Antibacterial Sensitivity Test of Crude Extract of (*Curcuma zedoaria, Solanum virginianum*and *Stephania japonica)* &Resistant Pattern of Clinically Isolated Bacteria against Conventionally Used Antibiotics

A Thesis Report Submitted to the Department of Pharmacy, East West University in Partial Fulfillment of the requirements for the Degree of M. Pharm In "Clinical Pharmacy and Molecular Pharmacology".

Submitted To

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EAST WEST UNIVERSITY

Introduction

Medicinal plant

According to WHO A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or synthesis of useful drugs.

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Moreover, these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpaste (Ghani, 2003).

Characteristics of Medicinal Plants

Medicinal plants have many characteristics when used as a treatment, as follow:

• Synergic medicine- the ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

• Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

• Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Bassam,2016).

Traditional use of medicinal plants

Traditional medicine is the sum total of knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures that are used to maintain health, as well as to prevent, diagnose, improve or treat physical and mental illnesses. Medicinal plants have been used in traditional medicine for hundreds of years with reputation as efficacious

Declaration

Nasrin Akter, hereby declare that this thesis entitled "Antibacterial Sensitivity Test of Crude Extract of some medicinal plants & Resistant Pattern of Clinically Isolated Bacteria Against Conventionally Used Antibiotics" submitted by me to the Department of Pharmacy in Clinical Pharmacy and Molecular Pharmacology is a genuine authentic research work carried out by me under the guidance and supervision of Shamsun Nahar Khan Ph.D, Associate Professor, Department of Pharmacy, East West University. I also declare that the contents of this dissertation, in full or in parts have not been submitted to elsewhere for the award of any other degree.

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Department of Pharmacy

East West University

remedies although there may not be sufficient scientific data to substantiate their efficacy. Medicinal properties of the plants were known even to prehistoric men, the discovery of medicinal properties of plants was not certainly based on any scientific data or on the knowledge of chemical constituents of plants. The exploration of the chemical properties of the plants throughout the age was accomplished principally through careful observation, trial and error and the accidental discovery.

In this process, the human race, over the centuries, has created a vast heritage of knowledge and experience on medicinal plants in different cultures and civilizations. Most of such indigenous knowledge handed down, through the ages, by oral tradition. The major portion of the present day knowledge of the medicinal properties of the plants is thus the sum total of these observation and experience (Ghani, 2003). Phytotherapy seems to be an alternate system of medicine for the people residing in the suburban/ rural areas. According to the WHO, about 80% of the world's population relies on traditional medicine for their primary health care.

In some Asian and African countries, 80% of the population depend on traditional medicine for primary health care.In many developed countries, 70% to 80% of the population has used some form of alternative or complementary medicine (e.g. acupuncture).Herbal treatments are the most popular form of traditional medicine, and are highly lucrative in the international marketplace. Annual revenues in Western Europe reached US$ 5 billion in 2003-2004. In China sales of products totaled US$ 14 billion in 2005. Herbal medicine revenue in Brazil was US$ 160 million in 2007. Not many countries have national policies for traditional medicine. Regulating traditional medicine products, practices and practitioners is difficult due to variations in definitions and categorizations of traditional medicine therapies. A single herbal product could be defined as either a food, a dietary supplement or an herbal medicine, depending on the country. This disparity in regulations at the national level has implications for international access and distribution of products.

World Health Organization (WHO) and its Member States cooperate to promote the use of traditional medicine for health care. The collaboration aims are;

Certificate by Chairperson

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Prof. Dr. Chowdhury Faiz Hossain

Chairperson

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- To support and integrate traditional medicine into national health systems in combination with national policy and regulation for products, practices and providers to ensure safety and quality.
- To ensure the use of safe, effective and quality products and practices, based on available evidence;
- acknowledge traditional medicine as part of primary health care, to increase access to care and preserve knowledge and resources; and
- To ensure patient safety by upgrading the skills and knowledge of traditional medicine providers.(World Health Organization)

Infectious diseases world wide have been known to be a cause of morbidity, disability and mortality. Approximately 15 million people die each year due to infectious diseases – nearly all live in developing countries1. Indiscriminate and unconcerned use ofantibiotics has led to increased microbial resistance.

Consequently, newer agents have been brought in at increasedeconomic costs to the patient but they too have becomeinefficacious in due course and pose worldwide a great threat tohuman health. So, noble, emerging and re-emerging infectiousdiseases have become a focus for the development of new cost-effective drug in both developed and developing countries.

Nature has been a source of medical treatments for thousands ofyears and today plant based systems continue to play animportant role in the primary health care of 80% of the world'spopulation.

Antibiotics represent one of the most successful forms of therapy in medicine. But the efficiency of antibiotics is compromised by a growing number of antibiotic-resistant pathogens.

Antibioticresistance, which is implicated in elevated morbidity and mortality rates as well as in the increased treatment costs, is considered to be one of the major global public health threats and the magnitude of the problem recently prompted a number of international and national bodies to take actions to protect the public.

Certificate by Supervisor

This is certify that the thesis paper entitled "Antibacterial Sensitivity Test of Crude Extract of (*Curcuma zedoaria, Solanum virginianum and Stephania japonica*) & Resistant Pattern of Clinically Isolated Bacteria against Conventionally Used Antibiotics"

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Shamsun Nahar Khan Ph.D

Associate Professor

Department of Pharmacy

East West University

Bacteria:

The simplest organisms living on earth today are bacteria, and biologists think they closely resemble the first organisms to evolve on earth. Too small to see with the unaided eye, bacteria are the most abundant of all organisms (figure 1.1) and are the only ones characterized by prokaryotic cellular organization. Life on earth could not exist without bacteria because bacteria make possible many of the essential functions of ecosystems, including the capture of nitrogen from the atmosphere, decomposition of organic matter, and, in many aquatic communities, photosynthesis. Indeed, bacterial photosynthesis is thought to have been the source for much of the oxygen in the earth's atmosphere.

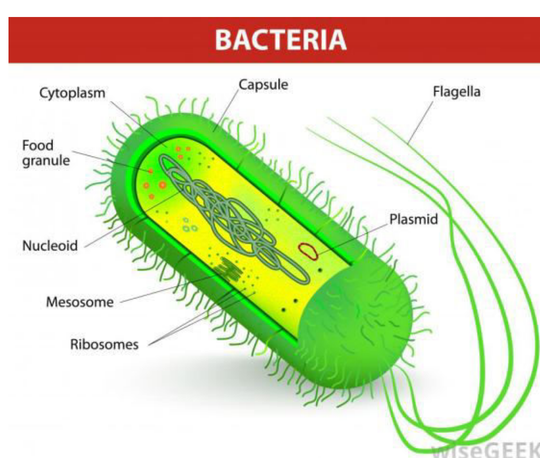

Figure 1.1: structure of bacteria

The major characteristics of Bacteria are based on their size, shape and Arrangements.

Bacterial Shape:

Bacteria are mostly simple in form and exhibit one of three basic structures: bacillus(plural,bacilli) straight and rod-shaped, coccus(plural, cocci) spherical-shaped, and spirillus (plural, spirilla) long and helical-shaped, also called spirochetes. Spirilla bacteria generally do not form associations with other cells and swim singly through their environments. They have a complex structure within their cell membranes that allow them to spin their corkscrew-shaped bodies which propels them along. Some rod-shaped and spherical bacteria form colonies, adhering end-to-end after they have divided, forming chains. Some bacterial colonies change into stalked structures, grow long, branched filaments, or

Acknowledgement

At the beginning I would like to remember the mercy and kindness of Almighty Allah for giving me that opportunity to study in this subject and the ability to complete my M. Pharm including this research appropriately.

I would like to pay my sincere thanks gratitude to supervisor Shamsun Nahar Khan Ph.D, Associate Professor, Department of Pharmacy, East West University for her master mind direction, constant supervision and support, optimistic counseling and continuous backup to carry out the research work as well as to prepare this dissertation. I am also thankful to my lab colleagues who helped me and supported me during the rigorous laboratory work to complete my thesis.

form erect structures that release spores, single-celled bodies that grow into new bacterial individuals. Some filamentous bacteria are capable of gliding motion, often combined with rotation around a longitudinal axis. Biologists have not yet determined the mechanism by which they move (Amelia , et al. 2015).

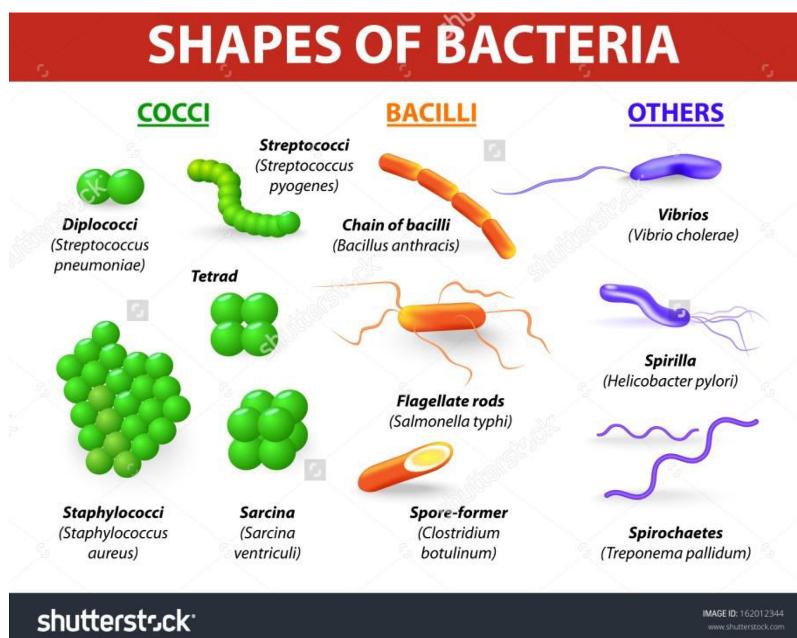

Figure 1.2: Shape of bacteria

Size

The unit of measurement used in bacteriology is the micron (micrometer).

1 micron (µ) or micrometer (µm) – one thousandth of a millimeter.

1 mill micron (mµ) or nanometer (nm) – one thousandth of a micronor one millionth of amillimeter.

1 Angstrom unit (Å) – one tenth of a nanometer.

The limit of resolution with the unaided eye is about 200 microns. Bacteria aresmaller which can be visualized only under magnification. Bacteria of medicalimportance generally measure 0.2 – 1.5 µm in diameter and about 3-5 µm in length.

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Bacterial Classification

Bacteria can be classified on the based

- ❖ Phenotypic
- ❖ Analytic
- ❖ Genotypic

Phenotypic Classification

➢ Microscopic morphology

– By Gram stain we can detect the organism,

– By the shape& size we can assume about the organisms i.e., rods (bacillus), spheres (cocci), curved or spiral,

➢ Macroscopic

– By Hemolytic properties on agar containing blood, pigmentation of the colonies, size and shape of colonies, smell and color.

➢ Serotyping

– By Antibody reactivity to specific antigens, can be classified.

➢ Antibiogram patterns

–Susceptibility to antibiotics can be classified the organisms.

➢ Phage typing

–Susceptibility to viruses that infect bacteria bacteriophages.

Analytic Classification

•Chromatographic pattern of cell wall mycolic acids is determine the type of bacteria

•Lipid analysis is the parameter of bacterial classification.

•By Proteomic analysis bacterial classification is also done.

–These techniques are labor intensive

–Require expensive equipment

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–Used primarily in reference laboratories

Genotypic Analysis

•Most precise method for bacterial classification is genotypic analysis.It can be done by

–Ratio of guanine to cytosine

–DNA hybridization

–Nucleic acid sequence analysis

•PCR method is another method for bacterial classification.

–Chromosomal DNA

–Ribotyping

–Plasmid analysis

Antibiotics

Antibiotics are powerful drugs, but they are not the cure for all the diseases. Antibiotics, also known as antimicrobial drugs, are drugs that fight infections caused by bacteria. They are not effective against viral infections caused by bacteria. They are not effective against viral infections like the common cold, most sore throats, and the flu.

Antimicrobial agent:

These are substancesproduced by microorganisms,which suppress the growth of or kill other microorganisms at very low concentrations.This definition excludes other natural substances which also also inhibit microorganisms but are produced by higher forms(e.g.antibodies) or even those produced by microbes but are needed in high concentration.

Now many antibiotics and their analogues have been synthesized,so both synthetic and microbiologically produced drugs need to be included together,however it would be more

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meaningful to use the term Antimicrobial Agent to designate synthetic as well as naturally obtained drugs that attenuate microorganisms.

Spectra of Antimicrobial Agent

Narrow-spectrum antibiotics:

Antimicrobial agents acting only on a single or limited group of microorganisms are said to have a narrow spectrum.For example,isoniazid is active only against mycobacteria.

Extended-spectrum antibiotics:

It is the term applied to antibiotics that are effective against gram-positive organisms and also against a significant number of gram negative bacteria.For example,ampicillin is considered to have an extended spectrum,because it acts against gram-positive and some gram negative bacteria.

Broad-spectrum antibiotics: It is the term applied to antibiotics that are effective against a wide variety of microbial species.

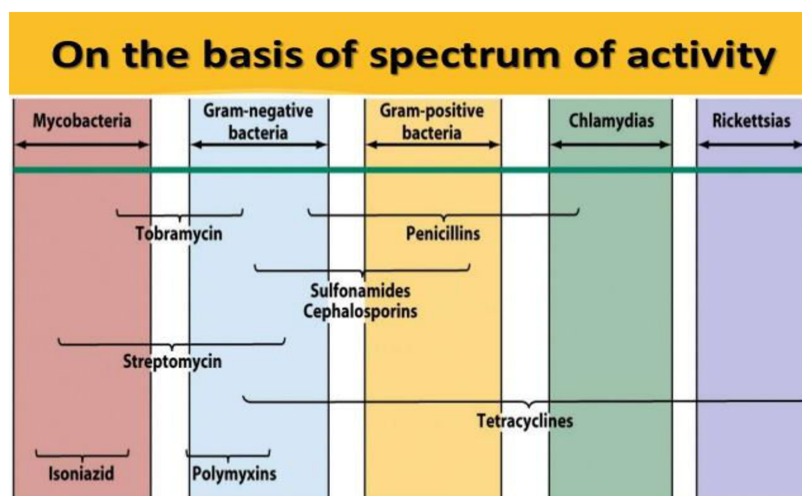

Figure 1.3: Spectrum of several antibiotics

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Mechanism of actions of antibiotics

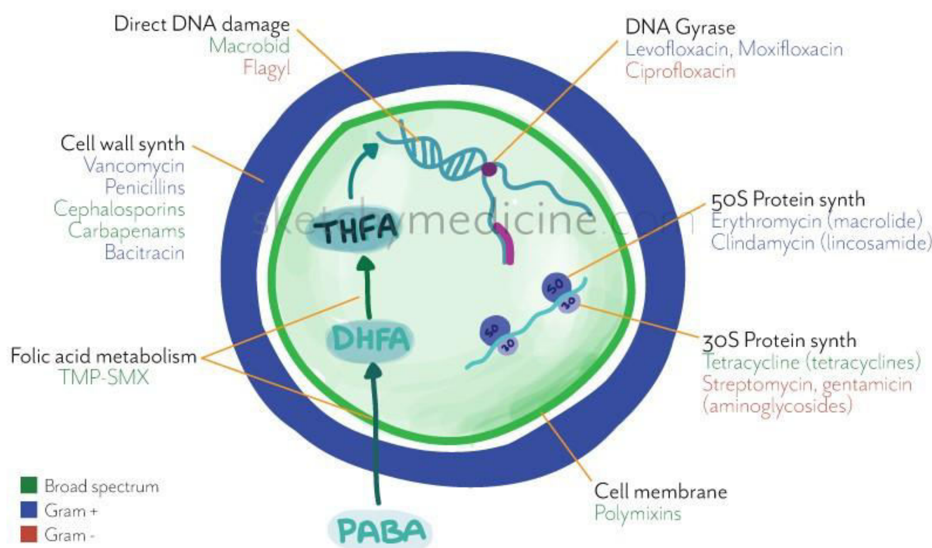

Figure 1.4: Site of antibiotic Action

Sulfonamides: Folic acid is synthesized from p-aminobenzoic acid(PABA),pteridine precursor.All the sulfonamides are synthetic analogs of PABA.Because of their structural similarity to PABA,the sulfonamides compete with this substrate for the bacterial enzyme, dihydrofolate synthetase.They thus inhibit the synthesis of bacterial folic acid.

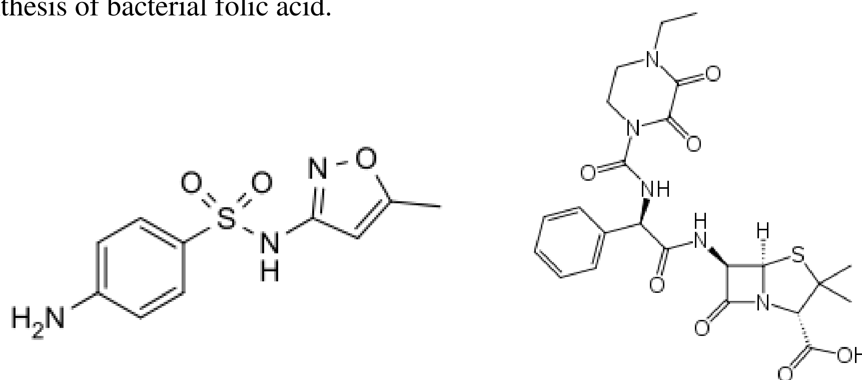

Figure1.5: Chemical structure Sulfamethoxazole and Trimethoprim

Co-Trimoxazole is combination therapy of trimethoprim with sulfamethoxazole.The synergistic antimicrobial activity of CO-Tromoxazole results from its inhibition of two sequential steps in the synthesis of tetrahydro folic acid, sulfamethoxazole inhibits the incorporation of PABA into folic acid;and trimethoprim prevents reduction of dihydrofolate to tetrahydrofolate.

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β-Lactam antibiotics: They inhibits the cell wall synthesis.They are penicillins,Cephalosporin,carbapenems,monobactams.

Penicillin: Penicillin's inactivate numerous proteins on the bacterial cell membrane. These penicillin binding proteins(PBPs) are bacterial enzymes involved in the synthesis of the cell wall and in the maintenance of the morphologic features of the bacterium.Exposure to these antibiotics can therefor not prevent cell wall synthesis,but also lead to morphologic changes or lysis of susceptible bacteria.Some PBPs catalyze formation of the cross –linkages between peptidoglycan chains.Penicillins inhibit this transpeptidase-catalyzed reaction,thus hindering the formation of cross links essential for cell wall integrity as a result of this blockade of cellwall synthesis.Example-Amoxicillin,ampicillin,cloxacillin.piperacillin etc.

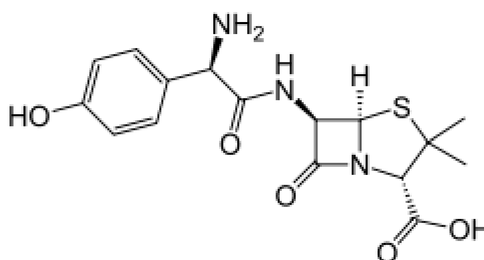
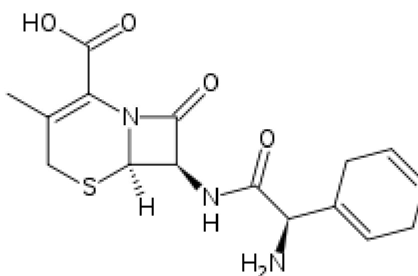


Figure 1.6: Chemical structure of Amoxicillin and Piperacilline

Cephalosporin: The Cephalosporin are β-lactam antibiotics that are closely related both structurally and functionally to the penicillin's.Cephalosporins have the same mode of action as penicillins.They are classified into four generation:

First generation: Cephradine, Cefazolin,Cephalexin.



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Second generation: Cefaclor,Cefuroxime,Cefoxitin.

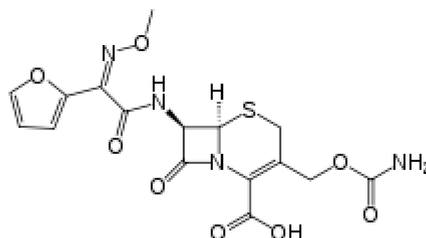

Figure 1.8:Chemical Structure of Cefuroxime

Third generation: Cefixim,Ceftriaxone,Ceftazidime.

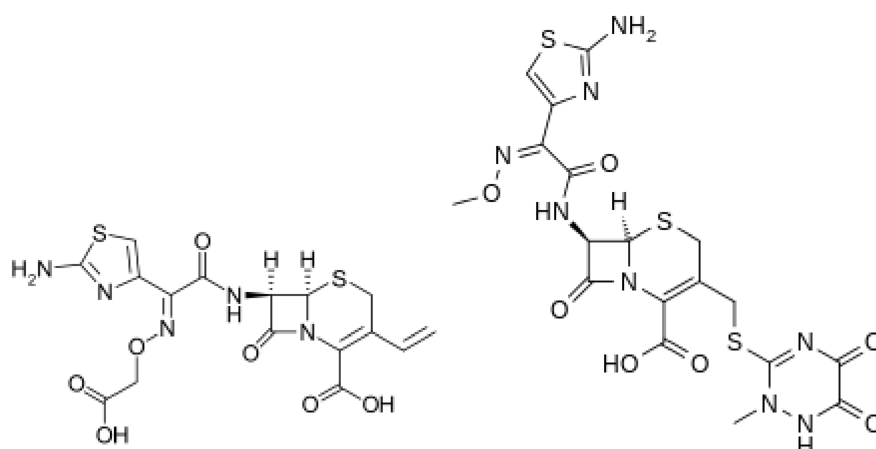

Figure 1.9: chemical structure of Cefixim and Ceftriaxone

Fourth generation: Cefepime.

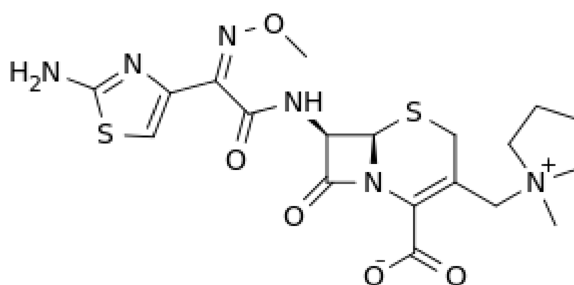

Figure 1.10:Chemical Structure of Cefepime

Quinolones: The quinolone antibiotics target bacterial DNA gyrase and topoisomerase IV.For many gram-positive bacteria, topoisomerase IV is the primary activity inhibited by the

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quinolones.In contrast,DNA gyrase is the primary quinolon target in many gram-negative microbes individual strands of double helical DNA must be separated to permit DNA replication or transcription.However,anything that separates the strands results in overwinding or excessive positive supercoiling of the DNA in frnt of the point of separation.The bacterial enzyme DNA gyrase is responsible for the continuous introduction of negative supercoils into DNA that both strands of the DNA be cut to permit passage of a segment of DNA through the break, the break is then resealed.

Quinolones are classified into four generation:

First generation: Nalidixic Acid

Second generation: Ciprofloxacin,Norfloxacin,Ofloxacin

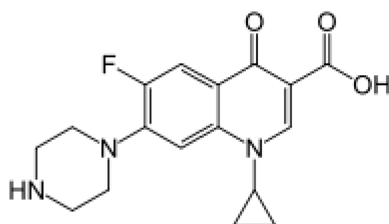

Figure 1.11:Chemical Structure of Ciprofloxacin

Third generation: Gatifloxacin,Levofloxacin,Moxifloxacin,Sparfloxacin

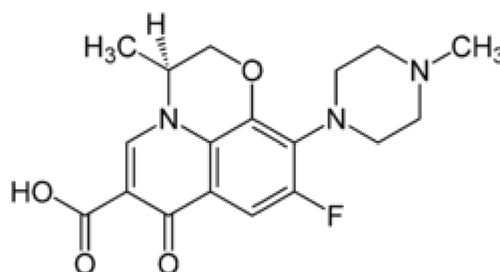

Figure 1.12:Chemical Structure of Levofloxacin

Fourth generation: Trovafloxacin

Protein synthesis inhibitors:

Tetracyclines,Aminoglycosides,Macrolides,Chloramphenicol,Clindamycin etc are protein synthesis inhibitors.

Abstract

In this research, we focused on the clinically isolated bacteria which were subjected to their sensitivity testing against Bangladeshi medicinal plants (*Curcuma zedoaria, Solanum verginianum, Stephania japonica*).These clinical isolates were also tested against conventional antibiotics to evaluate their resistance pattern. The clinical isolates were collected from Dhaka medical college, Dhaka, Bangladesh. The experiments showed that both gram positive and gram-negative bacteria were multi drug resistant against conventional antibiotics. The conventional antibiotic used in this study were Amoxicillin, Tetracycline, Vancomycin, Cefuroxime, Ciprofloxacin, Penicillin-G, Gentamicin, Ceftriaxone, Piperacillin, Tazobactam, Levofloxacin, Nitrofurantoin, Cefixim, Azithromycin, Cephradine, Erythremycin, Kanamycin, Cotrimozazol. Most of the bacteria (*Entarobactor spp. E.coli, Klebsiellla spp, Salmonella peratyphi, Staphylococcus aureus, Psedomonas spp.)* found resistance against conventional antibiotics.

Antibacterial sensitivity test against crude extract of those resistant bacteria in different doses were done. Methanolic extract *of Curcuma zedoaria (*100mg/ml, 200mg/ml, 400mg/ml*), Solanum verginianum (*100mg/ml, 200mg/ml, 400mg/ml*),Stephania japonica (*100mg/ml,200mg/ml,400mg/mland 500mg/ml*)* were used and found highly sensitive for those bacteria.

Crude extract of *Curcuma zedoaria* was effective at 400mg/ml dose and it's zone of inhibition were 10.12mm, 6.44mm, 7.60mm, 6.55mm, 7.71mm and 8.08mm respectively for *Entarobactor spp. E.coli, Klebsiella spp. Salmonella peratyphi, Staphylococcus aureus and Psedomonas spp.*

Crude extract of *Staphania japonica* was effective at 500mg/ml dose and it's zone of inhibition were 15.31mm, 6.56mm, 11.50mm,14.05mm and 9.93mm respectively against *Entarobactor spp. E.coli, Klebsiella spp. Salmonella peratyphi, Staphylococcus aureus* and *Psedomonas spp.*

And at 400mg/ml *Solanum virginianum* showed 8.12mm, 6.44mm, 7.60mm, 6.95mm, 7.71mm and 8.08mm zone of inhibition against *Entarobactor spp. E.coli, Klebsiella spp. Salmonila peratyphi, Staphylococcus aureus* and *Psedomonas spp.*

This study showed that these medical plants are showing potent activities with high sensitivity to the multi drug resistant bacteria, thus the pure compounds to be isolated from these medicinal plants may show novel antibacterial activities with new mechanism of actions.

Key Word: Antibiotics, Sensitivity, Resistant, Zone of inhibition, Clinical Isolated Bacteria, *Curcuma zedoaria, Stephania japonica*, and *Solanum virginianum*.

Aminoglycosides: Amikacin,Gentamicin,neomycin,Streptomycin etc are aminoglycoside antibiotics.Aminoglycosides diffuse through aqueous channels formed by porin proteins in the outer membrane of gram-negative bacteria to enter the periplasmic space.The primary intracellular site of action of the aminoglycosides is the 30S ribosomal subunit.Once inside the cell,aminoglycosides bind to polysomes and interfere with protein synthesis by causing misreading and premature termination of mRNA translation.

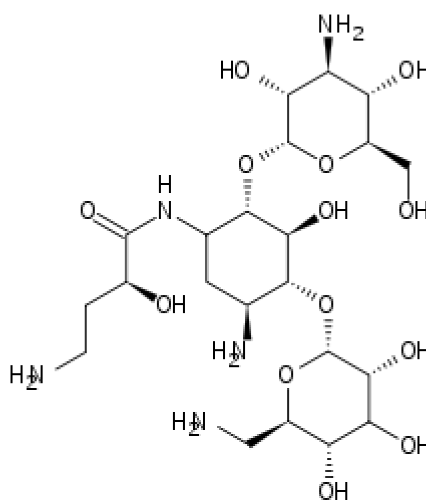

Figure 1.13:Chemical Structure of Amikacin

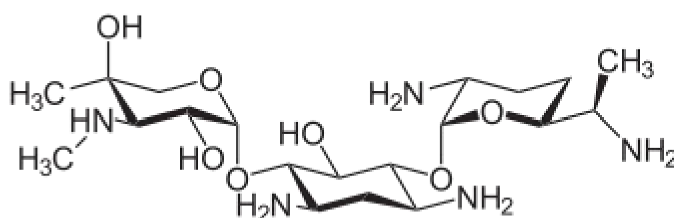

Figure 1.14:Chemical Structure of Gentamicin

Macrolides: Azithromycin,Clarithromycin,Erythromycinetc are macrolides antibiotics.They are bacteriostatic agents that inhibit protein synthesis by binding reversibly to 50S ribosomal subunits of bacteria.They inhibit the peptide formation and translocation of protein synthesis process .

Antibiotic Resistance

Antibiotic resistance is the ability of a microorganism to withstand the effects of anantibiotic. Today, almost all important bacterial infection in the India and throughout the world are

becoming resistant to antibiotics. Antibiotic resistance has been called one of the world's most pressing public health concerns. The rational use of antibiotics is the key to controlling the spread of resistance.

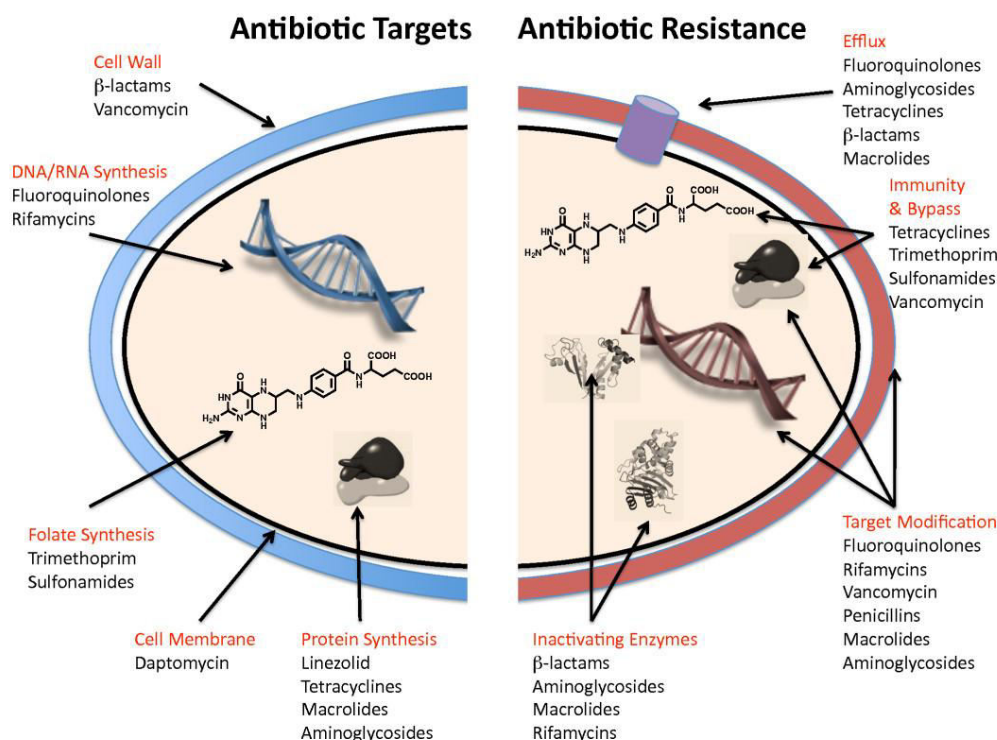

Figure 1.15: Site of bacterial resistant formation

Antibiotic-resistant bacteria that are difficult or impossible to treat are becoming increasingly common and are causing a global health crisis. Antibiotic resistance is encoded by several genes, many of which can transfer between bacteria. New resistance mechanisms are constantly being described, and new genes and vectors of transmission are identified on a regular basis (Jssica, et al. 2015).

Established mechanisms of antimicrobial resistant:

For an antibiotic to be effective, it must reach the target site in an active form, bind to the target, and interfere with its function. Thus, bacterial resistance to an antimicrobial agent can occur due to three general mechanisms.

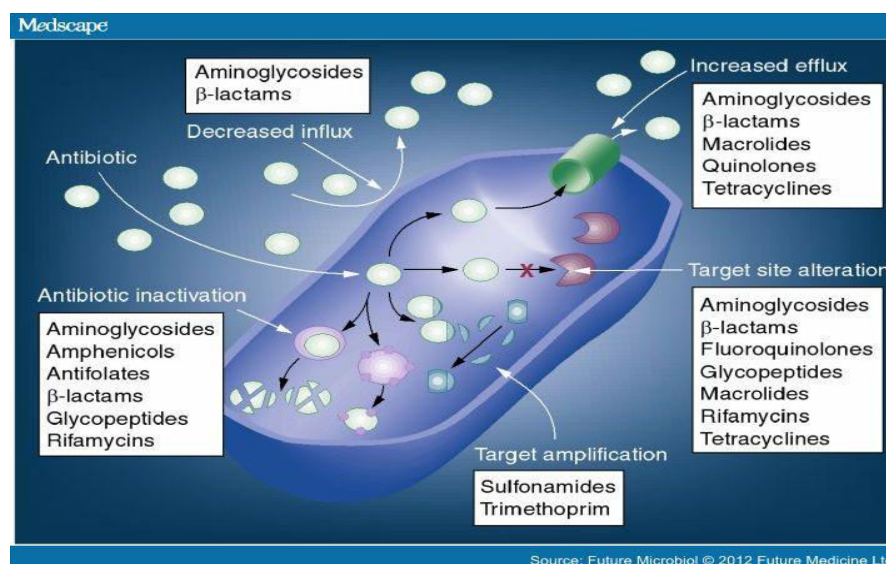

Figure 1.16: Mechanisms of antibiotic resistant

The drug does not reach its target

In Gram negative bacteria, many antibiotics enter the cell through protein channels called porins. Mutations or loss of these channels can prevent/slow the rate of antibiotic entry into a cell, effectively reducing drug concentration at the target site. If the drug target is intracellular and the drug requires active transport across the cell membrane, a mutation that interferes with the transport mechanism can confer resistance e.g. aminoglycosides. Bacteria can also transport antimicrobial drugs out of the cell through efflux pumps. Resistance to numerous drugs, including fluoroquinolones, macrolides, tetracyclines and beta lactam antibiotics, is mediated by this mechanism.

The drug is inactivated

Bacterial resistance to aminoglycosides can be due to a plasmid encoded aminoglycoside - modifying enzymes. Similarly, β-lactamase production is the most common mechanism of resistance to penicillins and other β-lactam drugs. Many hundreds of different β-lactamases have now been identified. A variation of thismechanism is failure of the bacterial cell to activate a prodrug e.g. lossof ability of M. tuberculosis to activate isoniazid (INH).

The target site is altered

This may be due to mutations in drug binding region of target enzyme e.g. fluoroquinolones, target modification e.g. ribosomal protection type of resistance to macrolides and acquirement of a resistant form of the susceptible target e.g., methicillin resistance in Staphylococcus Spp. Due to production of a low-affinity penicillin-binding protein (PBP).Strategies to prevent antimicrobial resistant in healthcare settings

Prudentantibiotic use: Antibiotics should be used only when they improve patient outcome. Not all infections need antibiotic treatment e.g. in patients with sore throat, benefit from antimicrobial therapy is small and is counterbalanced by the risk of adverse events like rash. Narrow spectrum agents should be used whenever possible. Broad spectrum agents should not be used as a cover for lack of diagnostic precision.

Antibiotics should be prescribed in optimal doses, regimens, and should be stopped when the infection is treated. Restrict the use of last line antibiotics for serious infections and only when simpler agents are likely to be ineffective. Whenever used for prophylaxis, antibiotics should be used for short courses and at appropriate times (e.g. during surgical prophylaxis, antibiotics should be given within an hour prior to incision) (Amelia, et al. 2015).

Prevention of infection:

Use of antimicrobials can also be reduced if infections areprevented in the first place. This can be achieved by improved use of vaccines and improved hygiene and infection control practices like compliance with hand washing protocols and aseptic techniques for catheterization. Catheters and drains should be removed when no longer needed.Clinicians should be familiar with local antibiotic sensitivity profiles and should comply with the local antibiotic guidelines.

A hospital antibiotic policy should be formulated based on local antimicrobial resistance data. Prescribers should be educated about the use of antibiotics, when not to use them and also the infection control strategies.

Hospitals should carry out surveillance of resistance patternshow much, where, in which organisms and to what antibiotics. Similarly antibiotic use pattern can be studied and these data can be used to devise targeted interventions to minimize antimicrobialuse.

Molecular Mechanisms of antibiotic Resistant:

Over the past decade, resistance to antibiotics has emerged as a crisis of global proportion. Microbes resistant to many and even all clinically approved antibiotics are increasingly common and easily spread across continents. At the same time there are fewer new antibiotic drugs coming to market. We are reaching a point where we are no longer able to confidently treat a growing number of bacterial infections. The molecular mechanisms of drug resistance provide the essential knowledge on new drug development and clinical use. These mechanisms include enzyme catalyzed antibiotic modifications, bypass of antibiotic targets and active efflux of drugs from the cell. Understanding the chemical rationale and underpinnings of resistance is an essential component of our response to this clinical challenge.

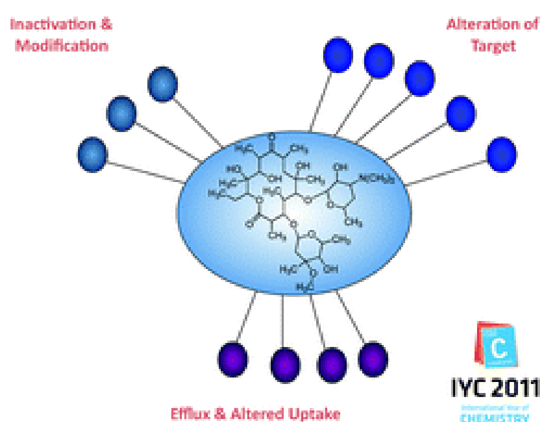

Figure 1.17:antibiotic targets and active efflux of drugs

The multidrug efflux systems contribute significantly to the increased resistance to multiple antibiotics in bacteria. A major challenge in developing efficacious antibiotics against drug resistant pathogens is to identify compounds that can counteract the efflux functions. The wealth of bacterial genomics information available suggests the presence of a variety of efflux systems in bacteria. Even a single bacterium may possess multiple efflux transporters of different families, with the overlapping substrate spectra. Accumulating evidence has

indicated that the MexXY multidrug efflux system is a primary determinant of aminoglycoside resistance in *Pseudomonas aeruginosa* (Jessica, et al. 2015).

The expression of bacterial multidrug efflux system is usually controlled by transcriptional regulators that either repress or activate the transcription of the multidrug efflux genes.

Plants Descriptions

2.1.*Staphania japonica:*

Figure 2.1: *Staphania japonica* plant

Stephania japonica is a climbing plant producing slender stems, that can become slightly woody when old, from a woody rootstock. (Hall,M.A. et al. 1997)

Climber or twiner, slender stems without prickles; dioecious. Leaves peltate, lamina circular to ovate or triangular, petiolate. Inflorescences up to 8 cm long. The leaves are sometimes harvested from the wild for medicinal purposes and to make a jelly.

E. Asia they are found in southern China, Japan, Korea, Indian subcontinent, Myanmar, Thailand, Laos, Malaysia, Indonesia, Philippines, New Guinea, Australia, Pacific. (Duraipandiyan,et al.2006)

2.1.1.Taxonomic Hierarchy of *Stephania japonica*

Kingdom:	Plantae
Subphylum:	Euphyllophytina
Infraphylum:	Radiatopses
Subclass:	Magnoliidae
Superorder:	Ranunculanae
Order:	Ranunculales

Family: Menispermaceae

Tribe: Menispermeae

Genus: Stephania

Species: ***S. japonica***

Common Name: Lektan (Bon.)

Other names

- ✓ Bengali: Akanadi, Nimuka, Maknadi.
- ✓ Chinese: Qian jin teng.
- ✓ Indonesia: Areuy geureung, Kepleng, Ginato bobudo
- ✓ Thai: Kon pit, Pang pon, Tap tao
- ✓ Vietnam: Thi[ee]n kim d[awf]ng, d[aa]y l[ox]i ti[eef]n.

Figure 2.2: Leaf of *Stephania japonica*

2.1.2.Use of *Stephania japonica*

- ✓ Plant used to cure itches.
- ✓ In Ayurveda, one of the three plants used as sources of "Patha," used in the treatment of urinary and heart related disorders.
- ✓ Used for skin sores, ulcers, furuncles, snake bites, stomach pains and leg edema.(Begum, et al. 1993)

- ✓ In northeast India, roots used for treatment of fever, diarrhea, dyspepsia and urinary diseases. Juice of root used for heart patients.
- ✓ In Japan and Taiwan, plant decoction used for malaria.
- ✓ In Indonesia, roots used for stomach aches. (Les, 2008)
- ✓ In Bangladesh, roots and leaves used for fever and diarrhea.
- ✓ Leaves applied on abscesses to facilitate pointing. Leaves macerate in water, mixed with molasses and drunk as cure for urethritis.
- ✓ Leaves also used for gastritis.
- ✓ Root paste used for vertigo and dysentery.
- ✓ Root tuber mixed with root juice of *Flemingia stricta* for asthma.
- ✓ Warmed root paste rubbed onto hydrocoeles (Senthamarai, et al.2012).

2.1.*Curcuma Zedoaria:*

Figure 2.3: *Curcuma zedoaria*

A leafy rhizomatous herb; rhizome pale yellow inside. Leaves with long petiols, lamina 30-60 cm long, oblong-lanceolate, clouded with purple along the midrib. Flowers in compact, oblong spike, 7.5-12.5 cm long, appearing before the leaves; flowers yellow. Coma bracts long, crimson or purple(Ankur, et al. 2015).

2.1.2.Taxonomic Hierarchy of *Curcuma Zedoaria*

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta

Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Lilianae
Order	Zingiberales
Family	Zingiberaceae
Genus	Curcuma L.
Species	Curcuma zedoaria

Others Name

- ✓ Bengali/Vernacular Name: Sothi.
- ✓ Tribal Name: Yak Cre Dong, aatega (Marma)
- ✓ English Name: Zedoary ,Indian Arowroot

Use of *Curcuma Zedoaria*

- ✓ Rhizomes and tubers are stimulant, carminative, stomachic, expectorant, demulcent, diuretic, rubefacient and cooling;
- ✓ use in flatulence and dyspepsia and as a corrector of purgatives; applied to bruises and sprains. (Ahmed., K. et al. 2003)
- ✓ It is used to clear the throat.
- ✓ Decoction of the rhizome is given in diarrhea;
- ✓ Together with long pepper, cinnamon and honey is given to relieve cough cold, fever and bronchitis.
- ✓ Fresh rhizome checks leucorrhoeal and gonorrhoeal discharges.
- ✓ Juice of the leaves is given in dropsy.

Essential oil from rhizome possesses antifungal and antibacterial activity. Curcumol and curdione contained in the rhizome have anticancer properties (Ghani, 2003). Chemical constiteunts: Rhizome contains an essential oil, sesquiterpenes, curcumol curcumadiol, furanodiene and its iso-derivatives, dehydrocurdione and zederone.

2.3.*Solanum vergininum:*

Figure 2.4: *Solanum virginianum*

Solanum virginianum commonly known as wild eggplant or nightshade plant, is a prickly herb found in most of the parts of the Asia and Australia of the world. It belongs to family Solanaceae, has spines throughout the plant. Fruits are globularand edible, flowers appear in cymes or sometimes solitary and are blue in colour, leaves are elliptical or ovate and are full of spines, stems appear green when young and brownishwhen matured. Various phytoconstituents have been found, the major constituents is alkaloid. It has vital role in various traditional as well as medicinal uses for curing internal and external physiological disorders.

2.3.1.Taxonomic Hierarchy *Solanum verginianum*

Kingdom: Plantae

Subkingdom: Viridiplantae

Super division: Embryophyta

Division: Tracheophyta

Class: Magnoliopsida

Superorder: Asteranae

Order: Solanales

Family: Solanaceae

Genus: *Solanum*

Species:*Solanum virginianum*

Other names:

- ✓ Bengali - Kantakari
- ✓ English-Yellow-berried Nightshade, Yellow berried nightshade, Febrifuge plant
- ✓ *Solanum surattense Burm.f.,*
- ✓ *S. xanthocarpum Schard. & Wendl.*

A very prickly diffuse bright green perennial herb, woody at the base; stem is zigzag; branches are numerous, the younger ones clothed with dense stellate tomentum; prickles are compressed, straight, yellow, glabrous and shining, often exceeding 1.3 cm. Leaves are usually 5-10 in numbers and 2.5-5.7 cm in length, ovate or elliptic, sinuate or sub pinnatified, obtuse or sub acute, stellately hairy on both sides, sometimes becoming nearly glabrous in age, armed on the midrib and often on the nerves with long yellow sharp prickles, base usually rounded and unequal-sided; petiole 1.3-2.5 cm long, stellately hairy. The berries are green and white strips when young but yellow when mature. They are 1.3-2 cm in diameter, yellow, or white with green veins, surrounded by the enlarged calyx. Seeds are 2.5 mm in diameter and glabrous. Calyx is nearly 1.3 cm long, densely hairy and prickly; tube short, globules. Lobes are 11 mm long, linear-lanceolate, acute and hairy outside. Filaments are 1.5 mm long, glabrous; anthers 8 mm long, oblong lanceolate, opening by small pores. Ovary is ovoid, glabrous (Muhammad, et al.2012).

Use of *Solanum verginianum:*

✓ Roots are diuretic and expectorant; employed in cough, asthma, chest pain and catarrhal fever.

- ✓ Fruit juice is useful in sore throat and rheumatism. Stem, flowers and fruits are carminative.
- ✓ Paste of the leaves is applied on painful joints to relieve pains. Seeds are given as an expectorant in asthma and cough.
- ✓ Decoction of the plant is useful in gonorrhoea. The plant also possesses cardioactive and antipyretic activities.
- ✓ Crude plant extract caused hypotension which has been attributed to release of histamine by some constituents.

Materials and methods

3.1.Resistant pattern of clinically isolated bacteria against conventionally used antibiotics

3.1.1. Equipments

- Petri dishes,
- Closed test tube,
- Beaker,
- 5ml vial,
- Rod spreader.
- Aluminum foil paper,
- Spatula,
- Glass container for agar,
- Gas burner,
- Forceps,
- Inoculating loop,
- Appendorf micropipette (Eppendorf, Germany),
- Appendorf micropipette tip,
- Appendorf tube,
- Autoclave (Hydroclave MC8,Barnstead International, USA),
- Laminar air flow (EQU/03-EHC,ESCO,USA),
- Incubator (BK4266),
- Hot air oven (YCO-N01,Germany industrial crop),
- Electric balance (ELH3000,shimadzu,Japan),
- Scissor,
- Masking tape,
- Permanent marking pen,
- Pencil,
- Scale,
- Powder free sterile hand gloves

- One time musk

3.1.2.Drugs and chemicals

- Nutrient agar(Hi-Media)
- Nutrient broth(Hi-Media)
- Mueller hinton agar(Hi-Media)
- MacConkey agar(Hi-Media)
- Ethanol,
- Amoxicillin,
- Tetracycline
- Vancomycin,
- Cefuroxime,
- Ciprofloxacin,
- Penicillin-G
- Gentamicin
- Ceftriaxone,
- Piperacillin Tazobactam
- Levofloxacin
- Nitrofurantoin
- Cefixim,
- Azithromycin
- Cephradine
- Erythremycin
- Kanamycin
- Cotrimozazol
- 0.9% NaCl solution
- Distil water

3.1.3.Microorganisms:

Microorganisms were collected from Microbiology Lab of Dhaka medical College.

- *Entarobactor spp.*

- *E.coli*
- *Klebsiellla spp*
- *Salmonella peratyphi*
- *Staphylococcus aureus*
- *Psedomonas spp*

3.2.Preparation of Media and Procedure

3.2.1.Preparation of Nutrient Broth:

13gm of dehydrate nutrient broth powder is required for 1000ml water. For this test 250ml of nutrient broth was required. 7gm nutrient broth was dissolved in distilled water up to 250ml.

3.2.2. Preparation of Nutrient Agar

28gm of dehydrate nutrient agar powder is required for 1000ml water. For this test every time 500ml of nutrient agar was required. 14gm nutrient agar was dissolved in distilled water up to 500 ml.

3.2.3. Preparation of Mueller hinton Agar:

38gmdehydrate agaris required for 1000ml of water.

3.2.4.Preparation of NaCl Solution

55.44gm NaCl is required for 1000ml water. 5.8gm of NaCl was dissolved in distilled water up to 100ml.

3.2.5.Agar Media Plate Preparation

Sterilization is most important for microbial test to avoid the contamination with other organisms and person. Agar media was needed for subculture, single colony isolation and sensitivity test of antibiotics. At first all Petri-dishes, test tubes, appendrof micropipette tips, apppendrof tubes, rod spreader and other glass wares were cleaned and after that sterilized by hot air oven at 150^0C for 1hour. For the inoculation of bacteria, nutrient broth and agar were required and for that agar solution was dissolved and sterilized by autoclave at 121^0C for 1hour and 30 minutes, after sterilization all were kept inside the luminary airflow. Prepared

nutrient agar was poured in each labeled sterile dish and screw cap test tube, then kept for few minutes to solidify. Agar should be poured in each dish in such a way that each dish contain 15-20ml agar.

3.2.6.Broth Media Tube Preparation

Broth media was needed for enrichment and inoculation of bacteria. After sterilization of broth media it kept at luminary air flow for cooling, after cooling dispense 5ml of broth each screw cap test tube with sterile pipette

3.3.Procedure

From the clinically isolated organisms(mother culture), for each organism took one loop full of organism and dissolved it in 5ml broth media containing test tube. After over night inoculation at 37^{0}C, turbid was growth and it was ready for single colony isolation. From the solution of organisms, the sterile loop containing microorganism was streaked across the surface of agar medium of Petri dish in zigzag pattern. It was kept overnight at 37^{0}C for inoculation. Mother cultures were preserved in refrigerator.

Next day one single colony was collected for the sensitivity test. That colony was dissolved in 0.9% NaCl solution and kept it at 37^{0}C for 2-4hours.

Figure 3.1. Hot air oven (YCO-N01,Germany industrial crop)

Figure 3.2. Autoclave (Hydroclave MC8,Barnstead International, USA)

3.3.1.Pouring Media on Plate

Media was poured in following ways:

1 .Agar was poured on the base of the petri dish and lid was kept open for a while that allows the excess water to evaporate from the surface of agar plate and made it dry.

2. Agar was poured in each dish in such a way that dish contains 15-20ml agar, and it was very crucial for the size of zone of inhibition since shallow layer of agar will produce a larger zone of inhibition than a deeper layer.

Figure 3.3. Laminar air flow (EQU/03-EHC,ESCO,USA)

3.3.2. Inoculation of the Agar Plate

A sterile tip was placed on the micropipette and took 0.5ml suspension from the inoculums tube was taken and poured on the middle of the plate. Then with the help of a sterile rod spreader the drop of bacterial suspension was spread uniformly by sterile throughout the entire agar surface. Then the micropipette tip was discarded. After that kept it to dry, then the antimicrobial agent disk was added to the plate, it was done placing disk .

3.3.3..Incubation of the Plate

After inoculation of the agar plate, petri dishes were kept into incubator because it provides environmental condition($35^0C±2^0C$)for the growth of microorganism.After overnight incubation (18-24hr) plates was kept outside of incubator and result was observed.

Figure 3.4. Incubator (BK4266),

3.3.4.Measuring Zone of Inhibition

The agar plates which were kept outside of the incubator, the diameter of zone of inhibition was measured by using slide calipers in millimeter(mm). Usually measurement is done by naked eye and it is done by holding the plate a few inches above a black, nonreflecting surface illuminated with reflected light. Result has to record in result sheet. If the diameter of the zone cannot be read, then measurement from the center of the disk to a point on the circumference of the zone where a distinct edge is present(the radius) has to done and

multiplied the measurement by 2 to determine the diameter. Growth on the edges of the disk is considered as 0mm.

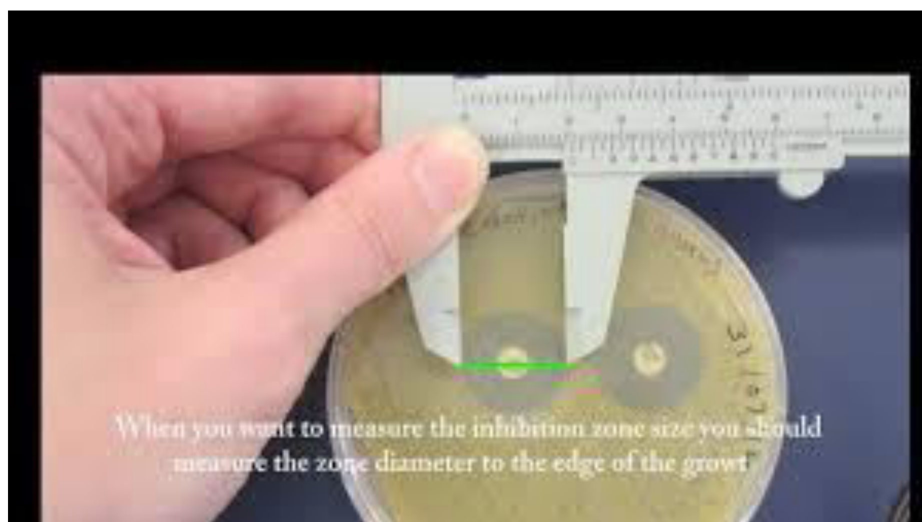

Figure 3.5. Measurement of zone of inhibition

3.3.5.Placement of the antibiotic disks

✓ We Placed the appropriate antimicrobial-impregnated disks on the surface of the agar, using sterile forceps to dispense each antimicrobial disk one at a time,.

✓ To add disks one at a time to the agar plate using forceps, placed the agar plate on the template. Sterilized the forceps by cleaning them with ethanol and allowing them to air dry, then igniting.

✓ Using the forceps carefully removed one disk from the cartridge.

✓ Partially removed the lid of the petri dish. Placed the disk on the plate and gently press the disk with the forceps to ensure complete contact with the agar surface. Replaced the lid to minimize exposure of the agar surface to room air.

✓ Continue to place one disk at a time onto the agar surface until all disks have been placed as directed.

✓ Once all disks are placed, replaced the lid, invert the plates, and placed them in a 37°C air incubator for 16 to 18 hours. After overnight incubation plate was taken out from incubator and measured the zone of inhibition with slide calipers.

3.4. Disk diffusion method

This method is used to determine the susceptibility or sensitivity of various microorganisms against antimicrobial agents. Here pathogenic microorganisms are grown in laboratory settings and disk impregnated with antimicrobial agent has to apply on the culture plate and growth of micro organism around the disk has to observe. Disks should not be placed closer than 24 mm (center to center) on the agar plate. Ordinarily, no more than 12 disks should be placed on a 150-mm plate or more than 5 disks on a 100-mm plate.

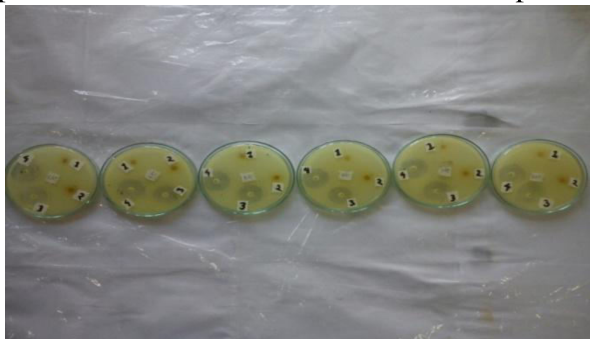

Figure 3.6. Antibacterial activity of Crud extract of *Solanum virginianum*

Disks should be avoided placing it close to the edge of the plate as the zones will not be fully round and can be difficult to measure.

Each disk must be pressed down with forceps to ensure complete contact with the agar surface or irregular zone shapes may occur.

If the surface of the agar is disrupted in any way (a disk penetrating the surface, visible lines present due to excessive pressure of the spreader against the plate during inoculation, etc.) the shape of the zone may be affected.

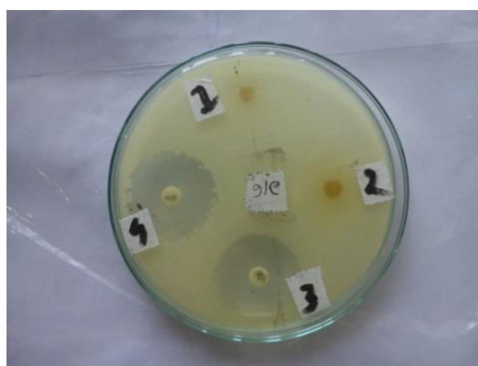

Figure 3.7. Antibacterial sensitivity test of crud drug(*Curcuma Zedoaria*)

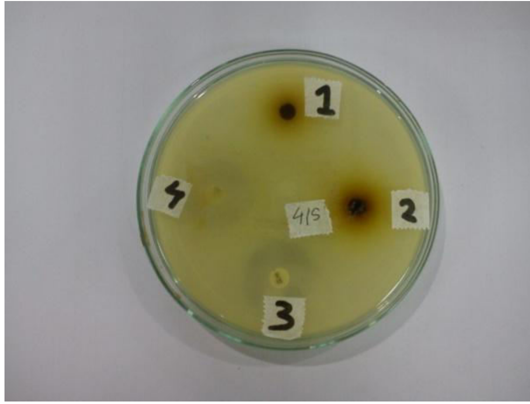

Figure 3.8. Antibacterial sensitivity test of crud drug(*Curcuma Zedoaria*)

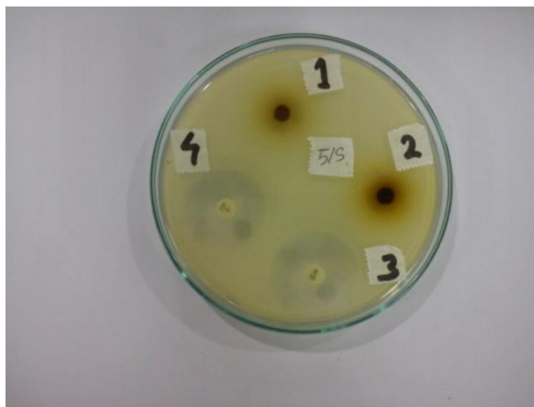

Figure 3.8. antibacterial sensitivity test of *solanum vergininum*

3.5.Antimicrobial Sensitivity test of Methanolic crud Extract of *Curcuma zedoaria, Solanum verginінum* and *Stephania japonica*

3.5.1.Equipments:

- Petri dishes,
- Closed test tube,
- Beaker,
- 5ml vial,
- Rod spreader.
- Aluminum foil paper,
- Spatula,
- Glass container for agar,
- Gas burner,
- Forceps,
- Inoculating loop,
- Appendorf micropipette (Eppendorf, Germany),
- Appendorf micropipette tip,
- Appendorf tube,
- Autoclave (Hydroclave MC8,Barnstead International, USA),
- Laminar air flow (EQU/03-EHC,ESCO,USA),
- Incubator (BK4266),
- Hot air oven (YCO-N01,Germany industrial crop),
- Electric balance (ELH3000,shimadzu,Japan),
- Scissor,
- Masking tape,
- Permanent marking pen,
- Pencil,
- Scale,
- Powder free sterile hand gloves
- One time musk
- Filter Paper

3.5.2.Chemicals:

- Nutrient agar(Hi-Media)
- Nutrient broth(Hi-Media)
- Mueller hinton agar(Hi-Media)
- Ethanol,
- 0.9% NaCl solution
- Distil water

3.5.3.Plant Extract

Methanolic extract of

- *Curcuma zedoaria,*
- *Solanum vergininum*
- *Stephania japonica*

3.5.4.Dose of crud extract:

- 100mg/ml
- 200mg/ml
- 400mg/ml
- 500mg/ml

3.5.5.Preparation of Curd Drug Disc:

Cured extract was weighted as per dosage form .dissolved in methanol for proper distribution. Use sterile micro pipette and filter paper(6mm in diameter). After prepare leave it for drying.

3.5.6.Microorganisms:

Microorganisms were collected from Microbiology Lab of Dhaka medical College.

- *Entarobactor spp.*
- *E.coli*
- *Klebsiellla spp*

- *Salmonella spp*
- *Staphylococcus aureus*
- *Psedomonas spp*

3.5.7.Preparation of Media and Procedure

3.5.7.1.Preparation of Nutrient Broth:

13gm of dehydrate nutrient broth powder is required for 1000ml water. For this test 250ml of nutrient broth was required. 7gm nutrient broth was dissolved in distilled water up to 250ml.

3.5.7.2.Preparation of Nutrient Agar:

28gm of dehydrate nutrient agar powder is required for 1000ml water. For this test every time 500ml of nutrient agar was required. 14gm nutrient agar was dissolved in distilled water up to 500 ml.

3.5.7.3.Preparation of Mueller hinton Agar:

38gmdehydrate agaris required for 1000ml of water.

3.5.7.4.Preparation of NaCl Solution:

55.44gm NaCl is required for 1000ml water. 5.8gm of NaCl was dissolved in distilled water up to 100ml.

3.5.7.5.Agar Media Plate Preparation:

Sterilization is most important for microbial test to avoid the contamination with other organisms and person. Agar media was needed for subculture, single colony isolation and sensitivity test of antibiotics. At first all Petri-dishes, test tubes, appendrof micropipette tips, apppendrof tubes, rod spreader and other glass wares were cleaned and after that sterilized by hot air oven at 150^0C for 1hour. For the inoculation of bacteria, nutrient broth and agar were required and for that agar solution was dissolved and sterilized by autoclave at 121^0C for 1hour and 30 minutes, after sterilization all were kept inside the luminary airflow. Prepared nutrient agar was poured in each labeled sterile dish and screw cap test tube, then kept for

few minutes to solidify. Agar should be poured in each dish in such a way that each dish contain 15-20ml agar.

3.5.7.6.Broth Media Tube Preparation:

Broth media was needed for enrichment and inoculation of bacteria. After sterilization of broth media it kept at luminary air flow for cooling, after cooling dispense 5ml of broth each screw cap test tube with sterile pipette.

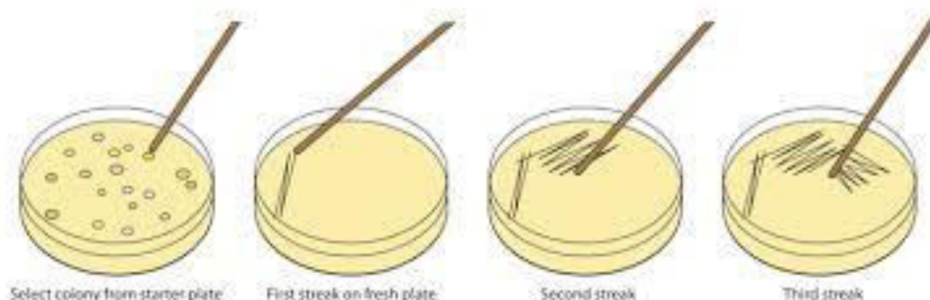

Figure 3.5.1.Single colony striking.

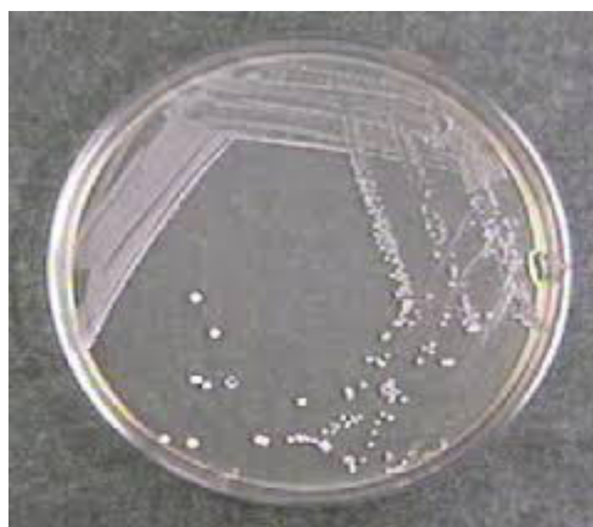

Figure 3.5.2. Single colony isolation

3.5.7.7.Procedure:

From the clinically isolated organisms(mother culture), for each organism took one loop full of organism and dissolved it in 5ml broth media containing test tube. After over night inoculation at 37^0C, turbid was growth and it was ready for single colony isolation. From the

solution of organisms, the sterile loop containing microorganism was streaked across the surface of agar medium of petri dish in zigzag pattern. It was kept overnight at 37^0C for inoculation. Mother cultures were preserved in refrigerator.

Next day one single colony was collected for the sensitivity test. That colony was dissolved in 0.9% NaCl solution and kept it at 37^0C for 2-4hours.

Figure 3.5.3. Hot air oven (YCO-N01,Germany industrial crop),

Figure 3.5.4. Autoclave (Hydroclave MC8,Barnstead International, USA)

3.5.7.8.Pouring Media on Plate:

Media was poured in following ways

1 .Agar was poured on the base of the petri dish and lid was kept open for a while that allows the excess water to evaporate from the surface of agar plate and made it dry.

2. Agar was poured in each dish in such a way that dish contains 15-20ml agar, and it was very crucial for the size of zone of inhibition since shallow layer of agar will produce a larger zone of inhibition than a deeper layer.

Figure3.5.5. Laminar air flow (EQU/03-EHC,ESCO,USA),

3.5.7.9.Inoculation of the Agar Plate

A sterile tip was placed on the micropipette and took 0.5ml suspension from the inoculums tube was taken and poured on the middle of the plate. Then with the help of a sterile rod spreader the drop of bacterial suspension was spread uniformly by sterile throughout the entire agar surface. Then the micropipette tip was discarded. After that kept it to dry, then the antimicrobial agent disk was added to the plate, it was done placing disk .

3.5.7.10.Incubation of the Plate:

After inoculation of the agar plate, petri dishes were kept into incubator because it provides environmental condition($35^0C \pm 2^0C$) for the growth of microorganisms .After overnight incubation (18-24hr) plates was kept outside of incubator and result was observed.

Figure 3.5.6. Incubator (BK4266

3.5.7.11.Measuring Zone of Inhibition:

The agar plates which were kept outside of the incubator, the diameter of zone of inhibition was measured by using slide calipers in millimeter(mm). Usually measurement is done by naked eye and it is done by holding the plate a few inches above a black, nonreflecting surface illuminated with reflected light. Result has to record in result sheet. If the diameter of the zone cannot be read, then measurement from the center of the disk to a point on the circumference of the zone where a distinct edge is present(the radius) has to done and multiplied the measurement by 2 to determine the diameter. Growth on the edges of the disk is considered as 0mm.

3.5.7.12.Disk diffusion method:

This method is used to determine the susceptibility or sensitivity of various microorganisms against antimicrobial agents. Here pathogenic microorganisms are grown in laboratory settings and disk impregnated with antimicrobial agent has to apply on the culture plate and growth of micro organism around the disk has to observe. Disks should not be placed closer than 24 mm (center to center) on the agar plate. Ordinarily, no more than 12 disks should be placed on a 150-mm plate or more than 5 disks on a 100-mm plate.

Disks should be avoided placing it close to the edge of the plate as the zones will not be fully round and can be difficult to measure.
Each disk must be pressed down with forceps to ensure complete contact with the agar surface or irregular zone shapes may occur.
If the surface of the agar is disrupted in any way (a disk penetrating the surface, visible lines present due to excessive pressure of the spreader against the plate during inoculation, etc.) the shape of the zone may be affected.

3.5.7.13.Placement of the antibiotic disks:

- ✓ We Placed the appropriate antimicrobial-impregnated disks on the surface of the agar, using sterile forceps to dispense each antimicrobial disk one at a time,.
- ✓ To add disks one at a time to the agar plate using forceps, placed the agar plate on the template. Sterilized the forceps by cleaning them with ethanol and allowing them to air dry, then igniting.

- ✓ Using the forceps carefully removed one disk from the cartridge.
- ✓ Partially removed the lid of the petri dish. Placed the disk on the plate and gently press the disk with the forceps to ensure complete contact with the agar surface. Replaced the lid to minimize exposure of the agar surface to room air.
- ✓ Continue to place one disk at a time onto the agar surface until all disks have been placed as directed.
- ✓ Once all disks are placed, replaced the lid, invert the plates, and placed them in a 37°C air incubator for 16 to 18 hours. After overnight incubation plate was taken out from incubator and measured the zone of inhibition with slide calipers.

Results and Discussions

4.1.1 *Escherichia coli* resistance pattern

*Escherichia coli*have been subjected to the evaluation of sensitivity against antibiotics. The samples were collected from the urine, wound swab and pus. The samples were subjected to sensitivity test against various conventionally used antibiotics by observing zone of inhibition which was produced by the antibiotics. Though *E.coli* is highly susceptible to resistanceagainst antibiotics, some *E. coli* found sensitive against all the antibiotics e.g amoxicillin, tetracycline, ceftriaxone andPiperacillin Tazobactam. Among all these antibiotics amoxicilli, tetracycline,showed zone of inhibition 10.11m and 9.02mm respectively. Third and fourth generation of antibiotics showed a good sensitivity against this *E. coli.*

Though cotrimoxazole is used widely as a choice of drugs for the urinary tract infections but in all cases it showed resistance. The detail result of this antibacterial study has been presented at tabular form.

Name of Patient: Parvin
Sex: Female
Age: 30 years
Sampled from: Wound Swab

Table 4.1.1:Antibacterial Sensitivity Study of *E. coli*against conventional antibiotics

Antibiotics	Zone of inhibition(mm)
Amoxicillin	10.11
Tetracycline	9.02
Vancomycin	Resistant
Cefuroxime	Resistant
Ceprofloxacin	Resistant

Antibiotics	Zone of inhibition(mm)
Penicillin-G	Resistant
Gentamycin	Resistant
Ceftriaxone	4.19
Piperacillin Tazobactam	5.3
Levofloxacine	Resistant
Nitrofurantoin	4.4
Cefixime	Resistant
Azithromycin	Resistant
Cephradine	Resistant
Erythromycin	Resistant
Kanamycin	Resistant
Cotrimozazol	Resistant

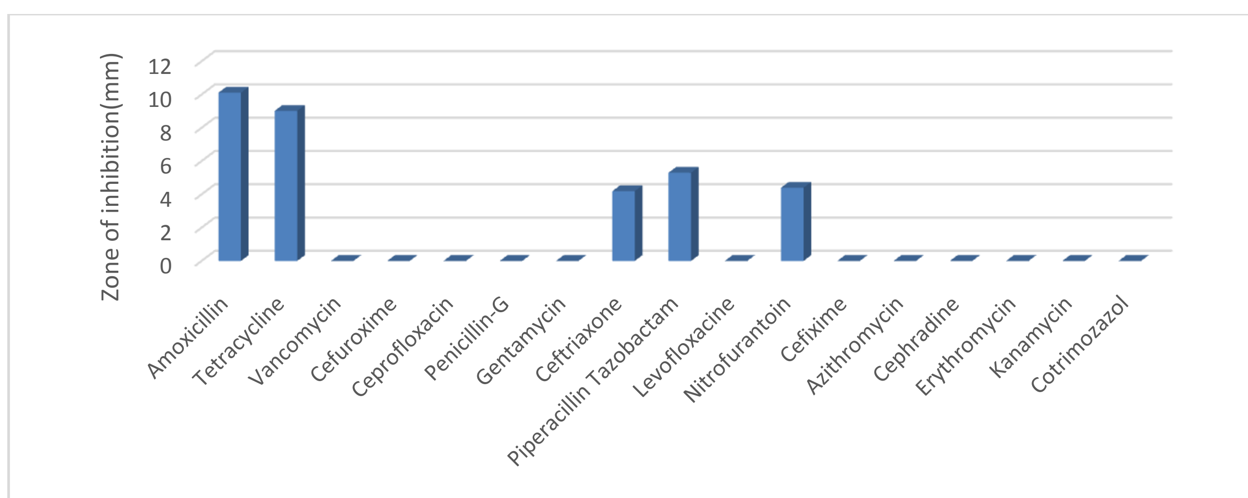

Figure 4.1.1. A graphical presentation of conventional antibiotics against *E-coli*

Name of Patient: Sohrab

Sex: Male

Age: 25 years

Sampled from: Pus

Table 4.1.2:Antibacterial Sensitivity Study of *E. coli* against conventional antibiotics

Antibiotics	Zone of inhibition(mm)
Amoxicillin	Resistant
Tetracycline	5.1
Vancomycin	4.2
Cefuroxime	Resistant
Ceprofloxacin	Resistant
Penicillin-G	Resistant
Gentamycin	9.98
Ceftriaxone	21.92
Piperacillin Tazobactam	8.44
Levofloxacine	12.21
Nitrofurantoin	12.30
Cefixime	Resistant
Azithromycin	Resistant
Cephradine	Resistant
Erythromycin	Resistant

Antibiotics	Zone of inhibition(mm)
Kanamycin	11.65
Cotrimozazol	Resistant

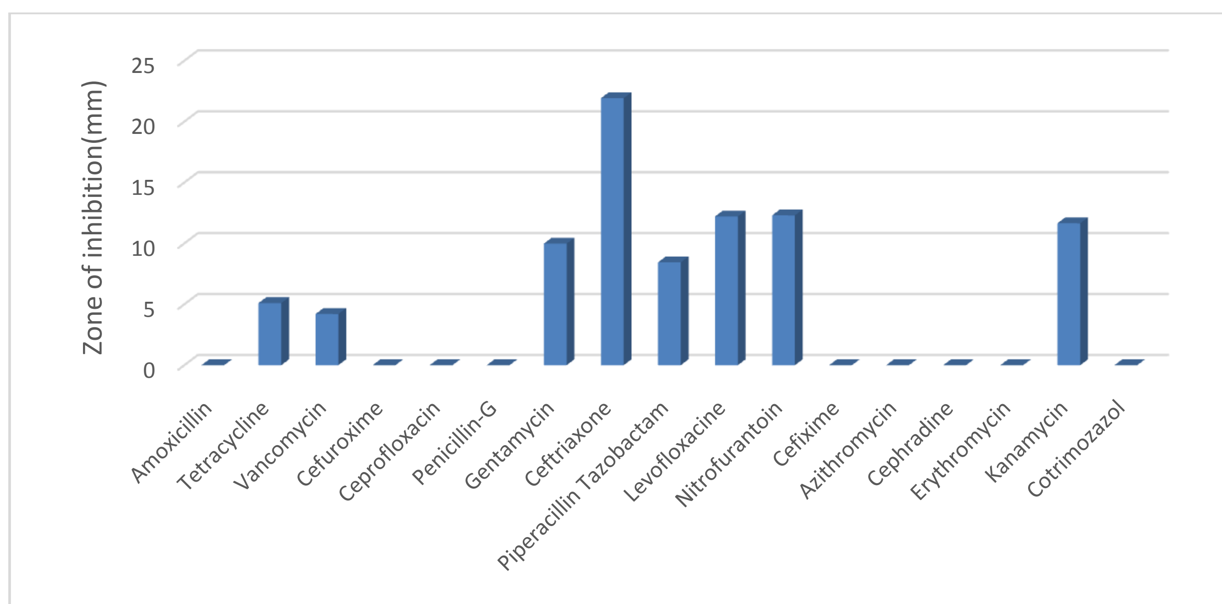

Figure 4.1.2: A graphical presentation of conventional antibiotics against *E-coli*

We know that *Escherichia coli* is one of the most frequent causes of many common bacterial infections, including cholecystitis, bacteremia, cholangitis, urinary tract infection (UTI), and traveler's diarrhea, and other clinical infections such as neonatalmeningitis and pneumonia.

These infections are treated with third generation cephalosporins (eg.Ceftriaxone, fluroquinolones, nitrofurantoin and co-trimoxazole). Antimicrobials known to be useful in cases of traveler's diarrhea include doxycycline, trimethoprim/sulfamethoxazole (TMP/SMZ), fluoroquinolones.Urinary tract infections caused by E. coli are usually treated with cephalosporins, fluroquinolones, macrolides, co-trimoxazole, nitrofurantoin, amoxicillin.

In our study we used penicillins (amoxicillin,piperacillin), first generation cephalosporins, second generation cephalosporin(cefuroxime),third generation cephalosporins, fluroquinolones, aminoglycosides, macrolides, co-trimoxazole, vancomycin, nitrofuratoin. These antibiotics shows sensitivity to the organisms except co-trimoxazole and vancomycin.

4.1.2.*Entarobactor spp.*resistance pattern

An Entarobactor spp have been subjected to the evaluation of sensitivity against antibiotics. The sample was collected from vaginal swab of a 50 year old female patient. The sample was subjected to sensitivity test against various conventionally used antibiotics and by observing zone of inhibition is produced by the antibiotics. In this particular case study only few of the antibiotics e.g. Ciprofloxacin, Azithromycin, Erythromycin, Kanamycin and Cotrimazazol showed slide sensitivity against this clinical isolates. Rest of the antibiotics showed resistance with no zone of inhibition. The detail result of this antibacterial study has been presented in the Table.

Name ofPatient: Sohrab
Sex: Male
Age: 25 years
Sampled from: Pus

Table 4.1.3:Antibacterial Sensitivity Study of *Entarobactor spp* against conventional antibiotics.

Antibiotics	Zone of inhibition(mm)
Amoxicillin	Resistant
Tetracycline	Resistant
Vancomycin	Resistant
Cefuroxime	Resistant
Ceprofloxacin	Resistant
Penicillin-G	Resistant
Gentamycin	Resistant
Ceftriaxone	Resistant

Antibiotics	Zone of inhibition(mm)
Piperacillin Tazobactam	12.76
Levofloxacine	Resistant
Nitrofurantoin	Resistant
Cefixime	Resistant
Azithromycin	Resistant
Cephradine	Resistant
Erythromycin	Resistant
Kanamycin	Resistant
Cotrimozazol	Resistant

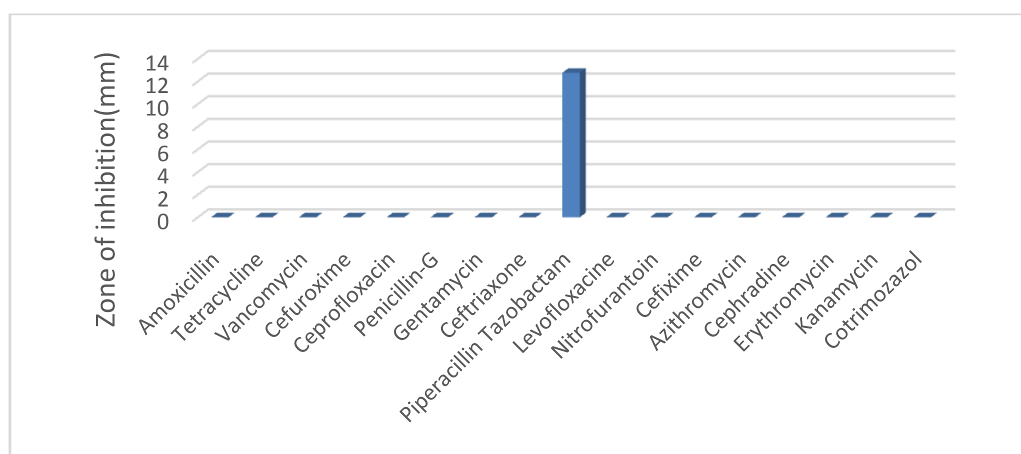

Figure 4.1.3. A graphical presentation of conventional antibiotics against *Enterococci spp.*

Important clinical infections caused by *Enterococcus spp.* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis. In this study, we found that the patient was completely resistant against most of the antibiotics except Piperacillin Tazobactam. Combination therapy with a cell wall–active agent (eg, penicillins, vancomycin)

and an aminoglycoside (eg, gentamicin, streptomycin) is necessary to adequately treat enterococcal endocarditis.

4.1.3.*Klebsiella spp*resistance pattern.:

Klebsiella spp. is one of the world's most dangerous superbugs;and becoming resistant to virtually every antibiotic available today.

The bacterium is non-motile, rod-shaped, Gram-negative, opportunistic, and gammaproteobacterium of the family *Enterobacteriaceae*; and it is a close relative of many familiar genera,such as *Citrobacter, Escherichia, Enterobacter, and Salmonella*(Minyahil,2015).

A Klebsiella spp. have been subjected to the evaluation of sensitivity against antibiotics.

The sample was collected from urine of 80 years old female patient. The sample was subjected to sensitivity test against various conventionally used antibiotics and by observing zone of inhibition is produced by the antibiotics. In this particular case study only three antibiotics e.g. Penicillin-G*,* Cefixime andCotromazazol *s*howedresistance against this clinical isolates, rest of the antibiotics e.g. showed sensitivity with zone of inhibition. The detail result of this antibacterial study has been presented in the Table.

Name of Patient: Sohrab
Sex: Male
Age: 25 years
Sampled from: Pus

Table 4.1.4: Antibacterial Sensitivity Study of *Klebsiella.spp* against conventional Antibiotics.

Antibiotics	Zone of inhibition(mm)
Amoxicillin	5.32
Tetracycline	17.75
Vancomycin	18.39
Cefuroxime	5.82
Ceprofloxacin	25.11
Penicillin-G	Resistant
Gentamycin	16.54
Ceftriaxone	11.37
Piperacillin Tazobactam	18.63
Levofloxacine	14.47
Nitrofurantoin	18.25
Cefixime	Resistant
Azithromycin	13.69
Cephradine	8.64
Erythromycin	21.28
Kanamycin	12.51
Cotrimozazol	11.02

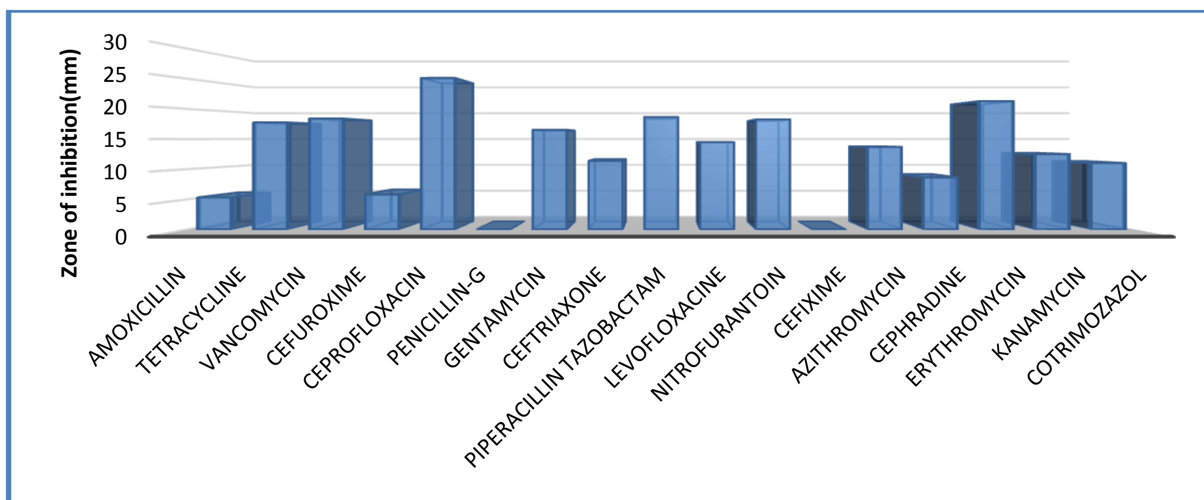

Figure 4.1.4: A graphical presentation of conventional antibiotics against *Klebsiella spp.*

Klebsiella organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, meningitis, diarrhea and soft tissue infections. *Klebsiella* species have also been implicated in the pathogenesis of ankylosing spondylitis and other spondyloarthro pathies.

Agents with high intrinsic activity against *K pneumoniae* should be selected for severely ill patients. Examples of such agents include third-generation cephalosporins (eg, ceftriaxone), carbapenems (eg, imipenem/cilastatin), aminoglycosides (eg, gentamicin, amikacin), and quinolones. These agents may be used as monotherapy or combination therapy.

Other antibiotics used to treat susceptible isolates include piperacillin, ceftazidime, cefepime, levofloxacin, norfloxacin, gaitfloxacin. For Nosocomial *K pneumoniae* pneumonia, antibiotics with high intrinsic activity are used. A regimen that includes imipenem, third-generation cephalosporins, quinolones, or aminoglycosides may be used alone or in combination.

For that we tried to cover the antibiotics range which are usually used for the treatment of *Klebsiella spp*.infections; and our study showed that penicillins(amoxicillins,piperacillins)cephradine (1^{st}generation cephalosporin),cefuroxime(2^{n}generation cephalosporin),ceftriaxone,cefixim (3^{rd}generation cephalosporin),cefepime (4^{th}generation cephalosporin),ciprofloxacin(2^{nd}generation fluroquinolone), levofloxacin (third eneration fluroquinolone),co-

timoxazole,vancomycin,erythromycin are resistant to this clinical isolate which we were collected.

4.1.4 *Salmonella peratyphi* resistance pattern

Enteric fever is a serious bacterial infection caused by *Salmonella enterica*serovars *Typhi* (*S.Typhi*) and *Paratyph*i *(S. Paratyphi)*, *S.Typhi* is more prevalent than *S.* Paratyphi A globally, with the best estimates predicting approximately 21 and 5 million new infections with each serovar per year, respectively. Both *S.typhi* and *S.paratyphi* A are systemic pathogens that induce clinically indistinguishable syndromes (Muhammad., A. et al.2012).

However, they exhibit contrary epidemiologies, different geographical distributions, and different propensities to develop resistance to antimicrobials. Additionally, they are genetically and phenol typically distinct, having gone through a lengthy process of convergent evolution to cause an identical disease.

Name of Patient: Saiful

Sex: Male

Age: 12 years

Sampled from: Blood

Table4.1.5:Antibacterial Sensitivity Study of *Salmonella spp.* against conventional antibiotics

Antibiotics	Zone of inhibition(mm)
Amoxicillin	26.11
Tetracycline	20.41
Vancomycin	14.44
Cefuroxime	24.01

Antibiotics	Zone of inhibition(mm)
Ceprofloxacin	30.1
Antibsiotic	Zone of inhibition(mm)
Penicillin-G	Resistant
Gentamycin	Resistant
Ceftriaxone	31.10
Levofloxacine	24.35
Nitrofurantoin	16.33
Cefixime	25.0
Azithromycin	20.1
Cephradine	18.52
Erythromycin	14.03
Kanamycin	17.53
Cotrimozazol	26.42
Penicillin-G	Resistant

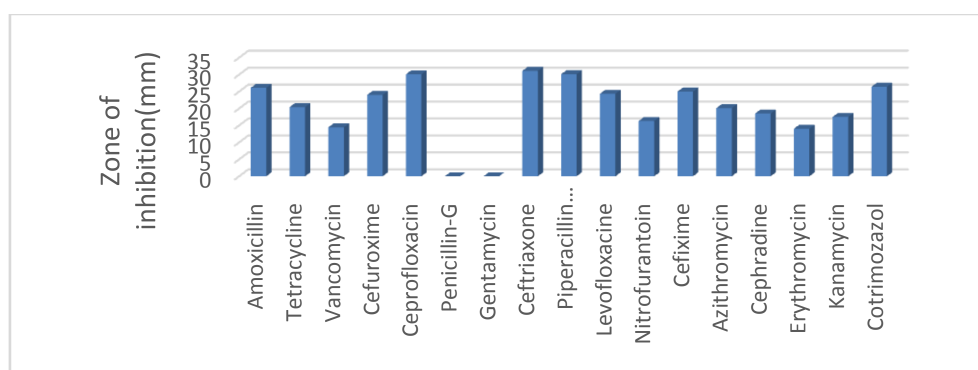

Figure 4.1.5: A graphical presentation of conventional antibiotics against*Salmonella spp.*

4.1.5. *Staphylococcus aureus*

The ability of *Staphylococcus aureus* to adhere to the extracellular matrix and plasma proteins deposited on biomaterials is a significant factor in the pathogenesis of orthopaedic-device related infections. *S. aureus* possesses many adhesion proteins on its surface, but it is not known how they interact with each other to form stable interactions with the substrate.

A novel method was developed for extracting adhesions from the *S. aureus* cell wall, which could then be further analyzed. The protocol involves using a FastPrep instrument to mechanically disrupt the cell walls resulting in native cell walls. Ionically and covalently bound proteins were then solubilized using sodium dodecyl sulphate (SDS) and lysostaphin, respectively. Western blot analysis of covalently bound proteins using anti-protein A and anti-clumping factor A sera showed that *S. aureus* produces most surface proteins in early growth, and less in post-exponential and stationary growth. Immuno-gold labelling of protein A, and clumping factor A was observed all over the bacteria and showed no distinct surface distribution pattern. However, this labelling showed expression of surface associated proteins varied in a growth-phase dependent and cell-density dependent manner.

Name of Patient: Naila
Sex: Female
Age: 20 years
Sampled from: Blood

Table 4.1.6:Antibacterial Sensitivity Study of *Staphylococcus aureus* against conventional antibiotics

Antibiotics	Zone of inhibition(mm)
Amoxicillin	Resistant
Tetracycline	Resistant
Vancomycin	15
Cefuroxime	Resistant

Antibiotics	Zone of inhibition(mm)
Ceprofloxacin	30
Penicillin-G	Resistant
Gentamycin	20
Ceftriaxone	12
Piperacillin Tazobactam	30
Levofloxacine	18
Nitrofurantoin	Resistant
Cefixime	Resistant
Azithromycin	Resistant
Cephradine	Resistant
Erythromycin	Resistant
Kanamycin	Resistant
Cotrimozazol	Resistant

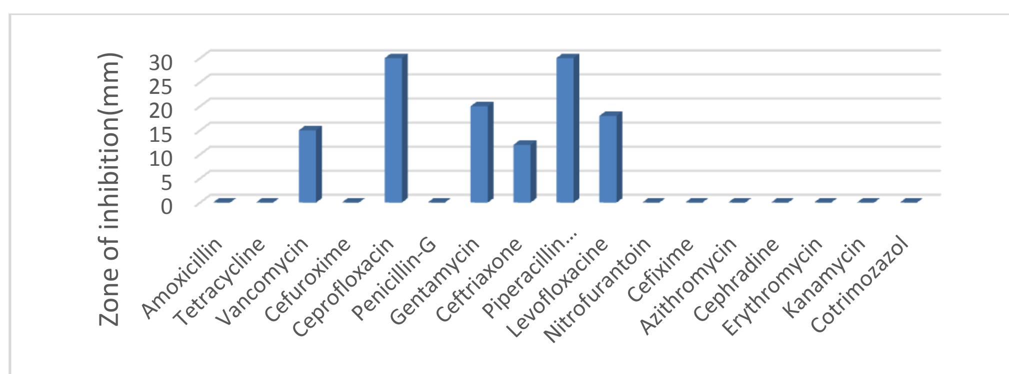

Figure 4.1.6:A graphical presentation of conventional antibiotics against *Staphylococcus aureus.*

4.1.6.*Pseudomonas spp*resistance pattern

Pseudomonas aeruginosa is a gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium that has an incredible nutritional versatility. It is a rod about 1-5 µm long and 0.5-1.0 µm wide. *P. aeruginosa* is an obligate respire, using aerobic respiration (with oxygen) as its optimal metabolism although can also respire anaerobically on nitrate or other alternative electron acceptors. *P. aeruginosa* can catabolize a wide range of organic molecules, including organic compounds such as benzoate. This, then, makes *P. aeruginosa* a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage, and hospitals. In all oligotropic aquatic ecosystems, which contain high-dissolved oxygen content but low plant nutrients throughout, *P.aeruginosa* is the predominant inhabitant and this clearly makes it the most abundant organism on earth .

P. aeruginosa is an opportunistic human pathogen. It is “opportunistic” because it seldom infects healthy individuals. Instead, it often colonizes immune-compromised patients, like those with cystic fibrosis, cancer, or AIDS. It is such a potent pathogen that firstly, it attacks up two thirds of the critically-ill hospitalized patients, and this usually portends more invasive diseases. Secondly, *P.aeruginosa* is a leading Gram-negative opportunistic pathogen at most medical centers, carrying a 40-60% mortality rate. Thirdly, it complicates 90% of cystic fibrosis deaths; and lastly, it is always listed as one of the top three most frequent Gram-negative pathogens and is linked to the worst visual diseases. Furthermore, *P.aeruginosa* is a very important soil bacterium that is capable of breaking down polycyclic aromatic hydrocarbons and making rhamnolipids, quinolones, hydrogen cyanide, phenazines, and lactins. It also exhibits intrinsic resistance to a lot of different types of chemotherapeutic agents and antibiotics, making it a very hard pathogen to eliminate (Muhammad, et al. 2012).

Name of Patient: Nazem
Sex: Male
Age: 10 years
Sampled from: Pus

Table 4.1.7:Antibacterial Sensitivity Study of *Pseudomonas spp* against conventional antibiotics

Antibiotics	Zone of inhibition(mm)
Amoxicillin	Resistant
Tetracycline	Resistant
Vancomycin	Resistant
Cefuroxime	Resistant
Ceprofloxacin	Resistant
Penicillin-G	Resistant
Gentamycin	Resistant
Ceftriaxone	Resistant
Piperacillin Tazobactam	9
Levofloxacine	Resistant
Nitrofurantoin	Resistant
Cefixime	Resistant
Azithromycin	Resistant
Cephradine	Resistant
Erythromycin	Resistant
Kanamycin	Resistant
Cotrimozazol	Resistant

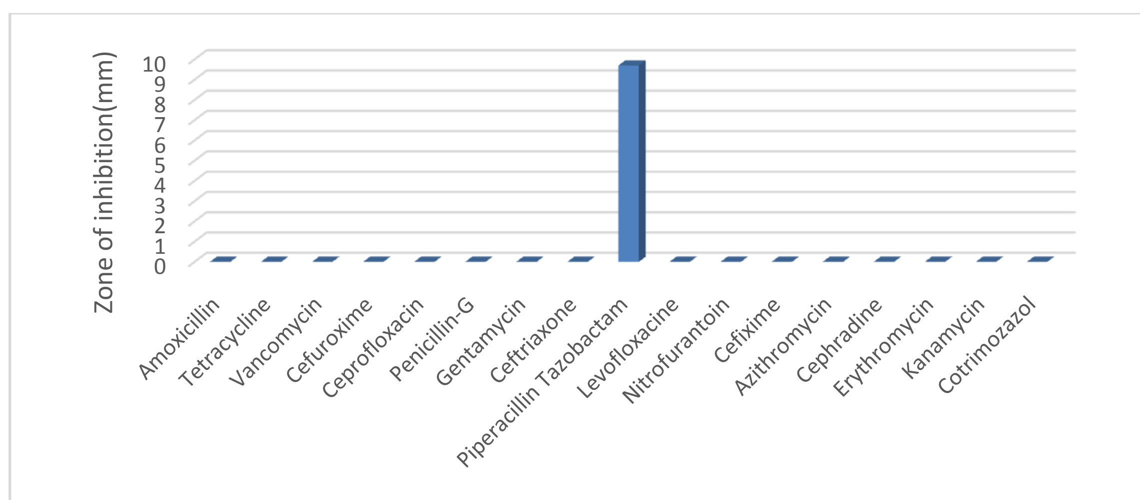

Figure 4.1.7: A graphical presentation of conventional antibiotics against *Pseudomonas spp*

Name of Patient: Elius

Sex: Male

Age: 75 years

Sampled from: Urine

Table 4.1.8:Antibacterial Sensitivity Study of *Pseudomonasspp.* against conventional antibiotics

Antibiotics	Zone of inhibition(mm)
Amoxicillin	16.1
Tetracycline	Resistant
Vancomycin	24.4
Cefuroxime	18.2
Ceprofloxacin	19.8
Penicillin-G	28.1
Gentamycin	20.0

Antibiotics	Zone of inhibition(mm)
Ceftriaxone	14.5
Piperacillin Tazobactam	28.1
Levofloxacine	24.7
Nitrofurantoin	Resistant
Cefixime	19.8
Azithromycin	17.4
Cephradine	Resistant
Erythromycin	Resistant
Kanamycin	23.9
Cotrimozazol	Resistant

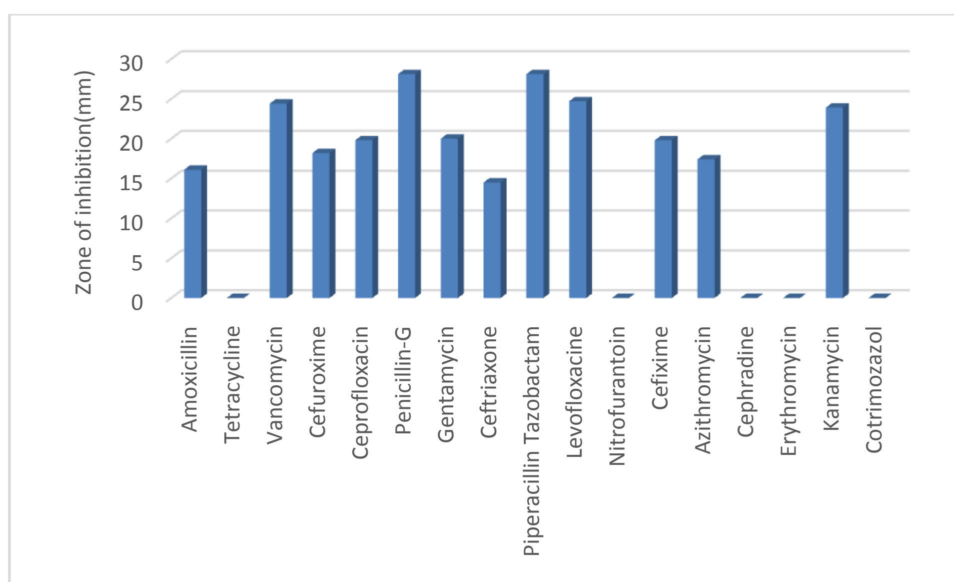

Figure 4.1.8: A graphical presentation of conventional antibiotics against *Pseudomonas spp.*

Antibacterial Sensitivity of Cured Extract

4.2.1Antibacterial sensitivity test of Methanolic Extract of *Curcuma zedoaria*

This test is determined the sensitivity of curcuma against clinically isolated human pathogens. Antibacterial effect of the methanolic extractof the plant *Curcuma zedoaria* studied in different doses (100mg/ml, 200mg/ml and 400mg/ml) of the crude extract, using positive controls (*Erythromycin, Ceprofloxacin*) and Methanol as negative control.

Negative control:

Because of methanolic extract we used methanol as a negative control. Sterile filter paper(6mm in diameter) was used.For each and every single organism we use different disc for negative control.

Result:

- We use double disc for single dose and average them.
- Measured the zone of inhibition with slide calipers.

Table 4.2.1: Sensitivity test of*Curcuma zedoaria.*

Name of the Bacteria	Dose			Positive Control		Negative Control
	100mg/ml (Average)	200mg/ml (Average)	400mg/ml (Average)	*Erythromycin*	*Ceprof-loxacin*	Methanol
Entarobact-or spp.	Nil	9.13 mm	10.12mm	22mm	22mm	Nil
E.coli	Nil	6.52mm	6.44mm	31mm	27mm	Nil
Klebsiella spp.	Nil	6.38mm	7.60mm	25mm	21mm	Nil
Salmonella peratyphi	Nil	Nil	6.55mm	25mm	21mm	Nil
Staphyloco-ccus aureus	Nil	Nil	7.71mm	25mm	21mm	Nil
Psedomons spp.	Nil	6.50 mm	8.08mm	30mm	28mm	Nil

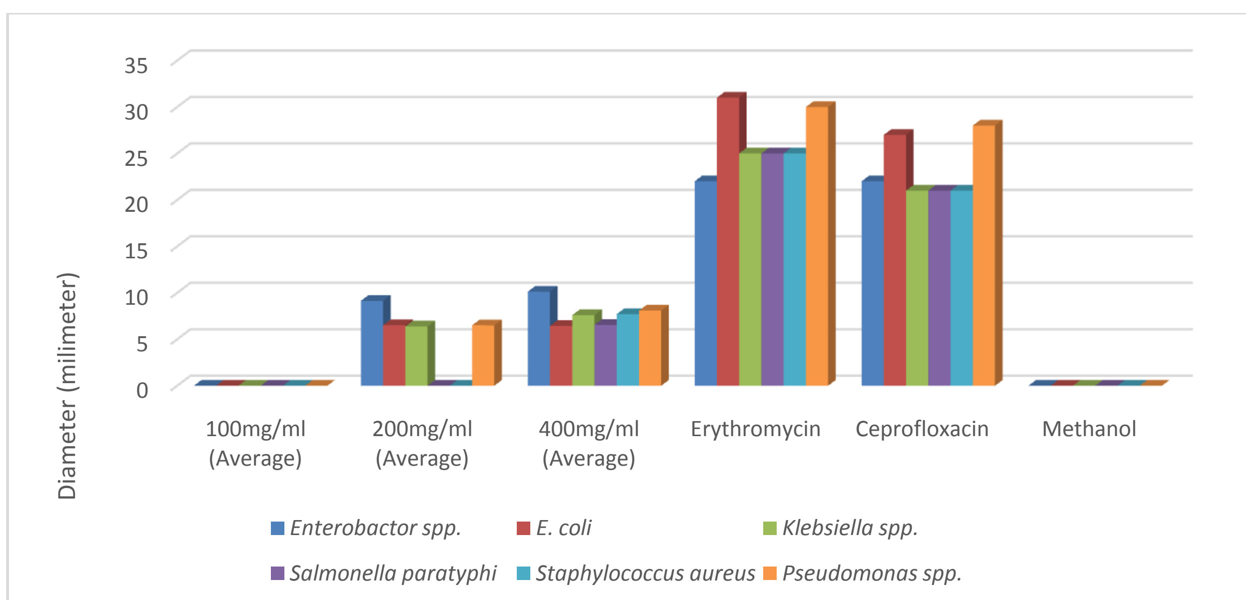

Figure4.2.1: Graphical Presentation of antibiotic sensitivity of plant extract of *Curcuma zedoaria by* Disc diffusion method at different doses

4.2.2. Antibacterial sensitivity test of Methanolic Extract of *Staphania japonica.*

This test is determined the sensitivity of *Staphania japonica*against clinically isolated human pathogens. Antibacterial effect of the methanolic extract of*Staphania japonica*studied in different doses (100mg/ml, 200mg/ml and 250mg/ml and 500mg/ml) of the crude extract, using positive controls (*Erythromycin, Ceprofloxacin*) and Methanol as negative control.

Table4.2.2 : Sensitivity test of *Staphania japonica.*

Name of the Bacteria	Dose				Positive Control		Negative Control
	100mg/ml (Average)	200mg/ml (Average)	250mg/ml (Average)	500mg/ml (Average)	*Erythromycin*	*Ceprofloxacin*	Methanol
Entarobactor spp.	8.12mm	9.53 mm	14.12mm	15.31mm	28mm	31mm	Nil
E.coli	Nil	Nil	6.44mm	6.56mm	18mm	25mm	Nil
Klebsiella spp.	7.04mm	8.41mm	10.10mm	11.50mm	26mm	30mm	Nil
Salmonila peratyphi	Nil	Nil	11.02mm	14.05mm	22mm	19mm	Nil
Staphylococcus aureus	7.51mm	8.31mm	9.41mm	9.93mm	25mm	30mm	Nil
Psedomonas spp.	6.53mm	7.03mm	9.08mm	10.03mm	21mm	25mm	Nil

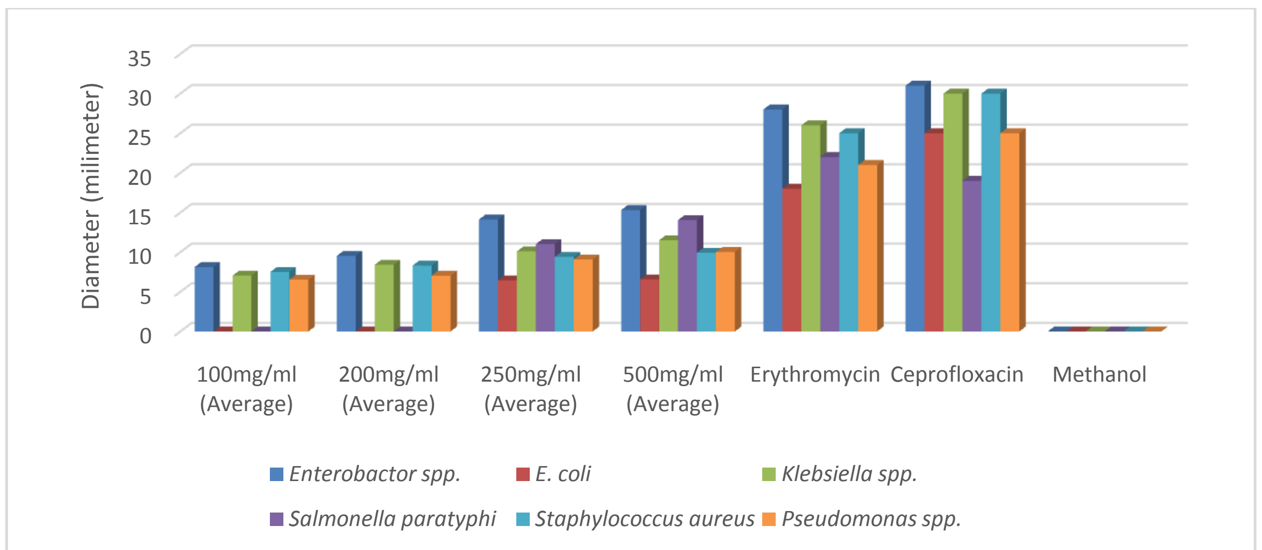

Figure4.2.2: Graphical Presentation of antibiotic sensitivity of plant extract of *Steaphania japonica by* Disc diffusion method at different doses

4.2.3 Antibacterial sensitivity test of Methanolic Extract of *Solanum verginianum.*

This test is determined the sensitivity of *Solanum verginianum* against clinically isolated human pathogens. Antibacterial effect of the methanolic extract of*Solanum verginianum*studied in different doses (100mg/ml, 200mg/ml and 400mg/ml) of the crude extract, using positive controls (*Erythromycin, Ceprofloxacin*) and Methanol as negative control.

Table4.2.3: Sensitivity test of *Solanum Verginianum*

Name of the Bacteria	Dose			Positive Control		Negative Control
	100mg/ml (Average)	200mg/ml (Average)	400mg/ml (Average)	*Erythromycin*	*Ceprofl-oxacin*	Methanol
Entarobactor spp.	Nil	8.51mm	8.12mm	22mm	22mm	Nil
E.coli	Nil	Nil	6.44mm	31mm	27mm	Nil
Klebsiella spp	Nil	6.38mm	7.60mm	21mm	23mm	Nil
Salmonella peratyphi	Nil	Nil	6.95mm	25mm	21mm	Nil
Staphylococcus aureus	Nil	Nil	7.71mm	25mm	21mm	Nil
Psedomons spp.	Nil	6.50 mm	8.08mm	30mm	28mm	Nil

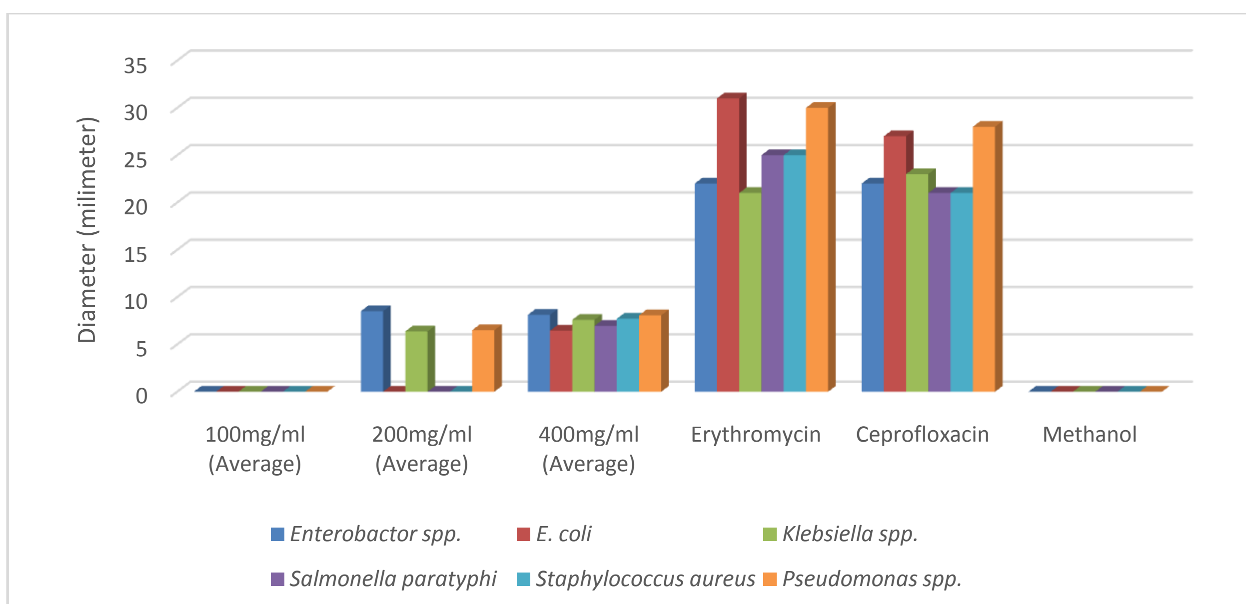

Figure 4.2.3: Graphical Presentation of antibiotic sensitivity of plant extract of *Solanum Vergininum by* Disc diffusion method at different doses

Discussion

From the result we can said that most of the bacteria are multi drug resistant. From sensitivity test of cured drug we observed that the few of multi drug resistant bacteria are sensitive against crud extract of different plants. If we do further test we can develop a new molecule for resistant bacteria.

Conclusion

Infectious diseases are the second-major cause of death worldwide and the third-leading cause of death in economically advanced countries. Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent and represent a priority health problem; hence, the problem of antibiotic resistance needs an urgent response. Developing a new antibiotic can take years and millions of dollars. Therefore, in the meantime, the rational use or retrieval of old antibiotics, like co-trimoxazole and other may be a short-term solution. Here, we focus our analysis on a natural antibiotic compound produced by several crud extract on,*E.coli, Salmonella spp, Kleblisella spp.* and *Pseudomonas* species, exerting a powerful bactericidal activity against a wide range of Gram-negative and Gram-positive bacteria.

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