



**RESEARCH ARTICLE**

**Isolation and Characterization of Bacterial Contaminants of Salad Vegetables**

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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 pathogenic microorganism associated with human diseases. A significant portion of enteric pathogens can persist on the surface and proferate. Proliferation of these dangerous pathogens can increase the likelihood of food borne disease associated with fresh or minimally processed produce. Fresh sample of radish, cucumber and cabbage collected from different markets and vendors in Bahadrabad, Haridwar, Uttrakhand, India. Viable bacterial counts on the surface of salad vegetables were studied using spread plate agar dilution method. Bacterial load ranges from  $3.0 \times 10^6$  to  $1.0 \times 10^7$  cfu/ml on NAM plates and  $2.1 \times 10^7$  to  $1.2 \times 10^9$  cfu/ml on MacConkey plates. The dominant bacteria were *Staphylococcus aureus*, *Listeria* spp. and *Shigella* spp. found in all samples. *Staphylococcus aureus* was isolated from majority of the samples. In our present study we found *Staphylococcus aureus* (50%), *Listeria* spp. (45%) and *Shigella* spp. (5%) respectively.

**KEYWORDS**

Salad Vegetables, Pathogenic Microorganism, Spread Plate Method, Viable Bacterial Count

**INTRODUCTION**

Salad vegetable can be defined as a food made primarily of a mixture of raw vegetables and/or fruits. Health benefits of salad vegetables are many, owing to the various vegetables present in them. The salad vegetables include tomatoes, cucumber, carrots, green chilli, cabbage and lettuce. They are sold in almost every market and can be seen hawked around by traders. Salad vegetables are a good source of antioxidants and phytonutrients. They are low in calories and are rich in complex carbohydrates, vitamins and minerals. These are good source of beta carotene, vitamin C and fibre.<sup>1</sup> Radish, cucumber and cabbage have beneficial health applications, they also clear the body toxin and purify the blood.

It also used in urinary disorder, cardiovascular condition, leucoderma, blood pressure, diabetes, fever etc. In Cato the Elder's work de agricultura ("on agriculture"), he suggested that women could prevent disease in their private parts by bathing in urine obtained from those who had frequently eaten cabbage.<sup>2</sup>

The extent of contamination depends on several factors that include, among other, use of untreated wastewater and water supplies contaminated with sewage for irrigation, post-harvest handling. Using of manure promotes the growth of crops and vegetables also contain a large number of pathogenic microorganisms.<sup>3,4,5</sup> Manure contains a large number of pathogenic microorganisms including *Salmonella*, *Escherichia coli* 0157:H7, *Bacillus anthracis*, *Brucella* spp., *Listeria monocytogens*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Klebsiella* spp. (Alice, 1997). *E. Coli* 0157 outbreaks were

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associated with apple cider, lettuce, radish, and other mixed salads.<sup>6</sup> However, cabbage has been linked to outbreaks of some food borne illnesses, including *Listeria monocytogenes*<sup>7</sup> and *Clostridium botulinum*. *Shigella* species are able to survive in shredded cabbage.<sup>8</sup> The major bacterial disease shaded are the various enteric diseases, diarrhoea, anthrax, salmonellosis, listeriosis, crown's disease, neurological disorders, antritis, etc..<sup>13,14</sup>

Salad vegetables should clean properly, as they are generally eaten raw or partially cooked. If these are not cleaned properly, these become source of food borne illness. Most pathogens in salad vegetables do not cause product spoilage, even at relatively high population. In the absence of spoilage signs, salads are consumed because they are perceived as safe. Several studies have associated outbreak of food poisoning with consumption of raw vegetable salad.<sup>15</sup> The presence of *E.coli*, *Enterobacter* spp., *Salmonella* spp., *Shigella* spp. and *Pseudomonas aeruginosa* has been reported in salad vegetables.<sup>16</sup>

Human pathogens that cause food borne infection or food poisoning can be prevented by the use of these antibiotics or their modified forms either as food preservative or as a normal antibiotic to cure food borne bacteria infection.<sup>17,18</sup>

In the present study we have determined the presence of bacteria, as well as their antibiotic susceptibility, in fresh salad vegetables sold in Bahadrabad region, come under district of Haridwar, Uttrakhand, India.

## MATERIAL AND METHODS

### Collection of Samples

A total of 9 samples of radish (3), cucumber (3) and cabbage (3) were collected in sterile polythene bags from different markets and vendors in Bahadrabad, Haridwar, Uttrakhand, India.

### Isolation and Identification of Bacteria

A 25gm of vegetable samples were weighed and rinsed in a 250ml beaker containing 100ml of sterile distilled water. The rinsed water samples were diluted  $10^{-1}$  to  $10^{-7}$ . 0.1ml of each dilution

was spread on Nutrient agar medium and MacConkey agar medium. Then the plates were incubated at 37°C for 24h to 48h for isolation of bacteria. Total viable counts were determined by counting colonies growing on NAM plates and both red and non-red colonies growing on MacConkey plates. On the basis of morphological characteristics, colonies on NAM plates and red- non red colonies on MacConkey plates were selected for Gram's staining. Biochemical tests- catalase, indole test, Methyl red, Voges Proskauer test, H<sub>2</sub>S production gas production, citrate utilization test were performed.

## RESULTS

### Total Viable Count

The salad vegetables showed a wide variation in total viable count ranging from  $2.1 \times 10^7$  to  $1.2 \times 10^9$  (on NAM plates) and  $3.0 \times 10^6$  to  $1.0 \times 10^7$  (on MacConkey plates).

### Cultural Characteristics and Biochemical Identification of Isolated Strains

Many bacterial types were isolated, golden yellow colour colonies from NAM plates and non-red colonies from MacConkey plates that grew with golden yellow on Mannitol salt agar were Gram positive, catalase positive, methyl red positive, indole test negative were taken as *Staphylococcus aureus*; White colonies from NAM plates and non-red colonies from MacConkey plates that were Gram positive, catalase positive, motility positive and carbohydrate fermentation positive, methyl red negative and indole negative taken as *Listeria* spp.; White colonies from NAM plates and non-red colonies from MacConkey plates that were Gram negative, methyl red positive, voges-proskauer-negative, H<sub>2</sub>S production negative, citrate negative were taken as *Shigella* spp.

In our present study the percentage occurrence of bacterial isolates were *Staphylococcus* (50%), *Listeria* spp. (45%) and *Shigella* spp. (5%) respectively. During the study of Antibiotic sensitivity of different Antibiotics on *Staphylococcus* spp., *Listeria* spp. and *Shigella* spp., following result was obtained.

Table 1: Table showing total viable count of salad vegetables

Sampling No.	Date of Sampling	Site of Sampling	Type of sampling	Total Viable Count (cfu /ml) on NAM plates	Total viable count(cfu/ml) on MacConkey plates
1.	30/1/2015	A	Cabbage	3.3x10 <sup>7</sup> SD2.4x10 <sup>8</sup>	–
2.	30/1/2015	A	Cucumber	2.1x10 <sup>7</sup> SD1.13x10 <sup>8</sup>	–
3.	30/1/2015	A	Radish	2.7x10 <sup>7</sup> SD2.5x10 <sup>8</sup>	1.0x10 <sup>7</sup> SD 2.3x10 <sup>8</sup>
4.	10/2/2015	B	Cabbage	1.2x10 <sup>8</sup> SD2.1x10 <sup>9</sup>	4.0x10 <sup>6</sup> SD 1.4x10 <sup>6</sup>
5.	10/2/2015	B	Cucumber	1.4x10 <sup>8</sup> SD5.9x10 <sup>9</sup>	3.0x10 <sup>6</sup> SD 0
6.	10/2/2015	B	Radish	1.5x10 <sup>8</sup> SD3.6x10 <sup>9</sup>	3.0x10 <sup>6</sup> SD 0
7.	24/2/2015	C	Cabbage	2.2x10 <sup>8</sup> SD6.3x10 <sup>8</sup>	–
8.	24/2/2015	C	Cucumber	1.2x10 <sup>9</sup> SD3.1x10 <sup>10</sup>	1.0x10 <sup>7</sup> SD 0
9.	24/2/2015	C	Radish	4.8x10 <sup>8</sup> SD1.6x10 <sup>9</sup>	1.0x10 <sup>7</sup> SD 0

A= Vendor-I Kaliyar Road, Bahadrabad

B= Vendor-II Kaliyar Road, Bahadrabad

C= Peeth Bazaar, Bahadrabad

(-) = No growth

CFU = colony forming unit

SD= Standard deviation

Table 2a: Identified bacteria in different salad vegetables (On NAM)

Table 2b: Identified bacteria in different Salad vegetables (On MacConkey)

Sample Site	Sample	<i>Staphylococcus aureus</i>	<i>Listeria</i> spp.
A	A1	-	+
	A2	+	+
	A3	-	+
B	B1	+	-
	B2	+	-
	B3	+	-
C	C1	-	+
	C2	+	+
	C3	-	+

Sample Site	Sample	<i>Staphylococcus aureus</i>	<i>Listeria</i> spp.	<i>Shigella</i> spp.
A	A1	-	+	-
	A2	+	-	-
	A3	+	-	+
B	B1	+	-	-
	B2	-	+	-
	B3	+	-	-
C	C1	-	+	-
	C2	+	-	-
	C3	+	-	-

A1= Cabbage, A2= Cucumber, A3= Radish,  
Positive = (+), Negative= (-)

Table 3a: Antibiotic sensitivity (ZOI) against *Staphylococcus aureus*

Antibiotics	Concentration mg/ml	<i>Staphylococcus aureus</i> ZOI (mm)
Amoxicillin (AM)	10	-
Cefazolin (CF)	30	-
Cephalexin (CP)	30	-
Roxithromycin (TH)	30	-
Cefadroxil (CD)	30	-
Erythromycin (E)	15	-
Ciprofloxacin (CL)	5	12
Vancomycin (Vn)	30	-
Ofloxacin (OF)	5	-
Sparfloxacin (SP)	5	-
Ampicillin (I)	10	-
Cloxacillin (V)	5	-

(-) = No zone of inhibition

Table 3b: Antibiotic sensitivity (ZOI) against *Listeria spp*

Antibiotics	Concentration Mg/ml	<i>Listeria spp.</i> ZOI (mm)
Ampicillin (AS)	20	-
Co-Trimoxazole (BA)	25	-
Cephalexin (PR)	30	-
Tetracycline (TE)	30	16
Cefotaxime (CF)	30	18
Ciprofloxacin	5	32

(RC)		
Levofloxacin (QB)	5	24
Linezolid (LZ)	30	24
Cloxacillin (CX)	1	-
Roxithromycin (AT)	15	-
Lincomycin (LM)	2	-
Gentamicin (GM)	10	-

(-) = Not measurable

Table 3c: Antibiotic sensitivity (ZOI) against *Shigella spp*

Antibiotics	Concentration Mg/ml	<i>Shigella spp.</i> (ZOI) mm
Ampicillin (AS)	20	9
Co-Trimoxazole (BA)	25	11
Cefotaxime (CF)	30	21
Piperacillin (PC)	100	10
Chloramphenicol (CH)	30	19
Ciprofloxacin (RC)	5	30
Ceftizoxime (CL)	30	21
Tetracycline (TE)	30	12
Ofloxacin (ZN)	5	-
Gentamicin (GM)	10	-
Amikacin (AK)	30	-
Gatifloxacin (GF)	10	19

(-) = Not measurable

*Staphylococcus aureus* showed resistance to all antibiotics except Ciprofloxacin and maximum zone of inhibition was 12mm (Table 3a). *Listeria* spp. Showed sensitivity to all antibiotics but less sensitive to tetracycline, Cefotaxime, Ciprofloxacin, Levofloxacin and Linezolid (Table 3b). *Shigella* spp. sensitive to all antibiotics but higher sensitive to ofloxacin, gentamicin and amikacin (Table 3c).

## DISCUSSION

Freshly consumed vegetables especially those used in salad mixture, have been implicated in food poisoning and thus hazardous to the health of the consumers. This could be linked to the fact that most of these salad vegetables are consumed without being subjected to thermal process or even through washing.<sup>19</sup> In this study, the total viable count was appeared on NAM and MacConkey agar by spread plate count method.<sup>20</sup>

In the present study we determined bacterial contaminants of salad vegetables (cabbage, cucumber and radish) from different sites. Among the radish analyzed, sample sourced from Peeth Bazar, Bahadrabad had the highest total viable count of  $4.8 \times 10^8$  cfu/ml. Radish are usually harvested from the soil hence can become contaminated by pathogenic organisms in soil. All the bacteria was isolated in this study have previously isolated from salad vegetables in other studies in Abroad and other places. Among the three different salad vegetables analyzed, cucumber samples had the lowest bacterial load. Cabbage samples had the highest bacterial load of  $2.2 \times 10^8$  cfu/ml. The highest total viable count for cucumber samples sourced from Peeth Bazar, Bahadrabad with a load of  $1.2 \times 10^9$  cfu/ml. This result was higher than the bacterial load of  $2.4 \times 10^7$  cfu/g reported by Aboh *et al.*, (2011). Similarly, the bacterial load of  $1.9 \times 10^6$  cfu/g reported by Abdullahi and Abdulkareem (2010).<sup>9</sup>

All the salad samples carried gram positive as well as gram negative bacteria. Rajvanshi, A. (2010) identified *Bacillus* (24.5%), *E.coli* (11.8%), *Pseudomonas* (11.8%), *Staphylococcus* (10.9%), *Enterobacter* (9.0%), *Streptococcus*

(6.4%), *Klebsiella* (5.4%) and *Citrobacter* (3.6%). Similarly, bacteria of belonging to the same genera were also identified by Eni *et al.*, 2010<sup>20</sup> in vegetables sampled from Sango, Ota, Nigeria. But in our present study we found *Staphylococcus* (50%), *Listeria* spp. (45%) and *Shigella* spp. (5%) respectively.

The presence of *Staphylococcus aureus* and *Listeria* spp. observed in all samples of salad vegetables which are predominant. The detection of *S.aureus* is of serious public health importance because of its ability to cause a wide range of infections especially food borne intoxication.<sup>16</sup> Contamination with *S.aureus* has been linked to carriage in nasal passages of food handlers or by infected workers. The presence of *S.aureus* and some Gram negative rods have been reported to contaminate some salad vegetables such as carrots, cucumber, tomato and radishes.<sup>21</sup> *Shigella* spp. are non-lactose fermenter usually associated with water contamination. Contamination with these organisms could arise from washing vegetables with contaminated water or handling of vegetables by infected workers. *Shigella* spp. has been frequently found in salad and dairy products. It is a principal agent of bacterial dysentery.

## CONCLUSION

The present study showed that there were high bacterial load present in salad vegetables taken from different sites (vendor-I, Kaliyar road Bahadrabad. vendor-II, Kaliyar road Bahadrabad and Peeth bazaar, Bahadrabad). Especially salad vegetables collected from vendor-II, Kaliyar road Bahadrabad has high number of bacteria because of waste water running around the vendor. The dominating bacteria were *Staphylococcus aureus*, *Listeria* spp. and *Shigella* spp. were reported in salad vegetables due to poor handling of these salad vegetables. *S. aureus* showed that they are resistance to all antibiotics except ciprofloxacin. While the practice of consumption of salad cannot be stopped on nutritional grounds, therefore measures should be taken to spread awareness amongst the vendors about the safe and hygienic practise and Government agencies can take initiative in this direction to lay out

guidelines for selling of fresh fruits and vegetables.

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