Is Raw Eaten Vegetables and Salads are Safe for Consumption? A Microbiological Investigation in the Context of Food Safety

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ABSTRACT

Salad vegetables are not only a food adjunct but also augment the nutritional value of the diet. Lactuca sativa (Lettuce), Cucumis sativus (cucumber), Lycopersicon esculentum (tomato), Spinach oleracea (spinach), Brassica oleracea(cabbage), Raphanus sativum (radish), Daucus carota (carrot) etc. are the most common vegetable, used in salad. It attracts microbial growth due to improper food processing techniques including both pre and post harvest technology practiced in India. Food-borne pathogens such as Listeria monocytogenes, Salmonella sp., Shigella sp., Campylobacter sp., Staphylococcus aureus, Clostridium botulinum, Escherichia coli etc are the main cause of enteric diseases associated with salads. The present study aimed to evaluate the potential hazard of microorganism associated with raw eaten salad vegetables, and highlighted the importance of proper processing before consumption. There is need to aware the public and farmers on the risk involved in the use of contaminated water and untreated manure during production, that is reflected in the health issues associated with the consumption of raw salads

Keywords: Salad vegetables, Raw eaten, Fresh produce, Microorganism, Pathogens

1. INTRODUCTION

1.1 Salad Vegetables

Salad vegetables arethe heterogeneous ready-to-eat (RTE) dish (eaten raw) and are important component of nutritional diet, providing vitamins and minerals and phyto-nutrients (Taban and Halkman, 2011). Salad vegetables are consumed in large quantities around the world (Nastou et al., 2012). Epidemiological evidence has clearly shown that diets based on fruits and vegetables, with high contents of natural antioxidants, contribute to reduce mortality from cardiovascular and cerebrovascular diseases, although their protective effect on cancer risk is less conclusive (Alia et al., 2003). In recent years many countries have taken initiative to encourage people to eat fresh fruits and vegetables. Leafy green vegetables (spinach, cabbage, lettuce and salad leaves (all

varieties), fresh herbs (cilantro, basil, parsley, chicory) were identified as the commodity group of highest concern from a microbiological safety perspective (FAO/WHO, 2008).Fresh salad vegetables and leafy vegetables are ready-to-eat (RTE) produce for healthy and convenient meals are considered as safe-to-eat by consumers (Lin et al., 1996; Oliveira et al., 2011). This commodity grouping was considered to include all vegetables of a leafy nature and of which the leaf is the intended for consumption such as lettuce (all varieties), spinach, cabbages, chicory, leafy fresh herbs (e.g. cilantro, basil, and parsley) and watercress (FAO/WHO, 2008).

RTE products are very attractive to consumers looking for healthy and convenient food. The microbiological safety of these foods is of special concern due to the absence of lethal treatments during processing. During harvesting, the superficial microbiota of vegetables comprises mainly Gram negative saprophytes, but pathogenic microorganisms can also be found. Vegetables may harbor pathogenic *Escherichia coli* and *Salmonella* sp. enteric bacteria involved in large food borne outbreaks worldwide, causing symptoms of gastroenteritis, and chronic infections. *Listeria monocytogenes* is a psychrotolerant and ubiquitous microorganism that causes listeriosis can contaminate RTE vegetables (Oliveira et al., 2011). Oranusi and Olorunfemi (2011), reported the presence of *Salmonella* sp*., Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus* sp*., Proteus* sp*., Lactobacillus* sp*., Klebsiella, Enterobacter, Micrococcus* sp*., Aspergillus niger, Mucor* and *Penicillium* sp. in ready to eat product.The microbiological safety of RTE is of great concern due to the absence of proper lethal treatments during processing (Oliveira et al., 2011).

1.2. Global Status of salad vegetables and fresh produce

The global production of fruit and vegetables grew by 94% from 1980 to 2004. In Mumbai, India, with its growing urban population travelling long distances to the place of work, it has become increasingly popular to eat healthy raw salad vegetables, fruits and sprouts preferentially to any other fast food. WHO, street-vended food survey revealed that fruits and vegetables from 86% of the total market (WHO, 1997).The United States of America being a major provider of fresh produce, approximately 35% of the fresh produce it consumes is imported. Given the role of fresh produce in a healthy diet, it is critical that these foods are as safe as possible. The safety of fresh produce is a global issue covering both the countries that import fresh fruits and vegetables and the countries that supply them. In many instances countries both export and import produce (FAO/WHO, 2008). Consumption of salad vegetables and fresh produce has increased significantly over the past two decades, because of the health benefits of consumers and they are more concerned about their health, due to this demand a large variety of domestic and imported produce has become available in all seasons (Lin et al., 1996; Cordano and Jacquet, 2009; Taban and Halkman, 2011; Olaimat and Holley, 2012). In Nigeria, the consumption of RTE fruits

consumption increases, as they are easily accessible, convenient and cheaper than whole fruits. The increased consumption with the associated risk of disease, to which consumers may be exposed, is a matter of great concern (Oranusi and Olorunfemi, 2011).

2. SOURCES OF MICROBIAL CONTAMINATION

2.1 pre harvesting

Salad vegetables such as lettuces, cabbage, tomato and, spinach, carry the risk of microbial contamination because of the usage of untreated irrigation water or sewage (Carey et al., 2009; Taban and Halkman, 2011; Kumar, 2012). Fields on which livestock or wild animals have grazed are more likely to be contaminated with enteric pathogens. Some bacteria can survive in agriculture soils for many months. *Salmonella* and *Listeria monocytogenes* could survive for months in sewage sludge applied to agricultural soils. In the case of fresh produce, events occurring before the crop is even planted can affect bacteriological quality and safety of the final product.The choice of a growing location is probably the initial contributing factor that will affect safety (Brackett, 1999). Type of water source also influence the microbiological quality of produce, wastewater contaminate vegetables with pathogenic microorganisms (Brackett, 1999; Solomon et al., 2002; Kumar, 2012). *Escherichia coli, Salmonella* sp.*, Campylobacter* sp. and *Shigella* sp. contaminate fruits and vegetables through contact with contaminated water or sewage (Oranusi and Olorunfemi, 2011; Kumar, 2012). Contamination with *E. coli* O157:H7 and *Salmonella* occurred on farms through the use of contaminated irrigation water and manure (Chang and Fang, 2007).

2.2 Post harvesting

Many factors comprises of contamination of vegetables. Pathogens may contaminate the vegetables and fruits during washing, peeling, slicing, trimming, packaging, and handling (Oranusi and Olorunfemi, 2011).As with production and harvesting, the level of worker awareness, training and motivation toward maintaining food safety can have dramatic effects on microbiological safety. Because fresh fruits and vegetables are raw commodities, they are inherently subject to potential contamination.Transportation by consumers also affects microbial safety of salad vegetables (Brackett, 1999).Most produce is grown in a natural environment, therefore, vulnerable to contamination with pathogens from multiple sources, including agricultural and post-harvest water, ill workers, the presence of wild or domestic animals or animal waste, and unsanitary equipment and facilities (FAO/WHO, 2008).

3. MICROORGANISMS IN SALAD VEGETABLES

Escherichia coli, *Clostridium botulinum, Salmonella, Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus*, grow on lettuce, cucumber, carrot, red cabbage, tomatoes, and a variety of

salad vegetables(Abdul-Raouf et al., 1993; Lin et al., 1996) and have been associated with food borne illness for decades (Borch and Arinder, 2002; Velusamy et al., 2010).*Listeria monocytogenes, Staphylococcus aureus, Salmonella enterica* and *Escherichia coli* are known as common food borne pathogenic microorganisms (Kim et al., 2013). Most of the pathogens are not newcomers, and food-borne outbreaks or infections are not a rare event (Harris et al., 2001; Velusamy et al., 2010).The symptoms of staphylococcal food poisoning had already been described in 1936, one of the earliest food poisoning caused by *Bacillus cereus* was reported in 1906 (Borch and Arinder, 2002).Cross-contamination is an important factor of food-borne illness. Fruits have been associated with outbreaks in many countries; microorganisms include bacteria, fungi, viruses and parasites. Contamination of norovirus is also reported with salad product. Salmonellosis has been associated with consumption of cut watermelon causes outbreaks in the United States of America (Oranusi and Olorunfemi, 2011). Fruits and vegetables become contaminated with pathogenic microorganisms while growing infields, orchards, vineyards or greenhouses, or during harvesting, post-harvest handling, processing, distribution, and preparation. Vegetables and fruits produce have potential to harbor pathogenic microorganisms, but *Shigella* sp*., Salmonella, enterotoxigenic* and *enterohemorrhagic*, *Escherichia coli, Campylobacter* sp*., Listeria monocytogenes*, *Yersinia enterocolitica, Bacillus cereus, Clostridium botulinum,* and parasites such as *Giardia lamblia*, *Cyclospora cayetanensis*, and *Cryptosporidium parvum* are of greatest public health concern (Beuchat, 2002).

4. INCIDENCE OF PATHOGENS

The WHO defines foodborne illnesses as diseases, usually either infectious or toxic in nature, causedby agents that enter the body through the ingestion of food (Velusamy et al., 2010). In the last two decades foodborne illness outbreaks and cases associated with fresh produce have rapidly increased. Fresh produce (fresh, cannedor processed) causes 20 million illnesses (24%) costing 38.6 $ billion every year in the US (Olaimat and Holley, 2012). Food borne pathogenic bacteria are causative agent of illness and death for many people every year, at great economic cost and human suffering (Borch and Arinder, 2002; Olaimat and Holley, 2012). Enterohemorrhagic *E. coli* infections in the USA incur about $1 billion in costs each year (Boyacioglu et al., 2013). In Taiwan, contamination of fresh cut vegetables with *E. coli* O157:H7 is reported. In the USA, eight lettuce-associated outbreaks were reported with food borne pathogens, including *E. coli* O157:H7 and *Salmonella* from 1973 through 1997. Salmonellosis caused by many *Salmonella serovars* has been found in humans and animals. The overall rate of infection ranged from 15 to 20/100, 000 population, during each year in the USA. The outbreaks caused by *Salmonella* sp. were frequent, both in Korea (20.7%) and in Japan (14.2%) *S. enteric serovars typhimurium* and *S. enterica serovars enteritidis* are the most frequently isolated serovars from food-borne outbreaks throughout

the world (Chang and Fang, 2007). In 2005 alone 1.8 million people died from diarrhoeal diseases (Velusamy et al., 2010).

Escherichia coli O157:H7 has caused numerous outbreaks of food-borne illness associated with leafy green vegetables. Abdul-Raouf et al. (1993) were the first to provide evidence of *E. coli* O157:H7 growth on cut leafy vegetable (McKellar and Delaquis, 2011). In 1995, an outbreak of *E. coli* O157:H7 infection involving at least 29 people in Montana was epidemiologically linked to consumption of leaf lettuce (Roever, 1998). The source of contamination was not determined. However, the lettuce was irrigated with surface water, and investigations of area grocery stores revealed unsanitary lettuce was irrigated with surface water, and investigations of area grocery stores revealed unsanitary leaf lettuce handling methods.In the Same year, in 1995, outbreak of *E. coli* O157:H7 was reported associated with iceberg lettuce; itwas not known how the lettuce was contaminated. However, it is possible that cross-contamination from meat products may have occurred during food preparation or storage (Roever, 1998). *E. coli* O157*, Salmonella* sp. and *L. monocytogenes* outbreaks have been linked to apple, tomatoes, celery, lettuce, radish, watermelon, orange and other mixed salads (Beuchat, 2002). *E. coli* O157:H7 causes20, 000 infections and more than100deaths each year in the United States (Michino, 1999). From 1982 to 2002, 21% of outbreaks are caused by *E. coli* O157:H7 in the United States and from 2000 to 2004, fresh produce was the second most identified vehicle causing *E. coli* O157 foodborne illness outbreaks (Olaimat and Holley, 2012).

In Japan, July 1996, an outbreak of *Escherichia coli* O157:H7 infection occurred among school children in Sakai City, Osaka, Between May and December1996, there were 9, 451 cases and 12 deaths reported in 16 total outbreaks linked to the consumption of raw radish sprouts (Michino, 1999; Olaimat and Holley, 2012). In USA and Canada, October 2006, 199 persons infected with the outbreak strain of*E. Coli*O157:H7 associated with spinach, 3 deaths were reported to Centers for Disease Control and Prevention (CDC) from 26 states. 51% of these cases were hospitalized and 16% developed acute renal failure (CDC, 2006; Olaimat& Holley, 2012). Enterohemorrhagic *E. coli* O157:H7 is a causative agent of hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Chang and Fang, 2007). The most recent *E. coli* outbreak in world linked to contaminated fenugreek sprouts resulted in over 50 deaths and over 4, 000 hospitalizations in 16 countries (Boyacioglu et al., 2013).

Interest in healthy diets increases the consumption of ready-to-eat vegetables salad. Apart from the consumption, people had changed their life style due to their busy schedule, results in shortage of time for home cooking that has led many people to buy RTE. A six year study was conducted in Santiago, Chile to estimate the presence of *L. monocytogenes* in vegetable salad. The finding of the

study showed that the raw-eaten-salad vegetables was contaminated with *L. monocytogenes*, and this was the first report on *L. monocytogenes* contamination of vegetable salads in Chile (Cordano and Jacquet, 2009). Most of the reported outbreaks were caused by pathogenic bacteria, especially *Escherichia coli* O157:H7 and*Salmonella*, pathogens *E. coli* O157:H7 and *Salmonella* are commonly found in wide variety vegetables (Chang and Fang, 2007).

5. SURVIVAL OF PATHOGENS

Gastrointestinal illness caused by enteroinvasive, enterotoxigenic and enteropathogenic, *Escherichia coli, Escherichia coli* O157:H7 are capable of growing at 8°C. *Escherichia coli* O157:H7 can grow at 8°C, a temperature below which ready-to-eat (RTE) meals and lightly processed salad vegetables may be exposed for several hours during marketing (Abdul-Raouf et al., 1993). Fruits and vegetables are an extraordinary source of micronutrients, vitamins and fiber and prevent vitamin C and vitamin A deficiencies; but they are exposed to microbial contamination via contact with soil, dust and water or by post-harvest processing.However, fruits became harbor of wide of pathogenic microbes (Oranusi and Olorunfemi, 2011; Taban and Halkman, 2011). Salad vegetables are mostly contaminated with *Escherichia coli*, *Salmonella* sp*., S. aureus, Enterobacter* sp*., Klebsiella* sp*., S. typhi, Serratia* sp*., Providencia,* and *P. aeruginosa, Cryptosporidium oocysts* and *Listeria monocytogenes,* that causes several diseases such as diarrhea, kidney failure, typhoid fever, food poisoning, paratyphoid fever, pneumonia, skin infection, respiratory disease (e.g. sinusitis), urinary tract infection, mastitis, phlebitis, meningitis, cryptosporidiosis etc. Washing and rinsing of some type of fruits and vegetables prolong shelf-life by reducing the number of microorganisms on the surfaces. However, only a portion of pathogenic microorganisms may be removed with this simple treatment (Beuchat, 2002; Oliveira et al., 2011; Kumar, 2012). Contamination with enteric bacterial pathogens like enterohemorrhagic, *E. coli* or *Salmonella* sp., leafy greens (spinach and lettuce) will support the survival of enteric bacterial pathogens because currently there is no completely effective killing step during the harvesting, processing and packaging steps(Boyacioglu et al., 2013).

E. coli O157:H7 is a hardy pathogen that can survive long periods of time in water, especially at cold temperatures. *Escherichia coli* O157:H7 has been considered to be an important pathogen that can cause serious illness with symptoms ranging from bloody diarrhea to hemolytic uremic syndrome. In contrast to other food-borne pathogens, *E. coli* O157:H7 is more tolerant of some organic acids and it can survive well in acidic food and beverages. Studies determined that it can survive in refrigerated acid foods for weeks (Eribo and Ashenafi, 2003).

Listeria monocytogenes is the pathogen of listeriosis and is transmitted to susceptible individuals by consumption of contaminated foods. Cases of human listeriosis that have been associated with

the consumption of raw vegetables are likely, in part, due to contamination by manure from ruminants. *L. monocytogenes* is known to grow on a variety of vegetables at refrigeration temperatures. Pathogens, along with spoilage microorganisms, may contaminate fruits and vegetables via several different routes and at several points throughout the pre and post-harvest system. Potential pre-harvest sources of microorganisms include soil, feces, irrigation water, water used to apply fungicides and insecticides, dust, insects, inadequately composted manure, wild and domestic animals, and human handling. Post-harvest sources include feces, human handling, harvesting equipment, transport containers, wild and domestic animals, insects, dust, rinse water, ice, transport vehicles, and processing equipments (Beuchat, 2002).

Pathogen *Listeriamonocytogenes* is a ubiquitous bacteria, with high mortality rate and can be present in associated with decaying vegetation and soil (Roever, 1998).The major population groups at risk for invasive listeriosis are the immune-compromised peoples such as pregnant women, new born babies, elderly people and AIDS patients (Jamali et al., 2013).*Escherichia coli*and *Salmonella*sp. are causative agent of food borne outbreaks in many countries, causing gastroenteritis, and even chronic infections (Oliveira et al., 2011). Epidemiologic evidence suggested that raw celery, tomatoes, and lettuce may have been vehicles of *L. monocytogenes* infection (Beuchat and Brackett, 1991; Jamali et al., 2013).

L. monocytogenes is widely diffused in the environmenton a wide range of vegetation (Beuchat and Brackett, 1991; Farber and Peterkin, 1991), and contaminate the vegetables during cultivation and post-cultivation as well as during handling or distribution; therefore fresh vegetables pose a potent risk of contamination as the eaten raw. Salad has another risk of contamination through preparation, distribution and storage (Olaimat and Holley, 2012). Beuchat and Brackett, (1991) reported the growth of *L. monocytogenes* on tomatoes at 10°Cand 21°C. The rate of death was slower at 10°C compared with that at 21°C and, also observed that death of *L. monocytogenes* at pH < 4.8 in cabbage juice was more rapid at refrigeration (5°C) temperature compared with 30°C. *L. monocytogenes*cells are dies at low pH (4.1) of tomato juice (Beuchat and Brackett, 1991). A six year study was conducted in Santiago to estimate the presence of *L. monocytogenes* in vegetable salad. The finding of the study showed that the salad sample was contaminated with *L. monocytogenes,* and it causes the contamination of vegetable during cultivation and post-cultivation as well as during handling or distribution, therefore fresh vegetables pose a potent risk of contamination as the eaten raw. Salad has another risk of contamination through preparation, distribution and storage (Olaimat and Holley, 2012). Listeriosis is a relatively rare but serious disease with high fatality rates (30%) compared with other food borne microbial pathogens, and its pathogen have capability to survive under the stress conditions such as high salt concentration, low temperature and low pH (Ponniah et al., 2010; Sant'Ana et al., 2012).

6. CONCLUSION

Increased global production, distribution and consumption of fresh produce and inconsistent application of ideal agricultural practices increase the incidence of foodborne illness or diseases. The present study revealed the potential hazard of raw eaten salad vegetables and indicates the importance of proper lethal treatments before consumption and the need of adoption of hygienic practices by food processors vendors. Proactive and practical education programs are needed in process, i.e., from the field to the consumer's plate and effective risk assessments of the microbial hazards associated with salad crops, which are eaten raw. Mesophilic bacteria, indicator organisms (Coliforms) and pathogens (*Salmonella* and *Listeria monocytogenes*) were present in the food. The vegetables and fruits get contaminated with pathogenic microorganisms while growing in fields or orchards or during harvesting, post harvesting handling, processing and distribution. Contamination of raw eaten salad vegetables by pathogenic microorganism may present a potential health hazard to consumers, and potential public health threat. However, pre-harvest and post-harvest practices play significant roles as sources of contamination.Street vended food had more microbial contaminants because it is difficult to attest to the hygiene of vendors. Vendors and consumers are advised to wash fresh fruits properly before processing; fruits should be handled with sanitized hands. Adaptation and application of Hazard Analysis and Critical Control Point (HACCP) can reduce the chance of contamination and eliminate the pathogenic microorganisms.

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