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Bacteriological quality of lettuce and tomato associated with consumer's hygienic practice in Lafto vegetable and fruit market, Addis Ababa city, Ethiopia

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This is to certify that the thesis prepared by Tofik Kedir, entitled: Bacteriological quality of lettuce and tomato associated with consumer's hygienic practice in Lafto vegetable and fruit market, Addis Ababa city, Ethiopia and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of Tables

List of Abbreviations

ACC Aerobic colony count

AFSSA French Agency for Food Safety

AMB Aerobic mesohpilic bacteria

APC Aerobic Plate Count

APHA American Public Health Association

BGLB Brilliant green lactose bile

CCFH Codex Committee on Food Hygiene

CFU/g Colony forming unit per g

E. coli *Escherichia coli*

EFSA European Food Safety Authority

FAOFood and Agricultural Organization

FC Fecal coliform

HOClhypochlorous acid

HPA Health protection agency

ICMSF International commission standard for food

LogCFU/g Logarithm of colony forming unit per gram of sample

MPN Most probable number

NMKL Nordic committee on food analysis

PPMParts per million

RSVraw salad vegetables

S. aureus *Staphylococcus aureus*

SD standard deviation

Spp. Specious

TCTotal coliform

TVCTotal viable count

WHOWorld Health Organization

Abstract

Background:-In the health conscious society of 21st century, vegetables become fundamental portion of human diet. They provide us essential vitamins, dietary fiber,minerals, antioxidants and vitamin C. However, due to its high water solubility and heat sensitivity, the vitamin is vulnerable to loss during thermal processing. During production, collection, transportation and preparation the vegetables usually

contaminated with pathogens from human or animal origin. Particularly vegetables requiring minimal or no further processing before consumption are one of the main sources of food born pathogen.

Objective:-This study was conducted to determine the bacteriological quality of lettuce and tomato associated with consumer's hygienic practice in lafto vegetable and fruit market in Addis Ababa city, Ethiopia.

Methods:-A cross-sectional study was conducted from January 2020 to August 2021 in Lafto vegetable and fruit market in Addis Ababa city, Ethiopia. A total of 60 samples of vegetables; 30 samples of lettuce and 30 samples of tomato were purchased, packed in zipper bag and transported to Ethiopian Public Health laboratory with the aid of sterilized dish in the ice box.The samples were appropriately diluted, analyzed and calculated for their microbial loads, mean, frequency, percentage and compared with international standards.Drug susceptibility test was also done for*Staphylococcus aureus and*Salmonellaspecies.

Results:-Sixty eight percent of total samples had aerobic colony count ≥ 5Log CFU/g; while 57% of samples had total coliform ≥ 4Log CFU/g and 28% of samples had fecal coliform ≥2Log CFU/g which were above permissible level for consumption without further processing.*E. coli*,*S.aureus* and *Salmonella*were isolated from 11%, 16.7% and 5% of total the sample respectively. All *S.aures*isolets were sensitive to gentamicin and 80% isolates were sensitive to Tetracycline/Chloramphenicol/Erythromycin whereas Ampicillin/Penicillin/Cefoxittine drugs were 100% resistance to*S.aureus*. *Salmonella* species isolates were 100% sensitive to Gentamicin and Co-trimoxazole and 100% resistance to Ampicillin/Tetracycline/Erythromycin.

Conclusion:-The present study clearly shows that the bacteriological quality of lettuce and tomato sold in the market had high level contamination of aerobic colony count, coliforms and *Staphylococcus aureus* species. Therefore vegetables need to be washed and disinfected before consumption.

Key words:-coliforms, colony count, bacteriological quality, lettuce and tomato

1- Introduction

1.1- Back ground

In the health conscious society of 21st century, vegetables become fundamental part of human diet. They provide us essential vitamins, minerals, dietary fiber and antioxidants (1).

World Health Organization and European Food Safety Authority recommended the intake of at least five servings of vegetables and fruits per day (2). However during production, collection, transportation and preparation the vegetables usually contaminated with pathogens from human or animal origin (3). Food borne pathogens are causing a great number of diseases with significant effects on human health and economy(4). Food borne diseases can be classified in to two major categories depending on the responsible agent: food-borne infections and food-borne poisonings/intoxications (5). Food borne infections are caused by the entrance of pathogenic microorganisms contaminating food into the body (6).*Salmonella, Campylobacter, E. coli O157* are commonbacteria that cause food borne infections(7).Food borne intoxication is caused by consumption of food containing toxins such as Staphylococcal intoxication, emetic poisoning of *Bacillus cereus*, botulism, toxigenic molds, poisonous mushrooms, and biogenic amines(8).particularly Vegetables that require little or no further processing before consumption have been linked to the agent for transmission of infectious microorganisms (9). Fresh vegetables have been recorded in a number of outbreaks of foodborne disease caused by bacteria. The number of individual bacteria that must be present to cause actual human disease varies with the type of organism as well as the age and condition of the host. Bacteria reproduce easily and quickly if the environmental conditions meet their specific requirement (3, 10).Chlorine in the form of hypochlorous acid (HOCl) and chlorine concentration of 100 ppm is major sanitizing agent used for disinfection of vegetables. However, the use of chlorine does not ensure elimination or even an efficient reduction in pathogen levels (3).

1.2- Statements of the problem

The 2007 WHO/FAO Expert Meeting agreed to a set of six criteria, which should be used to rank the commodities of concern as identified by the Codex Committee on Food Hygiene. The

meeting concluded that leafy vegetables currently presented the greatest concern in terms of microbiological hazards (11).

Currently there has been an increase in the number of outbreaks of illness associated with the consumption of vegetables and fruits (12).

The world's largest reported vegetable borne outbreak to date occurred in Japan in 1996 and of the over 11,000 people affected. The outbreak involved the death of three school children and was caused by *E. coliO157:H7* (1).The young, the old, the immune compromised and the pregnant consumers have potentially a higher risk of bacterial, viral or protozoan infection than other groups (9).

In Ethiopia the risk associated with exposure to outbreak of food-borne disease like contaminated vegetable is primarily due to lack of knowledge,attitude and practiceabout personal and environmental sanitation,particularly during the wetseason (9).

Lettuce and tomato are sold under low personal and environmental hygienic conditions in the market and roadsides. They are also commonly consumed in raw, without further processing in vegetable and fruit juice houses and in households in most parts of Ethiopia; these can certainly increase the chance of pathogenic microbial contaminations. As a result several outbreaks have been occurred in Addis Ababa city which were received broad media coverage, raising concerns about the potential safety of vegetables (12).

In Ethiopia there is also no continuous survey/assessment of food quality and safety in the market where fresh vegetables and fruits are sold. Although the potential safety risk of fresh vegetables has been increased year to year only limited researches are done in this area. Thus, this study was conducted to determine the bacteriological quality of lettuce and tomato associated with consumer hygienic practice in Lafto vegetable and fruit market, Addis Ababa city, Ethiopia.

1.3- Significance of the study

This study will primarily help for consumer to have better understanding and awareness about bacteriological quality of vegetables and fruits.The findings of this study can also be used by government regulatory authorities to develop disease prevention initiatives. Furthermore, the findings will serve as initial data for future research on the bacteriological quality of lettuce and tomato in Addis Ababa city.

2- Literature review

A research was done in Dhanbad city, India, by Mritunjay and Kumar in may 2017 in microbiological quality of raw salad vegetables consumed.All samples in this study were contaminated by total coliforms.*E. coli* and *salmonella* were isolated in 16.7% and 4% of the total samples respectively (13).

Another similar study was done in Sao Paulo, Brazil by Froeder, Martins, Landgraf, Franco, and Destro in May 2007 in microbial quality of minimally processed vegetables. Seventy three percent of samples had fecal coliform count higher than 10^2cfu/g and Salmonella was detected in only 3% of the samples (14).

Bohaychuk, Bradbury, Dimocketal.had studied a microbiological survey of fresh produce from farmers' markets in Alberta, Canada Feb 2009. According to this study 8.2% of the totalsamples that included spinach, carrots, green onions and lettuce were contaminated by*E. coli*butnot detected from any of tomatoes sample.*Salmonella* was also not detected from any of the total samples (15).

Tango, Khan, Wei and Hussain evaluated the microbiological quality and safety of fresh vegetables and fruits at retail level in Korea in January 2018. Aerobic plate count and total coliform were ranged from 1.7 to 10.6 log cfu/g and 2.2 to 7.9 log cfu/g respectively. *Staphylococcus aureus* were isolated in 1.4%of fresh samples. Three lettuce samples were contaminated by *Escherichia coli* bacteria with a bacterial load ranging from 2 to 4 log cfu/g and *Salmonella* spp. were not isolated in any of these fresh products (16).

Another study was also undertaken to assess microbial quality of fresh vegetables irrigated with polluted waters from Msimbazi River in Dares Salaam City Tanzania in 2018 Aug by Kayomboand Mayo. Vegetables from all the markets including super market were highly contaminated with fecal coliforms of up to 10^8cfu/kg (17).

Bacteriological quality of vegetables sold in Sango Ota, Nigeria was assessed by Obaigeli, Eni, Oluwawemitan and Solomon in May 2010. *Staphylococcusaureus*, *Salmonella spp*.and *Escherichia coli* were isolated in29.2%, 12.5% and 4.2% of total samples respectively (18).

In Ghana Microbial quality of ready-to-eat vegetable salads vended in the central market area of Tamale was assessed by Abakari, Cobbina and Yeleliere in 2018. *Escherichia coli*, *Salmonella*spp. and *Shigella* spp. were isolated in 96.7% ,73.3% and 76.7% of vegetable salads, respectively (19).

In Ethiopia, Addis Ababa city also a similar study was conducted by Ketema F in January 2017 to evaluate the bacteriological quality of locally prepared vegetable salad. Vegetable salad samples had a bacterial load of 6.06 log cfu/g, which was greater than the International Commission of Microbial Standards for Food (2).

Ogeneh,Oyarekua and Edeh had studied bacteriological count of commonly consumed foods and vegetables from food vendors in a market in Enugu, Nigeria.*Staphylococcusaureus*, *Salmonella* spp, *Escherichia coli* and *Shigella* spp. were isolated from the food samples (20).

Woldetsadik ,Drechsel , Keraita , Itanna , Erko and Gebrekidan had studied Microbiological quality of lettuce irrigated with wastewater in Addis Ababa, Ethiopia. The mean fecal coliform count of lettuce was ranged from 3.46-5.03 log10MPN per 100 g (37).

Another study was done to evaluate the bacteriological contamination of fresh vegetables farms aroundAwetuRiver in Jimma town by Weldezgina and Muleta in 2016. *Staphylococcusaureus*24.0% while *Salmonella* spp. 20.7% were isolated from the total samples (21).

Another research was also done by Tesfay and his colleagues on Microbial Quality of Fresh Lettuce Irrigated with Untreated Waste Water around Aksum University, Central Zone of Tigrai Region.Coliforms from lettuce sample were detected ranging from 3.207×10^{3} - 4.063×10^{5}cfu/ml and *salmonella spp*. was isolated in 100% of the lettuce samples (22).

In Similar Study, December 2018 by Temesgen and Kibret, bacteriological contamination of tomato irrigation farm along Abay river of Bahir Dar town, Ethiopia, the mean aerobic platecounts was4.72 cfu/g(23).

3- Objectives

3.1- General objective:-

To assess Bacteriological quality of lettuce and tomato associated with consumer's hygienic practice in Lafto vegetable and fruit market, Addis Ababa city, Ethiopia

3.2- Specific Objectives:-

- To assess the personal hygiene and sanitary practicing habits consumers during preparation of these products.
- To determine the aerobic plate count and fecal coli forms status of lettuce and tomato.

- To screen the existence of *salmonella* and *S. aurous* from lettuce and tomato.
- To determine Antimicrobial Susceptibility Patterns of Salmonella and S. aurous Isolates

4- Materials and Method

4.1- Study Design

The design of the study was cross sectional study withsystematic random sampling technique to evaluate the bacteriological quality of lettuce and tomato sold in the market.

4.2- Description of the study site and Period

The study was conducted in Addis Ababa city;Lafto Vegetable & Fruit Market from January 2020 _ August 2021. Addis Ababa, founded in 1887 G.C, is the largest and the capital city of Ethiopia, lies on the central plateau at an altitude of 2,400 meters above sea level. Its relative location is found at 9° N latitude &38° E longitude demarcation.The city is home to 25% of the urban population in Ethiopia and is one of the fastest growing cities in Africa but providing clean water to only 44% of the population and sewerage services to less than 30%.Following the Coronavirus (COVID-19) pandemics, AtikiltTera, a former fruit and vegetable market in Piassa, temporarily shifted to Jan Meda's fields and stayed there for more than five months. Eventually, it was moved toLafto Vegetable & Fruit Market, southwestern Addis Ababa. The market contains 980 compartmentalized shops accommodating 516 small-scale vendors, 46 distributors and 24 farmers.

4.4- Source of Sample; All vegetable and fruit shops in Lafto vegetable and fruit market

4.5- Study sample;from 315 vegetable and fruit retailers60 vegetable and fruit selling shops were selectedby using systematic random sampling technique

4.6- Sample Size andSampling

By using systematic random sampling technique from 315 vegetable and fruit retailersa total of 60 samples; 30 samples of lettuce and 30 samples of tomato were selected, purchased, packed with zipper bag and transported to the laboratory with the aid of sterilized dish in the ice box at different marketing day during study period. A total of one hundred twenty (120) questioners for consumers were also distributed for the assessment of hygienic and treating habits of raw vegetables.

4.7- Inclusion and exclusion criteria;From the samples, fresh vegetables were included in the study whereas physically damaged and spoiled vegetables were excluded from the study

4.8- Variables

A. Dependent Variable

- Bacteriological quality of lettuce and tomato

B. Independent Variable

- Storage environment of vegetables and fruits
- The quality of water used for washing vegetables
- Personal hygiene of vegetable consumers

4.9- Data Collection

4.9.1- Structured Questionnaire

Structured questionnaire determining the factors related to hygienic and treatment habits of consumers before consumption of raw vegetables were distributed to consumers. Awareness about microbial contamination and its health risks, Types of disinfectant, storage condition, type of water they used for washing fresh vegetables by consumers was assessed.

4.9.2- Laboratory-based experiment

Thirty samples of lettuce and thirty samples of tomato werepurchased, packed with zipper bag and transported to the Ethiopian public health food microbiology laboratory with the aid of sterilized dish in the ice box at different marketing day for analysis, isolation, and identification of microorganisms.

4.9.3- Sample Preparation for Bacteriological Analysis;from each collected sample 25g was aseptically weighted and homogenized for 3 minutes in 225 ml of sterile 0.1 percent (w/v) buffered peptone water.Using 9ml sterile saline solution blank as diluents and by transferring one ml of dilution from homogenized sample, 10^{-2} to 10^{-5}ten-fold serial dilutions were prepared.The homogenate was used to count, isolate, and characterize bacteria groupsfrom the samples (24).

4.9.4- Aerobic Plate Count (APC); in adequately leveled Petri dishes withplate count agar, one ml of homogenized serial diluted sample from 10^{-2} to 10^{-5}were pour plated .The plates were allowed to solidify and incubated at 30°C for 72 hours. Following incubation the Petri dish containing 30 to 300colonies was selected, counted withcolony counter, calculated for dilution factor and express as colony forming units per gram (25).

4.9.5-Enumeration of total coli forms;Approximately 5ml oftryptone soya agar previouslycooledto 45.0±1.0°C was added to appropriately labeled petri dishes and Pre-

incubatedfor 1-2 hoursat 20 – 25 °C (room temperature)thencovered with 10 - 15 ml of violet red bile agar at a temperatureof45.0±1.0°C.One ml aliquots from each dilution (10^{-2},10^{-3}, 10^{-4}, 10^{-5}) was aseptically transferred to each Petri dishes and Incubated at 37°C for 24 ± 3 hours in an inverted position. Typicalcolonieswerecounted and selectedcolonieswere confirmed by testing for production of gas in Brilliant Green Lactose Bile broth. A loop full of inoculum from all presumptive-positive violet red bile agar petri dish was Inoculated into tubes with 5ml Brilliant Green Lactose Bile brothwith inverted durham tubes and incubated at 37°C for 24 hours. A Brilliant Green Lactose Bile broth tube was observed for gas formation in the durham tubes. All positive BGLB broth tubes were considered positive for coliform confirmation (26).

4.9.6- Fecal coliforms: Approximately 5 ml of tryptone soya agar previously cooled to 45.0 ± 1.0 °C was added to appropriately labeled petri dishes and Pre-incubated for 1-2 hours at 20 – 25 °C (room temperature) then covered with 10 - 15 ml of violet red bile agar at a temperature of 45.0 ± 1.0 °C. One ml aliquots from each dilution (10-2,10-3, 10-4 , 10-5) was aseptically transferred to each Petri dishes and Incubated at 44.5°C for 24 hours and colony counted by using colony counter. Then, confirmatory test for fecal coliforms was done by taking 5 colonies from each presumptive-positive violet red bile agar petri-dish and inoculated into tubeswhich contained 5ml of EC broth with invertedDurham tubes and incubated at 44.5°C for 24hrs. Confirmation was obtained by gas production and the result was reported as the most probable number (MPN) per gram of samples (26).

4.9.7- *Escherichia coli* spp.;The procedure was continued from each positive E.C broth used during fecal coliforms detection. A loop full of inoculums was streaked on Eosin Methylene Blue (EMB) agar by using a sterilized loop and incubated at 44°C for 24 hours. Due to the fermentation of lactose, Escherichia coli colonies were distinguished by their typical green metallic shine. Potential positive plates were streaked on nutrient broth and incubated at 44°C for 24hrs, then0.3mlofkovac'sreagentwasadded and checked for dark red color at top of test tube (25, 36).

4.9.8- *S. aureus*; appropriate dilution of 0.1 mL aliquot of was spread-plated in duplicate on pre solidified plates of Mannitol Salt Agar and incubated at 37°C for 24 hrs. Yellow colonies on Mannitol Salt Agar plates were counted, calculated and picked aseptically and transferred to 5 mL nutrient broth for further purification and incubated at 37°C for 24–36 hours. Then, to obtain

distinct colonies, a loop of culture from the nutrient broth was streaked on the pre-solidified surface of nutrient agar and incubated at 37°C for 24–36 hours. Finally, the distinct colonies werecharacterized by using gram staining microbiological method. Gram positive cocci with clustered arrangement under the microscope were subjected to catalase, coagulase and hemolysis tests for confirmation (24, 36 and 27).

4.9.9- *Salmonella* spp.;In the vegetable samples, *Salmonella* spp. may be present in low numbers and may be injured. pre-enrichment (buffered peptone water) was used and incubated at 37°C for 24 hours for pre-enrichmentto diminish the risk of obtaining false negative results.From each pre-enriched samples, one ml transferred in to tubes containing 10 ml of RappaportVassiliadisbroth and thoroughly mixed for two minutes. Following mixing up, tubes were incubated at 41.5□C for 48 hours. A loop full of culture from the Rappaport Vassiliadis broth streaked onto Xylose Lysine Deoxycholate (XLD) agar and incubated at 37□C for 24 hrs for Salmonella.

Typical colonies which had slightly transparent zone of reddish color and a black center, a pink-red zone surrounding the colonies, were considered as presumptive *Salmonella*. Presumptive colonies from XLD agar were picked aseptically, streaked onto nutrient agar for purification, and incubated at 37°C for 24 hours. Pure colonies were transferred aseptically into TSB slants as stock cultures and stored at 4-5°C in the refrigerator. The pure cultures then subjected to biochemical tests like Citrate utilization test, Motility test, Lactose fermentation, H2S gas production test, Lysine Iron agar test and Urea hydrolysis test (27,36).

4.9.10- Antimicrobial Susceptibility Testing for Salmonellaspp. and S. aureus; antimicrobial susceptibility testing for*Salmonella* spp. and *S. aureus*was performed on Mueller Hinton Agar (Oxoid) plates following the standardized disk diffusion techniques as described by Clinical Laboratory Standard Institute (25).To determine the drug susceptibility pattern of these isolates the following ten commonly prescribed drugs were used: ampicillin (10 g), gentamycin (10 g), chloramphenicol (30 g), tetracycline (30 g), erythromycin (15 g), co-trimoxazole (25 g), Cefoxittine (30 g), ciproflaxin (5 g), and penicillin (10 g).Staphylococcus aureus (ATCC25923) and Escherichia coli (ATCC 25922) were used as reference strains for quality control of the antibiotics used (27).

4.10- Data quality assurance

In order to assure its quality, data was collected by two microbiologists who have basic knowledge on the public health microbiology.Zipper bag, sterile container and ice pack used for transportation of samples and the samples were processed and analyzed immediately as much as possible.

4.11- Statistical Analysis

The data was entered and analyzed using statistical Package for SPSS Version 23. Microbial loads, mean, frequency and percentage were analyzed and compared with international standards

4.12- Ethical consideration

Ethical clearance was obtained from Addis Ababa University,College of Health Science, Department of Medical Laboratory Sciences, ethical review Committee prior to the study. Then a letter informing about the study was written to EPHI food microbiology department from ethical Committee.

4.13- Operational definitions

Coliform: Coliforms are Gram-negative, rod-shaped facultative anaerobic bacteria (28)

Contamination: Contamination is undesired introduction of microbial into food so that it does not meet acceptable food hygiene standards (29).

Fecal coliform: are a sub-group of total coliform bacteria (that portion of the coliform group which produce gas from lactose in a multiple tube procedure liquid medium within 24 hours in a water bath maintained at 44.5 °C) that are present in large quantities in the intestines and feces of people and animals (30).

Food quality: Food quality is the totality of features and characteristics of a product that determines its value or acceptability to consumers (31).

Food safety: Food safety Assurance that food if free of any substance that maythreatenpersonal health and will not cause harm to the consumer when it is prepared and/or consumed according to its intended use (31).

Indicator organisms: are microorganisms which may not be pathogenic but are used to evaluate the presence of pathogenic microorganisms (32).

Pathogens: Organisms that cause disease (33).

Total coli form counts (TCC): Group (comprising all aerobic and facultative anaerobic, gram-negative, non-spore-forming, rod-shaped bacteria that ferment lactose with gas formation within 48 hours at 35°C) (34).

5- Results

5.1 Demographic and Educational status of respondents;from a total 0f 120 vegetable consumers interviewed 83 (69.2 %) were females and 37 (30.8 %) were males. Majority, 69(57.5 %) of the respondents had elementary educational status and 45(37.5%) were High school and above. The rest 6(5%) were illiterate.

5.2 Personal hygiene and vegetable treatment habits of respondents; Cleaning habits of consumers' during preparation of vegetable salad was 61.7%and only 43.3% of consumers used cleaning agent with water. Only48.3% of the respondents had cleaning habits of hand after using toilet.

Table- 1; Hygiene and Handling practice of vegetable consumers in lafto vegetable and fruit market, Addis Ababa city, Ethiopia, 2021

Hygiene and Handling practice		**Frequency**	**Percent**
Temporary storage site	Refrigerator	58	48.3%
	Shelf	62	51.7%
Water source for vegetable salad preparation	Tape water	119	99.2%
	Well	1	0.8%
Cleaning habit of the consumer during processing	Yes	74	61.7%
	No	46	38.3%

Cleaning agent used during processing	With water only With water and disinfecting agent	42 32	56.75% 43.25%
Frequency of cleaning	Once Twice Three and more	31 25 18	42% 33.8% 24.2%
Cleaning habits of hand after using toilet	Yes No	58 62	48.3% 51.7%
Cleaning of hand after using toilet with	Water only water and soap	29 29	50% 50%

5.3 Mean microbiological counts;All samples had countable number of aerobic colony count with mean of 6.90log CFU/ g for lettuce samples and 4.352log CFU/ g for tomatosamples. Mean total colony count was 4.80log CFU/ g for lettuce samples and 2.10log CFU/ g for tomato samples

Table- 2;Mean microbiological counts (log CFU/ g) of selected samples purchased from lafto vegetable and fruit market, Addis Ababa city, Ethiopia, 2021

Bacteriological Count	**Lettuce**			**Tomato**		
	Minimum	Maximum	Mean ±SD	Minimum	Maximum	Mean ±SD
ACC	5.47	8.12	6.90 ± 0.50	2.90	5.95	4.35 ± 0.73
TC	<1	6.74	4.80 ± 1.1	<1	4.78	2.10 ± 1.40
FC	<1	4.50	2.27 ± 1.50	<1	3.50	1.2 ± 0.58

ACC, aerobic colony count; TC, total coliform; FC, fecal coliform; S.D, standard deviation

5.4 Mean *Staphylococcal, Escherichia colispp*. and*Salmonella* counts; seven samples of lettuce and three samples of tomato had countable number of *Staphylococcus aureus*. Out of 60 samples of lettuce and tomato *Salmonella* was detected in 3(10%) of lettuce samples

Table 3; Mean*Staphylococcal* and***Escherichia coli spp.***counts (log CFU/ g) of selected samples purchased from lafto vegetable and fruit market, Addis Ababa, Ethiopia, 2021

	Sample with Countable number of colony (n)	Minimum	Maximum	Mean
Staphylococcus aures in lettuce	7	3.74	6.07	4.7±0.96
Staphylococcus aures in tomato	3	4.30	4.66	4.5±0.18
Total	10	3.74	6.07	4.6±0.80
Escherichia coli spp *in lettuce*	5	2.3	3.30	2.90±0.47
Escherichia coli spp in tomato	1			2.40
Total	6	2.30	3.30	2.83±0.47

Percentage of bacteriological contaminated samples;alllettuce and 36.7% of tomato samples had aerobic colony count greater than 10^5cfu/g of sample. ninety percent of lettuce and 23% of tomato had total coliforms greater than 10^4/g of samples.

Table 4; Percentage of bacteriological contaminated samples (ICMSF reference standard used)

Types of vegetable Samples tested	Total number of samples tested	Aerobic colony count $>10^5$	Total Coliform $>10^4$	Fecal Coliform $>10^2$
Lettuce	30	30 (100%)	27 (90%)	14 (46.7%)
Tomato	30	11 (36.7%)	7 (23%)	3 (10%)
Total	60	68%	56.7%	28.33%

Antibiotic susceptibility pattern; all *Salmonella spp.* and*S. aureus* and isolates were sensitive to gentamycin and all were resistance to ampicillin

Table 5: Antibiotic susceptibility pattern of *Salmonella* spp. and *S. aureus*isolated from vegetable samples.

Antimicrobial agent	***S. aurous* (n = 10)**				***Salmonella spp*. (n = 3)**			
Disc content (μg)	S	%	R	%	S	%R	%	
Ampicillin(10)	0	0	10	100	0	0	3	100
Tetracycline(30)	8	80	2	200	0	3	100	
Penicillin (10)	0	0	10	100	0	0	3	100
Chloramphenicol (30)	8	80	2	20	2	66.7	1	33.3
Ciprofloxacin (5)	4	40	1	10	2	66.7	1	33.3
Gentamycin (10)	10	100	0	0	3	100	0	0
Cefoxittine (30)	0	0	10	100	2	66.7	1	33.3
Co-trimoxazole(25)	2	20	8	80	3	100	0	0
Erythromycin (15)	8	80	2	20	0	0	3	100

Where S stands for sensitive and R for resistance

6:- Discussion

Tomato and lettuce are vegetables which can be eaten raw as vegetable salads. However if they are not carefully prepared they become hazardous for human health. From 120 respondents on temporary storage site of vegetables 62(51.2%) uses shelf instead of refrigerator, which may contribute to contamination and growth of hazardous microorganisms. Also significant number(38.3%) of respondents have no cleaning habits of vegetables during processing where the lettuce samples of all urban farming site of Addis Ababa had high fecal coliforms exceeding the recommended threshold (35).

From those who had habits of cleaning vegetables before consumption 56.8% uses water only and the rest 43.2% uses water with disinfecting agent. Majority (56.6%) of the respondents clean

vegetables with water only which cannot minimize microbes in satisfactory level. Using water with Proper cleaning agent or sanitizer can reduce microbes in significant level. Frequency of cleaning is also one of important factor in minimizations of microbes especially for vegetables which can be consumed in raw.

Cleaning habits of hand after using toilet is also very important factor in reduction of fecal oral transmission of microbes. Unfortunately more than half (51.7%) of the respondent had not habits of washing hand after using toilet and also from those who have hand washing habit only half of them uses water with soap.

Bacteriological count of vegetables; the bacteriological quality of samples in this study was different for lettuce and tomato. In all types of bacteriological count lettuce samples had higher count than tomato samples. This may be due to lettuce has broad and rough surface area which could be easily contaminated by bacteria compared to tomato which has narrow and smooth exposed outer surface.

Aerobic colony count for Lettuce samples in this study was ranged from 5.5Log cfu/g to 8.0Log cfu/g with amean of 6.90Log cfu/g.According to health protection agency, for raw, ready to eat food commodities such as vegetablesalad, ACC are likely to be much higher, between 6Logcfu/g and 8Log cfu/g. Except one sample the rest 96.6% of lettuce samples were included in the range of 6Log cfu/g to 8Log cfu/g which could be easily spoiled if not properly washed under storage. All lettuce samples in this study had aerobic colony count ≥5Log cfu/g which is above permissible level for consumption without further processing (39).

This indicates that all the lettuce samples were highly contaminated by bacteria. The result of this study is comparable with previous study done by weldezina and Muleta, 2008 where ACC was ranged from 5.95Log cfu/g to 9.42Log cfu/g with a mean of 6.94Log cfu/g and higher than four-kling et al., 2016 with amean of 5.50Log cfu/g(21,38).

Aerobic colony count for tomato in this study was ranged from 2.90Log cfu/g to 5.95Log cfu/g with amean of 4.35Log cfu/g which is lower than similar studies in jimma, Ethiopia with a mean of 5.3Log cfu/g (4). This may be due to aweturiver water in jima town which is highly contaminated with bacteria with mean ACC of 8.58 isused without any treatment for irrigation of vegetables that may be eaten raw is a possible preharvest source of contamination to fresh vegetables (21).

Twenty seven (90%) of lettuce and 7(23%) of tomato samples in this study had total coli form count above permissible level for consumption without further processing (39). The total coli form count of lettuce samples in the present study was higher than similar study in Beirut,four-kling et al., 2016 with a mean of 3.89 ±2.19(38).and lower than another study in Jima, Ethiopia by Ketema et al., 2014 with a mean of 5.2 ± 0.6 (2). The total coli form count of tomato in this study was lower than another similar study by Sahile et al., 2019 with a grand mean of 6.46Logcfu/g (12).Fecal coli form count of tomato was comparable with similar study inCôte d'Ivoire byCoulibaly-Kalpy et al., 2017in which the average load was 2.23Log CFU/g(40).

E. coli is Gram-negative, facultative anaerobic,rod shaped bacteria which is naturally found in the intestinal tracts of all warm-blooded animals, including humans.Although most *E. coli* strains are harmless, certain strains are pathogenic and cause diseases such as urinary tract infection, watery diarrhea, meningitis, bloody diarrhea and sepsis (42). *E. coli* is a specific indicator of faecal contamination to assess the safety and quality of food and water (41, 42).In this study about 16.7% samples of lettuce and 3.3% samples of tomato were contaminated by this organism. All counts in this study were greater than 2.oLogcfu/which is above permissible level for fresh vegetables(Gulf standard), which indicates that these vegetables were focally contaminated by human or animal origin. This study was in consistence with similar study in India (43) and greater than another study in Canada (44).

Staphylococcus aureus is a bacterium which is commonly present in human skin, nasal passage and throat without causing any discomfort. It has the ability to produce several enterotoxins that are responsible for food poisoning. The temperature range for the bacterium to form toxin is from 10 to 45°C and optimal at around 35 to 40°C. Hence, normal refrigeration temperature can restrict the formation of toxin (41). *Staphylococcus aureus*indicates contamination by food handler. High counts indicate that growth has occurred and possible presence of enterotoxins; thus can indicate a potential health hazard for inadequately processed raw vegetable consumers.About Sixteen percent (n=10) of samples in this study, 7(23.3%) samples of lettuce and 3(10%) samples of tomato were contaminated by *S.aures* with a mean of 4.6±0.8Log cfu/g and all positive samples were above permissiblelevel of gulf standard, which was an indication of poor handling practice may be at the stage of harvesting, transportation or selling.

Salmonella was also isolated from three (10%) lettuce samples which must not be detected in 25 g of any vegetable samples (Gulf standard).this study was higher thananother similar study done in Sao Paulo, Brazil (45).unless vegetables are properly washed and decontaminated with right type and concentration of sanitizing agent before consumption; raw vegetable consumers are under risk of food born pathogen due to the presence of high number of Staphylococcus and salmonella species in these vegetables sold in the market. All *S.aureus*isolates were sensitive to gentamicin and 80% isolates were sensitive to tetracycline/chloramphenicol/erythromycinwhereas ampicillin/penicillin/Cefoxittinedrugs were 100% resistance. *Salmonella* species isolates were 100% sensitive to Gentamicin and Co-trimoxazole, 100% resistance to ampicillin/tetracycline/erythromycin.

7- Limitations of the study

- Due to resource limitation and other reasons the study is conducted on bacteriological quality of fresh vegetables i.e. the study focuses on more of indicator organisms, each pathogenic microorganism was not identified.

8- Conclusion

The present study clearly shows that the bacteriological quality of lettuce and tomato sold in the market had high level contamination of aerobic colony count, coliforms and *Staphylococcusaureus*. According to this study vegetable consumers are under the risk of food borne pathogens due to the presence of high number of *Staphylococcus aureus* and *salmonella* species in the tested vegetable samples.Consumer's awareness on washing and sanitization method before consumption of raw vegetables was also very low. In conclusion the result shows that before consumption, vegetables need to be washed, cleaned and disinfected.

9- Recommendation

Safe processing and handling of vegetables and hygiene of venders and consumers can improve the quality of vegetables.

Innervations mechanisms should be identified and implemented by government regulatory bodies and strict measure to control and minimize health risk associated with vegetables consumption should be imposed.

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Code No. _____

APPENDEX - I: Participant Information Sheet

Name of Investigator:TofikKedir

Institution Name:Addis Ababa University

Title of Project:Bacteriological quality of lettuce and tomato associated with consumer's hygienic practice in selected markets in Addis Ababa city, Ethiopia

Introduction: In the health conscious society of 21st century, vegetables form an integral part of human diet. They provide us essential vitamins and minerals, dietary fiber and antioxidants and also contribute large quantities of vitamin C, but the vitamin's high water solubility and sensitivity to heat makes it susceptible to loss during thermal preparation. However During growth, harvest, transportation and further processing and handling the produce usually contaminated with pathogens from human or animal sources. Especially vegetable requiring minimal or no further processing prior to consumption have been implicated as vehicles for transmission of infectious microorganisms. Outbreaks of illness caused by bacteria, viruses and parasites have been linked epidemiologically to the consumption of a wide range of vegetables and, to a lesser extent fruits.

Purpose of the study:

This study will primarily help to consumer to have better understanding and awareness about bacteriological quality of freshly eaten vegetables and to vegetable retailer and vendor to stay competent in the market and also they benefit consumer by preventing disease spreading through consumption of fresh vegetables. Government regulatory bodies (Ethiopian Standard authority and Ethiopian Food, Medicine and Health Care Administration and

Control Authority) can also use the study result for designing appropriate disease prevention strategies. Moreover, the study will be used as an initial data for future researches on bacteriological quality of vegetables in Addis Ababa city.

Procedure:if you decide to participate in the study, you will answer the questions in the space provided to you.

Benefits:benefit consumer by preventing disease spreading through consumption of bacterial contaminated lettuce and tomat.

Confidentiality: The information that we collect from this research will be kept confidential. Information about you that will be collected from the study will be stored in a file, which will not have your name on it, but a code number assigned to it. It will be kept under lock and key, and it will not be revealed to anyone except the principal investigator and the data collectors.

Right to refuse or withdraw

You have full right to refuse from participating in this research if you do not wish to participate.

Whom to contact

If you have any questions contact with the following address:

Contact Address:

TofikKedir= Cell Phone: +251 910700220, email=tofikkedir28@gmail.com, or

KassuDesta= Cell phone:+251 911 10 70 99, email= kassudesta2020@gmail.com

Code No._____

APPENDEX - II: Consent Form

I have been informed about a study. For this study I have been requested to answer questions about my personal hygiene and my processing habits during preparation of lettuce and tomato for consumption. It has been read to me all the information stated in the introductory part and I have had an opportunity to ask any ambiguous question. I got satisfactory answer for all of my concerns. I have fully understood and gave my consent to give answer for questions asked to me.

It is therefore, with full understanding of the situation that I gave my informed consent and cooperate in the course of the study.

Participant's name_____ Sign. _____Date_____

If finger print (illiterate): name of the independent witness, and

Participant's name_____ Finger print._____Date_____

Name of researcher, date and signature of researcher

TofikKedir_____ _____/____/_____ (dd/mm/yy) _____

Code No._____

APPENDEX III

ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Questionnaire format sheet to assess quality of vegetables in lafto vegetable and fruit market in Addis Ababa to be fulfilled by Vegetable consumers

Name of data collector: _____

Sub-city of vender: _____

First of all thank you for your voluntary participation

Thick the box for your correct answer

1. What is the educational status of vegetable consumer?

☐ - Illiterate

☐ - Elementary

☐ High school and above

2. What type of vegetable he/she consume?

☐ - Lettuce

☐ - Tomato

☐ - Both types

4. Temporary storage site

- Shelf

- Refrigerator

5. Water source for vegetable salad preparation

- Tap water

- Well

- Spring

6. Cleaning habit of the consumer during processing

- Yes

- No

7. Cleaning agents used during processing

- With water only

- With water and disinfection agent

- Other

8. Frequency of cleaning

- Once

- Twice

-Three and more

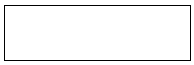
9. Cleaning of hand after using toilet

- Yes

- No

10. Cleaning of hand after using toilet by using

- Water and soap



- Water only

THANK YOU

ኮድቁጥር----------

<u>አባሪ - I፡ የተሳታፊመረጃቅጽ</u>

የመርማሪውስም፡ ቶፊቅከድር

የተቋሙስም፡- አዲስአበባዩኒቨርሲቲ

የፕሮጀክቱርዕስ፡ በአዲስአበባከተማበተመረጡገበያዎችከተጠቃሚዎችንፅህናጋርየተቆራኘየሰላጣእናየቲማቲምየባክቴሪያጥራት

የተከበሩተሳታፊዎችበጥናቱውስጥእንዲሳተፉእጋብዝዎታለሁ::የጥናቱንፕሮጀክትመቀላቀልሙሉበሙሉበውሳኔምርጫዎላይየ
ተመሠረተነው::ከመወሰንዎበፊትጥናቱለምንእንደተደረገእናለእርስዎምንእንደሚያካትትእነግርዎታለሁ::የዚህጥናትዋናዓላማ<u>በ
አዲስአበባበዋናዋናገበያዎችላይያለውንየአትክልትየንጽህናአያያዝናጥራትለማሻሻልነው</u>::በዚህጥናትውስጥለመሳተፍምንምገንዘ

ብአያገኙም።የእርስዎተሳትፎሙሉበሙሉበፈቃደኝነትነው።መሳተፍካልፈለጉበዚህጥናትውስጥላለመሳተፍሙሉመብትአለዎት
።

ኮድቁጥር----------

<u>አባሪ - II፡ የስምምነትቅጽ</u>

ስለጥናቱተነግሮኛል.
ለዚህጥናትሰላጣእናቲማቲምለምግብነትበሚዘጋጅበትጊዜስለየግልንፅህናእናየማቀነባበሪያልማዶቼጥያቄዎችንእንድመልስተጠይ
ቄያለሁ።በመግቢያውላይየተገለጹትመረጃዎችበሙሉተነብበውልኛልእናማንኛውንምአሻሚጥያቄለመጠየቅእድሉንአግኝቻለሁ
።ለጥያቄዎቼሁሉአጥጋቢመልስአገኘሁ።ሙሉበሙሉተረድቻለሁእናምለተጠየቁኝጥያቄዎችመልስለመስጠትፈቃዴንሰጥቻለሁ
።ስለዚህሁኔታውንሙሉበሙሉበመረዳትበመረጃላይየተመሰረተፈቃድሰጥቼበጥናቱሂደትውስጥየተባበርኩት።

ኮድቁጥር----------

አባሪ 3የአማርኛመጠይቅ

አዲስአበባዩኒቨርሲቲአዲስአበባዩኒቨርሲትየጤናሳይንስኮሌጅሜዲካልላብራቶሪሳይንስክፍል

በአዲስአበባውስጥበዋናዋናገበያዎችላይያለውንየአትክልትጥራትለማጥናትበአትክልትሸማቾችየሚሞላመጠይቅቅጽ

የመረጃሰብሳቢውስም: - _____

የከፊዲትከተማክፍለከተማ: - _____

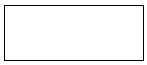
በመጀመሪያበፈቃደኝነትተሳትፎዎእናመሰግናለንለትክክለኛውመልስዎሳጥኑውስጥምልክትያድርጉ

1. ጾታ

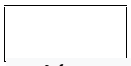
☐ -ወንድ

☐ -ሴት

2. የአትክልትሸማችየትምህርታዊሁኔታምንድነው?



ማንበብናመጻፍየማይችል/የማትችል



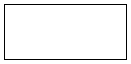
የመጀመሪያደረጃ

ሁለተኛደረጃትምህርትቤትእናከዚያበላይ

3. የሚጠቀመው/የምትጠቀመውአትክልትዓይነት

- ሰላጣ

- ቲማቲም

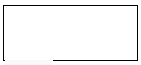


- ሁለቱምዓይነት

4. ጊዜያዊማከማቻቦታ

- መደርደሪያ

- ፍሪጅ



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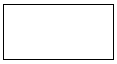
5. ለአትክልትዝግጅትእጥበየውሃምንጭ



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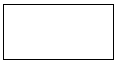
- ወራጅውሃ



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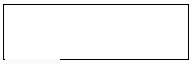
6. በአትክልትዝግጅትወቅትየሸማቹየማዕዳትልማድ



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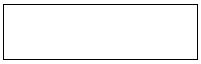

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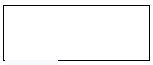
7. በዝግጅትጊዜጥቅምላይየዋሉየዕዳትወኪሎች

- በውሃብቻ

- በውሃእናበማጽጃወኪል



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8. የእጥበትድግግሞሽ

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-አንድጊዜ

- ሁለትግዜ

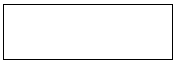
- ሶስትእናከዚያበላይ

9. መጸዳጃቤትከተጠቀሙበኋላየእጅየመታጠብልማድ

- አዎ

- አይ

10. መጸዳጃቤትከተጠቀሙበኋላየእጅምታጠቢያ



- በውሃእናበሳሙና

- በውሃብቻ

ለትብብሮ አመሰግናለሁ

Gulf standard for food stuffs

Item		n	c	m	M
Fresh fruits and vegetables to be consumed raw	Escherichia coli	5	2	10	10^2
	Salmonella	5	0	0	–
	Escherichia coli O157	5	0	0	–
	Listeria monocytogenes	5	0	0	–
	Staphylococcus aureus	5	2	10^2	10^3

n = Number of sample units to be examined. c = The maximum number of sample units allowed to have a microbiological criterion value greater than "m" and not to exceed the value of "M".
m = The acceptable microbial level in the sample unit; which separates the acceptable quality of marginal-quality acceptance. The product shall be acceptable if the value is equal to or less than "m"; if the value is above "m", the product is marginally acceptable or rejected. M = The maximum criterion value that should not be exceeded in any of "n" units. Sample unit

Declaration

I, the undersigned agree to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and conditions of the research publications office.

M.Sc. candidate: **TofikKedir (B.Sc.)**

Signature: _____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisor: **KassuDesta (MSc, PhD)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.

Advisor: **WaktoleGobena (BSc, MSc)**

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.