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Microbiological variation amongst fresh and minimally processed vegetables from retail establishers - a public health study in Pakistan

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Abstract

Fresh and minimally processed ready to eat vegetables are very attractive eatables amongst consumers as convenient, healthy and readily available foods, especially in the South Asian states. They provide numerous nutrients, phytochemicals, and vitamins but also harbor extensive quantity of potentially pathogenic bacteria. The aim of this study was to determine microbiological variation amongst fresh vegetables that were commercially available to the public at numerous retail establishments in Pakistan in order to present an overview of the quality of fresh produce. A total of 133 samples, collected from local distributors and retailers were tested for aerobic mesophilic and psychrotrophic, coliform and yeast and mould counts. Standard plating techniques were used to analyze all samples. Mesophilic count ranged from 3.1 to 10.3 log CFU/g with lowest and highest counts observed in onions and fresh cut vegetables. Psychrotrophic microorganisms count was as high as mesophilic microorganisms. Maximum counts for coliform were found in fresh cut vegetables with 100% samples falling over 6 log CFU/g. These results were consistent with yeast and moulds as well. In our study, *Escherichia coli* was determined as an indicator organism for 133 samples of fresh and minimally processed vegetables. Fresh cut vegetables showed the highest incidence of presumptive *E. coli* (69.9%). The results showed a poor quality of fresh vegetables in Pakistan and point to the implementation of good hygiene practices and food safety awareness amongst local distributors, food handlers at retail establishments.

1. Introduction

Fresh vegetables are an essential part of a balanced diet and there is much published evidence supporting nutritional and health gains linked to the consumption of raw or minimally processed vegetables. Aside from significant health and nutritional benefits, there has been a major change in the lifestyles and consumption patterns. With a wider range of food variety available, people now spend less time cooking at home. Such trends have led to popularity in consumption of raw or minimally processed vegetables that have become more convenient as ready to eat foods (Abadias *et al*., 2008). Factors influencing this upsurge in raw vegetable availability include geographical and climatic conditions, boost in international trade, enhanced preservation and storage and accelerated transportation together with year-round production that ensures continuous supply to consumers even during off season (Johnston *et al*., 2005). In Pakistan, most of the fresh produce (vegetables) originates locally. In comparison to other food commodities like meat, milk and other processed foods, fresh produce is readily available and low-cost which makes it an economical buy for most of the public hence increasing its consumption frequency.

However, these commodities can act as a vehicle for transmission of bacteria, parasites, yeast and moulds and many other types of viral pathogens. Contamination can occur during cultivation, irrigation, harvest, transport, storage and marketing and at the hands of consumers (Tournas, 2005). In developing countries, pre- and post-harvest processes and provisions contribute highly to contamination of fresh produce. For example, in Pakistan, a quarter of fresh produce is irrigated with wastewater (Pachepsky *et al*., 2011). The occurrence of

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foodborne related outbreaks by contaminated fresh vegetables has increased during recent years (Mukherjee *et al*., 2006). The most commonly linked pathogens to fresh produce include bacteria (*E. coli*, *Salmonella*), parasites (*Cyclospora*, *Cryptosporidium*) and viruses (Hepatitis A, Norwalk-like) (Tauxe *et al*., 1997) with *E. coli* -O157: H7 and *Salmonella* being the most abundantly found pathogens in outbreaks related to fresh produce. The Center for Disease Control and Prevention in the United States has reported amplification in the number of foodborne occurrence correlating to fresh produce around the world between 1995 to 2005 (Seow *et al*., 2012). In the US, all known foodborne outbreaks have been related to the fresh produce rising from <1 percent to a stirring 6 percent (Sivapalasingam *et al.*, 2004). OzFoodNet, an Australian disease surveillance network, has also reported an increase in illnesses caused by fresh produce (Angulo *et al*., 2008). Related studies have been carried out in several countries such as the US (De Roever, 1998; Olsen *et al*., 2000; Mukherjee *et al*., 2004; Mukherjee *et al*., 2006; Tournas *et al*., 2006; Oliveira *et al*., 2010) , the EU (Lund, 1993; Nygard *et al*., 2004; Johannessen *et al*., 2002; Emberland *et al*., 2007; Pezzoli *et al*., 2007;), Australia (Heard, 1999) and Japan (Gutierrez, 1997).

To our knowledge, there is a colossal amount of data available on fresh produce worldwide; however, there is limited published research in this area in Pakistan. The objective of the study was to determine the microbiological quality of fresh vegetables commercially available for consumers in twin cities (Islamabad, Rawalpindi) in Pakistan with the aim to guide future improvements in measures relating to food safety.

2. Materials and methods

2.1 Sample selection

Random samples (110) of nine different vegetables were collected from the local distributors in Rawalpindi and Islamabad during all four seasons of the year. The vegetables included tomato (16), carrot (12), green pepper (10), cucumber (13), radish (12), coriander (10), onion (13), lettuce (10) and cabbage (14) together with fresh cut vegetable samples (23). Fresh cut vegetables were selected from local vendors, restaurants and from open salad markets in the cities of Rawalpindi and Islamabad located in central region of Pakistan. Microbial analysis was performed in the Microbiology lab of Food Technology Department, PMAS-Arid Agriculture University Rawalpindi.

The markets chosen were those with high standing amongst the consumers where fresh produce was easily available thus assembling this study to be more productive. Sample units were selected randomly from various locations. Due to limited published data related to Pakistan that may imply specific vegetables linked to foodborne diseases, the samples analyzed were selected on the base of their commonalities amongst consumers.

The samples purchased from various distributors and markets were collected in re- sealable plastic bags which were then promptly transported to the laboratory. Samples that showed any visible ailment, damaged surface or were visibly compromised were discarded. Details of fresh-cut vegetables, including manufacturer, retailer, packing and best before date (if available) were documented systematically. All samples were examined within 24 h after the time of collection. Before the samples were taken out of the re-sealable plastic bags, the surface of the bags was sterilized with ethanol to prevent any cross contamination.

2.2 Sample preparation and microbial analysis

A sample of 25 g was added in 225 ml of 0.1% sterile peptone water (PW). It was homogenized for 2 min in a sterilized blender. Serial dilutions 1:10 were made from the produced homogenate. For aerobic mesophilic count (AM), the sample preparation was carried out following the methodology stated in FDA Bacteriological Analytical Manual. 100 µl of the prepared sample was pour plate in the Plate Count Agar (PCA) (Oxoid). Plates were then incubated at 37°C for 24 h. For psychrophilic count, the plates were prepared with the same methodology as mentioned for AM and incubated at 6°C for 6 days. Following this, the numbers of colonies formed were counted and results noted accordingly. The results were recorded in terms of CFU/ g (colony forming unit) (FDA BAM, 1998).

For coliform count, sample preparation was carried out as mentioned above. Suitable 1:10 dilutions of the produced homogenate were prepared using PW. For every dilution selected, 100 µl of the prepared sample was pour plate in chromogenic agar (Coliform/ *E. coli*: Oxoid). Afterward, all plates were incubated at specific conditions of 37°C for 24 h (FDA BAM, 1998). Subsequently, the red and pink colonies were counted. For yeast and mould count, preparation of samples was carried out as mentioned above. Suitable 1:10 dilutions of the produced homogenate were prepared using PW. For every dilution selected, 0.1 ml of the prepared sample was spread plate on Potato Dextrose Agar (PDA) (Oxoid). The plates were then incubated for 3-5 days at

25°C after which the colonies were counted (FDA BAM, 1998).

2.3 Pathogen analysis

The detection of *E. coli* was quantified using classical methodologies and the results were expressed in most probable number (MPN) following the methodology mentioned in FDA's Bacteriological Analytical Manual (FDA BAM, 1998).

2.4 Statistical analysis

The means obtained from the microbiological analysis of vegetables were analyzed by one-way ANOVA (Analysis Of Variance). To determine any significant difference amongst the means, they were subjected to Tukey test using Statistix 8.1 software (Analytical Software, FL, USA).

3. Results and discussion

Fresh vegetable quality depends on the use of adequate irrigation water together with good handling practices and appropriate storage. Processing steps like irrigation, harvesting, storage, handling, slicing cutting, shredding, and grading are all possible sources of contamination. Some of these microbes could spread during transport on whole and fresh-cut vegetables or even when these food commodities are not stored at recommended temperatures (1-5°C). Tables 1-4 show high populations of aerobic mesophilic, aerobic psychrotrophic, coliform and yeast and mould counts. Unanimously, the results suggested that the fresh and minimally processed vegetables had poor hygienic quality attained during multiple processes including harvesting, storage, transportation and lack of good handling practices.

The aerobic mesophilic count for fresh cut vegetables was 9.4 log CFU/g and a range of 7.1 to 10.3 log CFU/g (Table 2), depicting that all samples were unacceptable for consumption. The highest counts were found in the samples collected from street vendors and local restaurants. This was comparable to a survey conducted in India on 120 samples of fresh-cut vegetable and fruit salads collected from street vendors and had reported the aerobic mean counts ranging between 6-8 log CFU/g (Viswanathan and Kaur, 2001). These results were similar to the one obtained in this study and provided an overview of the unsanitary conditions predominant amongst street vendors.

Psychotropic microbes can multiply even during retail mostly when the food products are not stored at proper temperatures (1-5°C) (Abadias *et al*., 2008). Psychrotrophic counts in this study are very much comparable to the mesophilic counts (Table 2) and a similar trend was observed in a study by Abadias *et al*. (2008).

Overall, counts for whole vegetables varied significantly with cabbage, tomato and lettuce had the highest aerobic means of 7.4 and 7.2 log CFU/g with no significant difference amongst the means ($P<0.05$). Vegetables like onion, cucumber, and radish contained noticeably lower aerobic mesophilic, psychrotrophic and coliform bacteria (<6 log CFU/g). Other commodities showed consistently higher counts. The mean of coliform counts for whole vegetables ranged between 2.7 to 6.2 log CFU/g (Table 3). The highest means were found in cabbage and lettuce with 6.1 and 6.2 log CFU/g whereas most of the other vegetables fell under the range of 5 log CFU/g respectively.

Lettuce and cabbage show considerably high counts overall. A possible reason for this is due to the fact that leafy vegetables like these have high folds and the higher surface area which make more susceptible to trapping dirt, irrigation water or soil in the folds (Aycicek *et al*., 2006). This leads to a higher potential of microbial colonization on these types of vegetables and hence leading to higher risks of microbial pathogenesis. Furthermore, this phenomenon is enhanced by the fact that in Pakistan, it is common for many small-scale farmers to use untreated sewage water for irrigation, and as this water traps inside the folded leafy vegetables, it contributes to microbial growth.

Fresh cut vegetables had a mean coliform count of 8.0 log CFU/g which was lower than aerobic means. Mean value for Yeast and mould count (YMC) was lower than other aerobic counts (Table 4) amongst most of the selected commodities. Yeast and mould count (YMC) of onion was 3.9 log CFU/g whereas lettuce, cabbage, and tomato demonstrated the highest counts with means of 6.4 and 6.8 log CFU/g amongst whole vegetables respectively.

In our study, whole vegetables like cucumber, onion, radish and green pepper were found to have lower counts with onions having the lowest counts amongst all whole vegetables. The results aligned with a study conducted in the US. It concluded that green peppers and cucumbers had lesser counts as they have waxy, smooth and hard skin which does not allow microbes to reach inside and proliferate (Tournas, 2005). Lower counts in onions are due to their chemical composition. A study reported that

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Table 1. Quantitative analysis of aerobic mesophilic count (AM) of fresh vegetables collected from different retail establishers

		Percentage (%) of samples in indicated intervals						
	n	**$<10^{5a}$**	**10^5-10^6**	**10^6-10^7**	**10^7-10^8**	**$>10^8$**	**Range[b]**	**Geometric mean[b]**
Whole Vegetables	**110**							
Tomato	16	0.0	0.0	37.5	43.7	18.8	6.2-8.3	7.2±0.21[B]
Carrot	12	0.0	41.7	33.3	16.7	8.3	5.2-8.0	6.3±0.25[CD]
Green pepper	10	10.0	40.0	40.0	10.0	0.0	4.7-7.7	6.1±0.27[CD]
Cucumber	13	38.5	38.5	23.0	0.0	0.0	3.8-6.9	5.3±0.24[E]
Radish	12	8.3	41.7	50.0	0.0	0.0	3.9-7.0	5.8±0.25[DE]
Coriander	10	0.0	30.0	30.0	30.0	10.0	5.6-8.2	6.8±0.27[BC]
Onion	13	92.3	7.7	0.0	0.0	0.0	3.1-5.1	4.2±0.24[F]
Lettuce	10	0.0	20.0	20.0	20.0	40.0	5.3-8.7	7.2±0.27[B]
Cabbage	14	0.0	7.1	35.7	28.6	28.6	5.9-8.5	7.4±0.23[B]
Fresh cut vegetables	**23**	**0.0**	**0.0**	**0.0**	**4.3**	**95.7**	**7.1-10.3**	**9.4±0.18[A]**

Means represented by different superscript letters ([A, B]) are significantly different (P<0.05)
n: Number of samples
[a] : Range in CFU g^{-1} of result
[b] : Counts are provided in log CFU g^{-1} of result

Table 2. Quantitative analysis of aerobic psychrotrophic count (AP) of fresh vegetables collected from different retail establishers

		Percentage (%) of samples in indicated intervals						
	n	**$<10^{5a}$**	**10^5-10^6**	**10^6-10^7**	**10^7-10^8**	**$>10^8$**	**Range[b]**	**Geometric mean[b]**
Whole Vegetables	**110**							
Tomato	16	0.0	0.0	56.2	37.5	6.3	6.0-8.0	7.0±0.20[BC]
Carrot	12	0.0	33.3	33.3	16.7	16.7	5.3-8.2	6.6±0.25[C]
Green pepper	10	10.0	50.0	30.0	10.0	0.0	4.2-7.4	5.8±0.25[DE]
Cucumber	13	46.1	38.5	15.4	0.0	0.0	3.5-6.6	5.1±0.24[F]
Radish	12	8.4	58.3	33.3	0.0	0.0	3.6-6.7	5.5±0.26[EF]
Coriander	10	0.0	30.0	40.0	30.0	0.0	5.3-7.9	6.5±0.28[CD]
Onion	13	92.3	7.7	0.0	0.0	0.0	3.0-5.0	4.1±0.25[G]
Lettuce	10	0.0	20.0	20.0	40.0	20.0	5.4-8.7	7.0±0.28[BC]
Cabbage	14	0.0	0.0	35.7	50.0	14.3	6.1-8.2	7.3±0.23[B]
Fresh cut vegetables	**23**	**0.0**	**0.0**	**0.0**	**0.0**	**100.0**	**8.1-10.1**	**9.0±0.17[A]**

Means represented by different superscript letters ([A, B]) are significantly different (P<0.05)
n: Number of samples
[a] : Range in CFU g^{-1} of result
[b] : Counts are provided in log CFU g^{-1} of result

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Table 3. Quantitative analysis of coliform count (CC) of fresh vegetables collected from different retail establishers

	Percentage (%) of samples in indicated intervals							
	n	**<10^{4a}**	**10^4-10^5**	**10^5-10^6**	**10^6-10^7**	**>10^7**	**Range[b]**	**Geometric mean[b]**
Whole Vegetables	**110**							
Tomato	16	6.3	12.5	37.5	31.2	12.5	3.9-7.2	5.8±0.19[B]
Carrot	12	8.3	50.0	33.4	8.3	0	3.8-6.6	5.1±0.22[C]
Green pepper	10	70.0	30.0	0.0	0.0	0.0	2.7-5.0	3.7±0.21[D]
Cucumber	13	61.5	30.8	7.7	0.0	0.0	2.6-5.5	3.8±0.21[D]
Radish	12	50.0	33.3	16.7	0.0	0.0	2.5-5.5	4.2±0.22[D]
Coriander	10	10.0	50.0	30.0	10.0	0.0	3.4-6.3	4.9±0.24[C]
Onion	12	100.0	0.0	0.0	0.0	0.0	1.7-3.7	2.7±0.20[E]
Lettuce	10	0.0	10.0	20.0	60.0	10.0	4.4-7.1	6.2±0.24[B]
Cabbage	14	0.0	14.3	42.9	28.5	14.3	4.8-7.3	6.1±0.20[B]
Fresh cut vegetables	**23**	**0.0**	**0.0**	**0.0**	**4.3**	**95.7**	**6.9-8.7**	**8.0±0.15[A]**

Means represented by different superscript letters ([A, B]) are significantly different (*P*<0.05)
n: Number of samples
[a] : Range in CFU g^{-1} of result
[b] : Counts are provided in log CFU g^{-1} of result

Table 4. Quantitative analysis of yeast and mould count (YMC) of fresh vegetables collected from different retail establishers

	Percentage (%) of samples in indicated intervals							
	n	**<10^{5a}**	**10^5-10^6**	**10^6-10^7**	**10^7-10^8**	**>10^8**	**Range[b]**	**Geometric mean[b]**
Whole Vegetables	**110**							
Tomato	16	0.0	18.8	68.7	12.5	0.0	5.5-7.9	6.6±0.15[B]
Carrot	12	33.3	25.0	41.7	0.0	0.0	4.7-6.8	5.7±0.17[C]
Green pepper	10	30.0	40.0	30.0	0.0	0.0	4.6-6.7	5.6±0.19[C]
Cucumber	13	69.2	23.1	7.7	0.0	0.0	3.7-6.1	4.9±0.17[D]
Radish	12	91.7	8.3	0.0	0.0	0.0	3.8-5.1	4.6±0.17[D]
Coriander	10	10.0	40.0	50.0	0.0	0.0	4.6-6.3	5.8±0.19[C]
Onion	13	92.3	7.7	0.0	0.0	0.0	2.8-4.7	3.9±0.17[E]
Lettuce	10	10.0	10.0	60.0	20.0	0.0	4.4-7.7	6.4±0.19[B]
Cabbage	14	0.0	14.3	50.0	35.7	0.0	5.6-7.5	6.8±0.16[B]
Fresh cut vegetables	**23**	**0.0**	**0.0**	**0.0**	**26.1**	**73.9**	**7.4-9.2**	**8.4±0.12[A]**

Means represented by different superscript letters ([A, B]) are significantly different (*P*<0.05)
n: Number of samples
[a] : Range in CFU g^{-1} of result
[b] : Counts are provided in log CFU g^{-1} of result

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Chinese chive, scallions, and other *Allium* plants possessed natural antifungal activity (Yin and Tsao, 1999).

In this study, *E. coli* were detected in 30.1% of samples and the bacterial population varied between 1 to 6 log MPN/g (data not shown). The most contaminated samples were of fresh cut vegetables and none were detected in onion samples (Table 5). The contamination varied amongst other whole vegetables with carrot and lettuce showing the highest contamination of the bacterium. The contamination rate of *E. coli* found in our study was higher than the one reported by Sagoo *et al*. (2003) for fresh vegetable salads in the United Kingdom (1.3%) and by Abadias *et al*. (2008) for fresh cut vegetables in Spain (11.4%). A study reported high contamination of *E. coli* in samples ready to eat vegetables in Brazil (53.1%) (De Oliveira *et al*., 2011). Another similar study conducted in Brazil found a high percentage of *E. coli* amongst minimally processed vegetables (30%) (Prado *et al*., 2008).

Table 5. Occurrence of pathogens in randomly collected vegetables

		Percentage (%) of positive samples
	n	***E. coli***
Whole Vegetables	**110**	
Tomato	16	25.0
Carrot	12	41.7
Green pepper	10	10.0
Cucumber	13	23.1
Radish	12	16.7
Coriander	10	20.0
Onion	13	ND[a]
Lettuce	10	30.0
Cabbage	14	28.6
Fresh Cut Vegetables	**23**	**69.6**
Total	**133**	**30.1**

n : Number of samples
[a] : Not detected

Isolation and detection of *E. coli* are carried out to determine the sanitary conditions of the foods. There may be specific serotypes of this species that may be involved in foodborne diseases. However, in this study, no subtyping of *E. coli* was carried out. There have been numerous outbreaks relating to *E. coli* enterohemorrhagic (EHEC) strain and enteropathogenic (EPEC) strains (CDC, 2010).

There is very limited published data related to the quality of fresh produce in Pakistan Therefore results found in this study cannot be compared to any other data with similar geographical location. Studies related to raw food especially fruits and vegetables should be increased in Pakistan. This study revealed that the majority of whole and fresh-cut vegetables analyzed were of poor quality especially fresh cut vegetables. This indicates that there is a dire need for implementation of good hygiene practices. It is also suggested that the concerned authorities such as Punjab Food Authority should implement strict laws and checks for implementation of good hygiene practices together with training programs to create self-awareness amongst retailers and vendors.

Conflict of interest

There is no conflict of interest.

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