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Research Article

Bacteriological Quality Assessment of Fresh Lettuce and Tomato from Local Markets of Gondar, Ethiopia

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Abstract

Fruits and vegetables are source of nutrients, micronutrients, vitamins and fiber which are important for health and wellbeing of human. However, they are also well recognized as potential sources of bacterial infections. The aim of this study was to assess the bacteriological quality of fresh lettuce and tomato, 60 samples of tomato and lettuce were purchased from three places of the road side markets of Gondar town. Samples were analyzed for the prevalence of pathogenic microorganisms by standard plate count. All the samples were contaminated by pathogenic microorganisms although the level of contamination varied. *Escherichia coli* (65%) were the most dominant bacterial isolate associated with both tomato and lettuce. This was followed by *Pseudomonas aeroginosa* (61.7%), *Enterobacter aerogenes* (60%), *Staphylococcus aureus* (55%), *Klebsiella pneumoniae* (43%) and *Salmonella* species (10%). In all the samples tested, lettuce carried higher incidence of the pathogens than tomato. The handling practices of the retailers is generally low where most of the respondents use moistening of fruits and vegetables with contaminated water that promote the spread of pathogenic microorganisms. Poor hygienic practice is the main contributing factor for high level of contamination hence consumer's awareness on the dangers of pathogens and retailers training on how to protect fruits and vegetables from all sources of contamination is highly important.

Keywords: Lettuce, tomato, bacteriological quality, *Escherichia coli*, *Salmonella*, handling practices.

Introduction

Fresh fruit and vegetables are very essential components of the human diet and there is substantial evidence in favor of the health and nutritional benefits associated with the consumption of fresh vegetables. Their role in reducing the risk of lifestyle associated illnesses such as heart disease, diabetes and cancer has resulted in a further increase in their desirability and consumption (Legnani *et al.*, 2010). For example, tomato (*Lycopersicon esculentum*) is the second most important vegetable worldwide, in terms of the amount of vitamins and minerals it contributes to the diet (Kalia and Gupta, 2006). In order to benefit significantly from these health properties and other unspecified uses, the World Health Organization (WHO) recommends an intake of 400 g, or five to nine portions, of fresh fruits and vegetables per day (Matthews, 2006). The WHO has issued reports claiming that correct fresh produce intake alone could save 2.7 million lives a year and that 31% of heart disease cases are due to an insufficient intake of such foods (WHO, 2003; Johnston *et al*., 2006). There are a number of reports indicating that raw vegetables may harbor potential food borne pathogens.

Enteric pathogens have been found on a wide variety of produce including lettuce, tomatoes, and cantaloupes (Nutt *et al*., 2003). Outbreaks of *Salmonellosis* have been attributed to the consumption of contaminated tomatoes (Deza, 2003). Pathogens most commonly associated with fruit and vegetables include *Salmonella*, *Shigella*, *E. coli* 0157 H7, *Listeria*, *Campylobacter*, *Cryptosporidia* and viruses such as Hepatitis A. Specifically for lettuce the most severe microbiological threats are contamination with enterohemorrhagic *Escherichia coli* and *Salmonella* enteric (Anderson *et al.*, 2011). *Salmonella* was the most important pathogen in food borne outbreaks in Brazil between 2000 and 2012 (Gomes *et al*., 2013). One of the most commonly cited sources of outbreaks of food poisoning associated with fresh produce, is the contamination of lettuce with *Salmonella* species (Safe Food, 2007). For many countries, particularly developing ones, fruit and vegetable products have become valuable, making a considerable contribution to the economy as well as to the health of a country's population.

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For example, exports of fruits and vegetable products in Ethiopia have increased from 63,140 tons in 2009/10 to 166,000 tons in 2015/16. On the other hand Vegetables primary production of Ethiopia increased from 807,483 tons in 1998 to 1.63 million tons in 2017 growing at an average annual rate of 4.17%. Although raw-eaten fruits and vegetables have a primary role in the diet, they can cause foodborne diseases if they are contaminated (Allende *et al*., 2009; FAO/WHO, 2008). Research indicates that foodborne diseases have significantly increased during the recent years, due mainly to the consumption of raw-eaten fruits and vegetables (Abadias *et al*., 2008). Ethiopia, like many other countries is affected by burden of food-borne diseases due to contaminated foods (NCC, 2010).

There are a number of sources of microbial contamination, all of which must be controlled. Irrigation with poor-quality water is a major source of contamination to fruits and vegetables with foodborne pathogens. In Ethiopia the major producers of horticultural crops are small scale farmers, in which production being mainly rain fed and few under irrigation. Tomatoes, carrots, lettuce, beetroot, cabbage, spinach and Swiss chard are usually restricted to areas where irrigation water is available. Likewise, the use of raw animal manure for fertilizer can increase the threat of contamination of fruits and vegetables (Ackermann, 2010). However, as a consequence of the use of manure or manure derived organic fertilizers, it has been proposed that the microbiological quality of organic produce is lower than that of conventional production (Maffei *et al.,* 2013). Poor handling, storage, transportation and cleaning practices of the lettuce and tomato by retailers also contribute to vegetable contamination at the market (Beuchat, 1995). As a result, these organisms infest vegetables while still in the field, and are usually transmitted by contaminated wash water and spread by ineffective hygiene practices (Beuchat, 1996). Enteric pathogens and, consequently, their indicators (Coliforms in general, fecal coliforms and fecal streptococci) have received special attention as contaminants of horticultural products in recent years. Despite all potential risks, irrigated farming of high value crops is livelihood to many urban residents since it provides employment and income (Weldesilassie *et al.,* 2009). Most vegetables including lettuce is supplied by farmers who irrigate their crops using polluted river water or diluted wastewater, hence vegetables produced under such poor sanitation are vulnerable for contamination (Blaak *et al.,* 2015). However, information's on the microbial safety of food items in Ethiopia are limited (Habtu, 2011). This is a real gap that has to be filled by governing authorities and researchers. Unavailable information's on the microbial quality of food items such as fruits and vegetables may not motivate the governing authorities to take an action which reduces the risk of contracting infections due to the consumption of contaminated food items. Apart from the commercial and nutritional values of these raw-eaten vegetables and fruits, they can harbor pathogenic microorganisms that may potentially cause illness to consumers, if they are contaminated. Being tomato and lettuce are part and parcels of these produces, the present study aimed to determine the status of bacteriologic contamination on lettuce (*Lactuca sativa*) and tomato (*Lycopersicon esculeutum)* sold in three local and road side markets of Gondar town namely, 'Autoparko' (A), 'Kidame gebeya' (K), and 'Azezo' (Az) and to contribute to knowledge of the bacteriologic quality of these products. Lettuce and tomato which are usually consumed raw are selected as model crops to be assessed bacteriologically because of their importance as vehicle for different pathogens.

Materials and methods

Sample collection and preparation: The study was conducted at the University of Gondar, Ethiopia. Samples were collected from different places of Gondar town. By applying non-probability sampling method, a total of n=60 (30 tomatoes and 30 lettuces) samples were collected from the various retail markets of Gondar town. All samples were collected between September and February 2016 aseptically and transported to applied microbiology laboratory of University of Gondar in sterile plastic bag separately. Only tomatoes of good appearance were selected, whilst for lettuces the damaged, wounded and old leaves have been removed. Thus, only edible leaves or fruits were sampled. Analysis of these samples was conducted immediately.

Microbiological analysis: For microbiological analysis, 25 g of sample were aseptically taken from each sample (weighing 225 g each) and vigorously shaken in 225 mL of sterile Butterfield's phosphate buffer solution for 2 minutes. One milliliter of homogenized sample was diluted to ten folds using sterile Butterfield's phosphate buffer blank and then this was further diluted serially to (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}). These dilutions of fruit and vegetable samples were necessary to perform MPN counts and plating to obtain isolated colonies (JFDA, 2010).

Enumeration of Coliforms, Fecal Coliforms (Thermotolerant) and *E. coli:* Three tubes containing Lauryl Sulfate Tryptose (LST) (Merck) broth and Durham tube per dilution were inoculated using 0.1 ml aliquots from each dilution and incubated at 35°C for 48 h. The number of positive tubes per dilution (Positive tubes = growth/gas production) were recorded. Using the appropriate three-tube combination and the MPN tables the numbers of "presumptive coliforms" were determined. From each positive LST tube 0.1 mL aliquots were transferred into tubes containing 2% BGLB broth (Merck) and Incubated at 35°C for 48 h.

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Up to 5 suspicious colonies were transferred from each L-EMB plate to Plate Count Agar (PCA) slants and incubated for 18-24 h at 35°C and used for further testing. Gram stain was also being carried out. All cultures appearing as Gram-negative, short rods were tested for the Indole, Methyl red, Voges-Proskaur, and Citrate (IMViC) reactions and also re-inoculated back into LST to confirm gas production (JFDA, 2010). Aerobic mesophilic Count (AMC) was done by considering plates with colonies between 25- 250 and number of microorganisms were computed Based on FSSAI, 2012. All the positive tubes (obtained in MPN of lettuces and tomatoes) were sub-cultured onto the Eosin Methylene Blue (EMB) agar, Mannitol salt (MSA) agar, and Mac Conkey's agar, Nutrient agar, Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 18-24 h. Biochemical tests were done according to Kiiyukia (2003) and McFadden (1980). Pathogens such as *Pseudomonas* and *Salmonella* were detected based on standard techniques.

Antibiotic susceptibility test: The antibiotic susceptibility tests were carried out against the isolated bacterial strains with six antibiotics by disc diffusion method, compared with clinically isolated control-*E. coli* (CLSI, 2006). Antibiotic discs of Ampicillin (10 µg), Gentamicine (10 µg), Penicillin G (10 µg), Amikacin (30µg), Vancomycin (30µg) and Tetracycline (30 µg) was used for study. The inoculated plates were incubated at 37°C for 24 h and the inhibition zones were observed and measured either by calipers including the diameter of the disc. Interpretation of the results (susceptibility or resistance of the organisms) to each drug tested will be determined based on the published CLSI guidelines (CLSI, 2006). All the experiments were carried out in triplicate.

Data analysis: Variance analysis methods (ANOVA) have been used in interpretation of results with version 16, SPSS. T-Test was used in the evaluation of the significance of the difference between the groups. The significance between the values were evaluated at 95% confidence $p<0.05$.

Results and discussion

Bacteriological status of fresh Tomato and lettuce: All the samples tested were found to be contaminated although the level of contamination varied. The level of contamination was found to be highest in lettuce samples (Table.1) with the grand mean values of 3.2×10^5 (TAMC), 7.5×10^6 (TCC), 5.1×10^6 (TFC); and lowest in tomato (Table 2) with the grand mean values of 2.3×10^5 (AMC), 2.9×10^6 (TCC), 8.1×10^5 (TFC). The total *E. coli* count (TEC) level on lettuce collected from all the three markets were generally higher than (1.3×10^2 CFU/g, grand mean value) the TEC level on tomato (Table 2) of the same sampling area (5.5×10^1 CFU/g, grand mean value).

Woldetsadik *et al*. (2017) indicated that high fecal coliform levels exceeding the recommended thresholds have been found in Lettuce. Similar findings have been reported in sub Saharan cities (Amoah *et al.,* 2008).

The Mean Total Aerobic Mesophilic Count (TAMC), Total Coliform Count (TCC), Total fecal Coliform Count (TFC) and Total E.coli Count (TEC) for Tomato and Lettuce in AKAz: The total aerobic mesophilic count (TAMC) bacteria numbers on tomato and lettuce samples from all the three sites (AKAz) were ranged from 9.5×10^3 to 3.8×10^5 CFU/g and 1.9×10^5 to 3.9×10^5 CFU/g, respectively (Table 1). The lowest TAMC values for tomato were recorded at AKAz (9.5×10^3 CFU/g) and of lettuce were recorded at Kidame gebeya (1.9×10^5 CFU/g) (Fig. 1 and 2). The differences in TAMC levels on lettuce at the three sites were statistically significant (P=0.001). A site-by-site multiple comparison for lettuce samples in terms of TAMC levels between Autoparko and Kidame gebeya was statistically significant (P=0.015). However, TAMC level between Autoparko and Azezo (P=0.143), and Kidame gebeya and Azezo (P=0.280) sampling sites were not statistically significant. This shows that samples analyzed from Autoparko were much contaminated with TAMC even though Kidame gebeya and Azezo samples were almost having the same level of TAMC. For tomato, a site-by-site multiple comparison samples in terms of TAMC level between Autoparko and Kidame gebeya was statistically significant ($P=0.04 < 0.05$). However, TAMC level between Autoparko and Azezo ($P= 0.069 > 0.05$), and Kidame gebeya and Azezo ($P=0.825 > 0.05$) sampling sites were not statistically significant. This shows that samples analyzed from Autoparko were much contaminated with TAMC even though Kidame gebeya and Azezo samples were almost having the same level of TAMC.

The total coliform (TCC) bacteria numbers on tomato and lettuce samples from all the three sites (AKAZ) were ranged from 1.9×10^4 to 1.1×10^7 CFU/g and 9.3×10^5 to 1.1×10^7CFU/g respectively. The lowest TCC value for tomato and lettuce were recorded at Autoparko and Kidame gebeya or Azezo respectively (Fig. 3 and 4). The differences in total coliform contamination levels at the three sites were statistically significant (P= 0.001). TCC can be considered as hygiene indicator, especially for fecal contamination. Their presence indicates that pathogens might be present due to fecal contamination by human, animal, or water used for irrigation (Park *et al.,* 2013). A site-by-site multiple comparison for samples in terms of total coliform contamination levels between Autoparko and Kidame gebeya and Autoparko and Azezo were statistically significant (P= 0.002 and 0.001) respectively. However, total coliform contamination of lettuce between Kidame gebeya and Azezo sampling sites was not statistically significant (P=0.738) (Table 2).

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Table 1. Mean values ± (log S.D) of Total Aerobic Mesophilic Count, Total Coliforms Count, Fecal Coliform Counts and Total *E. coli* count for lettuce samples in AKAz.

Sample area	Lettuce			
	TAMC± (log SD)	TCC ± (log S.D)	TFC± (log S.D)	TEC (log S.D)
Autoparko (A)	$3.6 \times 10^5 \pm 4.6$	$7.0 \times 10^6 \pm 6.6$	$5.7 \times 10^6 \pm 6.7$	$1.8 \times 10^2 \pm 2.1$
Kidame gebya (K)	$2.9 \times 10^5 \pm 5.8$	$6.6 \times 10^6 \pm 6.7$	$8.3 \times 10^6 \pm 6.6$	$1.3 \times 10^2 \pm 2.1$
Azezo (Az)	$3.2 \times 10^5 \pm 4.5$	$8.8 \times 10^6 \pm 6.1$	$1.4 \times 10^6 \pm 6.5$	$9.3 \times 10^1 \pm 1.9$
Mean (grand)	$3.2 \times 10^5 \pm 4.5$	$7.5 \times 10^6 \pm 6.1$	$5.1 \times 10^6 \pm 6.5$	$1.3 \times 10^2 \pm 1.1$

Table 2. Mean values ± (log S.D) of Total Aerobic Mesophilic Count, Total Coliforms Count, Fecal Coliform Counts and Total *E. coli* count for Tomato samples in AKAz.

Sample area	Tomato			
	TAMC± (log SD)	TCC ± (log S.D)	TFC± (log S.D)	TEC (log S.D)
Autoparko(A)	$2.9 \times 10^5 \pm 5.1$	$1.7 \times 10^6 \pm 6.1$	$6.8 \times 10^5 \pm 5.9$	$4.5 \times 10^1 \pm 1.9$
Kidame gebya (K)	$2.0 \times 10^5 \pm 4.9$	$3.7 \times 10^6 \pm 6.6$	$6.7 \times 10^5 \pm 5.9$	$7.0 \times 10^1 \pm 2.2$
Azezo (Az)	$2.1 \times 10^5 \pm 4.7$	$3.2 \times 10^6 \pm 6.0$	$1.1 \times 10^6 \pm 5.4$	$5.0 \times 10^1 \pm 1.8$
Mean (grand)	$2.3 \times 10^5 \pm 4.7$	$2.9 \times 10^6 \pm 6.0$	$8.1 \times 10^5 \pm 5.4$	$5.5 \times 10^1 \pm 1.6$

Fig. 1. TAMC (CFU/g) on tomato in Autoparko (A), Kidame gebya (K), Azezo (Az).

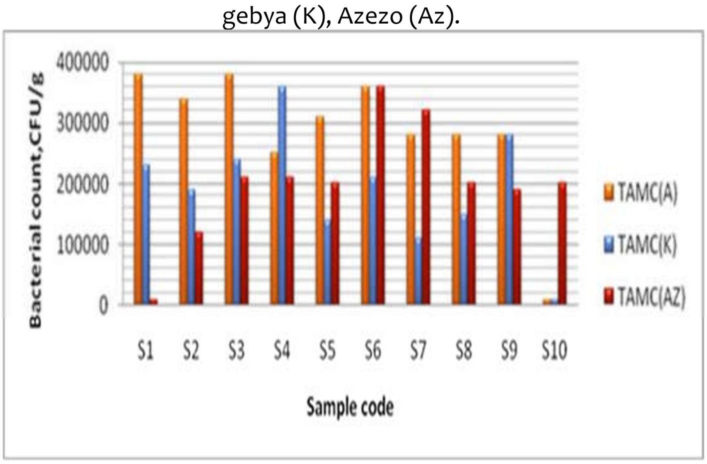

Fig. 3. TCC (CFU/g) on tomato in Autoparko (A), Kidame gebya (K), Azezo (Az).

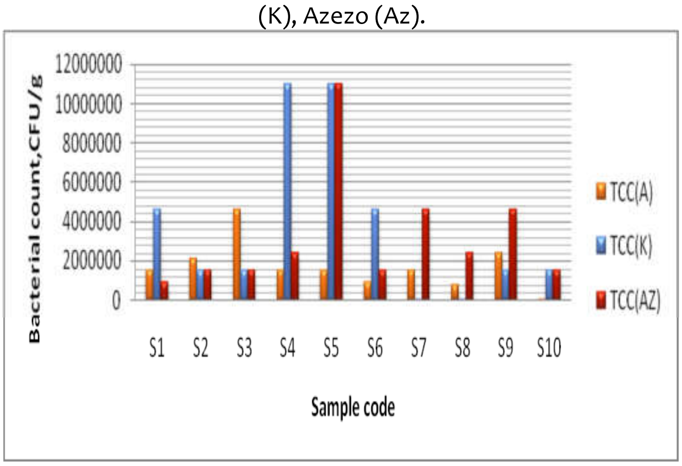

Fig. 2. TAMC (CFU/g) on lettuce in Autoparko (A), Kidame gebya (K), Azezo (Az).

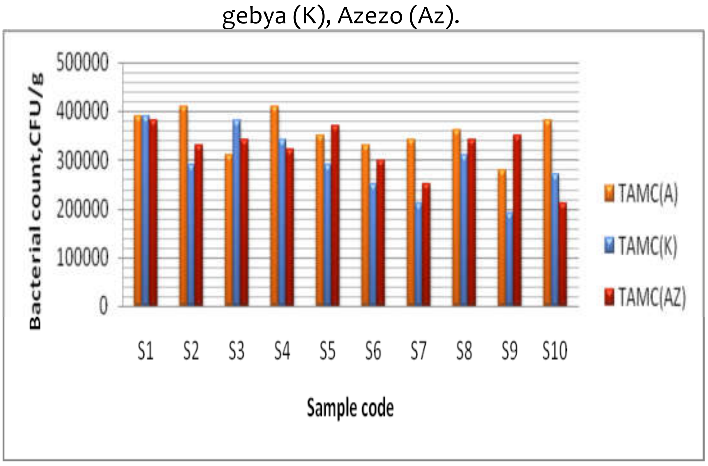

Fig. 4. TCC (CFU/g) on lettuce in Autoparko (A), Kidame gebya (K), Azezo (Az).

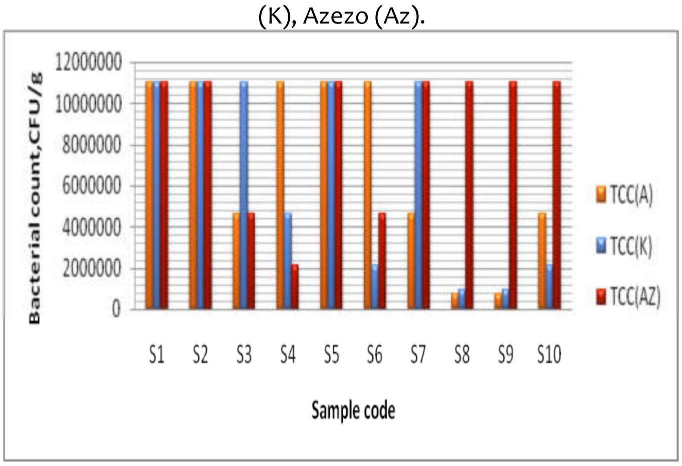

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Fig. 5. TFC (CFU/g) on tomato in Autoparko (A), Kidame gebya (K), Azezo (Az).

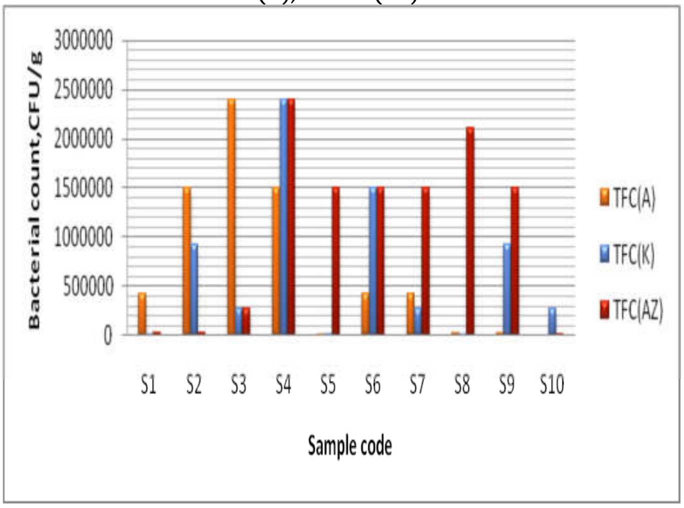
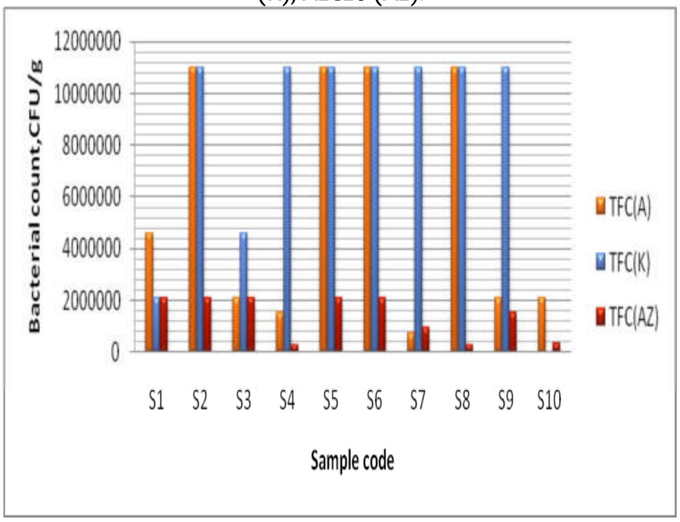


Fig. 6. TFC (CFU/g) on lettuce in Autoparko (A), Kidame gebya (K), Azezo (Az).



This shows that samples analyzed from Autoparko were much contaminated with total coliform even though Kidame gebya and Azezo samples were almost having the same level of contamination. In general the numbers of bacteria recorded in this study were still above the ICMSF (1998) limit of 10^3 to 10^5 CFU/100g wet weight of vegetables usually eaten raw. The TCC reported in this study is higher than the report of De Giusti *et al.* (2010): 10^2 - 10^4 CFU/g, it is however, lower than the findings of Viswanathan and Kaur, (2001): 10^6-10^9 CFU/g and it is in agreement with the reports of Aycicek *et al.* (2006): 10^4-10^7 CFU/g. The probable reasons for the discrepancy of results between this study and the reviewed studies may originate from a number of factors such as geographical variation, seasonal variation, time of sample collection, hygiene of the environment, handling practices and incubation time.

Fig. 7. TEC (CFU/g) on tomato in Autoparko (A), Kidame gebya (K), Azezo (Az).

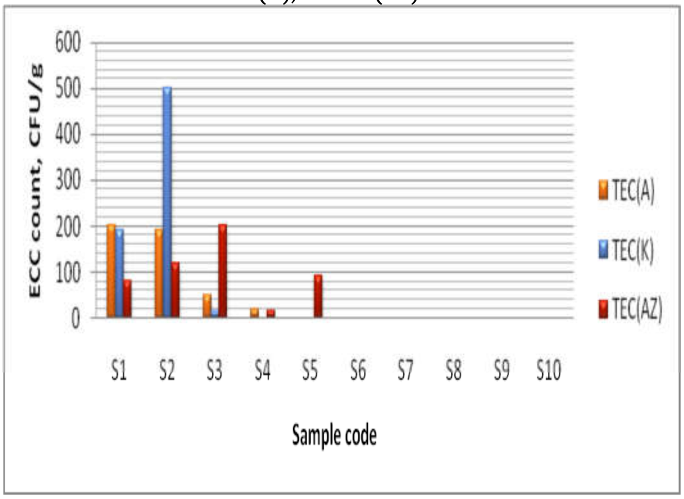
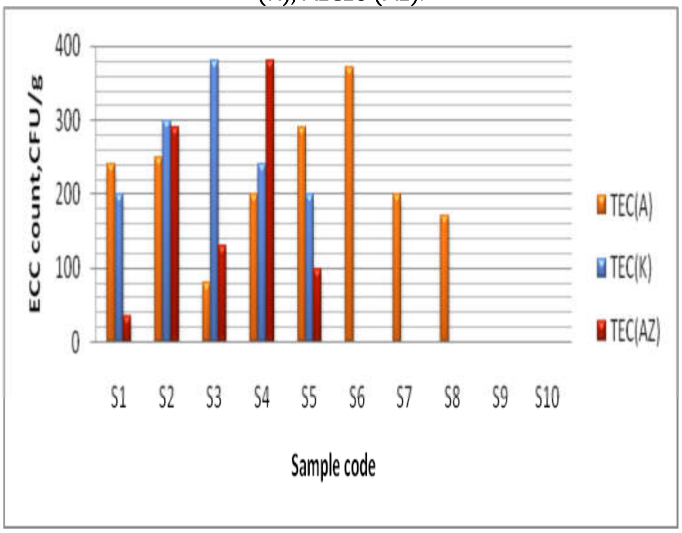


Fig. 8. TEC (CFU/g) on lettuce in Autoparko (A), Kidame gebya (K), Azezo (Az).



The presence of *E. coli* in the samples examined is an indicative of fecal contamination. According to the level of *E. coli*, (PHLS, 1996) guidelines, this study showed that 25%, 25%, and 50% of positive tomato samples (12/30) examined were contaminated with *E. coli* and were considered as "acceptable", "satisfactory" and "unsatisfactory" respectively. Likewise, 22.2% and 77.8% of positive lettuce samples (18/30) were contaminated with *E. coli* and were regarded as "acceptable" and "unsatisfactory" respectively. Therefore, unsatisfactory results (50% of tomato and 77.8% of lettuce) are outside of acceptable microbiological limits and are indicative of poor hygiene or food handling practices. The mean values of fecal coliform (TFC) bacterial contamination on tomato and lettuce from all the three sites ranged from 6.7 x 10^5 to 1.1 x 10^6 CFU/g and 1.4 x 10^6 to 8.3 x 10^6CFU/g respectively.

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Table 3. Frequency of occurrences of bacteria associated with tomato and lettuce in Autoparko, Kidame gebeya, and Azezo.

Sample type	Pathogens					
	P. aeruginosa	*S. aureus*	*K. pneumoniae*	*Salmonella* spp.	*E. aerogenes*	*E. coli*
Lettuce	19 (63.3%)	17 (56.3%)	14 (46.2%)	4 (13%)	16 (53.3%)	21 (70%)
Tomato	18 (60.5%)	16 (53.3%)	12 (40%)	2 (6.7%)	14 (46.7%)	18 (60%)
Total	37 (61.7%)	33 (55%)	26 (43.3%)	6 (10%)	30 (60%)	39 (65%)

Similarly, the highest values recorded for tomato and lettuce were the same at the three sites (2.4 x 10^6 CFU/g), but for lettuce same values were recorded (1.1 x 10^7) in Autoparko and Kidame gebeya. The lowest TFC values for tomato and lettuce were recorded at Autoparko (4.0 x 10^3 CFU/g) and Kidame gebeya (2.0 x 10^4 CFU/g) respectively (Fig. 5 and 6). The fecal coliform contamination levels of lettuce at the three sites (AKAz) were statistically significant. A site-by-site multiple comparison for samples of lettuce in terms of fecal coliform contamination levels between Azezo and Autoparko, Azezo and Kidame gebeya, and Autoparko and Kidame gebeya were statistically significant (P=0.015, 0.000 and 0.000 < 0.05) respectively. This shows that samples analyzed from the three sites were not equally contaminated with fecal coliforms. For tomato, a site-by-site multiple comparison for samples in terms of fecal coliform contamination levels between Azezo and Autoparko, Azezo and Kidame gebeya, and Autoparko and Kidame gebeya were not statistically significant (P=0.286, 0.724 and 0.977 > 0.05) respectively. This shows that samples analyzed from the three sites were equally contaminated with fecal coliform. This can be attributed to the fact that the incidence of coliforms may also be depended on the quality of water used for irrigation and handling along with the type of manure used. The mean values of *Escherichia coli* bacteria contamination on tomato and lettuce from all the three sites ranged from 1.0 x 10^2 to 2.3 x 10^2 CFU/g and 1.9 x 10^2 to 2.6 x 10^2CFU/g. Similarly, the highest and the lowest values were recorded at Kidame gebeya (5.0 x 10^2 CFU/g) (Fig. 7 and 8). The *E. coli* contamination levels for tomato and lettuce at the three sites were not statistically significant (P> 0.05). A site-by-site multiple comparison for samples of lettuce in terms of TEC levels between Azezo and Autoparko, Azezo and Kidame gebeya, and Autoparko and Kidame gebeya were not statistically significant (P=0.392, 0.605 and 0.732) respectively. This shows that samples analyzed from the three sites were equally contaminated with *E. coli.*

Frequency of occurrences of bacterial pathogens associated with tomato and lettuce in AKAz: The result showed that *Escherichia coli* (65%) were most predominant bacterial isolates associated with tomato and lettuce (Table 3). This was followed by *Pseudomonas aeruginosa* (61.7%), *Enterobacter aerogenes* (60%), *S. aureus* (55 %), *Klebsiella pneumoniae* (43 %), *Salmonella species* (10%).

In all samples tested, the result of this study showed that lettuce carried higher incidence of *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *Salmonella* species, *E. aerogenes*, and *E. coli* (63.3%, 56.7%, 46.7%, 13.3%, 53.3% and 70% respectively). Tambekar and Mundhada (2006) reported that the food borne illness associated with the consumption of fresh fruits and vegetables eaten raw sold at retail markets of Amaravati city, India and samples were also analyzed for the presence of dominant enteric bacterial pathogens were *Escherichia coli* (40%), followed by *Pseudomonas aeruginosa* (25%), *Salmonella* species (16%), *Staphylococcus aureus* (6%), *Klebsiella* species (3%) and *Enterobacter* species (1%).

A study conducted in Norway indicated that 8.9% of the Lettuce samples collected were contaminated by *E. coli* (Loncarevic *et al*., 2005). Solomon *et al.* (2003) showed in experimental study that manure contaminated with high numbers of *E. coli* could be a source of transmission of this pathogen to the plant tissue. However, in lettuce, 1.3% prevalence of *Salmonella* and 3.8% *E. coli* was reported by Ceuppens *et al.*, (2014). No any bacterial pathogens such as *Shigella* and *E. coli* were detected in any of the leafy vegetables and fruit samples collected from Canada (Denis *et al.*, 2016) and hence contamination of leafy vegetables by bacterial pathogens was found to be rare and sporadic occurrence. However, Salmonella was detected only at very low levels (P< 0.08%) in leafy vegetables (Denis *et al.*, 2016).

The dominance of *Pseudomonas aeruginosa* and coliforms amongst the bacterial genera identified from tomato and lettuce (Table 3) is not surprising as (Lund, 1992) reported that the majority of bacteria found on the surface of plants are usually Gram-negative and belong either to the *Pseudomonas* group or to the *Enterobacteriaceae.* The *Staphylococcus aureus* isolated was an indication of poor hygienic practices by both the farmers and sellers. *Salmonella* species were detected in 6 of 60 (10 %) fresh tomato and lettuce samples. The presence of *Salmonella* spp. in precut ready-to-eat herbs is legally unsatisfactory as this exceeds the food safety criteria in Regulation (EC) No. 2073/2005 (as amended). Ready-to-eat foods contaminated with *Salmonella* species are unsafe. They are considered to be detrimental to health and/or unfit for human consumption as they violate the food safety requirements (Article 14) of Regulation (EC) No.178/2002.

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Table 4. Antimicrobial susceptibility test results for bacterial isolates.

Antibiotic (disk potency)	Isolates (Inhibition zone diameter in mm)														
	P. aeruginosa			*E. coli*			*K. pneumoniae*			*Salmonella* spp.			*S. aureus*		
	I	S	R	I	S	R	I	S	R	I	S	R	I	S	R
Penicillin G (10µg)	-	-	8.3	-	-	7.9	-	-	8.7	-	-	-	-	-	23
Ampicillin (10µg)	-	-	8.7	-	-	8.3	15	-	-	-	17	-	-	-	-
Amikacin (30 µg)	-	-	13	-	-	8.5	-	27	-	-	15	-	-	-	-
Vancomycin (30µg)	-	17	-	-	-	21	-	-	20	-	-	-	-	22	-
Tetracycline (30µg	-	24	-	15	-	-	-	21	-	13	-	-	-	-	-
Gentamicine (10µg)	-	17	-	-	23	-	-	27	-	-	19	-	-	16	-

Note: R (Resistant), I (Intermediate), and S (Susceptible).

Table 5. Characteristics of respondents/retailers (n=45) in Autoparko, Kidame gebeya and Aezo of Gondar town.

Characteristic	Frequency	Percentage (%)
Sex		
Male	8	17.8
Female	37	82.2
Age group (Year)		
< 20	3	6.7
21- 30	7	15.6
31- 40	16	35.6
>40	19	42.2
Educational level		
Primary	11	24.4
Secondary	6	13.3
Tertiary	0	0
Illiterate	28	62.2
Experience in selling fresh produces (year)		
1- 2	15	33.3
3- 5	18	40.0
6- 8	9	20.0
>8	3	6.7

Antibiotic sensitivity test results for isolated bacterial pathogens: Based on the published CLSI guideline limits (CLSI, 2006), the results showed all isolates were completely susceptible to Vancomycin and Gentamicine (Table 4). On the other hand, all isolates showed complete resistance to Penicillin. *Klebsiella pneumoniae* showed susceptibility to Amikacin, Vancomycin, Tetracycline, and Gentamicine with the inhibition zones 27 mm, 20 mm, 21 mm, and 27 mm respectively, but intermediate for Ampicillin (15 mm) (Table 4). The inhibition zone diameter of *P. aeruginosa* was 8.3 mm, 8.7 mm, and 13 mm for Penicillin, Ampicillin, and Amikacin respectively, and is resistant to these antibiotics, but it was susceptible to Vancomycin, Tetracycline and Gentamicine (Table 4).

Biochemical profile and microbial study results of bacterial isolates: The isolated colonies such as *P. aeruginosa* displayed positive to citrate test but negative to Methyl Red test, Voges-Proskauer test, glucose, arabinose, lactose, sorbitol and sucrose fermentation tests. *E. coli* showed positive response to Indole test, Methyl Red test and the entire of all the carbohydrate fermentation tests. Based on the microscopic studies it was confirmed that, all the above identified coliform bacteria were found to be Gram negative except *S. aureus* as well as motile in nature, with the exception of *K. pneumoniae* and *S. aureus* were found to be non-motile.

Handling practices of retailers in AKAz: The results of the present study showed that 13 (28.9%) of the respondents pack and store the fruits in plastic sacks, 7 (15.6%) in baskets usually made from woven grasses, and 23 (51.1%) in wooden crates (Table 6). Almost none of the respondents have used plastic crates. Regarding the handling practices of retailers the result of this study showed that all the respondents (100%) have prolonged the shelf-life of unsold fruits and vegetables by moistening with water than using refrigeration.

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Table 6. Handling practices of retailers in Autoparko, Kidame gebeya, and Aezo of Gondar town.

Handling practices	Frequency	Percentage (%)
Kind of fruit and vegetable vended		
One kind	-	-
More than one kind	45	100
Styles of fruit and vegetable display		
Separately	-	-
Side by side	45	100
Source of fresh produces		
Farm gate	-	-
Selling point In the market	32	71.1
Delivered by the farmer	13	28.9
Washing before selling		
Yes	-	-
No	45	100
Actions for unsold (over 12 h) fresh produces		
Discard	-	-
Use refrigerator	-	-
Moistening with water	45	100
Containers used for transport and storage		
Plastic crates	2	4.4
Wooden crates	23	51.1
Plastic sacks	13	28.9
Baskets	7	15.6
Frequency of using containers		
For one round	-	-
For More than one round	45	100
Preference for cleaning containers		
Cleaned, after each round	-	-
No cleaning	45	100
Knowledge of fruit and vegetable hygiene/safety		
Yes	6	13.3
No	39	86 .7

Moistening fruits and vegetables with contaminated water and handling of produce by infected vendors and consumers in the marketplace promote the spread of pathogenic microorganisms (Beuchat and Ryu, 1997). The preferred packaging or storing materials were reported to be cheap and mostly available. And are used over and over again (100% of the respondents responded) until the materials are broken down and thorn into pieces. In all the three markets of Gondar, 45 (100%) of the respondents reported washing fruits and vegetables ahead of selling was not practiced. Hygienic conditions of fruits in the market need to be effectively improved to prevent them from any source of contamination. The results showed that all of the respondents (100%) prolonged the shelf life of unsold fruits and vegetables by moistening with water rather than using refrigeration. Moistening fruits and vegetables with contaminated water and handling of produce by infected vendors and consumers in the marketplace promote the spread of pathogenic microorganisms (Beuchat, 1999).

As it has indicated so far (Table 7), the retailers' knowledge on safe handling practices 39 (86.7%) respondents reported that they have no formal knowledge on safe handling practices and management. Regarding the education background of retailers, 28 (62.2%) are illiterate while 11(24.4%) had primary education and the rest 6 (13.3%) had secondary education. The observations indicate that there are both formal educational and post-harvest handling Knowledge gaps which in turn affect fruit and vegetable hygiene in the market. In order to instill awareness and change behavior regarding fruits and vegetables quality hygiene training is crucial (WHO, 2006). From the observation, all retailers used a single common balance for weighing different kinds of fruits; this may result in cross contamination. They lacked sanitary practices and personal hygiene was not observed. The displaying area for fruits and vegetables were found to be Unhygienic.

Unhygienic surroundings, improper waste disposal system and inadequate water supply attracts house-flies or fruit flies, probably further increases food contamination (Chumber *et al.*, 2007). Contaminated hands of vendor and perhaps lack of knowledge of hygienic practices and safety of food products are the main contributing factors of contamination (Fang *et al*., 2003). Even feces of animals like donkeys and humans were observed just around the displaying sites. Swarms of flies were also common.

Conclusion

The results in the present study clearly indicate the poor hygienic conditions of lettuce and tomato where many kinds of enteropathogenic bacteria were found. Such foods are hazardous to the consumers. The consumers are at risk of contracting food borne infections, therefore food producers, distributors and vendors are responsible for ensuring that their products meet all applicable food safety requirements protecting fruit/vegetable displaying sites from fecal contamination and containers used for displaying, transportation and storage facilities shall be kept clean and dry.

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