COLLEGEOF NATURAL SCIENCE

DEPARTMENT OF BIOLOGY-,

ISOLATION AND CHARACTERIZATION OF SPORE FORMING BACTERIA FROM PROCESSED AND CANNED PRODUCTS IN DEBRETABOR TOWN, AMHARA REGION

A THESIS SUBMITTED TO DEPARTMENT OF BIOLOGY, COLLEGE OF NATURAL SCEINCES FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS OF THE DEGREE OF MASTERS OF SCIENCE IN GENERAL BIOLOGY

BY: ASFAW GEBEYEHU

ADVISOR: MESERET GUTA (MSc. Asst. Prof)
CO ADVISOR: SHIFERAW DEMSSIE (MSc. Asst. Prof)

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JIMMA, ETHIOPIA

APPROVAL SHEET

As a thesis research advisor, I hereby certify that I have read and evaluated this thesis prepared under my guidance, by Asfaw Gebeyehu Worku entitled as "Isolation and characterization of spore forming bacteria from processed and canned food in Debretabor Town." I am recommended the paper to be submitted as fulfilling the requirement for the degree of Master of Science in Biology (General biology).

_____	_____	_____
Advisor	Signature	Date

As members of the board of examiners for the MSc thesis open defense examination, we certify that we have read and evaluated the thesis prepared by Asfaw GebeyehuWorku and examined the candidate. We recommended the thesis to be accepted as Fulfillment for the Requirements of the Degree of Master of Science in Biology (General biology).

_____	_____	_____
Chair Person	Signature	Date
_____	_____	_____
Internal Examiner	Signature	Date
_____	_____	_____
External Examiner	Signature	Date

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Abstract

Canned foods are not fully sterile from microbes. These food products are thermally processed and are very diverse. This can include low, medium and high viscosity liquids, and other solid canned food products. The primary objective of this study was to isolate and characterize spore forming bacteria from canned and processed foods available to consumers on the (super market and shops) Town. A total of 60 samples comprising 20 each of (fish, meat and powdered milk) were aseptically collected from Debretabor Town super markets and shops. Date of production, expiration and ingredients of each sample were recorded. The samples were homogenized and serial dilution was conducted and cultured on appropriate culture media following standard procedures. Isolates were further confirmed by using morphological and biochemical tests. Antimicrobial susceptibility test was performed for the isolated bacteria using disk diffusion method on Muller Hinton Agar plate. The result confirmed that two genera namely Bacillus and Clostridium were the most frequently isolated bacteria. Most of the isolates were resistant to Ampecilin, Cephalotin, Methicilin and Tetracycline. On the other hand these isolates were sensitive to Chloraphenicol, Erythromycin, Gentimycin and vancomycin. Both aerobic and anaerobic spore-former heat-resistant microorganisms which are isolated in this study have a role in poisoning and spoilage of canned foods and this might cause subsequent food borne outbreaks. Hence it is possible to recommend other researchers to come and do a research on the area and food factories to recheck their canned food products as well as have consider where they store the canned food products.

Keywords: spore forming, Bacillus, canned food, isolation, antibiotic resistance.

1. Introduction

1.1 Back ground of the study

There are various types of canned foods produced worldwide. These include canned meat and vegetable salads, powdered milk, canned baby foods, mayonnaise and salad dressing products, pickles, jams, jellies and related products, canned soups, canned meat and poultry products, canned sea food products, canned dry pack products, canned juices, fruit drinks and water, canned fruits and canned vegetables *(Gaze, 2005).*

In Africa canned food products have been widely circulated in the free market, including those found in supermarkets and traditional markets. Not only home industries have produced canned food, but also large industries. The existence of canned food began to be known to the public, although not widely *(ElShehawy, 2019).*

In Ethiopia There are also various types of canned foods that are locally produced and imported from abroad. The locally produced canned foods include, canned soups, canned fish, corned meat, and cookies. The imported canned foods include sardine, tuna, canned milk, canned meat, canned powdered milk, canned juices, fruit drinks and water (Yonas, 2013).The canned food that are found in Debre Tabour Town includes cookies, , canned milk, canned meat, canned powdered milk, water juice, fruits and vegetables.

Canned foods are subject to microbial spoilage. The range of food products that are thermally processed is very diverse. It can include low, medium and high viscosity liquids, some with particulates (Ranken, 2007). The effect of these different substrates on the heat resistance of a microorganism can be quite marked, with some proteins, fats and high total solids increasing the heat resistance by a factor of 2 or 3 when compared with the standard heat resistance of a similar microorganism in a broth (Gaze, 2005). The influence of these components on the heat resistance of microorganisms must, therefore, be carefully considered during product development. Spoilage of canned foods is usually caused by growth of microorganisms following leakage or under-processing. Leakage occurs from can defects arising from punctures or rough handling. Contaminated cooling water sometimes leaks to the interior through pinholes or poor seams and introduces bacteria that cause spoilage (Ranken, 2007). The presence of bacterial spore (Bacillus and Clostridium) in canned food is indicating in a more viscous or tighter packing in the

container. Under processed and leaking cans are of major concern and both pose potential health hazards (Ranken, 2007).

Based on acid content, canned foods are categorized as high acid, acid and low acid products. High acid canned foods include fruits and vegetable products, whose pH ranges from < 3.7 - 4.0, acid canned foods have pH 4.0 - 4.6 and low acid canned foods have pH> 4.6(Devos *et al*., 2009).. The pH values of low-acid canned foods provide an optimum pH for reproducing, outgrowth of spores and toxin production by bacteria. Canned foods that are considered to be low acid are meat and poultry products, vegetables, fish and milk products (Devos *et al*., 2009). These canned foods require proper handling of raw materials during production, adequate thermal treatment and proper handling of the product until it reaches consumers. The need for time/temperature control is primarily important to determine the potential for the survival of pathogenic microorganisms of concern, and the potential for the subsequent growth and/or toxin production. Improper heat treatment and process practices make these foods susceptible to bacterial spoilage (FDA, 2003).

Some bacteria, under stress conditions, can temporarily and reversibly change their genetic expression, leading to a new phenotype called spore. They are termed as spore forming bacteria (Mckenney *et al.,* 2013). Spores or endospores are differentiated cells, highly different to vegetative cells by their structure and especially their high resistance to physico-chemical agents.These sullied spores can undoubtedly get by more than a huge number of years, in view of their hard external covers, metabolically latent structures delivered by a portion of the vegetative bacterial cell the bacterial cell and methodology for endurance in unfriendly climatic conditions (Abecasis *et al*., 2013). Spores won't pick any single method to ruin the food possibly it takes numerous ways like vigorous, anaerobic or facultative oxygen consuming bacteria (Devos *et al*., 2009).Some of the microbes like *Clostridium botulinum* and *Bacillus cereus* cause food contamination(Mckenney *et al.,* 2013).

This study tried to isolate and characterize the bacteria in the canned foods and the fate of contaminating pathogens in the canned foods and to assess the distribution of drug resistance among the dominant bacterial groups isolated from the canned foods. The other reason that initiated the researcher to do a research on this title is that there was not a research done previously in this area and personal observations of some sick people after consumption of

canned food mainly babies and children. In addition conducting this research on this area would help to invite other researchers to come and perform further study on this title.

1.2 Statement of the problem

The WHO Food borne Disease Burden Epidemiology Reference Group provided in 2015 an estimate of global food borne disease incidence, mortality, and disease burden are in terms of aggravate situation (WHO 2015). The global burden of food borne hazards was 33 million in 2010; 40% affecting children under 5 years of age. The US CDC estimated that each year roughly 48 million people in the US gets sick, 128,000 are hospitalized, and 3,000 die from food borne diseases (WHO, 2015). The number of confirmed cases, hospitalizations and deaths caused by the most common food borne pathogens reported in the Food borne Diseases Active Surveillance Network, US, 2015. These major food borne pathogens also represent an important economic concern; the aid to cost more than $USD 3.5 billion in medical costs and a further $USD 304 million was paid by the European Commission for crop losses due to not selling the fresh product (WHO, 2015).

Bacteria are the most common cause of food borne diseases and exist in a variety of shapes, types and properties. Some pathogenic bacteria are capable of spore formation and thus, highly heat-resistant (e.g., *Clostridium botulinum*, *C. perfringens*, *Bacillus subtills*, *Bacillus cereus*) (Bacon, 2003). Spore forming bacteria that are present in packed food products are important because the formation of the spore by the bacterium allows it to be resistant to heat, freezing, chemicals, and other adverse environments that our food undergoes during processing and preparation. Spores can survive during food processing and after germination and out growth cause spoilage and even outbreaks of food borne illness. (Gonzalez *et al.*, 2002). Acute diarrheal illness is very common worldwide and estimated to account for 1.8 million childhood deaths annually, predominantly in developing countries (WHO, 2015). Consumption of canned food

products continue to be the risk contaminated with food borne diseases, so conducting studies on food borne pathogens and their antimicrobial resistance would help the users and other researchers deliver awareness about these processed and canned food. It is witnessed that Children who consume canned powdered milk products fell uncomforted and become sick so that the microbial states (spore formers) would be the possible cause of illness.

1.3 Research questions

After conducting this research, the following research questions were answered: -

- ✓ Which spore forming bacteria are most frequently occurred in canned food?
- ✓ To what extent canned products are contaminated by spore forming bacteria?
- ✓ What is the antimicrobial susceptibility pattern of isolated bacteria species?

1.4. Objective of the study

1.4.1. General objective

- ➢ The general objective of the study was to isolate and characterize spore forming bacteria from canned and processed food in Debre Tabor Town, South Gondar zone, Amhara region.

1.4.2. Specific objectives

- ✓ To isolate spore forming aerobic and anaerobic bacteria species from canned products.
- ✓ To characterize spore forming aerobic and anaerobic bacteria species from canned products.
- ✓ To determine the antimicrobial susceptibility pattern of the isolates

1.5 significance of the study

This study was conducted in Ethiopia, particularly Debretabor town to isolate and characterize spore forming bacteria in processed food products. In this study, different spore forming bacteria genera have been isolated from the collected food samples and these isolates might cause disease outbreak. Basic food safety measures and inadequate public awareness of hazards posed

by certain foods has severely hampered the deployment of precise scientific approach to this very series issue of public health and safety.

2. Review of related Literature

2.1. Microbiology of canned food

Foods have associated micro floras; certain microorganisms are usually found in certain food groups. These organisms gain entrance into the food during the canning operation, either from the soil, from the ingredients, or from equipment. On the basis of acidity classification of foods it is possible to make general statements relative to the microorganisms which are potentially capable of producing spoilage in canned foods (Jay, 2009).

2.1.1 PH and growth of Clostridium botulinum

For years, laboratories connected with the canning industry and others have studied *Clostridium botulinum*, its heat-resistance and processing recommendations for low-acid foods. While studying the growth requirements for *Clostridium botulinum*, it was found that the dividing line of acidity between products in which the organism would grow, and those in which it would not grow, was about pH 4.6; below this level, growth of the organism in a favorable medium is inhibited. Under other conditions, such as one in which nutrient value is low, growth may be inhibited, regardless of PH. As a practical matter, this means that products of pH levels higher than pH 4.6 must be processed *under steam pressure, at temperatures considerably higher than 212°F (100°C),* usually higher than 240°F (116^0C), in order to insure destruction of spores, while products at pH 4.6 or lower may be safely processed in an open bath at 212°F (100°C), (Jay, 2009).

Botulism

Botulism is an intoxication caused by a toxin produced in foods by the microorganism called *Clostridium botulinum*. This organism is a rod-shaped, spore forming bacillus. It originates in the soil in all parts of the world. *C.botulinum* is an anaerobic bacterium; it does not grow in the presence of free oxygen, or on surfaces which support the growth of many other types' of

bacteria. This bacterium produces an exotoxin which is the most deadly neuro-paralytic toxin known (Jay, 2009).

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Six types of *C. botulinum* have been described and are well known, i.e., Types A, B, C, D, E, and F. Each type produces a specific and somewhat different exotoxin, but each toxin causes similar symptoms. Anti-toxins or serums are specific to the particular type of toxin, but polyvalent vaccines are available. Intoxication is caused by ingestion of the exotoxin produced by the organism *C. botulinum*; it is not caused by the organism itself. The toxins are inactivated by heat in 10 minutes at 212°F (100°C).Types A, C and D are proteolytic that is, they produce an extremely foul and putrid odor, while Types B and E don't produce this odor.

According to Rhodehamel *et. al.* (1992), Type A was responsible for 60.1%of the confirmed outbreaks in the U.S. since 1990; Type B for 18.5%and Type E for 17.9%. Botulism occurs throughout the world because *C. botulinum* is widely distributed in nature and occurs in both cultivated and forest soils, bottom sediments of streams, lakes, and coastal waters, the intestinal tracts of fish and mammals, and gills and viscera of crabs and other shell fish (Smelt *et al*., 2010). Canned vegetables, sausages, meat products and seafood products have been the most frequent vehicle for human botulism. The types of foods involved in botulism vary according to food preservation and eating habits in different regions. Since any low-acid food can support growth and toxin formation, botulinum toxin has been found in a considerable variety of foods, including canned corn, peppers, green beans, beets, asparagus, mushrooms, ripe olives, spinach, tuna fish, salmon, chicken, chicken livers and liver pate, and in luncheon meats, ham, sausage, lobster, smoked fish, and stuffed eggplant. On the other hand High acid foods, such as fruits, tomato products, sauerkraut, vinegared foods, etc., are not susceptible unless some form of spoilage has resulted in removal of sufficient acid, thus permitting growth of *C. botulinum* (Smelt *et al*., 2010).

2.2 Factors affecting microbial growth

The factors that influence microbial growth can be divided in to two: namely intrinsic and extrinsic factors.

2.2.1 Intrinsic factors

Moisture content

Microorganisms need water in an available form to grow in food products. The control of the moisture content in foods is one of the oldest exploited preservation strategies. Food microbiologists generally describe the water requirements of microorganisms in terms of the water activity (a_w) of the food or environment. Water activity is defined as the ratio of water vapor pressure of the food substrate to the vapor pressure of pure water at the same temperature (Jay, 2000).Microorganisms respond differently to aw depending on a number of factors. Microbial growth, and, in some cases, the production of microbial metabolites, may be particularly sensitive to alterations in a_w. Microorganisms generally have optimum and minimum levels of aw for growth depending on other growth factors in their environments (Gonzalez *et al.,* 2012).

PH and acidity

It is well known that groups of microorganisms have pH optimum, minimum, and maximum for growth in foods. As with other factors, pH usually interacts with other parameters in the food to inhibit growth. The pH can interact with factors such as aw, salt, temperature, redox potential, and preservatives to inhibit growth of pathogens and other organisms. The pH of the food also significantly impacts the lethality of heat treatment of the food. Less heat is needed to inactivate microbes as the pH is reduced (Smelt *et al*., 2010).

Another important characteristic of a food to consider when using acidity as a control mechanism is its buffering capacity. The buffering capacity of a food is its ability to resist changes in pH. Foods with a low buffering capacity will change pH quickly in response to acidic or alkaline compounds produced by microorganisms as they grow. Meats, in general, are more buffered than vegetables by virtue of their various proteins (Smelt *et al*., 2010).

In general, pathogens do not grow, or grow very slowly, at pH levels below 4.6; but there are exceptions. Many pathogens can survive in foods at pH levels below their growth minima. It has been reported that *C. botulinum* was able to produce toxin as low as pH 4.2, but these experiments were conducted with high inoculum levels (10^3-10^4 CFU/g up to 10^6 CFU/g), in soy peptone, and with the presence of *Bacillus* species (Smelt *et al*., 2010).

Nutrient content

Microorganisms require certain basic nutrients for growth and maintenance of metabolic functions. The amount and type of nutrients required range widely depending on the microorganism. These nutrients include water, a source of energy, nitrogen, vitamins, and minerals (Jay, 2000).Varying amounts of these nutrients are present in foods. Meats have abundant protein, lipids, minerals, and vitamins. Most muscle foods have low levels of carbohydrates. Plant foods have high concentrations of different types of carbohydrates and varying levels of proteins, minerals, and vitamins. Foods such as milk and milk products and eggs are rich in nutrients. The microorganisms that usually predominate in foods are those that can most easily utilize the nutrients present (Nortermans *et al., 2013).* Food borne microorganisms can derive energy from carbohydrates, alcohols, and amino acids. Most microorganisms will metabolize simple sugars such as glucose. Others can metabolize more complex carbohydrates, such as starch or cellulose found in plant foods, or glycogen found in muscle foods (Nortermans *et al., 2013).*. Some microorganisms can use fats as an energy source.

Amino acids serve as a source of nitrogen and energy and are utilized by most microorganisms. Some microorganisms are able to metabolize peptides and more complex proteins. Other sources of nitrogen include, for example, urea, ammonia, creatinine, and methylamines. Examples of minerals required for microbial growth include phosphorus, iron, magnesium, sulfur, manganese, calcium, and potassium. In general, small amounts of these minerals are required; thus a wide range of foods can serve as good sources of minerals (Nortermans *et al., 2013).*

Generally, the simple carbohydrates and amino acids are utilized first, followed by the more complex forms of these nutrients. The complexity of foods in general is such that several microorganisms can be growing in a food at the same time (Scott *et al.*, 2007). The rate of growth is limited by the availability of essential nutrients. The abundance of nutrients in most foods is sufficient to support the growth of a wide range of food borne pathogens. Thus, it is very difficult and impractical to predict the pathogen growth or toxin production based on the nutrient composition of the food (Scott *et al., 2007*).

Biological structure

Plant and animal derived foods, especially in the raw state, have biological structures that may prevent the entry and growth of pathogenic microorganisms. Examples of such physical barriers include testa of seeds, skin of fruits and vegetables, shell of nuts, animal hide, egg cuticle, shell, and membranes (Meclure,2011).

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Redox potential

The oxidation-reduction or redox potential of a substance is defined in terms of the ratio of the total oxidizing (electron accepting) power to the total reducing (electron donating) power of the substance. In effect, redox potential is a measurement of the ease by which a substance gains or loses electrons (Loss, 2012). The redox potential (Eh) is measured in terms of millivolts. The major groups of microorganisms based on their relationship to Eh for growth are aerobes, anaerobes, facultative aerobes, and microaerophiles.*C. botulinum*is a strict anaerobe that requires an Eh of less than +60 mV for growth; however, slower growth can occur at higher Eh values (Loss, 2012). The relationship of Eh to growth can be significantly affected by the presence of salt and other food constituents. For example, in one study with smoked herring, toxin was produced in inoculated product stored at 15 °C (59 °F) within three days at an Eh of +200 to +250 mV(Morris, 2000). In this case, the major oxidant would be trimethylamine oxide, which becomes the electron acceptor for *C. botulinum*. The anaerobe *Clostridium perfringens* can

initiate growth at an Eh close to +200 mV; however, in the presence of increasing concentrations of certain substances, such as salt, the limiting Eh increases (Morris, 2000).

Naturally occurring and added antimicrobials

Some foods intrinsically contain naturally-occurring antimicrobial compounds that convey some level of microbiological stability to them. There are a number of plant-based antimicrobial constituents, including many essential oils, tannins, glycosides, and resins, that can be found in certain foods (Mossel, 2011). Specific examples include eugenol in cloves, allicin in garlic, cinnamic aldehyde and eugenol in cinnamon, allylisothiocyanate in mustard, eugenol and thymol in sage, and carvacrol (isothymol) and thymol in oregano. Other plant-derived antimicrobial constituents include the phytoalexins and the lectins. Lectins are proteins that can specifically bind to a variety of polysaccharides, including the glycoproteins of cell surfaces (Mossel, 2011).

Some animal-based foods also contain antimicrobial constituents. Examples include lactoferrin, conglutinin and the lactoperoxidase system in cow's milk, lysozyme in eggs and milk, and other factors in fresh meat, poultry and seafood. Lysozyme is a small protein that can hydrolyze the cell wall of bacteria. The lactoperoxidase system in bovine milk consists of three distinct components that are required for its antimicrobial action, lactoperoxidase, thiocyanate, and hydrogen peroxide (*Ranken*, 2007).

In addition to naturally-occurring antimicrobial compounds in foods, a variety of chemical preservatives and additives can extend the shelf life of food and/or inhibit pathogens, either singly or in combination. The selection and use of these preservatives is typically governed by food law regulation of a country or region of the world (*Ranken*, 2007). A number of criteria should be followed when selecting a preservative for a specific food application. Ideally, the preservative should have a wide spectrum of activity against the target spoilage organisms and pathogens expected to be encountered in the food. The preservative must be active for the desired shelf life of the food and under the expected formulation conditions in the food (Mossel, 2011).

2.2.2 Extrinsic factors

Types of packaging/atmospheres

Gases inhibit microorganisms by two mechanisms. First, they can have a direct toxic effect that can inhibit growth and proliferation. Carbon dioxide (CO_2), ozone (O_3), and oxygen (O_2) are gases that are directly toxic to certain microorganisms (Loss, 2012). This inhibitory mechanism is dependent upon the chemical and physical properties of the gas and its interaction with the aqueous and lipid phases of the food. Oxidizing radicals generated by O_3 and O_2 are highly toxic to anaerobic bacteria and can having an inhibitory effect on aerobes depending on their concentration. Carbon dioxide is effective against obligate aerobes and at high levels can deter other microorganisms (Loss, 2012). A second inhibitory mechanism is achieved by modifying the gas composition, which has indirect inhibitory effects by altering the ecology of the microbial environment. When the atmosphere is altered, the competitive environment is also altered. Atmospheres that have a negative effect on the growth of one particular microorganism may promote the growth of another. This effect may have positive or negative consequences depending upon the native pathogenic microflora and their substrate. Nitrogen replacement of oxygen is an example of this indirect antimicrobial activity (Loss, 2012).

Impact of temperature

All microorganisms have a defined temperature range in which they grow, with a minimum, maximum, and optimum. An understanding of the interplay between time, temperature, and other intrinsic and extrinsic factors is crucial to selecting the proper storage conditions for a food product. Temperature has dramatic impact on both the generation time of an organism and its lag period (Mossel, 2011).

Storage/holding conditions

Outbreaks of food borne illness that have resulted from cooling food too slowly are a practice that may permit growth of pathogenic bacteria. The primary concerns in this regard are the spore-forming pathogens that have relatively short lagging times and the ability to grow rapidly and/or that may normally be present in large numbers (Mossel, 2011). Organisms that possess such characteristics include *C. perfringens*, and *Bacillus cereus*. As with *C. perfringens*, food borne illness caused by *B. cereus* is typically associated with consumption of food that has supported growth of the organism to relatively high numbers. The FDA "Bad Bug Book" notes that "The presence of large numbers of *B. cereus* (greater than 10^6 organisms/g) in a food is

indicative of active growth and proliferation of the organism and is consistent with a potential hazard to health" (FDA 2001).

2.3 The antimicrobial susceptibility of isolates from canned food

Antibiotic resistance is not a recent phenomenon. On the contrary this problem was recognized soon after the natural penicillin was introduced for disease control and the bacterial strains found to harbor antibiotic resistance genes (Abdul et al., 2009). Antibiotics are products of the earth. More especially of soil they are by products of cellular metabolism of *Actinomycetes*, mainly *Streptomycetes* species, produce tetracycline, aminoglycosides (streptomycin, and its relatives), macrolides (etythromycin and its relatives), chloramphenicol, rifamycins, and most other clinically useful antibiotics that are not beta-lactam. But these resistant genes could be transferred to other soil bacteria (Harvey and Mason, 1998).

Recently, much emphasis has been placed on bacterial gene transfer and acquisition of antibiotic resistance in the environment. Studies have investigated that genes present in bacteria in manure could transfer to indigenous soil bacteria. Resistant isolates belonging to the *Bacillus cereus* group from farm soil and manure were isolated, and screened for tetracycline resistance genes. All isolates carried transposons, which could transfer to other Gram positive bacteria (Agers *et al.,* 2002).

Bacteria are now more mobile than ever before. Bacteria in every environment where antibiotics are used are constantly evolving and exchanging genes that confer resistance to antibiotics (Agers *et al.,* 2002). A bacterium that becomes resistant to antibiotics in a farm environment can easily find itself in canned foods through contamination and can later pass to the intestine of a person. Among the total isolates of *B. cereus,* 32 showed multidmg resistance and the most frequent pattern was Amp/ Cep/ Met PenG/ Sxt/ Tet. These resistance strains could harbor the gene from their natural environment and find themselves in canned foods through contamination. Almost all strains of *Bacillus cereus* in this study were susceptible to erythromycin, kanamycin chloramphenicol, streptomycin and vancomycin (Peter *et al., 2004).* In other studies from food poisoning incidents, *Bacillus cereus* were totally susceptible to amoxicillin-clavulanic acid, gentamycin and a good proportion were susceptible to penicillin, vancomycin and tetracycline (Peter *et al., 2004).*

3. Materials and Methods

3.1. Description of study area

The study was conducted in Debretabor Town which is one of the oldest towns in Southern Gondar Administration Zone situated in Amhara National Regional State of Ethiopia. It was established by AtsieSeyefeAread in 1335 G.C. The town is located at North latitude11°51′and 38°1′ East longitude. It is suited 100 kilometers southeast direction from Gondar town and 50 kilometers East direction of Lake Tana and 667 km direction along Addis Ababa. The town was the capital of Ethiopia under Emperor Tewodros II and Yohannes IV. The climate of the town is '*Dega' and 'woynadega'*. Average annual rain falls ranges between 1462_1533 millimeter.Currently the town has been serving as seat of south Gondar administration zone, Debre Tabor town administration and farta district. The town administration is subdivided into four locally administrative kebeles (The smallest administrative unit in Ethiopia) with a total population of 78,706. The expansions of commercial activities, health and educational institutions, and high fertility have duly increased the population of the town (EPA, 2014).

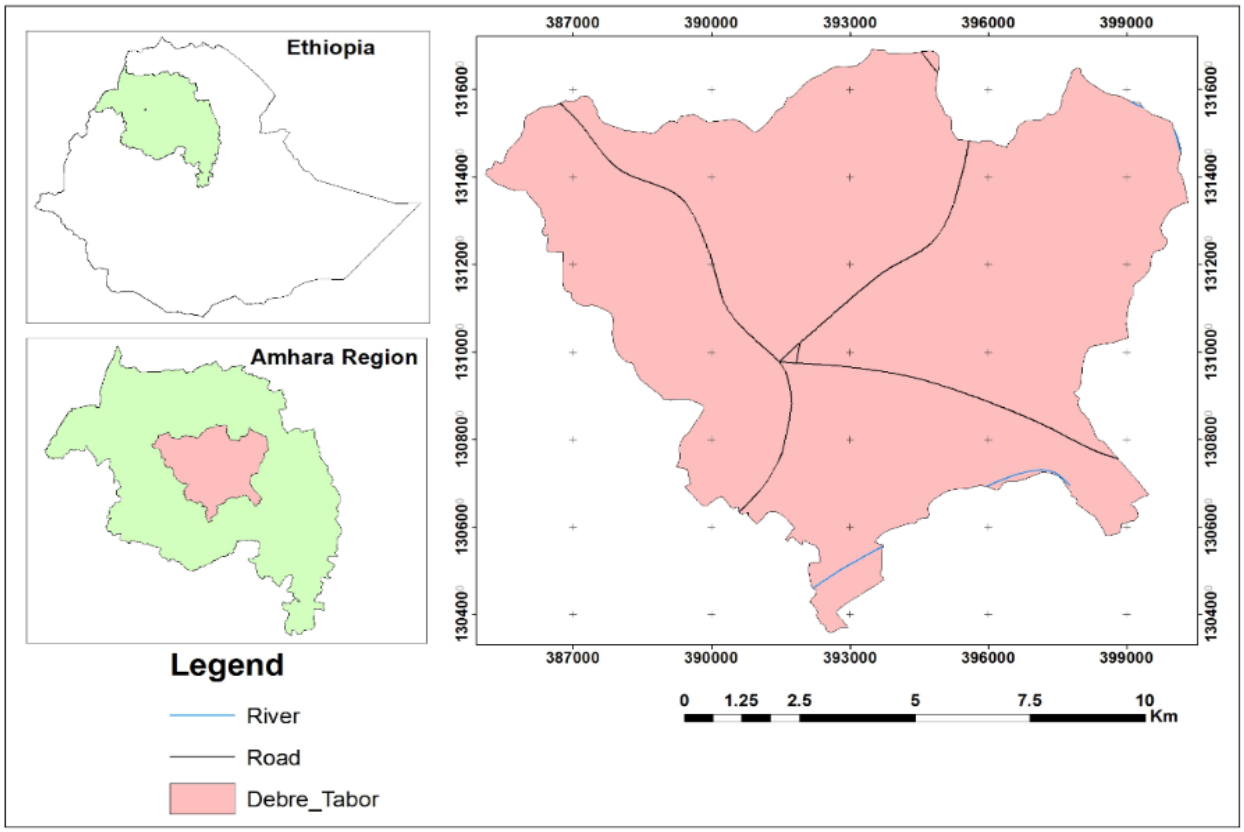

Figure 2 Map of Debre Tabor town (source Ethio GIS, 2014) IDCSO. 2014.

3.2 Study design and period

A cross sectional study design was conducted to isolate and characterize spore forming bacteria from canned food products (from January2023- June 2023).

3.3. Sample size determination

A total of 60 samples of canned foods comprising 20 each of (fish, meat, and powdered milk) were purposively collected from Debre Tabor supermarkets and shops since.

3.4 Sample collection and preparation

The PH of each sample was determined from the original sample using PH meter. Food samples were collected and transported to the Debre Tabor University in an ice box and stored at 4°C until testing. Before opening, cans were visually examined for presence of defects and physical damage observations were recorded. The non-coded end of the metal can was cleaned with alcohol-socked towel. This was flamed using a laboratory burner (Robert *et al*., 1996).

This was then followed by shaking the cans to mix their contents. A sterilized opening device was used to cut the desired size entry hole. Under aseptic conditions, 25g of samples were removed from the center and placed in to 225 ml of sterile bacteriological peptone water (BPW) and homogenized for 2 minutes. The homogenized samples were serially diluted and warmed. This was also done for all the three samples.

3.5 Bacteriological enumeration

From appropriate dilutions (prepared under 3.4), 0.1 ml aliquots were spread-plated in duplicates on pre-dried surface of Plate Count (PC) agar (Oxoid) plates. Plates were incubated at 30-32°C for (24-72) hours for aerobic spore former bacteria counting (cheesbrough, 2008). For the anaerobic spore former bacteria counting at 37°C for (24-72) hours then incubated in anaerobic jar (cheesbrough, 2008). Grams and spore staining techniques were conducted on cultures. Morphological and biochemical character of the isolates was examined. Motility was also observed by microscopic examination of the wet preparation of the isolate. Un-inoculated control plates were also incubated at the same temperature to check for sterility of plating media.

3.6 Floral analysis

After enumeration of the bacteria, about some colonies were picked randomly from countable plates and separately inoculated into tubes containing 5ml of Nutrient Broth (Oxoid). These were incubated at 32°C for aerobic isolates and at 37°C for the anaerobic isolates overnight. Cultures were purified by repeated plating and were characterized to the genus level using the following tests.

Cell morphology

From an overnight pure plate culture, colonies were picked and mounted on microscopic slide. The preparation was observed under light microscope using oil immersion objective. An old

Culture was used for observing spores. The morphological criteria considered during the Observations were:

- Cell shape: Regular: rod forms
- Cell arrangement: single, paired, cluster, tetrads
- Motility: motile, non-motile
- Spore: present

Biochemical test for bacteria isolates

The following biochemical tests were employed for identification of the different Bacteria isolates.

Oxidative-Fermentative (O/F) test

The utilization of glucose by each isolate was assessed by O/F test to identify microorganisms that metabolize glucose fermentatively or oxidatively or do not utilize glucose by either way. Ingredients (g/l): peptone 2g, yeast extracts 5g, NaCl 5g, K_2HPO_4 0.3g, glucose 109, agar 3g, distilled water 1000ml, bromothymol blue 0.08g, pH, 7.1. The freshly prepared medium (15 ml amount in 18 x 180mm test tubes) was immediately cooled under tap water to prevent oxygen from dissolving into the medium and inoculated by stabbing the culture with a sterile straight wire to the bottom. Acid formation and growth regions were interpreted after 2 to 5 days of incubation at 32°C (Gabey, 2010).

Hydrolysis of starch

This test is used to differentiate bacteria based on their ability to hydrolyze starch using the exoenzyme amylase. Soluble starch (6g) was dissolved in 60ml of distilled water and steamed for 10min. Melted sterile Nutrient agar (Oxoid) was mixed with soluble starch aseptically in proportion of 20ml starch in to 100ml of nutrient. Gram's iodine solution was prepared by dissolving 2g potassium iodide in 20ml of distilled water and 1g of finely ground iodine was added. The solution was allowed to stand overnight. Distilled water was added to make up to 300ml. A loopful of culture was inoculated along the diameter of the starch agar plate and incubated at 32°C. After incubation for 3 and 5 days, the growth was flooded with iodine solution. Formation of clear zone around growth indicated utilization of starch.

VogesProskaure Reaction (VP)

The VP test detects organisms that use the butylene glycol pathway and produce acetoin. When the VP reagents are added to MR-VP broth that has been inoculated with an organism that uses the butylene glycol pathway, the acetoin end product is oxidized in the presence of potassium hydroxide (KOH) to diacetyl. Creatine is also present in the reagent as a catalyst. Diacetyl then reacts to produce a red color. A loopful of culture was inoculated in to 5ml of MR-VP broth. The culture was incubated at 32°C for 3-5 days. Three ml of 5% alcoholic a-naphtol solution (5g-a-naphtol in 100ml of absolute alcohol) and three ml of 40 % (w/v) potassium hydroxide (KOH) were added into the culture. The mixture was shaken gently. Bright red colour was recorded as positive test (Workman, 2009).

Citrate test

The citrate test is used to determine the ability of a bacterium to utilize citrate as its sole source of carbon. Bacteria can break the conjugate base salt of citrate into organic acid and carbon dioxide. The carbon dioxide can combine with the sodium from the conjugate base salt to form a basic compound sodium carbonate. A pH indicator in the medium detects the presence of this compound by turning blue. A sterilized citrate agar was slanted in a test tube. Using sterilized needle the slant was streaked with the culture. The inoculated culture was incubated at 37°C for 7 to 14 days. Formation of blue colour on slant indicated utilization of citrates (Davis, 2007).

KOH test

This test was performed as proposed by Gregersen, (2011). One or two drops of 3% KOH solution were placed on a clean microscope slide. A colony was picked with a sterile bacteriological wire loop and stirred in the 3% KOH solution for 10 seconds to 2 minutes. The inoculating loop was raised slowly from the mass. When KOH solution became viscous, the thread of slime followed the loop for 0.5 to 2cm or more. Typically, this was observed in Gram negative bacteria. In case of no slime, watery suspension did not follow the loop, and this was seen in Gram-positive bacteria.

Catalase test

This test was used to determine those organisms that produced catalase enzyme. The young colonies were flooded with a 3% solution of H_2O_2. The formation of bubble indicated the presence of catalase enzyme (Clark, 2006).

Grams test

A primary stain (crystal violet) was added to heat fix the smear, followed by the addition of a mordant (Grams iodine), rapid decolonization with alcohol, acetone and finally counter staining with safranin (Beveridge, 1990).

Spore staining test

The spore was air dried and fixed on glass slide and covered with a square of blotting paper that fit the slide. The blotting paper was saturated with malachite green stain solution and steamed for five minutes (since steam can penetrate and break the spore to be easily stained), keeping the paper moist and adding more days. The slide was washed with tape water and counter stain with safranin for 30 seconds; blot dry.it was examined under the oil immersion lens for the presence of spores. Endospores were bright green and for viable cells brownish red to pink (May, 1999).

3.7Antimicrobial susceptibility testing for the isolated bacteria genera

3.7.1 Antimicrobial susceptibility testing for isolated aerobic genera

Antimicrobial susceptibility testing was done for 112 isolates. The slanted cultures were sub cultured and purified. From Nutrient Agar plates, two to three pure colonies were inoculated in to Nutrient Broth and incubated at 32°C for 18-24 hours. The growth was standardized to optical density of 0.5 McFarland Standard to bring the cell density to about 10^7 -10^8cfu/ml. The McFarland turbidity standard was prepared by mixing 0.1 ml $BaCl_2$ (1%) with 9.9ml H_2SO_4 (1%) (Jorgenson *et al*., 2011). Muller Hinton (MH) (Oxoid) plates were prepared and waned to room temperature for plating. A sterile cotton swab was dipped in to the standardized suspension and the excess was removed by turning the swab against the side of the container. The cultures were spread evenly over the entire surface of the Muller Hinton Agar plate by swabbing in three directions at 90° of each spreading .The plates were allowed to dry before applying antimicrobial discs.

The following antibiotic discs (all from Oxoid) were applied in this study: Erythromycin (Ery) (l5µg), Ampicillin (Amp) (10µg), Gentamycin (Gen) (10µg), Methicilin (Met) (5µg), Chloramphenicol (Chl) (30µg), Tetracycline (Tet) (30µg), Vancomycin (Van), (30µg), (10µg), and Cephalothin (Cep) (30µg)·

3.7.2 Antimicrobial susceptibility testing for isolated an aerobic isolates

The isolates were subjected to antimicrobial sensitivity testing. Disc diffusion technique was adopted and fresh blood agar media were used. Commercially prepared antimicrobial discs (Biological Limited, UK) impregnated with disc potent ampicillin (10µg), tetracycline (30 µg), chloramphenicol (30 µg), Erythromycin (25 µg), gentamycin (10µg), cephalothin (30µg), were used. A loopful of colony from each axenic culture was suspended in sterile saline to make a suspension with turbidity equals to that of 1% barium sulphate solution. This was enough to produce about 1.5 x 10^8cfu/ml (0.5 Macfarland standards). Three loopfuls were used to inoculate the agar surface by streaking method. After 10 minutes pre diffusion time, replicates of test drugs discs were aseptically placed onto the culture surface. These were an aerobically incubated at 35^{o}C for 18 hours. Incubation continued until 24 hours. The plates were examined for zones of inhibition at the individual discs. Zones were accurately measured and recorded using millimeter rule to the nearest whole number. The Table of National Clinical Laboratory Standards was consulted for the interpretation of the susceptibility patterns of the organism to the various drugs (Cheesbrough(2002).

3.8 Data Analysis

The data were analyzed using SPSS 21 version statistical software to compare the proportion of bacterial isolates from each food item. The data was analyzed using mean, standard deviation and variation.

4. Results and discussion

4.1 Results

4.1.1 Bacterial enumeration

A total of one hundred fifty six (156) aerobic and anaerobic bacteria isolates were isolated from fifty five canned foods and were characterized to various genera. Out of 60samples, 5 of them (2 from canned meat, 2 from canned fish and 1 from canned milk) had microbial load below detectable levels by the methods used in this study < 2 (log cfu/g).

The mean of spore forming counts in Sardine were 4.22. The mean pH value of Sardine was 5.8 (Table 1*).* Samples of Beef-in-Jelly had mean microbial count of 3.86 (Table 1).The mean pH value of Beef-in-Jelly was 5.70 (Table 1). The microbial load of Nido had mean microbial count of 2.79 (Table 1). The mean pH value of Nido was 6.17 (Table 1).Most of the canned food products were dominated by *Bacillus* and *Clostridium* isolates. Among these *Bacillus* isolates Bsp1, Bsp2 and Bsp3 were dominant in aerobic spore forming genera (Table 2) and anCsp1, anCsp2and anCsp3 were dominant for the anaerobic isolates (table 3).

Table 1: Mean counts (log cfu/g) of spore forming bacteria species of canned food samples

Group	Sample type	PH Mean ± SD	No of samples	spore forming (log cfu/g) bacteria genera	
				Mean ± SD	%CV
Fish	Sardine	5.84 ± 0.13	18	4.22 ± 0.64	15
Meat	Beef in Jelly	5.70 ± 0.11	18	3.86 ± 0.53	14
Powdered milk		6.17 ± 0.02	19	2.79 ± 0.23	8

4.1.2 Bacterial prevalence of canned food

The dominant bacillus isolates in the canned food items were Bsp1, Bsp2 and Bsp3, respectively (Table 2). These isolates were dominant across most canned food samples. The rest isolates namely Bsp4 and Bsp5 were relatively less prevalent (Table 2). From the canned food samples, fish had the maximum load of Bacillus species followed by meat and milk (Table2). The abbreviation for both *Bacillus* and *Clostridium* isolates was based on formative ability, enzyme production, oxygen utilization, the organ they infect, the disease they cause, proteolytic activity and based on their genera. For *Bacillus* isolates as Bsp1, Bsp2, Bsp3, Bsp4, Bsp5 and for *Clostridium as Csp1, Csp2, Csp3, Csp4.*

Table 2: Distribution of the dominant spore forming *Bacillus* isolates in canned food products

Isolates	Code	Canned food items			
		Beef	Fish	Milk	Total
Bsp 1	01	10	12	7	29
Bsp 2	02	7	15	2	22
Bsp3	03	11	10	2	23
Bsp4	04	1	1	5	7
Bsp5	05	9	3	2	

Total		38	41	18	95

Key: Bsp = *Bacillus* species

The most prevalent clostridium isolates were Csp1, Csp 2 and Csp4, respectively (Table 3) in most of the canned food samples. On the other hand Csp3 isolates were less prevalent in these canned food samples (Table3). From the canned food samples, the maximum Clostridium species counts were recorded in in fish followed by meat, and powdered milk (Table3).

Table 3: Distribution of the dominant *Clostridium* spore forming isolates in canned food products

Isolates	code	Canned food items			
		Beef	Fish	Milk	Total
Csp1	001	5	8	5	18
Csp2	002	6	8	3	17
Csp3	003	4	4	2	10
Csp4	004	4	9	3	16
Total		19	29	13	61

Key: Csp =*Clostridium* species

The result in (Table 4) implies that the isolates were aerobic, gram positive, rod shaped and spore forming. This character is mostly associated *bacillus* species.

Table 4: Biochemical and morphological characterization of aerobic spore forming isolates

Bacteria code	Morphology	Motility	spore	KOH test	Catalase test	Citrate test	O/F test	Gram rxn	VP test	Starch hydrolysis test
01	Rod shaped	Motile	present	_	+	+	+	+	_	_
02	Rod shaped	Motile	Present	_	+	+	+	+	_	_
03	Rod shaped	Non motile	Present	_	+	+	+	+	_	_
04	Rod shaped	Non motile	present	_	+	_	+	+	_	_
05	Rod shaped	Motile	present	_	+	+	+	+	_	_

In the table below (Table 5) the bacteria is characterized as anaerobic, gram positive, rod shaped and spore forming. Most of this character is associated with anaerobic *Clostridium* and *bacillus* species.

Table 5: Biochemical and morphological characterization of anaerobic spore forming isolates

Bacteria code	Shape	motility	Spore	KOH test	Catalase test	Citrate test	Gram rxn	VP test
001	Rod shaped	Non motile	Present	_	-	+	+	_
002	Rod	motile	Present	_	-	+	+	_

	shaped							
003	Rod shaped	Motile	Present	_	_	+	+	_
004	Rod shaped	Non motile	Present	_	-	_	+	+

4.1.3 Antimicrobial susceptibility pattern of aerobic spore forming isolates in canned food

A total of 47 aerobic spore forming *isolates were* isolated from the different canned products, were tested for their antimicrobial sensitivity. Among these, *Bsp1, Bsp2* and *Bsp3* were the dominant species. Of the 16 isolates of *Bsp1,* more than 50% were resistant to Ampicilin (68.7%), Cephalothin(87.5%), Methicilin(87.5%), and Tetracycline(62.5%).

All of the Bsp1 isolates were susceptible (>87.5%) to Erythromycin, Vancomycin and chloraphenicol (Table 6). Over 50% of *Bsp2* were resistant to Ampicilin (83.3%) and Cephalothin(91.7%). Most of these isolates were susceptible (>75%) to Erythromycin, Gentimycin, chloranphenicol, tetracycline and Vancomycin (Table 6).

Most of the isolates of *Bsp3 were* resistant to Ampicillin, Cephalothin, Methicillin, and Tetracycline (Table 6). However, almost all were sensitive to Chloramphenicol, Erythromycin, Gentamycin and Vancomycin (Table 6). Almost all of Bsp4 were susceptible all of the antibiotics used. On the other hand Bsp5 were resistant to Amp (66.7%) and this isolate was susceptible to all other used drugs.

Table 6: susceptibility pattern of aerobic spore forming bacteria in canned food

Bacteria code	Strain		Amp	Cep	Chl	Ert	Gen	Met	Tet	Van
Bsp1	16	R	11(68.7)	14(87.5)	1(6.25)	_	_	14(87.5)	10(62.5)	2(12.5)
		I	2(12.5)	_	_	_	2(12.5)	1(6.25)	1(6.25)	_
		S	3(18.8)	2(12.5)	15(93.75)	16(100)	14(87.5)	7(58.3)	5(31.25)	14(87.5)
Bsp2	12	R	10(83.3)	11(91.7)	1(8.3)	_	_	3(25)	2(16.7)	_
		I	_	_	_	_	_	2(16.7)	1(8.3)	_
		S	2(16.6)	1(8.3)	11(91.7)	12(100)	12(100)	1(6.25)	9(75)	12(100)

Bsp3	9	R	5(55.6)	6(66.7)	1(11.1)	_	1(11.1)	3(33.3)	4(44.4)	1(11.1)
		I	1(11.1)	_	_	_	_	1(11.1)	_	_
		S	3(33.3)	3(33.3)	8(88.9)	9(100)	8(88.9)	5(55.6)	5(55.5)	8(88.9)
Bsp4	7	R	2(28.6)	3(42.9)	1(14.3)	1(14.3)	_	2(28.6)	1(14.3)	1(14.3)
		I	_	_	1(14.3)	_	_	_	2(28.6)	_
		S	5(71.4)	4(57.1)	5(71.4)	6(85.7)	7(100)	5(71.4)	4(57.1)	6(85.7)
Bsp5	3	R	2(66.7)	1(33.3)	_	_	_	1(33.3)	1(33.3)	_
		I	_	_	_	_	_	_	_	_
		S	1(33.3)	2(66.7)	3(100)	3(100)	3(100)	2(66.7)	2(66.7)	3(100)
Total	47	R	30(63.8)	25(53.2)	4(8.5)	1(2.1)	1(2.1)	23(48.9)	18(38.3)	4(8.5)
		I	3(6.4)	_	1(2.1)	_	2(4.3)	4(8.5)	4(8.5)	_
		S	14(29.8)	22(46.8)	42(89.4)	46(97.9)	44(93.6)	20(42.5)	25(53.2)	43(91.5)

Key: Bsp = bacillus species, Amp=Ampecilin, Cephalothin,Chl = Chloraphenicol, Ert = Erythromycin, Gen =Gentimycin, Met=Methicilin, Tet= Tetracycline,Van= Vancomycin. R= resistant, S= susceptible, I= intermediate, and the numbers in the bracket are percentage values; the numbers outside the bracket are the isolates.

4.1.4 Antimicrobial susceptibility pattern of anaerobic spore forming isolates in canned food

A total of 36 anaerobic spore forming *isolates were* isolated from the different canned products, were tested for their antimicrobial sensitivity. The gram positive anaerobic spore forming isolates coded in Csp1 and Csp2 were resistant (>57.1%) to Ampecillin,Cephalotin and Tetracycline (table7). On the other hand Csp1 was susceptible (>71.4%) to chloraphenicol, Erythromycin and Gentimycin and Csp2 was susceptible (>70%) to chloraphenicol and Erythromycin (table7).

Csp3 was also susceptible (>75%) to Chloraphenicol, erythromycin and gentimycin and this species was resistant (>62.5%) to Ampecillin, Cephalothin and Tetracycline (table7). Csp4 was resistant (50%) to Genthimycin, (100%) to cephalothin and Tetracycline.this species was susceptible to Ampecillin (75%) and 100%) to Chloraphenicol and erythromycin (table7).

Table 7: Resistance and susceptibility pattern of anaerobic spore forming bacteria in canned food

Bacteria code	Strain		Amp	Cep	Chl	Ert	Gen	Tet
Csp1	14	R	8(57.1)	9(64.3)	2(14.3)	4(28.6)	1(7.1)	7(50)
		I	2(14.3)	3(21.4)	1(7.1)	_	3(21.4)	2(14.3)
		S	4(28.6)	2(14.3)	11(78.6)	10(71.4)	10(71.4)	5(35.7)
Csp2	10	R	7(70)	8(80)	3(30)	2(20)	6(60)	8(80)
		I	_	_	_	_	1(10)	_
		S	3(30)	2(20)	7(70)	8(80)	3(30)	2(20)
Csp3	8	R	5(62.5)	7(87.5)	_	_	2(25)	6(75)
		I	1(12.5)	_	_	_	_	_
		S	2(25)	1(12.5)	8(100)	8(100)	6(75)	2(25)
Csp4	4	R	1(25)	4(100)	_	_	2(50)	4(100)
		I	_	_	_	_	_	_
		S	3(75)	_	4(100)	4(100)	2(50)	_
Total	36	R	23(63.9)	28(77.8)	5(13.9)	6(16.7)	11(30.6)	25(69.4)
		I	3(8.3)	2(5.6)	1(2.8)	_	4(11.1)	2(5.6)
		S	10(27.8)	5(13.9)	30(83.3)	30(83.3)	21(58.3)	9(25)

Key: Csp= clostridium species, Amp= ampicillin, Cep= chloramphenicol, Ert= erythromycin, Gen =gentamycin, Tet= tetracycline, R= resistant, S= susceptible, I= intermediate, the numbers in the bracket shows percentage; the numbers outside the bracket are isolates.

4.2 Discussion

In this study bacillus was isolated from canned food products, this result is in agreement with the study conducted by (Boeye and Aerts,2009) in which among the gram positive isolates from previous study, *Bacillus* isolates obtained from canned fish milk and meat products, *B. firmus, B. cereus, B. licheniformis,* were the dominant. Studies showed that these species and other *Bacillus* species were often isolated from these canned food products. Another study also showed that strains of *B.sublilis, B.licheniformis* and *B.Cereus* were common inhabitants of the Pacific Ocean habitats (Elena *et al., 1999*). During canning process, spores of *Bacillus* species could survive the heat treatment whereas non-spore formers and heat sensitive bacteria would be eliminated. When product formulation is incapable of inhibiting spore germination, they could spoil the canned products (IFT/FDA, 2003). In another study using the simplified identification scheme, 115 strains of *Bacillus* species were isolated from spoiled canned foods. Most of the isolated strains belonged to the species *B.subtilis, B.megaterium* and*B. Brevis* (Kotzekidou, 2006). The flora of *Bacillus* species of the canned milk products was dominated by *B. cereus, B.pumilus* and *B. brevis.* A study in the assessment of the frequency and level of *Bacillus* species contamination in the Sardinian diet products, the most frequently isolated species were *B. cereus, B. coagulalls, B. subtilis* and *B. pumilus* (Cosentino*et al.,* 2007). These strains were isolated from human infections (Richter and Vedamuthu, 2001). If leftover milk consumed spores could germinate and infect the consumer. Most isolates of *Bacillus* species were reported as human infectious agents including *B.cereus, B.alme. B.laterosporus, B.subtilis, B. sphaericus. B.circulalls. B.brevis, B. licheniformis, B. macerans, B. Pumilus, and B. anthracis (*Kenneth, 2005).

In addition under this research the clostridium genera was isolated consisting of different species from the samples of canned and processed food and clostridium species are anaerobic that grow in the absence of oxygen . This is in agreement with the (Jay, 2013) that different Clostridium species found in commercially canned foods such as fish, meat vegetables and poultry. In another study botulinum toxin has been found in a considerable variety of canned foods, including canned corn, peppers, green beans, beets, asparagus, mushrooms, ripe olives, spinach, tuna fish, salmon, chicken, chicken livers and liver pate, and in meats, ham, sausage, lobster, sardines, smoked fish, and stuffed eggplant, powdered milk and cause neuroparalytic toxin that is caused by *clostridium botulinium* (Adams *et al., 2014*).

The isolates of most of these bacillus and clostridium species under this research showed drug resistance to ampicillin cephalothin and tetracycline. However these isolates were susceptible to erythromycin, chloramphenicol, and Vancomycin. In agreement with this result a study in Addis Ababa University most of the isolates from canned and processed meat, fish and powdered milk were B.lichenformis, B.cerus, B. macerans B.pmils, B.subtilus B.coagulans B.brevis and These isolates were resistant to Ampicillin, tetracycline Cephalothin, and Methicillin. However, most of these isolates were Sensitive to Chloramphenicol, Erythromycin, Gentamycin, Kanamycin, Polymyxin B, Streptomycin and Vancomycin (Yonas Chekole., 2013).

From this study different Bacillus and Clostridium isolates were found from the examined canned food samples. These microbes might be incorporated in to the foods during processing or the spore were not been sterilized during canning process or there is problem in the storage site in shops and super markets.

5. Conclusion and recommendation

5.1 Conclusion

This study confirmed that the dominant bacteria which were isolated from canned food products continue to dominate, so *Bacillus* and *Clostridium* species are the most frequently isolated bacteria from this study. Most of these isolates were resistant to Ampicillin, cephalothin and tetracycline. On the other hand these isolated microbes were sensitive to Chloramphenicol, Erythromycin and vancomycin. These canned foods are kept at room temperature in both market areas and the products might spoil before their expiry dates by these spore forming microbes.

Under this research both aerobic and anaerobic spore-former heat-resistant microorganisms have great role in poisoning and spoilage of various canned foods and this might cause subsequent outbreaks. The samples collected from Debretabor Town supermarkets and different shops provide information about some foods with spore forming aerobic and anaerobic microbial spore

formers. Thus, this study indicates that these canned foods could be under-processed, contamination raw materials or contaminated due to leakage by spore forming Bactria spores.

5.2 Recommendations

- Under this research different gram positive rod shaped genera (*Bacillus* and *Clostridium*) have been isolated from processed and canned food products.
- These isolates have a resistance to different antibiotics like ampicillin, tetracycline and cephalothin.
- It is possible to recommend here that Consumption of these canned and processed foods might cause disease outbreak against consumers and other researchers to come and do more researches in the area on these canned and processed food.
- Food manufacturing industries have to check back again and again their food processing, spore sterilizing and packing activities.
- The owners of supermarkets and shops have to consider storage site especially weather the food is exposed to external parameters (sun light, fridge, sharp devices etc.
- Finally consumers at each age level have to consider the canned foods side problems and at least they might reduce the risk through proper cooking.

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Annex

Images of lab activities