Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2016; 4(4D):1398-1401 **ISSN 2347-954X (Print)**
©Scholars Academic and Scientific Publisher
(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

Original Research Article

Biological burden of Raw Vegetables in Hail City

Ahmed Abu Rayyan[1]*, Hamid El .Nourain Hamdan[1], Walid Abu Rayyan[2], Ibrahim S. Majali[3]
[1]College of Public Health and Health Informatics, University of Hail, Hail, KSA
[2]Faculty of Medicine, University of Hail, Hail, KSA
[3]Al-Ghad International College for Applied Medical Sciences, Tabuk, Saudi Arabia

***Corresponding author**
Ahmed Abu Rayyan
Email: ahmadrayyan.yahoo.com

Abstract: This study aims to investigate the microbiological quality of fresh vegetables collected from several cultural ,agricultural sites, Supermarkets, Central vegetable market in Hail city during the summer2012. A total of 15 vegetable samples lettuce, Arugula and cucumber were tested to assess the biochemical and microbial contamination levels. The microbiological quality of fresh vegetables ranged from 4.3 to 10.4 $\log_{10}$ CFU/ g (aerobic bacteria); 0.71- 2.0 $\log_{10}$ CFU /g (coliforns); and 1.0 to 8.77 $\log_{10}$ CFU g^{-1} (E. coli) .Lettuce samples had significantly higher microbial loads including coliforms, E. coli and Aerobic plate count than Arugula and cucumber samples. In addition, samples from open markets had higher microbial loads, coliform, and E. coli than the samples collected from the other sites. Moreover, High percentage of E. coli was recovered from lettuce (43%) than those recovered in Arugula and cucumber samples (38 %) (19 %), respectively. Whereas significant coliforms were detected in lettuce samples (52%) compared to those from Arugula and cucumber samples (38%) (10%), respectively. This study showed that raw lettuce and Arugula which are usually used may contain hazardous pathogens that may affect human health.
Keywords: Fresh, produce, Total count, coliforms, Hail

INTRODUCTION:

Food contaminated with microorganisms may affect consumer's health. Microbial hazards continue to be one of the biggest threats to food safety [1]. Nutrients exuded from plant tissues support the growth of bacteria and fungi usually contaminate the surfaces of fruits and vegetables. The various types of microorganisms commonly found on vegetables are those that of coliforms or faecal coliforms groups, such as Klebsiella and Enterobacter [2]. Vegetables may also be exposed to many sources of contamination like contaminated sewage used in irrigation, Salmonella species and other pathogens are derived from raw and treated sewage. The use of untreated wastewater in irrigation represents an important route for transmission of these pathogenic organisms. The major pathogens associated with the use of highly polluted water are the faecal coliforms, E. coli [3]. Human pathogenic bacteria, such as Escherichia coli O157:H7, Salmonella spp., Listeria mono cytogenes, and Shigella spp., have been associated with food borne outbreaks involving fresh products [4].

Raw vegetables can harbor many microorganisms, which may be dispersed over the plant or appear as micro colonies embedded in the plant tissue [5, 6]. The majority of microorganisms associated with raw vegetables are non-pathogenic and gram negative organisms tend to dominate the bacterial population including Enterobacter spp. and other coliforms. During harvest and transport raw vegetables may be bruised resulting in the release of plant nutrients, providing nutrients for microorganisms present on the surface of the vegetable to grow. Vegetables can become contaminated with pathogenic organism during growth, harvest; postharvest handling or distribution [7, 8]. Most of raw vegetables are normally consumed without being cooked, so the possibility of food poisoning exists [9]. Unfortunately, the increase in consumption has been correlated with an increased frequency of outbreaks associated with these products.

MATERIALS AND METHODS

Collection of samples

15 samples each of lettuce, arugula and cucumber were collected from different retailers in Hail market, over a period of one month. The samples were taken to the laboratory for analysis and tested for

bacterial load. Sample units were chosen independently and randomly from the various locations

Samples preparation

Aerobic plate count

Approximately 25 g of each sample were placed in a sterile 250 ml beaker mixed with 225 ml of sterile 0.1% peptone water (PW) (Oxoid,) for 2 min. [10-11]. Appropriate 1:10 dilutions of the resultant homogenate or the rinse fluid were prepared using PW. Plate Count Agar (PCA) (Oxoid) ,plates were then inoculated with the selected dilutions using the spread technique for mesophilic plate count, the plates were placed in an incubator for 24 h at 37 °C. After incubation, plates with at least 30 colonies were counted ,results were reported in terms of $\log_{10}$ colony forming units per gram.

Enumeration of coliforms

Samples were prepared as described above. Appropriate 1:10 dilutions of the resultant homogenate or the rinse fluid were prepared using PW. For each selected dilution, 0.1 ml of sample was spread-plated onto (Brilliance E. coli/coliform; Oxoid). The plates were incubated at 37 °c for 24 h, after incubation the number of pink and purple colonies was counted; results were reported in terms of $\log_{10}$ colony forming units per gram.

Isolation of *Salmonella* spp.

Approximately 25 g of each sample were mixed with 225 ml of sterile buffered peptone water (BPW) (Oxoid) for 2 min. The homogenate or the rinse fluid was incubated for 24 h at 37 °C for pre-enrichment. Selective enrichment was then done by transferring 1 ml of pre-enrichment to 9 ml of tetrathionate (TT) broth (Oxoid), followed by incubating for 24 h at 42 °C, after incubation, one loopful of sample was taken from the TT broth and streaked on XLD agar (Oxoid) and (Salmonella shigella Agar; Oxoid) and incubated for 24 h at 37 °C. Typical colonies were picked and used for further identification via biochemical tests.

STATISTICAL ANALYSIS

The mean values obtained from the microbiological evaluation of vegetables were presented in form of simple tables and graphs. SPSS 16 for windows was used to analyze the data using ANOV test and the Turkey HSD as a post hock test.

RESULTS:

There was a significant difference in the total aerobic counts between lettuce and cucumber (p=0.003) while no significant relationship was observed between arugula and cucumber. Total coliforms in lettuce differ significantly from that detected in Arugula and cucumber (p=0.048) (p=0.003) respectively. (Table 3, 4) shows the significant differences between the groups of vegetables tested for total aerobic counts (p=0.009), and also significant difference for total coliforms was reported between vegetable groups (p=0.003), (Table 2). Samples obtained from super markets showed lower percentage of positives (23%), while those from venders had the highest positive among the samples tested (67%). Different types of organisms were isolated from the samples examined, the most common genera isolated were, Klebsiella spp. Which was the most frequently isolated from vegetables samples (55%), followed by Staphylococcus. Spp. (30%), Actinomycetes (10%) and E. coli (5%) were the least frequently isolated. All samples were negative for Salmonella spp.

Table 1: Mean $\log_{10}$ cfu/gram Aerobic plate count and Total coliform

Sample	Mean $\log_{10}$Aerobic plate count cfu/g	Mean $\log_{10}$ Coliform count cfu/g
Lettuce	6.02	2.31
Arugula	3.38	1.44
Cucumber	2.42	0.91

Table 2: The percentage of different species isolated from the tested samples

Species	% of isolates
Klebsiella	47
Staphylococcus	25
Actinomycetes	20
E.coli	08

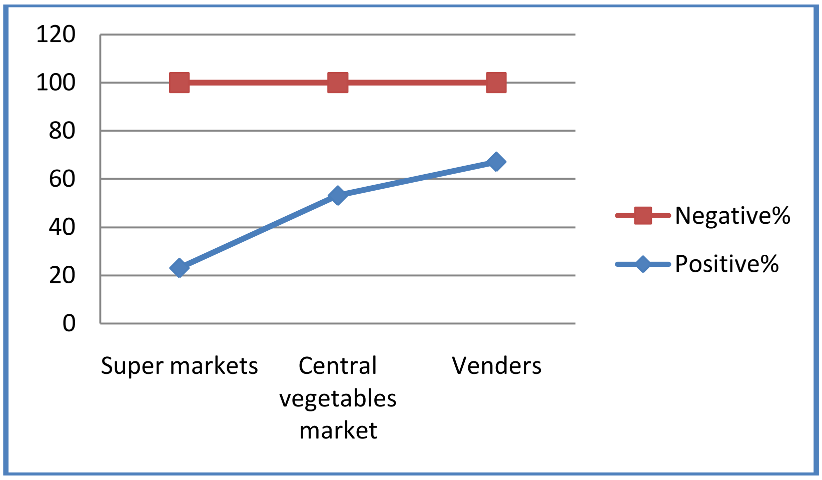

Fig.1: The percentage of positive and negative samples per site

Table 3: ANOVA Test

		Sum of Squares	df	Mean Square	F	Sig.
Total aerobic count	Between Groups	30.628	2	15.314	7.161	.009
	Within Groups	25.662	12	2.138		
	Total	56.289	14			
Total coliforms	Between Groups	5.011	2	2.506	9.548	.003
	Within Groups	3.149	12	.262		
	Total	8.161	14			

The mean difference is significant at the 0.05 level.

Table 4: Turkey HSD Post hock test

Dependent Variable	(I) vegetable	(J) vegetable	Mean Difference (I-J)	Std. Error	Sig.
Total aerobic count	Lettuce	Cucumber	3.39600*	.92487	.008
	Cucumber	Lettuce	-3.39600*	.92487	.008

Table 5: Turkey HSD Post hock test

Dependent Variable	(I) vegetable	(J) vegetable	Mean Difference (I-J)	Std. Error	Sig.
Total coliforms	Lettuce	Arugula	.87200*	.32399	.048
		Cucumber	1.40200*	.32399	.003
	Arugula	Lettuce	-.87200*	.32399	.048
	Cucumber	Lettuce	-1.40200*	.32399	.003

The mean difference is significant at the 0.05 level.

All the vegetables sampled in this study were contaminated with different bacterial species. However, the microbial load on vegetables varied with type and location from which the sample was drawn shows the Mean $\log_{10}$ cfu/gram Aerobic plate count and Total coliform for lettuce (5.06 and 2.68 $\log_{10}$cfu/gram),respectively. Microbial load for Arugula was 3.38 $\log_{10}$ cfu/gram for Total counts and 1.44 $\log_{10}$ cfu/gram for coliforms. (Table 1) samples from super markets showed the least counts while those obtained from vegetable markets and venders were highly contaminated. (Fig. 1)

Organisms isolated from vegetable samples:

Different morphological and biochemical tests were used to characterize the organisms isolated, many bacteria were identified and number of the different bacteria isolated from each of the samples varied as , Klebsiella spp were the most frequently isolated(47%) being present in 7of the 15 vegetables sampled, followed by Staphylococcus spp. (25%) Actinomycetes (20%) and E. coli (8%) were the least frequently isolated (Table 2).

DISCUSSION

The results obtained have shown that microorganisms are abundant on the surface of vegetables. The aerobic and coliform counts showed that Arugula, lettuce, and cucumber had high counts that are far above the specifications except tomatoes which had the least counts among the tested samples.

These high counts could be due to the poor hygiene practices through the production chain. Coliforms are usually indicators of intestinal contaminants from man and animals. This may not be too surprising since most often the source of watering the farms is not well controlled as this may add more microorganisms to the products. According to [12] bacterial load on leafy vegetables increase with time during storage which may impose dangers to consumers. The Gram positive Cocci and Bacilli sp. isolated were an indication of poor hygienic practices by both producers and retailers. The condition of sale makes the vegetables predisposed to contamination especially as practiced in local markets where the source of water in use is questionable [13]. The presence of Bacilli species in the four vegetables may be due to environmental factors,[14].

The presence of coliforms usually indicates probable presence of pathogenic organisms. The presence of coliforms in these vegetables may be due to the utilization, of organic fertilizers from domestic animals beside the low quality of the irrigation water in use [15] Demonstrated that E. coli can be transferred to vegetables even through iced water. These results are similar to those obtained by a related study[16], on the bacterial quality of vegetables which also showed a high count of total bacteria and coliforms. No salmonella was isolated from all tested samples. From the results obtained it was quite obvious that eating such vegetables can expose the consumer to health risks.

CONCLUSION

The results of this study indicate that the vegetables sold in the local markets are of inferior microbial quality and there was a significant difference between the leafy vegetables in the area. The way by which these vegetables are presented and in farm practices may have an important effect on their contamination.

ACKNOWLEDGEMENT:

My sincere thanks to the municipality environmental health officers for their appreciated cooperation to full fill this study

REFERENCES

1. Ali M.Al-Binali, M.A.-S.-F; Pattern of corrosive Ingestion in South Western Saudi Arabia. Saudi J.Gastroenterol. 2009; 15(1): 15-17.
2. ZhaoT., M. L; Health relevence of the presence of fecal coliforms in iced tea and in leaf tea. J.Food Protect., 1997; 60:215 - 218.
3. Sabiena F.A; Health Risks of Irrigation with untreated Urban Wastewater in the Southern Punjab,Pakistan, Research repot. Water Managemnet Institute, 2000.
4. Sivapalasingam S.F.C; Fresh produce:a growing cause of outbreaks of food borne illness in the United States,1973 through 1997. Journal of foof protection, 2004; 67:2342-2353.
5. Bhagwat A; Rapid detection of salmonella from vegetable rinse-water using real-time PCR. Food Microbiology, 2004; 21, 73-78.
6. Szabo E.C; Vegetables and vegetables products. In C. oir, Spoilage of processed foods;causes and Diagnosis. Waterloo: AIFST Inc, 2001.
7. I.G.Wilson, M. M; The Occurrence of Enteric pathogens and Aeromonas Species in organic vegetables. International Journal of Food Microbiology, 2001; 70(1-2): 155-162.
8. Beuchat L; Food safety issues:surface Decontamination of fruits and Vegetables eaten raw:A Review,. Geneva: Food safety unit ,WHO, 1999.
9. Acicek H.A; determination of total aerobic and indicator bacteria on sor wholesalers inAnkara,Turkey. Int.J.Hyg.Environ.Health, 2006; 209:197-201.
10. Marshall R; Standard Methods for the Examination of Dairy and Vegetable Products.16th edn.,. Washinton DC: American Public Health Association, 1992.
11. Vanderzant C, Splittstoesser D; Compendium of Methods for the Microbiological Examination of Foods,3rd.ed, Washinton : American Public Health Association, 1992.
12. Frank-Peterside N.A; Bacteria associated with spolilage of fluted pumpkins leaves and thier effect on chlorophyll contents. Nigerian Journal of Microbiology. 2006; 20(1): 751-756.
13. Caron D A; Green leafy vegetables. Edu.Hort.2004; 15: 22-36.
14. Godon R; The Genus bacillus in Hand book of Microbiology:Vol.1&2;. Cleeveland,Ohio: CRC press Inc. 1977; 1&2.
15. Kim J; Transfer of Escherichia coli O157:H7 to romaine lettuce due to contct water from melting ice. Journal of Food Protection, 2008; 71(2): 252-256.
16. Aliyu Y.B; Bacteriological quality of vegetables sold in some shops around Kano metropolis,Nigeria. Biological and Environmnetal Sciences Journal for the Tropics. 2005; 2(1): 145-148.