ADDIS ABABA UNIVERSTY

SCHOOL OF GRAGUATE STUDIES

PREVALENCE OF *SALMONELLA* AND *SHIGELLA* SPP. ON LETTUCE AND GREEN PEPPER AND THEIR DRUG RESISTANCE PATTERN.

BY

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ABBREVIAIONS AND SYMBOLS

APMIS- Acta Pathologica, Microbiologica et Immunologica Scandinavica.

BPW- Buffered Peptone water

CFU- Colony forming unit

CV- Coefficient of Variation

FSA -Food Standards Agency

H_2O_2- Hydrogen per oxide

H_2S- Hydrogen Sulphide

IFST- Institute of Food Science and Technology

KOH- Potassium hydroxide

LAB- Lactic acid bacteria

MDR- Multiple drug resistant

MSBB- Mannitol selenite broth base

SBB- Selenite broth base

Spp. - Species

SS Agar- Salmonella-Shigella Agar

TBB- Tetrathionate broth base

TSI- Triple Sugar Iron Agar

XLD medium- Xylose Lysine Deoxycholate medium.

UK- United Kingdom

USA- United States of America

ABSTRACT

The microbial load of lettuce and green pepper purchased from different supermarkets in Addis Ababa, Ethiopia, was analyzed. A total of 80 vegetable samples consisting of 40 lettuces and 40 green peppers were included in the study. More than 90% of the lettuce and the green pepper samples had aerobic mesophilic counts of ≥ log 6 cfu/g. Enterobacteriaceae were frequently encountered in these samples and about 97% of the lettuce and 58% of the green pepper samples had enterobacteraceae counts of ≥ log 5 cfu/g. Over 48% of the lettuce and 35% of the green pepper samples had coliform counts ≥ log 4 cfu/g. All vegetable samples harbored staphylococci and more than 92% of the lettuce samples and 80% of the green pepper samples harbored staphylococci with counts ranging from log 4 to log 6 cfu/g. About 42% of lettuce and 30% of green pepper samples had spore counts ≤ log 2 cfu/g. The majority of lettuce and green pepper samples harbored yeasts and mold and more than 88% of lettuce and 18% of green pepper samples had counts ≥ log 4 cfu/g.

The aerobic mesophilic flora of both lettuce and green pepper samples were dominated by Gram positive organisms, with *Bacillus* and *Micrococcus* being the most dominant species in the lettuce and green pepper, respectively. *Salmonella* was isolated from six (10%) samples consisting of two lettuce and four green pepper samples. *Shigella* was also isolated from fifteen (18.75%) samples of five lettuces and ten green peppers samples.

Resistance to antibiotics was seen in all isolates from the vegetable samples. All of the *Salmonella* and 97% of *Shigella* isolates showed resistance to penicillin. The majority of the *Salmonella* isolates showed susceptibility to polymixin B., gentamicin and ciproflaxin. Ampicillin resistance was observed in 42% of *Salmonella* and 79% of *Shigella* isolates. Multiple drug resistance (MDR) was observed in 8 and 24 isolates of *Salmonella* and *Shigella* isolates.

Key words/ phrases: - Addis Ababa; Lettuce; Green Pepper; quality; safety; *Salmonella*; *Shigella*, super market; vegetables.

1. INTRODUCTION

Fresh fruits and vegetables continue to be a public health concern from the standpoint of food safety. With the increase in customer demand for fresh/minimally processed, preservative-free/organic vegetables, and the increase in importation of fresh produce from countries where hygiene standards may be compromised , there has been heightened interest in outbreaks of human gastroenteritis that may be associated with such produce (Kirov , 1993). According to Chang and Fang (2007) the number of foodborne outbreaks due to consumption of contaminated fresh fruits and vegetables has been increasing for the last three decades. The incidences of these foodborne infections that are caused by bacterial pathogens continue to be a problem in industrialized nations and developing countries. These infections result in health and economic burdens in those countries and are especially severe in the young, older and immuno-compromised people.

Shortly after some major human pathogens were recognized as being spread from animal reservoirs, fresh fruits and vegetables emerged as new vehicles for the transmission of these zoonotic diseases (Brandl, 2006). Human infections traditionally are acquired via the ingestion of foods of animal origin which has now shifted to fresh produce as major vehicles of gastroenteritis (Chang and Fang, 2007). This becomes an important issue when coupled with the trend of people consuming more vegetables and fruits for health and nutritional reasons. Although the indigenous microflora in fruits and vegetables may be protective, cross contamination with foodborne and human pathogens may still occur during the production cycle and can originate from soil, insects, animals or humans. Irrigation with poor-quality water is one way that fruit and vegetables can become contaminated with foodborne pathogens. Groundwater, surface water, and human wastewater are commonly used for irrigation (McMahon and Wilson, 2001).

The risk of disease transmission from pathogenic microorganisms present in irrigation water is influenced by the level of contamination, the persistence of pathogens in water, in soil, and on crops, and the route of exposure (Steele and Odumeru, 2004). According to Chaidez et al. (2003) the mechanisms by which the pathogens reach the fresh produce are not fully understood; however, one hypothesis states that the fruits and vegetables become contaminated with water used at the dump tanks.

Specific examples include the use of polluted irrigation water or surface run-off water which can lead to the presence of pathogens on fruits and vegetables in the field. Like wise, the use of raw animal manure for fertilizer can increase the threat of contamination of fruits and vegetables (Nutt et al., 2003). As a result, the microbiological safety of fresh fruits and vegetables becomes a significant concern of both consumers and the food industry (Chang and Fang, 2007).

In the last several decades, the proportion of foodborne disease outbreaks in the United States associated with fresh produce rose from 0.7% in the 1970s to 6% in the 1990s (Johnston et al., 2006). There are a number of reports indicating that raw vegetables may harbor potential foodborne pathogens (Beuchat, 1996). *Listeria monocytogenes*, *Salmonella* (Doyle, 1990), and *Escherichia coli* (Nguyen-the and Carlin, 1994) have been isolated from raw vegetables. Enteric pathogens have been found on a wide variety of produce including lettuce, tomatoes, and cantaloupes (Nutt et al., 2003). As Deza et al. (2003) stated, outbreaks of salmonellosis have been attributed to the consumption of contaminated tomatoes. *S. enteric* serovar *Typhimurium* and *S. enteric* serovar *Enteritidis* are the most frequently isolated serovars from foodborne out breaks throughout the world.

The development of resistance is a global problem and is not restricted to special countries or bacterial species. The massive use of anti-microbial agents for medical and veterinary purposes creates a selective pressure, which leads to an increasing development of antibiotic resistance worldwide (Schroeter et al, 2004).

The presence of reliable information about the prevalence and susceptibility of foodborne pathogens to different antibiotics is important to improve the quality of antibiotics used for treatment, to influence the pattern of antibiotic usage and to assist governments to formulate policy for the supply and use of antibiotics.

There are a variety of methods used to reduce populations of microorganisms on whole and fresh-cut produce. Each method has distinct advantages and disadvantages depending upon the type of produce, mitigation protocol, and other variables. The best method to eliminate pathogens from produce is to prevent contamination in the first place. However, this is not always possible and the need to wash and sanitize many types of produce remains of paramount importance to prevent disease outbreaks (Parish et al., 2003).

Currently, there are a lot of local supermarkets that distribute fresh produce in Addis Ababa. But with current technology and packaging of fresh produce, microorganisms may be present at the time of purchase in grocery stores. As a result, these distributors should be aware of the microbiological status of fresh produce so that they could supply quality products that satisfy the local consumer and help them to go in to the international market. Besides, farmers, processors and consumers must be vigilant on food safety.

Information on the microbial safety of food items in Ethiopia is limited. There are very few reports on the microbial quality and prevalence of foodborne pathogens found on fresh produce in Ethiopia (Aberra Geyid et al., 1991).

1.1. Objectives

1.1.1. General Objectives

The general objective of this study is to investigate the microbial quality of fresh produce, lettuce and green pepper, and to asses the prevalence and antibiotic resistance of *Salmonella* and *Shigella* spp. isolated from lettuce and green pepper.

1.1.2. Specific Objectives

The specific objectives of the study are

1. to assess the microbial load of fresh lettuce and green pepper sold in local super markets in Addis Ababa,
2. to identify and characterize the dominant microorganisms,
3. to see the prevalence and the resistance pattern of *Salmonella* from lettuce and green pepper,
4. to evaluate the bacteriological safety and the resistance pattern of *Shigella* from lettuce and green pepper.

2. LITERATURE REVIEW

2.1. Vegetables

During the past three decades, the consumption of fresh fruits and vegetables has increased considerably based on nutritionists' recommendation to eat at least 5 servings per day. Although an increase in the consumption of fresh produce has contributed to the improvement of public health, it has also contributed to an increase in produce-related foodborne illness (Yuk et al., 2006). The quality of minimally processed lettuce is largely affected by the growth of microorganisms originating from preharvest environments. Therefore, the quality of these types of products would be affected by the way it was cultivated (Ponce et al., 2008).

Yuk et al. (2006) reported that the proportion of produce-related outbreaks increased from 0.7% in the 1970s (13 of 1857 food-associated outbreaks) to 6% in the 1990s (114 of 1788 outbreaks). Fruits and vegetables may be exposed to soil, insects, animals, water, and/or humans during growing or harvesting and in processing plants. Additionally, crops may be fertilized with the manure from domestic animals. Consequently, fruits and vegetables intended for raw consumption may be exposed to pathogenic bacteria, parasites, and viruses from animal excreta (birds, insects, rodents, and reptiles), from water (irrigation and rain), or from infected workers, manure, and food-processing facilities with poor sanitation. Therefore, methods that effectively and reliably reduce or eliminate pathogenic bacteria on fruits and vegetables are needed.

2.2. Microbiological evaluation of vegetables

The level of microorganisms in food items is essential as some of them are hazardous to health. There are a number of reports isolating *Aeromonas*, *Escherichia*, *Salmonella* and *Listeria* from raw vegetables, indicating that the raw vegetables may harbor potential food-borne pathogens (Schlech et al., 1983). The large number of total microorganisms and fecal-contamination indicators (*E. coli,* coliform, and enterococci) detected in the vegetable samples surveyed indicates a potential health hazard to consumers (Kim et al., 2004). The bacteria that are found belonged most frequently to the *Citrobacter-Enterobacter-Serratia* group of Enterobacteriaceae. Although usually regarded as human pathogens, these members of Enterobacteriaceae have also been recognized as inhabitants of soil and plants (Wright, et al., 1976). Thus, vegetables may serve as a reservoir from which the bacteria named above can colonize and infect a susceptible host (Ibenyassine et al., 2007).

According to McMahon and Wilson (2001), a range of commercially available organic vegetables were examined and no *Salmonella*, *Campylobacter*, *E. coli*, *E. coli* O 157, *Listeria* were found in any of the samples examined. *Aeromonas* species were isolated from 34% of the total number of organic vegetables examined. Although *Aeromonas* species are frequently detected in organic vegetables, the absence of accepted enteric pathogens was encouraging, and did not support the allegation of organic foods being of high risk due to the farming methods used (Wright et al., 1976).

2.3. Sources of contamination in the vegetable production chain

Vegetables can become contaminated with pathogenic organisms during growth, harvest, post-harvest handling, or distribution (McMahon and Wilson, 2001). Use of untreated wastewater in irrigation represents an important route for transmission of these pathogenic organisms. Raw vegetables are considered by some to represent an increased risk to public health when irrigation methods use untreated wastewater and no chemical treatments are employed to reduce the microbiological load on the raw product (Takeuchi et al., 2001). For example, contaminated irrigation water, the use of animal waste as fertilizer and the lack of hygiene on the part of foodhandlers make vegetables potential carriers of *Salmonella* spp. (Ruiz et al., 1987).

2.3.1. Use of waste water for irrigation

In many countries, urban wastewater is used to irrigate agricultural land. This way of disposing of urban sewage water has several advantages. Wastewater contains a lot of nutrients, which increase crop yields without use of fertilizer. Furthermore, sewage water is an alternative water source in arid and semi-arid areas where water is scarce. Some disadvantages are that wastewater can contain heavy metals, organic compounds, and a wide spectrum of enteric pathogens that may have a negative impact on the environment and human health (Ibenyassine et al., 2007).

The use of raw sewage to irrigate crops is an important mechanism that helps to propagate conditions conducive to cholera and typhoid fever (Castro-Rosas and Escartin, 2000). Increases in foodborne illnesses during the summer are not fully understood, although fresh produce likely plays a role since it is consumed in higher quantities during the summer (Ibenyassine et al., 2007). Furthermore, consumption of salad irrigated by wastewater has been found to be responsible for shigellosis in England. However, farming with wastewater is a source of income to many urban dwellers and is important in providing balanced diets and attaining urban food security. Thus, appropriate measures need to be developed to reduce health risks (Keraita et al., 2007).

2.3.2. Soil

It is known that soil is a recipient of solid wastes able to contain enteric pathogens in high concentrations. Pathogens that have been transported by wastewater can survive in soil or on crops (Ibenyassine et al., 2007). Although the role of soil as a reservoir of certain bacterial pathogens is not in question, recent findings show that soil may have a larger role in the transmission of enteric diseases than previously thought. Many of the diseases caused by agents from soil have been well characterized, although enteric diseases and their link to soil have not been so well studied (Santamariam and Toranzos, 2004)

2.3.3. Use of fertilizers

Manure and other animal wastes are widely used in agriculture, both organic and conventional. The use of manure as fertilizer, whether in organic or conventional agriculture, gives rise to concern about the possible contamination of produce with microbial pathogens, especially *Escherichia coli* O157 (IFST, 1999). However, the Soil Association recommendations for manure storage and treatment on organic farms may lead to enhanced reductions to the levels of pathogens in stored manures that are to be spread to land (Soil Association, 1999). The UK Food Standards Agency's (FSA) view is that there is currently no evidence to support the assertion that organic produce is more or less microbiologically safe than conventionally farmed produce (FSA, 2000).

2.4. Interaction of human pathogens with growing vegetables

Pathogens that have been transported by wastewater can survive in soil or on crops. The actual risk of disease transmission, however, is related to whether this survival time is long enough to allow transmission to a susceptible host. The crop and the field are the link between the pathogen in the wastewater and the potential for infection. The factors controlling transmission of disease are agronomic; examples of such factors are the crop grown, the irrigation method used to apply wastewater, and cultural and harvesting practices (Frost et al., 1995). Numerous opportunities exist for attachment and penetration of pathogenic bacteria into fresh produce in the field, as well as during harvesting, processing, and marketing, especially when a contaminated product is exposed to water or is damaged (Takeuchi et al., 2001).

2.5. Indicator microorganisms

To estimate food sanitary quality, the classical approach is based on the search for not only pathogenic microorganisms but also indicator microorganisms (Leclercq et al., 2002). The load of aerobic mesophilic microorganisms is among these which gives an estimate of total viable populations and is indicative of the endogenous microflora and the contamination undergone by the material (Ponce et al., 2008). The role of LAB (Lactic acid bacteria) on keeping quality of vegetables is not clear. LAB serve as a biocontrol agent in minimally processed refrigerated foods. LAB may exert antimicrobial effects due to one or more of the following mechanisms: lowering the pH, generating hydrogen peroxide, competing for nutrients, and possibly by producing antimicrobial compounds such as bacteriocins.

Coliforms are indicator of hygiene at the production stage and the maintenance of the cold chain. Fresh vegetables may be contaminated from soil, irrigation water, or improper handling. Contamination by microorganisms is a major factor in reducing the keeping quality and safety of many foods and rapid and reliable methods for assessing hygienic quality are urgently needed. One of the methods developed for this purpose in recent years has been assessing the numbers of active vegetative bacteria and yeasts in non-heat-treated samples. Members of the genus *Bacillus* are common contaminants of many foods and can be responsible for spoilage of the food or acute food-poisoning (Kelly and Kroll, 1987).

Yeasts and molds sometimes act as strict parasites and sometimes as latent parasites, depending on the plant resistance, the virulence of the strain, the competing microflora, and the ambient conditions. They may present a profound change in the rate of growth after harvest, when the plant resistance is diminished, and lead to rapid spoilage (Ponce et al., 2008).

2.6. Pathogens from vegetables

At one time, fresh fruits and vegetables were not considered food products that could support or be contaminated with human pathogens. However, this attitude has now changed. In a review of pathogenic microorganisms associated with raw vegetables, Beuchat (1996) listed those human pathogens that could survive and grow on lettuce and salad vegetables. There are confirmed foodborne outbreaks associated with the consumption of fresh lettuce products contaminated with *Escherichia coli* O157:H7 (Rajkowski and Fan, 2008).

Lettuce may become contaminated by pathogens from soil and irrigation water (Daiz and Hotchkiss, 1996). Irrigation of vegetables with polluted water and untreated wastewater is practiced worldwide. This practice is most common in the urban areas of low-income countries, which have no capacity to effectively treat wastewater and face increasing demands for fresh vegetables. Wastewater provides water and nutrients as important resources for irrigation, but has high levels of pathogens. In Ghana, high levels of faecal contamination have been reported in irrigation water and on vegetables grown in cities (Keraita et al., 2007).

According to Neimira (2003) salad vegetables, including fresh-cut lettuce can be a source of pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella,* and *Shigella* spp. *E. coli* O157:H7 is known to grow on shredded lettuce stored at 12 °C. *Escherichia coli* O157:H7 and *Salmonella* are commonly found in a wide variety of vegetables (including lettuce) and water (Chang and Fang, 2007). Among the more than 2500 Salmonella serotypes, *S. Typhimurium* and *S. Enteritidis* are the two most frequently reported serotypes (33% of isolates) from human sources in the United States (Cheung et al., 2007).

As reported by Chang and Fang (2007), in the US, eight lettuce-associated outbreaks were reported with foodborne pathogens, including *Escherichia coli* O157:H7 and *Salmonella* from 1973 through 1997.

2. 7. Health hazard from vegetables

The continuous rise in the number of outbreaks of foodborne illness linked to fresh fruit and vegetables challenges the notion that enteric pathogens are defined mostly by their ability to colonize the intestinal habitat (Brandl, 2006). Vegetables can become contaminated with such pathogenic organisms while growing, during harvest, from post-harvest handling, or during distribution (McMahon and Wilson, 2001).

Gastrointestinal infections are the most common diseases caused by enteric bacteria. Some examples are salmonellosis (*Salmonella* sp.), cholera (*Vibrio cholerae*), dysentery (*Shigella* sp.) and other infections caused by *Campylobacter jejuni*, *Yersinia* sp. and *Escherichia coli* O157:H7 and many other strains. Viruses are the most hazardous and have some of the lowest infectious doses of any of the enteric pathogens. Hepatitis A, hepatitis E, enteric adenoviruses, poliovirus types 1 and 2, multiple strains of echoviruses and coxsackievirus are enteric viruses associated with human wastewater. Among the most commonly detected protozoa in sewage are *Entamoeba histolytica*, *Giardia intestinalis* and *Cryptosporidium parvum* (Santamariam and Toranzos, 2004).

Fresh produce has been associated with numerous outbreaks of foodborne illness in North America in recent years (Niemira, 2003). Foodborne illnesses caused by *Salmonella* spp. have always been a significant health problem worldwide In Hong Kong, 22% of reported food poisoning outbreaks were caused by *Salmonella* during 2000–2004, and among all the *Salmonella* isolates, *S. Enteritidis*, *S. Typhimurium*, and *S. Derby* had been the top three serotypes during 1999–2004 (Cheung et al., 2007).

An increase in consumption of fresh fruits and vegetables worldwide has been paralleled by an increase in the number of foodborne illnesses attributed to fresh products. Numerous reports have indicated that raw vegetables may harbor potential foodborne pathogens (Beuchat, 1996). In particular, tomatoes, cantaloupes, and sprouts have been linked to outbreaks of salmonellosis (Guo et al., 2001), and outbreaks of illnesses caused by *Escherichia coli* O157:H7 have been associated with melon, apple cider, lettuce, and radish sprouts (Breuer et al., 2001). Moreover, coleslaw, cabbage, potatoes, radishes, bean sprouts, and cucumbers contaminated with *Listeria monocytogenes* have been linked to disease outbreaks (Shearer et al., 2001), and salad vegetables also may be contaminated with *Campylobacter* (Evans et al., 2003).

As reported by Chang and Fang (2007), in the US, eight lettuce-associated outbreaks were reported with foodborne pathogens, including *Escherichia coli* O157:H7 and *Salmonella* from 1973 through 1997. The overall rate of infection ranged from 15 to 20 per100, 000 populations during each year in the US. The outbreaks caused by *Salmonella* spp. were frequent both in Korea (20.7%) and in Japan (14.2%). *S. enteric* serovars *Typhimurium* and *S. enteric* serovars *Enteritidis* are the most frequently isolated serovars from foodborne out breaks throughout the world (Cheung et al., 2007).

Furthermore, according to Daiz and Hotchkiss (1996), a shigellosis outbreak caused by *Shigella sonnei* was associated with lettuce. Outbreaks of listeriosis have also been associated with lettuce. But no data preclude shredded lettuce as a vehicle for *E. coli* 0157:H7 outbreaks as the normal spoilage organisms present on vegetables can inhibit the growth of pathogens.

In developing countries, the morbidity and mortality rates of gastrointestinal tract infections from food borne bacteria have been difficult to establish. Most studies have only been able to gather data prospectively from isolated geographic sources, rather than from large point-source epidemics (Echeverria et al., 1994). For example, from 1991 to 2002, 21% of *Escherichia coli* O157: H7 outbreaks were from produce-related sources (Aruscavage et al., 2006).

2.8. Prevalence and drug resistance pattern of pathogens in produce

Vegetables may serve as a reservoir from which pathogenic microorganisms can colonize and infect a susceptible host (Ibenyassine et al., 2007). The contamination with human pathogens can occur during the growth of the produce using bovine manure fertilizer, contaminated irrigation water, contaminated wash water or cross contamination during the cutting of the lettuce. Even though these contamination levels are low, foodborne outbreaks linked to the consumption of lettuce do occur as cut lettuce can harbor and support the growth of foodborne pathogens.

In Taiwan, for example, one of 116 (0.86%) specimens of fresh cut vegetables was contaminated with *E. coli* O157: H7 (Chang and Fang, 2007). From 1991 to 2004, 21% of *E. coli* O157: H7 outbreaks were from produce-related sources (Aruscavage et al., 2006).

According to Neimira (2003) salad vegetables, including fresh-cut lettuce can be a source of pathogens such as *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella,* and *Shigella* spp. In their study on the prevalence of *Salmonella* in lettuce in US, Rajokowski and Fan (2008) showed that 2 out of 116 imported lettuce samples and one out of 142 domestic lettuces was positive for *Salmonella*. The prevalence of *Salmonella* on produce is slightly higher on piece with injury than with healthy tissue (Yuk et al., 2006).

Lee et al. (2001) reported that the frequencies of *Shigella sonnei* were less than 10% of shigellosis in the 1980s, but the isolation rate of *S. sonnei* sharply increased in the 1990s and accounted for over 95% of cases in Korea after 1998. Large outbreaks of *S. sonnei* infections have been reported from many parts of Korea during the period 1998 to 1999.

The development of resistance is a global problem and is not restricted to special countries or bacterial species. The incidence of human salmonellosis is rising in most of the countries where surveillance networks have been set up, and, along with this rising incidence, increasing rates of antimicrobial resistance have been reported worldwide. Studies by Schroeter et al. (2004) showed that in 2000–2002 roughly 65% of all *S. Typhimurium* isolates from foods showed a typically fivefold resistance (against ampicillin, chloramphenicol, streptomycin/spectinomycin, sulphonamides and tetracycline). Increasing antimicrobial resistance in *Salmonella* can limit the therapeutic options available for clinical cases that require antimicrobial treatment. This is the case for the clone of *S. typhimurium* DT104, characterized by chromosomal resistance to ampicillin, tetracycline, streptomycin, chloramphenicol and sulfonamides (Geimba et al., 2005).

Similarly, most strains of *Shigella* are becoming resistant to commonly used antibiotics (resistant to ampicillin, tetracycline, co-trimoxazole, streptomycin, chloramphenicol), and even to the most currently used antibiotics like mecillinam, ciprofloxacin. The development of resistance is so extensive that even nalidixic acid, the drug of choice in shigellosis, is fast losing its efficacy in *Shigella* infections**.** The antibiotic gentamycin, has a good anti-*Shigella* activity, but cannot be used freely because of its toxicity**.** This emergence of multiple drug resistant strains of *Shigella* has made treatment of dysentry difficult. Therefore, extensive work to look for newer antibiotics effective in diarrhoeal diseases, particularly caused by the drug resistant strains of *Shigella,* has been carried out very recently **(**Ahsan and Chowdhury, 1996).

Appropriate antimicrobial therapy for shigellosis reduces the duration and severity of dysentery. The use of ampicillin or trimethoprim combined with sulfamethoxazole has been proposed to treat shigellosis. However, high frequencies of resistance in the *Shigella* species to these first-line antimicrobial agents have been reported from many parts of the world (Lee et al., 2001).

2.9. Methods to reduce enteric bacterial infections and improve the microbiological safety

There are a variety of methods used to reduce populations of microorganisms on whole and fresh-cut produce. Each method has distinct advantages and disadvantages depending upon the type of produce, mitigation protocol, and other variables. The best method to eliminate pathogens from produce is to prevent contamination in the first place. However, this is not always possible and the need to wash and sanitize many types of produce remains of paramount importance to prevent disease outbreaks (Parish et al., 2003).

2.9.1. Washing with running tap water

Washing fresh fruits and vegetables with running tap water may remove soil and other debris, but it has a limited effect on surface microorganisms that occur at populations ranging from 10^3 to 10^9 CFU g^{-1} (Chang and Fang, 2007).

2.9.2. Disinfectants

A variety of disinfectant (chlorine, hydrogen peroxide, ozone, etc) have been used to reduce the bacterial population on fruits and vegetables (Chang and Fang, 2007). Several researchers have investigated the effectiveness of chemicals in killing pathogenic bacteria. But, microorganisms on the surface of raw produce may be difficult to remove due to entrapped or attached cells and porous surfaces (Sanglay et al., 2004). One approach to reduce contamination is to treat fresh produce with rinsing agents (Raiden et al., 2003). Among these, chlorination is widely practiced as a disinfection process for microbial control in water used to wash fruits and vegetables at packing houses. When properly applied, chlorine based products are efficient (Chaidezel et al., 2003). However, studies have shown that treatment of produce with chlorinated water has a limited bactericidal effect (Chang and Fang, 2007). Besides, microorganisms differ in sensitivity to disinfectants depending on the type of cell wall, membrane composition, age and cycle of growth, biofilm production and clump formation. A disinfectant is considered to have an effective microbicidal when it is capable of achieving 6-log, 4-log, and 3-log reduction of a bacteria, virus and protozoan, respectively. The official method has stated a reduction level of 5 log of selected surrogates for an effective sanitizer (Chaidezel et al., 2003).

2.9.2. Sanitizers

A variety of disinfectant (chlorine, hydrogen peroxide, ozone, etc) have been used to reduce the bacterial population on fruits and vegetables. However, besides their potential toxicity, they cannot completely remove or inactivate microorganisms of fresh produce. Hence, other sanitizers such as organic acids like acetic acid (vinegar) have been studied for their effectiveness to increase food microbiological safety (Chang and Fang, 2007).

2.10. Implications for control

Fresh fruit and vegetables have been identified as a significant source of foodborne bacterial-illness outbreaks in humans, especially when the vegetables are consumed raw. As a result, there has been a wealth of research on identifying and controlling hazards at all stages in the supply chain (Jougen, 2005).

To protect public health, the use of raw sewage in the irrigation of the vegetable culture must be prohibited. So it is necessary to consider the generalized program of wastewater treatment. Moreover, the requirements for treated wastewater must respect the sanitary standards for agricultural reuse. There is an urgent need for development and validation of standard methods of eliminating the pathogenic microorganisms from raw vegetables. Washing and sanitation steps are critical with produce because this type of food is often eaten raw (Ibenyassine et al., 2007).

With current technology and packaging of fresh produce, microorganisms are present at the time of purchase in grocery stores. Farmers and processors must be vigilant in food safety, but so must consumers. Proper storage and cleaning before eating will help prevent foodborne disease associated with fresh produce. The FSA (2000) recommends buying produce that is not bruised or damaged and buying cut produce that is refrigerated or packed in ice. It is also recommended that all produce be washed with water before consumption, even if it is prepackaged. The FSA (2000) suggests scrubbing produce capable of handling it, but does not recommend use of detergents. It is important to realize that produce safety is now a partnership that requires attention from farmers, processors, retailers, and consumers (Aruscavage et al., 2006).

3. MATERIALS AND METHODS

3.1 Collections of samples

A total of 80 fresh produce samples were purchased at different sampling days from different supermarkets (outlets) in Addis Ababa, Ethiopia between November, 2007-April 2008. These fresh produce varieties consisted of 40 lettuce and 40 green pepper samples. All samples were collected aseptically and immediately brought to the laboratory for microbiological analysis. Microbiological analysis was conducted within 1 to 3 hours of sample collection.

3.2. Microbiological analysis

For microbiological analysis, 25 g of sample was aseptically removed from each sample using a sterile scalpel and shaked in 225 ml of sterile 0.1% (w/v) bacteriological peptone water (Oxoid) for 1-3 minutes. Serial ten-fold dilutions were then prepared by transferring 1ml of the homogenized sample to 9ml diluent. Appropriate serial dilutions of the homogenates were then plated on a suitable agar medium.

3.2.1. Microbial enumeration

3.2.1.1. Aerobic mesophilic count

From appropriate dilutions, 0.1ml aliquots were spread-plated in duplicates on pre-dried surfaces of Plate Count Agar (Oxoid) plates. Colonies were counted after incubation at 30-32ºC for 48 hours.

3.2.1.2. Counts of Enterobacteriaceae

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Glucose Agar (Oxoid) plates. The seeded culture plates were incubated at 30-32ºC for 20-24 hours after which pink to red purple colonies with or with out haloes of bile precipitation were enumerated as members of *Enterobacteriaceae*.

3.2.1.3. Counts of coliforms

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Violet Red Bile Agar (Oxoid) plates. The culture media were incubated at 30-32ºC for 24 hours after which purplish red colonies surrounded by reddish zone of precipitated bile were counted as coliforms.

3.2.1.4. Counts of spore

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of nutrient agar plates, after heating suspension in water bath (80ºC) for ten minutes. Colonies were counted after incubation at 30-32ºC for 24 hours.

3.2.1.5. Counts of staphylococci

From appropriate dilutions, 0.1 ml aliquots were spread-plated in duplicates on pre-dried surfaces of Mannitol Salt Agar (Oxoid) plates. The culture plates were incubated at 30-32ºC for 36 hours after which yellow colonies were counted as staphylococci.

3.2.1.6. Counts of yeasts and moulds

From appropriate dilutions, 0.1 ml aliquots were spread-plated on Chloramphenicol-Bromophenol blue agar consisting of (g/l distilled water) yeasts extract (Oxoid) 5.0, glucose 20, choramphenicol 0.1, Bromophenol-blue 0.01, agar 15, pH 6.0–6.4. The plates were incubated at 25–28 º C for 4–5 days. Smooth, non-hairy colonies lacking extensions at margins under microscope were counted as yeasts.

3.2.2. Flora analysis

After enumeration of aerobic mesophilic bacteria, about 10 to 20 seemingly different colonies were picked randomly from countable plates and inoculated into tubes containing about 5ml Nutrient Broth No 2 (Oxoid). These were incubated at 30-32^{0}C over-night. Cultures were purified by repeated plating and preserved at 5^{0}C for further tests.

3.2.2.1. Cell morphology

From overnight pure broth culture, wet mount, Gram staining and spore staining was prepared on a microscope slide. The preparation was observed under light microscope using oil immersion objective. The morphological criteria considered during the observation were:

Cell Shape:	Regular: rods, cocci, coccoid forms
Cell arrangement:	Single, pairs, clusters, chains, and tetrads
Endospore:	Present, absent

3.2.2.2. Gram reaction (KOH test)

This test was done according to Gregerson (1978). 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 KOH solution for 10 seconds to minutes and the inoculating loop was then raised slowly from the mass. When KOH solution became viscous and the thread of slime followed the loop for 0.5 to 2cm or more, the isolate is considered as Gram-negative bacteria. In cases of no slime, the watery suspension did not follow the loop; the reaction was a characteristic of Gram-positive bacteria.

3.2.2.3. Oxidation Fermentation (O/F) test

The utilization of glucose by each isolate was assessed by O/F test to identify the isolates that metabolize glucose fermentatively or oxidatively or that did not utilize glucose by either way according to Hugh and Leifson (1953). The ingredients of the test medium were (g/1): Peptone, 2g; yeast extract, 1g; NaCl, 5g; K_2 HPO_4 , 0.2g; glucose, 10g: bromophenol blue, 0.08g; agar, 2.5g; distilled water, 1000ml, pH, 7.10.

15ml of the freshly prepared medium was dispensed into 18x180mm test tubes; and immediately cooled under tap water to avoid dissolution of oxygen in the medium. Loopfull of overnight culture of each isolate was inoculated into the medium by stabbing with a sterile straight wire to the bottom. Acid formation and growth regions after incubation at 30-32^{0}c for 2 and 5 days were interpreted as positive/negative O/F test.

3.2.2.4. Catalase test

Young colonies were flooded with a 3% solution of H_2O_2 (hydrogen peroxide). The formation of bubbles indicated the presence of catalase.

3.2.2.5. Cytochrome oxidase test

This test was conducted following the method outlined by Kovacs (1956). Freshly prepared reagent A and B were mixed in the ration of 2:3 immediately before use.

Reagents:

A) 1% -α –naphthol in absolute ethanol

B) 1% N,N-dimethyl-p-phenylenediammonium chloride in distilled water

The young colonies grown on Nutrient Agar plates were flooded with the mixture of A and B. The appearance of a blue color on the colonies within 30 seconds to 2 minutes indicated a positive reaction. Any very weak or dubious reaction that occurred after 2 minutes was ignored.

3.3. Isolation and characterization of *Salmonella* and *Shigella*

3.3.1. Primary enrichment

To test for the presence of *Salmonella* and *Shigella*, 25g of vegetable sample was mixed with 225ml of 1% Buffered Peptone Water (BPW). Ten ml of each sample was enriched in 50ml tryptone soy broth (Oxoid), homogenized and incubated at 37^0C for 18-24 hours for the metabolic recovery and proliferation of cells which could have been injured during processing or to bring the number of target organisms to a detectable level.

3.3.2. Secondary enrichment

The following broths were employed for secondary enrichment: Selenite Broth Base (SBB) (Oxoid) supplemented with sodium biselenite (4g/l), Tetrathionate Broth Base (TBB) (Oxoid) supplemented with iodine solution (20ml/1), Mannitol Selenite Broth Base (MSBB) (Oxoid) supplemented with sodium biselenite (4g/l), and Selenite Cestien Broth Base (SCBB) (Oxoid) supplemented with sodium biselenite (4g/l). The selective property of these broths lies in their ability to inhibit non-targeted microorganisms like Gram-positive bacteria and coliforms and permit the rapid multiplication of *Salmonella* and *Shigella*. After pre-enrichment in buffered peptone water, 1 ml of culture was transferred into separate tubes each containing 10 ml of SBB, 10ml of TBB, 10 ml of MSBB or 10ml of SCBB. The enrichment broths were incubated at 37^0C for 24 hours and TBB was incubated at 43^0C for 48 hours in water bath.

3.3.3. Solid media

MacConkey Agar No. 3 Salmonella-Shigella (SS) Agar and Xylose Lysine Desoxycholate (XLD) medium (all from Oxoid) were used for plating purpose. A loopful of culture from each selective enrichment broth was streaked separately on to each of the solid medium and incubated at 37^0C for 18-24 hours. Characteristic colonies from each selective medium were picked and further purified and tested biochemically. Uninoculated culture plates were incubated to check for sterility of the solid media.

3.3.4. Biochemical identification

Suspected *Salmonella* and *Shigella* colonies were picked and purified. Pure cultures were further tested for their minimum biochemical profile.

3.3.4.1. Triple sugar Iron Agar (TSIA) (Oxoid)

The butt was stabbed and the slant was streaked (pH 7.4 ± 0.2) and incubated at 37^oC for 18-24 hours, to detect fermentation of sucrose, glucose, lactose and the production of H_2S. For *Salmonella* the butt became yellow and gas was or was not produced and the slant showed a reaction that was alkaline or no change in some cases. H_2S was also produced. The Presence of alkaline (red) slant and acid (yellow) butt, without production of H_2S was considered as presumptive for *Shigella*.

3.3.4.2. Lysine Iron Agar (Oxoid)

The butt was stabbed and the slant was streaked (pH 6.7$\pm$0.2) with a loopful of the culture and incubated at 37ºC for 18-24 hours. *Salmonella* produced an alkaline reaction (purple color) throughout the medium. Due to the production of H_2S, an intense blackening of the medium was seen. The presence of alkaline slant and acid butt was considered presumptive for *Shigella*.

3.3.4.3. Urea Agar (Oxoid)

The slant was heavily streaked (pH 6.8$\pm$0.2) and incubated at 37ºC for 18-24 hours, to assess the hydrolysis of urea. Urease producing organisms hydrolyze urea to form ammonia and the medium may change to purple red. *Salmonella* and *Shigella* did not produce the enzyme urease and the color of the urea slant was unchanged.

3.3.4.4. Simmons Citrate Agar (Oxoid)

The slant was streaked (pH 7.0$\pm$0.2) and incubated at 37ºC for 18-24 hours, to investigate the utilization of citrate as the sole source of carbon. The slant changed to blue color for *Salmonella* and retained the original color for *Shigella.*

3.3.4.5. Mannitol broth (1%)

Ingredients: Mannitol,10gm; peptone,10gm; NaCl, 5gm; phenol red,0.024gm; distilled water,1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated in to the broth and incubated at 37ºC for 18-24 hours to test fermentation and production of gas. Durham's (fermentation) tubes were added to the carbohydrate tubes to detect the production of gas.

3.3.4.6. Sucrose broth (1%)

Ingredients: Sucrose, 10gm; peptone,10gm; NaCl, 5gm; phenol red, 0.024gm; distilled water,1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated in to the broth and incubated at 37ºCfor 18-24 hours to test fermentation and production of gas. Durham's (fermentation) tubes were added to the carbohydrate tubes to detect the production of gas.

3.3.4.7. Glucose broth

Ingredients: Glucose, 10gm; peptone, 10gm; NaCl, 5gm; phenol red,0.024gm; distilled water,1000ml and the pH was adjusted to 7.2. A colony was picked and inoculated in to the broth and incubated at 37ºC for 18-24 hours to test fermentation and production of gas. Durham's (fermentation) tubes were added to the carbohydrate tubes to detect the production of gas.

3.4. Drug susceptibility testing

Pure presumptive *Salmonella* and *Shigella* cultures were inoculated in to Nutrient Broth and the cultures were incubated at 32^0C for 18-24 hrs. After incubation, the cultures were adjusted to 0.5 McFarland to bring the cell density to approximately 10^7-10^8cfu/ml. The McFarland turbidity standard was prepared by mixing 0.1ml $BaCl_2$ (1%) with 9.9ml H_2 SO_4 (1%) (Andrew, 2001).

The isolates were tested for their susceptibility to different antibiotics on Mueller-Hinton agar (pH 7.4$\pm$0.2) following the standardized disc diffusion technique with Oxoid drug discs: Ampicillin (Amp),(10µg); Penicillin G, (Pen), (10iu); Ceftriaxone (Cef),(30µg); Streptomycin (Str),(10µg); Chloramphenicol (Chl),(30µg); Tetracycline (Tet),(30µg); Ciprofloxacin (Cip),(5µg); Polymyxin B (Pol. B),(100iu); Amoxicillin(Amo) (2µg); Kanamycin (Kan), (30µg); and Gentamicin (Gen), (10 µg). For the purpose of interpretation, those intermediate cases were considered sensitive.

3.5. Data analysis

Microsoft excel was used for data entry. Descriptive statistics was used to compute percentage and mean. To see if there was significant variation in counts within samples in each type of lettuce and green pepper, coefficient of variation (CV) was calculated. Significance was determined at the 10% level.

4. RESULTS

4.1. Microbial spectrum of lettuce

The microbial load (aerobic mesophilic count) of lettuce ranged from log 5 to log 9 cfu/gm and about 90% of the samples had counts higher than log 6 (Fig 1). A total of 229 bacterial strains were isolated from lettuces and characterized to various genera and bacterial groups (Table 1).

Table 1. Frequency distribution (%) of dominant bacteria in lettuce and green pepper purchased form supermarkets in Addis Ababa.

Sample type	No. of isolates	Gram-positives					Gram-negatives				
		Bacillus	Micro- coccus	Other Gram- positive rods	Staphylo- coccus	Total	Pseud- omonas	Aero- monas	Entero- bacteriaceae	Acineto- bacter	Total
Lettuce	229	30	17	8	1	56	16	13	8	7	44
Green pepper	378	30	32	18	4	84	8	3	4	1	16
Total	607	30	24.5	13	2.5	70	12	8	6	4	30

Generally, 56% of lettuce samples were dominated by Gram-positive organisms. The aerobic flora of lettuces was dominated by *Bacillus* spp. (30%) followed by *Micrococcus* (17%) and *Pseudomonas* (16%). Different microbial groups were found in all lettuce samples at varying levels (Fig. 1). Enterobacteriaceae and coliforms were encountered in about 75% and 48% of the samples, respectively with counts > log 4. About 42% of the samples had spore counts ≤ log 2 cfu/g. About 92% of the samples harbored staphylococci with counts ranging from log 3 to log 5 cfu/g. About 88% of the samples had yeast counts of ≥ log 4 cfu/g. Significant variation were noted in the counts of all microbial groups among the lettuce samples (CV=11.75–18.7) but variations within the aerobic mesophilic counts were not significant (CV = 9.84) (Table 2).

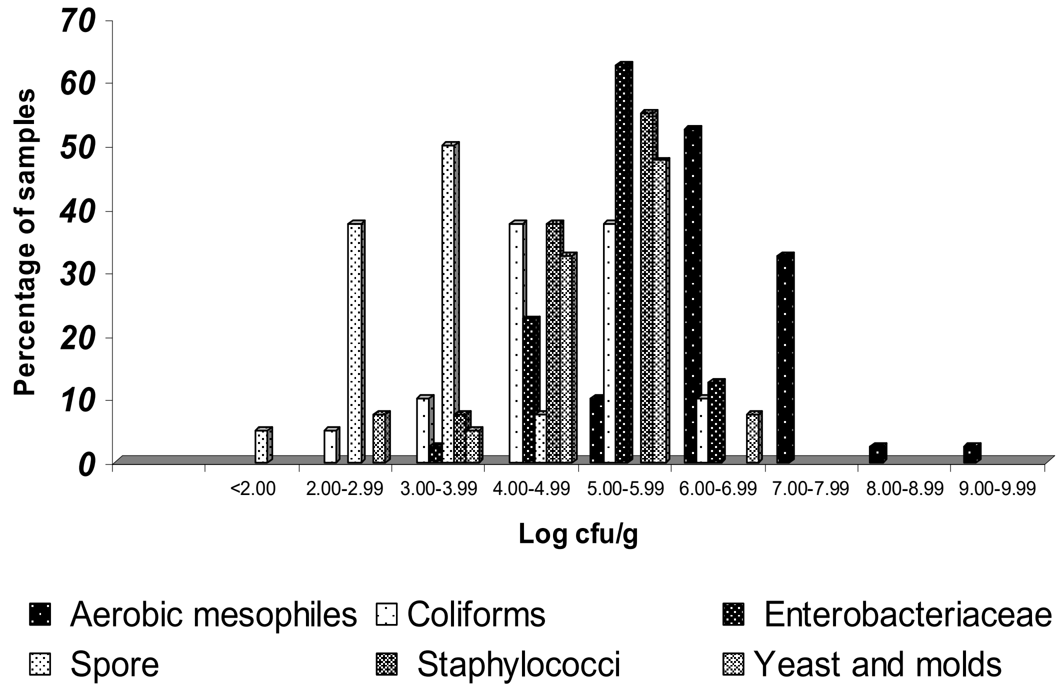


Fig. 1. Distribution of microbial counts (log cfu/g) in lettuce samples.

4.2. Microbial spectrum of Green peppers

A total of 378 bacterial strains were isolated from green pepper, and were characterized to various genera and bacterial groups (Table 1). Generally, 84% of green peppers were dominated by Gram-positive organisms. The aerobic mesophilic count of green pepper samples ranged from log 5 to log 9 cfu/g (Fig. 2). The aerobic flora was dominated by *Micrococcus* spp. (32%) followed by *Bacillus* spp. (30%) and Other Gram positive rods (18%). Different microbial groups were found in all green pepper samples at varying levels (Fig. 2). Almost all green pepper samples harbored enterobacteriaceae and yeasts with counts between log 2 and log 5 cfu/g. Over 70% of the samples contained coliforms and spore counts at levels > log 2 cfu/g. About 80% of the samples harbored staphylococci with counts ranging from log 4 to log 6 cfu/g. For counts of all microbial groups, variation within green pepper samples were significant (CV = 12.86 – 22.88) (Table 2).

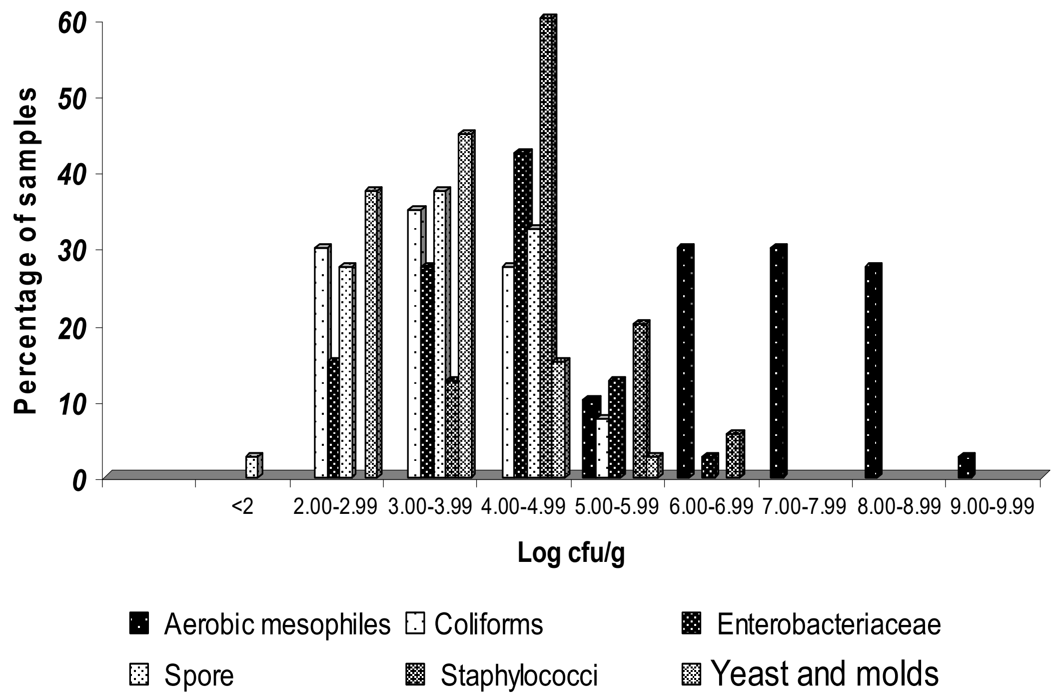


Fig. 2. Distribution of microbial counts (log cfu/g) in Green Pepper samples

Table 2. Microbial counts (log cfu/g) of lettuce and green pepper purchased from supermarkets in Addis Ababa.

	Lettuce					Green pepper				
Bacterial groups	Min	Max	Mean	S.D.	%CV	Min	Max	Mean	S.D.	%CV
Aerobic mesopiles	5.00	9.07	7.51	0.74	9.84	5.41	9.37	7.53	0.97	12.86
Coliforms	3.02	6.55	4.97	0.83	16.78	2.90	5.82	4.06	0.77	19.03
Enterobacteriaceae	3.79	6.37	5.08	0.59	11.75	3.00	6.01	4.84	0.88	18.19
Spore	<2.00	4.84	3.50	0.63	17.88	<2.00	4.99	3.47	0.79	22.88
Staphylococci	3.30	5.88	4.55	0.63	13.9	3.00	6.36	4.97	0.75	15.12
Yeast and molds	2.60	6.44	4.51	0.84	18.70	2.69	5.66	4.05	0.69	17.27

S.D.-Standard deviation; %CV-Percent coefficient of variation

4.3. Isolation and identification of *Salmonella* spp.

From a total of 80 lettuce and green pepper samples examined, six (7.5 %) samples were found positive for *Salmonella*. *Salmonella* was isolated from two (5%) samples of lettuce and four (10%) samples of green peppers. The overall prevalence of *Salmonella* isolates was 7.5% (6/80).

4.4. Isolation and identification of *Shigella* spp.

From a total of 80 lettuce and green pepper samples examined, fifteen (18.75%) samples were found positive for *Shigella*. *Shigella* was isolated from five (12.5%) samples of lettuces and ten (25%) samples of green peppers.

4.5. Antimicrobial resistance

4.5.1. *Salmonella*

Of the 12 *Salmonella* isolates subjected to antimicrobial susceptibility test, using a panel of eleven different antimicrobials, all were resistant to penicillin G., four isolates resisted ceftriaxone, three isolates showed resistance to streptomycin, five isolates were resistant to ampicillin, ten isolates were resistant to amoxicillin, none was resistant to polymixin B, gentamicin and ciprofloxacin and one isolate showed resistant to kanamycin, tetracycline, and chloramphenicol (Table 3). Eight isolates showed Multiple Drug Resistance (MDR). The isolates showed resistance to three, four and seven different antimicrobials. None of the resistance patterns was dominant. (Table 4).

Table 3. *Salmonella* and *Shigella* isolates resistant to the antibiotics tested.

Isolates	No. of isolates	No. of resistant strain										
		Pen	Str	Pol B	Tet	Amo	Cef	Gen	Cip	Amp	Kan	Chl
Salmonella	12	12	3	0	1	10	4	0	0	5	1	1
Shigella	29	28	10	7	12	24	12	1	1	23	2	4

Table 4. MDR pattern in *Salmonella* isolated from vegetables

No.antibiotic resisted	No. resistant isolates	The frequent pattern of resitance	
		No. of isolates	Resistance pattern
Three	5	1	Pen/Str/Amo
		3	Pen/Amo/Amp
		1	Pen/Str/Cef
Four	2	1	Pen/Str/Amo/Cef
		1	Pen/Amo/Amp/Kan
Seven	1	1	Pen/Str/Tet/Amo/Cef/Amp/Chl
Total	8		

4.5.2. *Shigella*

From 29 *Shigella* isolates subjected to eleven different antimicrobial agents, 28, 24 and 23 isolates were resistant to penicillin G, amoxicillin and ampicillin, respectively. Less than half of the isolates showed resistance to the other antimicrobials and only one isolate showed resistance to gentamicin and ciprofloxacin (Table 3). MDR was observed in 24 of the 29 *Shigella* isolates. The isolates showed resistance to three, four, five, six and seven different antimicrobials. The resistance was seen in 18 different patterns, and none of the resistance patterns was dominant. (Table 5).

Table 5.MDR pattern in *Shigella* isolated from vegetables.

No.antibiotic resisted	No. resistant isolates	The frequent pattern of resitance	
		No. of isolates	Resistance pattern
Three	6	5	Pen/Amo/Amp
		1	Pen/Tet/Amo
Four	4	1	Pen/Tet/Amo/Amp
		1	Pen/Pol B/Amo/Chl
		1	Pen/Amo/Cef/Amp
		1	Pen/Cef/Amp/Chl
Five	7	2	Pen/Pol B/Tet/Amo/Amp
		1	Pen/Str/Amo/Cef/Amp
		1	Pen/Pol B/Tet/Amo/Cef
		1	Pen/Str/Tet/Amo/Amp
		1	Pen/Amo/Cef/Amp/Chl
		1	Pen/Str/Amo/Cef/Gen
Six	4	1	Pen/Str/Pol B/Amo/Cef/Amp
		1	Pen/Str/Amo/Cef/Amp/Chl
		1	Pen/Str/Tet/Amo/Cef/Amp
		1	Pen/Str/Pol B/Tet/Amo/Amp
Seven	3	2	Pen/Str/Tet/Amo/Cef/Amp/Kan
		1	Pen/Str/Pol B/Amo/Cef/Cip/Amp
Total	24		

5. DISCUSSION

Various green vegetables are usually consumed raw. Information on the microbiology or nutritional quality of vegetable items in Ethiopia is limited (Aberra Geyid et al., 1991). Besides, low income and the low level of technology in the country have given no chance to the people to use suitable ways of packing and storage. (Gulelat Dessie and Mulgeta Taye, 2001).

The majority of lettuce and green peppers considered in this study had high microbial load and, in some cases, even pathogens were isolated. Cross contamination may occur while growing in field, during harvesting and post harvest handling, processing and distribution or a combination of these factors may contribute to high microbial counts. Furthermore, absence of safety measures to reduce the microbiological load on the raw product revealed inadequacies concerning quality and safety of these products.

The mean aerobic mesophilic counts of the lettuces in this study ranged between log 5 to log 9 cfu/g. These mean values were relatively higher than that reported by Ibenyassine et al. (2007) for lettuce in Morocco. Soriano et al. (2000) also reported counts of aerobic mesophiles for lettuces served in Spain university restaurants between log 3 to log 8 cfu/g. On the other hand, Blackburn et al. (1996) reported a mean aerobic mesophilic count of vegetable between log 2 to log 10. This mean value was remarkably higher than the mean count value obtained in this lettuce sample. Generally, high aerobic mesophilic count recorded in this lettuce samples in this study may be, therefore, due to it unhygienic handling.

In this study, the counts of Enterobacteriaceae obtained were also high in the lettuce sample in which 75% of the sample had counts ≥ log 4 cfu/g. Ibenyassine et al. (2007) also reported counts of Enterobacteriacea for lettuces in Morocco between log 5 to log 8 cfu/g. Coliforms were also encountered in lettuce in which around half of the sample had counts ≥ log 4 cfu/g. The mean values of counts of coliforms obtained from lettuces in this study were higher than that reported by Blackburn et al. (1996) for the enumeration of coliform bacteria in food which had mean count of coliforms as low as 1 cfu/g. Similarly, Soriano et al. (2000) reported lower mean counts of coliforms within the range of log 0.47 to log 3.38 cfu/g.

Since these groups of bacteria (Enterobacteriaceae and/or coliform) are not generally desirable because their potential of multiplication makes it hazardous for consumption, the presence of such high counts could indicate contamination by microorganism while growing in field, during harvesting and post harvesting handling, and distribution.

The mean values of counts of staphylococci obtained from lettuce were high in which more than 92% of the sample harbored staphylococci with counts ranging from log 3 to log 5 cfu/g. The lettuce samples were found to consist of *Staphylococcus*, that may cause staphylococcal food poisoning and it was reported that production of enterotoxin occurs at *Staphylococcus aureus* count of 10^6 cfu/g (Tatini et al., 1970). Staphylococci are common in products handled by hand, thus the high staphylococci load of lettuce observed in this study may be due to it unhygienic handling.

The mean values of counts of yeast and molds obtained were high in the lettuce samples in which about 88% of the sample had yeast counts of $\geq$ log 4 cfu/g. Therefore, the presences of such high counts of yeast indicate a short shelf life these vegetables would have either in shops or in consumer' home.

The mean bacterial spore counts of lettuce in this study were $\leq$ log 2 cfu/g. Kelly and Kroll (1987) reported a mean bacterial spore count of log 4.5 for enumeration of bacterial spores in food. This mean value was markedly higher than the one obtained in this sample. However, the potential of their germination makes them hazardous for consumption. If the level of contamination with B. cereus exceeds 10^6 cfu/g, it can play a role in the transmission of *B. cereus* food poisoning.

In general, a high count or aerobic mesophilic bacteria, Enterobacteriaceae, coliform, staphylococci, yeast and mold and bacterial spores indicates that the lettuce might be contaminated with microorganism during growth, harvesting, or distribution. Besides, inappropriate storage practices may result in proliferation of microorganism present. Therefore rapid and reliable methods for assessing hygienic quality are urgently needed.

There was high variability in the counts of all microbial groups within the samples of each lettuce. This shows the lack of washing and sanitation steps. In addition there is an increased potential for vegetables to become contaminated with pathogenic species during production and processing as there is no system of microbiological control of the raw vegetable or the processed one.

Salmonella spp. was isolated from two (5%) samples of lettuce and four (10%) samples of green peppers. *Salmonella* was not detected in a range of commercially available organic vegetable samples taken form Northern Ireland (McMahon and Wilson, 2001). Soriano et al. (2000) also failed to isolate *Salmonella* from lettuces served in Spain University restaurants. Ruiz et al., (1987) isolated *Salmonella* from 6.5% of vegetable sample in Spain. Rajkowski and Fan (2008) also isolated two *Salmonella* out of 116 imported lettuce sample and one out of 142 domestic lettuce indicating that the contamination with human pathogen can occur during the growth of the produce using bovine manure fertilizer, contaminated water or cross contamination during the cutting of the lettuce as the cut of lettuce can harbor and support the growth of food borne pathogen (nutrients from plant cellular material leakage). The detection of *Salmonella* from lettuce indicates possible contamination during production or processing of raw vegetables, which clearly support the allegation that raw vegetables are of high risk due to the farming method and processing used. It also shows the need for close supervision of processing and sanitation steps.

Salmonella isolates from vegetables in different parts of the world showed resistance to different antibiotics. The prevalence of resistant *Salmonella* isolates in this study to tetracycline (8%) was lower than those isolate from Malaysia (42%) (Yoke-kqueen et al., 2008) and from those isolates reported from Porto Algre, Brazil (53.4%) (Geimba et al., 2005). Similarly the resistance of these isolates to streptomycin (25%) was lower than those isolate from Malaysia (34%) (Yoke-kqueen et al., 2008) and Brazil (37%) (Geimba et al., 2005).

The resistance of *Salmonella* isolate in our study to gentamicn (0%) was lower than that of Brazil (13.7%) (Geimba et al., 2005) and also from Malaysia (9%) (Yoke-queen et al., 2008). On the other hand, 25% of our isolate showed resistance to chloramphenicol, which was higher than the results from Brazil (2.4%) (Geimba et al., 2005) and Malaysia (3%) (Yoke-kqueen et al., 2008). Similarly, 25% of our isolate showed resistance to kanamycin, which was higher than the results from Brazil (6%) (Geimba et al., 2005).

The prevalence of resistant *Salmonella* isolate in this study to ampicillin (42%) was higher than those isolates from Brazil (2.4%) (Geimba et al., 2005) and Malaysia (7%) (Yoke-kqueen et al., 2008). Similary, the resistance of these isolates to ceftriaxone (33%) was higher than those isolates from Malaysia (18%) (Yoke-kqueen et al., 2008). Resistance to most of the antibiotic could result in a serious public health concern.

The finding of 67% of MDR *Salmonella* in this study was higher than isolates from Malalyia (56.7%) and Brazil (23%) (Geimba et al., 2005). The investigation of multi-drug resistant *Salmonella* is revenant to gain an understanding of the epidemiology of emerging resistant *Salmonella* servovars.

Shigella was isolated from five (12.5%) samples of lettuces and ten (25%) samples of green peppers. *Shigella* was not detected in any of the lettuces served in Spain University restaurants (Soriano et al. 2000). Onyemeluk and Njoku-obi (1992) isolated

Shigella from 5.6% of the samples in Nigeria. The detection of *Shigella* from lettuces revealed inadequacy concerning quality and safety of these products.

Strains of *Shigella* isolates from fresh produce resistant to various commonly used antibiotics have been reported from various parts of the world (Onyemeluk and Njoku-obi, 1992). The resistance of *Shigella* isolates in this study to streptomycin (34%) is lower than that reported from Nigeria (52%) (Onyemeluk and Njoku-obi, 1992). Meanwhile, 41% of the isolates showed resistance to tetracycline, which was higher than that from Korea (4%) (Lee et al. 2001) and lower than that from Nigeria (52%) (Onyemeluk and Njoku-obi, 1992).

The prevalence of resistant *Shigella* isolates in this study to ciproflaxin (3.4%) was similar to those isolates from Korea (4%) (Lee et al. 2001).The resistance of the isolates to chlororamphenicol (29%) was lower than those isolates from Nigeria (52%) (Onyemeluk and Njoku-obi, 1992). On the other hand, the prevalence of resistant *Shigella* isolates in this study to ampicillin (79%) was higher than those isolates from Nigeria (52%) (Onyemeluk and Njoku-obi, 1992).

Multi-drug resistant *Shigella* has been reported to be increasing in incidence worldwide (Onyemeluk and Njoku-obi, 1992). The finding of 83% of MDR *Shigella* in this study was higher than those isolates from Nigeria (39.2%) (Onyemeluk and Njoku-obi, 1992).

The mean aerobic mesophilic counts of green pepper samples ranged from log 5 to log 9. cfu/g. These mean values were relatively higher than that reported by Ibenyassine et al. (2008) for green pepper in Morocco.

The mean value of Enterobacteriaceae recorded for green pepper sample was between log 2 and log 5. These mean values were relatively lower than those reported by Ibenyassine et al. (2007) for green pepper in Morocco which is between log 5 to log 8. Coliforms were encountered in more than 70% of the samples with counts at levels ≥

log 2 cfu/g. The mean value of counts of coliforms obtained from green peppers in this study was relatively higher than those reported by Blackburn et al. (1991) for coliform bacteria in food which had mean count of coliforms as low as 1 cfu/g.

More that 80% of the green pepper sample harbored staphylococci with counts ranging from log 4 to log 6 cfu/g. Thus, it may be hazardous because counts ≥ log 5 cfu/g are required to produce enough enterotoxin to cause food poisoning. Thus, the presence of such high counts of staphylococci in some green pepper samples can be a risk factor for the consumers in terms of staphylococcal food poisoning.

6. CONCLUSION AND RECOMMENDATION

Based on the findings obtained in this study, the following conclusions and recommendations are forwarded:

1. The majority of the samples we analyzed showed the presence of high microbial load, which indicated high contamination during harvesting, or distribution. Moreover, high variability in the counts of almost all microbial groups within the sample of each vegetable type might also indicate unhygienic handling and contamination during growth and distribution.

2. This study also pointed out a possible microbial safety problem from eating raw-consumed vegetables because they contained target pathogens. Thus to eliminate suspected pathogens washing and sanitation steps are critical.

3. Although vegetables are consumed in Ethiopia, there are no valid standard methods established for eliminating the pathogenic microorganisms from raw vegetables. Thus, there is an urgent need for development and validation of standards and quality control systems to improve their quality and safety of the consumer and to meet the export standards.

4. Microbiological safety of vegetables with respect to pathogenic microorganisms like *Salmonella* and *Shigella* is of paramount importance to ensure consumer safety.

5. Different degree of resistance to all antibiotics tested was observed in all the samples. Multiple drug resistance was also among the pathogenic isolates. Thus, monitoring the level of resistance of these pathogens to antibiotics used for treatment of infection is critical.

6. In order to minimize the risk of infection by pathogenic and resistant microorganisms, the danger of using of raw sewage in the irrigation of the vegetable culture must be appreciated.

7. Regulatory agencies should be established to monitor the sanitary condition and quality of raw vegetables.

8. There is a need to educate producers, handlers and consumers about the importance of hygienic handling and appropriate storage systems of these products.

REFERENCES

Aberra Geyid, Frew Tekabe, Asmamaw Tigre, Mulu Girma and Sisaynesh Assefa (1991). A preliminary study of the microflora level of some fruits and vegetables: pre- and post-preservation. *Ethiop. J. Health Dev.* **5**: 57-65.

Ahsan, M. and Chowdhury, A.K. (1996). Garlic extract and allicin: broad spectrum antibacterial agents effective against multiple drug resistant strains of *Shigella dysenteriae* type 1 and *Shigella flexneri,* Enterotoxigenic *Escherichia coli* and *Vibrio cholerae. Phytother. Res.***10**: 329-331.

Andrew, J.M. (2001). Determination of minimum inhibitory concentration. *J. Antimicrobial. Chemother.* **48**: 5-16.

Aruscavage, D., Lee, K., Miller, S. and Lejeune, J.T. (2006). Interactions Affecting the Proliferation and Control of Human Pathogens on Edible Plants. *J. Food Sci.* **13:** 281-292.

Beuchat, L.R. (1996) Pathogenic microorganisms associated with fresh produce. *J. Food Prot.* **59**: 204–216.

Blackburn, C. W., Baylis, C.L., and Petitt, S.B. (1996**).** Evaluation of petrifilm methods for enumeration of aerobic flora and coliforms in a wide range of foods. *Lett. Appl. Microbiol.* **22**: 137-1 40.

Brandl, M.T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Ann. Rev. Phytopathol*. **44**:367-392.

Breuer, T., Benkel, D.H., Shapiro, R.L., Hall, W.N., Winnett, M.M., Linn, M.J., Timothy, J.N., Barrett, J., Dietrich, S., Downes, F.P., Toney, D.M., Pearson, J.L., Rolka, H., Slutsker. L., and Griffin, P.M. (2001). A multi-state outbreak of *Escherichia coli* O157:H7 infections linked to alfalfa sprouts grown from contaminated seeds. *Emer.Infect. Dis.* **7(6):** 977-982.

Castro-Rosas, J., & Escartin, E.F. (2000). Survival and growth of Vibrio cholerae O1, *Salmonella typhi*, and *Escherichia coli* O157:H7 in alfalfa sprouts. *J.Food Sci.* **65(1):** 162-165.

Cheung, P.Y., Kwok, K.K., and Kam, K.M. (2007). Application of BAX system, Tecra UniqueTM *Salmonella* test, and a conventional culture method for the detection of *Salmonella* in ready-to-eat and raw foods. *J. Appl. Microbiol*. **103**: 219–227.

Chaidez,C., Moreno,M., Rubio,W., Angulo, M., and Valdez, B.(2003). Comparison of the disinfection efficacy of chlorine-based products for inactivation of viral indicators and pathogenic bacteria in produce wash water. *Int. J. Environ. Health Res.* **13 (3):** 295-302.

Chang, J., and Fang, T.J. (2007). Survival of *Escherichia coli* 0157:H7 and *Salmonella enterica serovars Typhimurium* in iceberg lettuce and the antimicrobial effect of rice vinegar against *E.coli* 0157:H7. *Food Microbiol.* **24:**745-751.

Diaz, C., and Hotchkiss, J. H. (1996). Comparative growth of *Escherichia coli* 0157: H7, spoilage organisms and shelf-life of shredded iceberg lettuce stored under modified atmospheres. *J. Sci. Food Agric.* **70**:433-438.

Deza, M.A., Araujo, M., and Garnido, M.J. (2003). Inactivation of *Escherichia coli* 0157:H7, *Salmonella entritidis* and *Listeria monocytogenes* on the surface of tomatoes by neutral electrolysed water. *Let. Appl. Microbiol.* **37**:482-487.

Doyle, M.P. (1990). Fruit and vegetable safety-microbiological considerations. *Hortsci.* **25:** 1478–1481.

Echeverria, P., Piyaphong, S., Bodhidatta, L., Hoge, W.C., and Tungsen, C. (1994). Bacterial enteric pathogens in uncooked foods in Thai markets. *J. Travel Medicine.* **1:** 63–67.

Evans, M.R., Ribeiro, C.D., & Salmon, R.L. (2003). Hazards of healthy living: Bottled water and salad vegetables as risk factors for Campylobacter infection. *Emer. Infect. Dis.* **9(10):** 1219-1225.

FSA. (2000). Position Paper: Food Standards Agency view on organic foods, 23 August, 2000. www.foodstandards. gov.uk/pdf_®les/organicview.pdf.

Frost, J.A., McEvoy, M.B., Bentley, C.A., Andersson, Y., & Rowe, B. (1995). An outbreak of *Shigella sonnei* infection associated with consumption of iceberg. *Emer. Infect. Dis.* **1(1):** 26-28.

Geimba, M.P., Tondo, E.C., and Brandelli, A. (2005). Antimicrobial resistance in *Salmonella enteritidis* isolated from foods involved in human foodborne outbreaks that occurred in the south of Brazil. *J. Food Safety.* **25:** 173–182.

Gregerson, G. (1978). Rapid method for distinction of Gram-positive from Gram-negative bacteria. *European J. Appl. Microbiol*. **5:**123-127.

Gulelat Desse and Mulugeta Taye. (2001). Microbial load and microflora of cassava (*Manahilot esculenta* Crantz) and effect of cassava juice on some food borne pathogens. *J. Food Tech .in Africa*. **6**: 21-24.

Guo, X., Chen, J., Brackett, R.E., and Beuchat, L.R. (2001). Survival of Salmonellae on and in tomato plants from the time of inoculation at flowering and early stages of fruit development through fruit ripening. *Appl. Environ. Microbiol.* **67(10):** 4760-4764.

Hugh, M. and Leifson, E. (1953). The taxonomic significance of fermentative versus oxidative Gram-negative bacteria. *J. Bacteriol.* **66**: 24-26.

Ibenyassine,K., Mahand, R.A., Karakomo, Y., Anajjar, B., Chouibani, M., and Enanaji, M.M. (2007). Bacterial pathogens recovered from vegetables irrigated by wastewater in Morocco. *J. Environ. Heal.* **9**: 42-47

IFST. (1999). Organic food. *Int. Food Safety News*. **8:** 2-6.

Johnston, L.M., Jaykus, L., Moll, D., Anciso, J., Mora, B., and Moe C.L. (2006).A field study of the microbiological quality of fresh produce of domestic and Mexican origin. *Int. J. Food Microbiol*. **112**:83-95.

Jongen, W. (2005). Improving the safety of fresh fruit and vegetables. *Belgian J. Brewing Biotechnol.* **13:** 642-656.

Kelly, A. and Krol, R.G.(1987**)** Use of the direct epifluorescent filter technique for the enumeration of bacterial spores *J. Appl Bacteriol.* **63:** 545-550.

Keraita, B., Konradsen, F., Drechsel, P., and Abaidoo, R.C. (2007). Reducing microbial contamination on wastewater-irrigated lettuce by cessation of irrigation before harvesting. *J.Trop. Med. and Int.Heal*. **12**: 8–14.

Kim, D.H., Song , H.P., Yook, H.S., Ryu, Y.G., and Byun, M.W. (2004). Isolation of enteric pathogens in the fermentation process of Kimchi (Korean fermented vegetables) and its radicidation by gamma irradiation. *Food Cont.***15**: 441-445.

Kirov, S.M. (1993).The public health significance of *Aeromonas* spp. in foods. A review. *Int. J. Food Microbiol.* **20**: 179–198.

Kovacs, N. (1956). Identification of *Pseudomonas pyocyanae* by the oxidase reaction. *Nature*. **178**: 703.

Lee J.C., Oh, J.Y., Kim, K.S., Jeong, Y.W., Cho J.W., Park, J.C., Seol S.Y., and Cho, D.T. (2001). Antimicrobial resistance of *Shigella sonnei* in Korea during the last two decades. *APMIS*. **109**: 228–34.

Leclercq, A., Wanegue, C., Baylac, P. (2002). Comparison of Fecal Coliform Agar and Violet Red Bile Lactose Agar for fecal coliform enumeration in foods. *Appl. Environ.Microbiol*. **68**:1631-1638.

McMahon, M.A., and Wilson, I.G. (2001). The occurrence of enteric pathogens and *Aeromonas* species in organic vegetables. *Int. J. Food Microbiol.* **70:**155-162.

Niemira, B.A. (2003). Radiation sensitivity and recoverability of *Listeria monocytogenes* and *Salmonella* on four lettuce types. *J. Food Sci*. **68**: 2784-2787.

Nguyen-the, C. and Carlin, F. (1994). The microbiology of minimally processed fresh fruits and vegetables. *Crit. Rev. Food Sci. Nutr.* **34:** 370–401.

Nutt, J.D., Li, X., Woodward, C.L., Zabala-Diaz, I.B., and Ricke, S.C. (2003). Growth kinetics response of a *Salmonella Typhimurium* poultry marker strain to fresh produce extracts. *Bioresource Technol*. **89**:313-316.

Onyemeluk, N.F. and Njoku-Obi, A.N. (1992). Shigellosis in Eastern Nigeria: Etiological frequency of serotypes and resistance to antibiotics. *Lett. Appl. Microbiol.* **14**: 266-270.

Parish, M.E., Beuchat, L.R., Suslow, T.V., Harris, L.J., Garrett, E.H., Farber, J.N., and Busta, F.F. (2003). Methods to Reduce/Eliminate Pathogens from Fresh and Fresh-Cut Produce. *Comp. Rev. Food Sci.Food safety.* **2**: 161-173.

Ponce, A.G., Aguero, M.V., Roura, S.I., Valle, C.E., and Moreira, R. (2008).Dynamics of indigenous microbial populations of butter head lettuce grown in mulch and on bare soil *J. Food Sci*. **00**: 1-7.

Raiden, R.M., Sumner, S.S., Eifert, J.D., and Pierson, M.D. (2003). Efficacy of detergents in removing *Salmonella* and *Shigella spp*. from the surface of fresh produce. *J. Food. Prot*. **66(12)**: 2210-15.

Rajkowski, K T., and Fan, X. (2008). Microbial quality of fresh-cut iceberg lettuce washed in warm or cold water and irradiated in a modified atmosphere package. *J. Food Safety*. **28:** 248–260.

Ruiz, B.G., Espiner, A.C. and Bolanos, M.S. (1987). Acomparative study of strains Of Salmonella isolated form irrigation waters, vegetables and human infections. *Epidem. Inf.* **98** : 271-276.

Sanglay, G.C., Eifert, J.D., and Sumner, S.S. (2004). Recovery of Salmonella spp. from raw produce surfaces using ultrasonification. *Foodborne Pathog. Dis.* **1(4)**: 295-9.

Santamariam, J., and Toranzos, A.G. (2004). Enteric pathogens and soil: a short review. *J. Int. Microbiol.* **6:** 5-6**.**

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J. and Hightower, A.W. (1983). Epidemic listeriosis—evidence for transmission by food. England. *J. Food Prot.* **52:** 203–206.

Schroeter, A., Hoog, B. and Helmuth, R. (2004). Resistance of *Salmonella* isolates in Germany. *J. Vet. Med.* **51**:389–392.

Shearer, A.E., Strapp, C.M., & Joerger, R.D. (2001). Evaluation of polymerase chain reaction-based system for detection of *Salmonella enteritidis*, *Escherichia coli* O157:H7, *Listeria* spp., and *Listeria monocytogenes* on fresh fruit and vegetables. *J. Food Prot.* **64(6):** 788-795.

Soil Association (1999). The Organic Food and Farming Report. Bristol: Soil Association.

Soriano, J.M., Rico, H., Molto, J.C., and Manes, J. (2000). Assessment of the microbiological quality and wash treatments of lettuce served in University restaurants. *Int. J. Food Microbiol*. **58**:123–12.

Steele, M., and Odumeru, J. (2004). Irrigation water as source of foodborne pathogens on fruit and vegetables. *J. Food Protection*. **67 (12)**: 2839-2849.

Takeuchi, K., Hassan, A.N., & Frank, J.F. (2001). Penetration of *Escherichia coli* O157:H7 into lettuce as influenced by modified atmosphere and temperature. *J. Food Prot.* **64(11):** 1820-1823.

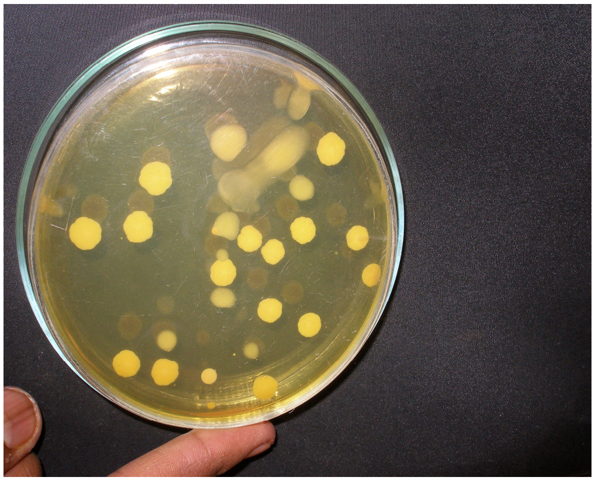
Tatini, S.R., Wasela, W.D., Jezeski, J.J. and Morris, H.A. (1970). Production of staphylococcal enterotoxin A in blue, break, Mozerrella and Swiss cheeses. *J. Dairy Science*. **56**: 429-435.

Wright, C., Kominos, S.D., & Yee, R.B. (1976). Enterobacteriaceae and *Pseudomonas aeruginosa* recovered from vegetable salads. *Appl. Environ. Microbiol.* **31(3):**453-454.

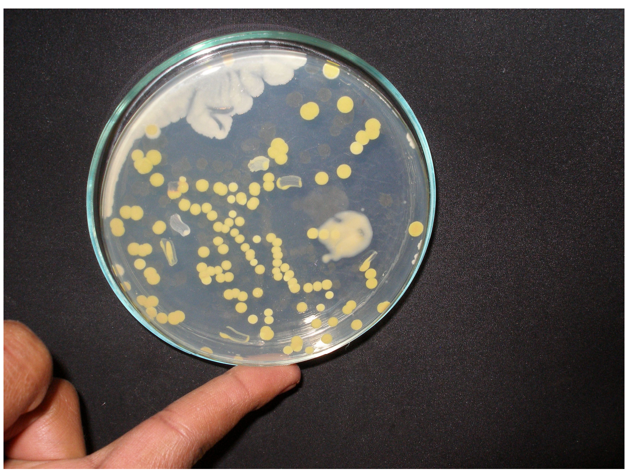
Yoke-Kqueen, C., Learn-Han, L., Noorzaleha, A.S., R. Son, A.S., Sabrina1, S., Jiun-Horng, S., and Chai-Hoon, K. (2008). Characterization of multiple-antimicrobial-resistant *Salmonella enterica* Subsp. *enterica* isolated from indigenous vegetables and poultry in Malaysia. *Let. in Appl. Microbiol*. **46**: 318–324.

Yuk, H.G., Bartz, J.A., and Schneider, K.R. (2006). The Effectiveness of sanitizer treatments in inactivation of *Salmonella* spp. from bell pepper, cucumber, and strawberry. *J. Food Sci.* **71**: 95-97.

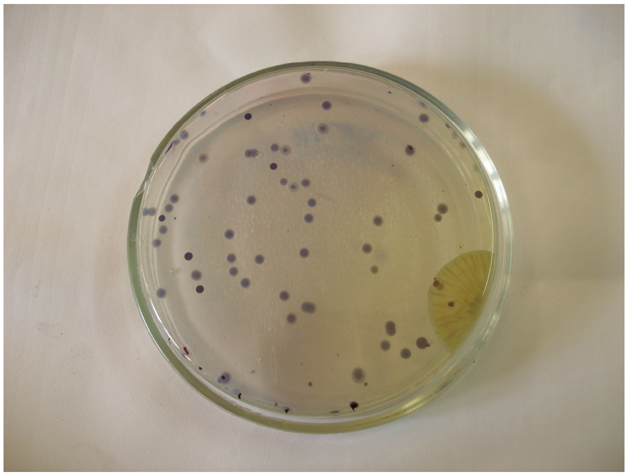
APPENDICES



Appendix 1: Staphylococcus count.

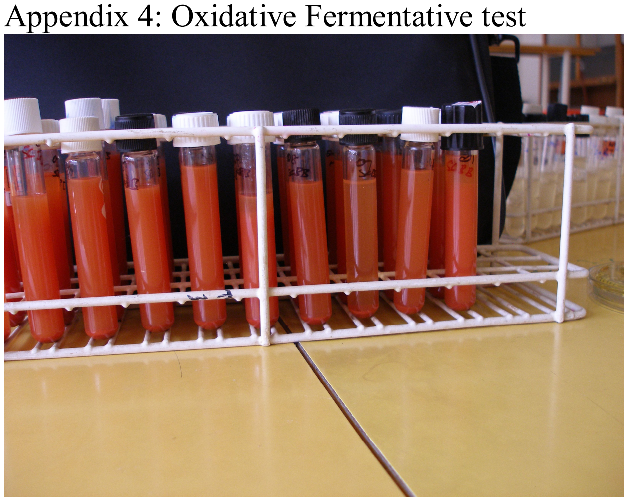


Appendix 2: Aerobic mesophilic count

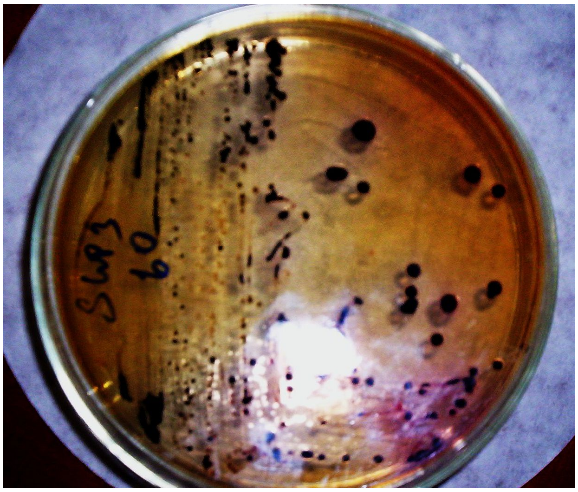


Appendix 3: Yeast and mold count

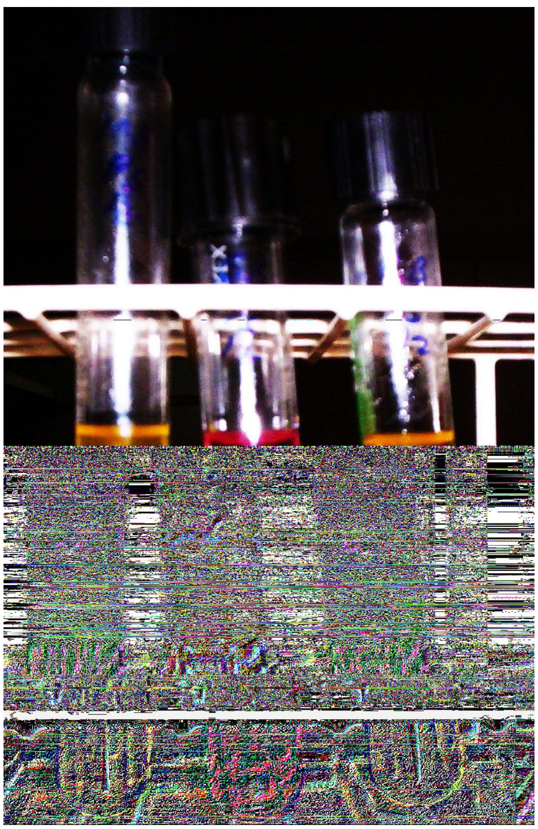
Appendix 4: Oxidative Fermentative test



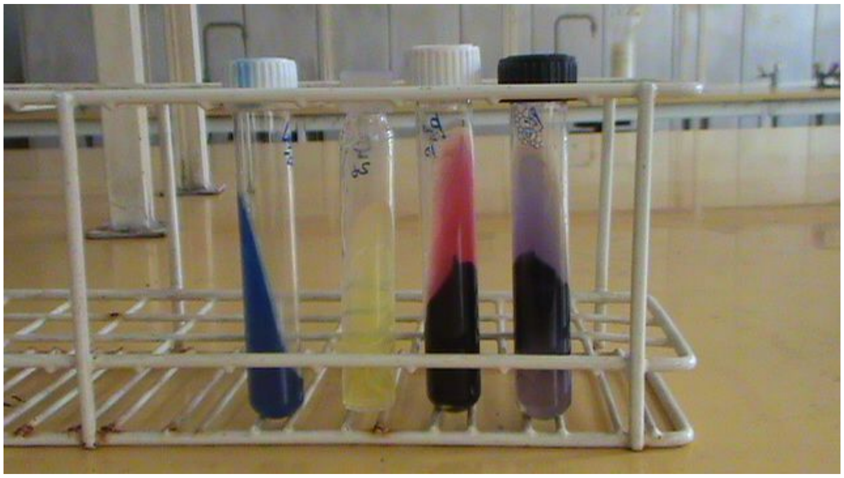
Appendix 5: Secondary enrichment test



Appendix 6: *Salmonella* spp. isolated from lettuce on XLD Agar.

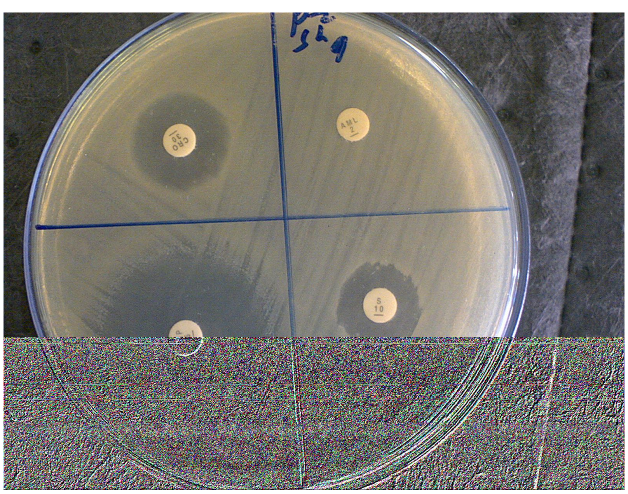


Appendix 7: Test for fermentation of carbohydrates.



Appendix 8: Biochemical tests for identification of *Salmonella* spp.

Appendix 9: Triple sugar iron agar tests for identification of *Salmonella* spp.



Appendix 10: Drug susceptibility testing.