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# Microbial Contamination of Ready-To-Eat Fresh Produce by Human Enteric Pathogens: The Global Burden of Foodborne Diseases

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**Abstract:- Human health is a major concern in the current scientific research areas. The research revolves around various diseases having a major impact on public health as well as country's economy. One of the major factors associated with increased number of disease outcome is consumptions of contaminated food. Consumption of microbiologically contaminated fresh food and their food products has been linked to several foodborne outbreaks involving *E. coli* and *Salmonella* enterica. Occurrence of raw and ready to eat fresh produce as potent source/vehicle for cause of human disease by pathogenic micro-organisms in not new. However, outbreaks of fresh produce based foodborne illness associated with enteric pathogens is less reported and documented. Outbreaks of food-borne illnesses linked with consumption of fresh produce, mainly caused by enteric bacteria such as *Salmonella* and pathogenic *E. coli* strains highlight important deficiencies in understanding the ecology of human enteric pathogens outside their animal hosts. The review focuses the recent finding on the interactions of enteric pathogens with fresh produce, the routes associated with contamination, and the phenotypic and genotypic associations leading to the persistence, colonization and change in the ecological and morphological aspects linked to plant-microbe interactions. The comprehensive overview may aid to discuss potential control strategies, which in turn ultimately can lead to management of the food borne illnesses linked to the fresh vegetables and fruits and safer food supply.**

***Keywords:*** *Food Safety, Fresh Produce, Food Borne Illness, E. Coli, Salmonella, Antibiotic Resistance.*

## I. INTRODUCTION

During the past two decades the consumption of fresh produce has been in increasing demand to maintain a healthy life style. Several vegetables are consumed fresh, raw or minimally processed to get the nutrient availability in higher amount such as fibers, vitamins etc [1]. However, consumption of contaminated produce can often lead to infection and disease development. People around the world are still affected and falling ill from consumption of contaminated food, with exerting heavy toll on both human health and nation's economy [2]. This is not only the case in developing countries with lack of proper sanitation and health but also in developed countries. In fact, over the last few years, several foodborne outbreaks have been linked with the consumption of fresh produce in many regions of the world including India. The fresh produce linked with the foodborne outbreaks were leafy vegetables, tomatoes, and cucumbers, etc. Potential foodborne pathogens, including *Listeria monocytogenes, Escherichia coli, Salmonella* and *Pseudomonas* strains have been reported to harbor in raw fresh produce [3]. However, in the light of several fresh-produce linked foodborne outbreaks, an emerging concern has been raised about the safety of vegetables and fruits due to their association with foodborne pathogens such as Shiga Toxin producing *E. coli* (STEC) and *Salmonella* [4]. More importantly, continuous rise in the number of foodborne outbreaks linked with the consumption of fresh produces has also raised questions whether plants are alternative non-animal hosts for *E. coli* and *Salmonella,* or whether they are simply matrices where these pathogens successfully colonize and persist.

More often, plants are not considered as hosts for enteric bacteria such as *E. coli* and *Salmonella*; these are often associated with mammalian or other animal hosts. However, in the light of several current and past outbreaks linked with the consumption of raw agricultural produce contaminated with pathogenic *E. coli* and *Salmonella*, plants may be considered as a common vector or even additional reservoirs for human enteric pathogens. Further, this raises several concerns about the mechanism, phenotypic/genetic traits, and virulence of *E. coli* and *Salmonella* associated with the non-animal host, i.e., plant. In contrast to the wealth of information available on the interaction of *E. coli* and *Salmonella* with their mammalian hosts, exploration of basic knowledge about their interaction with plants has just begun. A detailed understanding of the ecology, phenotypic traits, genetic diversity virulence traits exhibited by agricultural plants-origin *E. coli* and *Salmonella* may provide a possible explanation for their ability to survival in the plants with food safety issues.

Food safety a vital component for sustaining agriculture, economy and public health, but it also serves as a critical measure to achieve food security. However, global outbreaks of foodborne illnesses resulting from contaminated food have made microbiological safety and quality of food to public health. The problem of foodborne illnesses is not only in developing countries with poor hygiene practices or safety measurements but also in developed countries. Foodborne illnesses term used to any disease usually either infection or intoxicants acquired through consumption of contaminated food with microbial agents, toxins, or chemicals. Currently, foodborne illnesses comprise a large part of the morbidity or mortality worldwide and also pose a constant risk to human health globally. In India and other developing countries, foodborne illnesses are a significant health problem, but majorities of foodborne outbreaks are underreported or underestimated. Therefore, underestimation of foodborne illnesses or associated outbreaks in India affects the kind of measures and strategies strengthening of food safety and programs.

Understanding the biological threats and their sources is one of the greatest concerns associated with food commodities from prevention and control perspective, which can use to protect human health and enhance food-safety. Microbial agents that cause foodborne illnesses are known as foodborne pathogens which include bacteria, viruses, fungi, and protozoa. Although, viruses are primarily associated with foodborne illnesses, foodborne bacterial pathogens are the primary causes of hospitalizations and deaths associated with foodborne outbreaks [5, 6]. The majority of foodborne bacterial pathogens associated with foodborne outbreaks are *Campylobacter jejuni*, pathogenic *E. coli*, *Salmonella*, *Listeria monocytogenes*, *Clostridium perfringens* [7]. Among different food involved in foodborne outbreaks worldwide is food from animal-origin such as meat milk or milk product and food from plant-origin such as vegetables and fruits.

- *Human Enteric Bacterial Outbreaks Of Fresh-Produce*

Traditionally, foodborne bacteria pathogens are primarily linked with the food products of animal origin such as meat, egg, and milk products, etc. However, fresh vegetables, fruits, nuts or juice, which are consumed as raw, have been frequently implicated in foodborne outbreaks. The global production per annum of fruit and vegetables has increased by 94% from 1980 to 2004 and is still in rise [8]. Fresh fruits and vegetables, which are usually consumed without cooking or nominal processing, are important in a healthy diet. Over the last few years, several outbreaks of foodborne illnesses caused by bacterial pathogens such as pathogenic *E. coli*, *Salmonella*, *Listeria monocytogenes*, have been associated with the consumption of fresh vegetables and fruits such as lettuce, spinach, radish, sprouts, fenugreek, cantaloupes, and tomatoes [9]. Pathogenic *E. coli* strains, mainly Shiga-toxin producing *E. coli* (STEC) and *Salmonella* spp. are among the major human enteric bacteria which are frequently implicated in outbreaks of food-borne illnesses linked with consumption of fresh fruits or vegetables (Table 1). The true number of sick people in the outbreaks are likely higher than the number reported, and the outbreak may not be limited to the states with known illnesses. This is because many people recover without medical care and are not tested for *E. coli*.

Table 1 List of Food Borne Outbreaks Associated with Consumption of Leafy/Fresh Produce Contaminated with Enteric Pathogens

| **Bacterial strain** | **Produce type** | **Year** | **Number of cases** | **Country/Reference** |
|---|---|---|---|---|
| *E. coli* O157:H7 | Lettuce | 2005 | 135 | Sweden |
| *E. coli* O157:H7 | Spinach | 2006 | 205 | USA |
| *E. coli* O157:H7 | Lettuce | 2007 | 50 | Iceland, the Netherlands |
| *S. enterica* sv. Stanley | Alfalfa sprouts | 2007 | 44 | Sweden |
| *E. coli* O145 | Lettuce | 2010 | 27 | USA |
| *S. enterica* sv. Newport | Alfalfa sprouts | 2010 | 44 | [40] |
| *E. coli* O157:H7 | Romaine Lettuce | 2011 | 60 | USA |
| *Escherichia coli* O157:H7 | Spinach | 2012 | 33 | [41] |
| *E. coli* O26 | Clover sprouts | 2012 | 29 | [42] |
| Pathogenic *E. coli* | Watermelon | 2002 | 6 | [12] |
| *Salmonella* | Bitter gourd | 2013 | 43 | [14] |
| *Salmonella* | Salad | 2014 | 4 | USA* |
| *E. coli O96* | Lettuce, cucumbers | 2016 | 50 | USA* |
| *E. coli O157:H7* | Romaine Lettuce | 2019 | 167 | USA * |
| *Unknown* | Salad/Pickle | 2019 | 30 | India |
| *E. coli O157:H7* | Leafy Greens | 2020 | 40 | USA* |
| *Listeria monocytogens* | Fresh Express Packaged Salads | 2021 | 10 | USA* |
| *E. coli O157:H7* | Power Greens Packaged Salads | 2021 | 10 | USA* |

*The data presented in the table was obtained from current literature reviews, news articles, outbreak investigations and the Centers of Disease Control and Prevention [39].

*E. coli* is historically, among one of the most studied microorganisms which is treated as foundation for several biochemical, physiological and genetic concepts. *E. coli* occurs in diverse forms in environment, ranging from beneficial commensal strains to disease causing pathogens on human/animal hosts. Generally, non-pathogenic *E. coli* is harmless to human, but certain pathotypes of *E. coli* cause severe intestinal (diarrhea) and extra-intestinal (meningitis, urinary tract infections) infections in human. Gastroenteritis causing pathogenic *E. coli* strains categorized into six groups including enteropathogenic *E. coli* (EPEC) enteroaggregative *E. coli* (EAEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), and diffuse adherent *E. coli* (DAEC). The infections caused by *E. coli* is mainly results of fecal-oral route transmission. However, in the last few years, fresh produce such as lettuce, spinach, and cucumber associated foodborne outbreaks was caused by STEC, EPEC, ETEC and EHEC [10]. For example, in 2011, STEC O104:H4 has been associated with major foodborne outbreaks which were linked consumption of raw sprouts in Germany. In addition to Germany, STEC O104:H4 has been associated with several foodborne outbreaks in the United States, in the Europe, and other regions of the world. In India, a diarrheal outbreak caused by enterotoxigenic *E. coli* have also been linked to consumption of orange juice and watermelon [11, 12].

*Salmonella* is one the main Gram-negative bacteria which cause foodborne diseases worldwide. *Salmonella* infections are unfortunately a constant threat to human health globally. The genus *Salmonella* is divided into species; *S. enterica* and *S. bongori* [13]. *S. America*, which is further subdivided in more than 2500 serovars that mainly associated of foodborne illnesses or Salmonellosis, worldwide. The incidence of *Salmonella* infections has not declined in the last 15 years. Animal products such as poultry meat, eggs, and pork are mostly associated with *Salmonellosis*. However, a number of foodborne outbreaks reported globally have been associated with a variety of fresh produce such as sprouts, cantaloupes tomato, and fruit juice. For example, in 2002 and 2005, *S. enterica* serovar Newport has been implicated in tomato-associated foodborne outbreaks in Virginia. Likewise, basil related outbreaks of *S. enterica* serovar Senftenberg has been reported in the United States, United Kingdom, Denmark and in Netherland in 2007. In India, outbreaks of food poisoning caused by *Salmonella*, have been reported with consumption of potato-bitter gourd [14]. Interestingly, it has been demonstrated that *Salmonella* serovar Typhimurium can use plants as an alternative host.

- *Agricultural Plants as a Vehicle for Enteric Bacteria*

Plants are typically not act as a host for human enteric pathogens such as *Salmonella* and *E. coli*; that often-associated mammalian or other animal hosts. Previous foodborne outbreaks linked to fresh vegetables/fruit were initially suspected to results of cross-contamination during food-handling or preparation. However, outbreaks of fresh vegetables and fruits-borne illnesses across globally demonstrate that human enteric pathogens can enter the food chain at any point or can contaminate raw produce at any stage of production. Various studies comprising experimental models, natural environment and outbreaks associated data have shown the capability of the humans and plant pathogens to break the barriers amongst the kingdom resulting into adaptation to newer host for survival and cross-contamination [15, 16, 17]. Change in agriculture practices, food processing, consumer habits, and food surveillance are all possible explanatory factors which are associated with increases incidence of fresh produce-borne outbreaks. Interestingly, *E. coli* and *Salmonella* belong to Gram-negative family Enterobacteriaceae, which also contains a several important and widely occurring plant pathogens (Enterobacter, Erwinia, and Pseudomonas) that cause plant diseases. Recent studies also provided evidence that plant enterobacteria, *Klebsiella pneumonia* and *Pectobacteriu matrosepticum* shared a remarkably high proportion of their genome with human enteric pathogens. More interestingly, plant pathogens *K. pneumoniae* and *Pantoea agglomerans* which are endophytically colonize in the plants, have been reported to associate with opportunistic infections in both animal and human. Moreover, plant pathogens *Dickeya dadantii* and *Pantoea ananatis* have also been reported to cause disease in pea aphids and human respectively [18]. Thus, the taxonomic relatedness of these human and plant pathogens raises interesting questions about the possibilities for niche adaptation, horizontal exchange of genetic material or even host range expansion. The link between food-borne salmonellosis and food of animal origin is well established and considered regularly attention. The incidence of salmonellosis cases linked with consumption of food from animal origin has declined in recent years. However, the overall *Salmonella* infections linked with non-traditional sources of this pathogen has not declined [19]. These non-traditional sources of infection include fresh fruit and vegetables, spices, and nuts which are recognized as a potential vehicle for transmission of *Salmonella*. Further, the main habitat of *E. coli* is the lower gastro-intestine tract of mammals. However, several studies provided evidence that *E. coli* can persist and grow in extra-intestinal habitats including plants. In addition, in the light of several past fresh food-borne outbreaks suggested that the interactions of *Salmonella* and *E. coli* with fruits and vegetable are not uncommon [20]. Moreover, these outbreaks also suggested that plants could be common alternative vectors or even secondary reservoirs for transmission of *Salmonella* and *E. coli*.

- *Routes/Sources of Contamination*

Fresh produce can be contaminated with bacterial pathogens at multiple stages throughout its production and supply chain by direct contact with fecal waste during farming, cultivation, handling, wastewater irrigation and the use of biosolids or animal manure as fertilizer, and management practices [21]. Manure is commonly used for increasing the soil fertility and dispose to livestock waste, but enteric pathogens such as *E. coli* and *Salmonella* may persist in animal feces and can serve as potential source of contamination onto agricultural produce in the field (Fig. 1). In addition, contaminated water used form crop

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irrigation and pesticides spray, is considered a most important sources of inoculum of enteric pathogens in the agricultural field [22]. Studies reporting that *E. coli* O157:H7 when internalized in spinach plants sprinkled with contaminated water, survived in the leaf microenvironment/phylloplane for 14 days with an increase in titers and areas of colonization being observed over that time. These effects were not observed when spinach plants were inoculated by soil drench or tissue stabbing methods [23], which indicated that the spinach phylloplane is a supportive niche for *E. coli O157:H7* and likelihood of internalizing enteric pathogens increased when these organisms were introduced by water sprinkling systems instead of direct application into the soil. Further, many enteric bacteria such as *E. coli* and *Salmonella* have broad animal host range and bacteria may persist for a long time in the feces of that animal, which can serve as a source of enteric bacteria in the agricultural field. Moreover, post-harvesting processing of fresh produce using contaminated water or utensil may also help to internalize enteric bacteria in the agricultural plants depending upon produce type/water temperature [24]. The preparation and storage of fresh produce may also introduce *E. coli* and *Salmonella* during the processing environment. The pooling of raw agricultural produce also increases the possibility of cross-contamination in large-scale farming.

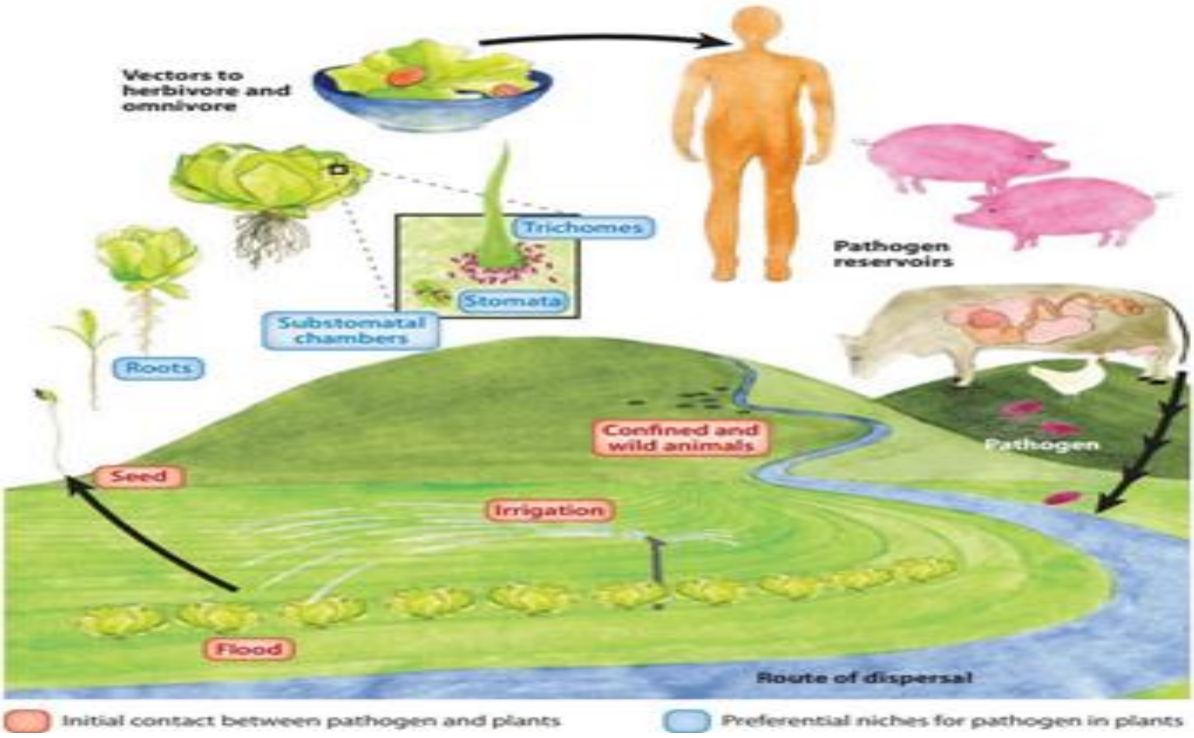

Fig 1 Sources and Infection Routes of Human Enteric Pathogens in Ecosystem [25]

➢ *Ecology and Phenotypic Diversity of Human Enteric Bacteria in Agricultural Plants*

Nonetheless, much information available on the interaction of enteric bacteria such as *E. coli* and *Salmonella* with their animal or human hosts, exploring the fundamental knowledge about their ecology, behaviors and phenotypic or genetic diversity has just begun. In the last few years, few studies have established that enteric pathogens can colonize and persist as epiphytic and endophytic life style on the surface and inner tissues of the agricultural plants, respectively. Recently, studies have also demonstrated that both *E. coli* and *Salmonella* needed an adaptation time before being able to survive and persist in plants, during the stress effects of harsh environmental conditions on their epiphytic life style [26]. To survive in the harsh environmental conditions, it is suggested that *E. coli* and *Salmonella* could reach to the inner tissues of plants to expand a relative protection against harsh conditions present on plant surface and thus acquired or enhance abilities to colonize in plants and develop resistant to antimicrobial actions [20].

In order to effectively survive and persist in the agricultural plants, human enteric bacteria such as *E. coli* and *Salmonella* need to grow over a range of different conditions of the host or surrounding environments. On the other hand, human enteric bacteria induce a response to host and environmental stress. The pH of agricultural plants and temperature of the environment may play an important role during the survival and persistence in non-animal hosts. Previously studies have demonstrated tolerance and survival of food-pathogens such as *Listeria monocytogenes* and *Salmonella* to non-optimal pH and temperature in a variety of foods and resistance to lethal effects of very low or high pH and temperature. Though, most of the studies examined the effects of pH and temperature on the tolerance of foodborne pathogens in different types of foods, little information is available evaluating the growth characteristics of human enteric bacteria survive and persist in agricultural plants under different environmental settings such as pH and temperature. Although, a recent study indicating that fresh produce-isolated *Salmonella enterica* serovar Cilantro did not colonize on plant surface under

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low temperature, but grow and survive at higher temperature. Another study also found that fresh-produce-origin *S. enterica* serotype Montevideo can grow successfully at non-optimal or acidic pH (~4.3) of tomatoes. This highlighting that understanding the response of enteric bacteria to pH of agricultural plants and temperature of the environment may help to elucidate their ecology in non-animal hosts or plants.

Generally, it is considered that non-hosts environments such as plants do not support the colonization and survival of human enteric pathogens. However, continuous increases in the microbial contamination of agricultural plants indicate that human enteric bacteria are able to colonize and survive in fresh produce during cultivation and post-harvest processing. Further, foodborne bacteria pathogens such as *E. coli* and *Salmonella* not only colonize or survive on the agricultural plant surface, but grow until the fresh produce is consumed by a new host. The most important strategies used by *E. coli* and *Salmonella* and other enteric bacteria during colonization or survival in the agricultural plants is the formation of biofilm. A biofilm is an assembled and structural organization of bacterial cells confined within a matrix of the EPS (extracellular polymeric substance) and which adhere to a living or inert surface. Several studies have demonstrated that biofilm formation is significantly associated with *E. coli* and *Salmonella* strains isolated from different sources such as clinical and animal. Biofilm formation behaviors of many bacteria including *E. coli* and *Salmonella* is significantly associated to enhance survival in natural environments, resistance to antimicrobial agents and during interaction with hosts. Recently, few studies indicate that *E. coli* and *Salmonella* and other foodborne bacteria pathogens are able to form biofilm on the surface of plants as well as in inner spaces of the plant tissues. It is also believed that bacterial cells confined in biofilm are more resistant to antibiotics and stress conditions [27], thus it is considered that biofilm formation is a survival strategy of *E. coli* and *Salmonella* to withstand on the surfaces of the agricultural plant under unfavorable conditions.

The production of curli fimbriae or thin aggregative fimbriae and cellulose in *E. coli* and *Salmonella* was found to be important components in the formation of extracellular polymeric matrix, which is essential during biofilm formation and persistence in various surfaces. Previous studies suggest that production of curli fimbriae and cellulose may be associated with the survival and persistence of *E. coli* and *Salmonella* in the food-environment. Evidences also suggest that production of cellulose and curli-fimbriae also offers protection to bacterial cells against harsh environmental conditions such as desiccation, osmotic shock, and UV radiation [28]. Conversely, expression of cellulose and curli-fimbriae in *E. coli* and *Salmonella* associated with their initial attachment in animal hosts or provides a physical barrier against the transmission of antimicrobial agents and compounds of the host immune response [29]. On the basis of the production of curli fimbriae and cellulose, both *E. coli* and *Salmonella* displayed different colony morphotypes; RDAR (red, dry and rough), indicating the production of cellulose and curli; b), PDAR (pink, dry and rough) indicating the production of cellulose only; c), BDAR (brown, dry and rough) indicating the expression of curli-fimbriae only; d), SAW (smooth and white) indicating no production of cellulose and curli-fimbriae. The distinct colony morphotypes such as BDAR and RDAR have been reported in *E. coli* and *Salmonella* strains isolated from clinical and animal sources [30]. The BDAR and RDAR morphotypes of *Salmonella* has been linked to increase their virulence or tolerance in long-term desiccation and nutrient depletion in biofilm [31]. However, limited information is available on the role of production of curli fimbriae and cellulose and related colony morphotypes in agricultural plant-related *E. coli* and *Salmonella*.

➢ *Genetic Diversity of Human Enteric Bacteria Associated with Plants*

In addition to various phenotypic factors such as curli fimbriae and biofilm formation; genotypic differences among pathogen strain or serovars was shown to play a large role in colonization and persistence on plants [18]. It is reported that when a cocktail of five different serovars (Montevideo, Michigan, Poona, Hartford, and Enteritidis) of *Salmonella* inoculated in to tomato plants, recovery of *Salmonella* from plant tissues revealed that serotypes Montevideo and Michigan were most prevalent; however, Poona, Hartford, and Enteritidis were not detected in any tissue samples of tomato [32]. Likewise, different serovars of *Salmonella* such as Cubana, Infantis, and Typhimurium showed internalization and colonization variability in alfalfa sprouts when seed was inoculated under same environmental conditions [33]. Further, when both *E. coli* O157:H7 and *E. coli* K12 were inoculated in seeds of alfalfa, colonization of *E. coli* O157:H7 was higher in alfalfa sprouts when compared to *E. coli* K12 [34]. In addition, when *E. coli* O157: H7 and *Salmonella* Typhimurium were inoculated in seeds of the lettuce plant, *E. coli* O157:H7 showed the highest probability of internalization and colonization in shoots of lettuce when compared to *Salmonella* Typhimurium [33].

In addition, during foodborne outbreaks related to fresh produces or other sources, investigation of strain subtyping of Pathogenic *E. coli* and *Salmonella* spp. is an important for source identification, diagnosis, and treatment. In addition, the possible relatedness of Pathogenic *E. coli* and *Salmonella* spp. from foods of plant-origin and those from animal-origin or clinical settings is a matter of concern. There are many molecular subtyping methods such as pulse-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST) which is used to discriminate *E. coli* and *Salmonella* spp. isolates beyond subspecies and strain level duet to their high discriminatory power. Though, PFGE is generally referred to as Gold Standard method for molecular subtyping, but it cannot be used for phylogenetic analysis.

On the other hand, MLST has emerged as a powerful tool for phylogenetic analysis as well as global epidemiology and genetic diversity of bacterial populations including *E. coli* and *Salmonella* spp. MLST based on the determination of the DNA sequences of a series of predetermined housekeeping genes of bacteria, which has been rapidly acquiring recognition as one of the most reliable molecular typing approaches currently available. MLST data provide an unambiguous characterization of a bacterial strain that can be comparable directly among laboratories via the online databases. A previous phylogenetic analysis of *E. coli* based on MLST has been shown that plant-associated covers most of *E. coli* diversity and they are also phenotypically distinct when compared with animal hosts. Very recently, a study based on MLST analysis of *Salmonella* isolates obtained from fresh produce in Turkey, found some sequence types of plant-origin *Salmonella* are widespread in the different environment and host organisms. Being one of the world's largest producers and exporter of raw agricultural produce and the association of fresh produce with foodborne illnesses, India needs a systematic analysis of foodborne *E. coli* and *Salmonella* for the early detection to these pathogens [35]. However, information about MLST analysis of plant-origin pathogenic/non-pathogenic *E. coli* and *Salmonella* spp. is not available in India.

- *Antimicrobial Resistance in Human Enteric Bacteria Associated with Fresh-Produce*

Emergence of outbreaks of foodborne illnesses linked with consumption of fresh produce, a growing food safety issues is related with the role of fresh food in human exposure to antimicrobial resistant bacteria [36]. Although, the prevalence and transmission of antibiotic resistance bacterial linked with clinical settings have been well studied, however, studies regarding antibiotic resistance profiling of bacteria from agriculture is limited. In the last few years, increasing evidence suggests that widespread use of antibiotics in non-clinical settings such as in agriculture has resulted in the rapid evolution and spread of antibiotic resistance as seen in clinical settings [37]. The prevalence of antimicrobial resistance in foodborne bacteria persist in an agricultural setting has great importance not only regarding human/animal health but also in indirect transfer of antibiotic resistance genes/plasmids to other pathogens or local plant microbiota via horizontal gene transfer. In 2014, a first-ever global report of WHO on antimicrobial resistance also emphasized that more data are urgently needed on the occurrence of antibiotic resistance in foodborne bacteria present in agriculture in different countries/system, in order to make a comparison between different countries and identify priority areas for intervention

Recently, a high degree of resistance to ampicillin, ciprofloxacin, nalidixic acid, or chloramphenicol has been reported in *E. coli* and *Enterococcus* spp. isolated from ready-to-eat salads [38]. A high degree of resistance to clinically important antimicrobials has been reported in *Salmonella* isolated from fresh vegetables. Thus, these studies demonstrated the prevalence of antibiotic resistance genes in enteric bacteria isolated form fresh vegetables or fruits. However, more systematic analysis or studies are required to better apprehend the possible role of fresh produce in human exposure to antimicrobial resistant bacteria as a repository of resistance genes or their contribution for the dissemination of bacteria resistant to critically important antibiotics.

## II. CONCLUSION

Several outbreaks of food-borne illnesses linked with consumption of fresh produce, mainly caused by *Salmonella* and pathogenic *E. coli* strains highlight important deficiencies in our understanding the ecology of human enteric pathogens outside their animal hosts. Although, several studies have been focused on the transmission of human enteric pathogens from fresh produce in the developed world, no documented report or data is available in India regarding the ecology, and genetic diversity of *Salmonella* and pathogenic *E. coli* strains colonize and persist in non-host or in the plants. Increased knowledge of both phenotypic and genotypic diversity may improve the fundamental question of the association between human enteric pathogens with plants and may provide important insights into their biology and ecological fitness. This may also have many applications in food safety such as assisting with the development of diagnostic tools for the identification and detection of foodborne pathogens. In addition, antibiotic susceptibility profiling of the of produce-related *Salmonella* and *E. coli* will help to understand the role of plants for the dissemination of bacterial strains resistant to clinically relevant antibiotics. More importantly, the obtained information from this analysis may aid in the development of novel preventive and control strategies, which in turn ultimately lead to management of the food borne illnesses linked to the fresh vegetables and fruits and safer food supply.

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## REFERENCES

[1]. Balali GI, Yar DD, Afua Dela VG, Adjei-Kusi P. Microbial contamination, an increasing threat to the consumption of fresh fruits and vegetables in today's world. International Journal of Microbiology. 2020 May 22;2020.

[2]. Lynch MF, Tauxe RV, Hedberg CW. The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities. Epidemiology & Infection. 2009 Mar;137(3):307-15.

[3]. Carstens, C. K., Salazar, J. K., & Darkoh, C. (2019). Multistate outbreaks of foodborne illness in the United States associated with fresh produce from 2010 to 2017. *Frontiers in microbiology*, *10*, 2667.

[4]. van Overbeek, L. S., van Doorn, J., Wichers, J. H., van Amerongen, A., van Roermund, H. J., & Willemsen, P. T. (2014). The arable ecosystem as battleground for emergence of new human pathogens. *Frontiers in Microbiology*, *5*, 104.

[5]. Chatziprodromidou IP, Bellou M, Vantarakis G, Vantarakis A. Viral outbreaks linked to fresh produce consumption: a systematic review. Journal of Applied Microbiology. 2018 Apr;124(4):932-42.

[6]. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson M-A, Roy SL, et al. Foodborne illness acquired in the United States—major pathogens. Emerging infectious diseases. 2011;17(1):7.

[7]. Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angulo FJ, et al. Attribution of foodborne illnesses, hospitalizations, and deaths to food 116 commodities by using outbreak data, United States, 1998–2008. Emerging infectious diseases. 2013;19(3):407.

[8]. Wirsenius S, Azar C, Berndes G. How much land is needed for global food production under scenarios of dietary changes and livestock productivity increases in 2030?. Agricultural systems. 2010 Nov 1;103(9):621-38.

[9]. Vemula SR, Kumar RN, Polasa K. Foodborne diseases in India–a review. British Food Journal. 2012 May 11.

[10]. Herman K, Hall A, Gould L. Outbreaks attributed to fresh leafy vegetables, United States, 1973–2012. Epidemiology & Infection. 2015;143(14):3011-21.

[11]. Lima Tribst AA, de Souza Sant'Ana A, de Massaguer PR. Microbiological quality and safety of fruit juices—past, present and future perspectives. Critical reviews in microbiology. 2009;35(4):310-39.

[12]. Pande T, Khan A, Pipersania R, Sethi S, Rath Y. Watermelon poisoning. Postgraduate Medical Journal. 2002;78(916):124-5.

[13]. Lamas A, Miranda JM, Regal P, Vazquez B, Franco CM, Cepeda A. A comprehensive review of non-enterica subspecies of Salmonella enterica. Microbiological research. 2018;206:60-73.

[14]. Kunwar R, Singh H, Mangla V, Hiremath R. Outbreak investigation: Salmonella food poisoning. medical journal armed forces india. 2013;69(4):388-91.

[15]. Bulgari D., Montagna M., Gobbi E., Faoro F. 2019. Green technology: Bacteria-based approach could lead to unsuspected microbe–plant–animal interactions. Microorganisms 7(2); 44; 16 p. DOI: 10.3390/microorganisms7020044.

[16]. Kim J.-S., Yoon S.-J., Park Y.-J., Kim S.-Y., Ryu C.-M. 2020. Crossing the kingdom border: human diseases caused by plant pathogens. Environmental Microbiology 22(7): 2485–2495. DOI: 10.1111/1462-2920.15028.

[17]. Sobiczewski, P., & Iakimova, E. T. (2022). Plant and human pathogenic bacteria exchanging their primary host environments. *Journal of Horticultural Research*, *30*(1), 11-30.

[18]. Holden N, Pritchard L, Toth I. Colonization outwith the colon: plants as an alternative environmental reservoir for human pathogenic enterobacteria. FEMS Microbiology Reviews. 2009;33(4):689-703.

[19]. Jackson BR, Griffin PM, Cole D, Walsh KA, Chai SJ. Outbreak-associated Salmonella enterica serotypes and food commodities, United States, 1998–2008. Emerging infectious diseases. 2013;19(8):1239.

[20]. Schikora A, Carreri A, Charpentier E, Hirt H. The dark side of the salad: Salmonella typhimurium overcomes the innate immune response of Arabidopsis thaliana and shows an endopathogenic lifestyle. PLoS One. 2008;3(5):e2279.

[21]. Rahman M, Alam MU, Luies SK, Kamal A, Ferdous S, Lin A, Sharior F, Khan R, Rahman Z, Parvez SM, Amin N. Contamination of fresh produce with antibiotic-resistant bacteria and associated risks to human health: a scoping review. International journal of environmental research and public health. 2022 Jan;19(1):360.

[22]. Iwu CD, Okoh AI. Preharvest transmission routes of fresh produce associated bacterial pathogens with outbreak potentials: a review. International journal of environmental research and public health. 2019 Nov;16(22):4407.

[23]. Mitra, R., E. Cuesta-Alonso, A. Wayadande, J. Talley, S. Gilliland, and J. Fletcher. 2009. Effect of route of introduction and host cultivar on the colonization, internalization, and movement of the human pathogen Escherichia coli O157:H7 in spinach. J. Food. Prot. 72:1521–1530.

[24]. Arah IK, Ahorbo GK, Anku EK, Kumah EK, Amaglo H. Postharvest handling practices and treatment methods for tomato handlers in developing countries: A mini review. Advances in Agriculture. 2016;2016.

[25]. Barak JD, Schroeder BK. Interrelationships of food safety and plant pathology: the life cycle of human pathogens on plants. Annual review of phytopathology. 2012;50:241-66.

[26]. Harapas D, Premier R, Tomkins B, Franz P, Ajlouni S. Persistence of Escherichia coli on injured vegetable plants. International journal of food microbiology. 2010;138(3):232-7.

[27]. Avila-Novoa, M. G., Guerrero-Medina, P. J., Navarrete-Sahagún, V., Gómez-Olmos, I., Velázquez-Suárez, N. Y., De la Cruz-Color, L., & Gutiérrez-Lomelí, M. (2021). Biofilm formation by multidrug-resistant serotypes of Salmonella isolated from fresh products: Effects of nutritional and environmental conditions. *Applied Sciences*, *11*(8), 3581.

[28]. Scher, K., Romling, U., & Yaron, S. (2005). Effect of heat, acidification, and chlorination on Salmonella enterica serovar Typhimurium cells in a biofilm formed at the air-liquid interface. *Applied and environmental microbiology*, *71*(3), 1163-1168.

[29]. Flemming H-C, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nature Reviews Microbiology. 2016;14(9):563

[30]. Milanov, D. S., Prunić, B. Z., Velhner, M. J., Pajić, M. L., & Čabarkapa, I. S. (2015). RDAR morphotype: A resting stage of some Enteroba cteriaceae. *Food and Feed Research*, *42*(1), 43-50.

[31]. Vestby LK, Møretrø T, Ballance S, Langsrud S, Nesse LL. Survival potential of wild type cellulose deficient Salmonella from the feed industry. BMC veterinary research. 2009;5(1):43.

[32]. Gurtler, J.B., Harlee, N.A., Smelser, A.M. and Schneider, K.R., 2018. Salmonella enterica contamination of market fresh tomatoes: a review. *Journal of food protection*, *81*(7), pp.1193-1213.

[33]. Aruscavage D, Miller SA, Lewis Ivey ML, Lee K, LeJEUNE JT. Survival and dissemination of Escherichia coli O157: H7 on physically and biologically damaged lettuce plants. Journal of food protection. 2008;71(12):2384-8.

[34]. Torres, A.G., Jeter, C., Langley, W. and Matthysse, A.G., 2005. Differential binding of Escherichia coli O157: H7 to alfalfa, human epithelial cells, and plastic is mediated by a variety of surface structures. *Applied and environmental microbiology*, *71*(12), pp.8008-8015.

[35]. Kohli C, Garg S. Food safety in India: an unfinished agenda. MAMC Journal of Medical Sciences. 2015 Sep 1;1(3):131.

[36]. Araújo S, Silva IA, Tacão M, Patinha C, Alves A, Henriques I. Characterization of antibiotic resistant and pathogenic Escherichia coli in irrigation water and vegetables in household farms. International journal of food microbiology. 2017;257:192-200.

[37]. Duffy E, Lucia L, Kells J, Castillo A, Pillai S, Acuff G. Concentrations of Escherichia coli and genetic diversity and antibiotic resistance profiling of Salmonella isolated from irrigation water, packing shed equipment, and fresh produce in Texas. Journal of food protection. 2005;68(1):70-9.

[38]. Campos J, Mourão J, Pestana N, Peixe L, Novais C, Antunes P. Microbiological quality of ready-to-eat salads: an underestimated vehicle of bacteria and clinically relevant antibiotic resistance genes. International journal of food microbiology. 2013;166(3):464-70.

[39]. List of Multistate Foodborne Outbreak Notices | CDC, https://www.cdc.gov/foodsafety/outbreaks/lists/outbreaks-list.html

[40]. Multistate Outbreak of Human Salmonella Newport Infections Linked to Raw Alfalfa Sprouts (Final Update) | Salmonella | CDC. Centers for Disease Control and Prevention, Centers for Disease Control and Prevention www.cdc.gov/salmonella/newport.

[41]. Multistate Outbreak of Shiga Toxin Producing Escherichia Coli O157:H7 Infections Linked to Organic Spinach and 119 Spring Mix Blend (Final Update). Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 10 Dec. 2012 www.cdc.gov/ecoli/2012/O157H7-11-12.

[42]. Multistate Outbreak of Shiga Toxin Producing Escherichia Coli O26 Infections Linked to Raw Clover Sprouts at Jimmy John's Restaurants (Final Update). Centers for Disease Control and Prevention, Centers for Disease Control and Prevention, 2 Nov. 2015 www.cdc.gov/ecoli/2012/O26-02-12.