

Research Article

Microbial load and prevalence of pathogens on surface of fresh vegetables in local market yards across Junagadh district of Gujarat

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ABSTRACT

The vegetables are being grown extensively in India. Minimally processed foods or other raw vegetables have been known to serve as vehicles of human disease for at least a century. Vegetables can be contaminated with microorganisms which are capable of causing human diseases. This study was designed to determine the microbial load and prevalence of pathogens on surface of fresh vegetables from local market yards across Junagadh district. Total 192 fresh raw vegetable sample's microbial load were assessed using the Total Plate Count (TPC) per gram of Vegetables surface. Prevalence of yeast, mould, *Escherichia coli*, *Salmonella* spp. and *Vibrio* spp. was found. Prevalence of *E. coli*, *Salmonella* spp. and *Vibrio* spp. were observed in most of the samples collected from market yards which could pose a health risk to consumers. Generally no pre-treatment was given to fresh produce before transporting to retailer and hence number of contaminants was so large. These high viable counts of local markets could be because of unhygienic handling condition of local market, exposure during transport, improper storage condition etc. and thus may add the load of organisms.

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INTRODUCTION

Raw vegetables have been known to serve as vehicle of human diseases for at least a century. Vegetables can become contaminated with microorganisms capable of causing human diseases while still on the plant in fields or orchards or during harvesting, transport, processing, distribution and marketing or in the home (Beuchat, 1998). Bacteria such as *Clostridium botulinum*, *Bacillus cereus* and *Listeria monocytogenes*, all are capable of causing illness and are normal inhabitants of many soils, whereas *Salmonella*, *Shigella*, *Escherichia coli* and *Campylobacter* reside in the intestinal tracts of animals, including humans and are more likely to contaminate raw vegetables through contact with faeces, sewage, untreated irrigation water or surface water (Abdelnoor *et al.*, 1983). Most of the contaminating flora are non-pathogenic and have a natural occurrence on the produce. However, pathogens from the human and animal reservoir as well as other pathogens from environment can be found at the time of consumption (Altokrusse *et al.*, 1997). The survival of enteric pathogens in

soil, manure, municipal wastes and irrigation water depends on different factors like relative humidity, microbial adhesion, rainfall, sunlight, etc. (De Roeve, 1998 and Francis *et al.*, 1999). Contamination with viruses or parasites can result from contact with faeces, sewage and irrigation water. Vegetables are purchased from local farmers or retail outlets for further preparation by street vendors. Numerous surveys have been carried out in many countries to determine the presence of pathogenic microorganisms on raw fruits and vegetables (Abdelnoor, 1983). In many instances, bacterial pathogens have not been detected. In other investigations, high percentages of samples have been found to contain bacteria capable of causing human disease. Results of these investigations show that the most common pathogens found on surface of vegetables are *L. monocytogenes*, *E. coli*, *Salmonella*, *V. cholerae*, *Staphylococcus* etc. While every effort should be made to prevent contamination of vegetables during production, transport, processing and handling, much improvement is still needed in some parts of the world if

hygienic production of vegetables is to be ensured. Furthermore, many microbial contaminants are part of the environment and vegetables may be inadvertently contaminated. The purpose of this work is to provide public health authorities with information on the surface decontamination of vegetables, and with guidelines and recommendations that can be provided to growers, processors and consumers.

MATERIALS AND METHODS

Sample collection and preparation:

A total of 192 samples of fresh vegetables and vegetables (tomato, brinjal, cluster bean, cabbage, okara, cauliflower, bottle gourd and chilli) was collected from four different local market yards (Visavadar, Mendarda, Vanthali and Junagadh). Three samples of each vegetable were collected twice at an interval of fifteen days. All the samples were collected in sterile bag put in universal container and transport to laboratory. From the total sample, half of the sample was rinsed thoroughly with the distilled water and rest was kept in neem extract for one minute before being washed with distilled water. These samples were further used for study.

Isolation of microorganisms:

For the isolation of microorganisms, different growth media were prepared namely Nutrient agar, Potato dextrose agar, Fluid selenite cystine medium (FSCM), Eosin methylene blue agar (EMB), Xylose lysine deoxycholate agar (XLD), Vibrio agar, Salmonella–Shigella agar (all from Hi Media, India) according to Manufacturer’s instruction, and were sterilized by autoclaving at 121°C for 15 min. Xylose lysine deoxycholate agar, Vibrio agar and Salmonella–Shigella agar, which do not require autoclaving, were sterilized by boiling for 15 min. One gram of each vegetable’s surface was weighed in sterile condition. Samples were serially 10 fold diluted. From first dilution (10^{-1}), 1 ml inoculation was poured in FSCM for enrichment of *Salmonella* and 0.1 ml was spread on EMB agar and Vibrio agar. After 24 h, from FSCM broth 0.1ml was spread on XLD and Salmonella–Shigella agar. These agar plates were incubated for 24 hrs at 37°C for colony formation. Each colony was isolated in a pure form for further studies

and identification by sub-culturing. Distinctive morphological properties of each pure culture such as colony form, elevation of colony and colony margin were observed.

Microbial load determination:

For determination of bacterial load, highest four dilutions (10^{-1} , 10^{-3} , 10^{-5} and 10^{-7}) were taken for analyses. While for determination of yeast and mould count (YC), spread plate technique was used. The plates were inverted and incubated at 37°C for 24 h for colony formation. The numbers of colonies were counted from N-agar plates which contained maximum 300 colonies and further colonies per gram were calculated. For YC the PDA plate was kept at room temperature for 3 days before counting the number of fungal colonies.

RESULTS AND DISCUSSION

The vegetables samples were collected from four randomly selected market yards of Junagadh district at different time intervals and analyzed for various parameters as below.

- Total plate count
- Yeast and mould count
- Qualitative detection of *E.coli*
- Qualitative detection of *Salmonella* spp.
- Qualitative detection of *Vibrio cholera*.

Total plate count:

Total plate count of fresh vegetables were in the range of 18.77×10^4 to 117.25×10^4 cfu/ g for market yards of Junagadh district (Table 1). The highest count was observed from the vegetables of Vanthali market yard and the lowest count was observed in Visavadar market yard. Out of eight vegetables, the highest microbial load was found on the surface of cauliflower in all market yard and lowest was found on surface of brinjal.

Yeast and mold count:

The range of yeast and mould count of fresh vegetables was between 4.81 to 44.15×10^2 cfu/ g from different market yards of Junagadh district (Table 2). The highest yeast and mould count was obtained in vegetables of Junagadh market yard and the lowest count was obtained in Visavadar market

Table 1 : Mean TPC (value x 10 ⁴) of vegetable samples collected from different regions of Junagadh district									
Location	Fresh vegetables								
	Brinjal	Cluster bean	Okra	Tomato	Cauli.	Cabbage	Chilli	Bottle gourd	Mean
Visavadar	14.56	12.46	15.45	18.995	82.5	37.5	36.69	34.16	31.54
Mendarada	42	55.2	30.45	89.99	111	57.33	63.83	18.49	58.54
Vanthali	15	14.49	1.115	64.9	150.5	92.83	91	48.49	59.79
Junagadh	3.52	11.86	33.	32	125	55.81	131.63	33.5	53.29
Mean	18.77	23.51	20.00	51.47	117.25	60.87	80.79	33.66	

yard. Out of eight vegetables, the highest yeast and mould was found on surface of chilli and lowest was found on the surface of brinjal.

Qualitative detection of *E.coli*:

Presence of *E. coli* on samples of vegetables ranged between of 0 to 66 per cent (Fig. 1). Vegetables collected from the market yard of Vanthali showed highest presence of *E.coli* while samples of Visavadar market yard showed lowest contamination of *E.coli*. Out of eight vegetables the highest contamination of *E.coli* was found on the surface of cauliflower in all market yard and lowest was found on the surface of brinjal.

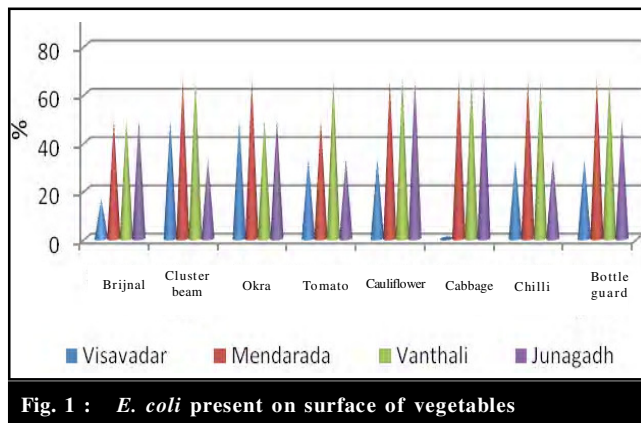


Fig. 1 : *E. coli* present on surface of vegetables

Qualitative detection of *Salmonella* spp.:

Salmonella spp. was found present in the range of 0 to 100 per cent of samples of vegetables. In Visavadar, market yard highest sample showed the presence of *Salmonella* spp. while in the Mendarada market yard lowest samples showed presence of *Salmonella* spp. Out of eight vegetables the highest *Salmonella* spp. found on surface of cabbage in all market yard and lowest was found on surface of Cluster bean

(Fig. 2).

Qualitative detection of *Vibrio cholerae*:

Vibrio cholerae was found in the range of 0 to 66 per cent of samples of vegetables. In Vanthali market yard highest sample showed the presence of *Vibrio cholerae*. While in Visavadar market yard’s sample showed lowest of *Vibrio*

Table 2 : Mean yeast and mould count (value x 10²) of vegetable samples collected from different region of Junagadh district

Location	Fresh vegetables								Mean
	Brinjal	Cluster bean	Okra	Tomato	Cauli.	Cabbage	Chilli	Bottle gourd	
Visavadar	4	25.98	21.23	5.51	15.5	11.33	47	13.16	17.97
Mendarada	10.15	26.35	33.33	6.83	31	27.91	56.6	15.66	25.98
Vanthali	2.5	47.13	12.63	10.49	52	35.5	41.66	8.99	26.37
Junagadh	2.59	46.5	24.28	8.52	61.5	29.83	31.35	13.16	27.22
Mean	4.81	36.49	22.87	7.84	40.00	26.14	44.15	12.75	

Table 3 : Mean TPC (value x10⁴) of vegetable samples collected from different regions of Junagadh district after the treatment with neem extract

Location	Fresh vegetables								Mean
	Brinjal	Cluster bean	Okra	Tomato	Cauli.	Cabbage	Chilli	Bottle gourd	
Visavadar	7.50	6.00	8.00	8.50	42.00	16.50	18.50	17.50	15.56
Mendarada	20.00	20.00	15.00	40.00	50.00	32.50	15.00	9.00	25.19
Vanthali	10.00	10.00	1.00	30.00	80.00	45.00	40.50	20.50	29.63
Junagadh	2.00	5.50	22.00	16.00	60.00	30.00	80.50	15.50	28.94
Mean	9.88	10.38	11.50	23.63	58.00	31.00	38.63	15.63	

Table 4 : Mean yeast and mould count (value x 10²) of vegetable samples collected from different regions of Junagadh district after the treatment with neem extract

Location	Fresh vegetables								Mean
	Brinjal	Cluster bean	Okra	Tomato	Cauli.	Cabbage	Chilli	Bottle gourd	
Visavadar	1	12.4	11.5	3.21	6.98	5.3	20.98	7.43	8.60
Mendarada	5.5	15	22.3	2.65	13.3	11.67	19.4	6.23	12.01
Vanthali	2	22.3	5.7	3.56	25	18.45	22.54	4	12.94
Junagadh	1.5	23	14.3	5	33.5	13.6	14.65	6.78	14.04
Mean	2.50	18.18	13.45	3.61	19.70	12.26	19.39	6.11	

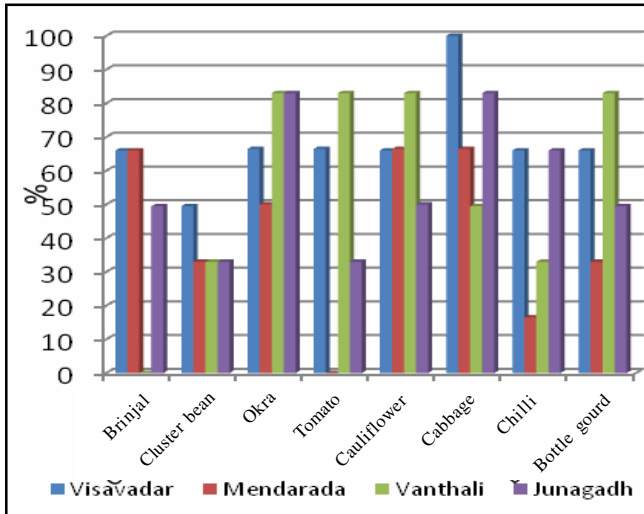


Fig. 2 : *Salmonella* spp. present on surface of vegetables

the pathogen on the surface of fresh vegetables (Table 3 and 4). Best reduction in obtained during the wash with Neem extract camperd to washing with tap water (Table 4, 5 and 6).

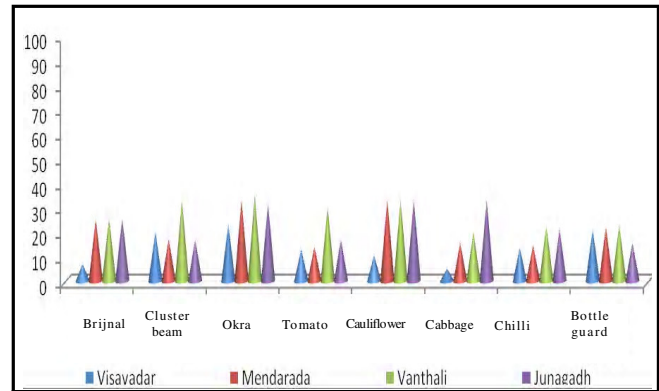


Fig. 4 : *E. coli* present on surface of vegetables after neem extract wash

cholerae. Out of eight vegetables the highest *Vibrio cholerae* was found on surface of cluster bean and lowest found on surface of cauliflower, cabbage and tomato (Fig. 3).

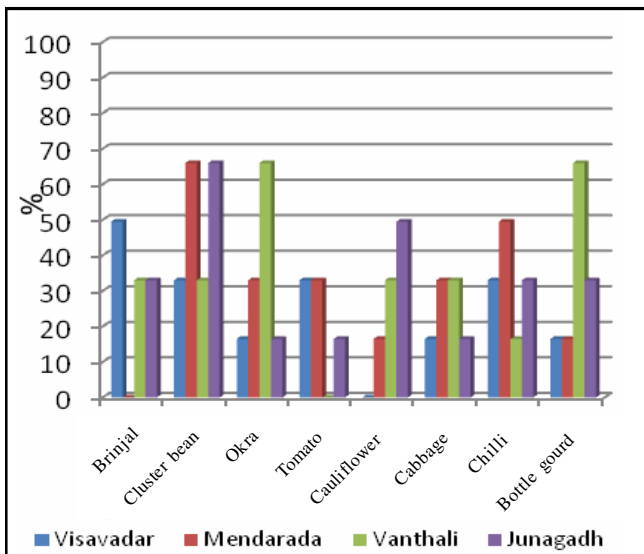


Fig. 3 : *Vibrio* spp. present on surface of vegetables

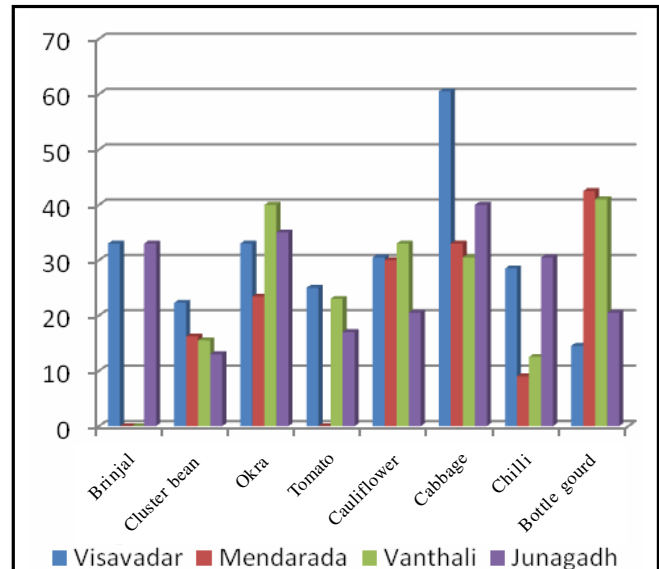


Fig. 5 : *Salmonella* spp. present on surface of vegetables after neem extract wash

Comparative studied of fresh and stored in refrigerator sample:

Comparative study showed that those vegetables samples stored in refrigerator for two days had to reduction of microbial load 30-50 per cent and also reduction of pathogen load as compared to fresh vegetables. The vegetables samples washed with tap water and Neem leaf extract water showed reduction in microbial load of 40-80 per cent and also reduced

Conclusion:

Fresh vegetables of local market harbour many pathogens indicating that contamination is due to subsequent handling, source of water, condition of local market yard, packaging, storage and transit. In India, majority of the people prefer to buy the local market’s fresh vegetables. However, the quality of fresh produce of the local market must be maintained in hygienic condition and proper handling, transport, storage must be controlled so that risk of contaminants decreases and chances of food borne outbreaks

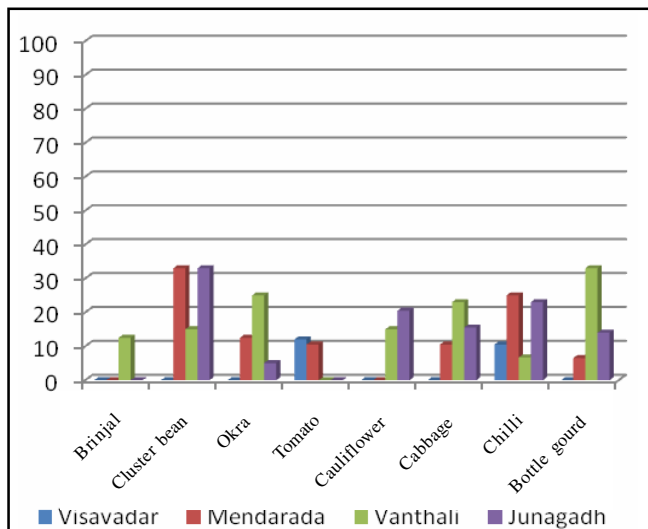


Fig. 6 : Represent the *Vibrio* spp. present on surface of vegetables after neem extract wash

can be minimized. These can be done by pre-treatment of

fresh produce by various anti-microbial agents to decrease the density of microbial contaminants from the surface of the fresh produce.

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