2021-07-26

ANTIMICROBIAL ACTIVITY OF ACTINOMYCTES AND DRUG RESISTANCE PROFILE OF COMMON BACTERIA ISOLATED FROM MUNICIPAL WASTE DUMP SITE INBAHIR DAR CITY, ETHIOPIA

AYALEW, FIKREMARIAM

BAHIR DAR UNIVERSITY

COLLEGE OF SCIENCE

DEPARTMENT OF BIOLOGY

ANTIMICROBIAL ACTIVITY OF ACTINOMYCTES AND DRUG RESISTANCE PROFILE OF COMMON BACTERIA ISOLATED FROM MUNICIPAL WASTE DUMP SITE INBAHIR DAR CITY, ETHIOPIA

BY:

FIKREMARIAM AYALEW YIMER

JUNE, 2021

BAHIR DAR UNIVERSITY, ETHIOPIA

,

Bahir Dar University
College of Science
Department of Biology

Antimicrobial activity of actinomycetes and drug resistance Profile of common bacteria isolated from municipal waste Dump site in Bahir Dar city, Ethiopia

By:
Fikremariam Ayalew Yimer

Advisor:
Baye Sitotaw (PhD)

Co-advisor:
Kindu Geta (PhD candidate)

A thesis Submitted to the Department of Biology of Bahir Dar University in Partial Fulfillment of the Requirements for the Degree of Masters in Biology (Applied Microbiology).

JUNE, 2021
Bahir Dar University, Ethiopia

Approval sheet

Submitted by:

Fikremariam Ayalew _____ _____

Name of Student Signature Date

As a thesis advisor, I certify that I have read and evaluated this thesis prepared under my guidance by Fikremariam Ayalew entitled **"**Antimicrobial activity of actinomycetes, and drug resistance profile of bacteria isolated from municipal waste dump site in Bahir Dar city, Ethiopia and I recommended the thesis paper to be submitted as fulfilling the requirements for the degree of master in Applied Microbiology.

Approved by all listed below:

1. Baye Sitotaw (PhD) _____ _____

Name of advisor Signature Date

2. Kindu Geta (Ph.D. Candidates) _____ _____

Name of co-advisor Signature Date

3**.** _____ _____ _____

Name of Internal Examiner Signature Date

4**.**_____ _____ _____

Name of External Examiner Signature Date

5. _____ _____ _____

Name of chairman Signature Date

Declaration

I declared that the work in this Thesis "**Antimicrobial activity of actinomycetes and drug resistance Profile of common bacteria isolated from municipal waste dump site in Bahir Dar city, Ethiopia**" was performed by me in the Department of Biology Stream of Micro Biology. This thesis has been submitted in partial fulfillment of the requirements for the award of a Master's Degree in Biology (Applied Microbiology) of Bahir Dar University.

Declared by:

Name: Fikremariam Ayalew Yimer
Signature:_____
Date: _____

Acknowledgment

In all circumstances I wish to express my sincere gratitude from the bottom of my heart, to my advisor Dr. Baye Sitotaw and co-advisor Kindu Geta (PhD) candidate for their unreserved and constructive guidance, advices and comments starting from the development of the thesis proposal to the safe completion of the thesis paper. I am also grateful to Mr. Misganew in assist the laboratory activity & contribute in Lab orientation at the beginning of laboratory activity.

My heartfelt thank is extended to my Classmates for sharing their accumulated knowledge about my activities and, share their materials and equipment in my laboratory activities. I am also grateful to the store man of Biology Departments and chemistry Department in losing their break time to give the laboratory media and reagents and other resources. My grand thank goes to my families for their moral and material supports while doing the research which was quit significant for the success of the work.

Table of contents

List of tables

List of Figures

List of Abbreviations and Acronyms

AMR:	Antimicrobial Resistance
APHI:	Amhara Public Health Institution
ARB:	Antimicrobial Resistant Bacteria
ARGs:	Antimicrobial Resistant Genes
AST:	Antimicrobial Susceptibility Test
CLSI:	Clinical and Laboratory Standards Institute
EPA:	Environmental Protection Agency
HGT:	Horizontal gene transfer
MBC:	Minimum Bactericidal Concentration
MDR:	Multi drug Resistance
MGEs:	Mobile genetic elements
MHA:	Mueller Hinton Agar
MIC:	Minimum Inhibitory Concentration
NA:	Nutrient Agar
SCA:	Starch Casein Agar
SW:	Solid Waste
UNEP:	United Nations Environment Program
USEPA:	United States Environmental Protection Agency

Abstract

The emergence of multidrug resistance (MDR) at an alarming rate among the microbial population has been a public health concern. Globally, finding new antimicrobials that can kill multidrug-resistant pathogens is thus a hot research area. Bacteria, specifically actinomycetes, are the most prolific producers of antibiotics. Municipal waste has been a reservoir for multidrug resistance pathogens due to the unwise disposal of leftover drugs from households. This study aimed to identify multidrug resistance and antimicrobial-producing bacteria in a municipal solid waste soil from dump site in Bahir Dar, Ethiopia. Thirty soil samples were randomly collected from the municipal waste dump site in three rounds from February up to June, and analyzed by using SPSS soffit ware version 26. Actinomycetes and other bacteria were isolated from these samples using the spread plate method on starch casein, and nutrient agar medium respectively. The isolates were screened for antimicrobial activity against at least one bacterial test strain by perpendicular streaking. Isolates showing antimicrobial activity were further screened for better potential using an agar well and disk diffusion method from an ethyl acetate crude extract of a bacterial culture. Isolates showing both antimicrobial activity and drug resistance were identified using cultural methods. Thirty nine bacterial isolates were subjected to different antibiotic disks to test antibiotic susceptibility tests by the standard Kirby-Bauer's disc diffusion method. Regarding the drug resistance profile, of a total of Thirty nine bacterial isolates, 38.4% were resistant; 21.74% of isolates were intermediate and 39.86% of bacterial isolates were susceptible to selected antibiotic disks and generally 46.15% of the bacterial isolates were multidrug resistance, from this the most MDR isolates were *S. aureus* & *E.coli*. According to this study, for chloramphenicol and ciprofloxacin most isolates were susceptible, but for amoxicillin and Nalidixic acid most bacterial isolates were resistance. *S.aureus* and *E.coli* were shows the most resistant from the other isolate. From a total of 70 actinomycetes, 20 (28.57%) showed antimicrobial activities with the inhibition zone ranging from 0.0±0.0 up to 26.0±1.0 mm on average, in both agar well and disk diffusion methods. Twenty effective actinomycetes were selected for MIC & MBC, and they have the value of MIC and MBC between 250µg/ml to 500250µg/ml. Based on antibacterial activities, two isolates were selected for the MIC and MBC tests against *E.coli* and *S. aureus*. From the present study it could be conclude that sebatamite municipal waste site was good source of antibiotic producing actinomycetes and it also contain most abundance of multidrug resistance. Further work needs to identify all antibiotic producing and drug resistance microorganism up to species level by using molecular characterization.

Key words: Antimicrobials, Actinomycetes, bacteria, drug resistance, waste dump sites.

1: INTRODUCTION

1.1. Background of the study

Municipal waste can be described as waste collected by or for municipalities and handled by them. It includes household waste, including bulky waste, related industrial and commercial waste, office buildings, organizations, and small businesses, as well as farm and garden waste, street sweeping, litter containers, and market cleaning waste if handled as household waste. The concept excludes waste from municipal sewage and treatment networks, as well as waste from the activities of construction and demolition. This indicator is measured in kilograms per capita and thousands of tonnes. It is possible to categorize urban waste into two groups (solid waste and liquid waste).

Antibiotic producing bacteria, especially actinomycetes, are a large group of phylogenetically related, filamentous, and aerobic Gram-positive bacteria that occur in natural and manmade environments (Aghamirian and Ghiasianm, 2009). They are widely distributed in soil, lakes, fresh and marine water bodies, sediment, manure, and compost, as well as waste sites and food products. They are one of the major groups of the soil population. However, their diversity and distribution may vary depending on the physical, chemical, and geographical factors of the soil (Gurung *et al*., 2009).

Secondary metabolites (specialized metabolites, poisons, by-products, or natural products) are organic compounds generated by bacteria, fungi, plants, or actinomycetes that are not directly involved in the organism's normal growth, development, or reproduction. Instead, they generally mediate ecological interactions, which may produce a selective advantage for the organism by increasing its survival ability or fecundity. Among these organisms, actinomycetes play a significant role in the production of antibiotics. This is because they have a great capability to synthesize and provide a wide variety of bioactive substances which are confirmed in numerous institutional and industrial laboratories for commercial and medical values (Sundaramoorthi *et al*., 2011).

Antibiotics are secondary metabolites that are isolated, extracted, and originally formed by most of the different types of soil microorganisms and used in a broad range of pathogens (Skold, 2011). There are so many different potential sources where antibiotics can be discovered, including

medicinal herbs (Ferrer *et al*., 2017) of which soil still remains the most important target site for most researchers in their efforts to discover new antibiotics that have pharmaceutical values. It is obvious that, most of the antibiotics used today were discovered from soil microbes (Sandhya *et al*., 2012). The antibiotic diversities in the soil depend on the availability of nutrients, soil type, temperature, pH, moisture, and aeration of the soil (Nasfi *et al*., 2018).

Recently, the discovery of new bioactive compounds from terrestrial actinomycetes has greatly decreased, whereas the rate of re-isolation of known compounds has increased. Thus, it is important to find less exploited genera of rare actinomycetes from unexplored or underexplored habitats that can produce novel antimicrobial compounds (Wadetwar and Patil, 2013). This is true due to the geographical variation in different parts of the world, which leads to variations in the soil type. That results in variations in the type of isolated Actinomycetes species and bioactive metabolites. Waste dumping sites are one habitat that is rich in microorganisms. This is because the majority of the refuse dump composition is biodegradable waste, which comprises nutrients with various components for their growth and metabolism (Chetan *et al*., 2017). Recently, waste dumps have received more attention from the scientific community in the search for industrially and medically important microorganisms that can produce unique biologically active enzymes, metabolites, and novel commercially important products (Mandal *et al*., 2019). Therefore, there is an immense possibility to screen these industrially and biotechnologically important actinomycetes and other bacteria from Sebatamite Municipal waste dumpsite for the production of secondary metabolites like antibacterial in Bahir Dar City, Ethiopia.

On the other hand, the emergence and spread of Antimicrobial Resistance (AMR) among pathogenic bacteria has been a growing problem for public health in recent decades (Rozman *et al*., 2020). Also, several studies in different parts of the world have reported the existence of Antimicrobial Resistant Genes (ARGs) and/or Antimicrobial Resistant Bacteria **(**ARB) from landfill leachate and solid waste disposal environments, with some being confirmed as pathogenic strains (Efuntoye *et al*., 2011; LaPara *et al*., 2011).

The occurrence of discarded antimicrobial agents and transferable (mobile) resistant genes within the surrounding environment is by far the most contributing factor to the emergence of antimicrobial resistance (AMR) organisms such as bacteria (Cycon *et al*., 2019). The effectiveness of antimicrobial agents against environmental microorganisms is undeniable, and some microbes use resistant determinants (genes) to avoid the threats posed by these antimicrobial agents

(Efuntoye *et al*., 2011).Resistance genes can develop either due to mutations (e.g. addition, deletion, substitution, etc.) or as a result of selection pressure by the utilization of antimicrobial agents (You *et al*., 2018). Recently, waste dumpsites have received more attention from the scientific community in the search for industrially and medically important microorganisms that can produce unique biologically active enzymes, metabolites, and novel commercially important products (Mandal *et al*., 2019). Furthermore, a wide variety of antibacterial producing and pathogenic microorganisms have been also reported to be present in these organic wastes ; Therefore, there is an immense possibility to screen diversified bacterial strains from Sebatamite municipal waste dumpsite in Bahir Dar town, Ethiopia.

1.2. Statement of the problem

Antimicrobial drugs used for therapeutic purposes for human, veterinary, and agricultural purposes favor the survival and spread of resistant organisms. Some antibiotics, for example, penicillin, erythromycin, and methicillin, which were effective against bacterial infectious diseases, are now less effective due to the acquisition of resistance to such antibiotics (Raja *et al*., 2010). Methicillin and vancomycin-resistant strains of S*taphylococcus aureus* and others cause an enormous threat to the treatment of serious infections. Because of this, treatment is becoming more complex and options for therapy are often limited worldwide. To overcome this problem, immediate replacement of existing antibiotics is necessary and the production of novel drugs that have unique mechanisms of action against drug-resistant pathogens is also important (Sundaramoorthi *et al*., 2011).

Around the world, improper disposal and continuous dumping of solid waste introduces several hazardous items such as heavy metals, expired pharmaceutical waste, residual antimicrobial agents, and pathogenic microorganisms that are thrown into common dump sites with refuse (Borquaye *et al*., 2019). In particular, the expired pharmaceutical and residual antimicrobial agents that are discharged randomly into the dumpsite exert a selection pressure in favor of resistant bacteria by killing or inhibiting the growth of susceptible bacteria (Wu *et al*., 2017; You *et al*., 2018), which often results in evolutionary changes (mutations) in the bacterial population. This leads to the mass increase of resistant bacteria as the competing microbiota has been wiped out by the dumped pollutants. The resistant strains of bacteria can also be accelerated and spread by the transfer of resistant genes among species and genera through horizontal gene transfer (HGT) with mobile genetic elements (MGEs) (Yu *et al*., 2016).

The study sites mainly focused on waste dumping sites, because waste dumping sites are habitats that provide a rich source of microorganisms. Because the majority of the refuse dump composition is biodegradable waste which contains nutrients with various components for their growth and

metabolism (Chetan *et al*., 2017). Due to this, recently, waste dumps have received more attention from the scientific community in the search for industrially and medically important microorganisms that can produce unique biologically active enzymes, metabolites, and novel commercially important products (Mandal *et al*., 2019).

Various studies on the prevalence of bacteria of public health importance on municipal waste dump sites, for example, have recently been reported (Achudume and Olawale, 2007; Addo *et al*., 2015; Williams and Hakam, 2016; Song'oro *et al*., 2019).According to Awisan *et al*. (2011), bacteria associated with clinical diseases such as *Staphylococcus aureus, pseudomonas aeruginosa, Klebsiella pneumoniae,* and *Escherichia coli* are aerobic and opportunistic bacteria in the Irisan dumpsite associated with clinical diseases, antibiotic-resistant enteric isolates (*Salmonella* spp., *Shigella* spp.*), and* Vibrio. Besides this, many studies have also recognized the importance of municipal solid waste as an important source of mobile genetic elements, antibiotic-resistant genes, and resistant microbes (Yu *et al*., 2016; Wu *et al*., 2017; You *et al*., 2018). Recent studies of Hrenovic *et al*. (2019) also confirmed that of all types of environments, the dumpsite soil probably has the largest and most divergent resistance which includes both bacteria with intrinsic and acquired antibiotic resistance.

In fact, geographical conditions and types of waste generated in one location vary from any other; and since microbial proliferation depends on the geographical conditions and available nutrients; it is logical that risks to public health from one municipal dumpsite cannot be the same elsewhere. Despite the poor solid waste management practice in most municipalities of the underdeveloped countries like Ethiopia, no scientific reports that that performed to test for antibacterial producing and antimicrobial-resistant bacteria from dumpsites sebatamite dumped municipal waste in Bahir Dar town. Since, antibiotic resistance profiles of the pathogenic bacteria may vary from country to country, regionally and, locally and can also change rapidly with time; as such, they need to be monitored and managed closely because of their public health implications and impacts (Manyi-Loh *et al*., 2018).

Therefore, this study is mainly aimed at the antibacterial-producing actinomycetes and drug resistance profile of isolated bacteria from sebatamite dumped municipal waste soil in Bahir Dar Town, Ethiopia. Further work needs to identify all antibiotic producing and drug resistance microorganism up to species level by using molecular characterization and antibiotic resistance profiles of the pathogenic bacteria may vary from country to country, regionally and, locally and can also change rapidly with time; as such, they need to be monitored and managed closely because of their public health implications and impacts

1.3. Objectives of the study

1.3.1. General objective

- To isolate and characterize antibacterial producing actinomycetes and drug resistance profile of common bacteria from Sebatamite, dumped municipal waste soil in Bahir Dar City, Ethiopia.

1.3.2. Specific objectives

- To isolate and characterized antibacterial producing actinomycetes from sebatamite dumped municipal waste soil,
- To evaluate the antagonistic activities of actinomycetes against some selected human pathogenic bacterial strains,
- To determine the drug resistance profiles of the bacterial isolates to some selected antimicrobial agents.

1.4. Significance of the study

The findings of the present study will be useful to many new researchers by serving as a framework and providing vital information on the occurrence of antibacterial producing actinomycete and give vital information about the occurrence of drug resistance bacteria from waste dump soil to study further in a similar area. This discovery may be significant for the scientific community as they look for new bioactive secondary metabolites, such as antibacterial from actinomycetes, from various sources. Besides, finding the antibacterial actinomycetes and drug resistance of bacteria that produce new antibacterial producing actinomycetes from waste dumping sites may support the existing drugs prescribed for clinical infections. Furthermore, this discovery isolates antimicrobial-resistant bacteria, which is critical for understanding the potential effects on the environment, public and animal health, and future intervention measures and to generate local knowledge input to tackle drug resistance.

2: LITERATURE REVIEW

2.1. Definition and classification of municipal solid waste

Any garbage or refuse sludge from a wastewater treatment plant, a facility treatment plant, or a pollution control facility, in addition to other discarded material, resulting from industrial, commercial, mining, and agricultural operations, as community activities, is taken into account solid waste. Almost everything we do generates some form of garbage. Solid waste includes refuse and trash from domestic, industrial, commercial, and institutional establishments such as hospitals, as well as market waste, yard waste, and street sweeping, which can be hazardous or nonhazardous.

Solid waste (SW) sources and types are numerous and vary in composition. According to Arukwe, (2012), the composition of SW is defined as the varying types of waste materials that it consists of and their characteristics. The composition of solid waste can vary from country to country, regionally, and locally because of the variation in population numbers, rapid urbanization, and the change in the living standards of the community. As reported by the United Nations Environment Program (UNEP), (2010), food waste, paper, plastics, textiles, rubber, leather, garden waste, wood and charcoal, glass, metals, ash and soil, and others are the main components of solid waste in Bahir Dar City.

2.2. Microorganisms producing antimicrobial compounds

There have been several antibiotics obtained and studied in the past, particularly from members of the Streptomyces genus (Tiwari and Gupta, 2012). It is estimated that the majority of antibiotics (70%) were discovered from the Streptomyces species alone during the1950s and 1960s (Tishkov, 2001). Such figures help us frame the obstacles facing future drug development efforts. The ABL database identifies over 8000 antimicrobial products of which Streptomyces alone produces about 45.6 percent (Lazzarini *et al*., 2001) while other members of actinomycetes genera produce (16.00%). Because many of the fungal products are either plant toxins or inhibitors of mammalian enzymes, fungi only produce 21.5 percent. Sixteen-point nine percent (16.9%) was produced by other bacteria (Lazzarini *et al*., 2001; Tiwari and Gupta, 2012).

2.3. Actinomycetes

2.3.1. Biology of actinomycetes

Actinomycetes are Gram-positive bacteria, belonging to the class Actinobacteria characterized by the substrate and aerial mycelium (Olano *et al.*, 2009; Hotam *et al*., 2013a). The hyphae or mycelia are non-septet, but septa may be observed in some species such as *Nocardia brevicatena* (Li *et al*., 2016). The fruiting bodies may be branching, straight, or spiral-shaped and become spherical, cylindrical or oval spores as reviewed in (Mukesh, 2014). Actinomycetes possess a rigid cell wall an organization that upholds the cell and thwarts it from bursting during high osmotic pressure. They have high guanine and cytosine (G+C) ratio of the DNA (>55mol %). They present one of the largest taxonomic units among the 18 foremost ancestries currently recognized within the domain bacteria (Prudence *et al.*, 2020; Hotam *et al*., 2013a).

The majorities of actinomycetes are aerobic or facultative anaerobes and have growth temperatures between 25^{o}C and 30^{o}C. However, thermophilic species grow at about 50^{o}C (Mukeshi, 2014) and certain actinomycetes namely *Actinoplanes, Amycolatopsis, Catenuloplanes, Dactylosporangium, Kineospora, Microbsispora, Micromonospora, Actinomycetospora, Nonomuraea* (Nair and Abraham, J. (2020) are slow-growing and are difficult to cultivate.

2.3.2. Ecology of actinomycetes

Actinomycetes are widely distributed in nature, but primarily in soil (George *et al*., 2012; Rinoy *et al*., 2012; Lekhak *et al*., 2018). They constitute a significant component of the microbial populations in most soils (10^{6}-10^{7} colony forming units per gram of soil) (Rinoy *et al*., 2012). Their distribution in the soil is influenced by geographical location, temperature, soil type, pH, organic matter content, agricultural activities, aeration, nutrient availability, moisture content, and soil vegetation (Arifuzzaman *et al*., 2010; Rinoy *et al*., 2012). Species distribution decreases as soil depth increases (Takahashi and Omura, 2003; Hotam *et al*., 2013a). These organisms play an important role in the recycling and mineralization of nutrients in the soil by degrading complex compounds which other organisms cannot degrade (Rinoy *et al*., 2012). Such species are found mostly in compost. They can fix nitrogen, solubilize nutrients, immobilize nutrients, and produce siderophores and biological control agents (Kekuda *et al*., 2010; Rinoy *et al*., 2012). The most dominant actinomycetes in the soil are the genus *Streptomycetes* although, others like *Norcardia,*

Microbispora, *Micromonospora*, *Actinomyces*, *Actinoplanes*, and *Streptosporangium* have also been isolated (Hotam *et al*., 2013b).

2.3.3. General Characteristics of Actinomycetes

Actinomycetes that can generate antibiotics have distinct characteristics, such as heterotrophic in nature, strict saprophytes, and some of them are plant and animal parasitic or mutually beneficial associations. They play various roles, such as nutrient recycling, when considering the role of Actinomycetes, and they are aerobic and others are anaerobic. Actinomycetes Species like *Frankia* require very specialized media for growth and conditions for incubation (Attwell and Colwell, 1984). The typical bacteriological media used in the laboratory, such as nutrient agar, trypticase agar, blood agar, brain heart infusion agar, and starch casein agar, are important for emerging actinomycetes. To enable differentiation and the production of characteristic spores and pigments, actinomycetes require unique media (starch casein).

Either one of these media is not commercially available and must be prepared using colloidal chitin, soil extract, and plant material decoctions in the laboratory. When the organism is sub-cultured on a more acceptable growth medium, such as oatmeal or inorganic salts, starch agar, pale, smooth, hard colonies of Streptomyces species on nutrient agar may be transformed into bright yellow colonies with powdery white aerial mycelium and spirals of arthrospores. Outgrowths from a mycelium spore or fragments develop into hyphae that penetrate the agar (substrate mycelium) and hyphae that branch repeatedly and cement together to form a rough, leathery colony on the surface of the agar. The colony's density and consistency are depending upon the medium's composition.

2.3.4. Isolation of actinomycetes

Isolation of actinomycetes from the soil sample is a very difficult tax because antibiotic producing actinomycetes are slow-growing and many factors need to be considered during isolation processes (Jiang *et al*., 2016). These include chemical pretreatment of the samples, use of specific selective media, the addition of antibacterial and antifungal agents in the media and culture conditions (Sharma *et al*., 2014).

Physical pretreatment of soil samples: Pretreatment of soil samples using physical treatments enhance isolation of a new strain of actinomycetes from soil samples includes air drying, dry heating, moist incubation at 120$_{o}$C for 1hour (h) and desiccation, differential centrifugation, rehydration and centrifugation, sucrose gradient centrifugation, Cellulose infiltration, pollen baiting

and drying methods (Sharma *et al*., 2011). Physical treatment of soil by air drying aids in the isolation of new strains of actinomycetes such as *Microbispora* and *Streptosporangium*, which can help to eradicate undesired bacteria, particularly non-spores (Hayakawa, 2007). Rare actinomycetes spores have been found to be more resistant to desiccation and heating than other microbe spores, particularly Gram-positive bacteria spores (Kavitha *et al*., 2010). Centrifugation of a phosphate buffered soil sample releases zoospores. Centrifugation thus increases the isolation of motile actinomycetes, which can float in the supernatant whereas non-motile actinomycetes are retained in the sediments during centrifugation. (Yamamura *et al*. (2005) used a sucrose gradient centrifugation approach to improve *Nocardia* spp. isolation from a soil sample. Putting the barrier between the culture of actinomycetes and agar apparent prevent the growth of non-filamentous bacteria by undelaying agar allowing only filamentous bacterial penetration of the underlying agar (Hayakawa, 2007). Contaminants bacteria can be eliminated by pollen grains of pinus bait. Bacteria colonize pollens floating on the outward of soil suspension. Unwanted bacteria are excluded by dehydrations of sporangia and the spores are liberated upon immersion in water.

Chemical treatment of soil sample: The use of chemical methods to enhance isolation of new strains of actinomycetes was reported by previous researchers (Zhang, 2011). Spores of some genera of actinomycete such as *Streptosporangium* and *Microbispora* can withstand treatment with sporicidal chemicals such as phenol, benzethonium chloride and chlorhexidine gluconate (Hong *et al*., 2009). The addition of macromolecules to the growth media, such as calcium carbonate, humic acid, casein, and chitin, has been shown to boost actinomycete development while inhibiting or hindering contaminating bacterial and fungal colonies (Kavitha *et al*., 2010). Actinomycetes use these inorganic and organic compounds as carbon and nitrogen sources (Zhang, 2011).

Pretreatments of soil with antimicrobial agents: The use of antibacterial and antifungal agents such as anisomycin, Cycloheximide, gentamicin, kanamycin, nalidixic acid, novobiocin, nystatin, penicillin, primaricin, polymyxin, rifampicin, streptomycin, tunicamycin and vancomycin in the isolation media enhances the selection of members of the family Actinomycetales (Hong *et al*., 2009; Zhang, 2011). Actinomycetes produced these antimicrobial agents in their natural habitat during the scarcity of nutrients to suppress the growth of other organisms to enable them to compete within the environment. The genes encoding the enzymes that synthesize antimicrobial agents and either secondary metabolite are found on chromosomes organized in form of gene clusters. Resistance genes of actinomycetes to its products are located either at the beginning or at the end of the cluster, often in both positions. Therefore, the addition of these antimicrobial agents in the media enhances selective isolation of the actinomycetes by eliminating unwanted micro-organisms.

Selective media for isolation of new strains of actinomycetes: Different selective media have been developed for the isolation of different actinomycetes species from samples collected from different ecosystems or habitats (Jiang *et al*., 2016). These include Starch-casein medium, Humic acid-vitamin agar, Starch casein nitrate agar (SCS), Hair hydrolysate vitamin agar (HHVA); Bennet's agar (BA), Arginine-glycerol salt (AGS) medium, Chitin medium, Modified Benedict's medium, Soybean meal-glucose medium, Gauze's agar medium, Czapek's agar medium, Egg albumen medium, Glucose-asparagine medium, Glycerolasparaginate agar 2, Chitin agar, Coal-vitamin agar, Mineral salt (MS) medium, Yeast extract-malt extract agar, M3 agar medium, Asparagine agar, Glycerol-glycine agar, Starch yeast casein agar (SYCA), Actinomycetes Isolation agar (AIA), Humic Acid vitamin gellan gum (HVG), Tap water yeast extract agar (TWYE), Coal vitamin agar (CVA), Asparagine-glucose agar medium as reviewed in (Mukesh, 2014).

2.4. Identification of actinomycetes

In any screening system, the taxonomic characterization of actinomycetes containing new metabolites is an essential step. However, in many cases, taxonomic analysis of the microorganism is conducted only when the metabolite that it generates is of great importance, i.e. when a definition (PFGE), Amplified ribosomal DNA restriction analysis (ARDRA), Random amplified polymorphic DNA (RAPD) and genus-specific primers would be useful for modern characterization of actinomycetes (Sharma *et al*., 2014). Of the producing microbe is required for patent application.

The conventional methods for identification of actinomycetes are based on morphological and biochemical observations. Colony morphology (example; smooth, rough etc) can be observed under dissecting microscopes (Mukesh, 2014). Actinomycetes spore shapes and arrangement can be observed under light and electron microscopes using slide culture (Kavitha and Vijayalakshmi, 2007), and coverslip technique. Strains are observed for several characteristics such as presence or absence of aerial mycelium, fragmentation or non-fragmentation of the substrate and aerial mycelium, presence of sclerotia, spore chain morphology and colour of spore mass (Kavitha and Vijayalakshmi, 2007).

Biochemical characteristics of different actinomycetes species have been studied by many researchers. The most widely used tests are catalase test, oxidase test, gelatin, starch hydrolysis, urea test, nitrate reduction, esculin degradation, Citrate utilization, Indole test, Triple Iron sugar test, Methyl red test, Voges-proskauer test. However, Biochemical identification of actinomycetes

using different Analytical Profile Indexes (API) kits were also reported; such as API 20A (bioMerieux, Marcy l'Etoile, France) kit (Flora *et al*., 2015), API ZYM kit (bioMerieux, Marcy l'Etoile, France), API® Coryne and Rapid ID 32 A kits (Biomérieux) have also been reported to be useful for identification of actinomycetes species. Although phenotypic identification remained the gold standard, so far trustworthy classification of actinomycetes may not be possible using conventional methods. Thus, rapid molecular methods including Restriction fragment length polymorphism (RFLP), Pulse field gel electrophoresis

2.5. Screening of actinomycetes for new antimicrobial compounds

Search for new antibiotics effective against resistant pathogenic microbes is presently an important area of research as reviewed in (Mukesh, 2014; Basha, and Rao. 2017). Screening for the production of new bioactive compounds from isolates obtained from less explored ecosystems or geographical area is a possible way to obtain novel bioactive compound. There are no best screening methods, the success of any method depends on the selection of appropriate test procedure, where samples are obtained and the number of isolates tested (Atsede, 2011). Several logical strategies for discovering novel bioactive chemicals, such as focused screening and culture-based screening, have proven to be useful (Tiwari and Gupter, 2012).The cultural-based technique which is the conventional method for novel antibiotic screening involves primary screening by cross streak method, right angle streak method, and agar plug method. Modification of these procedures was also reported by some researchers (Ensieh *et al.*, 2015). The isolates that showed activities during primary screening were subjected to secondary screening techniques to quantify activities of the antimicrobial metabolites. The results of primary screening are confirmed by secondary screening of active actinomycetes. According to the literature, there may be inconsistencies between primary (solid medium) and secondary (liquid medium) screening activities. Antimicrobial activity in a solid medium may not translate to antimicrobial activity in a liquid medium, and vice versa (Mukesh, 2014).

2.6. Antibiotic producing Actinomycetes and other Bactria

Actinomycetes produce secondary metabolites some of which are bioactive compounds (including antimicrobial agents). These have been used in the treatment of infections for decades (Ashforth *et al*., 2010; Hotam *et al*., 2013a). Since the discovery of streptomycin in 1943, from *Streptomyces griseus*, several antibiotics have been isolated from actinomycetes with broad-spectrum activities (Bérdy, 2012; Mukesh, 2014) such as tetracycline, cephamycins, erythromycin, and many others.

The mechanisms by which actinomycetes, for example, *Streptomyces* to produce these important antibiotics is not yet understood, but is thought to be due to the higher content of guanine and cytosine (G+C) of the DNA (>55mol %) (Kurtbök. 2012). Laskaris *et al.* (2010) reported that these organisms produce antibiotics to kill off potential competitors in their natural habitat. Indeed, actinomycetes have provided more than half of the naturally occurring antibiotics discovered to date and continue to be screened for useful compounds (Rinoy *et al*., 2012). About more than 23,000 bioactive secondary metabolites were identified from microorganisms in which more than 10,000 (45%) of these bioactive compounds are produced by actinomycetes. Among actinomycetes spp, Streptomyces species produced 7,600 (76%) of these bioactive compounds (Olano *et al*., 2009; Carvalho *et al*., 2016).

Several studies were done on antibiotic-producing bacteria isolated from dumpsite soil. Actinomycetes were isolated from waste dump soil in Kenya by Abebe Bizuye *et al*. (2018) and showed antibacterial activity *E. coli, S. boydii*, *S. typhi,* and *V. cholerae*. Sethi *et al*. (2013) isolated *Penicillium* chrysogenum from soil and observed their maximum antibacterial activities with a zone of inhibition against *Staphylococcus aureus* (17mm), *Pseudomonas aeruginosa* (11mm), *Escherichia coli* (19.8mm), and *Klebsiella pneumonia* (8.2mm). Wadetwar and Patil, (2013) also isolated *Streptomyces* spp. from the soil and have antimicrobial activity against *B. subtilis, B. cereus, S. aureus, E. coli*, P. *vulgaris, P. aeruginosa, C. albicans,* and *A. niger.* The extract of *Streptomyces* spp. isolated from plastic dumpsites in India showed antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Duddu and Guntuku, 2016).

On the other hand, Bacillus *subtilis* extract was effective only for *Staphylococcus aureus* and *Pseudomonas aeruginosa* with a zone of inhibition of 13.4mm and 13.8mm respectively. Hakim *et al*. (2018) isolated *Bacillus* species from waste dump soil in Pakistan and showed antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pneumoniae, Salmonella typhimurium, Shigella flexneri, Klebsiella pneumonia, Vibrio cholerae, Pseudomonas aeruginosa Escherichia coli, Acinetobacter baumannii*. Mandal *et al*., (2019) were isolated *Bacillus* species from the solid waste dumpsite in Bangladesh against *Escherichia coli* and *Salmonella paratyphi*. Sura *et al*., 2017 also isolated *Bacillus* species from a local soil sample of Iraq with a varying zone of inhibition against *E. coli, S. aureus, K. pneumonia, P.aeruginosa*. Singh *et al*. (2012) and Rafiq *et al*. (2018) also confirmed the antibacterial activities of *Bacillus* species against *Escherichia coli* and *Staphylococcus aureus* from the soil in India and Pakistan. The *Bacillus* species isolated from food wastes in Nigeria also have antibacterial activities against

Pseudomonas aeruginosa, *Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia*, *Salmonella typhi*, *Bacillus cereus*, *Corynebacterium diphtheria*, and *Shigella dysentriae* (Ozabor and Fadahunsi, 20l9).

2.7. Mechanisms of antibiotic action

For many antibiotics, the mechanism of action is not understood fully. However, it is known that antibiotics can act in the following ways: 1) inhibit cell wall synthesis, 2) inhibit protein synthesis, and 3) inhibit nucleic acid synthesis (Kathleen *et al*., 1994). The differences in cellular structure among bacterial species can lead to resistance to certain antibiotics. For example, Gram-negative bacteria exhibit high intrinsic resistance to many antibiotics because of the nature of their cell wall, which restricts the absorption of many molecules to movements through openings called porins. Perhaps even more important, when β-lactamase is present in the periplasmic space, the antibiotic remains outside the cell, where the enzyme, which is too large to enter even through an unmodified porin, can reach and inactivate it (Tortora *et al*., 2010). Acquired resistance can arise either through mutation or horizontal gene transfer. The presence of the antibiotic in question leads to selection for resistant organisms, thereby shifting the population towards resistance. The major mechanisms of acquired resistance are the ability of the microorganisms to destroy or modify the drug, alter the drug target, reduce uptake or increase efflux of the drug, and replace the metabolic step targeted by the drug (Kathleen *et al*., 1994).

2.7.1. Inhibitors of cell wall synthesis

A bacterium's cell wall is made up of a macromolecular network called peptidoglycan. In Gram-positive organisms, the peptidoglycan layer is thick and may have a thin layer of teichoic acid outside the peptidoglycan. In contrast, Gram-negative organisms have a thin single layer of peptidoglycan covered by a complex outer membrane layer composed of lipopolysaccharides, lipoproteins, and phospholipids. There are two major groups of cell wall synthesis inhibitors, the β-lactams, and the glycopeptides antibiotics. As bacterial cell walls are wholly unlike the membranes of eukaryotes, they are an obvious target for selectively toxic antibiotics. The β-lactams include the penicillins, cephalosporins, and carbapenems. These agents bind to the penicillin-binding proteins (PBP's) that cross-link strands of peptidoglycan in the cell wall. In Gram-negative cells, this leads to the formation of fragile spheroplasts that are easily ruptured; in Gram-positive cells, autolysis is triggered by the release of lipoteichoic acid. The mechanism of β-lactam resistance is via the action

of the β-lactamases. These enzymes catalyze the hydrolysis of the β-lactam ring and, thereby, inactivating these antibiotics and development of resistance by many bacteria (eg, *S.aureus, Neisseria gonorrhoeae, Pseudomonas* sp, *Bacteroides fragilis*, and some enteric Gram-negative bacilli).

2.7.2. Inhibitors of protein synthesis

Protein synthesis is a common feature of all cells. Bacterial and eukaryotic ribosomes differ in both size and chemical composition. Eukaryotic cells have the 80S (with 60S and 40S subunits) ribosomes; prokaryotic cells have 70S ribosomes. Thus, antibiotics that affect protein synthesis can have a selective effect on sensitive bacteria without affecting human cells. Among the antibiotics that interfere with protein synthesis are chloramphenicol, erythromycin, streptomycin, and tetracycline (Kathleen *et al*., 1994). Many types of antibiotics inhibit bacterial protein synthesis. These drugs take advantage of structural differences between bacterial ribosomes and eukaryotic ribosomes. The aminoglycoside antibiotics are a group whose mechanism of action is not completely understood. The three major groups of aminoglycosides are streptomycin, neomycin, and kanamycin (Greenwood, 2000). The antibacterial activity of aminoglycosides is directed primarily against aerobic Gram-negative bacilli; there is little activity against anaerobes and Gram-positive bacteria (streptococci).

2.7.3. Inhibitors of nucleic acid synthesis

Rifampin is the most well-known antibiotic derivative from the rifamycin family. These drugs are structurally related to the macrolides and inhibit the synthesis of mRNA (Muleta and Assefa. 2018). Microorganisms may develop resistance to rifampin rapidly in vitro as a one-step mutation; this also occurs in vivo. For this reason, rifampin should not be administered alone, except for short-term chemoprophylaxis. This characteristic is probably an important 20 factor in its ant tubercular activity because the tuberculosis pathogen is usually located inside tissues or macrophages (Muleta, A., & Assefa, F. (2018). The quinolones are a chemically varied class of broad-spectrum antibiotics widely used to treat many diseases, including gonorrhea and anthrax. Drugs in this class include nalidixic acid, norfloxacin, and ciprofloxacin (Kathleen *et al*., 1994). Quinolones inhibit bacterial growth by acting on DNA gyrase which is responsible for cutting DNA strands, thus preventing supercoiled DNA and topoisomerase IV.

2.8. Factors affecting the production of antimicrobial compound by actinomycetes

2.8.1 Nutritional factors

Production of antimicrobial compounds or secondary metabolites by actinobacteria greatly depends on the nutritional component of the media (Ababutain *et al*., 2013). Minor changes in the composition of nutrients (media) have an impact on the mass and value of secondary metabolites (Rajeswari *et al.,* 2015). Organic matter, salinity, relative wetness, pH, and vegetation are major parameters that can affect the formation of secondary metabolites by actinomycetes in solid or broth medium, according to Arasu *et al*. (2014) and Ghorbani-Nasrabadi *et al*. (2013).Studies on the physiological and biochemical parameters to increase the production of antibiotics showed that different sources of carbon, nitrogen, phosphate sources and other nonessential metabolites have a significant relationship between nutrient depletion and biosynthesis of secondary metabolites (Arasu *et al*., 2014). Antibiotic accumulation begins to increase in many cases only after the nutrients are depleted. In candihexin production, the addition of a nitrogen source in the idiophase, returns the fermentation to the trophosphase and production is reduced.

2.8.2 Cultural conditions factors

Factors other than nutritional parameters, such as oxygen, temperature, agitation and light were also reported to affect antibiotic production (Arasu *et al*., 2014) during the fermentation process. These factors need to be optimized for optimum antimicrobial by particular species or strain of actinomycetes. Fermentation is the primary mechanism producing antimicrobials (Harms *et al*., 2017). If there is a high concentration of oxygen then fermentation does not occur, causing inhibition of the development of antimicrobials. Temperature can affect the capacity of the microorganism to proliferate and effectively metabolize.

2.9. Antimicrobial susceptibility Profiles of bacteria isolated from waste dump sites.

Drug-resistant bacteria are being studied in solid waste dumps all over the world (Idahosa *et al*., (2017) isolated potential bacterial isolates (*Escherichia, Shigella, Staphylococcus, Salmonella., Bacillus, Enterococcus, Clostridium, Proteus, Klebsiella, and Pseudomonas* from rural and urban market dumpsites of Nigeria and tested antimicrobial susceptibility against all isolates to the

selected antimicrobial agents. The isolates were susceptible to ciprofloxacin, pefloxacin, tetracycline, and gentamycin, while streptomycin, erythromycin, augmentin, and ampicillin were the most ineffective antibiotics. Similarly, *Klebsiella* sp., *Bacillus subtilis, Streptococcus* sp., *Escherichia coli, Klebsiella mobilis, Staphylococcus* sp., *Micrococcus* sp., *and Pseudomonas aeruginosa* were isolated by Eghomwanre *et al*., (2016) from Nigeria's contaminated soils and sediments and confirmed that the majority of the isolates were resistant to spectrum, chloramphenicol, sparfloxacin, ciprofloxacin, amoxicillin, augmentin, gentamycin, pefloxacin, ofloxacin, and streptomycin antimicrobial agents. *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella aerogenes, Klebsiella pneumonia, Pseudomonas aeruginosa, and Serratia marcescens* bacterial isolates were tested for their susceptibilities to 10 antimicrobial agents and most of them were resistant (Oviasogie and Agbonlahor, 2013).

Adekanle *et al*., (2014) also isolated *Serratia* spp., *pseudomonas aeruginosa, Klebsiella oxytoca, Acinetobacter* spp., *Proteus* spp., *Escherichia coli, Klebsiella* spp., *Staphylococcus aureus,* and aerobic spore bearers, and the majority of the isolates were resistant to cloxacillin, penicillin, and tetracycline antibiotics. *Escherichia* spp., *Klebsiella* spp., *Staphylococcus* spp., *Bacillus* spp., *Pseudomonas* spp., *Proteus* spp., *Micrococcus* spp., *Streptococcus* spp., and *Neisseria* spp. have also been isolated from Nigeria's dumpsites as multidrug-resistant bacteria (Odum *et al*., 2020). Mwaikono *et al*., (2015) also isolated bacteria (*Escherichia*, *Pseudomonas*, *Enterococcus*, *Bacillus*, *Shigella* spp.) from a municipal dumpsite, and all of them were resistant to penicillin G, ceftazidime, cefotaxime, Amoxycillin /clavulanic, nalidixic acid, and cefoxitin antimicrobial agents. *Aeromonas* sp., *Arthrobacter* sp., *Corynebacterium* sp., *Pseudomonas* spp., and *Streptococcus* spp. were isolated and exhibited resistance to amoxicillin, augmentin, cefuroxime, and erythromycin antibiotics (Owolabi, and Hekeu, 2014). Song'oro *et al*. (2019) isolated *Escherichia*, *Klebsiella*, *Serratia*, *Shigella*, *Pseudomonas*, Citrobacter spp., *Enterobacter, Salmonella*, *Staphylococcus*, *Bacillus*, *Yersinia*, *Vibro cholera*, *Providencia*, *Morganella,* and *Proteus* spp. and showed resistance against ampicillin, ceftazidime, cefotaxime, amoxicillin, and cefepime antibiotics.

2.10. The resistance of microorganisms and how it is acquired

Antimicrobial resistance (AMR) refers to a microorganism's resistance to an antimicrobial agent to which it was previously susceptible. Martinez's (2009) findings demonstrate that the development of drug-resistant strains of microorganisms is caused by the fact that many antibiotics are bacteriostatic rather than bactericidal. Generally, microorganisms acquire antibiotic resistance either by genetic

change or by non-genetic mechanisms (or both). Non-genetic resistance (adaptive), for example, occurs when microorganisms that cause tuberculosis persist in the tissues out of reach of antimicrobial agents or when certain strains of bacteria temporarily change to L-forms that lack most of their cell walls (Prakasam *et al*., 2017).

However, genetic resistance in bacteria is due to either a change in the bacterial chromosome (intrinsic) or by the acquisition of extra chromosomal DNA (acquired) (Li *et al*., 2010). Chromosomal resistance is because of a mutation in chromosomal DNA that alters the synthesis of ribosomal proteins. In contrast, extra chromosomal resistance is usually due to the presence of R-plasmids, or R-factors. These plasmids can bear as many as six or seven genes, each of which confers a different antimicrobial resistance. It can also easily be transferred from one strain or species of bacteria to another through horizontal gene transfer (transduction and conjugation), which is the main resistance mechanism in most bacteria (Nesme and Simonet, 2015). Finally, a large pool of resistant genes has been created and an increased burden on society by transferring drug-resistant bacteria from environmental to human pathogens.

2.11. Multidrug resistance in microbiology

Antimicrobial resistance displayed by a type of microbe to at least one antimicrobial agent in three or more antimicrobial categories is known as multiple drug resistance (MDR) or multi resistance. Antimicrobial categories are antimicrobial agent classifications based on their method of action and specificity to target organisms (Magiorakos, *et al*., 2012). MDR bacteria that resist multiple drugs are the most dangerous to public health; other forms include MDR viruses and parasites.

The designations extensively drug-resistant (XDR) and pandrug-resistant (PDR) have been coined to describe different levels of MDR in bacteria. Extensively drug-resistant (XDR) bacteria are resistant to all antimicrobial drugs except those in two or fewer antibacterial groups. Pandrug-resistant (PDR) bacteria are non-susceptible to all antimicrobial drugs across all antimicrobial categories (Magiorakos, *et al*., 2012). The criteria were published in the journal Clinical Microbiology and Infection in 2011 and are freely available on the internet (Magiorakos, *et al*., 2012).

2.11.1 Bacterial resistance to antibiotics

Antimicrobial resistance has allowed certain microbes to live for thousands of years. They achieve this by spontaneous mutation or DNA transfer. This mechanism allows some bacteria to resist the action of antibiotics, rendering them useless (Bennett, 2008). In order to achieve multi-drug resistance, these microorganisms use a variety of mechanisms, including no longer relying on a glycoprotein cell wall, enzymatic deactivation of antibiotics, decreased cell wall permeability to antibiotics, altered antibiotic target sites, efflux mechanisms to remove antibiotics (Nikadio, 2009), and increased mutation rate as a stress response (Nikadio, 2009).

2.12. Mechanism of Antibiotic Resistance and their Genetic basis

There are many mechanisms that bacteria exhibit to protect themselves from antibiotics. These resistance mechanisms can be biochemical and genetic aspects (Džidić *et al*., 201) (Table 1.3). Resistance of pathogens to drugs is determined by the presence of specific genes and / or mutations (Li *et al*., 2018). Resistance to antibiotics basically can occur by (1) modification of a drug target results in the inability of the drug to bind to its biological target thus rendering the drug unable to kill the bacteria, (2) active efflux results in the intracellular dilution of drugs making the extruded drugs unavailable for their inhibitory action or (3) prevent cellular entry of drug into the inside of the bacterial cell; and (4) enzymatic inactivation of the drug results from the metabolic degradation of the drug into a form that is rendered ineffective in inhibiting bacterial growth (Wright, 2011; Kumar and Varela, 2013).

Table 1: Biochemical aspects of antibiotic resistance mechanisms

Mechanisms of resistance	Description	Resistance gene	Reference
Target modification	Alteration in the primary site of action can arise from mutations at the target gene resulting in altered target structure.	rpoB1,Altered penicillin binding proteins (MecA genes2)	(Davies and Davies, 2010)
Enzymatic inactivation of drug	Antibiotics are inactivated by enzymatic hydrolysis, group transfer and redox process	Gene β- lactamases (bla), by *Enterobacteriaceae*, amino glycoside modifyingenzymes	(Kumar, 2017)

Efflux pumps	Trans-membrane transport proteins, used for exporting specific metabolites and xenobiotic toxic substances out of the cell	tetA gene in *E. coli* (tetracycline gene)	(Džidić *et al*., 2008)
Change in membrane permeability	Intrinsic ability to restrict the entry of small molecules	*P. aeruginosa* Lipopolysaccharide (LPS)	**(Kumar, 2017)**

2.12.1. Genetic aspect of antibiotic resistance mechanisms

Genetic aspect of antibiotic resistance mechanisms can be classified as intrinsic resistance and acquired resistance. Pathogens being initially drug resistant represents intrinsic (natural or de novo) characteristic feature of an organism, which allows bacteria to tolerate the encountered antibiotic (Wright, 2010). Acquired bacterial antibiotic resistance can result from a mutation of cellular genes or both the acquisition of foreign resistance genes. This type of resistance is much more important and significant for clinical aspect because of the possible spread of resistant genes through a sensitive microbial population (Chroma and Kolar, 2010). There are two main ways of acquiring antibiotic resistance: a) through mutation in different chromosomal loci and b) through horizontal gene transfer (i.e. acquisition of resistance genes from other microorganisms) such as bacterial conjugative plasmids, transposable elements and integrons systems.

2.12.2. Enzymatic Drug inactivation mechanisms

As Kumar, (2017) reports that the development of β-lactamases, which hydrolyze the β- lactam ring of β-lactam antibiotics, results from an enzymatic drug inactivation mechanism mediated by the hydrolysis process. Penicillins, cephalosporins, monobactams, and carbapenems are examples of beta-lactam antibiotics (Cag *et al*., 2016). They all have the same beta-lactam ring and work by attaching to and inactivating penicillin-binding proteins (PBPs), which are crucial for the bacterial cell wall construction (Meletis, 2016).

Genes encoding β -lactamases (bla) can be found on the chromosome (e.g. AmpC β -lactamase) or on mobile genetic elements such as plasmids (TEM-1 β -lactamase, SHV-1 (sulfhydryl variable active site), CTX-M (cefotaxime degrading enzyme) and transposons, or as part of integrons in these transferable elements (Chroma and Kolar, 2010; Kumar, 2017).

β -lactamase synthesis remains the most important mechanism of resistance to β -lactam drugs in Gram-negative bacteria. Extended β -spectrum lactamases are one type of these enzymes (ESBLs). TEM-1, TEM-2, and SHV-1 have evolved over time to hydrolyze and create ESBLs from a wide range of extended spectrum cephalosporins. The most common ESBLs are TEM, SHV, and CTX-M.

2.13. The epidemiology of antibiotic resistance

Antimicrobial resistance (AMR) has emerged as a major medical concern, especially in low- and middle-income countries (LMICs) (World Health Organization, 2014; Laxminarayan *et al*., 2013). Antibiotic use is rising in LMICs as earnings rise, antimicrobials become more accessible, and hospital stewardship and over-the-counter sales are poorly controlled. Multidrug-resistant (MDR) microorganisms are emerging and spreading in community and hospital settings as a result of this. The cumulative incidence of community-acquired Extended-Spectrum Beta-Lactamase (ESBL) generating Escherichia coli and Klebsiella pneumoniae infections is increasing over time, according to hospital data from LMICs (Ansari *et al*., 2015). The frequency of AMR organisms causing hospital-acquired infections (HAI) in ICUs in LMICs is substantially greater than in the United States, according to a recent report from the International Nosocomial Infection Control Consortium (INICC) (US).

The difference in mortality between individuals with and without the condition of interest, known as attributable mortality, is a significant measure used to evaluate the burden of AMR. In the United States, mortality from AMR infection is predicted to be 6.5 percent, resulting in 23,000 deaths each year (Center for Disease Control and Prevention and U.S. Department of Health and Human Services). The number of deaths caused by antibiotic-resistant bacteria is estimated to be around 25,000 per year in the European Union (European Centre for Disease Prevention and Control and European Medicines Agency, 2009).

In LMICs, there is a scarcity of data on AMR-related mortality. In ICUs in Colombia, Peru, and Argentina, mortality due to ventilator-associated pneumonia is estimated to be 17 percent, 25 percent, and 35 percent, respectively, and is linked to a high percentage of AMR pathogens (Cuellar *et al.,* 2008). In Tanzania, mortality due to ESBL and methicillin-resistant Staphylococcus aureus (MRSA) is reported to be 27 percent and 34 percent, respectively, in newborn sepsis (Kayange *et al*., 2010) This has been used to calculate that ESBL and MRSA are responsible for 58,319 deaths in India alone (Laxminarayan *et al.,* 2013).

A joint attempt to standardize AMR surveillance systems has been launched. In an effort to harmonize the surveillance systems of AMR, a joint initiative between the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Prevention and Control (CDC) have developed standard definitions of multidrug-resistance (MDR) (Magiorakos *et al*., 2012).

2.14. Diffusion methods

The Disk-diffusion method is one of the standardized methods used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. The test is performed by inoculating Mueller-Hinton agar plates with a standardized inoculum (1-2 × 10^8 CFU/mL) of the test microorganism. Then, commercially available filter paper discs of 6 mm in diameter, containing the test compound at the desired concentration, are placed on the agar surface. The Petri dishes are incubated under suitable conditions (18-24 hours at 35^{o}C). Inhibition growth zones around each drug are measured to the nearest millimeter. The Antibiogram provides qualitative results by categorizing bacteria as susceptible, intermediate, or resistant according to the annually published guidelines by CLSI (Balouiri *et al*., 2016).

The Agar well diffusion method is one of the standard methods used worldwide to evaluate the antimicrobial activities of extracts. Similar to the disc diffusion method, the agar plate surface is inoculated by spreading a volume of standardized (1.5× 108 CFU/mL) microbial inoculum over the entire agar surface. After this, an aseptically punched hole with a sterile cork borer with a diameter of 6 mm and the necessary amount of the antimicrobial agent or solution with the desired concentration is injected into the well.

3: MATERIALS AND METHODS

3.1. Description of the study area

Bahir Dar is the capital of Amhara National Regional State and covers a total area of 152,600 km^2 and is located at 11” 38’N, 37” 10’E on the South of Lake Tana where the Blue Nile River starts. The elevation reported for the city is about 1801 meters above sea level (QCB, 2010). The area receives an average annual rainfall ranging between 850mm to 1250mm with minimum and maximum average daily temperatures of 10℃ and 32℃, respectively (BoARD, 2006). The study was conducted specifically on Sebatamite dumping municipal waste soil from February 2021 to June 2021. According to the old master plan, this research area is located 7 kilometers outside of the city and covers 22 hectares. However, at the moment, people live less than a kilometer away from the dump area. The study's geographical coordinates are 11° 32 "° 32 37 North latitude and 37° 23 East to 37° 23 East longitude, with a mean elevation of 1,790 meters above sea level. The city of Bahir Dar produces more than 98.8 tonnes of waste daily. The dumped waste is a mixture of residential (54), commercial (24.2), institutional (17), and street sweeping (3.56) (Kassahun Tassie, 2018).

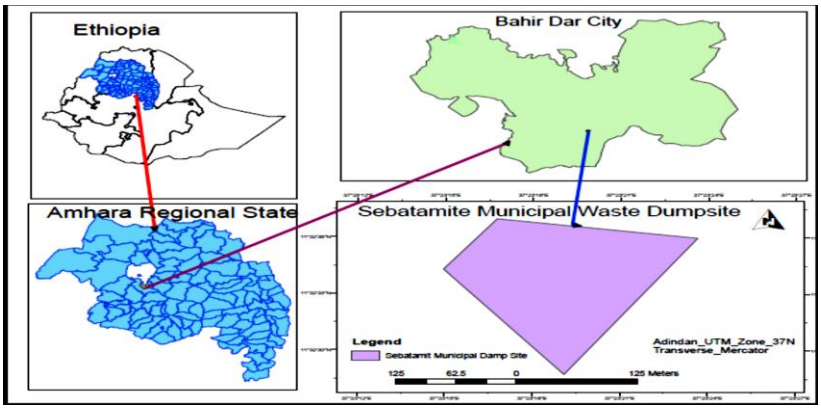


Figure 1: Map of the study area.

3.2. Study design and period

The study was carried out using cross-sectional research design, to isolate and characterize antibiotic-producing actinomycetes and drug-resistant profile of common bacteria from the dumpsite soil. Also, it was designed to assess the bacterial isolates for their antibacterial activities

on pathogenic bacterial strains through a laboratory-based investigation. The activities were studied from February to June, beginning with data collection and ending with thesis presentation.

3.3. Sample collection

A total of 30 waste soil samples were randomly collected from 5 sub sites or sapling points (six samples per sampling point) from Sebatamite dump municipal waste at the depth of 5-10 cm three times using standard methods (Aghamirian and Ghiasian, 2009). The soil samples were excavated by using a sterilized pickax, collected in zip-lock polythene bags, labeled and transported to the research laboratory of Microbiology, department of Biology, Bahir Dar University, using an icebox. Then the soil samples taken from the study site were mixed in biosafety to have one representative sample, (Rinoy *et al*., 2012 and Ekeke and Okonwu, 2013) and air-dried at room temperature, then crushed by using a mortar and pestle. After this, it was processed and stored at 4 °C for further studies.

3.4. Isolation of actinomycetes and other bacteria

Isolation of actinomycetes was carried out according to the method described by (Arifuzzaman *et al*., 2010 and George *et al*., 2012). The soil samples were air-dried at room temperature (Rotich *et al*., 2017). 1g of air-dried soil sample was suspended in 9mL of sterile saline water and made into the stock solution by diluting 1g of sieved soil in 9 mL of sterile saline water and shaking it well with a vortex mixer. From the stock solution, serial dilutions were made from one-tenth stepwise to a 10^{-9} dilution. A volume of 0.1 ml of suspension from 10^{-3} up to 10^{-7} dilutions was taken and spread evenly by using a sterile glass spreader aseptically over the surface of sterile starch casein agar medium plates. On the other hand, for isolation of total bacteria, serially diluted samples were spread on sterilized nutrient agar medium, and Eosin methyl blue (EMB), salmonella, Shigella (SS) agar, Mannitol salt agar medium were also used for the isolation of *E.coli, Salmonella-Shigella and S. aureus* respectively as a selective medium. For each dilution, triplicate plates were used and starch casein agar plates were incubated at 30°C for 7 days. Nutrient agar plates and the other agar plates were incubated for 24 hours at $37\ ^{0}$c in an inverted position (Narendra *et al*., 2010).

After incubation, morphologically distinct actinomycete colonies on the starch casein agar and nutrient agar plates were picked and further sub-cultured on their respective isolation media. The actinomycete colonies were purified by the streak plate method (Reddy *et al*., 2011). Once the pure colonies were obtained, each colony was further identified based on its characteristics, like smell,

colony morphology, the color of hyphae, shape, and the presence or absence of aerial and substrate mycelium. The selected and identified actinomycetes were then transferred from the plate to a starch casein agar slant and incubated at 27 °C for 7 days, while other bacterial plates on nutrient agar were incubated for 24 hours. After incubation, morphologically distinct actinomycetes colonies and other bacteria colonies were collected, purified, and maintained on starch casein agar slants and nutrient agar slants at 4°C for further analysis and subsequent characterization (Narendra *et al*., 2010). It can also be successfully stored as a glycerol stock at -20°C and used for further research.

3.5. Source of the test bacterial pathogens and inoculum preparation.

Standard strains of Gram-positive bacteria (*Staphylococcus aureus, Streptococcus pyogenes, and Enterococcus faecalis*) and Gram-negative bacteria (*Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae*) were obtained from the Amhara Public Health Institution and kept at 4°C in the laboratory for further studies. Bacteria used for the study was prepared by inoculating isolates into the nutrient broth and incubating them at 37°C for 24 - 48 hours. The culture turbidity was adjusted to 0.5 McFarland using sterile normal saline (CLSI, 2020).

3.6. Procedures for inoculum preparation and inoculation

To follow the procedures for inoculum preparation and inoculation first, From an 'overnight culture', 4-5 morphologically similar test bacterial colonies were suspended in 5 mL nutrient broth and incubated for 4 hours at 37°C and compared to the level of 0.5 McFarland standards, which is approximately equivalent to 10^6–10^8 CFU/ml. After adjusting the turbidity, a sterile cotton swab was dipped into the suspension and streaked over the entire surface of the prepared medium by rotating the plate at 60^o to ensure the even distribution of the inoculum (Prashith *et al*., 2012).

3.7. Primary screening

Primary screening on bacteria was performed using the cross streak method as described by (Raja and Prabakaran, 2011). A seven - day old culture of actinomycetes and a 24 hour old culture of other bacteria were inoculated as streak lines on starch casein agar medium and Nutrient agar medium respectively.

The inhibitory metabolite-producing ability of the isolates was preliminarily screened *in vitro* against selected cultures of human pathogenic bacteria using the perpendicular streak plate method.

The isolates to be tested for antibiotic production were streaked horizontally across the diameter of the Muller-Hinton agar (MHA) medium and incubated for 7 days at 28 2°C.After incubation, clinical bacterial strains, viz., *S. aureus, E. coli, P. aeruginosa, S. pyogenes,* and *K. pneumoniae and Enterococcus faecalis,* which were adjusted to the level of 0.5 McFarland standards, were streaked vertically (at a 90° angle) very close to the screened one from left to right respectively. The plates were further incubated at 37°C for 24 hours and a zone of inhibition was indicated between the antibiotic-producing isolate and the test organisms that were considered to be positive for antibiotic production (Wadetwar and Patil, 2013).

3.8. Secondary screening

A. Production of crude extracts:-

Twenty (20) actinomycetes and two (2) other bacterial isolates were selected for secondary screening in a small scale submerged fermentation state. Two hundred milliliters of Starch Casein, and Nutrient Broth was dispensed into a 500ml Erlenmeyer Flask/250, into which a loop full of seven days and 24 hours grown isolates was inoculated and incubated on a platform shaker (New Brunswick Scientific), at 200 rpm at room temperature for 10 days, according to (Remya and Vijayakumar, 2008; Dhanasekaran *et al*., 2009). After ten days of incubation, the content of the incubation flask's content was filtered through Whatman No.1 filter paper. An equal volume of ethyl acetate (1:1) was then added to the culture filtrates and shaken vigorously for 1 hour and the solvent phase that presumably contained an antibiotic compound was separated from the aqueous phase in a separator funnel (Assistant, Germany). The ethyl acetate phase that contains antibiotics was evaporated and concentrated by using a water bath in study of rotary vapor at a temperature of 60^{o}C (Remya and Vijayakumar, 2008).

3.8.1. Secondary screening by using the disc diffusion method.

Antibiotic activity of the crude extract from culture filtrates of each isolate was evaluated by using disc diffusion assays (Hassan *et al.,* 2001). The extracts of each isolate were selected for antibacterial activity using the disc diffusion method with some modifications (Mandal *et al*., 2019). The inoculum was prepared by mixing a few (4-5) morphologically identical colonies with 5 mL nutrient broth and incubated for 4 hours at 37°C. The bacterial suspension was compared to the 0.5 McFarland standards, which corresponds to $1.5x10^{8}$ CFU/mL. After adjusting the turbidity, bacterial test strains, viz., *S. aureus, E. coli, P. aeruginosa, S. pyogenes, K. pneumoniae, Enterococcus faecalis,* were swabbed uniformly on a sterile MHA medium using a sterilized cotton swab and left for 5-10 minutes to absorb the moisture. Sterile whatmanpaper No.1 discs having a 6

mm diameter were impregnated with 30 µl of the extracts. Discs were treated with supernatant and the selected standard antibiotic disc was applied in triplicate to a pre-inoculated MHA medium and left for 2 hours at 4°C to diffuse the metabolite and then incubated at 37°C for 24 hours without inverting the plates. Gentamycin (30µg) is used as positive controls and ethyl acetate is used as negative controls. After incubation, the zone of inhibition (mm) around each disc was measured and recorded (Balouiri *et al*., 2016).

3.8.2. Secondary screening by using the agar well diffusion method.

The inoculum was prepared using the disc diffusion method. Six mm diameter wells were made on the prepared plates using a sterile cork borer. A volume of 0.1 ml of extract was carefully added into each well and allowed to diffuse for 1-2 hours and incubated at 37 °C for 24 hours. The sterilized ethyl acetate was filtered and used as a negative control and Gentamycin as a positive control. After incubation, the zone of inhibition around each well was measured and recorded. The experiment was conducted in triplicate (Narendra *et al*., 2010).

3.9. Determination of MIC and MBC concentration

The concept of MIC is the lowest concentration of an antibacterial agent necessary to inhibit visible growth, while minimum bactericidal concentration (MBC) is the minimum concentration of an antibacterial agent that results in bacterial death. The closer the MIC is to the MBC, the more bactericidal the compound. Broth two-fold serial dilution method was used to determine the MIC and MBC (Andrews, 2001). Two clinical isolates, one from Gram-positive (*Staphylococcus aureus*) and one from Gram-negative (*Escherichia coli*) were selected. For this experiment, 12 sterilized screw-capped test tubes were used. One ml of nutrient broth was added to the 1-10 test tubes and 2 mL into the test tube 11 (broth control). 1 mL of the crude extract solution was dispensed into test tube 1 up to test tube 10 with a serial dilution technique by mixing and changing the micropipette tips at each dilution and 2 mL to test tube 12 (crude extract control). Finally, one ml was discarded from test tube 10. 0.1 ml of standardized inoculum of the clinical isolate was added into test tubes 1-10 and incubated at 37 °C for 18-24 hours. After incubation, the MIC value was determined by observing the growth of bacteria in the test tube. From the above test tubes with no turbidity, 0.1 ml was spread over the surface of the MHA plates. After incubation at 37°C for 24 h, the MBC was determined by observing the colonies.

3.10. Antimicrobial Susceptibility Test

The standard Kirby-Bauer`s disc diffusion method was performed to determine the antimicrobial resistance profiles**.** The bacterial inoculum was prepared by suspending four to five (4 up to 5)

morphologically identical colonies in 5 mL nutrient broth (HiMedia, India) and being incubated for 24 hours at 37°C. After adjusting the turbidity, the surface of the prepared MHA medium (Accumix, India) was evenly inoculated with bacterial suspension using sterile cotton swab. Antibiotic discs (Tetracycline (30 µg), Gentamicin (10 µg), amoxicillin (2 µg), Ciprofloxacin (5 µg), Nalidixic acid (30 µg), and Chloramphenicol (30 µg) that selected from different antibiotic class were carefully placed on the surface of Muller-Hinton agar medium previously inoculated with a broth culture of the test bacterial isolates. The plates were incubated at 37°C for 24 hours and the diameter of inhibition around the discs was measured to the nearest millimeter and interpreted as sensitive (S), intermediate (I), or resistant (R) according to the defined breakpoints of the Clinical and Laboratory Standards Institute (CLSI, 2020). For routine quality control (QC) of antimicrobial susceptibility tests, *Staphylococcus aureus* (ATCC® 25923), *Pseudomonas aeruginosa* (ATCC® 15442™), *Escherichia coli* (ATCC® 25922), *Klebsiella pneumonia (*ATCC® 4352), *Enterococcus faecalis* (ATCC® 51299™), and *Streptococcus pyogenes* (*ATCC® 19615™*) were used. MDR was determined based on three or more than three antibiotic class resisted by selected isolates.

3.11. Characterization of actinomycetes and other bacterial isolates

The potential isolates selected from the primary and secondary screening for the production of antimicrobial agents were characterized by morphological, biochemical, and physiological test**s** (Cappuccino and Sherman, 2014). Identification was done by comparing the characterization of the isolates with Bergey's Manual of Determinative Bacteriology (Bergey and Holt, 1994).

3.11.1. Morphological characterization

Macroscopic study: Morphological characteristics of the isolates were studied by growing the isolates on Starch Casein Agar medium, Nutrient agar medium, EMB agar medium, SS agar medium and Mannitol salt agar medium. Seven-day and two-day old culture of actinomycetes and other bacteria isolates were inoculated into each of the media by the streak plating technique and incubated at 28^{o}C for 7 days and 37^{o}C for 2 days, respectively. The macroscopic features of the active and other bacteria isolates observed were colony color, aerial mycelium, substrate mycelium, pigment production and colony surface (Singh *et al*., 2009).

Morphological characterization by microscopic method:

Morphological characterization by Gram staining: Isolates were characterized by gram staining (Williams *et al*., 1993).

3.11.2. Biochemical characterization tests

Thirty nine other bacterial isolates and 20 actinomycetes isolates were tested in a biochemical test of starch hydrolysis (Remya and Vijayakumar, 2008). Methyl red – Voges – Proskauer test, Catalase test, Indole test, Urease test, Citrate utilization test (Cheesebrough, 2006), Triple sugar iron test (Vlab, 2011), and SIM tests were done to identify the isolates.

3.11.3. Physiological characterization

The physiological characters of isolates were studied based on temperature tolerance and resistance to sodium chloride.

Temperature tolerance:-In the physiological characterization of the temperature tolerance of the isolates were determined based on starch casein and nutrient agar medium. A loop full of the test isolates from a 7 days and 2 days old culture was taken and serially diluted from 10^{-1} – 10^{-8} in sterile distilled water; it was agitated with a vortex and 0.1 ml of the suspension was taken and inoculated with the spread plate technique. The experiment was done in duplicate and the colony was counted with a log colony-forming unit after incubating the isolates at 25, 30, 37, and 45°C. The optimum temperature for maximum growth was determined by visual examination of the growth and the results were recorded (Laidi *et al*., 2006).

Growth with sodium chloride:-The isolates were tested for levels of tolerance to Sodium chloride on Nutrient Agar (Oxide) is supplemented with 5%, 7%, and 10% sodium chloride. Agar plates were inoculated with test isolates with the streak plate technique. The experiment was done in duplicate. The plates were incubated at 30^{o}C for 7 days and observations were made to record the highest concentration of salt that allowed growth (Santhi *et al*., 2010).

3.12. Statistical analysis

Data were analyzed using the statistical package to social science (SPSS) version 26 software. Results of the antibacterial activities of the metabolites were evaluated by measuring the diameter of the inhibition zone (mm). The data collected from the secondary screening method were analyzed using descriptive statistics and reported as Mean ± SD after three repeats of the experiment and presented by a table. The antimicrobial resistance profiles of the bacterial isolates were reported as susceptible (S), intermediate (I), or resistant (R) according to the annually published microbiological breakpoints by the (CLSI, 2020). The results were presented by tables.

4: RESULTS AND DISCUSSION

4.1. Result

4.1.1. Isolation of actinomycetes and other bacteria

Isolation of actinomycetes: Totally, seventy morphologically distinct colonies of actinomycetes were isolated on starch casein agar medium, and screened for antibacterial activities. Only 20 colonies were screened for primary screening of the total number of colonies.

Isolation of other bacteria: -A total of thirty nine bacterial isolates were obtained from Sebatamite dumped municipal waste soil, which included bacterial species belonging to seven (7) genera. Among these, the Gram-positive isolates were *Bacillus, Staphylococcus,* and Gram-negative isolates *Escherichia, Klebsiella, Pseudomonas, Shigella,* and *Salmonella*. *Staphylococcus* had the highest prevalence of 13 (27.66%). The next abundance of isolates was *Escherichia* with 11 (23.40%). The least occurring isolates were *Bacillus, Shigella, and Pseudomonas* 2(4.26%) for each as shown in the figure (2).

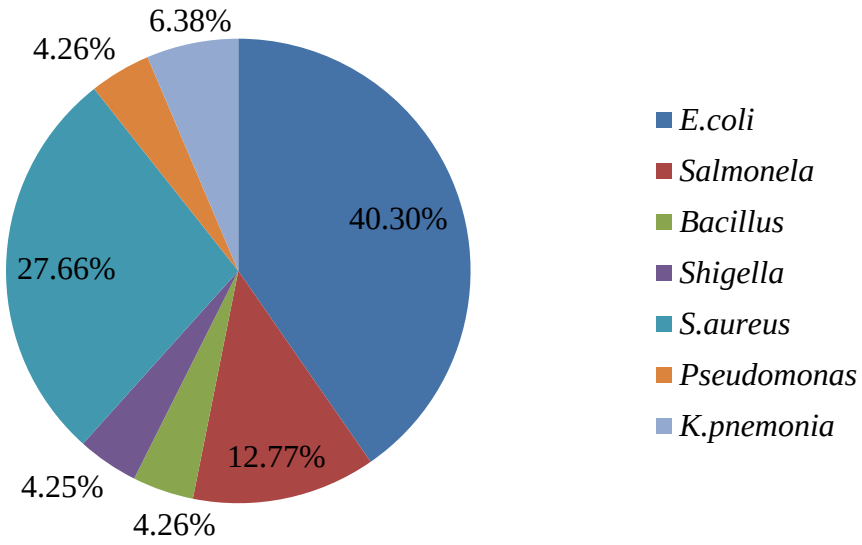


Figure 2: The Frequencies of bacterial isolates in dumped municipal wastes Soil

4.1.2. **Primary screening of antibacterial activity of actinomycetes**

The seventy different isolates of actinomycetes were subjected to antibacterial activity in primary screening by the dot spot method and the perpendicular streak method. From these isolates, only 20 isolates of actinomycetes showed antibacterial activities, while the other 50 isolates of

actinomycetes were no shows inhibition zone against test strains of *Staphylococcus aureus, Klebsiella Pneumonia, Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes,* and *Enterococcus faecalis (*Table2).

Table 2: Antibacterial activity of actinomycetes, against test strains

S. no	Isolate	Test strains						
	Code	*S. aureus*	*K. pneumoniae*	*E. coli*	*S. pyogenes*	*P. aeruginosa*	*Enterococcus Faecalis*	show broad-spectrum against 6 test strain
1	AC.2	12	-	-	-	-	11	2 (33.33%)
2	AC.4	16	-	14	16	-	12	4 (66.66%)
3	AC.7	10	-	-	-	-	-	1 (16.66%)
4	AC.10	11	-	-	-	12	10	3 (50 %)
5	AC.11	-	-	-	-	-	13	1 (16.66%)
6	AC.15	-	-	-	13	-	12	2 (33.33%)
7	AC.23	9	-	-	-		19	2 (33.33%)
8	AC.31	14	-	15	-	-	-	2 (33.33%)
9	AC.37	-	-	-	-	14	-	1 (16.66%)
10	AC.40	-	-	10	-	-	-	1 (16.66%)
11	AC.42	9	-	19	-	-	-	2(3.33%)
12	AC.44	13	-	-	-	-	-	1(16.66%)
13	AC.46	17	11	12	-	15	18	5 (83%)
14	AC.48	-	-	11	12	-	15	3 (50 %)
15	AC.49	9	-	-	-	16	-	2 (33.33%)
16	AC.51	-	-	10	-	-	-	1 (16.66%)
17	AC.59	-	-	-	11	-	-	1 (16.66%)
18	AC.60	-	-	-	12	-	-	1 (16.66%)
19	AC.63	-	-	17	14	-	-	2 (33.33%)
20	AC.68	12		-	-	17	-	2 (33.33%)
Total Inhibition Zone		11 (15.7 %)	1(1.43%)	8(11.43%)	6 (8.57%)	6 (8.57%)	7(10%)	

Key: + = inhibition zone; −= denotes no clear zone

In primary screening, the total percentage of inhibition by actinomycetes against the test strain was *S. aureus* 11 (15.7%), *K. pneumoniae* 1(1.4%), *E. coli* 8 (11.1.43%), *P. aeruginosa* 6 (8.57%), *Streptococcus pyogenes* 6 (8.57%), and *Enterococcus faecalis* 7 (10%). The total of twenty (28.57%) of actinomycetes showed antibacterial activity against any one of the tested bacteria. 50 (71.43%) actinomycetes isolates not showed antagonistic activity.

4.1.3. Secondary screening of selected actinomycetes and other bacteria

A. **antagonistic activity by disk diffusion method**: Twenty actinomycetes and 2 other bacterial isolates were screened for antibacterial activity against the pathogenic test strains by the disc

diffusion method. Fermentation was performed on 20 effective actinomycetes and two other bacterial isolates based on the results of primary screening. The results of ethyl acetate crude extracts of other bacteria and actinomycetes for antimicrobial activities in the disk diffusion method were presented in (Table 3). These crude extracts showed activity against a minimum of 1 test strain, and a maximum of 5 test strains.

Table 3: Antagonistic activity of actinomycete and other bacterial isolates by disk diffusion method

	isolate code	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*	Streptococcus pyogenes	*Pseudomonas Aeruginosa*	*Enterococcus faecalis*
1	AC2	11.3±2.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	17.0±1.0
2	AC4	22.7±1.5	0.0±0.0	23.0±1.0	23.0±1.0	0.0±0.0	23.0±1.0
3	AC7	13.3±1.5	. 0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
4	AC10	23.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	22.0±1.0	23.0±1.0
5	AC11	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	24.0±1.0
6	AC15	0.0±0.0	0.0±0.0	0.0±0.0	24.0±1.0	0.0±0.0	24.3±1.2
7	AC23	24.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	24.7±1.5
8	AC31	23.3±0.6	0.0±0.0	23.3±1.5	0.0±0.0	0.0±0.0	0.0±0.0
9	AC37	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	16.3±14.2	0.0±0.0
10	AC40	0.0±0.0	0.0±0.0	23.0±1.5	0.0±0.0	8.0±13.9	0.0±0.0
11	AC42	23.0±1.0	0.0±0.0	22.3±1.5	0.0±0.0	0.0±0.0	24.3±1.2
12	AC44	23.3±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
13	AC46	25.0±1.0	24.3±1.5	24.7±1.2	0.0±0.0	25.0±2.0	24.0±1.0
14	AC48	0.0±0.0	0.0±0.0	24.0±1.0	23.7±1.5	0.0±0.0	23.0±1.0
15	AC49	24.3±1.5	0.0±0.0	0.0±0.0	0.0±0.0	24.7±1.2	0.0±0.0
16	AC51	0.0±0.0	0.0±0.0	25.6±1.0	0.0±0.0	0.0±0.0	0.0±0.0
17	AC59	0.0±0.0	0.0±0.0	0.0±0.0	25.0±2.2	0.0±0.0	0.0±0.0
18	AC60	0.0±0.0	0.0±0.0	0.0±0.0	26.0±1.0	0.0±0.0	0.0±0.0
19	AC63	0.0±0.0	0.0±0.0	24.3±1.5	24.0±1.0	0.0±0.0	0.0±0.0
20	AC68	24.3±1.5	0.0±0.0	0.0±0.0	0.0±0.0	24.0±1.0	0.0±0.0
21	TB15	24.0±1.0	0.0±0.0	20.0±1.0	0.0±0.0	0.0±0.0	22.0±1.0
22	TB24	22.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	24.3±1.5	0.0±0.0
23	Total	12.9±11.3	1.1061	9.5±11.6	6.6±10.9	6.5±10.8	10.4242

Key: AC = actinomycete isolate; TB = other bacterial isolate;

As shown in table 3 in disk diffusion, the crude extracts of actinomycetes and other bacteria isolates have shown antibacterial activities against 6 test strains with zones of inhibition ranging from 0.0±0.0 mm to 25.0±1.0 mm against *Staphylococcus aureus*, from 0.0±0.0 mm to 24.3±1.5 mm against *Klebsiella Pneumoniae,* from 0.0±0.0 mm to 25.6±1.0 mm against *Escherichia coli,* from 0.0±0.0 mm to 26.0±1.0 mm against *Streptococcus Pyogenes,* from 0.0±0.0 mm to 25.0±2.0 mm against *Pseudomonas aeruginosa* and from 0.0±0.0 mm to 24.7±1.5 mm against *Enterococcus*

faecalis. And 2 other bacterial isolates were also screened for antibiosis disk diffusion against 6 test strains. These two isolates have also shown different inhibition zones against the strain by varying measurements, like 24.0±1.0 mm, and 22.0±1.0 mm against *Staphylococcus aureus*, from 0.0±0.0 to 20.0±1.0 against *E.coli***,** from 0.0±0.0 to 24.3±1.5 against *Pseudomonas*, mm, and from 0.0±0.0 to 22.0±1.0 against *Enterococcus.*

Antagonistic activity by agar well diffusion method: The agar well diffusion method is used for antimicrobial evaluations. In this study, 20 potential actinomycete isolates and two isolates of other bacteria were tested for their antagonistic activities against 6 different bacterial strains (table 4).

Table 4: Antagonistic activity of crude extracts of selected isolates by agar well diffusion

	isolate code	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*	Streptococcus pyogenes	*Pseudomonas Aeruginosa*	*Enterococcus faecalis*
1	AC2	18.7±2.1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	20.0±1.0
2	AC4	22.7±1.5	0.0±0.0	22.0±1.0	21.3±1.5	0.0±0.0	23.0±5.2
3	AC7	20.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
4	AC10	24.3±1.5	0.0±0.0	0.0±0.0	0.0±0.0	20.7±	20.7±1.5
5	AC11	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	20.3±1.5
6	AC15	0.0±0.0	0.0±0.0	0.0±0.0	20.7±0.6	0.0±0.0	20.0±1.0
7	AC23	22.7±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	22.0±1.0
8	AC31	21.3±1.5	0.0±0.0	21.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0
9	AC37	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	21.3±2.1	0.0±0.0
10	AC40	0.0±0.0	0.0±0.0	20.0±2.0	0.0±0.0	0.0±0.0	0.0±0.0
11	AC42	21.0±2	0.0±0.0	16.7±5.9	0.0±0.0	0.0±0.0	0.0±0.0
12	AC44	19.7±1.5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
13	AC46	22.0±1.0	23.0±1.0	23.3±0.6	0.0±0.0	21.7±1.2	21.3±1.5
14	AC48	0.0±0.0	0.0±0.0	21.3±1.5	20.0±1.0	0.0±0.0	19.7±1.1
15	AC49	21.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	21.7±1.5	0.0±0.0
16	AC51	0.0±0.0	0.0±0.0	21.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0
17	AC59	0.0±0.0	0.0±0.0	0.0±0.0	21.3±1.5	0.0±0.0	0.0±0.0
18	AC60	18.3±0.6	0.0±0.0	0.0±0.0	20.3±1.2	0.0±0.0	0.0±0.0
19	AC63	0.0±0.0	0.0±0.0	22.7±1.5	20.7±0.6	0.0±0.0	0.0±0.0
20	AC68	21.7±0.6	0.0±0.0	0.0±0.0	0.0±0.0	22.3±1.2	0.0±0.0
21	TB15	20.3±1.5	0.0±0.0	19.3±0.6	0.0±0.0	0.0±0.0	19.3±0.6
22	TB24	20.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	20.7±0.6	0.0±0.0
23	Total	13.3±10.3	1.1±4.8	8.5±10.4	5.6±9.3	5.8±9.6	8.7±10.3

Key: AC = actinomycete isolate; TB = other bacterial isolate

As shown in table 4, the crude extracted isolates of actinomycetes have shown antibacterial activities against 6 test strains with zones of inhibition ranging from 0.0±0.0 mm to 24.3±1.5 mm

against *Staphylococcus aureus,* from 0.0±0.0 mm to 23.0±1.0 mm against *Klebsiella Pneumoniae,* from 0.0±0.0 mm to 23.3±0.6 mm against *Escherichia coli,* from 0.0±0.0 mm to 21.3±1.5 mm against *Streptococcus Pyogenes,* from 0.0±0.0 mm to 22.3±1.2 mm against *Pseudomonas aeruginosa and* from 0.0±0.0 mm to 23.0±5.2 mm against *Enterococcus faecalis*. And 2 other bacterial isolates were also screened for antibiosis via agar well diffusion against 6 test strains. These two isolates have also shown different inhibition zones against the strain by varying measurements, like 20.0±1.0 mm, and 20.3±1.5 mm against *Staphylococcus aureus,* 0.0±0.0 mm, and 19.3±0.6 mm against *Escherichia coli,* 0.0±0.0 mm, and 20.7±0.6 mm against *Pseudomonas aeruginosa,* and 0.0±0.0 mm, and 19.3±0.6 mm against *Enterococcus faecalis*

4.1.4. Determination of MIC and MBC

To determine the MIC and MBC of selected isolates that were observed from a test tube 1, up to 10 equal amounts of Nutrient Broth (1ml) and a test strain (0.1mi) were added. Then after 1000µg was added in test tube one, 500 µg was added in the test tub two, 250 µg was added in test tube Three, 125 µg was added to the test tube. Four, 62.5µg, were added to the test tube, Five, 31.25 g was added to test tube sex, 15.27 g to test tube seven, 7.81 g to test tube eight, 3.9 g to test tube nine, and 2 was added to test tube ten the values of MIC was observed. The MIC of the crude extract solution of AC.4 was 250 µg /ml against *Staphylococcus aureus* and 500 µg /ml against *E.coli.* The MIC of the crude extract solution of AC.46 was 250 µg /ml against *E.coli* and *Staphylococcus aureus*. And also, the MBC of the crude extract solution of AC.4 was 250 µg /ml against *Staphylococcus aureus and* 500 µg /ml against *E.coli*. The MBC of crude extract solution of AC.46 was 500 µg /ml against *Staphylococcus aureus* and *E.coli (*table 5).

Table 5: The MIC and MBC values of isolates AC.4 & AC.46 of actinomycetes against S. aureus and E.coli.

	MIC		MBC	
Isolates	*Staphylococcus aureus*	*Escherichia coli*	*Staphylococcus aureus*	*Escherichia coli*
AC.4	250µg /ml	500µg /ml	250µg /ml	500µg /ml
AC.46	250µg /ml	250µg /ml	500µg /ml	500µg /ml

4.1.5. Antibiotic resistance profile of bacterial isolates

As there is no guideline for breakpoints for *Bacillus* in the CLSI guidelines and these species are mainly gram-positive bacteria like *Staphylococcus* spp., the breakpoints of *Staphylococcus* spp. were used as the criteria for interpretation. The breakpoints of *E.coli* are recommended and used for other Enterobacteriaceae such as *Shigella* & *Salmonella* Spp. (CLSI, 2020) (table 5& Appendix V).

Table 6: Antibiotic resistance profile of bacterial isolates from dumped municipal waste soil

Isolate type	No of isolates	TET.		GET		AMO.		CIPRO.		NA.		CHLOR.		Frequency of resistant	% of resistance
		R	% of R	R	% of R	R	% of R	R	% of R	R	% of R	R	% of R		
E.coli	11	R(6)	54.50%	0	0	R(11)	100%	0	-	R(11)	100%	0	0	28	42.4
Salmonella	6	0	0	0	0	R(6)	100%	0	0	R(4)	66.70%	0	0	10	15.2
Bacillus	2	0	0	0	0	R(2)	100%	0	0	0	0	0	0	2	28.6
Shigella	2	R(1)	50%	0	0	-	-	0	0	R(2)	100%	0	0	3	21.4
S. aureus	13	R(6)	46.20%	0	0	R (13)	100%	0	0	R(10)	76.90%	0	0	29	44
Pseudomonas	2	R(1)	50%	0	0	R(2)	100%	0	0	-	-	0	0	3	37.5
K. pneumonia	3	-	-	R(2)	100%	-	-	0	0	R(3)	100%	0	0	7	46.7
Total	39	**14**		**2**	**0**	**34**		**0**	**0**	**30**		**0**	**0**	**82**	
Activities of susceptibility in (%)		35.90%		5.12%		87.20%		0.00%		76.90%		0.00%		35.04%	

Key: R=resistanr;TET=Tetracyclun;GET=Gentamicine;AMO.=Amoxicillin:CIPRO.=Ciprofloxacin: NA=Nalidixic acid; CHLOR.= Chloramphenicol

Based on the antimicrobial resistance profiles of the bacterial isolates, of a total of 39 bacterial isolates, 35.9% were resistant, against tetracycline; 87.1% 0f isolates were resistant against amoxicillin but not susceptible;75% of isolates were susceptible against ciprofloxacin, but there were no isolates that resistant against to ciprofloxacin; 69.23% of bacterial isolates were resistant against to nalidixic acid and 64.1% of total bacterial isolates were susceptible against to chloramphenicol, but there were no isolates that resistant to chloramphenicol. As shown in table 6, *E.coli* isolates were resistant to amoxicillin, Nalidixic acid, and as well as tetracycline in the lower case, but are more susceptible to gentamycin, ciprofloxacin, and chloramphenicol. The *Staphylococcus* isolates showed resistance to, amoxicillin, and nalidixic acid but were susceptible to ciprofloxacin, chloramphenicol, and tetracycline. *Salmonella* isolates showed resistance to, amoxicillin and nalidixic acid but were susceptible to tetracycline, ciprofloxacin, chloramphenicol

and gentamycin. *Shigella* isolates are resistant to nalidixic acid, but are susceptible to tetracycline, ciprofloxacin and chloramphenicol. *Klebsiella pneumoniae* isolates are resistant to all except chloramphenicol and ciprofloxacin. *Bacillus* isolates are resistant to amoxicillin, and nalidixic acid, but are susceptible to tetracycline, chloramphenicol and ciprofloxacin. *Pseudomonas* isolates are resistant to amoxicillin and nalidixic acid, but are susceptible to ciprofloxacin, chloramphenicol and tetracycline.

Generally, of 39 bacterial isolates, the frequency of resistant, intermediate and susceptible were 34.45%, 21.74%, and 39.86% respectively; to antibiotic disks of tetracycline, gentamycin, ciprofloxacin, nalidixic acid and chloramphenicol. Individual isolate genera, however, had their own resistance to the various antibiotic disks.

From thirty-nine bacterial isolates, 18 (46.15%) had multidrug resistance to the antibiotic disks of tetracycline, gentamycin, amoxicillin, ciprofloxacin, nalidixic acid and chloramphenicol (which were taken from 5 antibiotic class). The MDR of selective bacterial isolates was determined as the following (table 7).

Table 7: The number and percentage of multidrug resistance bacterial isolates from sebatamite dumped municipal waste.

Isolates	R_0 = N (%)	R_1 = N (%)	R_2 = N (%)	R_3 = N (%)	R_4 = N (%)	R_5 = N (%)	MDR≥3
E.coli =11	0	0	8(63.6)	3(27.3)	1(9.1)	0	4(36.4%)
Salmonella=6	0	2(33.3)	2(33.3)	1(16.66)	1(16.66)	0	2(33.33%)
Bacillus =2	0	1(50)	0	1(50)	0	0	1(50%)
Shigella=2	0			2(100)			2(100%)
S. aureus=13	0	0	6(46.15)	4(30.77)	2(15.38)	0	6(46.15%)
Pseudomonas=2			1(33.3)	1(50)	0	0	1(50%)
K. pneumoniae=3	0	0	1(33.33)	2(66.66)			2(66.66%)
Total		3	18	14	4		**18**
%		7.70%	46.15%	38.90%	10.26%		**46.15%**

Key: MDR= Multi-drug resistant, R_0 =Isolates susceptible to all antibiotic class, R_1 =Isolates resistant one antibiotic class, R_2 = Isolates resistant to two antibiotic class, R_3 = Isolates resistant to three antibiotic class, R_4 = Isolates resistant four antibiotic class, R_5 = Isolates resistant to Five antibiotic class.

4.2. Discussion

4.2.1. Isolation of actinomycetes and other bacterial isolates.

The most significant bioactive chemicals for the treatment of infectious disorders are antibiotics. However, due to the emergence of multidrug-resistant organisms, there are now fundamental problems in the treatment of infectious diseases. As a result of the global burden of multidrug-resistant infections, there has been a growing interest in finding effective treatments (Abo-Shadi, 2010).

In the present study, among 30 waste soil samples collected, 70 actinomycetes isolates were isolated on the basis of different colony characteristics (colony shape, size, margin, color, opacity, elevation, and texture) from sebatamite dumped municipal waste soil samples. As compared to the previous reports by Atsede Mulat (2011), 30 soil samples were isolated from different sources and by the other reports Adamu (2020), fifty six actinomycetes isolates were isolated from different sources. On the other hand, 39 bacterial isolates were isolated from this dumped municipal waste soil in solid agar medium (EMB, Mannitol salt agar, SS agar and nutrient agar).

Various similar works have also reported the existence of bacteria belonging to these genera from various parts of the world. The recovery of members of the genera *Staphylococcus*, *Bacillus*, *Micrococcus*, *Pseudomonas*, *Klebsiella*, *Citrobacter*, *Proteus*, and *Escherichia*, species from the dumpsite was in agreement with the previous reports conducted in India (Chetan *et al*., 2017), Nigeria (Oluyege *et al*., 2017), Ghana (Borquaye *et al*., 2019), and Kenya (Song'oro *et al*., 2019) on municipal waste dumpsites.

Besides this, Chetan *et al*. (2017) additionally isolated *Serratia*, *Arthrobacter*, *Streptococcus*, *Corynebacterium*, and *Aeromonas* species from solid waste dumpsite. Similarly, Song'oro *et al*. (2019) also isolated *Vibrio cholera*, *Enterobacter*, *Serratia*, *Shigella*, *Salmonella*, *Providencia*, *Yersinia*, *Morganella* species from the dumpsite soil. This difference might happen as a result of differences in the complex of disposed wastes, physicochemical parameters of the dumpsite soil, and geographical and seasonal variations of the study areas. In fact, the heterogeneity of the dumpsite environment results in a heterogeneous population of soil bacteria (Chikere *et al*., 2011).

4.2.2. **In-*vitro* screening and evaluation of bacteria for antibacterial-production**

Seventy actinomycete isolates were isolated from Sebatamite municipal waste dumpsite and screened for antibiotic-production against bacterial strains using perpendicular streak plate method and dot spot method. Among these, only twenty (28.6%) isolates showed antibacterial activity against at least one of the tested bacteria. While, the remaining bacterial isolates were found to be incapable of exhibiting antibacterial activity against the various test organisms this might be due to, their natural tendency to do not produce antimicrobial substances.

The results obtained from primary screening indicated that twenty of these isolates showed antibacterial activity against Gram-positive bacteria and against Gram-negative tested bacteria. This diverse *in-vitro* antagonistic effect might be due to the multiple modes of actions of the tested bacterial isolates against bacterial strains. This result (28.6%) of actinomycete isolates was higher than 21.88%,and 26.7% and less than 59.09% from previous reports (Bzuye *et al*., 2013;Abo-Shadi, *et al*., 2010; Thakur *et al*., 2007).The difference might be the potentials of isolates, types of test strain methods of testing, sours of isolates and so on.

Observation of clear inhibition zones around the wells on the inoculated plates is an indication of antimicrobial activities of antibiotics extracted from actinomycetes against test organisms. In the present study the inhibition zone, the results in this study ranged from 0.0±0.0 mm to 24.3±1.5 mm against *Klebsiella Pneumoniae*, ranges from 0.0±0.0 mm to 25.0±1.0 against *S.aureus,* from 0.0±0.0 mm to 25.6±1.5 mm against *Escherichia coli,* from 0.0±0.0 mm to 26±1.0 mm against *Streptococcus Pyogenes,* from 0.0±0.0 mm to 25±2.0 mm against *Pseudomonas aeruginosa, and ranges from 0.0*±0 to 24.7±1.5 against *Enterococcus faecalis*. In Ethiopia's Gondar Town (B. Bizuye *et al*., 2013), they reported a 0-40 mm inhibition zone of crude extracts against selected test organisms isolated from the soil, Gurung, (2009), reported 0-18 mm inhibition zone of crude extracts against selected test organisms, And Atsede Muleta (2011) reported a 5-24mm inhibition zone of crude extracts against selected test organisms isolated from a different source. So the inhibition results in the present study were less than those reported by B. Bizuye *et al*., 2013,and higher than Gurung, (2009) but nearly identical to those reported by Atsede Muleta (2011).

4.2.3. The MIC and MBC values of selected isolates against *S.aureus* & *E.coli.*

In the present study the MIC and MBC values of the isolate by ethyl acetate crude extract were found between 250µg/ml and 500 µg/ml for different isolates selected isolates against different test

strain strains. In this study, the actinomycetes isolates of (AC.4), MIC values in *Staphylococcus aureus* and *E.coli* were 250µg/ml, and 500µg/ml respectively. While the MIC value of actinomycete Isolate (AC.46) in *Staphylococcus aureus* was 500µg/ml, and in E.coli was 250µg/ml. And the MBC value of the crude extract of isolate (AC.4) in Staphylococcus aureus was 250 g/ml, and the MBC value of (AC.4) in *E.coli* was 500 g/ml, while the MBC value of the actinomycete crude extract isolate (AC.46) in both *Staphylococcus aureus* and *E.coli* was 500 g/ml.

When comparing the results with the previous studies as reported by Mikayel *et al*., 2017, it showed the ethanol crude extract was bactericidal (MBC/MIC ≤ 2), or bacteriostatic (MBC/MIC ≤ 4). Actinomycetes isolate KBMWDSb6 (M6) produced bactericidal activity against *E. coli* ATCC 25922, *E. coli* 2966, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923, and bacteriostatic activity against *P. aeruginosa* 2929, *S. aureus* 2876. Actinomycetes isolate BRWDSc (SP) also produced bactericidal activity against *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 and was bacteriostatic against *S. aureus* 2876 and *S. aureus* ATCC 25923.

The value of MIC and MBC of the crude extract solution this study was not fit with previous report of Adamu, (2020), reported at 150 to 620µg/mL. This could be due to the concentration process of the extract, and nature of solvent which was done using evaporation (Tara *et al*., 2009), or variation in the test organisms used (Carvalho *et al*., 2016). Vangadeesh *et al*., 2011 stated that the MIC of the crude extract could also be affected by several parameters, including organism vulnerability, microorganism form, concentration and bioactive metabolite form, cultural media composition, incubation temperature, and time. This results in a value lower than that of Tara *et al*. (2009), who reported the MIC values of 1000 µg/mL.

4.2.4. Antimicrobial susceptibility profiles of the bacterial isolates

With regard to the antimicrobial susceptibility test profiles of the bacterial isolates from Sebatamite municipal waste dumpsite, of a total of 39 bacterial isolates, 35.9% were resistant, and 30.77% of the bacterial isolates were susceptible against tetracycline; 38.5% of isolates were susceptible against gentamicin;82.1% 0f isolates were resistant against amoxicillin but not susceptible;75% of isolates were susceptible against ciprofloxacin, but there were no isolates that resistant against to ciprofloxacin; 69.23% of bacterial isolates were resistant against to nalidixic acid and 64.1% of total bacterial isolates were susceptible against to chloramphenicol, but there were no isolates that resistant to chloramphenicol. *E.coli* isolates were resistant to amoxicillin, Nalidixic acid, and as well as tetracycline in the lower case, but are more susceptible to gentamycin, ciprofloxacin, and

chloramphenicol. The *Staphylococcus* isolates showed resistance to, amoxicillin, and nalidixic acid but were susceptible to ciprofloxacin, chloramphenicol, and tetracycline. *Salmonella* isolates showed resistance to, amoxicillin and nalidixic acid but were susceptible to tetracycline, ciprofloxacin, chloramphenicol and gentamycin. *Shigella* isolates are resistant to nalidixic acid, but are susceptible to tetracycline, ciprofloxacin and chloramphenicol. *Klebsiella pneumoniae* isolates were resistant to all except chloramphenicol and ciprofloxacin. *Bacillus* isolates are resistant to amoxicillin, and nalidixic acid, but are susceptible to tetracycline, chloramphenicol and ciprofloxacin. *Pseudomonas* isolates are resistant to amoxicillin and nalidixic acid, but are susceptible to ciprofloxacin, chloramphenicol and tetracycline.

Majority of the bacteria (*Staphylococcus*, *Pseudomonas, Klebsiella*, and *Escherichia* species) isolated during the course of this finding were reported earlier by (Obire *et al*., 2002; Sayah *et al*., 2005; Achudume and Olawale, 2007; Williams and Hakam, 2016; Idahosa *et al*. 2017: Borquaye *et al*., 2019) as potential pathogens from the dumpsite that may capable of causing disease in humans which is in agreement with the results of this finding. The presence of these potential pathogens reported in the present investigation might be attributed to the disposal of complex wastes that are originated from various sources to the municipal waste dumpsite and results contamination of the dumpsite soil environment.

In general, ciprofloxacin and Chloramphenicol were effective antimicrobial agents against to all of the bacterial species which correlates the results of Mwaikono *et al*., (2015). Chloramphenicol was also effective to most of the bacterial isolates except. A study done in Nigeria by Oviasogie *et al*. (2010) was reported agreement ideas with the present study most of the bacterial isolates were intermediate and resistant to tetracycline. This implies the high occurrence of these disposed antimicrobial agents on the dumpsite soil and in turn, changes the bacterial pressure from the dumpsite environment.

Regarding to multi-drug resistant of bacterial isolates, from the total of 39 bacterial isolates 18 (**46.15%)** of them were MDR isolates. Previously Multidrug-resistance among dumpsite isolates has been commonly reported. All the bacteria isolated from the dumpsites in Abraka showed multiple drug resistance patterns. These finding are similar to those of Oviasogie and Agbonlahor (2003) and Odjadjare *et al.* (2012). The high rate of multidrug resistance among the dumpsites isolates is suggestive of the introduction of resistant strains through the observed indiscriminate defecation and disposal of wastes at these dumpsites. The isolates must have originated from sources associated with high antibiotic use (Odjadjare *et al*., 2012).

5: **Conclusion and Recommendation**

5.1 CONCLUSION

Generally, in this study, both antibiotic-producing and drug-resistant bacteria were isolated, characterized, and identified from Sebatamite municipal waste dumpsite based on laboratory investigation. From the present study, it can be summarized that 20 actinomycetes isolates possessed an *in-vitro* antibacterial activity against Gram-positive (*S. aureus, Streptococcus pyogenes, & Enterococcus*) Bacteria and Gram-negative (*E.coli, Klebsiella Pneumonia, Pseudomonas*) bacterial strains by using disk diffusion, and agar well diffusion methods. Besides this, *Staphylococcus, Pseudomonas, Klebsiella, Shigella, salmonella,* and Escherichia isolates were isolated as drug-resistant bacteria and tested their antibiotic susceptibility against some antibiotic Disk. Ciprofloxacin and Chloramphenicol were the most effective antimicrobial agents against all isolated bacterial species with results of 75%, and 64.1% respectively. In the present study, bacterial isolates were exhibited more resistance towards amoxicillin and intermediate to another antimicrobial. Out of 39 bacterial isolates 18(46.15%) were multi-drug resistant. Furthermore, this study determines the minimum inhibition concentration and minimum bactericidal concentration of two more potent antibacterial activities of actinomycete isolates against *S. aureus* and *E.coli.*

5.2 Recommendation

The following recommendations are formulated based on the findings of this study:

- Although, the isolation, characterization, and identification of some actinomycetes & other bacteria, based on morphological, physiological, and some biochemical characterization, up to genus levels further study needs to characterized and identified isolates up to species levels by using molecular characterization.
- Since most commonly used drugs for the treatments of the infection caused by microorganism's especial opportunistic organisms are currently in needs, more study should be carried out not only from waste dump areas but other soil samples from different areas and ecosystems within Bahir Dar town.

6. References

Ababutain, I.M., Zeinab, K.A., Nijla, A.A. (2013). Optimization of environmental and nutritional conditions to improve growth and antibiotics productions by Streptomyces sp., isolated from Saudi Arabia Soil. *International Research Journal of Microbiology*, **4**(8): 179-187

Abebe Bizuye.,Bii, C., Erastus, G., and Maina, N. (2017). Antibacterial metabolite prospecting from Actinomycetes isolated from waste damped soils from Thika, the central part of Kenya. *Asian Pacific Journal of Tropical Disease* **7**(12): 757-764.

Abo-Shadi, M. A. A. R., Sidkey, N. M., & Al-Mutrafy, A. M.(2010). Antimicrobial agent producing microbes from some soils rhizosphere in Al-Madinah Al-Munawwarah, KSA.

Achudume, A. C. and Olawale, J. T.(2007).Microbial pathogens of public health significance in waste dumps and common sites. *Journal of Environmental Biology* **28**(1): 151-154.

Adekanle. M. A., Oluremi, A. S., and Akindele, A. A. (2014).Bacteria isolated from waste-dump sites soil at Osogbo, Osun State. *Journal of Microbiology and Biotechnology Res*earch **4**(3): 66-69.

Aghamirian, R.M, and Ghiasianm A.S. (2009).Isolation and characterization of medically important aerobic actinomycetes in the soil of Iran, *Open Microbiology Journal* **3**:53-7.

Andrews, M. (2001).Determination of minimum inhibitory concentrations, *Journal of Antimicrobial Chemotherapy* 48: 5-16.

Ansari SNepal HPGautam RShrestha SNeopane PGurung GChapagain ML (2015) Community acquired multi-drug resistant clinical isolates of Escherichia coli in a tertiary care center of Nepal Antimicrobial Resistance and Infection Control 4:15.

Arasu, M.V., Thankappan, S. R., Naif, A. A.,Veeramuthu, D., Savarimuthu, I., Paul, A., Sun-Ju, K. V., Aldous J., Kyung, D. L., Ki, C. C.(2014).Nutritional requirements for the production of antimicrobial metabolites from *Streptomyces. African Journal of Microbiology Research* . **8**(8): 750-758

Arifuzzaman, M., Khatun, M. R., and Rahman, H.(2010). Isolation and screening of actinomycetes from Sundarbans soil for antibacterial activity. *African Journal of Biotechnology* **9**(29): 4615-4619.

Arukwe, A., Eggen, T., and Möder, M.(2012). Solid waste deposits as a significant source of contaminants of emerging concern to the aquatic and terrestrial environments a developing country case study from Owerri, Nigeria. *Science of the Total Environment* **438**(1): 94-102.

Atsede, M.(2011). *Isolation and Screening of Antibiotic Producing Actinomycetes from Soil,* (Doctoral dissertation, Addis Ababa University)

Attwell, R.W., and Colwell R.R. (1984).Thermo actinomycetes as terrestrial indicators for estuarine and marine waters . In: **Biological, biochemical and biomedical aspects of actinomycetes**, pp. 441-472, (L. Ortiz Ortiz and L.F. Bojalil, eds.). Academic Press Inc.

Awisan, J., Demicais Jr, V., Badiang, A., Cariño, L.,and Ferrer, D. M. (2011). Aerobic pathogenic and opportunistic bacteria of public health significance in Irisan dumpsite soil .

Balouiri, M., Sadiki, M., and Ibnsouda, S. K. (2016) . Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis* **6(**2): 71-79.

Basha, S. C., and Rao, K. R. S. S. (2017).An intrinsic assessment of bioactive potentiality of mangroves actinomycetes. *J Entomol Zool Stud*, **5(**1): 20-26.

Bennett, P. M. (2008). Plasmid encoded antibiotic resistance: acquisition and transfer of antibiotic resistance genes in bacteria. *British journal of pharmacology*,**153**(1):347-357.

Bergey,D. H., and Holt, J.G. (1994). **Bergey`s manual of determinative bacteriology**. 9th ed. Baltimore: William and Wilkins.

Bizuye,A., Moges, F., and Andualem, B. (2013). Isolation and screening of antibiotic-producing actinomycetes from soils in Gondar town, North West Ethiopia . *Asian Pacific journal of tropical disease*, **3**(5):375-381.

Borquaye, L.S., Ekuadzi, E., Darko, G., Ahor, H.S., Nsiah, S.T., Lartey, J.A., Mutala, A.H., Boamah, V.E.,and Woode, E. (2019).Occurrence of Antibiotics and Antibiotic-Resistant Bacteria in Landfill Sites in Kumasi, Ghana. *Hindawi Journal of Chemistry* 1-10.

Cappuccino, J.G., and Sherman, N.(2014). **Microbiology: A Laboratory Manual**. 10th ed. Pearson Education Inc. San Francisco.

Carvalho,T., and Van Der Sand, S. (2016).Evaluation of antimicrobial activity of the endophytic actinomycete R18 (6) against multiresistant Gram-negative bacteria. *Anais da Academia Brasileira de Ciências*, **88**(1): 155-163.

Cheesbrough, M. (2005).*District laboratory practice in tropical countries, part 2*. Cambridge university press.

Chetan, D.M., Raghavendra, H.L., and Prithviraj, H.K. (2017).Isolation and Characterization of Bacteria from Solid Waste, *International Journal of Research and Scientific Innovation* **4**(5): 63-68.

Chikere, C. B., Okpokwasili, G. C., Chikere, B. O. (2011).Monitoring of microbial hydrocarbon remediation in the soil. *Journal of 3 Biotechnology* **1**(3): 117-138.

Chroma,M., and Kolar, M. (2010). Genetic methods for detection of antibiotic resistance: focus on extended-spectrum β-lactamases. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* **154(**4): 289-296.

Cuellar LEFernandez-Maldonado ERosenthal VDCastaneda-Sabogal ARosales RMayorga-Espichan MJCamacho-Cosavalente LACastillo-Bravo LI.(2008). Device-associated infection rates and mortality in intensive care units of Peruvian hospitals: findings of the International Nosocomial Infection Control Consortium Revista Panamericana De Salud Pública **24**:16–24.

Cycon,M., Mrozik, A., and Piotrowska-Seget, Z. (2019). Antibiotics in the Soil Environment Degradation, and Their Impact on Microbial Activity and Diversity, *Frontiers Microbiology* **10**(338): 1-45.

Davies, O. F., Adeleye, I. A., Wang, P.G. (2015).Hyoscyamine-producing marine *Actinomycetes* from Lagos Lagoon sediment.*Asian Pacific Journal of Tropical Biomedicine* **5**(3):196-201.

Dhanasekaran, D., Thajuddin, and Panneerselvam, A. (2009). Distribution and Ecology of Antagonistic Streptomyces from Agriculture and Coastal Soil in Tamil Nadu, India . *Journal of Culture collections* **6**(1): 10-20.

Duddu, M. K., and Guntuku, G. (2016).Isolation, screening and characterization of antibiotic-producing actinomycetes from Kapuluppada plastic waste dumping yard, Visakhapatnam . *International journal of pharmacy and pharmaceutical sciences* **8**(11): 221-229.

Dziri, R., Klibi, N., Alonso, C. A., Jouini, A., Ben Said, L., Chairat, S., and Torres, C. (2016). Detection of CTX-M-15-producing Escherichia coli isolates of lineages ST131-B2 and ST167-A in environmental samples of a Tunisian hospital. *Microbial Drug Resistance* **22**(5):399-403.

Efuntoye, M. O., Bakare, A. A., and Sowunmi, A. A. (2011).Virulence factors and antibiotic resistance in *Staphylococcus aureus* and *Clostridium perfringens* from landfill leachate . *African Journal of Microbiology Research* **5(**23):3994–3997.

Emmanuel, I., Joseph, N., Kingsley, E. I., Egbebor, E. M.,and Lawrence, E. (2011). Antibiotic susceptibility profiles of enteric bacterial isolates from dumpsite utisols and water sources in a rural community in Cross River State, Southern Nigeria. *Nature Science* 9: 46-50.

Ensieh, S., Mona, S., Vida, T. (2015). Antibacterial activity of some Actinomycetes isolated from soils of Alborz province, Iran.Progress in Biological Sciences. 5(2): 159-167.

Ferrer, M., Méndez-García, C., Rojo, D., Barbas, C., Moya, A .(2017). Antibiotic use and microbiome function. *Biochemical Pharmacology* **134**: 114-126.

Flora, D. O., Adeyemi, A. I., & George, W. P. (2015).Hyoscyamine-producing marine Actinomycetes from Lagos Lagoon sediment. *Asian Pacific Journal of Tropical Biomedicine*, **5**(3):196-201.

George, M., Anjumol, A., George, G., and Hatha, A. M. (2012). Distribution and bioactive potential of soil actinomycetes from different ecological habitats . *African Journal of Microbiology Research* **6**(10): 2265-2271.

Ghorbani-Nasrabadi, R., Greiner, R., Alikhani, H.A., Hamedi, J., Yakhchali, B. (2013).Distribution of Actinomycetes in different soil ecosystems and effect of media composition on extracellular phosphatase activity. *Journal of Soil Science and Plant Nutrition.***13** (1): 223-236.

Gurung, T. D., Sherpa, C., Agrawal, V. P., and Lekhak, B. (2009).Isolation and characterization of antibacterial actinomycetes from soil samples of Kalapatthar, Mount Everest Region. *Nepal Journal of Science and Technology* **10:** 173-182.

Hakim, A., Bhuiyan, F. R., Iqbal, A., Emon, T. H., Ahmed, J., and Azad, A. K. (2018). Production and partial characterization of dehairing alkaline protease from Bacillus subtilis AKAL7 and Exiguobacterium indicum AKAL11 by using organic municipal solid wastes . *Heliyon*, **4**(6): 646.

Harms, H., König, G. M., and Schäberle, T. F.(2017). "Production of antimicrobial compounds by fermentation" in *Antibiotics* (New York, USA: Springer), 49–61

Hasaneen, M., El-Sayed, A., and Sabry, S. (2018). Identification, Characterization and Optimized Antimicrobial Production of Streptomyces thinghirensis Isolate. *Journal of Agricultural Chemistry and Biotechnology* **9**(12): 263-268.

Hayakawa, Y., Shirasaki, S., Kawasaki, T., Matsuo, Y., Adachi, K.,and Shizuri, Y. (2007).Structures of new cytotoxic antibiotics, piericidins C 7 and C 8. *The Journal of antibiotics*, **60**(3):201-203.

Hong, K., Gao, A. H., Xie, Q. Y., Gao, H., Zhuang, L., Lin, H. P., ... and Ruan, J. S. (2009). Correction: Hong, K. et al. Actinomycetes for Marine Drug Discovery Isolated from Mangrove Soils and Plants in China. Mar. Drugs 2009, 7, 24–44. *Marine Drugs*, **7**(4):495-496.

Hrenovic, J., Ivankovic, T., Durn, G., Dekic, S., Kazazic, S., and Kisic, I. (2019). Presence of carbapenem-resistant bacteria in soils affected by illegal waste dumps . *International Journal of Environmental Health Research* **29**(2): 154-163.

Idahosa I. B., Obueh, H. O., Aigbekaen, M. O., Odesiri-Eruteyan, E. A. (2017). Characterization of Potential Pathogenic Bacterial Isolates from Urban and Rural Market Dumpsites . *International Journal of Microbiology and Biotechnology* **2**(3): 129-134.

Jiang, Y., Li, Q., Chen, X.,and Jiang, C. (2016).Isolation and cultivation methods of Actinobacteria. *Actinobacteria-basics and biotechnological applications*,39-57.

Kassahun Tassie. (2018). Household Behavior and Demand for Better Solid Waste Management Services: A Case of Bahir Dar City, Amhara National Regional State, Ethiopia. *Journal of Waste Recycling* **3** (1):1-15.

Kavitha, A.,Vijayalakshmi, M., Sudhakar, P., and Narasimha, G. (2010). Screening of actinomycetes strains for the production of antifungal metabolites. *African Journal of Microbiology Research*, **4**(1): 027-032.

Kayange NKamugisha EMwizamholya DLJeremiah SMshana SE (2010) .Predictors of positive blood culture and deaths among neonates with suspected neonatal sepsis in a tertiary hospital, Mwanza-Tanzania BMC Pediatrics **10**:39.

Kipre, B. G., Guessennd, N. K., Koné, M. W., Gbonon, V., Coulibaly, J. K., and Dosso, M. (2017). Antibacterial activity of the stem bark of Tieghemella Heckelii Pierre ex . A Chev against methicillin-resistant Staphylococcus aureus . *BMC complementary and alternative medicine* **17**(1):1-5.

Kumar, S., and Varela, M. F. (2013).Molecular mechanisms of bacterial resistance to antimicrobial agents . *Chemotherapy* **14**:522-534.

Laidi RF., Kansoh AL., Elshafi AM., and Cheikh B. (2006). Taxonomy, identification, and biological and activity of a novel isolate of streptomycestendae. *Arab Journal of Biotechnology* **9**:427-436.

LaPara, T. M., Burch, T. R., McNamara, P. J., Tan, D. T., Yan, M., and Eichmiller, J. J. (2011). Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into Duluth-Superior Harbor. *Environmental Science and Technology* **45**(22):9543–9549.

Laxminarayan RDuse AWattal CZaidi AKWertheim HFSumpradit NVlieghe EHara GLGould IMGoossens HGreko CSo ADBigdeli MTomson GWoodhouse WOmbaka EPeralta AQQamar FNMir FKariuki SBhutta ZACoates ABergstrom RWright GDBrown EDCars O (2013) .Antibiotic resistance-the need for global solutions The Lancet Infectious Diseases 13:1057–1098.

Li, D., Yu, T., Zhang, Y., Yang, M., Li, Z., Liu, M., and Qi, R.(2010).Antibiotic resistance characteristics of environmental bacteria from an ox tetracycline production wastewater treatment plant and the receiving river . *Journal of Applied and Environmental Microbiology* **76**(11): 3444–3451.

Li, Y., Cao, X., Ge, H., Jiang, Y., Zhou, H., and Zheng, W. (2018).Targeted surveillance of nosocomial infection in intensive care units of 176 hospitals in Jiangsu province, China. *Journal of Hospital Infection*, *99*(1), 36-41.

Loupy, K. M., Lee, T., Zambrano, C. A., Elsayed, A. I., D'Angelo, H. M., Fonken, L. K.,and Lowry, C. A. (2020). Alzheimer's Disease: Protective Effects of Mycobacterium vaccae, a Soil-Derived Mycobacterium with Anti-Inflammatory and Anti-Tubercular Properties, on the Proteomic Profiles of Plasma and Cerebrospinal Fluid in Rats. *Journal of Alzheimer's Disease*, (Preprint), 1-23.

Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... and Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, **18**(3):268-281.

Mandal, C., Tabassum, T., Shuvo, J.M.,and Habib, A. (2019). Biochemical and molecular identification of antibiotic-producing bacteria from waste dumpsite soil . *Journal of Advanced and Biotechnology Experimental Therapeutics* **2**(3): 120-126.

Manyi-Loh, C., Mamphweli, S., Meyer, E., Okoh, A.(2018). Antibiotic Use in Agriculture and Its Consequential Resistance in Environmental Sources: Potential Public Health Implications. *Molecules* **23**(795): 1-48.

Marcone, G. L., Binda, E., Reguzzoni, M., Gastaldo, L., Dalmastri, C.,and Marinelli, F. (2017). Classification of Actinoplanes sp. ATCC 33076, an actinomycete that produces the glycolipodepsipeptide antibiotic ramoplanin, as Actinoplanes ramoplaninifer spp. nov. *International journal of systematic and evolutionary microbiology*, **67**(10):4181-4188.

Martinez, J. L., (2009).Environmental pollution by antibiotics and by antibiotic resistance determinants. *Journal of Environmental Pollution* 157: 2893–2902.

Mikayel, G., Margarit P., Armen T. (2017). Antimicrobial activity of some plant materials used in Armenian traditional medicine. *BMC complementary and alternative medicine* **17**(1):1-9.

Moura, A., Pereira, C., Henriques, I., Correia, A. (2012). Novel gene cassettes and integrons in antibiotic-resistant bacteria isolated from urban wastewaters. *Research in microbiology* **16**(32), 92-100.

Mukesh, S. (2014). Actinomycetes: Source, Identification, and Their Applications.*International Journal of Current Microbiology and Applied Sc*iences. **3**(2): 801-832.

Muleta, A., and Assefa, F. (2018).Isolation and screening of antibiotic producing actinomycetes from rhizosphere and agricultural soils. *African Journal of Biotechnology*, **17**(22):700-715.

Mwaikono, K. S., Maina, S., and Gwakisa, P. (2015). Prevalence and antimicrobial resistance phenotype of enteric bacteria from a municipal dumpsite, *Journal of Applied and Environmental Microbiology* **3**(3): 82-94.

Narendra, K., Ravi, K.S., Mishra, S.K., Singh, A.K., and Pachouri, U.C. (2010).Isolation and screening of soil actinomycetes as a source of antibiotics active against bacteria.*International Journal of Microbiology Research*. **2**:12-6.

Nesme, J., and Simonet, P.(2015). The soil resistome: a critical review on antibiotic resistance origins, ecology, and dissemination potential in telluric bacteria. *Environmental Microbiology* **17**(4): 913-930.

Nikadio, H. (2009). Efflux-mediated drug resistance in bacteria: an update. *Drug*, **69**:12.

Nyandjou, Y. M. C., Yakubu, S. E., Abdullahi, I. O., and Machido, D. A.(2019). Multidrug resistance patterns and multiple antibiotic resistance index of salmonella species isolated from Waste Dumps in Zaria Metropolis, Nigeria. *Journal of Applied Sciences and Environmental Management* **23**(1): 41-46.

Obire, O., Nwaubeta, O., and Adue, B. N. (2002).Microbial community of a waste-dump site. *Journal of Applied Sciences and Environmental Management* **6** (1): 78-84.

Odum, E. I., Idise, O. E., and Ogogo, D. I. (2020).Multidrug resistant bacteria in dumpsite soils within Abraka, delta state, Nigeria. *Fudma Journal of Sciences* **4**(2): 639-644.

Olano, C., Mendez, C., and Salas, J.A. (2009).Antitumor Compounds from Marine Actinomycetes. *Marine Drugs* 7: 210-248.

Oluyege, J.O., Orjiakor, P.I., Omolara, A.R. (2017). Antimicrobial activities of ethyl acetate extracts of fungi isolated from waste dumpsites in Ado-Ekiti, Nigeria. *International Journal of Scientific Innovations* **1** (1): 015-021

Oviasogie, F. E., Ajuzie, C. U., Ighodaro, U. G. (2010).Bacterial analysis of soil from waste dump site. *Archives of Applied Science Research* **2**(5): 161-167.

Oviasogie, F. E., and Agbonlahor, D. E.,(2013). The burden, antibiogram and pathogenicity of bacteria found in municipal waste dumpsites and on waste site workers in Benin City. *Journal of Medicine and Biomedical Research,* **12**(2): 115-130.

Owolabi, J. B., and Hekeu, M. M.(2014). Heavy metal resistance and antibiotic susceptibility pattern of bacteria isolated from selected polluted soils in Lagos and Ota, Nigeria. *International Journal of Basic and Applied Sciences* **14**(6): 6-12.

Prakasam, C., Poongothai, E., Siddharthan, N.,and Hemalatha, N. (2017). Isolation, identification, enumeration, and antibiotic profiling of microbes from soil contaminated with hospital waste dumbing. *Journal of Pharmaceutical and Biological Sciences* **5**(3): 126-133.

Prashith K.T.R., Shobha, K.S., Onkarappa, R., Goutham, S.A., and Raghavendra, H.L. (2012) .Screening of Biological Activities of a *Streptomyces* Species Isolated from the soil of Agumbe, Karnataka, India. *International Journal of Drug Development and Research* **4** (3): 104-114.

Quantification and Characterization (QCB) (2010).Waste Quantification and Characterization Bahir Dar, Quantification and Characterization Bahir Dar (QCB), Bahir Dar, Ethiopia

Rajeswari, P., Polpass, A. J., Richa, A., Solomon, R. D. J., (2015).Characterization of saltern based Streptomyces sp.And statistical media optimization for its improved antibacterial activity.*Frontier in Microbiology*. 5:1-11.

Reddy NG., Ramakrishna DPN., and Raja Gopal SV. (2011).Morphological, physiological, and biochemical studies of marine Streptomyces rochei (MTCC 10109) showing antagonistic activity against human pathogenic microorganisms. *Asian J Biol Sci* **4**:1-14.

Remya, M., and Vijayakumar, R.(2008). Isolation and characterization of marine antagonistic actinomycetes from west coast of India. *Medicine and biology* **15**(1): 13-19.

Rinoy, V., Nishamol, S., Suchithra, R., Jyothy, S.,and Mohamed, H.A.A. (2012). Distribution and Antibacterial Activity of Actinomycetes from Shola Soils of Tropical Montane Forest in Kerala, *Journal of Environment* **01** (03): 93-99.

Rotich, M. C. (2018). *Bio-prospecting for broad spectrum antibiotic producing actinomycetes isolated from virgin soils in Kericho county* (Doctoral dissertation, JKUAT-COHES).Vangadeesh *et al*., 2011

Rozman, U., Duh, D., Cimerman, M., and Turk S.S.(2020). Hospital wastewater effluent: hot spot for antibiotic-resistant bacteria. *Journal of Water, Sanitation, and Hygiene for Development* 1-8.

RRaja, A., Prabakaran, P.,and Gajalakshmi, P. (2010). Isolation and screening of antibiotic-producing psychrophilic actinomycetes and its nature from Rothang hill soil against viridian's Streptococcus sp. *Research Journal of Microbiology*. **5**:44-9.

Sandhya, M. V. S., Ramyakrishna, E., Divya, P., Kumar, A. P., Karthik, R., Yazein, E. (2012).Isolation of antibiotic-producing bacteria from soil. *International Journal of Applied Biology and Pharmaceutical Technology* **6** (1): 46-51.

Santhi VS, Jose PA, and Solomon RDJ., (2010). Isolation, and characterization of antagonistic actinomycetes from mangrove sediments. *International Journal of Current Research* **3**:020-023.

Sayah, R. S., Kaneme, J. B., Johnson, Y., Miller, R (2005) Patterns of antimicrobial resistance observed in *Escherichia coli* isolates obtained from domestic and wild – animal faecal samples, human septage, and surface water. *Applied and Environmental Microbiology* **71**(3): 1394–1404.

Sethi, S., Kumar, R., and Gupta, S. (2013). Antibiotic production by microbes isolated from soil. *International Journal of Pharmaceutical Sciences and Research* **4**(8): 2967-2973.

Sharma, D., Kaur, T., Chadha, B. S., and Manhas, R. K. (2011).Antimicrobial activity of actinomycetes against multidrug resistant Staphylococcus aureus, E. coli and various other pathogens. *Tropical journal of pharmaceutical research* **10**(6): 801-808.

Sharma, M., Dangi, P., and Choudhary, M..(2014). Actinomycetes: source, identification, and their applications. *Int J Curr Microbiol App Sci*, **3**(2), 801-832.

Singh, L. S., Mazumder, S., and Bora, T. C. (2009). Optimisation of process parameters for growth and bioactive metabolite produced by a salt-tolerant and alkaliphilic actinomycete, Streptomyces tanashiensis strain A2D. *Journal de mycologie médicale*, **19**(4): 225-233.

Skold, O.(2011).**Antibiotics and Antibiotic Resistance**. Hoboken, New Jersey: Wiley and Sons, Inc.

Song'oro, E., Nyerere, A., Magoma, G., and Gunturu, R. (2019).Occurrence of Highly Resistant Microorganisms in Ruai Wastewater Treatment Plant and Dandora Dumpsite in Nairobi County. Kenya. *Advances in Microbiology* **9**(5): 479-494.

Sulaimon, A. M.,Akinwotu, O. O., and Amoo, O. T. (2015). Resistance of bacteria isolated from Awotan dumpsite leachate to heavy metals and selected antibiotics. *Int. J. Res*, **2**, 8-17.

Sundaramoorthi, C., Vengadesh, P.K., Gupta, S., Karthick, K., and Tamilselvi, N. (2011). Production, and characterization of antibiotics from soil-isolated actinomycetes. *International Research Journal of Pharmacy* **2**:114-8.

Tara, D.G., Chringma, S., Vishwanath, P.A., Binod, L. (2009).Isolation and Characterization of Antibacterial Actinomycetes from Soil Samples of Kalapatthar, Mount Everest Region. *Nepal Journal of Science and Technology* **10**:173-182.

Thakur, D.,Yadav, A., Gogoi, B. K., and Bora, T. C., (2007).Isolation and screening of Streptomyces in soil of protected forest areas from the states of Assam and Tripura, India, for antimicrobial metabolites. *Journal de Mycologie Medicals*, **17**(4): 242-249.

Tishkov, S.(2001). Bioactive products from Actinomycetes antibiotics, enzyme inhibitors , immunomodulators. *Innovative aspects in biotechnology of prokaryotes . National bank for industrial microorganisms and cell cultures, Sofia*:111-138.

Tiwari, K., and Gupta, R. K.(2012). Rare actinomycetes: a potential storehouse for novel antibiotics. *Critical reviews in biotechnology*, **32**(2): 108-132.

UNEP. (2010).Assessment of the Solid Waste Management System in Bahir Dar Town and the Gaps identified for the Development of an ISWM Plan.Forum for Environment.

USEPA. (2016).Advancing sustainable materials management: fact sheet. (Accessed date: 20November 2020).

Valgas, C., Souza, S. M. D., Smânia, E. F., Smânia Jr, A. (2007).Screening methods to determine the antibacterial activity of natural products. *Brazilian journal of microbiology* **38**(2): 369-380.

Wadetwar, N., R and Patil, T., A.,(2013).Isolation and characterization of bioactive actinomycetes from the soil in and around Nagpur. *International Journal of Pharmaceutical Sciences and Research* 4(4): 1428-1433.

Williams, J.O., Hakam, K. (2016). Microorganisms associated with dump sites in Port Harcourt Metropolis, Nigeria. *Journal of Ecology and the Natural Environment* **8**(2):9-12.

World Health Organization, (2014). Antimicrobial resistance: global report on surveillance

Wu, D., Huang, X.-H., Sun, J.-Z., Graham, D. W., and Xie, B.,(2017). Antibiotic resistance genes and associated microbial community conditions in aging landfill systems, *Environmental Science and Technology* **51**(21): 12859–12867.

Yamamura,H., Hayakawa, M., Nakagawa, Y., Tamura, T., Kohno, T., Komatsu, F., and Iimura, Y. (2005). Nocardia takedensis sp. nov., isolated from moat sediment and scumming activated sludge. *International journal of systematic and evolutionary microbiology*, **55**(1):433-436.

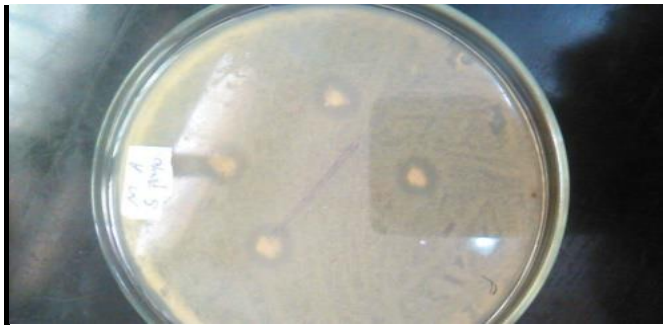
You, X., Wu, D., Wei, H., Xie, B., and Lu, F. (2018).Fluor quinolones and β-lactam antibiotics and antibiotic resistance genes in autumn leachates of seven major municipal solid waste landfills in China. *Environment International* **113**: 162–169.

Yu, Z., He, P., Shao, L., Zhang, H., and Lu, F. (2016).Co-occurrence of mobile genetic elements and antibiotic resistance genes in municipal solid waste landfill leachates: a preliminary insight into the role of landfill age. *Water Research* **106**: 583–592.

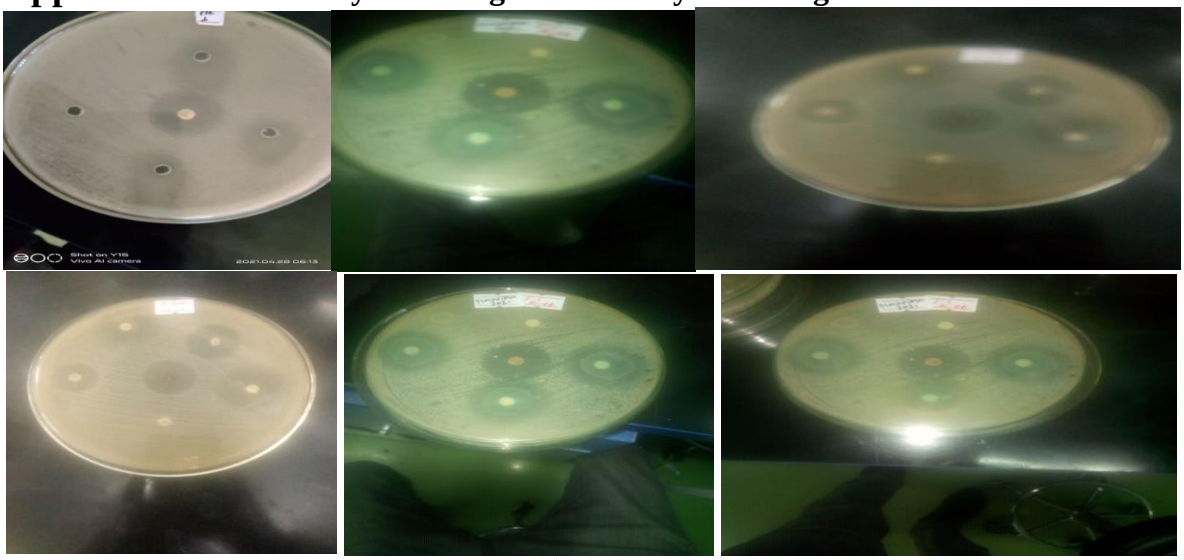
Zhang, J., and Zhang, L.(2011).Improvement of an isolation medium for actinomycetes. *Modern Applied Science*, **5(**2): 124.

7. Appendixes

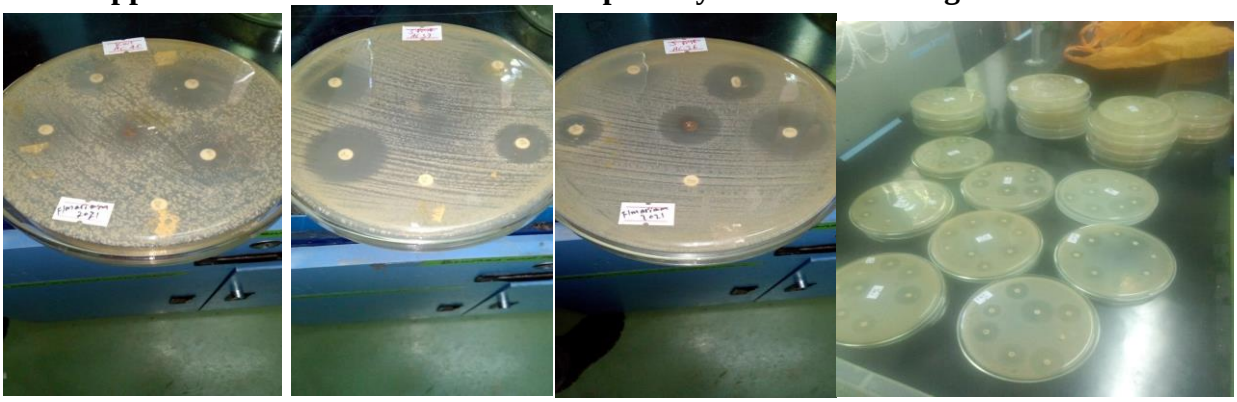
Appendix I: Some primary screening of isolates by do Spot method



Appendix II:-Secondary screening of isolates by disk & agar well Diffusion methods



Appendix III: - Some Antibiotic susceptibility Test of isolates against some strains



APPENDIX IV:-Characterization of actinomycetes and other bacteria

Table 1: Colony characteristics of actinomycete isolates

Isolate Codes	Colony characteristics							
	Shape	Size	Margin	Color	Opacity	Elevation	Surface	Texture

IA2	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA4	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA7	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA10	Filamentous	Large	Entire	White	Opaque	Raised	Rough	Moist
IA11	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA36	Corkscrew	Large	Entire	White	Opaque	Raised	Rough	Moist
IA37	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA38	Filamentous	Large	Entire	White	Opaque	Raised	Rough	Moist
IA40	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA42	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA44	Corkscrew	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA45	Filamentous	Large	Entire	White	Opaque	Raised	Rough	Moist
IA46	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA48	Corkscrew	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA49	Corkscrew	Large	Entire	White	Opaque	Raised	Rough	Dry
IA51	Corkscrew	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA59	Filamentous	Large	Entire	White	Opaque	Raised	Rough	Moist
IA60	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA61	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist
IA63	Filamentous	Large	Entire	White	Opaque	Raised	Smooth	Moist

IA: isolate actinomycete

Table 2: Morphological, physiological, and biochemical characteristics of the isolated actinomycetes

Isolate codes	Gram reaction	Cell shape	Catalase test	Motility	Indole test	Methyl red test	Voges Proskauer	Citrate utilization	Starch	Urease test	Fermentation of D-	Lactos	Sucros	H_2S	Gas from glucose	**Probable bacteria**
IA2	+	Rod	+	−	−	+	+	+	+	+				+	−	*Streptomycetes*
IA4	+	Rod	+	−	−	+	+	+	+	+				+	−	*Streptomycetes*
IA7	+	Rod	+	−	−	+	+	+	+	+				+	−	*Streptomycetes*
IA10	+	Rod	+	−	−	+	+	+	+	+				+	−	*Streptomycetes*
IA11	+	Rod	+	−	−	+	+	+	+	+				+	−	*Streptomycetes*
IA36	+	Rod	+	+	−	+	+	+	+	+				+	−	Actinomycetes
IA37	+	Rod	+	−	−	+	+	+	+	+				+	−	*Streptomycetes*
IA38	+	Rod	+	−	−	+	+	+	+	+				+	−	*Streptomycetes*

IA40	+	Rod	+	−	−	+	+	+	+	+	+	−	*Streptomycetes*
IA42	+	Rod	+	−	−	+	+	+	+	+	+	−	*Streptomycetes*
IA44	+	Rod	−	+	−	+	+	+	+	+	+	−	Actinomycetes
IA45	+	Rod	+	+	−	+	+	+	+	+	+	−	*Streptomycetes*
IA46	+	Rod	+	+	−	+	+	+	+	+	+	−	*Streptomycetes*
IA48	+	Rod	−	−	−	+	−	+	+	+	+	−	*Streptomycetes*
IA49	+	Rod	−	−	−	+	−	+	+	+	+	−	Actinomycetes
IA51	+	Rod	−	−	−	+	−	+	+	+	+	−	Actinomycetes
IA59	+	Rod	+	+	−	+	+	+	+	+	+	−	*Streptomycetes*
IA60	+	Rod	+	+	−	+	+	+	+	+	+	−	*Streptomycetes*
IA61	+	Rod	+	+	−	+	+	+	+	+	+	−	*Streptomycetes*
IA63	+	Rod	+	+	−	+	+	+	+	+	+	−	*Streptomycetes*

Key: + (Positive) ; - (negative); **IA:** isolate actinomycete

Table 3: Colony characteristics of bacterial isolates obtained from Sebatamite Municipal waste dumpsite.

Isolate Codes	Colony characteristics							
	Shape	Size	Margin	Color	Opacity	Elevation	Surface	Texture
IB1	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB2	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB3	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB4	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB5	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB6	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB7	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB8	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB9	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB10	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB11	Bacilli	Large	Entire	White	Opaque	Convex	Smooth	Moist
IB12	Bacilli	Large	Entire	White	Opaque	Raised	Smooth	Moist
IB13	Bacilli	Large	Entire	White	Opaque	Raised	Smooth	Moist
IB14	Bacilli	Large	Entire	White	Opaque	Raised	Smooth	Moist
IB15	Rod	Medium	Irregular	Yellow	Opaque	Convex	Rough	Dry
IB16	Bacilli	Large	Entire	White	Opaque	Raised	Smooth	Moist
IB17	Rod	Small	Entire	White	Translucent	Convex	Smooth	Moist
IB18	Rod	Small	Entire	White	Translucent	Convex	Smooth	Moist
IB19	Rod	Small	Entire	White	Translucent	Convex	Smooth	Moist

IB20	Rod	Small	Entire	White	Translucent	Convex	Smooth	Moist
IB21	Rod	Small	Entire	White	Translucent	Convex	Smooth	Moist
IB22	Rod	Small	Entire	White	Translucent	Convex	Smooth	Moist
IB23	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB24	Rod	Medium	Irregular	Yellow	Opaque	Convex	Rough	Moist
IB25	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB26	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB27	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB28	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB29	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB30	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB31	Cocci	Large	Entire	Yellow	Opaque	Convex	Smooth	Moist
IB32	Bacilli	Large	Entire	Pink	Opaque	Umbonet	Smooth	Moist
IB33	Bacilli	Small	Entire	Pink	Opaque	Umbonet	Rough	Dry
IB34	Bacilli	Small	Entire	Pink	Opaque	Umbonet	Wrinkle	Dry
IB35	Bacilli	Small	Entire	Pink	Opaque	Umbonet	Wrinkle	Dry
IB36	Rod	Medium	Entire	Cream	Opaque	Raised	Smooth	Moist
IB37	Rod	Medium	Entire	Cream	Opaque	Raised	Smooth	Moist
IB38	Rod	Medium	Entire	Cream	Opaque	Raised	Smooth	Moist
IB39	Rod	Medium	Entire	Cream	Opaque	Raised	Smooth	Moist

IB; isolate bacteria

Table 4: Morphological, physiological, and biochemical characteristics of the isolated gram-positive and gram-negative bacteria from the study area

Isolate codes	Gram reaction	Cell shape	Catalase test	Motility	Indole test	Methyl red test	Voges-Proskauer	Citrate	Starch	Urease test	Fermentation of D-Lactos	Fermentation of Sucro	H_2S from	Gas from	**Probable bacteria**
IB1	−	Bacilli	+	+	+	+	−	−	−	−			+	+	*E.coli*
IB2	−	Bacilli	+	+	+	+	−	−	−	−			+	+	*E.coli*
IB3	−	Bacilli	+	+	+	+	−	−	−	−			+	+	*E.coli*
IB4	−	Bacilli	+	+	+	+	−	−	−	−			+	+	*E.coli*
IB5	−	Bacilli	+	+	+	+	−	−	−	−			+	+	*E.coli*

IB6	−	Bacilli	+	+	+	+	−	−	−	−	+	+	*E.coli*
IB7	−	Bacilli	+	+	+	+	−	−	−	−	+	+	*E.coli*
IB8	−	Bacilli	+	+	+	+	−	−	−	−	+	+	*E.coli*
IB9	−	Bacilli	+	+	+	+	−	−	−	−	+	+	*E.coli*
IB10	−	Bacilli	+	+	+	+	−	−	−	−	+	+	*E.coli*
IB11	−	Bacilli	+	+	+	+	−	−	−	−	+	+	*E.coli*
IB12	−	Bacilli	+	+	−	+	−	+	−	−	+	−	*Salmonella* spp.
IB13	−	Bacilli	+	+	−	+	−	+	−	−	+	−	*Salmonella* spp.
IB14	−	Bacilli	+	+	−	−	−	+	−	−	+	−	*Salmonella* spp.
IB15	+	Rod	+	+	−	+	+	−	+	+	+	−	*B. subtilis*
IB16	−	Bacilli	+	+	−	+	−	+	−	−	+	−	*Salmonella* spp.
IB17	−	Bacilli	+	+	−	+	−	+	−	−	+	−	*Salmonella* spp.
IB18	−	Bacilli	+	+	−	+	−	+	−	−	+	−	*Salmonella* spp.
IB19	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB20	−	Bacilli	+	−	+	+	−	−	−	−	−	−	*Shigella* spp.
IB21	−	Bacilli	+	−	+	+	−	−	−	−	−	−	*Shigella* spp.
IB22	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB23	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB24	+	Rod	+	+	−	−	+	−	+	+	+	−	*B. cereus*
IB25	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB26	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB27	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB28	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB29	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB30	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB31	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB32	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB33	−	Bacilli	+	+	−	−	−	+	−	−	−	−	*Pseudomonas* spp.
IB34	−	Bacilli	+	+	−	−	−	+	−	−	−	−	*Pseudomonas* spp.
IB35	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB36	+	Cocci	+	−	−	+	+	+	±	+	−	−	*S. aureus*
IB37	−	Rod	+	−	−	−	+	+	−	+	−	+	*K. pneumoniae*
IB38	−	Rod	+	−	−	−	+	+	−	+	−	+	*K. pneumoniae*

IB39 − Rod + − − − + + − + − + *K. pneumoniae*

Key: + (Positive) ; **-** (negative); **IB**; isolate bacteria

Appendix V: Antibiotic resistance profile of bacterial Isolates

Table 5: Antibiotic susceptibility testing of total bacterial isolates

NO.	Isolates	Tetra cyclone 30µg	Gentamycin 10µg	Streptomycin 30µg	Vancomycin 30µg	Amoxicillin2µg	Ciprofloxacin 5µg	Nalidixic Acid 30µg	Chloramphenicol30 µg	Presumptive
1	TB.1	23(S)	14 (I)	11 (R)	-	9 (R)	23(I)	11(R)	31 (S)	*E.coli*
2	TB.2	23 (S)	20 (S)	13 (I)	-	8 (R)	23 (I)	11(R)	31(S)	*E.coli*
3	TB.3	13 (I)	13(I)	19 (S)	-	9 (R)	30 (S)	11(R)	35 (S)	*E.coli*
4	TB.4	11 (R)	20 (S)	19 (S)	-	8 (R)	27 (S)	11(R)	33 (S)	*E.coli*
5	TB.5	10 (R)	14 (I)	10 (R)	-	0 (R)	26(S)	10(R)	16 (I)	*E.coli*
6	TB.6	13 (I)	22 (S)	21 (S)	-	0 (R)	23(I)	11(R)	15(I)	*E.coli*
7	TB.7	10(R)	12(R)	18 (S)	-	0 (R)	25(S)	9(R)	25 (S)	*E.coli*
8	TB.8	13(I)	22(S)	19 (S)	-	8 (R)	29 (S)	12(R)	28 (S)	*E.coli*
9	TB.9	10(R)	13(I)	12 (R)	-	9 (R)	32 (S)	1O(R))	16 (I)	*E.coli*
10	TB.10	11(R)	14(I)	16 (S)	-	9 (R)	32 (S)	10 (R))	27 (S)	*E.coli*
11	TB.11	10(R)	23(S)	11 (R)	-	7 (R)	24(I)	12 (R)	33 (S)	*E.coli*
12	TB.12	23 (S)	14 (I)	14 (I)	-	0 (R)	22(I)	12 (R)	13(I)	*Salmonella*
13	TB.13	12 (I)	13 (I)	11 (R)	-	0 (R)	21(I)	20 (R)	25 (S)	*Salmonella*
14	TB.14	104(I)	14(I)	9 (R)	-	0 (R)	32 (S)	12 (R)	15 (I)	*Salmonella*
15	TB.15	16 (I)	19(S)	-	10(R)	0 (R)	24 (S)	21 (S)	23 (S)	*Bacillus*
16	TB.16	21 (S)	19 (S)	11 (R)	-	9(R)	23 (I)	19 (S)	16 (I)	*Salmonella*
17	TB.17	22 (S)	17 (S)	10 (R)	-	0 (R)	22(I)	10 (R)	29 (S)	*Salmonella*
18	TB.18	23 (S)	19 (S)	11 (R)	-	0 (R)	33(S)	20 (S)	29 (S)	*Salmonella*
19	TB.19	11 (R)	20 (S)	-	10(R)	10(R)	30 (S)	12 (R)	30 (S)	*S. aureus*
20	TB.20	21 (S)	20 (S)	13(I)	-	9 (R)	25 (S)	14 (I)	29 (S)	*Shigella*
21	TB.21	20 (S)	13 (I)	11(R)	-	0 (R)	30 (S)	13 (R)	28 (S)	*Shigella*
22	TB.22	10 (R)	21 (S)	-	10 (R)	0 (R)	24 (S)	12 (R)	17 (I)	*S. aureus*
23	TB.23	14 (I)	14(I)	-	11 (R)	9 (R)	30 (S)	13 (R)	28 (S)	*S. aureus*
24	TB.24	15 (I)	19 (S)	-	11 (R)	0 (R)	19(I)	20 (I)	25 (S)	*Bacillus*
25	TB.25	25 (S)	13 (I)	-	12 (R)	0 (R)	23 (S)	20 (I)	30 (S)	*S. aureus*
26	TB.26	15 (S)	21 (S)	-	11 (R)	0 (R)	23 (S)	12 (R)	16 (I)	*S. aureus*
27	TB.27	11(R)	14 (I)	-	10 (R)	0 (R)	30 (S)	12 (R)	28 (S)	*S. aureus*
28	TB.28	22 (S)	13(I)	-	16 (I)	0 (R)	22 (S)	19 (I)	17 (I)	*S. aureus*
29	TB.29	12 (R)	23 (S)	-	10 (R)	10 (R)	30 (S)	13 (R)	36 (S)	*S. aureus*
30	TB.30	16 (I)	11 (R)	-	11 (R)	9 (R)	30 (S)	19 (I)	30 (S)	*S. aureus*
31	TB.31	25 (S)	10 (R)	-	11(R)	10 (R)	25 (S)	14 (R)	35 (S)	*S. aureus*
32	TB.32	23 (S)	14 (I)	-	11(R)	0 (R)	30 (S)	10(R)	28 (S)	*S. aureus*
33	TB.33	27(S)	14 (I)	-	-	0 (R)	29 (S)	13 (R)	28(S)	*Pseudomonas.*
34	TB.34	11 (R)	20(S)	-	-	0 (R)	30 (S)	10(R)	27 (S)	*Pseudomonas.*
35	TB.35	22 (S)	13(I)	-	12(R)	0 (R)	30 (S)	12 (R)	27 (S)	*S. aureus*
36	TB.36	27(S)	13 (I)	-	11(R)	0	32 (S)	13(R)	33 (S)	*S. aureus*
37	TB.37	-	13 (I)	9 (R)	-	-	18 (I)	13(R)	30 (S)	*K. pneumonia*
38	TB.38	-	12(R)	11 (R)	-	-	30 (S)	12(R)	30 (S)	*K. pneumonia*
39	TB.39	-	11 (R)	10 (R)	-	-	35 (S)	9 (R)	36 (S)	*K. pneumonia*