

## Assessment of Microbial Burden on Vegetable Salads for Food Safety and Human Health

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### Abstract:

Plates of mixed greens salads have become fundamental piece of individuals' eating routine all around the globe. They are normally consumed raw and with no treatment or careful washing; henceforth they have been accounted for to become vehicles for the transmission of pathogenic microorganism related with human diseases. Therefore, in present study fresh samples of salads consisting of various fruits and vegetable salads were collected from different areas in vicinity Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala (HR), India and evaluated for bacterial loads using standard microbiological techniques. Most of samples showed presence of high amount mesophilic bacteria. Some samples further showed the presence of pathogens like *Escherichia coli*, *Salmonella spp.* and *Staphylococcus spp.*, which were confirmed by using various biochemical test like triple sugar iron test, catalase test and sugar fermentation test etc. Most of microorganisms were found to be susceptible towards various antibiotics used in the study. The study may be helpful to policy makers in planning of food safety and health security in future for food borne disease free livelihood, sustainable development, economy and mankind.

**Keywords:** *Escherichia coli*, *Salmonella spp.*, *Staphylococcus spp.*, Antibiotic susceptibility test, Triple sugar iron test, Simmons citrate test, Catalase test, Food security, Human health.

## INTRODUCTION

In recent years, because of health interest and renewed diet trends fruits salad, vegetable salad and mix salads are gaining increasing importance in human diet and becoming integral part of the human diet (Desbordes, 2003). Moreover for weight and health conscious persons mix salads are very beneficial owing to their high vitamin and fiber contents (Ramteke et al., 2016). Over the last twenty years, researches in human nutrition have shown that a balanced diet rich in fruits and vegetables ensures good health and can reduce the risk of certain diseases (Dègnon, 2018). As a result, consumption of these salads has increased worldwide (Eraky et al., 2014). Ready-to-eat salads are mostly prepared from raw fruits and vegetables. But, a poor hygienic quality of salads results mainly from a lack of hygiene and non-compliance of the good practices and often leads to large number of food poisoning (Froder et al., 2007). New cut vegetables are viewed as a potential danger since the event of pathogens can't be rejected and the item is consumed without preparing. In this manner,

ready to-eat serving of mixed greens vegetables could be a potential pathogen source if not cleanly handled before utilization (Mritunjay and Kumar, 2017a). Furthermore salads are typically served at room temperature, which can aggravate the problem of contamination by various microbes (Garg et al., 1990).

A significant contributing variable in these raw horticultural products are contamination by creature or human waste and utilization without a preparing step that will remove related bacterial pathogens. One potential source of entry of microorganisms into foods grown from the ground is by natural introduction with take-up happening through either due to explicit morphological structures in the plant or potentially through breaks in tissues that happen because of punctures, wounds, cuts and parts (Johnston et al., 2005). These abuses to the organic product or vegetable can happen during growing or harvesting, furthermore preparing conditions and inappropriate handling contribute generously to the passage of bacterial pathogens into the item (Maffei et al., 2016). The regular food borne pathogens related with raw leafy foods incorporate *Clostridium spp.*, *Escherichia coli*, *Shigella spp.*, *Campylobacter jejuni*, *Staphylococcus aureus*, *Salmonella spp.*, and *Bacillus cereus* and so forth (Kumar et al., 2006). These are a portion of the basic microorganisms that cause food related disease. Microorganisms, for example, *Salmonella* species, *S. aureus* and *E. coli*, which can be passed on by food to humans, cause food contamination and food borne sickness, for example, tuberculosis, typhoid fever and cholera (Foskett et al., 2003). Similar studies led in different parts of the world have additionally recognized vegetables prepared by food sellers particularly by street food vendors to be exceptionally contaminated with fecal material and harmful micro-organisms (Henseler, 2005; Olsen, 2005; Amoah et al., 2006). Accordingly, food safety has become a genuine concern and a major focus for some researchers in recent years. Likewise, the enthusiasm of general society on sanitation issues is on the domination around the world (WHO/FAO, 2015). Regardless, food safety related issues keep on enduring over the globe and stay an incredible test (Victor et al., 2017). Therefore, the present study was focused on assessment of microbial load on raw salads served with food in Mullana city of Ambala district in Haryana, India for food safety and public health. Mainly study was conducted to assess the presence of enteric pathogenic bacteria like *E. coli*, *S. typhi* and *Staphylococcus spp.*

## MATERIALS AND METHODS

The present study was carried out in Mullana area around M.M. (Deemed to be University) in Ambala district of Haryana state, India (30.1502°N, 77.0242°E). The study was conducted during the period from February to March, 2019. Samples were collected on different days and different vendors. Samples were collected early in the morning in sterilized zip lock polypropylene (PP) bags and transported to the laboratory as soon as possible. The samples were analyzed within 2hrs of collection. The samples included various ready-to-eat fruits and vegetable salads, like carrot, beetroot, capsicum, cabbage, tomato, radish and cucumber etc. A total of 20 samples were collected from 10 different areas serving ready to eat salads to the customers. Integrated samples of vegetables salads (25g each) was blended with 0.1% buffered peptone water (250ml), from which 1:10 serial dilutions were made. Total aerobic plate counts were done using standard methods by plating dilutions on tryptone soya agar, where dilutions was plated in duplicate on plate count agar (PCA HiMedia Lab.) after bacteriological analytical manual (BAM, 2020). Appropriate dilutions were then enumerated for presence and confirmation of *E. coli*, *Salmonella spp.* and *Staphylococcus spp.* For isolation and selection of *E. coli*, MacConkey agar was used; whereas Brilliant Green agar (BGA), Mannitol salt agar (MSA) and Blood agar were used for isolation of *Salmonella spp.* and *S. aureus* respectively.

After spreading on agar plates, the inoculated plates were incubated at requisite time-temperature combinations and the total numbers of colonies was determined as a colony forming unit per ml (CFU ml<sup>-1</sup>) of sample (FAO, 1979; USFDA, 2001). Total number of microbes per gram of salad was calculated accordingly. Based on their morphological contrasts, the microbes were separated for additional investigations. Pure cultures were stored using glycerol stocks at -20°C for further examinations. Finally, the standard biochemical tests i.e. Triple sugar iron agar (TSIA) test, Simmons citrate agar (SCA) test, Catalase test, Sugar fermenting test (glucose, sucrose, maltose and lactose) were performed to confirm the identity of all the isolates using standard methods (FAO, 1979). Thereafter antibiotic susceptibility test was also conducted to assess resistivity and susceptibility of

isolated bacteria towards various antibiotics using standard method (Upadhyay, 2016a). For preparation of bacteriological media, analytical grade chemicals and standard culture media (HiMedia Lab.) were used. Standard borosil make laboratory glasswares were used throughout the experimentation (Upadhyay, 2016b). Every care has been taken to avoid cross contamination during laboratory work and standard laboratory safety rules has been followed. The number of colonies per plate was converted to colony forming unit (CFU/g).

## RESULTS

The results of total plate count and detection of various pathogens from different sampling areas of the site of investigation was recorded carefully (Table 1). The samples collected from various locations were found to be contaminated with high number of mesophilic bacteria and these tallies surpassed as far as possible limits suggested by Indian Food Safety and Standards Act, 2006 of Food Safety and Standards Authority of India. Samples collected from area number 3 were found to be most contaminated followed by area number 6; however, samples collected from area number 1 were found to be least contaminated ( $22 \pm 2.38$ ). During detection of pathogens from collected samples reflected the occurrence of *E. coli*, *Salmonella spp.* and *Staphylococcus spp.* The *E. coli* was found in most of the samples except area number 1, 4 and 5. Whereas *Salmonella spp.* was detected only in samples from area number 7 and 9. The samples collected from area number 4, 8 and 9 was found to be contaminated with *Staphylococcus spp.*, however, samples from area 9 was found to be contaminated with all the pathogens used in the study (Table 1). Further biochemical test like triple sugar iron agar test, simmons citrate agar test, catalase test and sugar fermenting test confirmed the presence of all these pathogens in samples from area number 9 (Fig. 1A-G). The *in vitro* antibiotic susceptibility was tested through disc diffusion assay and showed significant result through noticeable zone of inhibition (Fig. 1H; Table 2).

**Table 1: Total bacterial (mesophilic) counts of various samples (Log CFU/g) and identification.**

| Sampling area | CFU at different dilution factor |                  |                  |                  |               | Pathogenic bacteria species |                       |                           |
|---------------|----------------------------------|------------------|------------------|------------------|---------------|-----------------------------|-----------------------|---------------------------|
|               | 10 <sup>-2</sup>                 | 10 <sup>-4</sup> | 10 <sup>-6</sup> | 10 <sup>-8</sup> | Mean $\pm$ SD | <i>E. coli</i>              | <i>Salmonella sp.</i> | <i>Staphylococcus sp.</i> |
| Area 1        | 9                                | 5                | 4                | 4                | 22 $\pm$ 2.38 | ND                          | ND                    | ND                        |
| Area 2        | 10                               | 7                | 3                | 4                | 24 $\pm$ 1.82 | D                           | ND                    | ND                        |
| Area 3        | 21                               | 15               | 11               | 8                | 55 $\pm$ 5.61 | D                           | ND                    | ND                        |
| Area 4        | 13                               | 8                | 6                | 5                | 32 $\pm$ 5.16 | ND                          | ND                    | D                         |
| Area 5        | 9                                | 9                | 5                | 3                | 26 $\pm$ 3.55 | ND                          | ND                    | ND                        |
| Area 6        | 18                               | 14               | 7                | 4                | 43 $\pm$ 6.39 | D                           | ND                    | ND                        |
| Area 7        | 11                               | 8                | 8                | 3                | 30 $\pm$ 3.31 | D                           | D                     | ND                        |
| Area 8        | 8                                | 9                | 6                | 5                | 28 $\pm$ 1.82 | D                           | ND                    | D                         |
| Area 9        | 8                                | 9                | 5                | 3                | 25 $\pm$ 2.75 | D                           | D                     | D                         |
| Area 10       | 10                               | 7                | 8                | 5                | 30 $\pm$ 2.08 | D                           | ND                    | ND                        |

Sampling size, 20; D, Detected; ND, Not detected



**Figure 1: Biochemical test and susceptibility assay of bacteria collected from samples of area number 9 showing positive triple sugar iron test (A), simmons citrate agar test (B), catalase test (C), sugar fermentation test using various sugars (D, glucose; E, maltose; F, lactose; G, sucrose) and antibiotic susceptibility test (H).**

## DISCUSSION

Utilization of ready-to-eat fresh fruits and vegetables has expanded around the world, prompting increment in episodes brought about by food borne pathogens. In the Indian subcontinent, raw fruits and vegetables are generally consumed without washing or other cleaning methodology, in this manner prompting new food safety threats (Mritunjay and Kumar 2017b). It has been reported that breaking the protective epidermal barrier of fruits and vegetables before eating using various methods like peeling or slicing not only increased nutrient availability but also provided large surface areas for microbial growth and decreased product shelf-life. Besides, mechanical harms caused to cells during handling not just constrains the shelf-life of raw fruits and vegetables yet in addition gave more passage to food borne pathogens (Badosa et al., 2008). The variation in results collected from various areas may be due to the difference in the hygiene conditions adopted by different vendors and time taken by various vendors before actual serving and removing of skin of fruits and vegetables. Earlier also various studies reported high mesophilic counts on salad vegetables (Badosa et al., 2008; Seo et al., 2010; Oliveira et al., 2011). Another possible reason for difference in results may be due to composition of salads collected from different area. Previously also it has been reported that difference in composition of salads lead to variations in microbial load of salads (Pingulkar et al., 2001; Mritunjay and Kumar 2017a). Present study further confirms this trend of results.

**Table 2: *In vitro* antibiotic susceptibility tests through disc diffusion assay for different bacteria species using various antibiotics (zone of inhibition or clearance in mm).**

| S. No. | Name of antibiotics | <i>E. coli</i> | <i>Salmonella sp.</i> | <i>Staphylococcus sp.</i> |
|--------|---------------------|----------------|-----------------------|---------------------------|
| 1.     | Nalidixic acid      | < 10           | 26                    | < 10                      |
| 2.     | Ciprofloxacin       | 38             | 35                    | 14                        |
| 3.     | Colistin            | 10             | 10                    | NA                        |
| 4.     | Aztreonam           | NA             | NA                    | 12                        |
| 5.     | Meropenem           | NA             | 38                    | > 40                      |
| 6.     | Chloramphenicol     | 28             | 27                    | 34                        |
| 7.     | Amoxyclav           | 14             | 16                    | 22                        |
| 8.     | Cefpodoxime         | 20             | 21                    | NA                        |
| 9.     | Rifampicin          | 11             | 10                    | 12                        |
| 10.    | Tigecyclin          | 25             | 22                    | 24                        |
| 11.    | Netilmicin          | 31             | 20                    | 20                        |
| 12.    | Cefoparazone        | 34             | 25                    | NA                        |
| 13.    | Linezolid           | < 10           | 33                    | < 10                      |

NA, Not applied

The high frequency of coliforms for example *E. coli* in the greater part of the samples might be credited to contact of fruits and vegetables with soil, dust, water, utilization of filthy utensils for serving, cross contamination from spoiled vegetables, or the utilization of grimy handling utensils like cutters and plates (Denis et al., 2016; Abakari et al., 2018). Presence of *E. coli* in plates of mixed greens showed conceivable fecal contamination of nourishment and poor handling practices (Tambekar et al., 2007). A few farmers utilize animal compost or fecal deteriorate as a manure to enhance soil quality which may increment coliform tally (Geldreich et al., 1962). Further, handling of food with contaminated hands and utensils used of untreated waste water for washing purposes lead to the unhygienic status of salads (Greig et al., 2007). Even type of water used for irrigation of these fruits and vegetables also found to influence coliform count of salads, as it was often seen that some farmers especially in the big cities used sewage or untreated water directly from municipality storms for irrigation purpose of vegetables (Pagadala et al., 2015). It has been reported that some strains of *E. coli* could cause gastroenteritis, diarrhea and lead to food borne illness in humans in humans when ingested (Baker et al., 2016). The *E. coli* contamination was found to be influenced by the location in which sampling carried out.

The presence of *Staphylococcus spp.* might be due to its carriage in nasal passages of food handlers or infected workers (El-Shenawy et al., 2014). The *Salmonella spp.* and *Staphylococcus spp.* in vegetables occurred because of its washing with contaminated water and handling by infected workers, vendors and consumers in the market place (Garcia et al., 1987; Mritunjay and Kumar, 2015). Some previous studies also reported about contamination of *Staphylococcus spp.* and *Salmonella spp.* in various food samples (Mudgil et al., 2004; Kumar, 2012). Hand washing analyses of workers have also been reported to contain *Salmonella spp.* which indicated that even food handlers could contaminate salads (Lambrechts et al., 2014). As most of the food vendors and handlers were not using hand gloves while preparing, packing and serving fruits and vegetables. Some past examinations have shown that *Salmonella* cross-contamination happened as often as possible using contaminated vegetables that were inappropriately cleaned and unsanitized (Mritunjay and Kumar, 2017a). The survival and entry of these enteropathogens have been shown in crops, especially carrots, lettuce etc. irrigated with contaminated sewage (WHO, 1998). Another possible reason for contamination by these pathogens might be due that the vendors were sold egg and egg products have been implicated in contamination with various *Salmonella spp.* (Kumar et al., 2006). As it was noticed most of the bacteria strains recovered during investigation was susceptible to available antibiotics corroborated to earlier studies for antibiotic susceptibility and resistivity being conducted towards isolated microorganisms (Eromo et al., 2016). According to a report of the European Union Commission Regulation (2007), effect of microbiological hazards such as *Salmonella spp.* on food safety is now a major public health concern worldwide.

## CONCLUSIONS

Taking everything into account, the high bacterial burden and presence of pathogens like *E. coli*, *Salmonella spp.* and *Staphylococcus spp.* in the samples of mixed greens could serve as a pointer for need to advance awareness about the conceivable well being risks that could be because of poor treatment of these vegetables. Therefore, along these lines the requirement for administrative bodies to guarantee that microbiological standards are set up and followed by farmers and vendors for the handling and distribution while serving of mixed greens vegetables. Thankfully most of the pathogenic organisms were found to be susceptible towards various antibiotics tested while doing antibiotic susceptible testing of isolated microorganisms. But everyone should be careful while eating salads consisting of fresh, raw fruits and vegetables as various foods related illness and outbreaks were reported due to consumption of raw salads. Therefore, present study was conducted to assess the microbial load of salads consisting of raw and minimally processed fruits and vegetables collected from Mullana city of district Ambala, Haryana, India for delivering message among societies towards food safety and human health through cleanliness and germ-free joining hands approaches.

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