

Prevalence of total coliforms, faecal coliforms and *E. coli* in Rawalpindi vegetable markets

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ABSTRACT

Nutritional value of vegetables and high prices of meat and meat originated food compel common people to consume plant originated food particularly salad vegetables. Microbial population of vegetable surfaces contains a large number of pathogenic bacteria including members of Enterobacteriaceae like *Escherichia coli* (*E. coli*). A survey was conducted in three major markets of Rawalpindi, Pakistan. Tomato, lettuce, cabbage and cucumber samples were collected from three shops of each market. Each vegetable was analysed as unwashed and washed for total coliforms, faecal coliforms and *E. coli* by FAO (Food Quality Manual). About two hundred and fifty *E. coli* isolates were preserved, serotyped for presence of O157 serotype. Total coliforms, faecal coliforms and *E. coli* count exceeded the permissible limits in most samples. The highest Total coliforms were associated with cabbage (3.78 log₁₀ cfu/g). Cucumber was the least contaminated by Total coliforms (2.15 log₁₀ cfu/g). *E. coli* was detected in tomato, lettuce, cucumber and cabbage. Washed samples showed reduced bacterial population. Seventy six isolates of *E. coli* were biochemically characterized and serotyped for O157 antigen. A majority of strains could not be identified by serotyping. These findings conclude with high potentially pathogenic microbial load on salad vegetables and urge for preventive action on priority basis.

Keywords: *Escherichia coli*; Total Coliforms; Faecal Coliforms; Vegetables; Serotyping; Microbial Population

1. INTRODUCTION

Foodborne illness has recently gained much attention worldwide due to its deleterious effects on human health and consequentially on national economy. Worldwide increased consumption of fresh vegetables in the form of raw and minimally processed salads has resulted in increase in foodborne outbreaks which sometimes may be fatal [1]. Food trade among various countries may bring in additional food safety challenges by allowing extensive spread of unhygienic food, and predominantly produce [2].

Foodborne outbreaks are witnessed by people of developed countries like the United States, Japan and Germany [3,4,5]. Coliform bacteria, particularly *E. coli*, are an index of water and food sanitation. Some of the strains are pathogenic like *E. coli* O157:H7 and are able to produce serious illness in humans including diarrhoea, vomiting, severe abdominal pain, hemorrhagic colitis (HC), and the acute hemolytic uremic syndrome (HUS) [6,7].

Foodborne illnesses seldom come into notice in developing countries like Pakistan. The proposed study is thought to assess the risk related to consumption of contaminated salad vegetables by detecting total coliforms, faecal coliforms and *E. coli* associated with salad vegetables sold at retail shops of Rawalpindi. Data generation for health policy makers and to have a view of difference between contamination level of unwashed and washed

vegetables were also the research objectives.

2. MATERIAL AND METHODS

Three markets of Rawalpindi were randomly selected *i.e.* Khannapul vegetable market, Chungi 22 vegetable market and Rajabazar vegetable market. Four salad vegetables (1 kg each) *i.e.* tomato, lettuce, cucumber and cabbage were sampled from three random markets of the city.

2.1. Sample Analysis

Each sample was coded and analysed for total coliforms, faecal coliforms and *E. coli* as (a) unwashed (b) washed. Sample was prepared and serially diluted in Butterfield phosphate buffer according to method described in FAO Manual (1992) [8].

Diluted Butterfield phosphate buffer and all media required were autoclaved at 121°C and 15 pounds/sq. inch pressure for 15 - 20 minutes in Harvey Sterile Max autoclave (USA).

2.2. Presumptive Test for Total Coliforms and Faecal Coliforms

One ml blended sample was put in Pyrex screw cap test tubes containing 9 ml sterile Lauryl sulphate Tryptose (Oxoid) broth and inverted Durham tubes and incubated at 35°C for 24 - 48 hrs in Binder incubator. Production of gas in inverted Durham tubes was the presumptive test for coliforms presence in the sample.

2.3. Confirmatory Tests for Total Coliforms

Positive cultures in LST tubes were transferred to sterile BGLB (Oxoid) tubes and incubated at 35°C for 24 - 48 hrs in incubator. Gas production in inverted Durham tubes confirmed the presence of coliforms in sample. Their number was assessed by counting confirmed positive test tubes of consecutive three dilutions using MPN technique.

2.4. Confirmatory Test for Faecal Coliforms

Positive LST culture was transferred to sterile 10 ml EC medium and incubated for 48± hours at 45.5°C in GFL 1086 waterbath (Germany). MPN of faecal coliforms was calculated from positive EC tubes for consecutive three dilutions.

2.5. Biochemical Test for *E. coli*

E. coli isolates were confirmed by performing tests for indole production, methyl red reactive compound tests, Voges Proskauer reactive compound tests, and citrate utilization. IMViC pattern of ++-- and -+-- was interpreted

as Biotype I and II *E. coli* strains, respectfully. Some strains showing atypical characteristics were Gram stained.

All isolated strains of *E. coli* were tested for catalase production as described by Collins and Lyne (1980) [9].

2.6. Serotyping O157

In order to serotype the Enterohemorrhagic strain of *E. coli*, among the isolated strains, O157 Oxoid (DR120M) Dryspot Latex detection kit was used.

All isolates from fresh produce were coded as VB (Vegetable Bacteria) and given a number. All *E. coli* isolates from salad vegetables were preserved for short term on Nutrient agar (Sigma) slants and long term by freeze-drying in 10% skim milk (Difco).

2.7. Statistical Analysis

All experiments were performed in triplicate. The means, standard deviation and standard error of all experiments was determined. Graphs preparation and standard error calculation was performed in Excel 2010. Bi-factorial Analysis of Variance (ANOVA) was applied to know the significance of washed and unwashed vegetables of different markets. Pairwise T test was applied (Statistix 8.1) to assess the difference between each vegetable in unwashed and washed conditions. Total Coliforms, Faecal Coliforms, *E. coli* values were also converted to log 10 before graph preparation.

3. RESULTS AND DISCUSSION

A total of 36 samples were collected from their respective shops and markets and coded after transporting to the laboratory.

3.1. Samples Collection

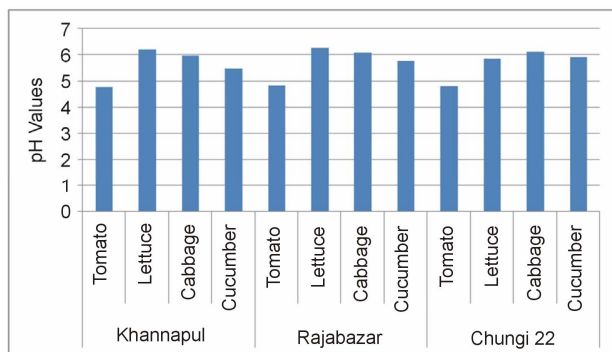
Table 1 indicates details of the samples collected and the code given in the Food Microbiology Laboratory of Grain Quality Testing Laboratory (GQTL), National Agriculture. Research Centre, Islamabad.

3.2. Sample Analysis: pH Studies

Lowest pH values were recorded for tomatoes while cabbage and lettuce blended samples showed highest pH values in overall survey **Figure 1**. Rawalpindi city contained vegetables of pH 4.75 (FMS105abc, tomato, Khannapul) to 6.23 (FMS217abc, lettuce, Rajabazar). This result is similar to the findings of Dobricevic *et al.* (2005) [10] and Chutichudet *et al.* (2011) [11]. pH values of cabbage and lettuce were found to be close to one another while that of cucumber and tomato varied differently from other vegetables tested. Cabbage analysed for pH values indicated that it ranged from 5.6 - 5.94 while cucumber

Table 1. Details of samples collected from different markets of Rawalpindi.

S.NO	Sample Code	Vegetable Type	Market
1.	FMS105abc	Tomato	Khanapul
2.	FMS106abc	Cucumber	Khanapul
3.	FMS107abc	Cabbage	Aabpara
4.	FMS202abc	Cucumber	Rajabazar
5.	FMS209abc	Tomato	Rajabazar
6.	FMS216abc	Cabbage	Rajabazar
7.	FMS217abc	Lettuce	Rajabazar
8.	FMS258abc	Tomato	Chungi22
9.	FMS265abc	Cabbage	Chungi22
10.	FMS277abc	Cucumber	Chungi22
11.	FMS282abc	Lettuce	Khannapul
12.	FMS283abc	Lettuce	Chungi22

**Figure 1.** pH of blended vegetable samples isolated from different markets of Rawalpindi.

ranged from 5.09 - 6.41. This result could not be compared with other results as pH values related to blended cabbage sample in sterile distilled water was lacking in literature. Average pH of cucumber was found to be 5.7 by Barathi *et al.* (2001) [12]. It was noted that vegetables with high pH values harboured more microorganisms as compared to vegetables with low pH values. This trend was also found by Weissinger *et al.* (2000) [13].

3.3. Total Coliforms

Rawalpindi markets revealed results that cabbage from Khannapul was highly contaminated followed by lettuce, cucumber and least count was found on tomatoes **Figure 2**. Data for Total coliforms showed non significant ($p < 0.05$) difference between three markets of Rawalpindi. It also shows Rajabazar vegetable market analysis for salad vegetables contamination with total coliforms. Cucumber was found with highest number coliforms followed by tomato and cabbage while cucumbers were least contaminated. Chungi No. 22 vegetable market exhibited cabbage as most contaminated salad vegetable while cucum-

ber was least contaminated. Tomato and lettuce contained almost equal number of coliforms. Washing has significantly ($p < 0.05$) reduced the number of coliforms on cabbage and lettuce but non-significance difference was noted for cucumber and tomato in chungi 22 vegetable market. This result resembles to the outcome of investigation conducted by Manios *et al.* (2006) with minor difference in total coliform counts [14]. Al-Lahham *et al.* (2003) also found tomato fruit contaminated with total coliforms [15]. These types of figures were also obtained by Johnston *et al.* (2006) and Machado *et al.* (2006) [16, 17]. Analysis of variance revealed that washed samples showed significantly ($p < 0.05$) decreased number of total coliforms in all vegetables. This result is similar to the findings of Yasmeen *et al.* (2008) and Ibrahim and Jude-Ojei (2009) [18,19].

3.4. Faecal Coliforms

Results of faecal coliforms exhibited presence of faecal contamination in all samples collected from all vegetable markets of Rawalpindi and the results revealed that lettuce contained maximum number of faecal coliforms. Machado *et al.* (2006) found faecal count more than 10^2 cfu/g on lettuce which is less than the count obtained in present study faecal load (maximum values 10^6) [17]. Stine *et al.* (2005) also concluded results indicating microbial load on fresh vegetables that resembled current findings [20]. Cabbage was associated with least number of faecal population **Figure 3**. This variation may be due to involvement of some additional factors like pesticides residues. Usually cabbages and radish are heavily sprayed to control vegetable pests [21]. Washed samples showed significantly ($p < 0.05$) lowered faecal contamination as compared to unwashed cucumber and cabbage. Rajabazar vegetable market were found with all samples contaminated with faecal coliforms. Among them lettuce contained highest faecal population while cabbage and tomato showed lower counts. Cabbage and lettuce vegetable samples of Chungi 22 vegetable market contained maximum number of faecal coliforms while cucumber and tomatoes showed lower results for faecal contamination. Pairwise T test showed that each washed vegetable harbor significantly ($p < 0.05$) less count than unwashed. Bifactorial ANOVA revealed non significant difference ($p < 0.05$) between different markets of Rawalpindi for faecal count. This finding shows similarity with Xu and Warriner (2005) and Jensen *et al.* (2010) [22,23].

Washing with sterile distilled water significantly ($p < 0.05$) reduced faecal contamination in all samples tested except cucumber. This result may be due to the presence of faecal coliforms in internal parts of cucumber fruit **Figure 2**. These faecal coliforms may have entered through variable sources like faeces, contaminated irrigation water, animal wastes used as fertilizer, grazing animals,

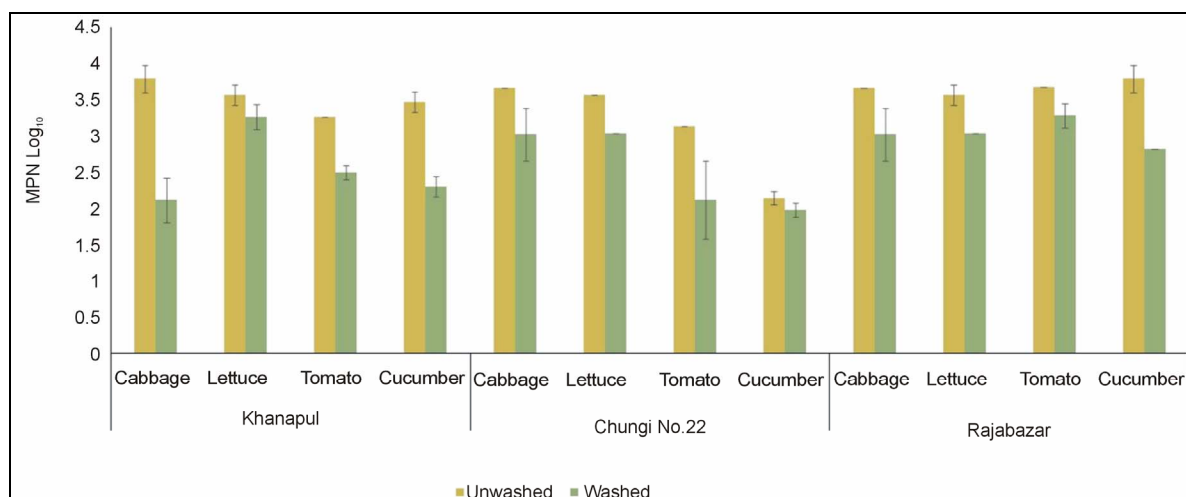


Figure 2. Total coliforms of unwashed and washed (log₁₀ MPN± S.E) salad vegetables of three vegetable markets of Rawalpindi.

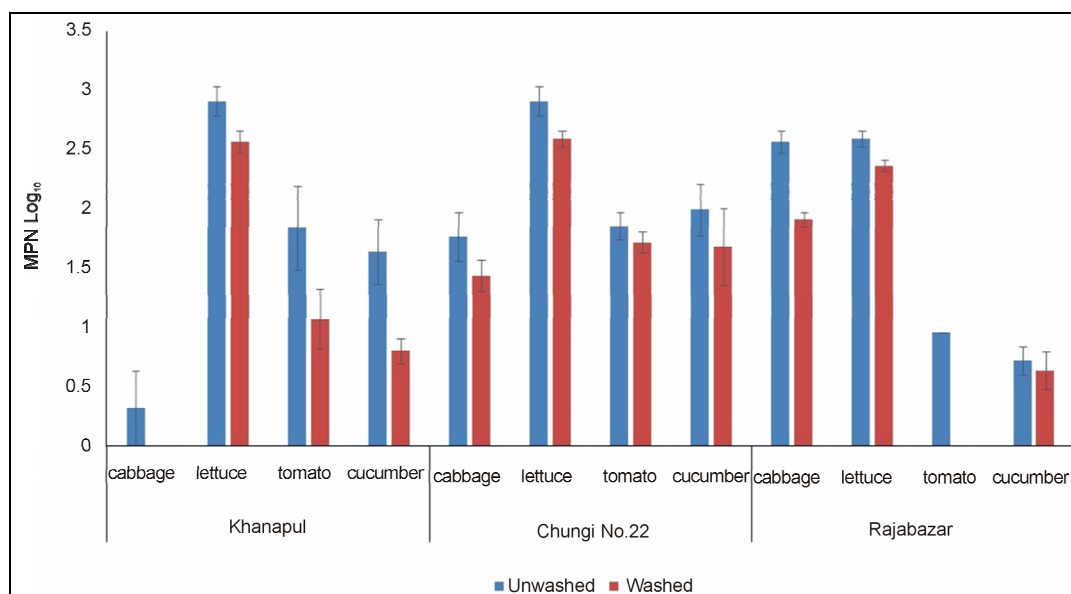


Figure 3. Faecal coliforms (log₁₀ MPN± S.E) of unwashed and washed salad vegetables of Rawalpindi vegetable markets.

birds faecal droppings and human contact. The high count may also be result of contamination during transportation from fields to mandis and from mandis to retail outlets.

Heinonen-Tanski *et al.* (2007) found no enteric pathogens present on cucumbers samples. This is contradictory result to present may be because of human urine application to the soils where cucumber was grown by researchers [24].

3.5. *Escherichia coli*

Rawalpindi markets analysed for *E. coli* count showed that Khannapul vegetable market contained maximum *E. coli* count for lettuce and cabbage contained significantly ($p < 0.5$) lowest counts among all vegetables tested **Fig-**

ure 4. Rajabazar vegetable market harboured lettuce being more contaminated than other vegetables while cabbage was observed with lower counts. Chungi 22 vegetable market cabbage was thickly contaminated with *E. coli* while cucumbers contained lower count. Same results were obtained by Mukherjee *et al.* (2004) and Solomon *et al.* (2003) [25,26]. Simeos *et al.* (2001) reported presence of *E. coli* in all samples of selected commodities [27]. Present results show a contradiction with the findings of Valentin-Bon *et al.* (2008) for presence of *E. coli* on lettuce [28]. The variation may have developed because of different ecological and sanitary condition of two countries. Washing significantly ($p < 0.05$) reduced *E. coli* population of three markets of Rawalpindi studied.

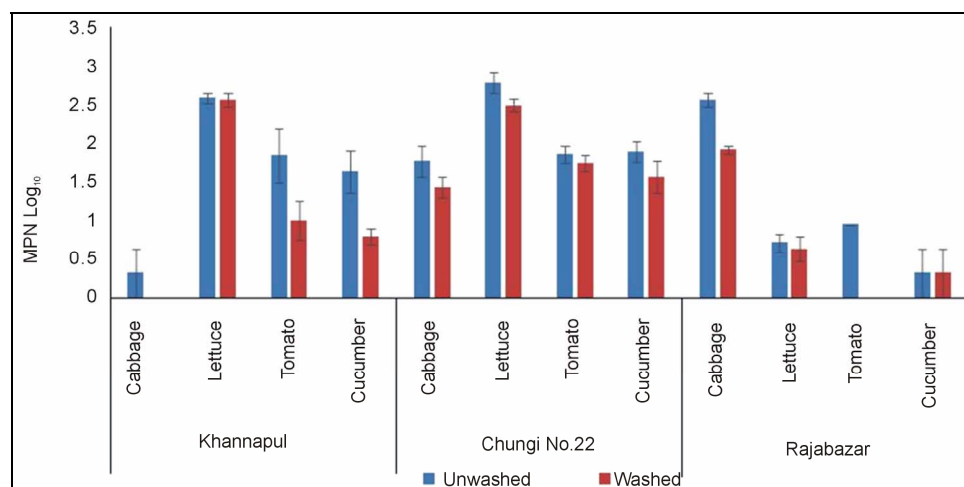


Figure 4. *E. coli* of unwashed and washed salad vegetables of three vegetable markets of rawalpindi.

Two factor analysis of variance for *E. coli* showed non significant ($p < 0.05$) difference between three markets of Rawalpindi.

It was also found that *E. coli* was more associated with lettuce. This association may be due the fact that leafy greens provide more surface area for contamination. *E. coli* count in tomato and cucumber was lower than the values of *E. coli* contamination detected by Bharathi *et al.* (2001) [12]. It is probable that *E. coli* attaches itself to the cut bruised area of vegetables more rapidly as compared to other pathogens.

Faecal coliforms number in most of samples was equal to the count of *E. coli* but in some cases faecal count was higher than *E. coli* count and is similar to the previous research work [28].

3.6. Biochemical Tests of *E. coli*

Khannapul vegetable market showed 18 *E. coli* isolates belonged to *E. coli* Biotype II while 7 were Biotype I. One *E. coli* strain (91VB) showed atypical characteristics and was confirmed as *E. coli* isolate after Gram staining and microscopy. Twenty eight *E. coli* isolates were obtained from Rajabazar of Rawalpindi. Nineteen *E. coli* isolates were Biotype I and 7 biotype II. Among 23 isolates of *E. coli* 19 were Biotype I, three were biotype II and one (161VB) showed atypical characteristics. All isolates produced catalase. **Figure 5** shows catalase production by *E. coli* 157VB isolate of Chungi 22 vegetable market.

Indole production was noted in most of the isolates of each market. All *E. coli* strains were gas producing. These results are similar to the characteristics exhibited by strains by other researchers [29]. The difference may be result of *E. coli* isolates from different origin or genetic descendent from *Shigella*. *Shigella* cannot produce indole and *E. coli* isolates might possessed multivariant genetic origin [30]. Chungi 22 market indicated high

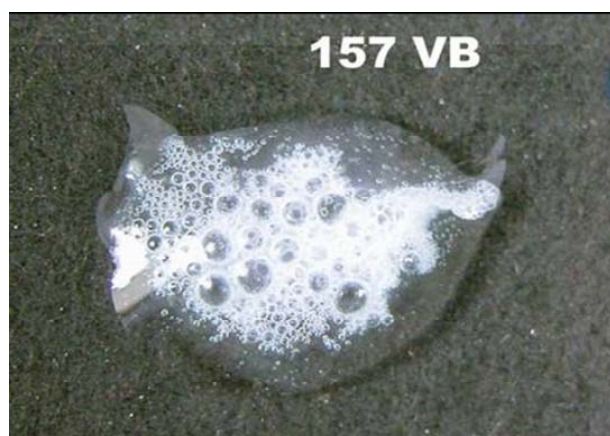


Figure 5. Catalase production by *E. coli* isolate 157 VB.

percentage of *E. coli* Biotype I (82.%) as compared Biotype II (13.04%) while 73% *E. coli* isolates obtained from Khannapul vegetable market were of Biotype II (**Figure 6**).

3.7. O157 Serotyping

Serotyping results showed that 4 strains belonged to *E. coli* O157 (89VB, 92VB, 101VB and 112VB). Positive agglutination was noted for O157 serogroup while negative agglutination indicated presence of non O157. Stephen *et al.* (2008) did not isolate any O157:H7 strain [31]. This contradiction may be due to difference of sampling area and source of bacteria on salad vegetables.

4. CONCLUSION

It was concluded on the basis of present research findings that *E. coli* is highly prevalent in local vegetable markets of Rawalpindi, Pakistan. There was lack of proper sanitary conditions in retail outlets. A proper check and balance system was needed. Washing reduced the

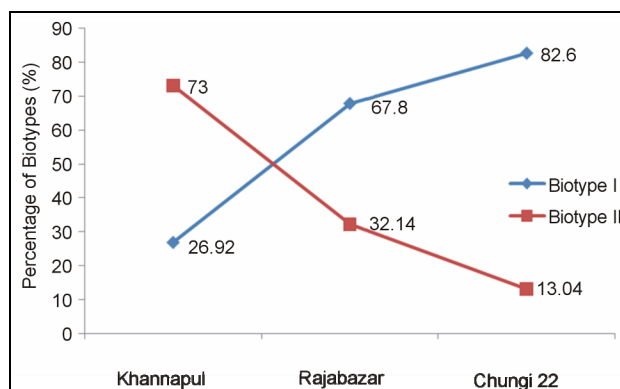


Figure 6. Percentage of biotypes of *E. coli* in three different vegetable markets of Rawalpindi.

microbial load on vegetables. The conclusions drawn from the results propel recommendations that a comprehensive sampling plans should be made, a regular survey of vegetable markets should be conducted after every three months and the faecal coliforms sources should be removed on priority. Cross contamination should be avoided by food handlers, distributors and field workers. Awareness must be created among public and political elites.

5. ACKNOWLEDGMENT

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