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### RESEARCH ARTICLE

## COMPARATIVE STUDY ON BACTERIAL LOAD FROM POST-HARVEST VEGETABLES SOLD AT ROAD SIDE AND SUPERMARKET: IN CASE OF JIMMA TOWN, SOUTHWESTERN ETHIOPIA.

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Bacterial load, raw vegetables, unsafe marketing environment, pathogenic bacteria

#### **Abstract**

Vegetables are important protective food and highly beneficial for the maintenance of health and prevention of diseases. However, during growth, harvest, transportation and further handling the product can be contaminated with pathogens from animal and human sources. This study was aimed to assess the bacteriological load of post-harvest vegetables sold at Jimma town markets. A total of 32 vegetable samples were purchased from Ajip and kochi of Jimma town, specifically from road side and supermarket and analyzed for their bacteriological loads following standard bacteriological methods. Result showed that the total aerobic count in cabbage samples ( $2.34 \times 10^9$  to  $2.75 \times 10^9$  cfu/ml) and carrot samples ( $2.18 \times 10^9$  to  $2.5 \times 10^9$  cfu/ml) taken from the road sides were higher than the total aerobic count for cabbage samples ( $1.41 \times 10^9$  to  $1.78 \times 10^9$  cfu/ml) taken from supermarket and carrot samples ( $1.65 \times 10^9$  to  $1.72 \times 10^9$  cfu/ml) taken from the shops. The two pathogenic bacteria namely, *S. aureus* and *E. coli* were identified from all samples. Samples taken from both shops and road sides were contaminated by pathogenic bacteria. The cabbage and carrot might be contaminated as a result of handling by farmers or retailers. Improper handling and improper hygiene might lead to contamination of raw cabbage and carrot and this might eventually affect the health of the consumers. It is necessary and important that both the farmer who harvests the vegetables into bags for transportation, the marketers and consumers take necessary and appropriate precautions in preventing contamination and eating of contaminated vegetables.

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#### **Introduction:-**

Vegetables are a complex group of wide varieties of different types of crops. Most vegetables are annual or biennials, completing their life cycle in one or two years respectively. It has diverse form of propagation: by seed or vegetative parts. Many vegetables can be grown under a wide range of conditions, while others have more specific requirements for water, temperature and light (Shanmugavenu, 1989). Vegetables are considered as the leafy outgrowth of plants or plant shoots. These include plants or plant parts used in making soup and served as integral parts of main meal (Yusuf *et al.*, 2004). Vegetables can also be regarded as the edible component of plants, such components includes leaves, stalk, roots, tubers, bulbs, flowers and seed (ICMSF, 1998). They are important for food and highly beneficial for the maintenance of health and prevention of diseases. They contain

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valuable food ingredients which are essential for the proper function of the body and also contain various medicinal and therapeutic agents and are valued mainly for their high vitamin and mineral content. Studies have evaluated the association of fruit and vegetables consumption with the reduction of risk of specific diseases (Hung *et al.*, 2004).

Production practices, growth conditions and the location of the edible part during growth can affect their bacterial status, which may affect the health of consumers. Possible sources of these pathogens are soil, faeces (manure, both of human and animal origin), water (irrigation, cleaning), animals (including insects and birds), handling of the products, harvesting and processing equipment, and transportation (Aycicek *et al.*, 2006; Amoah, 2005; Beuchat, 2002).

The occurrence of bacterial populations on vegetables is recognized as a source of potential health hazard to man and animals. This is due to their production of bacteriotoxins compounds which are capable of inducing several critical clinical symptoms in man following ingestion or inhalation; even though they differ in their degree and manner of toxicity. The contamination of vegetables by bacteria could also be as a result of poor handling practices in food supply chain, storage conditions, distribution, marketing practices and transportation (Effiuvwevwere, 2000). Most of raw vegetables are normally consumed without being cooked, so the possibility of food poisoning exists (Aycicek *et al.*, 2006). The increase in consumption has been correlated with an increased frequency of outbreaks of illness associated with fresh vegetables (McMahon and Wilson, 2001).

Numerous outbreaks of illness caused by bacteria have been linked epidemiologically to consumption of fresh vegetables. Increased recognition of fresh vegetables as suspected vehicles of human illness in industrialized countries (Altekruse *et al.*, 1997; Bean and Griffin, 1990) may probably result in increased numbers of associated or confirmed cases in the future. Fresh vegetables recently have a significance source of human pathogens and human contaminants that pose a potential threat to human health worldwide and the contamination is special concern. Because most vegetables likely to be consumed raw without cooking (European Commission, 2002).

Growing and marketing of fresh vegetables in Ethiopia has been complicated by postharvest losses both in terms of quality and quantity between harvest and consumption. In Jimma town, vegetables such as cabbage and carrot are common supplementary diets. The market in the town supply vegetables from the nearby farm lands and far farm lands that cross many hundred kilometers. The problem can be enhanced from poor management of vegetables in the markets. Jimma's town market conditions that favor contamination can be raised from poor hygiene of the vendors, using bacteriologically unsafe containers, poor handling practice and unhygienic environmental conditions such as sanitarily unsafe marketing environment. The consequences of the problems are increasing loss of vegetables due to bacterial spoilage and human illness in Jimma town. However, in Jimma town, especially Ajip and Kochi local markets there are no sufficient scientific information on bacterial load of the mentioned vegetables. Therefore, the present study was aimed to assess the bacteriological load of fresh vegetables sold at Jimma town market by considering the aforementioned gap.

## **Material and Method:-**

### **Description of the study area:-**

The study was conducted in Jimma town, located 353 km southwest of Addis Ababa, and the capital city of Ethiopia. The town's geographical coordinates are 7°41'N latitude, 36°50'E longitude, and an average altitude of 1,780 m above sea level. It lies in the climatic zone locally known as "Woyna Daga" (1,500-2,400 m above sea level) which is considered to be ideal for agriculture as well as human settlement. The town is generally characterized by warm climate with a mean annual maximum temperature of 30 °C and a mean annual minimum temperature of 14 °C (Alemu *et al.*, 2011).

### **Study Design and Period:-**

Comparative cross sectional study design was conducted. Samples were collected from the two different local markets (road side and shop), Ajip and Kochi, in Jimma town. The study was conducted from March 2014 to May 2014.

### **Sampling Techniques:-**

A representative samples were selected by simple random sampling.

**Table 1:-** Sampling details of vegetables from Ajip and Kochi local markets.

Site		Vegetable sample	
Kochi		Cabbage	Carrot
	k-SH	4	4
	K-ST	4	4
Ajip	A-SH	4	4
	A-ST	4	4
Total		16	16

K-SH: - Samples taken from Kochi local market from the shop, K-ST: - Sample taken from Kochi local market from the street, A-SH: - Samples taken from Ajip local market from the shop, A-ST: - Sample taken from Ajip local market from the street.

#### Sample Collection:-

Random sampling procedure was adopted to collect the samples from Ajip and Kochi market. Two commonly used vegetables, namely carrot and cabbage, were selected for the present investigation. The vegetables were purchased and polythene zip bags were used for the collection of samples. Samples were transported aseptically to Microbiology Laboratory Department of Biology, Jimma University for further analysis. The polythene bags with vegetables were kept in the refrigerator at -14°C until it was used.

#### Sample preparation for microbiological analysis:-

For sample preparation, 25g of the samples were aseptically removed from each sample, shredded into small pieces and vigorously shaken in 225ml of sterile 0.1%(w/v) peptone water for 3 min separately to homogenize the sample (Shalini,2010). In addition, a serial dilution was made. A 10 mL of water sample was also mixed with 90 mL of peptone water using vortex mixer. Finally, appropriate serial dilutions of the suspension were spread-plated on a suitable solid media.

#### Bacterial Counts

Colonies on the Petri dishes were counted. Bacterial species were identified by comparing their morphological characters of known taxa and gram staining techniques and then confirmed by the pattern of biochemical reactions using the standard methods (Cheesbrough, 2006). Some of the Gram-negative rods were identified by indole and H<sub>2</sub>S production, gas formation, citrate utilization, motility and urease test. For Gram-positive bacteria catalase tests was used (Cheesbrough, 2006).

#### Result:-

A total of thirty two samples of cabbages and carrots were analyzed in this study. Based on cultural, morphological and biochemical testing, *S.aureus*, *Salmonella* spp, *Streptococcus* spp, *pseudomonas* sp, *E. coli* and *L.monocytogen* were identified (Table 3).

The results obtained from the study associated with the post harvest vegetables showed that total mean aerobic count for cabbage ranged from  $1.41 \times 10^9$  to  $1.78 \times 10^9$ cfu/ml while total mean coliform count ranged from  $1.5 \times 10^9$  to  $1.66 \times 10^9$ cfu/ml for the samples taken from the shops. The total mean aerobic count for cabbage ranged from  $2.34 \times 10^9$  to  $2.75 \times 10^9$ cfu/ml while the total mean coliform count ranged from  $2.4 \times 10^9$ to  $2.58 \times 10^9$ cfu/ml for the samples taken from the road sides. For carrot, the total mean aerobic count ranged from  $1.65 \times 10^9$  to  $1.72 \times 10^9$ cfu/ml while the total mean coliform count ranged from  $1.7 \times 10^9$  to  $1.92 \times 10^9$  for the samples taken from the shops. The total mean aerobic count ranged from  $2.5 \times 10^9$  to  $2.75 \times 10^9$ cfu/ml while total mean coliform count ranged from  $2.47 \times 10^9$  to  $2.58 \times 10^9$  those samples taken from the road sides.

**Table 2:-** Bacterial mean colony count (cfu/ml) for each post harvest vegetable.

Sample	Location	Total Aerobic Counts (cfu/ml)	Total Coliform Counts (cfu/ml)
Cabbage	K-SH	1.78x10 <sup>9</sup>	1.5x10 <sup>9</sup>
	K-RS	2.34x10 <sup>9</sup>	2.4x10 <sup>9</sup>
	A-SH	1.41x10 <sup>9</sup>	1.66x10 <sup>9</sup>
	A-RS	2.75x10 <sup>9</sup>	2.58x10 <sup>9</sup>
Carrot	K-SH	1.65x10 <sup>9</sup>	1.92x10 <sup>9</sup>
	K-RS	2.5x10 <sup>9</sup>	2.47x10 <sup>9</sup>
	A-SH	1.72x10 <sup>9</sup>	1.7x10 <sup>9</sup>
	A-RS	2.18x10 <sup>9</sup>	2.42x10 <sup>9</sup>

Based on morphology and biochemical tests six bacteria were isolated from cabbage and carrot samples. Morphologically, two spherical shape bacteria namely *S.aureus* and *Streptococcus* spp. and four rod shaped bacteria such as *Pseudomonas* spp., *E.coli*, *Salmonella* spp. and *L.monocytogen* were identified. Based on gram reaction, three gram positive bacteria such as *S.aureus*, *Streptococcus* spp. and *L.monocytogen* and three gram negative bacteria were isolated. This result showed that all bacterial isolates were catalase positive except *Streptococcus* spp.

**Table 3:-** Bacterial isolates based on morphology and biochemical test from Kochi and Ajip local markets.

Identified bacteria	Morphological characters	Biochemical test	
		Gram reaction	Catalase test
<i>S. aureus</i>	spherical	+	+
<i>Streptococcus</i> spp.	spherical	+	-
<i>Pseudomonas</i> spp.	bacilli (rod)	-	+
<i>E. coli</i>	bacilli (rod)	-	+
<i>Salmonella</i> spp.	bacilli (rod)	-	moderate(+)
<i>L. monocytogen</i>	bacilli (rod)	+	+

(-) Gram negative and catalase positive (+) , Gram positive and catalase negative

This result (table.4) showed that *S. aureus* and *E. coli* were isolated from Ajip and Kochi local markets shops and road sides. The prevalence of *S. aureus* in cabbage and carrot samples in the two markets have shown the degree at which such a *S. aureus* proliferate and compete successfully in respect of the other bacteria. *Salmonella* was observed only in Ajip and Kochi shops. *Pseudomonas* was only observed in cabbage samples.

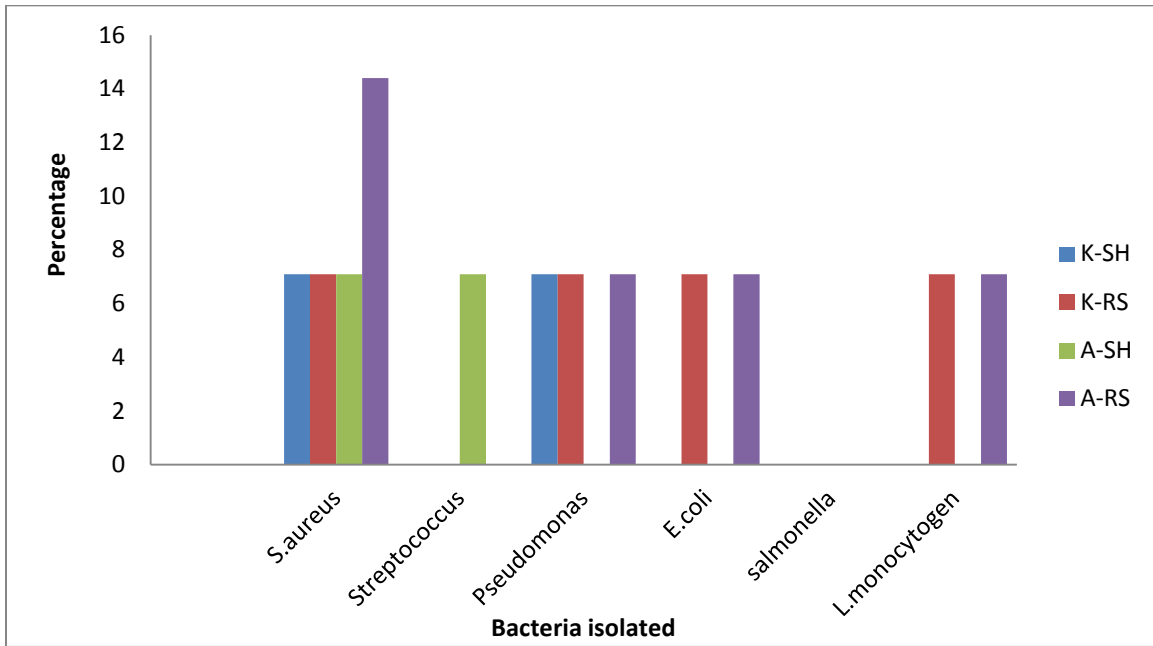
**Table 4:-** Prevalence of six bacteria isolated from vegetables sampled in Ajip and Kochi local market, Jimma town.

Bacteria isolates	Vegetable Samples							
	Cabbage				Carrot			
	K-SH	K-ST	A-SH	A-ST	K-SH	K-ST	A-SH	A-ST
<i>S.aureus</i>	+	+	+	+	+	+	+	+
<i>Streptococcus</i> sp.	+	-	+	-	-	+	-	+
<i>Pseudomonas</i> sp.	-	+	-	+	-	-	-	-
<i>E.coli</i>	+	-	+	+	+	+	+	+
<i>Salmonella</i> sp.	+	-	-	-	-	-	+	-
<i>L.monocytogen</i>	-	+	-	+	-	-	-	-

(-) indicate that no isolate, (+) indicate the presence of bacteria

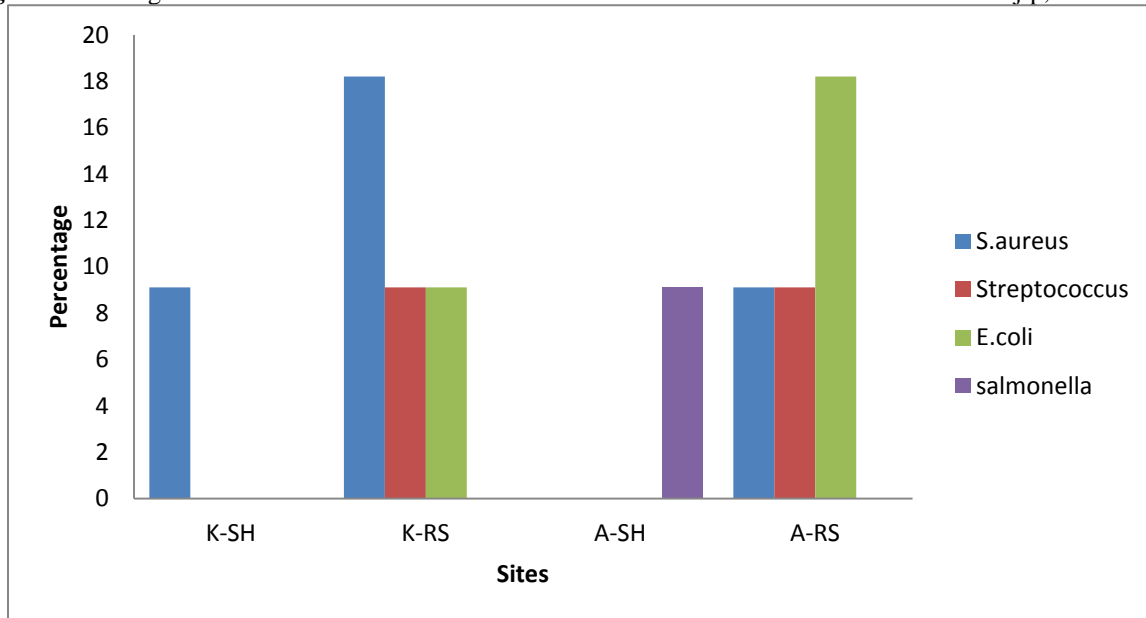
This result (figure.1) showed that (35.7%) of bacteria were isolated from samples taken from Ajip local market road sides followed by (28.6%) of bacteria were isolated from Kochi local market road sides. However; the frequency of occurrence of bacterial isolates from Kochi shops was (21.4%) as compared to the frequency of bacteria isolated from Ajip shops (14.3%).

**Fig 1:-** Percentage of occurrence of bacteria associated with contaminated Cabbage in Kochi and Ajip local market, Jimma town.



The percentage of occurrences of bacteria associated with contaminated carrot samples taken from Kochi and Ajip shops and road sides. This result showed that (36.4%) of bacteria were isolated from samples taken from Ajip local market road sides. Similarly, (36.4%) of bacteria were isolated from Kochi local market road sides. However; the frequencies of occurrence of bacterial isolates from Ajip shops was (18.2%) as compared to the frequency of bacteria isolated from Kochi shops (9.1%).

**Fig. 2:-** Percentage of occurrences of bacteria associated with contaminated carrot in Kochi and Ajip, Jimma town.



The frequency of occurrence of bacterial isolates from the road sides 18(72%) were higher than that of the shops 7(28%) table.5. In the shops, the samples were kept on the shelves which prevent vegetables from fecal contamination from the soil. *S. aureus* 6(24%) were most predominant bacterial isolates associated with contaminated vegetables samples from road sides and 4(16%) samples taken from the shops. This was followed by

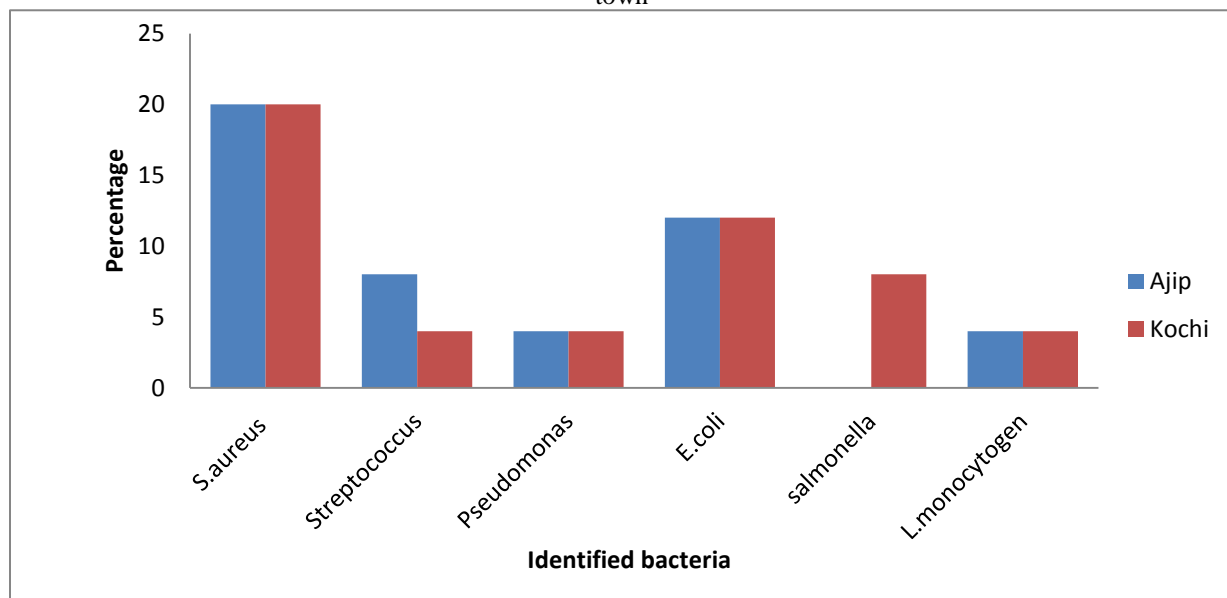
*E. coli* 5(20%) were detected in vegetable samples taken from road sides and 1(4%) from the shops. However, *Pseudomonas* spp., *Streptococcus* spp. and *L.monocytogen* were not detected in the samples taken from the shops.

**Table 5:-** Frequency of occurrences of bacteria associated with contaminated vegetables in shops and road side, Jimma town.

Identified bacteria	Frequency (%)	vegetable samples		Site	
		Cabbage (%)	Carrot (%)	Shop (%)	Road side (%)
<i>S.aureus</i>	10(40)	5(20)	5(20)	4(16)	6(24)
<i>Streptococcus</i> sp.	3(12)	1(4)	2(8)	-	3(12)
<i>Pseudomonas</i> sp.	2(8)	2(8)	-	-	2(8)
<i>E.coli</i>	6(24)	3(12)	3(12)	1(4)	5(20)
<i>Salmonella</i> sp.	2(8)	1(4)	1(4)	2(8)	-
<i>L.monocytogen</i>	2(8)	2(8)	-	-	2(8)
Total	25(100)	14(56)	11(44)	7(28)	18(72)

The percentage of occurrences of bacteria associated with contaminated vegetables from Ajip and road Kochi is shown in (Fig. 3). This result showed that the percentage of bacteria isolated in vegetable samples taken from Kochi local market (52%) was higher than the percentage of bacteria isolated from Ajip local market (48%).

**Fig. 3:-** Percentage of occurrences of bacteria associated with post harvest vegetables from Ajip and Kochi, Jimma town



### Discussion:-

In this study the total mean aerobic count for cabbage ranged from  $1.41 \times 10^9$  to  $1.78 \times 10^9$  cfu/ml while total mean coliform count ranged from  $1.5 \times 10^9$  to  $1.66 \times 10^9$  cfu/ml for the samples taken from the shops. The total mean aerobic count for cabbage ranged from  $2.34 \times 10^9$  to  $2.75 \times 10^9$  cfu/ml while the total mean coliform count ranged from  $2.4 \times 10^9$  to  $2.58 \times 10^9$  cfu/ml, for samples taken from the road side. For carrot, the total mean aerobic count ranged from  $1.65 \times 10^9$  to  $1.72 \times 10^9$  cfu/ml while the total mean coliform count ranged from  $1.7 \times 10^9$  to  $1.92 \times 10^9$  cfu/ml those samples taken from the shops. The total mean aerobic count ranged from  $2.5 \times 10^9$  to  $2.75 \times 10^9$  cfu/ml while total mean coliform count ranged from  $2.47 \times 10^9$  to  $2.58 \times 10^9$  cfu/ml those samples taken from the road sides. However, the result reported by (Adebayo *et al.*, 2012), the pathogenic bacteria associated contaminated vegetables showed that the total aerobic count for cabbage ranged from  $1.43 \times 10^6$  to  $2.10 \times 10^6$  cfu/ml while the total coliform count ranged from  $3.10 \times 10^6$  to  $4.2 \times 10^6$  cfu/ml. The total aerobic count for carrot ranged from  $2.07 \times 10^6$  to  $2.20 \times 10^6$  cfu/ml while total coliform count ranged from  $2.8 \times 10^6$  to  $3.0 \times 10^6$  cfu/ml. In comparison, the present results showed that there were high microbial load than the result presented by the above microbiologists. This was probably due to the climatic condition and altitude of the two vegetables market towns (Jimma, Ethiopia and Uyo metropolis, Nigeria),

the difference in the people, the hygienic status of the marketing sites and storage rooms, the hygienic status of the retailers, the duration of storage periods and the place and distance in which vegetables were came.

In the current study *S. aureus* was highly prevalent in both cabbage and carrot samples in the two markets followed by *E.coli* and salmonella spp. was the least abundant bacteria were observed. The prevalence of *S. aureus* in almost all the vegetables in the two markets have shown the degree at which such a microbe proliferate and compete successfully in respect of the other bacteria. This finding was in conformity with that of Uzeh *et al* (2009), who reported that, the presence of *S. aureus* in carrots, cucumber, cabbage and lettuce at food outlets within Lagos Metropolis, Northern Nigeria. And also it is in agreement with the work of Erin (2010), who reported that *Pseudomonas spp.*, *Staphylococcus spp.* and *Streptococcus spp.* were the major bacterial species that were generally present on all vegetables in Kaduna markets, Northern Nigeria.

The presence of *S. aureus*, pathogenic bacteria has public health concern and significance. The cabbage and carrot might be contaminated as a result of handling by farmers or retailers. Improper handling and improper hygiene might lead to contamination of raw cabbage and carrot and this might eventually affect the health of the consumers. Most of bacteria isolated in this study are those commonly found in soil and water. The presence of the most frequently isolated index of food quality and indicators of fecal contamination such as *E.coli* and salmonella sp. reported in this study are an indication of fecal contamination of fresh cabbage and carrot as a result of unhygienic handling (Omemu and Bakole, 2005).

This study showed that (36.4%) of bacteria were isolated from samples taken from Ajip local market road sides. Similarly, (36.4%) of bacteria were isolated from Kochi local market road sides. However; the frequencies of occurrence of bacterial isolates from Ajip shops was (18.2%) as compared to the frequency of bacteria isolated from Kochi shops (9.1%). This difference may due to the hygiene of marketing area, sanitation of the retailers, the storage rooms and the duration of storage periods.

The results showed that *S. aureus* 6(24%), *E. coli* 5(20%), *Streptococcus spp.*,3(12%), *pseudomonas sp.* and *L.monocytogen* 4(16%) totally 18(72%) were found in the road side. However, salmonella was not detected from road sides. On the other hand, half of identified pathogenic bacteria such as *S. aureus* 4(16%), *Escherichia coli* 1(4%), and salmonella 2(8%), totally 7(28%) were detected from the samples taken from the shops. This result was in agreement with Viswanathan (2001), reported that the presence of salmonella, *S.aureus*, fecal *E.coli* and *pseudomonas spp.* in cabbage and carrot.

### **Conclusion:-**

This study showed that vegetables produced in Jimma town represent a bacteriologically unsafe for consumers. It is necessary and important that both the farmer who harvests the vegetables into bags for transportation, marketers and consumers take necessary and appropriate precautions in preventing the risk. From this study, some of isolated bacteria have serious public health risk while others fasten spoilage of the vegetables. High numbers of these pathogenic bacteria in post harvest vegetables would lead to the consumer's illness with attendant symptoms and consequences of the particular or combined microbial presence. Reduction of risk for human illness associated with fresh vegetables can be better achieved through controlling points of potential contamination in the field during harvesting, during processing, storage or distribution in the retail markets food services facilities or at home. Generally, post harvest vegetables of road side are harboring many bacterial contaminants and pathogens as compared to of shops, indicating that these are protected from contamination while subsequent handling, packaging, and storage. Thus, one should buy fresh vegetables from Shop, as they are bacteriologically safe. It is therefore urgent to sensitize the consumers and retailers about vegetable contamination problems. Further studies could be recommended to evaluate the bacterial pathogens at strain level and their antimicrobial resistance pattern.

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