition to the galloyl glycosides. Nature offers a practically limitless supply of extremely different structures through these and comparable couplings of two or more natural components to one another. But it should be noted that not every tannin necessarily consists of a galloyl unit or derivative. The so-called condensed tannins, which are produced from flavanoid precursors, contain examples of this type[66].

2.3.6 **Terpenes (terpenoids)**

Terpenes are the largest class of secondary metabolites and are primarily composed of many isoprene units (many isoprene units) that are joined to one another in a variety of ways[67]. Terpenes are the most prevalent group of secondary metabolites, and they combine numerous five-carbon isoprene units produced by hydrocarbons[68]. Terpenoids exhibit notable pharmacological properties, including anti-inflammatory, antimalarial, antibacterial, antiviral, cholesterol synthesis inhibition, and anti-cancer actions[69]. One of the most common and chemically diverse categories of natural products is terpenes. They are combustible unsaturated hydrocarbons that are typically present in liquid form in oleoresins, resins, and essential oils[70]. Numerous plants make volatile terpenes to entice particular pollinating insects and to prevent some plants from being eaten by animals[71]. Some plants develop terpenes that are less volatile but have a strong bitter or toxic flavor. Terpenoids can also have medicinal properties such as anticarcinogenic, anti-malarial, anti-ulcer, hepaticidal, antimicrobial, or diuretic activity, as well as the sesquiterpenoid anti-malarial drug artimisinin and the diterpenoid anticancer medicine taxol[72].

The majority of terpenoids are lipophilic and interact with biomembranes and membrane proteins through terpenes. Terpenes are generally cytotoxic to a variety of organisms, including bacteria, fungus, insects, and vertebrates, and are frequently employed in herbal medicine to treat infections[73]. Terpenoids are considered to be altered terpenes in which methyl groups have been added, deleted, or relocated. Terpenoids and steroids that have been known to be effective against S*taphylococcus aureus*[74]. These substances also possess anticarcinogenic qualities[75].Depending on how many carbon atoms they contain, terpenoids are classified as monoterpenes, sesquiterpenes, diterpenes, sesterpenes, and triterpenes. The

majority of terpenoids, which vary in their structural makeup, are physiologically active and used across the globe to treat a wide range of illnesses. Terpenes are used in many flavorings and excellent scents because of their pleasant odour. Medicines for malaria like artemisinin and similar substances are made from terpenes and their derivatives. Terpenoids, meantime, have a variety of uses in the production of foods, medications, beauty products, vitamins, hormones, and other products.

2.4 Epidemiology of STI

Sexually transmitted infections (STIs) carry a heavy burden of uncertain health, social, and financial ramifications. Because many people may feel stigmatized when discussing STIs, many of them remain hidden. Nearly 37 million people worldwide were HIV positive in 2017, and 66% of cases, 68% of new adult HIV infections, 92% of new pediatric infections, and 72% of all AIDS-related deaths occurred in sub-Saharan Africa. With that in mind, the main focus of an African STI containment strategy should be on prevention and related knowledge. According to WHO data, the burden of disease and death caused by STDs across the globe jeopardizes quality of life, sexual and reproductive health, and the health of newborns and children. In addition to causing cellular alterations that precede certain cancers, sexually transmitted infections also indirectly aid in the sexual transmission of HIV.

Sexually transmitted infections negatively impact people's general well-being and place a significant financial burden on households and national health systems, particularly in middle- and low-income nations. For instance, syphilis, trichomoniasis, gonorrhea, and chlamydia are the four sexually transmitted infections that an estimated 500 million people get each year. There are more than 530 million individuals who have herpes simplex virus

type 2 (HSV2). One of the most prevalent STIs, HPV infection affects over 290 million women worldwide.

2.5 Symptoms of Sexually Transmitted Infections

Vaginal discharge, burning or discharge from the urethra in men, genital ulcers, and abdominal pain are common signs of sexually transmitted infections. Not all STIs cause symptoms, or they may only cause minor ones. Thus, it is feasible to be infected without realizing it. You can still teach it to other people, though. In the event that symptoms exist, they may consist of the following: an odd odor coming from the penis or vagina, sores or warts on the genital area, painful or frequent urination, itching and redness in the genital area, blisters or sores in or around the mouth, anal itching, soreness, fever, and abdominal pain.

2.6 Diagnosis of Sexually Transmitted Infections

Treating the indications or symptoms of a collection of illnesses as opposed to a single disease is the goal of the syndromic approach to the diagnosis and treatment of sexually transmitted infections (STIs). This has been acknowledged as the preferred method of management since it enables the treatment of one or more conditions that frequently occur simultaneously. STI screening can support STI management and prevention and offer a chance for further health promotion and education during any and all medical visits. Whenever feasible, STI prevention and screening ought to be incorporated into regular medical visits. Sexually active people should discuss the risks of STIs and the necessary testing with their healthcare provider. Given that many STIs typically don't cause symptoms. Certain STIs can be identified by a physical examination, microscopic analysis of a sore, or

by swabbing fluid from the penis, anus, or vagina. Other STI types can be diagnosed with blood tests. It is crucial to perform a comprehensive ano-genital examination and obtain a good sexual history. Inquiries about symptoms, recent sexual history, sexual orientation, type of sex (oral, vaginal, or anal), potential pregnancy (female), use of contraceptives (including condoms), history of recent antibiotic use, drug allergies, and recent international travel should all be included in the history.

STI diagnostic tests with high accuracy are commonly utilized in affluent nations. These are particularly helpful in the diagnosis of infections that show no symptoms. Diagnostic tests, however, are mainly unavailable in low- and middle-income nations. When testing is offered, it's frequently pricy, difficult to access geographically, and results are frequently delayed for patients. As a result, care or treatment may not be completed and follow-up may be hindered. There are currently rapid and affordable blood tests available for STIs. In certain settings with limited resources, this test is already in use. The test requires little training, is accurate, and can yield results in 15 to 20 minutes. Numerous rapid tests for additional sexually transmitted infections (STIs) are being developed and could enhance STI diagnosis and treatment, particularly in environments with limited resources.

2.7 Treatment of Sexually Transmitted Infections

There are currently effective treatment available for a number of STIs,. The three bacterial STIs (chlamydia, gonorrhea, and syphilis) and the single parasitic STI (trichomoniasis) can typically be cured with currently available, efficient single-dose antibiotic regimens. The best treatments currently available for HIV and herpes are antivirals, which slows the progression of the illness but are unable to reverse it. Immune system modulators such as interferon and

antiviral drugs can aid in the fight against hepatitis B and reduce liver damage. The number of treatment options for sexually transmitted infections (STIs) has decreased due to the rapid increase in antibiotic resistance, particularly gonorrhea. Gonorrhea has become a multidrug-resistant organism due to its decreased susceptibility to the "last line" treatment option, which is oral and injectable cephalosporins, as well as its previously demonstrated resistance to penicillins, sulphonamides, tetracyclines, quinolones, and macrolides. Although less common, antimicrobial resistance also exists for other STIs, so prevention and early treatment are essential. STI case handling Rather than using laboratory testing to guide treatment, low- and middle-income countries rely on syndromic management, which is based on the identification of consistent groups of symptoms and easily recognized signs (syndromes). With this method, which frequently uses clinical algorithms, medical professionals can identify a particular infection based on symptoms they have seen. Simple, same-day treatment is guaranteed by syndromic management, which also spares costly or unfeasible diagnostic testing. But this method ignores infections that show no symptoms at all.

2.8 The Challenges Associated with Sexually Transmitted Infections.

2.8.1 High cost of Multiplex diagnostic nucleic acid amplification tests

Tests for nucleic acid amplification (NAATs) have completely changed how STIs are diagnosed. NAATs enable STI screening to be incorporated outside of the typical STI outpatient clinic setting, such as in the context of regular HIV care. Additionally, NAATs make STI screening significantly simpler by providing the patient with options for both self- and home-collection. High-income nations have widely adopted NAATs due to their convenience in sample collection. Businesses are starting to introduce NAATs that can

identify several pathogens in a single specimen. STI screening has historically relied on serology, pathogen cultivation, and direct light microscopic visualization. Although the modalities of these tests have a high specificity, their sensitivity was frequently low. Although the development of highly sensitive and specific tests has benefited greatly from the amplification of pathogenic DNA or RNA, most low- and middle-income countries cannot afford it. The identification of the causative agent of a STI-related syndrome, such as urethritis, vaginal discharge, or genito-ulcerative disease, may also benefit financially from this.

2.8.2 **Shortages of Out-Of-Patent Antibiotics**

An example of sThe first-line treatment for syphilis, benzathine penicillin G (BPG), was said to be globally short-handed in 2017. Future shortages of first-line antibiotics for gonorrhea, trichomoniasis, and chlamydia can also be anticipated because these medications are off-patent[76].Syphilis makes up just 1% of BPG prescriptions; rheumatic heart disease is the most common indication for BPG. The only treatment that is thought to be safe for expectant mothers to prevent congenital syphilis is BPG. The active pharmaceutical ingredient was produced in just three factories, all located in China, due to the low profit margins and high production costs of BPG. The stock-out risk has significantly increased as a result. Two of the manufacturers recently stopped producing because of governmental regulations and environmental concerns[77]. BPG has been identified by the WHO as a necessary medication with a high stock-out risk. In order to guarantee acceptable quality, safety, and efficacy standards of BPG supplied by international agencies (such as the Global Fund to Fight AIDS, Tuberculosis, and Malaria), it has invited manufacturers to apply for WHO pre-qualification.

Demand-side measures include prioritizing appropriate syphilis treatment and strengthening national BPG forecasting and procurement systems.

2.9 Antimicrobial Resistance

Antimicrobials are used to cure infections in people, animals, and plants by killing or preventing the growth of bacteria[78]. Each antimicrobial kind targets a distinct anatomical feature or physiological function unique to its target bacterium. Antimicrobials can either be wide, focusing on a characteristic that all members of a group of bacteria share, or narrow, influencing a characteristic that is exclusive to one or a few species within a group. Antimicrobials that work well against one species or group of organisms do not work as well when applied to another species or group that does not possess the targeted characteristic[79]. Antimicrobial resistance (AMR) is a global issue that has detrimental long-term repercussions due to the fact that antibiotic resistance is developing more quickly than new treatments are being developed. Although STIs caused by antibiotic resistance are a global public health problem, front-line healthcare professionals frequently fail to recognize their significance.

According to the World Health Organization's (WHO) first general global report on antimicrobial resistance, antibiotic resistance isn't just a concern for the future; it's already happening everywhere. Since no significant new antibiotics have been developed in the last 30 years, this is even more concerning. Furthermore, this report specifically notes decreased

susceptibility in 36 countries and treatment failures owing to resistance to extended spectrum cephalosporins, the last-resort treatments for gonorrhea, in 10 countries. Control of gonococcal infections was given priority in the World Health Organization (WHO) policy report on STIs, in part due to the threat of diseases that would become incurable due to medication resistance[80]. Other prevalent STIs have also been linked to treatment resistance, many of which can lead to silent illness and contribute to the spread of drug-resistant organisms worldwide[81]. Numerous promising antibacterial treatments could become worthless due to the quick rise in antibiotic resistance[82]. A bacterium's capacity to endure the effects of antibiotics is referred to as bacterial resistance. The inability of medicines to treat infectious diseases for which they were intended is a developing global issue known as antibiotic resistance.

Now that the WHO has warned that the world is "running out of antibiotics," concerns about the rise of antibiotic resistance worldwide are intensifying. Antimicrobial-resistant microbes have recently presented a significant challenge to the management of clinical contagious disorders, leading to a progressive rise in the incidence of hospital acquired infections[83]. As bacterial infections are one of the main causes of sickness and death, antibiotic resistance continues to grow globally, putting the ability to treat common infectious diseases at risk. Because of its likelihood to spread globally and the resulting shortage of therapeutic options, antibiotic resistance is an important concern in health care[84].

A genetic mutation that dramatically affects a pathogenic microbe's morphology or physiology giving it the ability to avoid or resist the effects of an antimicrobial drug is what causes AMR, a natural process[85]. The resistance to antibiotics, happens when bacteria develop a resistance to antibiotic medication. Because of this, the resistant strain sustains

exposure to the antibiotic while other strains do not have this mutation. The resistant microorganism can then reproduce and divide, passing on the genetic advantage. In some cases, horizontal gene transfer between species can also occur[86]. An infected person or host, whether human, animal, or plant, can transmit their anti-microbial-resistant strain to others by direct or indirect transmission, such as through food products, the environment, or direct contact between animals and humans[87]. Over time, the resistant strain dominates the species, rendering the antimicrobial drug largely useless. Inhibiting particular antimicrobial pathways, such as cell wall construction, nucleic acid synthesis, ribosome activity, protein synthesis, folate metabolism, and cell membrane function, frequently results in the development of antibiotic resistance[88].

Because they are available over the counter, without a prescription, and through unregulated supply chains, antibiotic misuse is made easier in developing nations[89]. Poverty is a significant root cause of antimicrobial usage in developing countries, and noncompliance with antimicrobial use has various effects on resistance[90]. However, even among the wealthy, some patients miss doses unintentionally or on purpose, particularly when signs and symptoms start to fade after an initial positive therapeutic response[91]. Other times, such when a severe side effect occurs, people stop taking their medication only to end up in the hospital again with a reoccurring illness caused by a more virulent and resistant strain of the microorganism[92]. These efforts expose remaining organisms to sub-therapeutic levels of antibiotics, increasing the likelihood that they may develop resistance.

In developing nations, where people frequently obtain antibiotics without a prescription and through uncontrolled supply chains, self-medication is a popular practice[93].Threats posed by gram-negative and gram-positive resistant bacterial strains have pushed researchers to look

into new antibiotic classes and cutting-edge methods to combat the issue of resistance[94]. If prompt and proactive action is not taken, we are quickly moving into a post-antibiotic world in which routine diseases and minor wounds can once again be fatal.

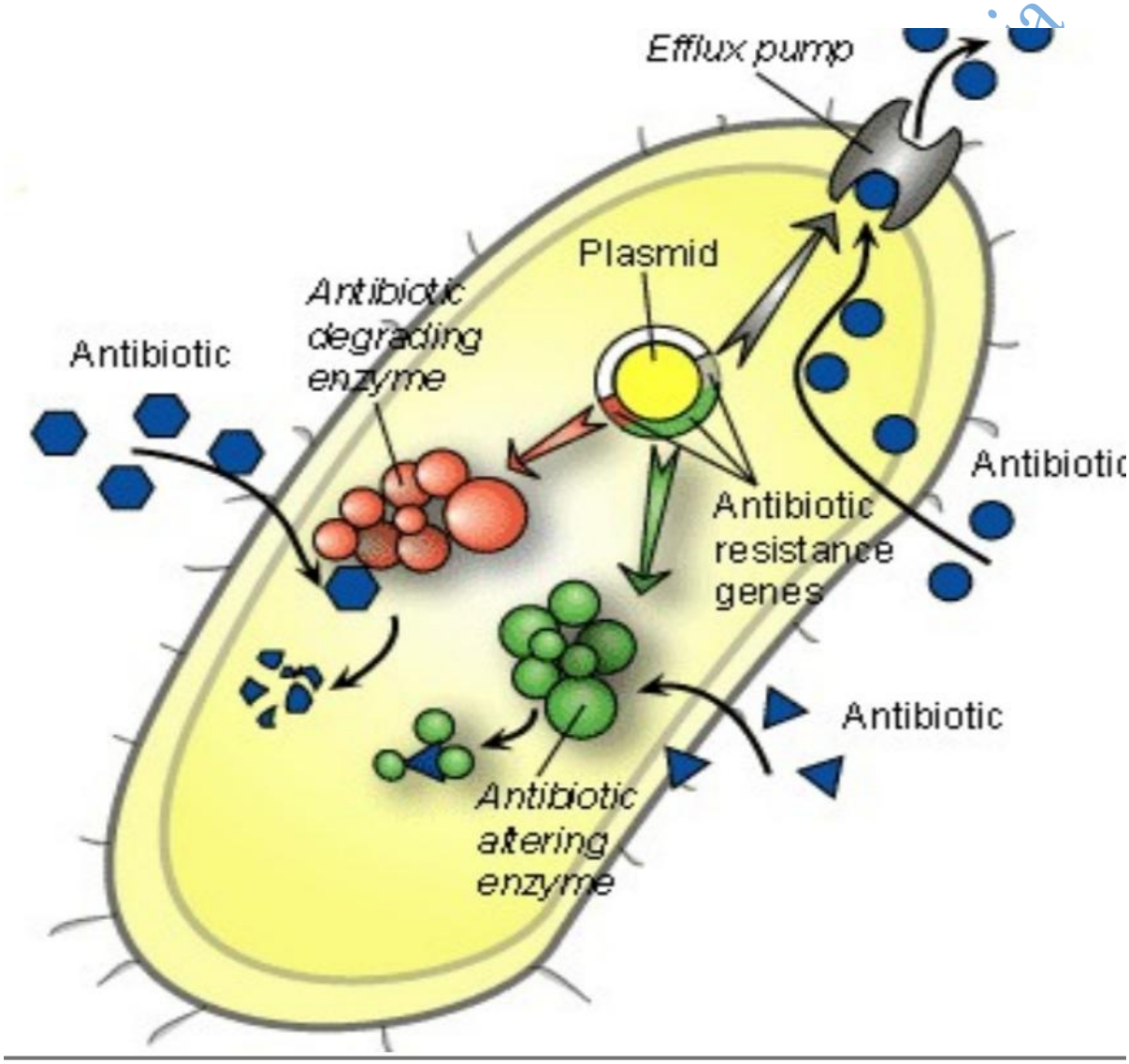

Figure 1.1. Antimicrobial resistance mechanisms[95]

2.10 Bacterial Isolates

Bacteria, viruses, fungus, and protozoa are all considered microorganisms, sometimes known as microbes. They can be found in either a commensal (which does not cause disease) or pathogenic (which causes infection) connection with humans, animals, plants, water, soil, and the air.

2.11 The Bacteria

The simplest, oldest, and most prevalent form of life on earth is bacteria. Additionally, they are the only species having prokaryotic cellular structure. Bacteria were widespread for more than 2 billion years before eukaryotes first formed in the Earth, and they are still present in the earliest rocks from which fossils have been found, which 3.5 to 3.8 billion years old are. Early oxygen-producing photosynthetic bacteria (cyanobacteria) changed the earth's atmosphere, resulting in a high level of bacterial and eukaryotic diversity. Both production and the cycling of the materials necessary for all other life-forms depend heavily on bacteria. Only bacteria are capable of fixing nitrogen from the atmosphere.

There are currently 5000 known types of bacteria, but there are undoubtedly many thousands more that need to be properly identified. The structural variations between various bacteria are negligible even when seen under an electron microscope in comparison to other groups of

organisms. Since morphological distinctions between bacteria are so negligible, the majority of bacteria are categorized according to their metabolic and genetic traits. Because the features of these organisms frequently change depending on their development conditions, bacteria can only be accurately classified when they are cultivated on a specified medium.

On Earth, bacteria are omnipresent and coexist with eukaryotes. Numerous other, harsher settings would be fatal to any other type of life. Yet, bacteria can survive in many of these. Bacteria can survive in settings that would kill most other species, such as hot springs that would roast other organisms, hypersaline environments that would dry out other cells, and atmospheres full of deadly gases like methane or hydrogen sulfide. These hostile environments resemble those on the early Earth, when life first emerged, perhaps. Bacteria most likely developed the ability to live in these challenging environments early on and have continued to do so even as the rest of the environment has changed. Antibiotic residue-filled environments impose selective pressure and aid in the emergence of microorganisms that are resistant to antibiotics. Numerous studies have concentrated on antibiotic-resistant bacteria that are frequently grown in laboratories due to the possible health concern.

2.11.1 ***Staphylococcus aureus***

Staphylococcus is a gram positive, non-motile organism that is both a commensal bacterium and a human pathogen. This group of organisms are often fastidious, facultative anaerobes without spores, observed under the microscope in clusters, pairs, and occasionally short chains. *S. aureus* colonizes about 30% of the population of people[96]. *Staphylococcus aureus* is a pathogen that colonizes the skin and mucous membrane of the anterior nares, gastrointestinal tracts, perineum, genitourinary tracts, and pharynx. It is widespread,

adaptable, and extremely contagious[97]. It is the cause of a variety of illnesses in both humans and animals that have a serious effect on public health. Significant public health implications were presented by host specialization, the capacity to acquire and lose virulence and resistance genes, as well as the possibility for zoonotic transmission[98]. Clinically, *S. aureus* is the most dangerous staphylococci member and the cause of a wide range of illnesses, including food poisoning, endocarditis, necrotic pneumonia in children, and skin infections (such as folliculitis, furuncles, impetigo, wound infections, and scalded skin syndrome)[99], Soft-tissue infections, toxic shock syndrome, purpura fulminans, endocarditis, osteomyelitis, food poisoning, and urinary tract infections are a few examples of medical conditions. A research of a cohort of individuals visiting a sexually transmitted infections (STI) clinic described other body areas of *Staphylococcus* colonization, including the vaginal area[100].

Infertile couples' semen cultures had a prevalence of 75% and high vaginal and endocervical swabs had a prevalence of 38.7% *S. aureus*[101]. *S. aureus* was shown to be the most common vaginal pathogen (57.33%) among local infertile women, followed by *Escherichia coli* (25.33%), according to another study[102]. The presence of genes for antibiotic resistance such mecA, vanA, staphylococcal exotoxins, and others that promote the onset of the disease process, immune evasion, and host tissue damage are what contribute to the disease's severity[103]. *S. aureus* (28.1%) and S. saprohyticus (13.9%) were the most prevalent pathogens discovered and have detrimental effects on sperm motility and morphology in a research of a total of 140 sperm samples obtained from the University of Benin Teaching Hospital[104].

Increased rates of community-associated (CA)-MRSA infection have been reported among HIV-positive people[105], sexually active males who have sex with men (MSM), and people

who engage in high-risk sexual practices (as indicated by improper or infrequent condom usage). However, the pathogenic *staphylococcal* infection has considerably increased over the past 20 years due to an increase in infections linked to healthcare and caused by strains that are resistant to antibiotics[106]. The emergence of antibiotic resistance in *S. aureus* was first noted in the middle of the 1940s, when a strain of the bacteria produced penicillinase, a hydrolyzing enzyme that allowed it to resist penicillin[107]. Public health is particularly at risk due to the sharp increase of antibiotic-resistant bacteria. MRSA stands for methicillin-resistant *S. aureus*, a type of bacteria that has the capacity to withstand all β-lactam medications, including penicillins and cephalosporins. A non-native gene that codes for a penicillin-binding protein (PBP2a) with a markedly reduced affinity for β-lactams is typically acquired to confer resistance.

Since only patients and medical staff were initially exposed to these resistant strains, they were given the label nosocomial associated penicillin resistant *S. aureus*. However, resistant strains that were not clearly linked to the hospital strains' risk factors were eventually discovered among community members. As a result, from the late 1940s to the early 1960s, penicillin resistance grew. Methicillin, a semi-synthetic homologue of penicillin, was then introduced into clinics as a preferred medication for the management of *S. aureus* infection[108]. However, within a year of its debut as a preferred strategic medication for the treatment of *S. aureus* infection, methicillin resistance in *S. aureus* was reported. Community-associated methicillin-resistant *S. aureus* (CA-MRSA) colonization and infection have become more common worldwide since the 1990s[109]. The development of a genomic island containing the mecA methicillin resistance determinant is the cause of methicillin-resistant *S. aureus* (MRSA). Consequently, many critically necessary antibiotics' therapeutic effectiveness is

decreased, and the length of hospital stays is increased[110]. This is a follow-up to the discovery of a methicillin resistance gene that codes for a penicillin-binding protein that is less susceptible to all beta lactam antibiotic classes[111].

2.11.2 ***Candida specie***

A yeast fungus called *Candida* is a component of the human muco-cutaneous flora. *Candida albicans, glabrata, tropicalis, stellatoidea, parapsilosis, catemilata, ciferri, guilliermondii, haemulonii, kefyr, and krusei* are a few of the 200 or more species that exist. In 20–50% of healthy, asymptomatic women, the lower vaginal tract flora contains *Candida species*[112]. 75% of women get vaginitis at least once in their lifetimes, with *Candida* being the primary cause[113]. A fungal infection of any species from the genus *Candida* (one genus of yeasts) is known as candidiasis, thrush, or yeast infection. The most typical cause of candidiasis in humans is *Candida albicans*. In vaginal specimens, non-albicans including *C. glabrata* and *C. tropicalis* are also present[114]. According to reports, women on broad-spectrum antibiotics, pregnant women, those with diabetes, and women living with HIV/AIDS had increased carrier rates[115]. The main method of transmission involves sexual behavior as well as non-sexual contact such as handling contaminated medical supplies, wearing or exchanging garments by teenagers, and others[116].

Several other factors, such as pregnancy, the use of oestrogen-rich medications and oral contraceptives, uncontrolled diabetes mellitus, prolonged use of broad-spectrum antibiotics that destroy good and beneficial bacteria, allowing yeast overgrowth, poor dietary practices, and poor personal hygiene, can also be linked to an increased rate of *C. albicans* colonization of the vagina[117]. Many medical professionals think that nylon underwear and tight, insulating

garments make women more susceptible to vaginal candidiasis by raising the perineum's temperature and moisture levels. A study of African women found that those who wore tight clothing had a higher prevalence of Vulvovaginal candidiasis caused by *Candida albicans* than those who wore loose clothing[118]. Douching, constantly wet vulva from tight clothing, chemicals coming into contact with the vagina from scented tampons, poor personal hygiene, and prolonged use of antibiotics that kill the benign and beneficial bacteria that allow yeast overgrowth are additional physical factors that can cause genital tract infections[119].

Candidiasis includes a variety of infections, from minor illnesses like vaginitis and oral thrush to more serious, sometimes fatal conditions[120]. Candidiasis can also affect male genitalia and is a fairly common cause of vaginal discomfort (vaginitis)[121]. In 85%–90% of VVC instances, it is solitary[122]. Recent investigations revealed that non-albicans *Candida* are now regarded as pathogens due to the rise in the frequency with which they are isolated from clinical specimens. Symptoms include pain or soreness in the vagina, discomfort during sexual activity, pain or discomfort when peeing, and abnormal vaginal discharge[123]. Although most cases of vaginal candidiasis are minor, some women might develop serious infections characterized by vaginal wall fissures, edema, and redness[124].

To continue existing within the host and spread disease when the chance presents, *C. albicans*[125]. Azole-based medications are often used to treat *Candida* infections. [126]. . The level of resistance to these medications is rising, though. Fluconazole is inherently resistant to several *Candida* species, such as *C. glabrata* and *C. krusei*, and most fluconazole-sensitive isolates of *C. dubliniensis* encode multidrug transporters that cause fluconazole resistance during clinical therapy[127]. Since speciation and antifungal susceptibility testing of *Candida*

isolates are not frequently performed for clinical purposes, this is mostly due to the use of over-the-counter (OTC) medications and empiric regimens to treat these infections[128].

2.11.3 ***Escherichia coli***

One of the most common species discovered in human semen and genitourinary diseases, particularly epididymitis, is *Escherichia coli.* [129]. Closely connected hosts, such as pets and romantic partners, frequently share *E. coli* strains, whether they are dangerous or not[130]. In communal settings, uropathogenic *Escherichia coli* (UPEC) can be sexually transmitted and, in the absence of sexual interaction, can also be spread among family members[131]. These organisms were considered relative aetiologies for the symptoms the patients were experiencing because they (together with *Lactobacilli)* have previously been linked to urinary tract infections with unusual symptoms[132].

Infection with one of these pathotypes can cause enteric/diarrheal disease, urinary tract infections (UTIs), and sepsis/meningitis, three main clinical syndromes. Enteropathogenic *E. coli* (EPEC), enterohaemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and diffusely adherent E. coli (DAEC) are the six well-defined groups of intestinal pathogens[133]. ExPEC is the new name for the E. coli pathotypes linked to extraintestinal infections[134]. R-factor on plasmids, resistance genes on the chromosomes, synthesis of β -lactamase, and extended range β -lactamase enzymes are among the factors frequently causing E. coli resistance[135].

2.11.4 ***Klebsiella pneumoniae***

Since its discovery in 1883 by German pathologist Carl Friedländer, who later gave it the generic name *Klebsiella* in honor of German bacteriologist Edwin Klebs[136]. It is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultatively anaerobic, rod-shaped bacillus of the Enterobacteriaceae family that grows as a mucoid lactose fermenter on MacConkey agar[137]. Using classifications from the Centers for Disease Control and Prevention, the site of infection associated with the bacteremia was identified as pneumonia, urinary tract infection, meningitis, incisional wound infection, other soft tissue infection, intra-abdominal infection, and primary bloodstream infection[138].

One of the agents that can cause bacterial vaginitis is *Klebsiella pneumonia,* which is multiresistant to medicines, particularly beta-lactam drugs[139]. *K. pneumonia* is an opportunistic pathogenic bacteria that has been confirmed by bacteriologists to infect people of all ages. It is also one of the nosocomial pathogenic agents that can easily grow and multiply in a variety of environments, including soil and hospitals, and is the primary cause of urinary infections, wounds, and burns[140]. High morbidity and death rates from *Klebsiella* infections point to the organism's virulence characteristics, as well as its high level of antibacterial agent resistance that is encoded on several plasmids[141].

According to numerous studies, *K. pneumoniae* is capable of producing widely distributed beta-lactamases that may withstand a variety of antibiotic actions by using a variety of resistance mechanisms[142]. Bacteria have developed highly effective methods for clonal expansion and the distribution of resistance characteristics, and they employ a variety of ways to evade the effects of antimicrobial drugs[143]. The prevalence of the resistant organisms

is now a global issue, and there are few effective treatment options available[144]. Testing to identify which antibiotics will treat this infection is necessary because the recommended treatment changes as the organism develops resistances, making it difficult to treat *K. pneumoniae* infections due to the emerging resistance to traditional antibiotics that were once effective against them.[145].

2.12 Antibiotics

The word "antibiosis," which means "against life," was used to create the name antibiotic. Antibiotics used to be thought of as chemical molecules made by one type of bacteria that are poisonous to other microorganisms[146]. A chemical produced by a single microorganism or of biological origin that, at low concentrations, can hinder the growth of or be harmful to other bacteria was the original definition of an antibiotic because of this idea[147]. Modern times have changed this concept to also include antimicrobials that are partially or entirely manufactured synthetically.

While some medicines can totally eradicate other germs, others can just stop their growth. Bacteriostatic substances prevent bacterial growth, whereas bactericidal substances kill bacteria[148]. Despite the fact that the term "antibiotic" often refers to an antibacterial, antibiotic compounds are classified as either antibacterial, antifungals, or antivirals depending on the type of microorganisms they combat[149]. Penicillin was accidentally discovered in soil-dwelling fungus *Penicillium notatum* in September 1928 by late English bacteriologist Sir Alexander Fleming. Its discovery was first reported in 1929, and the first human clinical trials were conducted in 1940[150].

2.12.1 **Classification of Antibiotics**

Because there are exploitable metabolic variations between the organism and the host, it is possible to create chemotherapeutic drugs that are toxic for infectious organisms but safe for the host[151]. This results in many antibiotic classes that act on various metabolic targets and pathways. These include agents that stop the production of bacterial proteins (such as aminoglycosides), block the creation of cell walls and nucleic acids (such as beta-lactams), and damage the structure of cell membranes (such as polymyxins). There are various classification categories for antibiotics, but the most popular ones are based on their molecular makeup, modes of action, and range of activity[152]. Others include the method of administration (oral, topical, and injectable). It is typical for antibiotics from the same structural class to exhibit a similar pattern of efficacy, toxicity, and allergic-related side effects.

The first significant antibiotic, "penicillin," was discovered and developed in the 1920s. It was then introduced into the human health care system in the 1940s, and it has since changed how bacterial infections are managed and combated[153]. Antibiotics' antibacterial activity isn't quite completely selective, though. While combating pathogenic bacteria, they also combat the beneficial microbiota that all humans require and possess, such as those in the gastrointestinal tract[154]. Therefore, the prescription and administration of any given antibiotic is based on the total intended benefit while taking into account the associated negative effects. Because of this, it is essential to comprehend the mode of action of any antibiotic before introducing it into our system for delivering healthcare, and current molecular biological techniques have greatly contributed to clarifying our understanding in this regard. Based on their chemical or molecular composition, the following kinds of antibiotics are frequently

used: Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides, and Oxazolidinones[155].

2.13 Antimicrobials and Antibiotics

Antibiotics, which fight bacteria and treat bacterial infections, are a type of preventive medicine. Antibiotic resistance is created when bacteria alter their forms in response to frequent antibiotic use. Generally speaking, antimicrobial resistance to medications used to treat diseases brought on by other bacteria like parasites, viruses, and fungus like Candida and malaria. Hence Antimicrobials are one of the few treatments available for severe bacterial infections in humans, and they play a significant role in human medicine. If left untreated, serious infections are likely to cause considerable morbidity or fatality. Multidrug resistance is also a result of illnesses that are related to the site of the infection, such as pneumonia or meningitis, or the host, such as a newborn or an immunosuppressive medication. The use of such antibacterial agents is maintained because the decline in their efficacy brought on by the evolution of resistance has a substantial negative impact on human health, particularly for those who have infections that pose a serious risk to their lives. These are the treatment options for major bacterial infections in humans, which are crucial to human medicine. Untreated infections could result in substantial morbidity or fatality. Multidrug resistance could also happen occasionally.

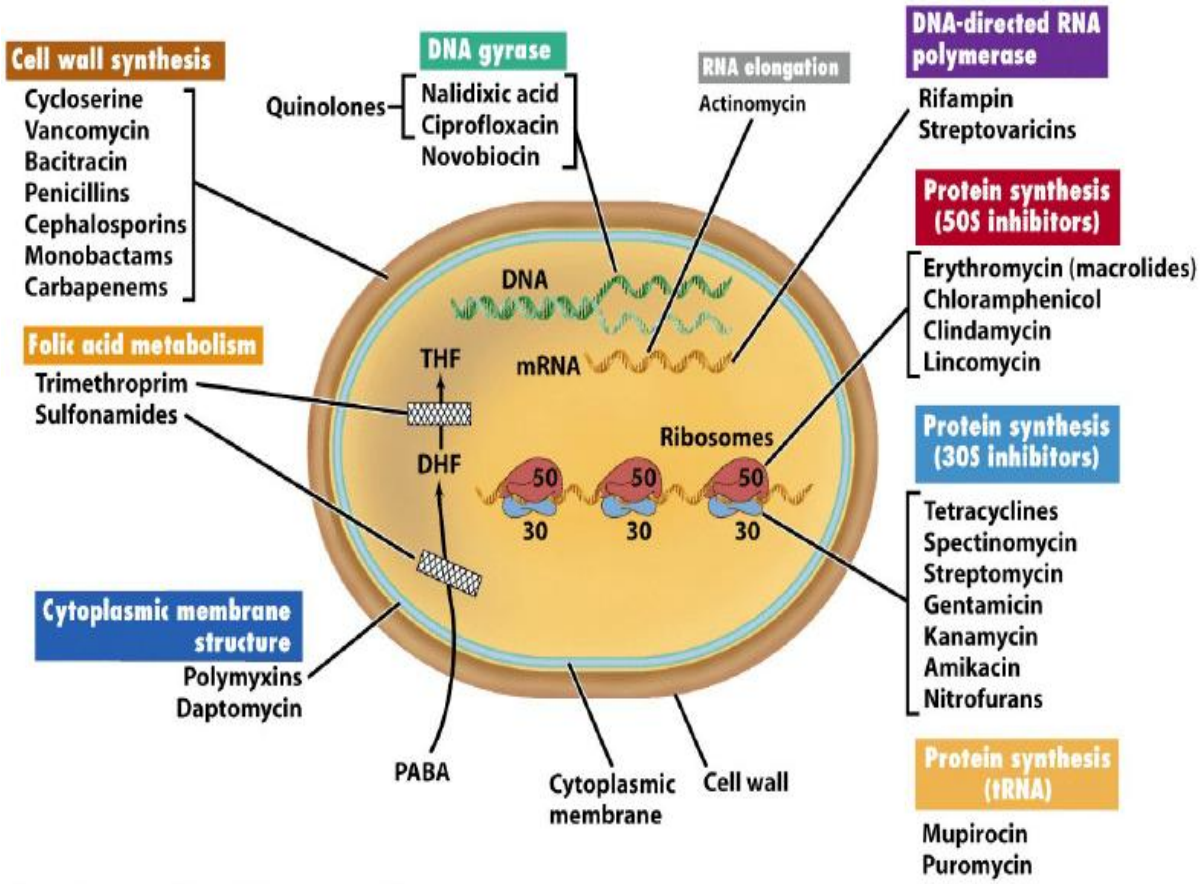

Figure 20-14 Brock Biology of Microorganisms 11/e

Figure 2.2 Classes of antibiotics/antibacterial agents and their modes of action on bacteria[156]

2.13.1 **Antibiotics Targeting Cell Wall**

The cell wall of bacteria is comprised of peptidoglycan, which is composed of long sugar polymers. Transglycosidases work on the peptidoglycan to cross-link the glycan strands, and the peptide chains extend from the sugars in the polymers to create linkages from one peptide to another[157]. In the presence of penicillin binding proteins (PBPs), glycine residues cross-link the D-alanyl-alanine part of the peptide chain[158]. The cell wall is strengthened by this cross-linking. Glycopeptides and β-lactams prevent the production of cell walls.

- Beta-Lactam Antibiotics - The PBPs are the main targets of β-lactam drugs. It has been proposed that the D-alanyl D-alanine section of the peptide chain, which is typically bound by PBP, is mimicked by the β-lactam ring. PBP interacts with the β-lactam ring and isn't used in the production of fresh peptidoglycan. The lysis of the bacterium is caused by the rupture of the peptidoglycan layer.

- Glycopeptides bind to the D-alanyl D-alanine part of the peptide side chain of the precursor peptidoglycan subunit. Vancomycin, a potent antibiotic, stops this D-alanyl component from binding to the PBP, hence inhibiting the formation of cell walls[159].

2.13.2 **Inhibitors of Protein Biosynthesis**

First, a process known as transcription uses the information in bacterial DNA to create a messenger RNA (m-RNA) molecule. Then, a process known as translation creates the proteins found in m-RNA using the macromolecular structure known as the ribosome. Ribosomes and cytoplasmic factors help to catalyze the synthesis of proteins. The 30S and 50S subunits of the ribonucleoprotein make up the bacterial 70S ribosome[160]. The 30S or 50S component of the bacterial ribosome is the target of antimicrobials that prevent protein production[161]

- Inhibitors of the 30S subunit - Aminoglycosides (AGs) are positively charged molecules that bind to the negatively charged OM to produce huge holes, which then allow antibiotics to enter the bacterium. The bacterial ribosome is the major target of the action; in order to enter, it must pass through the cytoplasmic membrane, requiring an active, energy-dependent bacterial transport mechanism that needs oxygen and an active proton motive force. Due to these factors, AG are ineffective against anaerobic bacteria and only function under aerobic circumstances. Since these AG allow for higher penetration of AG within the cell and at low dosages, they work in synergy with antibiotics that block the formation of cell walls (such as -lactam and glycopeptides). Through hydrogen bonds, AGs engage with the 30S subunit's 16S r-RNA close to the A site. They result in mRNA translation being misinterpreted and ending too soon. Tetracyclines, such as tetracycline, chlortetracycline, doxycycline, or minocycline, act upon the conserved sequences of the 16S r-RNA of the 30S ribosomal subunit to prevent binding of t-RNA to The Site[162].

- Chloramphenicol is one of the inhibitors of the 50S subunit and interacts with the conserved regions in the 23S r-RNA's peptidyltransferase cavity. As a result, it prevents t-RNA from binding to the ribosomes A site, inhibiting the production of proteins.[163].

- Macrolides target the conserved sequences of the peptidyltransferase core of the 23S r-RNA of the 50S ribosomal subunit to alter the early stage of protein synthesis, known as translocation[164]. As a result, incomplete peptide chains prematurely separate. Streptogramin B, macrolides, and lincosamides all exhibit a comparable mode of action.

- Oxazolidinones: Linezolid, a member of this group's innovative class of antibiotics that is entirely synthetic, was just recently approved. Several phases of protein synthesis are disrupted by oxyazolidinones: both (i) bind to the 50S subunit's 23Sr RNA to reduce protein synthesis, and (ii) block 70S inhibition via interacting with peptidyl-t-RNA[165].

2.13.3 **Inhibitors of DNA Replication**

Fluoroquinolones (FQ), a type of quinolone, prevent the bacterial DNA gyrase enzyme from nicking double-stranded DNA, adding negative supercoils, and then resealing the nicked ends. When the strands separate to enable replication or transcription, this is required to prevent excessive positive supercoiling of the strands. Two A subunits and two B subunits make up the DNA gyrase. The DNA is cut by A subunit, B subunit adds negative supercoils, and A subunit subsequently reseals the strands. The FQs interfere with the A subunit's ability to cut and reseal strands by binding to it with a high affinity. Topoisomerase IV is a

prominent target of action in Gram-positive bacteria because it nicks and separates the daughter DNA strand following DNA replication. Greater potency against Gram-positive bacteria might result from greater affinity for this enzyme. Mammalian cells lack DNA gyrase or topoisomerase IV in favor of topoisomerase II, which has a low affinity for FQ and is therefore less hazardous to cells[166].

2.13.4 **Folic Acid Metabolism Inhibitors**

Both sulfonamides and trimethoprim block different processes in the metabolism of folic acid. Combining trimethoprim and sulpha medications, which operate at various points along the same biosynthetic pathway, results in synergy and a lower rate of resistance mutation. Sulfonamides have a higher affinity for the enzyme than the natural substrate, p-amino benzoic acid, and inhibit dihydropteroate synthase in a competitive manner. Trimethoprim is an agent that works later in the production of folic acid and inhibits the dihydrofolatereductase enzyme[167].

2.13.5 **Prevention of Accumulation of Antimicrobials**

In order to do this, either the absorption or efflux of the antibiotic from the cell must be increased.

- Modifications in Outer Membrane Permeability - Drug molecules can enter a cell through self-uptake, diffusion through porins, and diffusion through the bilayer. The OM of Gram-negative bacteria houses the porin channels. Only porins allow tiny hydrophilic compounds (such as -lactams and quinolones) to pass through the OM. Because there are fewer porin channels, less -lactam and FQ antibiotics enter the cell,

which increases resistance to these antibiotic families. Low-OM permeability is the cause of Pseudomonas aeruginosa's acquired resistance to all antibiotic classes.

- Efflux pumps - Membrane proteins that maintain low intracellular concentrations of antibiotics while exporting them from the cell are known as efflux pumps[168]. Before these antimicrobials reach their target, efflux mechanisms pump them out of the cell again at the same rate at which they enter[169]. Unlike porins, which are located in OM, these pumps are found in the cytoplasmic membrane. All types of antibiotics, with the exception of polymyxin, can cause the activation of efflux systems[170]. Antibiotics may be specific to efflux pumps. The majority of them are multidrug transporters that may pump a variety of unrelated antibiotics, including macrolides, tetracyclines, and FQ. As a result, they greatly aid in the development of multidrug resistance organisms[171].

- Modification of the Target Molecule - A frequent mechanism of resistance involves natural variances or acquired changes in the target sites of antimicrobials that inhibit drug binding. Target site modifications frequently result from a bacterial gene's chromosomal spontaneous mutation. Minor changes to the target molecule can have a significant impact on antibiotic binding since antibiotic interactions with targets are typically extremely selective.

    - A change in the ribosome's 30S or 50S subunits causes the body to become resistant to medications that interfere with protein synthesis, such as macrolides, tetracycline, chloramphenicol, and AGs. To inhibit protein synthesis, AGs bind to the 30S ribosomal subunit, whereas chloramphenicol,

macrolides, lincosamides, and streptogramin B bind to the 50S ribosomal subunit[172].

- PBP Alteration - The synthesis of β-lactamases is a preferred mechanism for the development of resistance to Gram-negative bacteria, whereas the modification of the PBP is a preferred strategy for resistance to Gram-positive bacteria. Penicillin-binding protein mutations cause a decreased affinity for β-lactam drugs. This process explains why *Streptococcus pneumoniae* and *Enterococcus faecalis* are resistant to penicillin and ampicillin, respectively. The insertion of a mobile genetic element called "*staphylococcal* cassette chromosome mec" into the chromosome of *Staphylococcus aureus* that contains the resistance gene mec A is also linked to *Staphylococcus aureus*' resistance to methicillin and oxacillin.[173]. mec A gene produces the novel penicillin-binding protein PBP2a, which is necessary to alter the native staphylococcal PBP. PBP2a has strong β-lactam antibiotic resistance. All -lactam antibiotics, streptomycin, tetracycline, and in certain situations, erythromycin can be used to treat S. aureus strains that are resistant to methicillin[174].

- Modified peptidoglycan precursors - Glycopeptides, such as vancomycin or teicoplanin, can impede the formation of cell walls in Gram-positive bacteria by binding to D-alanyl-D-alanine residues. As a result of the conversion of D-alanyl-alanine to D-alanyl-lactate, glycopeptides become resistant to them since they are unable to cross link with them.[175]. The strains of E. faecalis and Enterococcus faecalis are highly resistant to teicoplanin and vancomycin (Van

A-type resistance). Van B and Van C type resistance exhibits vancomycin resistance but is teicoplanin susceptible[176].

- Quinolones bind to the DNA gyraseA subunit, which causes mutated DNA gyrase and topoisomerase IV to cause FQ resistance. DNA gyrase and topoisomerase IV, which are both encoded by the genes gyr A and gyr B and par C and par E, are modified as part of the resistance process[177]. FQ cannot bind as a result of replication failure caused by mutations in the genes gyr A and par C.
- Tetracycline resistance-imparting ribosomal protection mechanisms.
- RNA polymerase mutations that provide rifampicin resistance.

2.14 The Application of Herbal Combinations as Antimicrobial Agents

Because they can provide prospective molecules and combinations of bioactive substances that can be utilized, herbal mixtures have the potential to be therapeutically advantageous in the management of human STIs[178].Infectious infections cause close to 50,000 deaths per day worldwide. Microorganisms that can thrive in warm, wet, dark areas of the human body, such as the mouth and anus, are what cause infectious diseases. Such infections are still a serious global health issue[179]. Sexually transmitted infections (STIs) are the most deadly infectious disorders, and they are brought on by aberrant commensal microbial overgrowth[180]. Infectious disorders brought on by microorganisms are becoming more prevalent, especially in the majority of developing nations. The health care industry has paid close attention to the

hunt for natural antimicrobial agents due to their perceived efficacy, safety, and lack of adverse effects[181].

This is due to the fact that the historically utilized medicinal herbs have demonstrated pharmacological activity[182]. Plant extracts and their active components are used extensively in therapeutic medicine[183]. Based on their antibacterial properties, medicinal plants have been the subject of a number of reports. In recent years, many formulations of the chemically discovered chemicals and physiologically active molecules from plant extracts have been suggested for commercial usage[184]. This may be due to the way that natural products interact with infections to prevent or minimize damage to other crucial molecules or the host's function[185]. There is a need to create and conduct scientific research on new antibacterial agents with unique modes of action as the prevalence of infectious diseases rises[186].

Despite the fact that pharmaceutical corporations have created a variety of antimicrobial medications, the rise of microorganism resistance to these drugs remains a serious issue. Antimicrobial resistance affects the supply of antibiotics used to treat and enhance human health[187]. Utilizing antibiotic resistance inhibitors derived from plants is one way to decrease antibiotic resistance[188]. It has been observed that antimicrobials derived from herbal extracts have the potential to be effective treatments for infectious disorders[189].Many variables, such as inadequate or improper therapeutic therapy or a high prevalence of disease coupled with an inability to start prevention initiatives, can lead to the development of resistance to the antimicrobial drugs[190]. The misuse of antibiotics can potentially contribute to antibiotic resistance.

It is well known that herbs create a variety of chemicals to defend themselves from infections. Therefore, it is anticipated that herbal extracts exhibiting target sites in addition to those used by antibiotics will be effective against infections that have developed resistance to those drugs[191]. Herbs having chemicals that could be used to make novel antimicrobial medications should either prevent or stop the growth of bacteria and have a low or nonexistent toxicity toward host cells[192]. The herbal mixtures investigated in this study are those perceived to have antimicrobial properties based on the studied and local uses of the herbs, in Nigeria. Based on research and traditional applications of the plants in Nigeria, the herbal mixes under investigation in this study are those that are thought to have antibacterial characteristics. Therefore, the objective of this experiment was to find those herbal extracts that can prevent the growth of particular microbes. The outcomes of this screening could be a useful tool in the search for an effective method of treating sexually transmitted illnesses.

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Chapter Three

Methodology

3.1. Purchase of Herbal Mixture

Six different herbal mixtures (three NAFDAC registered ones and three unregistered ones) acclaimed to be used for STI treatment namely, Bakaida Flusher, Weakness Private Parts (WPP) mixture, Prebens General diseases herbal tonic, Legend STD Herbal Mixture, Kambest STD Infection Flusher and Med Bunch STD Eradication Herbal Mixture were purchased from different Health and Wellness stores in Ibadan, Oyo state. The herbal medicines were labeled samples A to sample F (Table 1) and stored at specified temperature on the label until use.

3.2. Source of Test Isolate

Pure stock cultures of four test organisms were collected having confirmed with biochemical tests and obtained through High Vaginal Swab (HVS) from the microbiological laboratory of University College Hospital (UCH), Ibadan Nigeria. The organism includes *Staphylococcus aureus*, *Candida albican*, *Klebsiella pneumoniae* and *Escherichia coli*. The Mueller Hinton agar slope were streaked with a sterile inoculation loop to freshen all microbial stock cultures, which were then kept at 4ºC. The organisms were then subcultured using the streak plate method in freshly prepared petri plates. Each of the strains were subjected to pathogenicity test to confirm that they are pathogenic strains of the microorganisms.

3.3. Preparation of Herbal Mixture

To avoid contamination, the herbal preparations' sealed bottle was cleaned with 70% ethanol before being opened. The sterile mixture obtained were preserved in sterile universal bottles and frozen at 4°C. Every assay were carried out in a sterile setting. Each of the concentrate were then diluted to various concentration starting from 100mg/ml to 66.7mg/ml, 50mg/ml and 40mg/ml. Afterwards, they were tested for their antimicrobial potency on the following bacterial isolates: *Staphylococcus aureus*, *Candida albican*, *Klebsiella pneumoniae* and *Escherichia coli.* In the fifth well, distilled water served as the control.

3.4. Ethical Consideration

Research authorization was obtained from University College Hospital (UCH), Ibadan Nigeria.

3.5. Preparation of Media

MacConkey and Muller Hinton agar were prepared according to the manufacturer's recommendations, and sterilized at 121°C for 15 minutes at a pressure of 151 b/sq inch. The medium was allowed to cool to about 47-50°C before pouring into sterile petridishes and left to solidify[1]. It was stored in the refrigerator for subsequent uses.

3.6. Antimicrobial Activity Evaluation of the Herbs

Each herbal mixture extract's antimicrobial activity was evaluated using the agar well diffusion method. Pure isolate cultures were emulsified in peptone water, and the suspensions were compared to a McFarland standard of 0.5. After coating the Nutrient agar plate with

0.1ml of the standardized suspension of the test organisms to evenly cover the surface, the plate was allowed to dry for 15 minutes. A sterile 6mm cork borer was used to aseptically create five wells on the nutritional agar plate, with the fifth well serving as a control. Using a sterile automatic micropipette, 50µl of each local herbal mixture in concentrations ranging from 100 mg/ml to 66.7 mg/ml, 50 mg/ml, and 40 mg/ml were applied differently to the wells of various plates. Distilled water was put to the fifth well to serve as the negative control.

The petri plates were labeled, incubated at 37°C for 24 hours, and then left to stand for 30 minutes at room temperature to allow the appropriate diffusion of the herbal combination. The test bacteria's sensitivity to each extract was indicated by distinct zones of inhibition surrounding the wells. Using a transparent 30mm ruler, the diameters of the clear zones surrounding each well were measured[2]. To assure accuracy and reproducibility of results, the tests were run twice. The minimum inhibitory concentration needed to totally suppress a test organism for up to 48 hours of incubation, also known as the lowest concentration that can significantly reduce inoculum viability by more than 90% was recorded[3].

3.7. Antimicrobial Susceptibility Test of Bacteria with Conventional Antibiotics

The isolates were tested for antimicrobial disc susceptibility using the Kirby-Bauer technique on newly made Mueller-Hinton agar[4]. The suspension's turbidity was compared to the 0.5 McFarland standard after pure isolates were emulsified in a small amount of peptone water. On the agar plate, 0.1ml of the suspension was applied. Disk diffusion method was used to test for antibiotic susceptibility using antibiotic discs (Gram positive and Gram negative)[6]. Using sterile forceps, commercially available antibiotic discs were aseptically inserted on

each of the inoculated petri dishes. All susceptibility testing carried out included the indicator organisms *Staphylococcus aureus*, *Escherichia coli*, and *Klebsiella pneumoniae*. The sensitivity pattern was read after the plates had been incubated for 24 hours at 37°C.

The zones of inhibition shown on the agar plates that cause antibiotic susceptibility patterns are referred to as Toas antibiogram. The antibiotic discs used were Perfloxacin [10 µg], amoxicillin [30 µg], Rocephin [25 µg], Septrin [30 µg], Chloramphenicol [30 µg], Ciprofloxacin [10 µg], Sparfloxacin [10 µg], Augmentin [10 µg], Gentamycin [10 µg], Zinnacef [20 µg], Erythromycin [10 µg], Ampiclox [30 µg] and Tarivid [10 µg] according to CLSI guidelines[5]. On nutrient agar, the agar well diffusion method was employed to test the fungi's sensitivity. Each well received 50µl of antifungal dilution in various strengths. Ketoconazole and fluconazole were the antifungal medications used. The zones of inhibition were measured and recorded after the plates were incubated for yeast for five days at 25ºC[6].

3.8. Method of Data Analysis

Analytical Statistics Utilizing SPSS (Statistical Package for the Social Sciences) Version 23 software, descriptive statistical analysis was performed. To examine whether category variables were connected, the Chi-square test was used. A P value less than 0.05 was taken into consideration to be significant for all statistical tests.

3.9. Determination of the Microbial Quality of Herbal Mixture

To avoid contamination, the sealed bottles of herbal remedies were cleaned with 70% ethanol before being opened. The herbal mixtures were mixedd by gently inverting the bottles several times. The conventional plate count method was used to assess the microbiological quality[7].

There were counts of *Salmonellae*, total fungi, and total bacteria. For the purpose of counting the different types of organisms, each liquid solution was diluted 10 times using serial dilution before being plated out on a new medium plate. Eosine Methylene Blue agar was used for the fecal indicator E. Coli count, Xylose Lysine Desoxycholate agar was used for the Salmonellae count, and nutrient agar was used to count the total bacteria and Sabouraud Dextrose agar was used for total fungal count.

The local herbal remedy was then pipetted into 9ml of sterile peptone water. The last three dilution (10^3) was innoculated on already prepared agar plates. The growth of bacteria was monitored on the agar plates after an 18–24 hour incubation period at 37°C. For 3 to 5 days, the Sabauraud dextrose agar was incubated at room temperature to encourage fungal development. To stop the development of bacteria, streptomycin was added in the fungal plates. Both the standard bacterial and fungal counts were taken. Colony forming units (cfu) were used to report colony numbers[8]. Based on colony appearance, Gram staining, and a series of biochemical assays, bacteria isolates were identified[9].

3.10. General Biochemical and Identification of Isolates

3.10.1. Gram Staining

Differential staining is used to categorize microorganisms as gram positive or negative. On a spotless, grease-free glass slide, thin smears of brand-new, pure bacteria cultures were applied. The slides were heated by passing them over a Bunsen burner flame after being allowed to air dry. The slides were placed on a rack over a sink, saturated with crystal violet for 30 seconds, rinsed with clean running water, saturated with iodine for 60 seconds, rinsed with water, decolored with 95% alcohol for 30 seconds, counter stained with safranin for 30

seconds, rinsed with clean running water, allowed to air dry, and then examined with an oil immersion lens. While gram negative bacteria stained red, gram positive bacteria did not.

3.10.2. Microscopy for Fungal Culture

It looks for spores and conidia, whether they are septate or aseptate. A small amount of the fungal culture was taken off the edge and teased into the lactophenol cotton blue using a sterile needle after a drop of lactophenol blue had been put to a clean, grease-free glass slide. Covering the smear with a cover slip allowed the microscope's 10X and 40X objectives to be used to see it.

3.10.3. Biochemical test

- **Citrate Utilization Test**

This test is used to determine which organisms use citrate as their only source of carbon. A sterile wire loop zigzag infected a citrate agar slant with the test organism, and it was then incubated at 37°C for 48 hours. Afterward, positive and negative controls were noted[10].

- **Indole Test**

This test measures an organism's capacity to convert the amino acid tryptophan to pyruvate and indole. The test organism is cultured aerobically for 24 hours at 37 °C after being innoculatedd into 5 ml of sterile peptone water. By gently shaking the broth culture while adding 0.5 ml of Kovac's reagent, the generation of indole was assessed. Results were then documented.

- **Motility Test**

This test determines whether or not an organism is motile. The test was conducted by sticking a sterile stabbing needle into the butt of the test organism and injecting it into a Sulphide Indole Motility (SIM) solution. After that, both positive and negative controls were noted.

- **Catalase Test**

This test distinguishes between Staphylococci that produce catalase and those that do not, like streptococci. H_2O_2 is broken down by catalase into hydrogen and water. This test was conducted by emulsifying the test organism in a drop of distilled water on a glass slide devoid of grease, then adding 3% hydrogen peroxide. The presence of gas bubbles indicates a poor outcome.

- **Sugar Fermentation**

It was studied whether using sugar (glucose, sucrose, and lactose) as the only source of carbon might produce gas, acid, or both at once. The ability of the organism to utilise sugar as a source of carbon is shown by the formation of acid or gas. A color-changing indicator (Bromocresol, 0.3 ml) was also added to the peptone water after 1 gram of peptone had been dissolved in 220 ml of de-ionized water. The peptone water was divided equally among the three sterile conical flasks. The available sugar (2.2 g) was separately dissolved in the peptone water, and 10 ml of the resulting mixture was dispensed into test tubes containing an inverted, clean Durham tube. The test tubes were then sterilized in an autoclave, and the corresponding isolates were inoculated aseptically into the sugar solution and incubated for 48 hours at 37 °C. Results were then documented.

- **Oxidase test**

This test identifies the source of the cytochrome oxidase enzyme and is employed to separate Pseudomonas, which is oxidase positive, from all other enteric bacteria. The procedure involved smearing the test organism on the filter paper after moistening it with a few drops of the oxidase reagent (tetra-methyl-p-phenylene diamine dihydrochloride). An oxidase test is positive if the color is deep purple; otherwise, the test is negative.

3.11. Phytochemical Screening of the Herbal Mixture

To determine the phytochemicals in the herbal blend, phytochemical screening was done. This was carried out at The Polytechnic of Ibadan's Central Research Laboratory. Standard techniques were used to conduct phytochemical screening on the herbal mixture to look for secondary metabolites[11]. Saponins, tannins, alkaloids, steroids, flavonoids, terpenoids, chalcones, phenol, glycosides, and phytochemicals were among the substances that were examined for. Standard techniques were used to do both qualitative and quantitative screening of the phytochemicals in the extracts to determine the components[12].

3.11.1. Qualitative Determinations

- **Test for Taninns**: In a test, 1ml of the extract was boiled in 20ml of water for 5 minutes before being filtered. When a few drops of 0.1% ferric chloride were added, a green or blue-black coloring was seen, indicating that tannin was present[13].
- **Test for Phlobatannins**: Phlobatannins were confirmed by the formation of a crimson precipitate after boiling 2ml of each herbal mixture's extract in 1% aqueous hydrochloric acid.

- **Test for Saponin**: A little amount of the extract—about 5ml—was cooked in 20ml of distilled water before being filtered. To create a stable, long-lasting froth, 10ml of the filterate was combined with 5ml of distilled water and forcefully shaken. Three drops of extra-virgin olive oil were added to the foam, which was quickly shaken before being checked for the creation of an emulsion, which indicates the presence of saponin.
- **Test of Flavonoids**: To 5ml of each extract, 3ml of a 1% aluminum chloride solution was added. The presence of flavonoids was indicated by the yellow coloration that was seen. To the aforementioned mixture, 5ml of a diluted ammonia solution was added before adding concentrated H_2SO_4. On standing, a yellow color vanished. The yellow tint that vanished when the person stood up suggests that flavonoids were detected in the sample.
- **Test for Steroids**: Each sample's 2ml extract first received 2ml of acetic anhydride, which was then carefully followed by 2ml of H_2SO_4. When steroids are present, the color shift from violet to blue or green indicates their existence.
- **Test for Terpenoids (Salkowski test)**: Each sample's 2ml extract first received 2ml of acetic anhydride, which was then carefully followed by 2ml of H_2SO_4. When steroids are present, the color shift from violet to blue or green indicates their existence.
- **Test for Cardiac Glycosides and Cardenolides (Keller – Killani test)**: Two milliliters of glacial acetic acid containing one drop of ferric chloride solution were added to five milliliters of each extract. With 1ml of concentrated sulfuric acid, this was downplayed. A brown ring at the interface denotes cardenolides' deoxysugar

properties, confirming the presence of cardenolides. The presence of glycoside is confirmed when a violet-green ring appears in the acetic acid layer behind the brown ring[14].

- **Alkaloids:** On a steam bath, 1ml of the extract was mixed with 5ml of 1% aqueous HCL before being filtered while still hot. The filtrate was treated with a few drops of Mayer's reagent (potassium mercuric iodide solution), Wagner's reagent (iodine in potassium iodide solution), or Dragendorff's reagent (potassium bismuth iodide solution) after distilled water was added to the residual. A positive test for alkaloids is the production of a cream color using Mayer's reagent and a reddish-brown precipitate using Wagner's and Dragendorff's reagent.
- **Anthraquinone:** 5ml of extract was combined with 10ml of benzene, filtered, and the filtrate was then given 5ml of a 10% NH_3 solution. Anthraquinones were detected when the mixture was shaken and showed signs of pink, red, or violet hue in the ammoniac (lower) phase.
- **Chalcones:** 5ml of each plant part's extract was combined with 2ml of an ammonia solution. Chalcone presence was established by the formation of a reddish tint.
- **Phenol:** A 30 ml test tube was filled with 5 ml of the extract, followed by 10 ml of distilled water. In addition, 30 minutes were given for 2 ml of ammonium hydroxide solution and 5 ml of concentrated amyl alcohol to react. The appearance of a blue green tint was interpreted as a sign that phenol was present[15].

3.11.2. Quantitative Determinations

- **Tannin**

A 50ml beaker was filled with 0.20g of the sample. 20 ml of 50% methanol was added, covered with parafilm, and incubated for one hour at 77–80°C in a water bath. It was vigorously shaken to achieve even mixing. In a 100 ml volumetric flask, the extract was quantitatively filtered using two layers of Whatman No. 41 filter paper, 20 ml of water, 2.5 ml of the folin-Denis reagent, and 10 ml of 17% Na_2CO_3 were then added and thoroughly mixed. The mixture was prepared as directed, with water thoroughly mixed in. After 20 minutes, a bluish-green tint will begin to appear. Similar to the 1ml sample above, working standard solutions of tannin in the 0–10ppm range were handled. After color development, a Spectronic 21D spectrophotometer was used to measure the absorbance of samples and standard solutions of tannic acid at a wavelength of 760 nm. Utilizing the formula, % Tannin was determined[16].

$$\%\text{TANNIN} = \frac{\text{absorbance of sample X average gradient factor X Dilution factor}}{\text{Wt. of Sample X 10,000}}$$

- **Alkaloids Procedure**

This is a titrimetric and distillation process. 20ml of 80% pure alcohol was added to 2g of finely ground sample in a 100ml beaker to create a smooth paste. The liquid was then transferred to a 250 ml flask, where 100 ml more alcohol and 1 g of magnesium oxide were added. The mixture was digested for 1.5 hours in a boiling water bath with periodic shaking under a reflux air condenser. While still hot, the liquid was filtered through a tiny buchner funnel. The residue was put back into the flask and digested once more for 30 minutes with

50ml alcohol. After the alcohol had evaporated, boiling water was added to make up for the lost alcohol. Three drops of 10% HCL were added after the entire amount of alcohol was eliminated. The entire solution was then placed into a 250 ml volumetric flask, to which 5 ml of potassium ferrocyanide solution and 5 ml of zinc acetate solution were added and thoroughly mixed to create a homogeneous solution. The flask was set aside for a short while, filtered through dry filter paper, and 10 ml of the filtrate was put into a separatory funnel where the alkaloids were aggressively extracted by shaking with five successive amounts of chloroform. The residue obtained was dissolved in 10ml hot distilled water and transferred into a kjeldahl tube with the addition of 0.20g sucrose and 10ml Conc.H_2SO_4 and 0.02g selenium for digestion to a colorless solution to determine %N by Kjeldahl distillation method. %Nitrogen got is converted to % total alkaloid by multiplying by a factor of 3.26 i.e % Total alkaloid = %N X 3.26

% Alkaloids = %N X 3.26

Allen's commercial organic Analysis[17]

- **Flavonoids Determination**

A glass rod was used to swirl 80ml of 95% ethanol into 0.50g of finely ground sample in a 100ml beaker to avoid lumping. The mixture was put into a 100ml volumetric flask after being filtered using a Whatman No. 1 filter and adjusted with ethanol. In order to create a magenta red colouring, 0.5g of magnesium turnings were added after 1ml of the extract and four drops of concentrated HCL through a dropping pipette were pipetted into a 50ml volumetric flask. From a 100ppm stock solution, standard flavonoid solutions ranging from 0 to 5 ppm were made, and they were similarly processed with HCL and magnesium turnings

as samples. A digital Jenway V6300 Spectrophotometer was used to measure the absorbance of the magenta red coloring of the sample and standard solutions at a wavelength of 520 nm. Using the formula, the percentage of flavonoid is determined.

$$\frac{\text{Absorbance of sample X average gradient factor X dilution factor}}{\text{Wt. sample X 10,000}}$$

Allen's commercial Organic Analysis[18]

- **Saponin Procedure:**

• Saponin Analysis was performed using Brunner's (1984) Spectrophotometric technique. 100ml of isobutyl alcohol was added to a 250ml beaker after 1g of the finely powdered sample was weighed in. To ensure even mixing, the mixture was shaken on a UDY shaker for five hours. A Whatman No. 1 filter paper was used to filter the mixture into a 100 ml beaker, and 20 ml of a 40% saturated solution of magnesium carbonate was then added. To get a clear, colorless solution, the mixture made with saturated MgCO3 was once more filtered through a Whatman No1 filter paper. A 50 ml volumetric flask was filled to the mark with distilled water after adding 2 ml of a 5% $FeCl_3$ solution and 1 ml of the colorless solution. For the blood red color to fully emerge, it was left to stand for 30 minutes. From saponin stock solution, standard Saponin solutions ranging from 0 to 10 ppm were created. Similar to how the 1ml sample above was handled, the standard solutions also received 2ml of the 5% $FeCl_3$ solution. After color development, a Jenway V6300 Spectrophotometer was used to measure the sample's absorbance as well as that of reference saponin solutions at a wavelength of 380 nm.

%Saponin = Absorbance of sample X gradient factor X dilution factor

Wt.of sample X10000

Direct Spectrophotometer determination of Saponin. Anal. Chem[19]

- **Determination of Glycoside**

Pipette 10 ml of extract into a 250 ml conical flask.On a vortex mixer, 50ml of chloroform was added and shaken for an hour. After filtering the liquid into a 100 ml conical flask, 2 ml of 2% sodium nitroprusside and 10 ml of pyridine were added, and the mixture was vigorously agitated for 10 minutes. Later, 3ml of 20% NaOH was added to create a brownish yellow hue. Glycoside standards with concentrations ranging from 0 to 5 mg/ml were created from a stock 100 mg/ml standard. The standards from 0 to 5 mg/ml underwent the same processing as the sample above. At a wavelength of 510 nm, the absorbances of the sample and the standards were measured using a Spectronic 21D Digital Spectrophotometer. The formula used to compute percent glucose was

Absorbance of sample X gradient factor X dilution factor

Wt. of sample X10000

- **Determination of Steroids**

In a 100ml beaker, 0.50g of sample extract was measured out. After 30 minutes of shaking on a shaker, 20 ml of a chloroform-to-methanol (2:1) mixture was added to dissolve the extract. The entire mixture was then filtered into a second dry, clean 100ml conical flask/beaker using Whatman No. 1 filter paper. The resulting residue was repeatedly cleaned

of steroids using a chloroform-methanol combination.5ml of alcoholic KOH was added to 1ml of the filtrate, which was pipetted into a 30ml test tube, and the mixture was vigorously agitated to create a homogeneous mixture. After that, the mixture spent 90 minutes in a water bath with a temperature of 37°C–40°C.It was brought to room temperature before 10 ml of petroleum ether and 5 ml of distilled water were added. On the water bath, this was evaporated until dry. The residue in the dry bottle received 6ml of Liebermann Burchard reagent, and an absorbance measurement at a wavelength of 620nm was made using a Spectronic 21D digital spectrophotometer. Standard Steroids were created from a 100 mg/ml stock steroid solution and handled in a manner comparable to the aforementioned sample. % Steroid was determined using the following formula:

$$\frac{\text{Absorbance of Sample X Gradient X Dilution Factor}}{\text{Wt of sample X 10000}}$$

Methods of Analysis of Analytical Methods Committee of Royal Society of Chemistry[20].

- **Determination of Cardenolides**

After properly measuring 0.50g of extract into a 100ml beaker, 50ml of chloroform was added to dissolve the extract. After the extract was completely dissolved in chloroform, 0.20 of sodium bicarbonate powder ($NaHCO_3$) was added to eliminate any free acids. The two layers of the mixture were then allowed to separate by being put to a 250ml Separatory funnel and vigorously shaken.It took 5 drops of acetic anhydride to clarify the mixture and remove any hazy suspensions. This was placed in a 100ml volumetric flask, filtered using a Whatman No. 1 filter paper, and prepared with chloroform to the appropriate mark. To

establish the gradient factor, standard cardenolides solutions of concentrations 0–10 mg/ml were made from a 100 mg/ml stock cardenolide solution.

% Cardenolide was calculated using the formula:

$$\frac{\text{Absorbance of sample X gradient factor X dilution factor}}{\text{Wt. of sample X 10000}}$$

Methods of Analysis of Analytical Methods Committee of Royal Society of Chemistry[21]

- **Determination of Phlobatannin**

In a 50ml beaker, 0.50g of sample extract was measured out.20ml of 50% Methanol was added, covered with parafilm, and incubated for one hour at 77-800C in a water bath. The mixture was thoroughly mixed and then filtered through a Whatman No. 1 Filter paper into a 50 ml volumetric flask using aqueous methanol as a rinse and distilled water to make up to the correct volume. A 50 ml volumetric flask was filled with 1 ml of the sample extract, 20 ml of water, 2.5 ml of the Folin-Dennis reagent, and 10 ml of 17% sodium carbonate. For 20 minutes, the mixture was fully homogenized.Phlobatannin stock solution containing 100 mg/ml was used to establish a standard concentration of 0 to 5 mg/ml, which was then handled similarly to the sample above. A Spectronic 21D spectrophotometer was used to measure the absorbances of the sample and standard solutions at 550 nm. The formula for calculating %Phlobatannin was used:

$$\frac{\text{Absorbance of sample X gradient factor X dilution factor}}{\text{Wt. of sample X 10,000}}$$

Methods of Analysis of Analytical Methods Committee of Royal Society of Chemistry[22]

- **Determination of Terpene**

A 50ml conical flask was filled with 0.50g of sample, 20ml of a 2:1 combination of chloroform and methanol, and it was thoroughly shaken before being let to stand for 15 minutes.Later, the combination underwent another 15 minutes of centrifugation.The resulting supernatant was discarded, and the precipitate was again washed with a 20ml solution of chloroform and methanol before centrifugation.

40ml of a 10% sodium deodocyl sulphate solution was used to dissolve the precipitate that resulted.At 30-second intervals, 1 ml of the 0.01 M ferric chloride solution was added to the mixture above; it was then shaken well and left to stand for 30 minutes.A 100 mg/l stock terpenes solution from Sigma-Aldrich chemicals, USA, was used to create standard terpenes with a concentration range of 0 to 5 mg/ml. On a digital spectrophotometer with a 510nm wavelength, the absorbances of the sample and standard Terpene concentrations were measured.The formula is used to determine the percentage of terpene:

$$\frac{\text{Absorbance of sample X gradient factor X dilution factor}}{\text{Wt. of sample X 10,000}}$$

- **Determination of Phenol**

To prevent lumping, 0.20g of the sample was weighed into a 50 ml beaker, 20 ml of acetone was added, and the mixture was well homogenized for 1 hour. After thoroughly mixing the mixture, it was filtered through Whatman No. 1 filter paper into a 100ml volumetric flask

and rinsed with acetone. A 50 ml volumetric flask was filled with 1 ml of sample extract, 20 ml of water, 3 ml of phosphomolybdic acid, 5 ml of 23% Na_2CO_3, and carefully mixed. The flask was then filled to the mark with distilled water and left to stand for 10 minutes to acquire a bluish-green color. Standard Phenol was created from a 100 mg/l stock solution from Sigma-Aldrich Chemicals, USA, with a concentration range of 0–10 mg/ml. The absorbances of the sample and standard phenol concentrations were measured using a digital spectrophotometer at 510 nm wavelength.The formula is used to determine the proportion of phenol:

$$\frac{\text{Absorbance of sample X gradient factor X dilution factor}}{\text{Wt. of sample X 10,000}}$$

- **Determination of Chalcones**

In a 100ml beaker, 0.50g of sample extract was measured out. After 30 minutes of shaking on a shaker, 20 ml of a chloroform-to-methanol (2:1) mixture was added to dissolve the extract. The entire mixture was then filtered into a second dry, clean 100ml conical flask/beaker using Whatman No. 1 filter paper.

The resulting residue was repeatedly cleaned of Chalcones using a chloroform-methanol combination. 5ml of alcoholic KOH was added to 1ml of the filtrate, which was pipetted into a 30ml test tube, and the mixture was vigorously agitated to create a homogeneous mixture. After that, the mixture spent 90 minutes in a water bath with a temperature of 37°C–40°C.It was brought to room temperature before 10 ml of petroleum ether and 5 ml of distilled water were added. On the water bath, this was evaporated until dry. The residue in the dry bottle

received 6ml of Liebermann Burchard reagent, and an absorbance measurement at a wavelength of 620nm was made using a Spectronic 21D digital spectrophotometer. From a stock steroid solution containing 100 mg/ml, standard chalcones with a concentration of 0 to 4 mg/ml were created and handled identically to the sample above. The formula used to compute % Chalcones is:

$$\frac{\text{Absorbance of Sample X Gradient X Dilution Factor}}{\text{Wt of sample X 10000}}$$

Endnotes

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Chapter Four

Results and Discussion of Findings

4.1 Results

In the present study, a total of 4 clinical specimens were obtained through High Vaginal Swab (HVS) technique at university college Hospital (UCH) Ibadan. These are *Candida albicans, Staphylococcus aureus, Escherichia coli* and *Klebsiella pneumoniae* and a total of six herbal products were tested for their activity against these clinical isolates.

Table 4.1 shows the information of the herbal mixture used with or without NAFDAC registration number. Table 4.2 shows the zone of inhibition exhibited by the registered herbal mixture (mm) against the clinical isolates causing STD. It is observed that the herbal mixtures at different concentrations did not inhibit the indicator organisms.

Table 4.3 shows the zone of inhibition exhibited by unregistered herbal mixture (mm) against clinical isolates. It was observed that all brands of the unregistered herbal mixtures did not inhibit *C. albicans, S. aureus, K. pneumonia* at the different concentrations used, but the unregistered herbal mixture sample A inhibited *E. coli* with inhibition zone of 19mm, 17.5mm and 14.5mm at concentration of herbal mixtures of 100mg/ml, 66mg/ml, 50mg/ml respectively but did not inhibit *E.coli* at herbal mixture concentration of 40mg/ml.

Table 4.4 shows the susceptibility pattern of clinical isolates to herbal mixtures. It is observed that the indicator organisms were not susceptible to the herbal mixture A which inhibited *E,coli.*

Table 4.5 shows the antibiotic sensitivity of standard clinical isolates to conventional drugs. Sparfloxacin and Gentamycin have the highest susceptibility while the least susceptibility was found in Chloramphenicol and Ampiclox, Pefloxacin, Gentamicin, Ampiclox, Augmentin, Chloramphenicol, Rocephin, Sparfloxacin and Ciprofloxacin inhibited *E.coli* at various concentrations while Ofloxacin, Erythromycin, Chloramphenicol, Ciprofloxacin inhibited *K. pneumoniae* at different concentrations. *S.aureus* was resistant to all antibiotic used.

Table 4.6 showed antifungal sensitivity of the standard clinical Isolates to antifungal agents. Ketoconazole and fluconazole inhibited the organism *C.albicans* with a zone of inhibition of 20 and 25 mm respectively. Table 4.7 and 4.8 shows the microbial counts of the registered and unregistered liquid herbal mixtures (Cfu/ml) respectively. The organism recovered in all the herbal mixtures included *Bacillus spp, S. aureus*, *P. aeruginosa*, *E.coli* and *Salmonella*. Herbal mixture E had the highest level of microbial contamination with a total of 12 organisms including *Bacillus spp, S. aureus*, *E.coli* and *Salmonella*. Herbal mixture A and D had the lowest level of contamination with a total of 2 organisms including *Bacillus spp* and *S. aureus* in herbal mixture D and *S. aureus in herbal mixture* A.

Table 4.9 shows the qualitative phytochemical analysis of the herbal mixtures and all the herbal mixtures contain all the phytochemical Tannin, Saponin, Alkaloid, Flavonoid, Steroid. All the herbal mixture did not contain the phytochemical Phenol. Herbal mixture A, E and F had Glycosides and Chalcones as an addition to their phytochemical compound while herbal mixture B has the phytochemical Chalcones. Herbal mixture C and D does not contain the phytochemical Glycosides and Chalcones. Herbal mixture A and C were the only herbal mixture that contained the phytochemical Terpenoid.

Table 5.0 shows the quantitative phytochemical analysis of the herbal mixtures and they all contain the phytochemical Tannin, Saponin, Alkaloid, Flavonoid, Glycosides, Chalcones, Terpenoid and Steroid in various concentrations

The herbalists who sold the medicines for this investigation advertised them as being effective for treating several STDs. Plastic bottles were used to package and sell every herbal product. The samples yielded a total of 35 bacterial isolates, including 13 Gram-positive and 22 Gram-negative species. The contaminants that were isolated the most frequently in the testing sample were *Staphyloccocus aureus* s (36.1%), followed by *Bacillus species* (30.5%). Other contaminants included *Escherichia coli* (19.4%), *Pseudomonas aeruginosa* (8.3%) and *Salmonella species* (5.5%). Every herbal mixture has at least one contaminant; 17% of the herbal mixtures had bacteria, 33% of the mixtures contained two, and 33% contained four. One sample had five pollutants that were retrieved.

In this investigation, just 17% of the samples evaluated had satisfactory microbiological quality. The degree of pathogenic Gram negative organism contamination of the items is a cause for worry. It was challenging to determine why some herbal samples, which had registration were superior to herbal mixes without registration because the registered samples were just as contaminated as the unregistered samples. Different types of zones of inhibition against the investigated antibiotics were formed by the isolates.

The agar diffusion method was used to assess the antibacterial activity of these herbal combination discs against test Gram-positive and negative bacteria after an antimicrobial susceptibility test revealed that some plant extracts display antimicrobial activity over time. *Escherichia coli*, one of the examined bacteria, was the only one for which one herbal blend was discovered to exhibit antibacterial activity.

Table 4.1: Details of the Herbal Medicinal Products

Sample	Herbal Medicinal Product	Nafdac Registeration No
A	Legend Std Mixture, Ghana	NA
B	Prebens General Disease Herbal Tonic	NA
C	Med-Bunch STD Eradication Flusher	A7-2124L
D	Bakaida Flusher	A7-4328L
E	Kambest STD Infection Flusher	A7-4829L
F	Weakness Private Parts Mixture	NA

(NA: not available).

Table 4.2: Zone of Inhibition Exhibited by Registered Herbal Mixture (mm) against Clinical Isolates Causing STI

		Sample Concentration			
Isolates Used	**Brand Codes**	**100mg/ml**	**66.7mg/ml**	**50g/ml**	**40mg/ml**
K. pneumoniae	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R
C. albicans	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R
E. coli	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R
S, aureus	C	R	R	R	R
	D	R	R	R	R
	E	R	R	R	R

R = Resistant, S = sensitive.

Table 4.3: **Zone of Inhibition Exhibited by Unregistered Herbal Mixture (mm) against Clinical Isolates Causing STI**

		SAMPLE CONCENTRATION			
Isolates Used	Brand Names	100mg/ml	66.7mg/ml	50g/ml	40mg/ml
K. pneumoniae	A	R	R	R	R
	B	R	R	R	R
	F	R	R	R	R
C. albicans	A	R	R	R	R
	B	R	R	R	R
	F	R	R	R	R
E. coli	A	19	17.5	14.5	R
	B	R	R	R	R
	F	R	R	R	R
S, aureus	A	R	R	R	R
	B	R	R	R	R
	F	R	R	R	R

R = Resistant, S = sensitive.

Table 4.4: Summary of the Susceptibility Pattern of Clinical Isolates to Herbal Mixtures

Herbal drugs	**A**	**B**	**C**	**D**	**E**	**F**
K. pneumonia	R	R	R	R	R	R
C.albican	R	R	R	R	R	R
E. coli	S	R	R	R	R	R
S, aureus	R	R	R	R	R	R
Total	1	-	-	-	-	-

R = Resistant, S = sensitive, - = No effect

Table 4.5: Antibiotic Sensitivity of Standard Clinical Isolates to Conventional Drugs.

	Concentration (µg)	*E. coli*	*Klebsiella*	*S. aureus*
AM	30µg	-	-	-
S	30µg	-	-	-
PEF	10µg	18	-	-
Z	20µg	-	-	-
SXT	30µg	-	-	-
GN	10µg	20	-	-
OFX	10µg	-	14	-
E	10µg	-	17	-
APX	30µg	8	-	-
AU	10µg	15	-	-
CH	30µg	17	8	-
R	25µg	16	-	-
SP	10µg	20	-	-
CPX	10µg	19	11	-

Key: - = No effect

PEF=Perfloxacin(10µg), AM= amoxicillin (30µg), R= Rocephin (25µg), SXT= Septrin (30µg), CH= Chloramphenicol (30µg), CPX= Ciprofloxacin (10µg), SP= Sparfloxacin (10µg), AU= Augmentin (10µg),GN= Gentamycin (10µg), Z= Zinnacef (20µg),E= Erythromycin (10µg), APX= Ampiclox (30µg) and OFX= Tarivid (10µg).

Table 4.6: Antifungal Sensitivity of Standard Clinical Isolates to Antifungal Drugs.

Concentration (µg)	*C. albicans*
Ketoconazole (10)	20
Fluconazole (10)	25

Table 4.7: Microbial Counts of the Registered Liquid Herbal Mixtures (CFU/ml).

Product Code	C	D	E
Bacillus sp	3	1	3
S.aureus	2	1	7
P. aeruginosa	1	-	-
E.coli	2	-	1
Salmonella	1	-	1
Fungi	-	-	-
Total	9	2	12

Key: - = No effect

Table 4.8: **Microbial Counts of the Unregistered Liquid Herbal Mixtures (CFU/ml)**

Product Code	A	B	F
Bacillus sp	-	1	3
S.aureus	2	-	1
P.aeruginosa	-	-	1
E.coli	-	2	2
Salmonella	-	-	-
Fungi	-	-	-
Total	2	3	7

Key: - = No effect

Table 4.9: **Qualitative Phytochemical Analysis of the Herbal Mixtures**

	Phytochemicals								
Sample I.D	**Tannin**	**Saponin**	**Alkaloid**	**Flavonoid**	**Terpenoid**	**Glycoside**	**Chalcones**	**Phenol**	**Steroid**
A	++	+	+	++	+	+	+	-	+
B	+	+	++	++	-	-	++	-	+
C	+	+++	++	++	+	-	-	-	+
D	+	++	++	++	-	-	-	-	+
E	++	++	++	++	-	+	+	-	+
F	+	+	++	++	-	+	++	-	+

+ represents a positive result and – represents a negative result.

Table 5.0: **Quantitative Phytochemical Analysis of the Herbal Mixtures**

	Phytochemicals								
Sample I.D	**Tannin**	**Saponin**	**Alkaloid**	**Flavonoid**	**Terpenoid**	**Glycoside**	**Chalcones**	**Phenol**	**Steroid**
A	0.145	0.213	0.041	0.207	0.032	0.032	0.082	0.000	0.027
B	0.142	0.143	0.064	0.525	0.205	0.000	0.524	0.000	0.030
C	0.130	0.194	0.051	0.402	0.401	0.000	0.000	0.000	0.021
D	0.123	0.154	0.043	0.374	0.000	0.000	0.000	0.000	0.014
E	0.150	0.143	0.031	0.308	0.000	0.025	0.054	0.000	0.019
F	0.156	0.321	0.014	0.420	0.000	0.019	0.045	0.000	0.015

4.2 Discussion of Findings

According to the WHO research, a significant portion of people in many developing countries have access to and use herbal remedies[1]. These are due in part to things like perceived efficacy, safety, and the lack of adverse effects[2]. The preparations in this study were all stated to be for sexually transmitted illnesses after an analysis of the indications for which they were prepared. The majority of time, herbal medicines contain soil and airborne germs and mold. Total aerobic bacteria should not exceed 10^5cfu/g, yeasts and molds should not exceed 10^3cfu/g, *enterobacteria* and other gram-negative organisms should not exceed 10^3cfu/g, and *Salmonella* and *E. coli* should not be present[3]. The samples had various levels of bacterial contamination. 90% of the samples evaluated in this investigation had microbial burdens that were higher than permitted by the regulatory agencies[4].

In addition to the high microbial loads, it was shown that the herbal samples contained undesirable species or diseases. These are gastrointestinal-related organisms that suggest the possibility of fecal contamination[5]. These contaminants might have been introduced when samples were being prepared and containers were being rinsed in contaminated water. Vegetables and other plant components have been reported to act as reservoirs for a variety of bacteria, including gastrointestinal diseases[6]. Poor production quality and harvesting techniques have also been linked to the occurrence of *Escherichia coli* and *Salmonella spp*[7]. Utilizing improperly cleaned plant components that have previously been in contact with manure are other possible causes. There is also *Pseudomonas aeruginosa*, a soil-born bacterium that can cause infections of the urinary tract, respiratory system,

wounds, and other organs. The presence of *Staphylococcus aureus* in the samples may be due to inappropriate harvesting, drying, storage, or handling practices, which may have an impact on their microbiological quality[8].

The fact that roots and vegetative plant parts that have been in contact with the soil and are included in the preparations are supported by the recovery of quite a few *Bacillus species*, the frequently predominant aerobic spore-forming bacteria naturally occurring micro flora of medicinal plants. The presence of organisms like *S. aureus* indicates that contamination may have happened during collection, post-harvest processing, and manufacturing due to handling by personnel who carry dangerous bacteria or common commensals. Since preparations typically include numerous pieces that come from various harvest sites, the inclusion of multiple pollutants in a single preparation as seen in this study is expected. Additionally, additional contamination and microbial growth may be brought on by storage and transit procedures. Failure to regulate the temperatures of liquid forms and finished herbal products also leads to microbial proliferation. The surroundings and tools used to make the preparations, as well as the containers used to package them, are additional potential sources of contamination. A perfect package shouldn't have any negative effects on the microbiological quality of the desired preparations[9].

The findings of this investigation imply that all of the isolated organisms had microbial counts that were considerable and could potentially serve as an infection source. Therefore, it is necessary for the relevant regulatory bodies to establish some regulations to control the manufacturing, usage, and distribution of herbal medicines. In order to effectively treat diseases, items must be safe, effective, and of high quality. Numerous research carried out in other nations have reported finding high levels of aerobic microorganisms in herbal remedies[10]. The organic chemicals and minerals contained in the plants used to make

herbal medicine gave nutrition to the microbes associated with the raw herbal material and may have helped the germs observed in this study multiply. These bacteria could cause the final product to degrade and have a different composition, which could result in herbal goods of lower quality. It's possible that using such subpar herbal medicines won't have the desired therapeutic effects. The therapeutic potential of a medicinal product may be diminished or destroyed by the presence of numerous microbial contaminants in some herbal samples, and the patient may even develop another ailment as a result. The use of antibiotics is crucial in the fight against bacteria that cause infectious diseases. However, widespread antibiotic abuse in the healthcare and poultry industries has led to the development of antibiotic resistance in many bacterial species[11].

The presence of tannin, saponin, alkaloid, flavonoid, terpenoid, glycoside, chalcones, and steroid in the herbal product may be responsible for the mixture's antibacterial characteristics. The herbal mixture contained a variety of phytochemical elements, including saponins, tannins, alkaloids, terpenoids, glycosides, and flavonoids, according to a qualitative phytochemical examination. These bioactive substances have been found to have bactericidal or fungicidal activities on the studied human pathogens and are naturally present in the majority of extracts. Previous research of a similar nature has demonstrated the antibacterial activity of some of the pharmacologically active components. Chemical and structural identification of these substances is now achievable thanks to improvements in procedures for the separation, purification, and identification of bioactive chemicals[12].

Endnotes

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Chapter Five

Conclusion and Recommendation

5.1 Summary of Findings

In order to treat and/or eradicate diseases brought on by resistant microorganisms, medicinal plants have been used as a potential source for new classes of antimicrobial medicines with novel mechanisms of action. Since ancient times, medicinal plants have been utilized to treat STIs. Some of these plants have shown notable or weaker antibacterial efficacies against the microorganisms linked to STIs.

One herbal mixture was shown to have good antibacterial activity and the potential to be employed as an alternative therapeutic option for the treatment of STIs after testing six randomly selected herbal mixtures against the microorganisms causing STIs. When applied against the test microorganism, practically all herbal mixtures showed a decrease in antibacterial effectiveness. *Escherichia coli* was the only bacterial pathogen against which the herbal blend shown antibacterial efficacy. Only 10% of the six products had antibacterial properties; none, however, have antifungal properties.

All of them had high levels of microbial contamination. It's possible that their production did not follow current good manufacturing practices. The plant extracts contained a variety of phytochemicals, including tannin, saponin, alkaloid, flavonoid, terpenoid, glycoside, chalcones, phenol, and steroids.

5.2 Conclusion

In order to treat and/or cure diseases brought on by resistant microorganisms, medicinal plants have been used as a potential source for new classes of antimicrobial medicines with novel mechanisms of action. Since ancient times, medicinal plants have been utilized to treat STIs, with some of them showing lower or notable antibacterial efficacies against the microorganisms linked to STIs. STIs are one of the most significant health issues in the world, and they are far more prevalent among underprivileged groups.

Only one herbal mixture (A) was found to have good antimicrobial activity and had the potential to be used as an alternative therapeutic option for the treatment of one of the organisms linked to STIs after evaluation of six herbal mixtures marketed as being effective against the pathogens causing STIs. Therefore, a medicinal plant must have the lowest MIC value for each examined microbe in order to be regarded a candidate plant with good antibacterial activity.

5.3 Recommendations

The microbiological quality of medicinal plants is determined by external and intrinsic factors. Extrinsic factors like humidity, harvest technique, post-harvest treatment, packaging, and storage conditions determine the extent and nature of microbial contaminants, while intrinsic factors determine the amount and type of beneficial microbial content for plant survival and growth. Since microbial contaminations in some plants can affect the concentration of their active ingredients, it is essential to accurately estimate the microbial load in raw materials in order to produce herbal extracts that are as pure as possible and have the greatest therapeutic potential.

Due to the deposition of fungicides, pesticides, and other microbes in medicinal plants, which are utilized as raw materials for the production of herbal extracts for use as dietary

supplements and herbal medicines, there may be quality and safety difficulties. People have largely relied on plants for their basic needs, including the production of life-saving medicines. However, because of human impact and unchecked wild assortment, therapeutic plants are at risk. It is therefore recommended that deliberate efforts toward training and development are essential for maintaining a stockpile of medicinal plant species.

As complex mixes derived from biological sources, herbal medical medicines require a lot of work to ensure a consistent and suitable quality. The pattern and concentration of ingredients in herbal medical products should be kept as constant as feasible by careful plant selection and a standardized production process as this is a requirement for reliable therapeutic benefits. From the selection of propagation material to the delivery of the finished product to the customer, quality must be incorporated throughout the entire process. To guarantee the microbiological quality and safety of the herbal medicinal goods, manufacturers of herbal products must effectively adhere to current good manufacturing practices. As a result, the standards of herbal medications sold on the market need to be constantly monitored and controlled. National regulatory organizations should create control procedures for the sale of these herbal medicines to stop or lessen consumption of goods that fall short of the basic requirements for quality. Additionally, campaigns associated with basic healthcare facilities or family health initiatives should be launched in the area to direct the right preparation of herbal medicines (avoiding microbial contamination).

5.4 Suggestions for Further Studies

Our study has a number of drawbacks. Swab culture is mostly used in clinical microbiological laboratories, and clinical diagnosis may not be accurate enough. The

sensitivity testing only comprised commonly used antibiotics; socio-demographic characteristics were not taken into account. Future research must address these issues, and appropriate procedures must be put in place to protect these life-saving medications for the long term. The potential antibacterial activity mechanism of the extracts must be identified.

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APPENDIX I

Samples of Herbal Mixtures Commonly Sold for STI Treatmen

APPENDIX II

Kambest Infection STD Flusher

APPENDIX III

ADIGGS WPP Herbal Mixture

APPENDIX IV

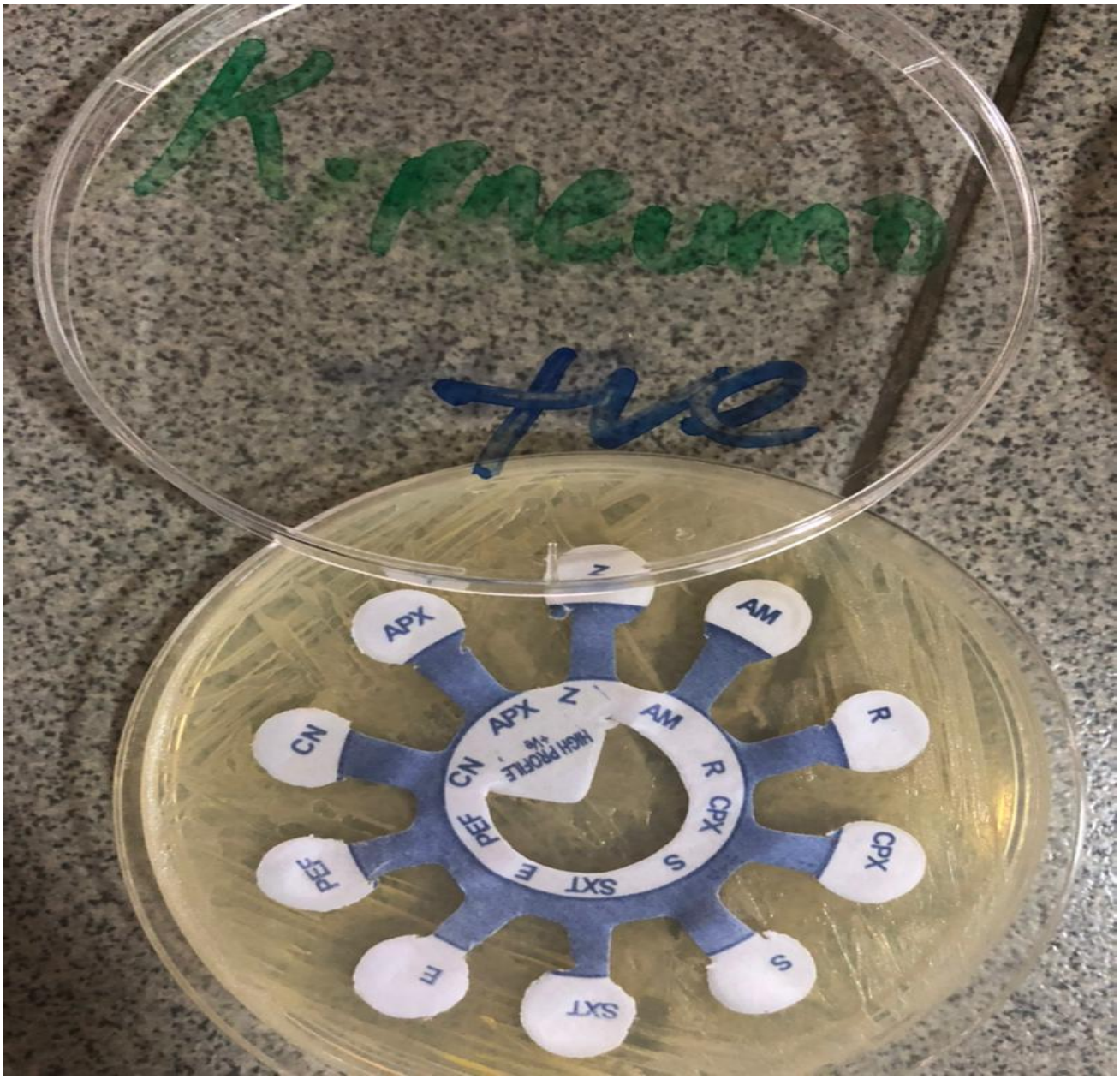

Plate Showing Test Organism Susceptibility to Common Antibiotics on Commercial Antibiotics Disk

APPENDIX V

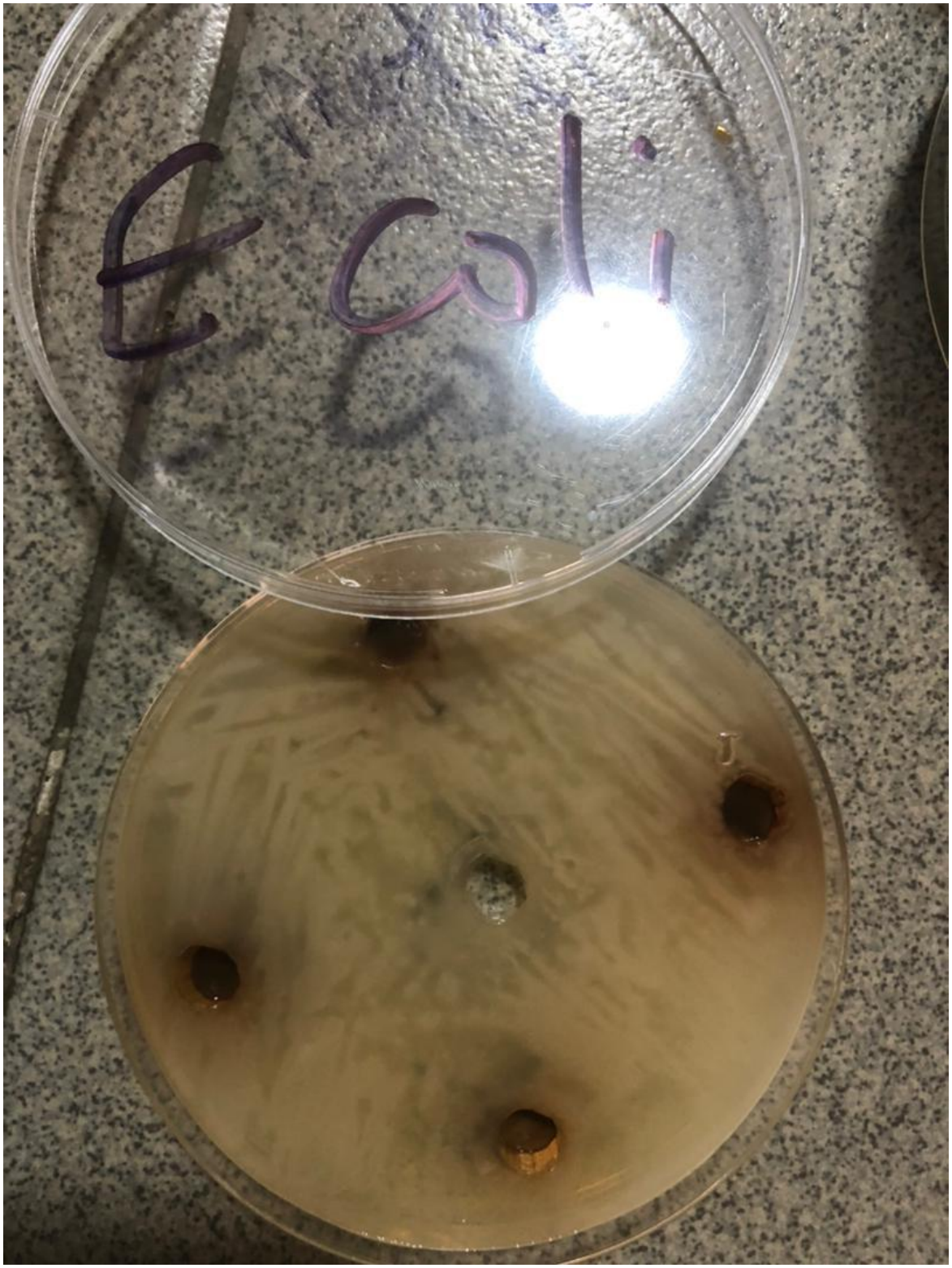

Plate Showing Test Organism

APPENDIX VI

Plate Showing Test Organism Susceptibility to Common Antibiotics on Commercial Antibiotics Disk

APPENDIX VII

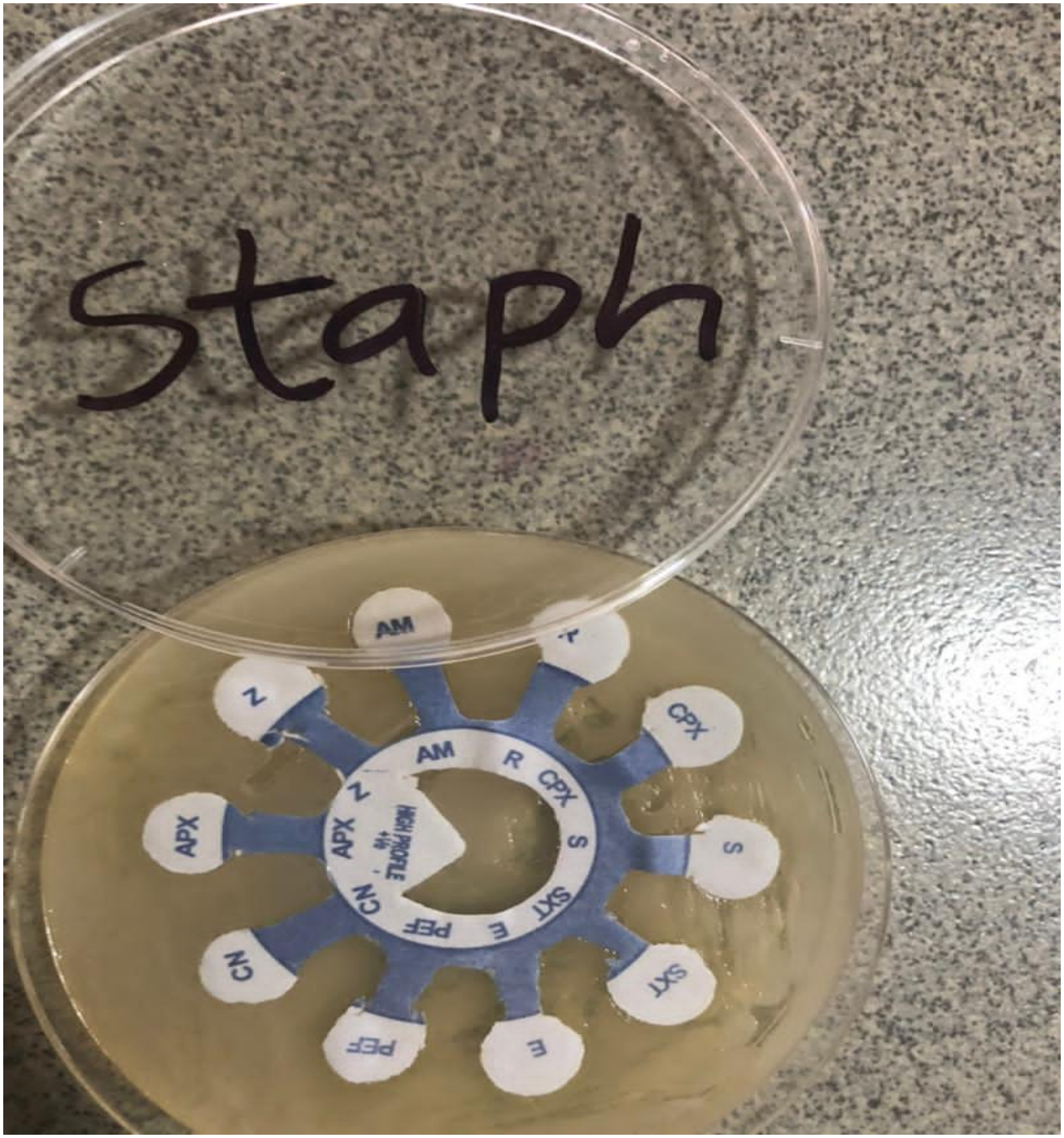

Plate Showing Test Organism Susceptibility to Common Antibiotics on Commercial Antibiotics Disk

APPENDIX VIII

Plate Showing Test Organism Susceptibility to Common Antibiotics on Commercial Antibiotics Disk

APPENDIX IX

Biodata

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OYSCOED Model High School, Oyo	2000-2003	.
School of Science, Oyo	2003-2006	SSCE
LAUTECH, Ogbomoso	2007-2015	BMLS

C. Working Experience:

I.	Adeoyo Hospital, Ringroad (Internship)	2016-2017
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III.	Union Diagnostics, Yaba, Lagos	2020
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Biotechnology Society of Nigeria

Microbiology Society of Nigeria

Signature **Date**

Originality Report

This is to certify that to the best of my knowledge, the content of this thesis is my own work. This thesis is free from plagiarism and has not been submitted for any degree or other purposes.

I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged.

Naimot Aramide, EMIOLA.