Review Article

Assessment of Pathogenic Micro-organisms Associated with Vegetable Salads

Sunil Kumar[1,*], Mukesh Yadav[1], Ashwanti Devi[1], Mahesh Uniyal[2], Vikas Kumar[1], Nirmala Sehrawat[1], Raj Singh[1]

[1]Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University), Mullana (Ambala), Haryana, INDIA.
[2]Department of MMICT and BM, Maharishi Markandeshwar (Deemed to be University), Mullana (Ambala), Haryana, INDIA.

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ABSTRACT

Fruits and salads are the healthy sources of nutrients for many people's regular meals served in restaurants, hotels and in the food points of various educational institutions. However, vegetable salads have been linked with outbreaks in many continents all over the globe, which resulted in several health issues. Reason behind the contamination of salads is primarily the poor hygiene management. Carrot, cucumber, onion, cabbage and tomato samples are most commonly sold salads by street vendors and restaurants and stated as common source of transmission. Aim of this study was to probe the prevalence of most common bacterial strains of the salad samples, which are served in and sold by local street vendors, canteens and restaurants. Present investigation revealed a serious concern of the variable bacterial loads in the salad and vegetable samples. Spreading awareness of the potential health risks were also discussed in the present study, which are associated with poor handling of these salads and vegetables.

Key words: Bacterial Culture, Bacteriological Load, Food Contamination, Pathogenic Bacteria, Salad.

Correspondence:
Dr. Sunil Kumar,
Assistant Profesor, Department of Biotechnology, Maharishi Markandeshwar (Deemed to be) University, Mullana, Ambala, Haryana, INDIA.

Email: sunilhr10h@gmail.com
ORCID: 0000-0002-0689-2244

INTRODUCTION

Salad vegetables are eaten raw, without being cooked, and without being washed or peeled, increasing the risk of food poisoning.[1] Pathogenic microbes cab contaminate vegetables and salads during handling, harvesting, through equipment, and via shipping containers.[2] Food poisoning is a big public health problem all over the world.[3] Certain high-risk factors are associated with human morbidity, mortality, and economic loss, like; absence of proper cooking, consumption without cleaning or washing the salads.[4,5]

In the recent years, requirement for freshly cut and ready to eat (RTE) salads has been improved, owing to the food value and health benefits as well.[6] Different organizations like; WHO suggested and encouraged the usage of salads and vegetables as healthy food.[7-9] Health beneficial values has enthralled much attention towards usage of salads.[7] Raw vegetable has been a congruent part of salads, which offered a vast variety of fiber inside, minerals, vitamins, and additional phyto-chemicals.[10] Salads are a rich source of phyto-nutrients, antioxidants, and have health shielding values to improvize overall human health.[11,12] Consumption of fiber rich meal are resulted in lesser risk of coronary cardiac disease, lower concentrations of blood cholesterol, chronic obstructive pulmonary disease (COPD), and in stroke protection, hypertension, abridged blood pressure, control of body weight, regulaton and control of glycemia control, and mentainance of good microflora of gut.[13,14] Vegetable salads have been seen with anticancer properties and also prevent cardiovascular disease (CVD) expansion.[15-17] Raw vegetables, green vegetables, tomatoes, cruciferous vegetables, carrots, and allium vegetables are different types of vegetables, which have been proven to impart protection against malignancies.[18] Phenolics are yet another important element, which is also derived from

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vegetables in the Mediterranean diet. Flavonoids are the specialized phenolics, which are considered as one of the fundamental bioactive compounds in fatching health promoting effects, like; cancer prevention, pro-apoptotic effects, anti-proliferative action.[19,20] Additional benefits like; prevention of rheumatoid arthritis, neurodegenerative diseases (Parkinson's and Alzheimer's disease) are also associated with ample usage of vegetables.[21-23] Decline in the risk of chronic disease have alo been observed in a few investigations where augmented usage of fruit and vegetable in the diet of older people, which associates a feasible nutritional target with enhanced immune system.[24] Routine consumption of vegetable and salads should be given continued attention to improve health and diminish the risk of acquisistion of disease.[25] Modern guiding principles support an intake of fiber rich diet in doubling and inclusion of plentiful vegetables and fruits servings.[26,27] This study was designed to investigate the common bacteriological and parasitological contamination sources and different factors associated with ready to eat and raw vegetables and salads.

Food-borne pathogens in vegetables salad:

Coliforms are Gram-negative, facultative anaerobic, oxidase negative, non-spore forming rods, which perform lactose fermentation with gas production after 48 hr at 35–37°C.[28] Coliform bacteria can be found in the guts of mammals and humans.[29,30] The genera *E. coli, Klebsiella, Citrobacter, Enterobacter,* and some speacies of *Serratia* together form a composition of entire coliform bacteria.[31,32] Many bacteria of nonfecal source are included in the total coliform clad; thus, the faecal coliforms are distinguished from total coliforms just by their ability to thrive at high temperatures of 44.5°C.[33] The bacteria total coliforms and *E. coli* are commonly utilized as an indicator of hygiene, particularly in the case of faecal contamination.[34] Their existence means that pathogens may be present as a result of human or animal faeces contamination.[35] Other coliforms can be found in atmosphere (soil, nutrient-rich waters, rotting plant matter) and faeces as well, and some of them can also thrive in different water delivery systems.[36]

Gastroenterological diseases by parasitic contamination of raw vegetables and salads is another vital aspect utilized to monitor the human health.[37] Common vegetables are purchased from different markets and street vendors of urban and rural areas. Sediments obtained from vegetables washings are examined by microscopy. Once the culture is grown on artifical media, genomic DNA is isolated from culture samples. Result from a study showed that 34% of the samples under investigation were found with contamination of one or more parasitic species.[38] Typical vegetables harboring parasitic contaminations are lettuce (29.5%), and tarragon leaves (2.3%). In lettuce isolates, *Giardia duodenalis* has been traced most commonly from (23.5%).[39,37] Other parasites have also been detetced in low frequencies from lettuce salads.[40] An urgent need is suggested by multiple investigations for tracing sources of parasitic contamination of salads at high detection level.[41]

In 1885, a German paediatrician named Theodor Escerichia discovered *E. coli* in the faeces of a child suffering from diarrhoea. *E. coli* is a gram-negative bacillus, which ferments lactose and glucose and produce steam and acids. In the human colon environment, *E. coli* is the most copious facultative anaerobic flora amongst others that colonises the latter. *E. coli* belongs to the faecal coliform community and is a more precise source of faecal contamination. Not all strains of *E. coli* are commensals, a few strains have shown the pathogenic properties, which become lethal in human, avian and other mammalian diseases and also in food-borne complications.[42] Some *E. coli* strains develop virulence traits that enable them to cause a wide range of diseases. There are many types of pathogenic *E. coli* strains that cause diarrheal outbreaks.[43] Differences in three structural antigens are used to classify different strains of *E. coli*: antigens H, O, and K. Human *E. coli* can be divided into three classes based on genetic and clinical criteria: pathogenic (enteric or diarrheagenic), commensal, and extraintestinal pathogenic *E. coli* (ExPEC).[44,45]

Diarrhoea causing most frequent agents in infants is *E. coli* (Diarrheagenic) strains, which are categorized under the EPEC (entero-pathogenic *E. coli*), ETEC (entero-toxigenic *E. coli*), a leading cause of travellers' diarrhoea, and enteropathogenic *E. coli* (EPEC, a major cause of travellers' diarrhoea.[46,47] Entero-haemorrhagic *E. coli* (EHEC), causes hemolytic uremic syndrome and hemorrhagic colitis, entero-invasive *E. coli* (EIEC), causes severe dysentery.[48,49] DAEC (diffusely adherent *E. coli*) and EAEC (entero-aggregative *E. coli*), are other toxin-producing types of *E. coli*.[50]

Microbiological investigation

Total Plate Count Method

SPC (Standard Plate Count) is one of the most frequently used tests to assess the quality of food in terms of microbial load. This is also recognized as 'total viable count' or 'aerobic plate count'.[51] However, the importance of SPCs, varies significantly depends up

on food item types and the its underging processing. In SPC methods, vegetable salads dilutions of 10^{-2}, 10^{-3}, and 10^{-4} are prepared using searial dilution with the help of sterile pipettes. Each dilution is pipetted into sterile Petri dishes in duplicate, with correct labeling of different concentrations. The dilution bottle is stirred atleast 5 times and allowed to rest for at least 3 min before being pipette into the Petri dish. 0.1 ml of each of dilution is transferred aseptically onto the nutrient/selective agar plate surface. A sterile L-shaped glass rod is used to spread the inocculum on the agar plate. Then at 35°C, the plates are incubated for 24-48 hr. Next day, total number of isolated colonies are counted and the total aerobic microorganism per gram are determined. Different dilution factors (10^{-2}, 10^{-3} and 10^{-4}) of vegetable salads samples were utilized for claculation of clony forming units (CFUs/ml). Each of the experiment are performed in duplicate from dilutions to colony count to rule out the handling error. Microbiological qualities of RTE salads and foods are assessed using microbiological guidelines to assist in the analysis and interpretation.[52]

Microbiological Quality Categories

Based on SPC, indicator organism levels, presence or number of pathogenic microbes, three microbiological rating divisions have been allocated (Figure 1). There are four levels of satisfaction: potentially dangerous, unsatisfactory, marginal, and optimal.[53]

- Potentially Dangerous – quantities in this range have the potential to cause food poisoning, and prompt action should be taken.
- Unsatisfactory – In this level, results are indicative of poor handling practices or hygiene and hence considered unacceptable food under microbiological limits.
- Marginal – Food items in this level are on the borderline of permissible range of microbiological quality limits, however, issues of hygiene cannot be rulled out.
- Satisfactory – This level states a satisfactory rank of microbiological quality.

Risk associated with contaminated vegetables salads:

Gastroenteritis in several cases of human have been attributed due to ingestion of contaminated vegetables in the last decade, with a rise in frequency.[54] Risk of contracting food-borne illness have mounted as consequence cosumption of raw or improper cooked vegetables, and hence considered as well recognised

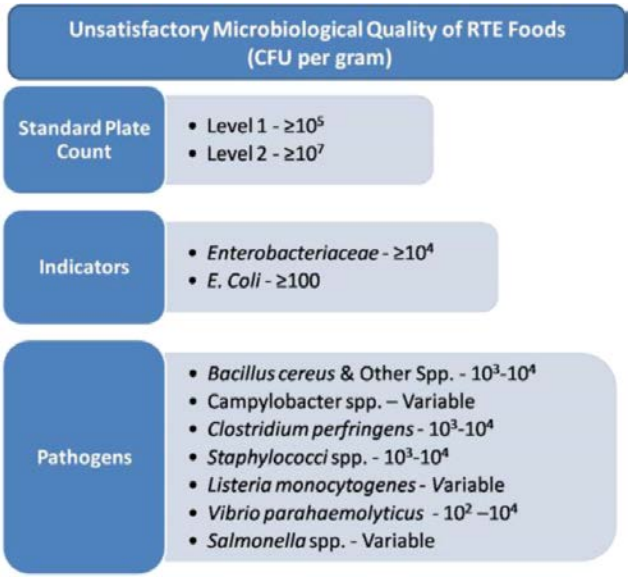

Figure 1: CFUs of different indicator and Pathogenic bacteria showing unsatisfactory microbiological quality of ready-to-eat Foods.

medium for the spread of pathogenic enteric bacteria in human.[47]

According to a recent study, vegetable salads like coriander leaf, cucumber, carrots, and tomato are often tainted with *Bacillus cereus, Aeromonas* spp., *Campylobacter, Salmonella* spp., *Escherichia coli, Staphylococcus* spp and *Shigella* spp.[55] An escalating graph of food-borne sickness has been seen due to under-cooked or raw vegetables resulting in the tansmission of most of the human gastrointestinal pathogens.[56] For safe management of food and to decrease the food associated dieseases, various tools have been developed for risk analysis.[57] Both the food processor industries and regulatory authorities utilize such tools to control the microbial risk to ensure microbiological quality control and safety for consumers.[58] Among various foremost elements of risk analysis, both statistical and scientific information are employed for risk assessment and estimation of probability of death and severe illness.[58,59] QMRA (Quantitative microbial risk assessment) is very crucial for the detection of microbial risks associated with specific food consumption, which also helps in providing the information of infection level coupled with a specific food intake.[60] This makes sure that the resources are decisively synchronized to curb the risk associated with food-borne microbes.

While the spoiling yeasts, bacteria, and moulds prevail in the raw vegetables microflora, other pathogenic parasites such as helminths, and protozoa with a capability of facilitating infections in human have also been recorded

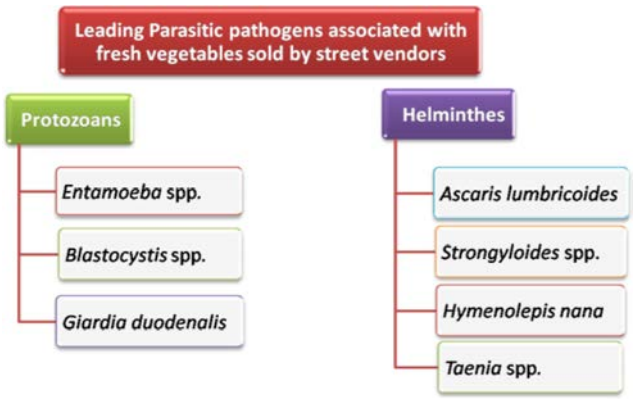

Figure 2: Leading Parasitic Pathogens of RTE Vegetable Salad.

(Figure 2). Minimally processed vegetable salads such as raw vegetables, infected by pathogenic bacteria, like; such as Listeria monocytogenes, *Salmonella*, and *E. coli,* may serve as carriers of bacterial agents to humans and they cause food safety issues, especially gastroenteritis as well as being vectors for traveler's diarrhoea and enterotoxigenic.[27]

Identification

Gram Staining

Following incubation, individual bacterial colonies on each plate are examined and registered based on their form, colour, texture, border, and phenotypic caharacteristic appearance. An isolated representative colony of the cultured sample is Gram stained. Gram staining is used to expose the cells' characteristic grouping and organisation. The gram stain is also known as differential stain as it has got the discriminatory power to distinguish and stain both Gram negative and Gram positive simultaneously on a single slide.[61] Purple color is acquired by Gram positive bacteria (GPB) due to its higher affinity towards crystal violet. When iodine is added, they maintain the crystal violet and purple color is retained after decolorization with acetone. However, Gram negative bacteria (GNB) lose crystal violet color when decolorized with acetone and stain pink due to counterstain Safranin.

Molecular Methods (MALDI–TOF MS)

MALDI-TOF MS (matrix-assisted laser desorption ionization-time of flight mass spectrometry) has recently emerged as a powerful method for routine clinical isolate detection.[62] Bacterial detection using MALDI-TOF MS has been tested quicker, less expensive more precised than traditional phenotypic or molecular approaches.[63] The manufacturer's adjusted score values is used to classify MALDI-TOF MS identifications: a score value of ≥ 2 suggested correct species recognition, a score value from 1.7 to 1.9 suggested the identification up to genus level, and a value of 1.7 indicated no identification or not reliable results. In this technique, overnight grown culture are picked from media plate and a thin smear of the colony is prepared over a steel plate of MALDI-TOF MS instrument. The sample is air dried for a while and 1µl of matrix (alphacyano-4-hydroxycinnamic aci is added over the pre-dried smear follwed by air dry. Now plate with prepared smear is inserted inside the instrument and instrument identified the microorganism using laser beams and analyzing softwares.[64]

DISCUSSION

The present study depicted the microbial variety and their burden in salad vegetables items sold in different canteens and restaurants and isolate and identify bacteria from salad vegetables items. High total viable count (TVC) indicates unsafe condition and therefore the occurrence of possible contamination. Data on vegetable spoiling bacteria and their survival potential is quite obscure and such informations could be of critical importance to determine vegetable shelf life and storage period, and enhance food quality and safety. The current investigation targetted the capacity of vegetable salad to either harbor or inhibit the progression of vegetable spoiling microbes. It is also critical to verify the ability of such microbes to cause pathogenicity to host due to consumption of these spoiled salads and vegetables to avoid the risk of food poisoning. As a result, the period of alive and cultivable microbes in vegetable salad samples are measured with suggestive value of examining product consistency and its ability to sustain micro-organisms. Therefore, present investigation attempted to provide the information on causative agents of food borne illnesses due to contaminated vegetable salads intake.

Finally, showing the potential of vegetables to affect microbial growth would go a long way toward ensuring food consistency, stability, and shelf life. A number of surviellance programs have been conducted previously to draw the demographic scenario of microbial contamination of salad vegetables. Despite of that, informations of contaminants' survival is still limited. Multiple antibiotic resistances were found among bacterial isolates on salad vegetables, according to previous report. Thus, intensive surveillance of isolates to detect emerging developing world is needed. Since salads do not need any additional heat treatment, vigorously washing of vegetables salads and

treatment of the latter with antibacterial chemicals for a comprehensive period of time is necessary to remove pathogens and dramatically reduce the microbial load.

Since the way vegetables were handled on wet markets was less hygienic, it was obvious that samples from wet markets yielded a higher proportion of bacteria. The surroundings and locations for vegetable displays in markets were filthy, and the handlers did not wear gloves when handling the vegetables. Contamination may occur as a result of improper handling and transporting in a contaminated container. Aside from that, market vegetables may have a long holding period, which can lead to the accumulation of pathogenic bacteria.

CONCLUSION

Finally, we conclude with the following recommendations for vegetables salad: Good hygiene practices should be promoted among all food services providers. Implementing and monitoring of safety regulators should be on top priority. Routine review of vegetables agricultural practices must be followed. Setting up local guidelines and food standards on local data are encouraged. Further studies are recommended on Salads and such food to trace sources of contamination. Association of transfer of antibiotic resistance with food borne bacteria are highly recommended to avoid the spread of the latter in food chain.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

CFU: Colony Forming Unit; **COPD:** Chronic Obstructive Pulmonary Disease; **CVD:** Cardio Vascular Disease; **DAEC:** Diffusely Adherent *E. coli;* **DNA:** Deoxyribo-Nucleic Acid; **EAEC:** Entero-Aggregative *E. coli;* **EHEC:** Entero-Haemorrhagic *E. coli;* **EPEC:** Entero-Pathogenic *E. coli;* **ETEC:** Entero-Toxigenic *E. coli;* **GNB:** Gram Negative Bacteria; **GPB:** Gram Positive Bacteria; **MALDI-TOF MS:** Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry; **QMRA:** Quantitative Microbial Risk Assessment; **RTE:** Ready To Eat; **SPC:** Standard Plate Count; **TVC:** Total Viable Count; **WHO:** World Health Organization.

SUMMARY

To summarize the present study, an array of microbial flora associated with vegetable salads was discussed in this study.Risk factors and levels of microbiological quality satisfaction are also discussed. Different phenotypic and molecular methods were discussed for correct identification of contaminating microbes. Good hand hygiene is highly recommended along with proper washing of vegetable salads before consumption.

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