CODEN [USA]: IAJPBB ISSN: 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

http://doi.org/10.5281/zenodo.1472796

Available online at: http://www.iajps.com Research Article

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS AND PLASMID PROFILE OF MICROBIAL ISOLATES FROM VEGETABLES COLLECTED IN JIMMA TOWN, ETHIOPIA

Eshetu Chilo*[1], Mulatu Gashaw[2], Haiymanot Tasew[2], Mio Ayana[2], Zeleke Mekonnen[2]

[1] Department of Biomedical sciences, Faculty of Public Health and Medical Sciences, Mettu University, P.O. Box 318 Mettu, Ethiopia.

[2] Department of Medical Laboratory Science And Pathology Institute of Health Sciences, Jimma University, P.O. Box 378, Jimma Ethiopia

Abstract:

Background: *Vegetables might have frequently been contaminated with different microbial agents including pathogens from soil, animal and human sources. They may be contaminated at any point during growing, harvesting, sorting, packaging, and storage. This study was aimed at assessing antibiotic susceptibility patterns and plasmid profile of microbial isolates from fresh vegetables collected in selected local market at Jimma town, southwest Ethiopia from Feb to April, 2015.*

Methods:*A cross sectional study design was conducted. A total of 150 fresh vegetable samples were collected on different days from different local markets. Five types of fresh vegetable comprising lettuce, cabbage, carrot, tomato, green pepper was collected from farmers and four purposively selected local markets. For microbiological analyses, 25g of sample was aseptically weighed and washed gently in 225ml of sterile 0.1% (w/v) peptone water (Oxoid) for 3 minutes. Total plate count, bacterial isolation, antimicrobial susceptibility tests and plasmid identification were performed.*

Results: *More than 80% of vegetable specimens collected showed viable counts of > 10^6CFU/g with ranges of 10^5-10^7CFU/g. A total of 102 bacterial isolates of eight genera were identified. Enterobacter spp. (21.60%) was the most dominant followed by Citrobacter spp. (20.6%), Klebsiella spp. (18.6%), Salmonella spp. (11.8%), E. coli (10.8%), Proteus spp. (9.8%), S. aureus (4.9%), and P. aeruginosa (2%). More than 90% of microbial isolates were resistant for Ampicillin and amoxicillin.All the S. aureus isolates were sensitive to oxacillin and vancomycin.Nearly 96% of the isolates were sensitive for ciprofloxacillinwhereas 33 % of the isolates showed resistance to oxytetracycline.. Resistance to nitrofurantoin, nalidic acid, streptomycin, chloramphenicol, cotrimoxazole, ceftriaxone, kanamycin and Gentamycin were 32%,26.8%,17.5%,13.4%,11.3%,11.3%,8.2%, 5% respectively.Plasmid was detected in 20 out of 91resistant isolate to at least one antibiotic.*

Conclusion: *Vegetables in Jimma local markets were significantly contaminated with potentially pathogenic bacteria and multiple antibioticsresistant. This necessitates following more strict hygienic practices along the chain of vegetables production and supply.Moreover, the current study results demonstrated that plasmids are one of the important ways to spread resistance to many antimicrobial agents.*

Key words: ***vegetable, microbial isolate, antibiotic resistance pattern, plasmid profile, Jimma***

***Corresponding author**:

Eshetu Chilo,

Department of Biomedical Sciences,
Faculty of Public Health and Medical Sciences, Mettu University,
P.O. Box 318 Mettu, Ethiopia.
Email: eshetuchilo@gmail.com

QR code

Please cite this article in press Eshetu Chilo et al., ***Antimicrobial Susceptibility Patterns and Plasmid Profile of Microbial Isolates from Vegetables Collected In Jimma Town, Ethiopia.,*** *Indo Am. J. P. Sci, 2018; 05(10).*

INTRODUCTION:

Background

Globally, food borne illnesses are accountable for significant morbidity and mortality. Factors contributing to the emergence of foodborne diseases are changes in human demographics and behavior, technology and industry, and international travel and commerce; microbial adaptation; economic development and land use; and the breakdown of public health measures[1, 2].

Fresh vegetable products have been implicated in a number of documented outbreaks of foodborne illness particularly in Europe, Japan, United States, and Canada. Outbreaks of illness caused by bacteria, viruses and parasites have been linked epidemiologically to the consumption of a wide range of vegetables. Surveillance of vegetables has indicated that these foods can be contaminated with various bacterial pathogens, including *Salmonella, Shigella, E. coli O157:H7,Listeria monocytogenes and Campylobacter*. However, the prevalence of foodborne pathogens on vegetables and their involvement in outbreaks are not well documented in developing countries[1, 3].

The emergence of antimicrobial resistance in disease-causing bacteria is a public health concern that poses unique communication challenges[4]. Antimicrobials are essential for treating infectious disease in both humans and animals. However, their improper use may lead to the emergence of new strains of bacteria that cannot be treated with commonly used antimicrobials. Sometimes, pathogens emerge that are resistant to multiple antimicrobials, making treatment extremely difficult. A further complication is that antimicrobials are commonly used in food animals, such as cattle, swine, and poultry, which are a common source of exposure to human pathogens linked to food[5, 6].

The prevalence of antimicrobial resistance among food borne pathogens is reported to have increased, probably as a result of selection pressure created by the use of antimicrobials in agriculture and animal health[7, 8].

Most dangerous microorganisms do not change the appearance of the food, so we usually can't tell that the food is contaminated with dangerous microor-ganisms by just looking, smelling or tasting it. Several surveys have demonstrated the presence of pathogenic enteric bacteria on produce and in unpasteurized fruit or vegetable juices and vegetable sampled during production or at retail markets in different parts of the world among them some survey were done in Addis Ababa, Gondar, Hawassa, Bahirdar, Bangladesh, Pakistan, South Africa [2, 7, 9-18].

However, data regarding microbiological safety, contamination level, antimicrobial property and presence of plasmid profiles of these organisms is important in guiding preventive measures. In developing countries like Ethiopia, such data is limited. Moreover these factors vary from local to national necessitating surveillance at any particular area of interest.

METHODS:

Study site. The study was conducted in Jimma Town, which is located at south west of Ethiopia, about 352 km from Addis Ababa, the capital of Ethiopia. Based on the 2007 Census conducted by the CSA, Jimma Zone has a total population of 2,486,155 of whom 1,250,527 are men and 1,235,628 women; with an area of 15,568.58 square kilometers, Jimma has a population density of 159.69. While 137,668 or 11.31% are urban inhabitants, a further 858 or 0.03% are pastoralists. A total of 521,506 households were counted in this Zone, which results in an average of 4.77 persons to a household[9](32). In the study area consumption of fresh vegetable is a common practice.

Study design and period. A cross-sectional study was conducted from Feb 2015 to April 2015 in Jimma town, southwest Ethiopia.

Sample Collection and Analysis. All vegetables including lettuce, cabbage, carrot, tomato, green pepper brought to Jimma town for sale during the study period was the target sample for this study. A purposive sampling technique was employed to collect a total of 150 vegetable samples. The entire sample was collected and put in sterile plastic containers, properly labeled and transportedwithin 3hrs of sample collection to the Microbiology Laboratory at Medical Laboratory and Pathology Department, Jimma University for microbiological analysis. Microbiological analysis was conducted within three hours of sample collection.

Data collection

A semi-structured questionnaire was used that contained questions concerning the pre- harvest and post-harvest vegetable contamination. The questionnaire was adapted from reviewing similar studies and prepared first in English language and was then translated into Amharic; and then pretested

for its appropriateness. The questionnaire was further modified after pretesting.Laboratory format was used to record the laboratory test results.

Microbiological analysiss: For microbiological analyses, 25g of sample was aseptically removed from each sample using a sterile spatula and gently shaken in 225ml of sterile 0.1% (w/v) bacteriological peptone water (Oxoid) for 3 minutes. Serial dilutions of 10^{-2}, $_{10}^{-3}$ were made and then 0.1ml of the suspension from each dilutions were plated in duplicate on a pre-dried surfaces of nutrient agar and average count were recorded after multiplying by reciprocal of dilution factor and reported as colony forming unit per gram[6].

For isolation of *Salmonella*spp and *Shigella* spp. Vegetable samples (25 g) was added to 225 ml bacteriological peptone water, gently shaken and the suspension incubated at 37^{o}C for 24 hours for the metabolic recovery and proliferation of cells. From this, 1ml of culture was transferred into tubes containing 10 ml of Selenite F Broth. Selenite F broth was incubated at 37^{o}C for 24 hours. After enrichment, culture from selenite F broth was separately streaked on plates of MacConkey Agar, Xylose Lysine Desoxycholate (XLD) medium (all from Oxoid). Characteristic colonies that are non-lactose fermenterswith black centers from each selective medium was picked, purified and tested biochemically on Kligler‟s Iron Agar (Oxoid), Lysine Iron Agar (LIA) (Oxoid), Urea Agar (Oxoid), oxidase, indole, Simmons Citrate Agar (Oxoid) and SIM Medium (Oxoid). For isolation and identification of *S. aureus,* a loop full of sample from the homogenate was inoculated on Manitol Salt Agar (MSA) and yellow colonies on MSA which was catalase positive and coagulase positive isolates was identified as *S. aureus*.

Drug susceptibility testing: The criteria used to select the antimicrobial agents tested was based on the availability and frequency of prescription for the management of bacterial infections in Ethiopia and WHO Recommended antimicrobials for surveillance of *Salmonella* and *E. coli* as they are the major food borne pathogen around the globe[6].
Antimicrobial susceptibility testing for microbial isolate was performed using the disk diffusion method and results were interpreted using the criteria of the CLSI (Clinical and laboratory standard institute). The antibiotics used were Ampicillin (10µg) ,Amoxicillin (10µg),Chloramphenicol(30µg) ,Streptomycin (10 µg) ,Oxytetracycline (30µg), Cotrimoxazole (1.25µg) ,Ciprofloxacillin (5µg) ,Gentamycin (10µg), Nalidic acid (30µg) Kanamycin (30µg), Ceftriaxone (30µg), Nitrofurantoin (300µg). Antibiotic susceptibility testing for *S. aureus* was determined for Erythromycin (E-15 µg), Oxacillin (Ox -1 µg), and Vancomycin (Va-30 µg) in addition to the above antibiotics. A standard reference strains *E. coli* (ATCC 25922) and S.aureus (ATCC 25923) with known sensitivity were used in this study. Interpretation of readings as sensitive, intermediate or resistant was made according to a standard chart

Screening for plasmidDNA
Sample preparation was performed by culturing isolates on Nutrient agar (NA). Colonies grown on NA were transferred to and grown in 12 ml of tryptic soya Broth (Oxoid) containing 50µg/ml ampicillin and incubated at 37°C for 24 hours. The plasmid DNA was extracted by using cold alkaline lysis method protocol described by C. Rohde & B. Henze[14]using sodium acetate as neutralizing agent. Agarose gel electrophoresis (1%) was run for 1.30hrs at 100V using horizontal gel electrophoresis apparatus. The plasmids were visualized with ultraviolet light.

Data Quality Control
The following measures was undertaken so as to control the quality of the data and laboratory investigation. Properly designed and pre-tested data collection instrument was used. Every day the collected data was cross checked for completeness, consistency and on site corrective action was made.

A standard operational procedure tools was strictly used for sample collection, transportation, processing and storage. Special emphasis was given during coding each culture media as well as the collected vegetable samples. Before use all disks, reagents and culture media was checked being at appropriate temperature and within specified shelf life. Antibiotic sensitivity test was performed according to clinical laboratory standard institute guide. *E. coli* strain ATCC 25922 and *S. aureus* strain ATCC 25923 was included. For plasmid isolation, optimization test were performed for gel concentration, running time and volt.

All media were checked visually before being inoculated for any change in appearance that could indicate contamination or deterioration.
Statistical Analysis
Data were organized and summarized in simple descriptive statistics methods. Moreover, all components of the data entered and analyzed using SPSS 20.0 computer software. Chi-square test (X^2)

results were used and a *p*-value of less than 0.05 was considered statistically significant.

Ethical Consideration

Ethical clearance wasobtained from Jimma University research and ethical review committee. The purpose and procedures of the study wasexplained to the respondents (vendors of vegetables) a verbal consent was obtained from all study participants. Privacy and confidentiality of the study participants response and laboratory test result was maintained.

RESULTS:

Table1: Socio-demographic characteristics of vender of vegetables of selected local markets of Jimma town Southwest Ethiopia, 2015

Variables	Frequency (n)	Percent (%)
Gender		
Male	6	20
Female	24	80
Total	**30**	**100**
Educational status		
No formal education	13	43.3
Primary education	11	36.7
Secondary education	6	20
Total	**30**	**100**
Market place		
Kochi	25	16.7
Bishishemerkato	25	16.7
Hirmatamerkato	25	16.7
Agip	25	16.7
From farmer	50	33.3
Total	**150**	**100**
Type of product		
Green pepper	30	20
Carrot	30	20
Lettuce	30	20
Cabbage	30	20
Tomato	30	20
Total	**150**	**100**
Occupation		
Merchant	20	66.7
Farmer	10	33.3
Total	**30**	**100**
Place of display		
Open market	21	70
Grocery	9	30
Total	**30**	**100**
Water source for washing		
Pipe water	19	63.3
Well water	1	3.4
Surface water	10	33.3
Total	**30**	**100**

Socio-demographic Characteristics

A total of 150(30 each) vegetable samples including lettuce, cabbage, carrot, tomatoes and green peppers were studied. A total of 30 vegetable venders were interviewed, 10 farmers and 20 merchant at different vendor and market days. The mean age of the respondents was 31.6 with standard deviation of 3.85 the minimum was 25 and maximum was 40. Majority (80%) of the vegetable vendors were female. With respect to educational status majority 43 % had no formal education, 36. 7 % had primary education while the rest 20% had secondary education (**Table 1**).

Table 2: Knowledge and practice related to vegetable farming for farmer respondents of vegetable venders Jimma town south west Ethiopia, 2015.

	Frequency (n)	Percent (%)
Put animals in fenced area		
Yes	10	100
No	-	
Harvest container used to carry other materials		
Yes	10	100
No	-	
Type of fertilizer used		
Decayed manure	9	90
Inorganic fertilizer	1	10
Water source for irrigation		
Pipe water	-	
Well water	-	
Surface water	10	100
Habit of washing harvest equipment		
Yes	10	100
No	-	
Hand washing before harvesting		
Yes	-	
No	10	100
Keep container off the ground		
Yes	-	
No	10	100

Almost all (9 out of 10) of the farmers used decayed manure as fertilizer while only one farmer used organic fertilizer to cultivate vegetables as soil amendment. Moreover all farmers used surface water for irrigation and washing purpose. Whereas all merchants used tap water for washing purposes and all the participants had latrine and reportedly all washed their hands after visiting toilet. All vegetable venders participated in the study had direct contact with vegetable with bare hand without washing their hands during vending and harvesting **(table-2**).

Table 3: Mean total aerobic mesophilic bacterial counts (CFU/g) of fresh vegetables by type of product collected from selected local markets Jimma town southwest, Ethiopia 2015.

AMC	type of product					Total
	green pepper	carrot	lettuce	cabbage	tomato	
10^5	4(13.3%)	4(13.3%)	2(6.7%)	3(10.0%)	3(10.0%)	16(10.7%)
10^6	25(83.3%)	25(83.3%)	26(86.7%)	26(86.7%)	26(86.7%)	128(85.3%)
10^7	1(3.3%)	1(3.3%)	2(6.7%)	1(3.3%)	1(3.3%)	6(4.0%)

Nearly 85% of vegetable samples had total viable counts of greater than10^6CFU/g with ranges of 10^5-10^7CFU/g for all vegetable types(**Table 3).**

Table 4: Prevalence of bacteria isolated from fresh vegetables collected in selected local market of Jimma town southwest Ethiopia, 2015

Microbial isolates	Frequency(n)	Percent (%)
E.coli	11	10.8
Salmonella spp	12	11.8
Proteus spp	10	9.8
Klebsiellaspp	19	18.6
Citrobacter spp	21	20.6
Enterobacter spp	22	21.6
Pseudomonas spp	2	2.0
S.aureus	5	4.9
Total	102	100.0

The presence *E.coli* in freshly consumed vegetables was indicators of poor sanitation condition and presence of potential pathogenic microorganisms such as salmonella. Moreover, detection of *Salmonella spp*in 25 gm of vegetable samples is considered as unacceptable for consumption. The presence of *S.aureus* in fresh vegetable samples indicates direct hand contact by venders of vegetables as these organisms are present on skin of as normal flora(**table-4**).

Table 5: Distribution of bacterial isolates by type of product from fresh vegetables collected in selected local market of Jimma town southwest Ethiopia, 2015

Microbial isolates	type of product green pepper	carrot	lettuce	cabbage	tomato	Total
E.coli	1(6.2%)	1(4.2%)	3(13.0%)	3(15.8%)	3(15.0%)	11(10.8%)
Salmonella spp	_	4(16.7%)	5(21.7%)	2(10.5%)	1(5.0%)	12(11.8%)
Proteus spp	1(6.2%)	2(8.3%)	1(4.3%)	2(10.5%)	4(20.0%)	10(9.8%)
Klebsiellaspp	3(18.8%)	5(20.8%)	4(17.4%)	3(15.8%)	4(20.0%)	19(18.6%)
Citrobacter spp	5(31.2%)	2(8.3%)	6(26.1%)	5(26.3%)	3(15.0%)	21(20.6%)
Enterobacter spp	4(25.0%)	7(29.2%)	4(17.4%)	3(15.8%)	4(20.0%)	22(21.6%)
P.auroginosa	_	1(4.2%)	_	1(5.3%)	_	2(2.0%)
S.aureus	2(12.5%)	2(8.3%)	_	_	1(5.0%)	5(4.9%)
Total	16(100.0%	24(100.0%	23(100.0%	19(100.0%	20(100.0%	102(100.0%

The difference in distribution of bacterial isolate by type vegetables and market place were not statistically significant (p-value >0.05) (table-5&6)

Table 6: Frequency distribution of bacterial isolates by market place from fresh vegetables collected in selected local market of Jimma town southwest Ethiopia, 2015

Microbial isolate	market place kochi	bishish	Hirma	agip	Total
E.coli	2(22.2%)	1(11.1%)	3(33.3%)	3(33.3%)	9(100.0%
Salmonella spp	2(25.0%)	2(25.0%)	2(25.0%)	2(25.0%)	8(100.0%)
Proteus spp	1(12.5%)	2(25.0%)	2(25.0%)	3(37.5%)	8(100.0%)
Klebsiellaspp	6(50.0%)	2(16.7%)	2(16.7%)	2(16.7%)	12(100.0%)
Citrobacter spp	5(31.2%)	4(25.0%)	4(25.0%)	3(18.8%)	16(100.0%)
Cnterobacterspp	4(25.0%)	4(25.0%)	3(18.8%)	5(31.2%)	16(100.0%)
Pseudomonas spp	0	0	0	2(100.0%)	2(100.0%)
S.aureus	2(40.0%)	0	0	3(60.0%)	5(100.0%)
Total	22(28.9%)	15(19.7%)	6(21.1%)	23(30.3%)	76(100.0%)

Antimicrobial susceptibility

More than 90% of microbial isolates were resistant for ampicillin and amoxicillin but *salmonella* and *proteus spps* were 100% resistant for ampicillin and amoxicillin. All the *S. aureus* isolates were sensitive to oxacillin and vancomycin. Nearly 96% of the isolates were sensitive for ciprofloxacillin whereas oxytetracycline was resisted by 33 % of the isolates. Resistance to nitrofurantoin, nalidic acid, streptomycin, chloramphenicol, cotrimoxazole, ceftriaxone, kanamycin and Gentamycin were 32%, 26.8%, 17.5%, 13.4%, 11.3%, 11.3%, 8.2%, 5% respectively(**table-7&8**).

Table 7: Antibiotic resistance patterns of gram negative rods isolatedfrom fresh vegetables sold in selected local market of Jimma town southwest Ethiopia, 2015

Bacterial isolate		Antimicrobial agents											
	Numb	**AM**	**AMX**	**C**	**S**	**SXT**	**OXT**	**CIP**	**GN**	**NA**	**K**	**CRO**	**F**
E. coli	11	7	7	4	4	-	6	3	0	4	1	4	6
	%	64	64	36	36		54.5	27		36	9	36	54.5
Salmonella spp	12	12	12	2	-	-	8	-	-	2	-	2	2
	%	100	100	16.6			66.7			16.6		16.7	16.7
Proteus spp	10	10	10	-	5	7	8	-	1	6	-	-	7
	%	100	100		50	70	80		10	60			70
Klebsiellaspp	19	19	17	2	4	2	1	-	1	6	1	1	7
	%	100	89.4	10.5	21.1	10.5	5.3		5.3	31.6	5.3	5.3	36.8
Citrobacter spp	21	20	20	-	2	1	5	1	-	4	2	2	6
	%	95.2	95.2		9.5	4.8	23.8	4.8		19.1	9.5	9.5	28.5
Enterobacter	22	21	21	3	2	1	4	-	3	2	4	2	3
spp	%	95.5	95.5	13.6	9	4.5	18		13.6	9	18	9	13.6
Pseudomonas	2	2	2	2	-	-	-	-	-	2	-	-	-
spp	%	100	100	100						100			
Total	97	91	89	13	17	11	32	4	5	26	8	11	31
	%	93.8	91.8	13.4	17.5	11.3	33	4	5	26.8	8.2	11.3	32

Key: AM=Ampicillin (10µg), AMX=Amoxicillin (10µg) ,C=Chloramphenicol(30µg) ,S=Streptomycin (10 µg), SXT= Cotrimoxazole (1.25µg) ,OXT=Oxytetracycline(30µg) ,CIP=Ciprofloxacillin (5µg) GN=Gentamycin (10µg) ,NA=Nalidic acid (30µg) ,K=Kanamycin (30µg),CRO=Ceftriaxone (30µg) F=Nitrofurantoin (300µg)

Table 8 Antibiotic resistance patterns of *S.aureus* isolatedfrom fresh vegetables sold in selected local market of Jimma town southwest Ethiopia, 2015

Bacterial isolate	Antimicrobial agents															
	Numb	AM	AMX	C	S	SXT	OXT	CIP	GN	NA	K	CRO	F	OX	VA	E
S.aureus	5	-	3	-	2	-	-	-	-	1	-	-	-	-	-	-
	%		60		40					20						

Key: AM=Ampicillin (10µg), AMX=Amoxicillin (10µg) ,C=Chloramphenicol(30µg) ,S=Streptomycin (10 µg), SXT= Cotrimoxazole (1.25µg) ,OXT=Oxytetracycline(30µg) ,CIP=Ciprofloxacillin (5µg) GN=Gentamycin (10µg) ,NA=Nalidic acid (30µg) ,K=Kanamycin (30µg),CRO=Ceftriaxone (30µg) F=Nitrofurantoin (300µg), OX=Oxacillin (1µg) ,VA=Vancomycin (30µg) ,E=Erythromycin (15µg)

Screening for plasmid

All (102) bacterial isolates were screened for the presence of plasmid. Out of 91 isolate resistant to at least one antibiotics plasmid were detected in 20 and plasmid were not detected in sensitive and intermediately sensitive isolate which were 8 and 3 in number respectively.

DISCUSSION:

Previous report from different parts of the world indicated the presence of indicator organism and potential pathogen and multiple antimicrobial resistance isolates in fresh vegetables sold in the open markets and supermarkets. Similarly our finding supported the finding of previous study in demonstrating the potential bacterial pathogens such as Salmonella spp and multiple antibiotic resistance isolate were also detected that could potentially serve as reservoir of resistant gene as evidenced by the presence of plasmid in resistant isolate.

The present study demonstrated heavy microbial contamination of fresh vegetables sold in the selected open markets with the ranges of total aerobic mesophilic counts between 10^5-10^7 CFU/g for all vegetable samples.There was no significant variation in microbial load of vegetable samples by market place, educational level, type of venders, type of products and storage condition.

Studies elsewhere have investigated the microbiological quality of street vended foods in different countries; high bacteria counts and a high incidence of food borne pathogens in such foods have been reported. The microbial load of vegetables in the current study area is higher compared to study done in Accra[19, 20]for tomato sample and comparable with study done in South Africa[7][8] and Nigeria[21, 22]Accra[13, 19]but lower compared to study done in Ghana, documented in the street foods of Kumasi [13, 23] and Bangladesh[16, 24]. The discrepancy between the present study and previous studies might be as a result of the variations in geographical locations, seasonal, climatic and environmental conditions, the kind of sample and sample size examined, the sampling techniques, methods used for detection of the microbial isolates and socioeconomic status.

More than 85% of vegetable samples had total viable counts of greater than 10^6CFU/g.Similar study conducted in Addis Ababa reported over 90% of the vegetable samples had aerobic mesophilic counts of ≥ log 6 CFU/g[12, 25]. The high microbial load of vegetable in this study could be due to fact that in both grocery and open markets vegetables were seen displayed on open stalls in close proximity to waste container without lids where flies are swarming all over the place, mostly close to open gutter, direct hand contact during both harvest and sell. Moreover all farmers used surface water for washing purpose.

Consumption of microbial contaminated fresh vegetables is food safety concern, as these product may represent a potential risk for the consumer's health, particularly in debilitated or immune compromised individuals[2, 17].Different researchers reported different result for varieties of microbial isolate from fresh product. In the present study a total of 102 bacterial isolates of eight genera were identified. *Enterobacter* spp. (21.60%) was the most dominant followed by *Citrobacter spp*. (20.6%), *Klebsiella spp*. (18.6%), *Salmonella* spp. (11.8%), *E. coli* (10.8%), *Proteus spp*. (9.8%), *Staphylococcus spp*. (4.9%), and *Pseudomonas aeruginosa* (2%). The prevalence of *E.coli* in the present study was lower compared to study done in Lebanon[18, 26] which reported prevalence of (42.30%) this could be due to sample type, sample size, climatic and seasonal variation. However the present study was comparable

with study done in Lebanon in demonstrating the presence of pathogenic microorganism in fresh vegetables consumed which are usually consumed raw and represent a risk for human health[18, 26].

Comparable results was reported from similar study conducted in Nigeria[21, 22]Jordan [20, 27] andSpain[27]. However, similar study from South Africa did not isolate *Salmonella*spp. and *Escherichia coli* in any of the vegetable samples[7, 8]which could be due to the difference in hygienic practice.

Guchi and Ashenafi reported isolation of *Salmonella* and *Shigella* from eight (10%) and 24 (30%) vegetable samples, respectively[12, 25].In addition other species including *Pseudomonas and staphylococci* were also isolated from lettuce and green pepper. Over 80% of the green pepper and lettuce samples harbored *staphylococci.*
Akter et al reported presence of *E. coli*, *S.* dysenteriae, *K. pneumonia*, *S. typhimurium, P.vulgaris* and gram-positive bacteria including, *S. aureus* in the fresh vegetable samples[16, 24].Moreover their study demonstrated that the fresh vegetable samples collected from local markets were heavily contaminated with resistant bacteria and is of special concern for human consumption.

The presence of antibiotic-resistant bacteria in fresh vegetables may constitute food safety concern since bacteria serving as a reservoir for resistance determinants may have great influence on resistance gene transfer in natural habitats, such as the human colon, fruit and vegetable surface[4, 28, 29]. More than 90% of microbial isolates were resistant for ampicillin and amoxicillin. All the *S. aureus* isolates were sensitive to oxacillin and vancomycin. Nearly 96% of the isolates were sensitive for ciprofloxacillin whereas oxytetracycline was resisted by 33 % of the isolates. Resistance to nitrofurantoin, nalidic acid, streptomycin, chloramphenicol, cotrimoxazole, ceftriaxone, kanamycin and Gentamycin were 32%, 26.8%, 17.5%, 13.4%, 11.3%, 11.3%, 8.2%, 5% respectively(table-7&8).

Antibacterial resistance is a worldwide threat and concerns have arisen about the involvement of commensal and pathogenic bacteria in the maintenance and spread of resistance genes[22](29).Results of the present study demonstrated multiple antibiotic resistance isolates from fresh vegetable samples which was comparable to a report from Nigeria[21](24) Addis Ababa[12](25), Jordan[20](22), Belgium[25](4),Spain[27]and Bangladesh[16](26).

Plasmids are extra-chromosomal pieces of DNA, which are capable of replicating independently of the genome, and are particularly important in the spread of antibiotic resistance genes[14](34).In the present study 91 resistant isolates were screened for the presence of plasmid. Among which only 20 out of 91 resistant isolates were found to contain plasmid. This finding is very low compared to similar study done in other parts of the world for example Study done in Australia to check the involvement of plasmids in the resistance to antibiotics observed in some of the isolates, plasmid DNA was extracted from all 86 isolates that were resistant to at least one antibiotic. Plasmids of varying numbers and sizes were found in 74.4% of resistant isolates, while 25.6% did not possess any plasmids[24](30). Another study conducted to determine bacterial Load of Fresh Vegetables and Their Resistance to currently used antibiotics in Saudi Arabia revealed presence of plasmid DNA in all a preselected multidrug-resistant isolates tested[18](31). Moreover study conducted by Akter et al reported presence of plasmid in 22 isolate tested for plasmid profile[16](26). The low prevalence observed in our study might be due to methodological difference, sensitivity of the tests and environmental factors.

CONCLUSION:

In conclusion, the current study revealed that more than 80% of vegetable samples had total viable count > 10^6 CFU/g. Thus, presence of *E.coli* in fresh vegetables samples were indicators of poor sanitation.Salmonella spp were detected in 11.8% fresh vegetables. The predominant bacterial isolate was *enterobacterspp* followed by *citrobacter spp*. It was depicted that detectionmore than 90 % of microbial isolatewas resistant for ampicillin and amoxicillin. Plasmid carriage was detected in 22% resistant isolates. Thus, resistance to multiple antibiotics might have been conferred by plasmids.

Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution:

Eshetu Chilo conceived the study, participated in the study design, data collection, data analysis, and drafted the paper for publication. Mulatu Gashaw and Haimanot Tasew participated in study design, sample collection, laboratory wok, data analysis, interpretation and preparation of manuscript. Zeleke Mekonnen and Mio Ayana participated in laboratory work during plasmid isolation and drafted the paper

for publication. All authors have read and approved the final paper.

ACKNOWLEDGMENTS:
The authors would like to thank Jimma University for financial and logistic support. The authors would like to extend their gratitude to the vendors of the vegetables for their willingness and cooperation during the study.

REFERENCES:

1. Altekruse S, Cohen M, Swerdlow D: **Emerging foodborne diseases**. *Emerging infectious diseases* 1997, **3**(3):285.
2. Kibret M, Tadesse M: **The bacteriological safety and antimicrobial susceptibility of bacteria isolated from street-vended white lupin (Lupinus albus) in Bahir Dar, Ethiopia**. *Ethiopian journal of health sciences* 2013, **23**(1):19-26.
3. SCF/CS/FMH/SURF/Final: **Risk Profile on the Microbiological Contamination of Fruits and Vegetables Eaten Raw .Report of the Scientific Committee on Food. Brussels - Belgium; 2002 Apr. http://europa.eu.int/comm/food/fs/sc/scf/index_en.html**. In.
4. Acar J, Rostel B: **Antimicrobial resistance: an overview**. *Revue Scientifique et Technique-Office International des Epizooties* 2001, **20**(3):797-807.
5. WHO: **Integrated surveillance of antimicrobial resistance: guidance from a WHO Advisory Group. World Health Organ Geneva.** . 2013.
6. Svara F, Rankin DJ: **The evolution of plasmid-carried antibiotic resistance**. *BMC evolutionary biology* 2011, **11**(1):1.
7. Nyenje ME, Odjadjare CE, Tanih NF, Green E, Ndip RN: **Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in Alice, Eastern Cape Province, South Africa: Public health implications**. *International journal of environmental research and public health* 2012, **9**(8):2608-2619.
8. Organization WH: **Integrated surveillance of antimicrobial resistance: guidance from a WHO Advisory Group**. 2013.
9. CSA: **Summary and statistical report of the 2007 population and housing census. population size by age and sex**. 2008.
10. Faruk MO, Akhter MZ: **Presence of coliforms and fecal coliforms in fast food items of local restaurants and fast food outlets of Dhaka city**. *Bangladesh Journal of Microbiology* 2012, **28**(1):49-51.
11. Farzana K, Akram MR, Mahmood S: **Prevalence and antibiotics susceptibility patterns of some bacterial isolates from a street-vended fruit product**. *Afr J Microbiol Res* 2011, **5**:1277-1284.
12. Guchi B, Ashenafi M: **Microbial load, prevalence and antibiograms of Salmonella and Shigella in lettuce and green peppers**. *Ethiopian journal of health sciences* 2010, **20**(1).
13. Halablab M, Sheet I, Holail H: **Microbiological quality of raw vegetables grown in Bekaa Valley, Lebanon**. 2011.
14. Bimboim H, Doly J: **A rapid alkaline extraction procedure for screening recombinant plasmid DNA**. *Nucleic acids research* 1979, **7**(6):1513-1523.
15. Amponsah-Doku F: **Bacterial contamination of lettuce and associated risk factors at production sites, markets, and street food restaurants in urban and peri-urban Kumasi**. Kwame Nkrumah University of Science and Technology; 2006.
16. Akter S, Rafiq-Un N, Rupa F, Bari M, Hossain M: **Antibiotic resistance and plasmid profiles in bacteria isolated from market fresh vegetables**. *Agric Food Anal Bacteriol* 2011, **1**(2):140-149.
17. Beuchat LR, Ryu J-H: **Produce handling and processing practices**. *Emerging infectious diseases* 1997, **3**(4):459.
18. Hassan SA, Altalhi AD, Gherbawy YA, El-Deeb BA: **Bacterial load of fresh vegetables and their resistance to the currently used antibiotics in Saudi Arabia**. *Foodborne pathogens and disease* 2011, **8**(9):1011-1018.
19. Mensah P, Yeboah-Manu D, Owusu-Darko K, Ablordey A: **Street foods in Accra, Ghana: how safe are they?***Bulletin of the World Health Organization* 2002, **80**(7):546-554.
20. Burjaq SZ, Shehabi AA: **Fresh leafy green vegetables associated with multidrug resistant E. coli**. *The International Arabic Journal of Antimicrobial Agents* 2013, **3**(2).
21. Adeshina GO, Jibo SD, Agu VE: **Antibacterial susceptibility pattern of pathogenic bacteria isolates from vegetable salad sold in restaurants in Zaria, Nigeria**. *J Microbiol Res* 2012, **2**:5-11.
22. Labro M-T, Bryskier J-M: **Antibacterial resistance: an emerging 'zoonosis'?***Expert review of anti-infective therapy* 2014, **12**(12):1441-1461.
23. Amponsah-Doku F, Obiri-Danso K, Abaidoo R,

Drechsel P, Kondrasen F: **Bacterial contamination of lettuce and associated risk factors at production sites, markets and street food restaurants in urban and peri-urban Kumasi, Ghana**. *Scientific Research and Essays* 2010, **5**(2):217-223.

24. Akinbowale OL, Peng H, Barton M: **Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia**. *Journal of Applied Microbiology* 2006, **100**(5):1103-1113.
25. Holvoet K, Sampers I, Callens B, Dewulf J, Uyttendaele M: **Antimicrobial resistance in E. coli isolated from lettuce, irrigation water and soil**. In: *18th Conference on Food Microbiology: 2013*: Belgian Society for Food Microbiology (BSFM); 2013.
26. Halablab M, Sheet I, Holail H: **Microbiological quality of raw vegetables grown in Bekaa Valley, Lebanon**. *Am J Food Technol* 2011, **6**(2):129–139.
27. Falomir M, Gozalbo D, Rico H: **Coliform bacteria in fresh vegetables: from cultivated lands to consumers**. *Current research, technology and education topics in applied microbiology and microbial biotechnology* 2010, **2**:1175-1181.
28. Teale C: **Antimicrobial resistance and the food chain**. *Journal of applied microbiology* 2002, **92**(s1).
29. Commission Er: **Risk profile on the microbiological contamination of fruits and vegetables eaten raw**. In.; 2002.