



Bacterial Load on Street Vended Salads in Jaipur City, India

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Abstract

Salad is a mixture of fresh vegetables and fruits; eaten raw or partially cooked that promotes good health but harbour a wide range of microbial contaminants. To determine the bacterial load on salads sold in Jaipur city, India, salads viz. carrot, coriander and cucumber were collected from different sampling sites. Viable bacterial counts on the surface of salads were studied after imposing following main and sub treatments. Main treatments were (i) washing with ordinary tap water and (ii) washing with warm tap water (40°C). The sub treatments were (i) peeling and (ii) no peeling.

In general, bacterial load on salads after washing varied from 0.320 to 1.38×10^4 cfu/ml water wash. Coriander had more bacterial load than carrot and cucumber, respectively. Warm water washing was significantly superior and dislodged 37.3%, 37.6% and 38.7% more bacteria from carrot, coriander and cucumber, respectively over ordinary tap water washing. Peeling, as expected, also reduced the bacterial load by 40.4 % and 31.3% over no peeling treatment in case of carrot and cucumber, respectively.

All the salad samples carried gram positive as well as gram negative bacteria; however, the population of the later was more. Bacteria belonging to ten genera were identified. *Bacillus* (24.5%) was frequently isolated followed by *E.coli* (11.8%), *Pseudomonas* (11.8%), *Staphylococcus* (10.9%), *Enterobacter* (9.0%), *Streptococcus* (6.4%), *Klebsiella* (5.4%) and *Citrobacter* (3.6%). Two useful genera were *Lactobacillus* (1.8%) and *Leuconostoc* (1.8%) also identified. These bacteria may enter in plant tissue through irrigation water and persist there.

The findings in the present study indicate poor handling practices and therefore the risk presented to the consumers suggest the need of spreading awareness and measures to improve microbial quality of salads. When anyone purchases fresh salads first of all ensures the safety measures.

Key words: Bacterial load, salad, carrot, coriander, cucumber.

Introduction

Salad can be defined as a food made primarily of a mixture of raw vegetables and/or fruits. Health benefits of salads are many, owing to the various vegetables present in them. Vegetables are a good source of antioxidants and phytonutrients. They are low in calories and are rich in complex carbohydrates, vitamins and minerals. Salads should be cleaned properly, as they are generally eaten raw or partially cooked. If these are not cleaned properly, these become source of food-borne illnesses.

Pathogens on edible plants present a significant potential source of human illness. A significant portion of enteric pathogens can persist on the surface and proliferate. Proliferation of these dangerous pathogens can increase the likelihood of food-borne disease associated with fresh or minimally processed produce. Fresh vegetables and fruits become contaminated with microorganisms during production, harvest, packing, and distribution (Bartz, Wei, 2003).

Several outbreaks of gastroenteritis have been linked to the consumption of contaminated fresh vegetable borne outbreak, occurred in Japan in 1996 in which 11,000 people affected and about 6,000 cultures were confirmed. The outbreak involved the death of three children and was carried by *Escherichia coli*. The most common bacterial

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enteropathogens associated with fruits and vegetables are *Salmonella* spp. (Thunberg et al., 2002). *E. coli*O157 outbreaks were associated with apple cider, lettuce, radish, alfalfa sprouts, and other mixed salads (Beuchat, 1996).

There are reports of food borne illness associated with the consumption of fruit juices at several places in India and elsewhere (Bhaskar et al., 2004; Chumber et al., 2007; Ghosh et al., 2007). Such juices have shown to be potential sources of bacterial pathogens notably *E. coli* 0157:H7, species of *Salmonella*, *Shigella*, and *S. aureus* (Buchmann et al., 1999)

In India the presence of coliforms and *staphylococci* in kinnow and mandarin juices in Patiala city could be reported (Ganguli et al. 2004). Similarly coliforms were observed, in fresh fruit and vegetable juices sold by the street vendors of Nagpur city (Titarmare, Dabholkar and Godbole, 2009)

As the salads viz. carrot, coriander and cucumber have a very high consumer preference and eaten raw or partially cooked due to health effect throughout the country. The present work was undertaken to determine the bacteriological quality of fresh salads sold by the street vendors of Jaipur city.

Materials and Methods

Salads viz. carrot, coriander and cucumber were collected aseptically from various local markets of Jaipur city and placed in sterile container, then transferred to the laboratory for sample analysis. The samples were analyzed within two hours from procurement. Total fifteen samples were collected from different sampling sites of Jaipur.

Bacterial counts were determined after imposing following main and sub treatments. Main treatments were (i) washing with ordinary tap water and (ii) washing with warm tap water (40⁰ C). The sub treatments were (i) peeling and (ii) no peeling.

For the treatments, 20g of salad sample was washed with 200ml ordinary tap water for two minutes. Then rinsed salad sample was divided into two parts equally, one part of salad sample (10g) without peeling and another part was peeled off, both kept in 100ml sterile distilled water in separate sterile 250ml conical flask, then placed on shaker for 10 minutes. Now, the isolation was carried out using tenfold serial dilution of rinsed water sample and spread on nutrient agar, MacConkey agar and MRS agar media plates. Similarly, 20g of salad sample was washed with 200ml warm tap water (40⁰ C) for two minutes. Then rinsed salad sample was divided into two parts equally, one part of salad sample (10g) without peeling and another part was peeled off, both kept in 100ml sterile distilled water in separate sterile 250ml conical flask, then placed on rotator shaker for 10 minutes. Now, the isolation was carried out using tenfold serial dilution of rinsed water sample and spread on respective media plates.

Pure cultures of different isolates were obtained by streak plate method on respective media. Isolates were characterized on the basis of morphological analysis, biochemical tests and Gram's reaction. Catalase, Indole test, Methyl-red, Voges Proskauer tests, TSI, H₂S production gas production, Citrate utilization tests and Lactic acid test were performed.

The experimental results are expressed as means \pm Standard Deviation (S.D.) of triplicate measurements. The results were processed using Microsoft Excel 2007 and the data were subjected to one way analysis of variance (ANOVA) and the significance of differences between samples means were calculated by XLSTAT 2010 (Addinsoft SARL, USA) software using Duncan's multiple range tests. *P* values \leq 0.05 were regarded as significant.

Results & Discussion

In general, bacterial load on salads after washing varied from 0.320 to 1.38 \times 10⁴ cfu/ml water wash. Microbial load ranged from 1.3 \times 10⁶ - 1.82 \times 10⁷ cfu/ml in fruits and vegetable juices sold in Nagpur city (Titarmare, Dabholkar and Godbole, 2009). Similarly, microbial load ranged from 1.3 \times 10⁶ - 3.0 \times 10⁷ cfu/ml on all the fruits and vegetables sampled from Sango, Ota, Nigeria (Eni, Oluwawemitan and Solomon, 2010).

Coriander had 28% and 31% more bacterial load than carrot and cucumber, respectively. Warm water washing was significantly superior and dislodged 37.3%, 37.6% and 38.7% more bacteria from carrot, coriander and cucumber, respectively over ordinary tap water washing. Peeling, as expected, also reduced the bacterial load by 40.4 % and 31.3% over no peeling treatment in case of carrot and cucumber, respectively.

All the salad samples carried gram positive as well as gram negative bacteria; however, the population of the later was more nearly 8.3%. Bacteria belonging to ten genera were identified. *Bacillus* (24.5%) was most frequently isolated followed by *E.coli* (11.8%) *Pseudomonas* (11.8%) *Staphylococcus* (10.9%) *Enterobacter* (9.0%) *Streptococcus* (6.4%) *Klebsiella* (5.4%) *Citrobacter* (3.6%) and 12.7% remain not identified. Bacteria of belonging to the same genera were also identified by Eni, Oluwawemitan and Solomon, 2010 in fruits and vegetables sampled from Sango, Ota, Nigeria.

Bacteria belonging to two useful genera were *Lactobacillus* (1.8%) and *Leuconostoc* (1.8%) also identified. These bacteria were isolated after peeling in case of carrot and cucumber and in coriander after warm water wash. These bacteria may enter in plant tissue through irrigation water and persist there.

The microorganisms present in fruits and vegetables are a direct reflection of the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce (Beuchat,1996).All the bacteria isolated in this

study have been previously isolated from fruits and vegetables in other studies, both in India and elsewhere (Tambekar and Mundhada, 2006). Bacteria enter intercellular spaces through natural apertures such as stomata, lenticels, broken trichomes and stem scars and may persist in healthy plant tissue (Bartz and Wei, 2003).

This study provides a general overview of the microbiological quality of fresh salads sold in Jaipur city, India. The results in the present study clearly indicate the poor hygienic conditions of these salads and the consumers are at risk of contacting food borne infections. While the practice of consumption of salads cannot be stopped on nutritional grounds, therefore measures should be taken to spread awareness amongst the vendors about the safe and hygienic practices and Government agencies can take initiatives in this direction to lay out guidelines for selling of fresh fruits and vegetables. As consumers, we need to recognize that food safety is important for fresh fruits and vegetables. Food from sources like super markets may be protected from contamination and spoilage during subsequent handling, packaging, storage and while it transit. Before purchase fresh salads considers the safety measures.

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Table1. Bacterial load on Salads (cfu × 10⁴/ ml of washing)

Treatments	Carrot	Coriander	Cucumber
Tap water washing		1.38 ± 0.367	
i) No peeling	1.038 ± 0.100		1.088 ± 0.043
ii) Peeling	0.774 ± 0.121		0.484 ± 0.077
Warm water washing		0.834 ± 0.281	
i) No peeling	0.682 ± 0.101		0.676 ± 0.052
ii) Peeling	0.396 ± 0.050		0.320 ± 0.030

*Values are mean ± SD and significant at (p < 0.05)

Table2. Characterization of isolates

Total No. of Isolates	110
Gram Positive Cocci	21
Gram Positive Bacilli	27
Gram Negative Bacilli	52
Catalase Positive	92
Indole Positive	16
MR Positive	24
VP Positive	33
Citrate Positive	42
TSI	54 (+/+), 12 (-/-), 44(-/+)
H ₂ S Production	04
Gas Production	37
Lactic Acid test	04

Table3. Identification of isolates on the basis of Gram's reaction and biochemical tests.

Name of bacteria	No. of bacteria (cfu × 10 ⁴ / ml of washing)		
	Carrot	Coriander	Cucumber
<i>E.coli</i>	04	05	04
<i>Enterobacter</i>	03	04	03
<i>Staphylococcus</i>	02	06	04
<i>Streptococcus</i>	03	02	02
<i>Pseudomonas</i>	05	04	04
<i>Klebsiella</i>	02	03	01
<i>Citrobacter</i>	01	01	02
<i>Bacillus</i>	08	13	06
<i>Lactobacillus</i>	02	-	-
<i>Leuconostoc</i>	-	01	01
Not identified	06	05	03