

Microbiological analysis of commonly consumed vegetables: A review on the ongoing studies

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Abstract

Vegetables are frequently consumed by people all over the world and hence, the microbiological quality of vegetable especially fresh produces are of significance for the sake of public health sustainability. Vegetables are spoiled by a range of microorganisms including bacteria and fungi. Among the bacterial species, *Corynebacterium*, *Pseudomonas* spp., *Listeria monocytogenes*, *Clostridium botulinum*, *Erwinia carotovora*, and *Xanthomonas campestris* are the common vegetable spoilage microorganisms while the fungal genera *Botrytis cinerea*, *Colletotrichum*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Fusarium*, *Penicillium*, and *Rhizopus* spp. are the prominent spoiling candidates. Understanding vegetable spoilage mechanism/ microbial pathogenesis is important. The harvesting and handling procedures, storage conditions of vegetables, processing, injury of the vegetable plant tissues, and plant resistance against the pathogens are also of significance in order to analyze the toxic metabolites in the affected sites within the vegetables as well as for the prediction of the foodborne illness outbreaks. Implementation of good agricultural practices (GAPs) and good manufacturing practices (GMPs) during vegetable cultivation, harvesting, sorting, storage, transportation, and distribution is important. Moreover, processing of vegetables prior to consumption; i.e., controlling contamination of microbes, a thorough wash with vinegar water, salt water, and other washing means are important preventive measures to ensure the prevention of microbial contamination. Furthermore, up-to-date knowledge on the vegetable spoiling microorganisms, the epidemiology of such microbial contamination, and possible sources and route of the contamination may attribute to the possible future perspective on food safety.

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1. Introduction

Because of the high contentment of vitamins like vitamins B, C, K, and the minerals (calcium, potassium, and magnesium), and due to their properties as dietary fiber, fresh vegetables and fruits are heavily consumed by people all over the world (Balali et al., 2020). As preventive care for chronic diseases like cardiovascular disease (CVD), diabetes, obesity, and micronutrient deficiencies, the consumption of fresh vegetables is highly recommended (Balali et al., 2020). However, microbiologically spoiled vegetables constitute a potential health hazard for humans since the spoiled raw and fresh vegetables often serve as vehicles for pathogenic bacteria, viruses, and parasites (Septembre-Malaterre et al., 2018; Tournas, 2005).

An array of pathogenic bacteria has been detected to contaminate fresh fruits and vegetables of which *Pseudomonas* spp., *Campylobacter jejuni* (gastrointestinal pathogen), *Bacillus cereus*, *Erwinia carotovora*, and *Xanthomonas campestris*, *Clostridium botulinum* (botulinum neurotoxin), *Clostridium perfringens* (produces toxins A-E, and are most prevalent in clinical samples excluding feces), *Escherichia coli* O157:H7 (mostly contaminating the fresh produces), *Listeria monocytogenes* (causing spoilage of the Ready-To-Eat; i.e., RTE foods), *Salmonella* spp. (resident microorganisms in most livestock and many wild animal intestines), *Shigella* (causing major epidemics in Africa, Asia, and in Central America), *Staphylococcus* spp. (producing staphylococcal enterotoxin A), *Acinetobacter*, *Alcaligenes*, *Cronobacter sakazakii*, *Aeromonas*, *Flavobacterium*, *Arcobacter*, *Lactococcus*, *Micrococcus*, and *Vibrio* spp. (causing gastroenteritis; *V. parahaemolyticus* being the most common cause of food-borne disease outbreak), *Yersinia enterocolitica*, parasites including *Cyclospora cayetanensis*, *Toxoplasma gondii*, and *Trichinella spiralis*, and viruses including Hepatitis A and Noroviruses are significant. (Balali et al., 2020; Tournas, 2005; Bintsis, 2017; NCBI, 2017).

Among various spoilage bacteria, *Pseudomonas* spp. have long been considered the most frequently isolated species from spoiled vegetables, probably by their capacity to produce the plant cell wall-degrading enzymes (PCWDEs) like pectinase, xylanase, cellulase, pectate lyase, and polygalacturonase, which are responsible for vegetable spoilage (Lee et al., 2013). Indeed, fresh vegetables are often consumed in their raw forms or sometimes they are slightly cooked in order to retain their vitamin and mineral contents; which unfortunately may result in various food-borne infections and disease outbreaks like typhoid fever, dysentery, diarrhoea, and cholera (Balali et al., 2020; Mir et al., 2018).

Due to the gradual increase in the world population, while there is an increasing demand for the consumption of fresh fruits and vegetables; such a scenario of microbial contamination of vegetables thus threatens the world's mass public health (Snyder and Worobo, 2018). Knowledge about diseases commonly linked with microbial contamination of vegetables is required especially for health professionals in order to find out the means of protecting the mass public

health from vegetable-oriented disease complications. Knowing about microbial contamination sources is another attribute of food safety. Studying the worldwide scenario of pathogenic microorganism-derived disease outbreaks may necessitate the implementation of an appropriate surveillance system of food safety, risk analysis (i.e., food safety policies, food legislation, food inspection, laboratory analysis, etc.), hazard analysis critical control point (HACCP) and the total quality management (TQM) in association to good agricultural practices (GAPs) and good manufacturing practices (GMPs) during vegetable farming, harvesting, distribution, and the food quality analysis (FQA) during processing (Attrey, 2017). Moreover, appropriate guidelines for reducing microbial contamination of vegetables are also needed to ensure the essentials of food safety. The present review, therefore, articulated the current knowledge on food-contaminating microorganisms based on the previously published literature.

2. Foodborne illness

Spoilage of various types of food items results in food insecurity with concomitant foodborne illness, and sometimes foodborne disease outbreaks around different regions of the world, which is also associated with huge economic loss. Foodborne illness is also termed as a foodborne disease (FBD), foodborne infection, or food poisoning (Noor & Feroz, 2017; CDC, 2014, Noor, 2019). Microbial pathogens implicated in most FBDs are Norovirus, non-typhoidal *Salmonella* spp., *Clostridium perfringens*, and *Campylobacter* spp. (Noor, 2019). As per a report published in 2020, FBD is a global public health problem affecting 600 million people annually (Faour-Klingbeil and Todd, 2019). Foodborne microbial hazards are pointed through (1) foodborne infections (mainly caused by *Salmonella* spp., *Campylobacter* spp., *E. coli*, and *Staphylococcus aureus*) (2) intoxications (mainly caused by *Clostridium perfringens*, *S. aureus*, and *B. cereus*), (3) toxi-coinfections (Noor, 2019; Bintsis, 2017; Scannell, 2011).

During foodborne infections, the invading bacterial cells remain alive, an incubation period is needed to colonize the host intestinal epithelial cells with concomitant release of toxins and other virulence factors including the biofilm-related adhesive genes (Noor, 2019; Bintsis 2017). In foodborne infections, since the incubation period is involved, the symptoms of FBD appear lately after food consumption time (Bintsis, 2017).

Foodborne intoxications result relatively quickly because no bacterial incubation is required; rather, this results from the release of bacterial toxins (Bintsis, 2017). During such intoxication, toxins instead of bacterial/ fungal cells are introduced into the individuals (Friedman & Rasooly, 2014). Botulinum neurotoxin, cholera toxins, Shiga toxin, and *Staphylococcus* enterotoxins are widely known bacterial toxins and the major fungal toxins include aflatoxin B, fumonisins, and ochratoxin A, all of which trigger foodborne intoxications (Noor, 2019; Friedman & Rasooly, 2013). According to the toxin production capacity, *E. coli* pathogens have already been subdivided as the enteropathogenic *Escherichia coli* (EPEC); Enterohemorrhagic *E. coli* (EHEC, also known as Shiga toxin-producing *E. coli* [STEC], causing hemolytic uremic syndrome; the strain was formerly known as verotoxin-producing *E. coli* [VTEC]); enterotoxigenic *E. coli* (ETEC); enteroaggregative *E. coli* (EAggEC); enteroinvasive *E. coli* (EIEC); and attaching and Effacing *E. coli* (A/EEC) (Bintsis, 2017).

During toxicoinfection, both the vegetative or the spores of *Bacillus cereus*, *B. anthracis*, *Clostridium perfringens*, etc.

are introduced into the consumer's body; and subsequently release the corresponding toxins like hemolysin BL, nonhemolytic enterotoxin, and cytotoxin K from *B. cereus* (Noor, 2019; Jovanovic et al., 2021). A prominent example of toxi-coinfection can be placed through the enterotoxins associated with *Bacillus cereus sensu lato (s.l.)* which includes foodborne pathogens (Prince & Kovac, 2022).

As stated earlier, microbial contamination of foods usually takes place during the stages of crop harvesting, processing, storage, shipping, preparation of foods, kitchen utensils, etc. (Noor & Feroz, 2017, Frazier, 2007, Todd, 2014). Hence, the control of food contamination by microorganisms can be done by identifying the contaminating microorganisms and their genetic makeup (i.e., the virulence genes), knowledge of microbial enzymes and toxins associated with food deterioration, and food protection by the legislative actions by specific regulatory bodies like Centers for Disease Control and Prevention (CDC), Center for Science in the Public Interest (CSPI), FoodNet, etc. (Noor & Feroz, 2017).

2.1 Pathogen identification

Ensuring food safety as well as conducting a risk assessment for transmission of emerging microorganisms not only during crop production, harvesting, storage and distribution, procession but also through the food chain is significant to secure mass public health. Besides the traditional isolation and enumeration system involving basic microbiology and biochemistry, technological advancements to monitor the levels of microbial contaminants by employing sensitive approaches like using the single-cell droplet microfluidic system, ELISA based techniques, DNA biosensor, use of microcontroller unit (MCU) for pathogenic signal processing, nanobiosensor, smartphone-based biosensor, spectroscopic techniques, PCR-based- and (loop-mediated isothermal amplification) LAMP-based methods, aptasensor, DNA microarray-based methods, next generation sequencing (NGS) combined with bioinformatic approaches, whole genome sequencing (WGS) of single isolates, single nucleotide polymorphism (SNP) analysis and genomic multi-locus sequence typing (MLST) for strain discrimination, detection of RNA/ DNA/ proteins, microextraction and chromatographic methods, and the detection of primary/ secondary metabolites would be highly effective (Saini et al., 2021, Jagadeesan et al., 2019).

Indeed, for the detection of microbial pathogens within food samples, traditional cultural and biochemical methods certainly impart the exact data about food spoilage; however, such detection techniques are labor-intensive as well as time-consuming (Noor & Maniha, 2019). Thus, the quantitative microbial risk assessment (QMRA), based on experimental and meta-analytical data, and the identification of virulent genes are regarded as a novel advancement in identifying microorganisms and their components and also the presence of opportunistic pathogens in foods especially, in case of opportunist waterborne infections (OWI) (Price et al., 2017; Noor & Maniha 2019).

Contamination and growth of spoilage microorganisms may limit the shelf life of vegetables and may account for the spreading and transmitting diseases, and the survival and/or growth of these pathogens in fresh vegetables and fruits are certainly a potential factor for instigating human/ animal illness (Rahman et al., 2016; Noor et al., 2015). As stated earlier, foodstuffs may endanger consumers to an increased risk of FBD, mainly due to ineffective removal/ inactivation of pathogenic microorganisms before consumption. There are lots of reports of ready-to-eat fruit and vegetable-related disease outbreaks so far (571 outbreaks between 1980 - 2016, causing 173 deaths out of 72,855 infected cases as of 2019) of which microbiologically (*Escherichia coli* and *Salmonella*, norovirus, and hepatitis A virus) contaminated leafy

green vegetables accounted for 51.7% outbreaks. (Machado-Moreira et al., 2019). Hence, identifying the nature and source of microbial contamination as well as the prevalence of microbial spores (which caused 31.8% of deaths) in these foodstuffs is of public health significance (Machado-Moreira et al., 2019; Alam et al., 2015).

2.2 Previous works on pathogen identification in fresh vegetables

An analysis of the last two decades reveals the continuous proliferation of a range of pathogenic microorganisms in fresh produce, resulting in foodborne disease outbreaks in different geographic locations around the world (Degaga et al., 2022, Łepecka et al., 2022, Arienzo, 2020, Akoachere et al., 2018, Mritunjay & Kumar, 2017, Noor & Feroz 2015; Rahman & Noor, 2012, Nipa et al., 2011, Cordano & Jacquet, 2009, Abadias et al., 2008, Sagoo et al., 2003). Prevalence of *S. aureus*, *Listeria monocytogenes*, *E. coli* was prominent, and even fecal coliforms were also detected in the raw salad vegetables (RSV) from their studies.

In 2020 in Fiche town, Central Ethiopia, a microbiological cross-sectional study on tomato (*Solanum copersicum*), cabbage (*Brassica oleracea*), green pepper (*Capsicum annuum*), lettuce (*Lactuca sativa*), and carrot (*Daucus carota*) revealed the prevalence of aerobic mesophilic bacteria on an average count of 10^5 colony-forming units (CFU)/g with 10^4 CFU/g of Enterobacteriaceae (Degaga et al., 2022). Cabbage samples were mostly contaminated with aerobic mesophilic bacteria (10^6 log CFU/g) while the minimum was in green pepper samples showed the least (10^4 log CFU/g). Of all samples, staphylococcal contamination was estimated at up to 11% (average) while *S. aureus* was the most prevalent (20%) microorganism in cabbage (20%), followed by lettuce by 15%. *Salmonella* spp. were also detected in up to 15% of the samples tested (Degaga et al., 2022). A study on the ready-to-eat (RTE) salad vegetables by Łepecka et al. (2022) showed the prevalence of mesophilic microorganisms up to 10^6 CFU/g, with the dominant presence of Enterobacteriaceae, *E. coli* (50% samples were contaminated), *Salmonella* spp. (in nearly 27% of the samples tested), *L. monocytogenes* (around 33%), *S. aureus*, yeast, and molds. Abundance of *Salmonella* spp. (67%) and *L. monocytogenes* were also observed in the RTE salad vegetables stored in supermarket chain shops in Lazio, Italy (Arienzo et al., 2020).

The study by Akoachere et al (2018) on salad vegetables in Fako Division, Cameroon, revealed the average bacterial contamination of the tested samples up to 10^6 CFU/g, total coliform counts ranging from 4 to more than 2400/g and fecal coliforms from 3 to 1100/g. *S. aureus* isolates were detected with a 35.4% prevalence, *Shigella flexneri* with a 25.6% prevalence, and *Entamoeba* spp. with a 21.7% prevalence while *Serratia marcescens* was identified in 8.5% of the total samples tested. Microbial contamination was noticed to be highest in lettuce and lowest in green pepper (Akoachere et al., 2018). The gene-based work; i.e., real-time polymerase chain reaction (qPCR) conducted by Mritunjay and Kumar, (2017) using 480 samples of RSV of 8 different categories revealed the presence of *E. coli* in 16.7% samples, pathogenic *E. coli* O157:H7 in 1.3% samples, *L. monocytogenes* in 3.5% samples, and *Salmonella* spp. in 4% samples tested. However, they didn't find the cabbage samples to be contaminated with any of these microorganisms (Mritunjay & Kumar, 2017).

Based on these findings, it is clear that the improvements in the agriculture status from a microbiological quality perspective are highly demanding since the public health risks within the mass population are caused by these pathogenic microorganisms which are initially harbored through the agricultural products, and as they are RTE products,

the processing may not be enough to ensure those products free from microorganisms. Hence, microbiological quality management of the agricultural products, detection of the specific microbial pathogens contaminating the agricultural products or RSV, and the prediction of possible FBD outbreaks are necessary (Noor & Feroz, 2015).

Earlier works showed the huge prevalence of contaminating microorganisms in fresh produces. Prevalence of fecal coliforms, *E. coli*, *S. aureus*, and *Listeria* spp. were found in the RTE vegetable samples in the work conducted by Rahman and Noor (2012). Additionally, *Klebsiella* spp. were enumerated in tomato samples up to 10^5 CFU/g, *Vibrio* spp. was estimated upon enrichment within a range of 10^4 to 10^7 CFU/g in carrot, lettuce, and tomato samples; and *Salmonella* spp. and *Shigella* spp. were estimated within 10^3 to 10^7 CFU/g and 10^4 to 10^8 CFU/g respectively, in all samples (Rahman & Noor, 2012). Nearly similar results were also reported by Nipa et al. (2011) using the same salad vegetables. A special emphasis was given to *L. monocytogenes* between 2000 and 2005 by Cordano and Jacquet (2009) who analyzed 717 samples of three types of RTE salads in Santiago, Chile. They found 88 *L. monocytogenes* isolates out of 347 frozen samples, and 22 isolates in 216 freshly supermarket products, which unravel the exceptional survival strategy of *L. monocytogenes* in refrigerated conditions (Cordano & Jacquet, 2009). During 2005-2006, a survey of fresh and minimally-processed fruit and vegetables, and sprouts was conducted in the Lleida area of Catalonia, Spain, using a total of 300 samples including 21 RTE fruits, 28 whole fresh vegetables, 15 sprout samples, and 237 RTE salads (Abadias et al., 2008). Fresh-cut vegetables revealed a high prevalence of microorganisms (especially *E. coli* and *Salmonella* spp.) in grated carrots, arugula, and spinach (Abadias et al., 2008). In the RTE vegetables, the presence of *Salmonella*, *L. monocytogenes*, and fecal coliforms are certainly unacceptable as is known from the very beginning (Sagoo et al., 2003). Nevertheless, the minimally processed cut and packaged salad actually have a great chance of exposure to a range of adversarial conditions during growth, harvest, preparation, and distribution, which may escalate the possibilities of microbial contamination (Sagoo et al., 2003).

2.3 Prevalence of drug-resistant microorganisms in food

Foods are known as vehicles in human exposure to drug-resistant bacteria (Mola et al., 2021). Anti-microbial resistance (AMR), including multi-drug resistance (MDR), is a global health public health issue. Several MDR microorganisms (i.e., resistant to at least one anti-microbial agent present in three or more anti-microbial classes) have been reported to be prevalent in foods of which *Salmonella* spp. are of major concern (Campos et al., 2019; Mesbah et al., 2017). Food-borne diseases caused by drug-resistant as well as MDR microorganisms impart risk also in the food production chain especially in case of the raw/ unprocessed foods and in fresh produces (Hashempour-Baltork et al., 2019). It's interesting to note that the drug-resistance trait even from non-pathogenic bacteria can be spread through the horizontal gene transfer to other microbial species (i.e., the onset of opportunistic pathogenesis caused by *Escherichia coli* and *Salmonella enterica*) which afterward may affect the gut microbiome upon consumption with the possible expression of the antibiotic-resistance genes (ARG) (Gekemidis et al., 2018). In addition, *Vibrio* spp., some species of *Aeromonas*, spores of *Clostridium botulinum* type F, *Campylobacter*, *Staphylococcus aureus*, *Listeria* spp., and *Shigella* are widely known foodborne MDR microorganisms (Maneilla-Becerra et al., 2019; Mola et al. 2021).

3. Food safety and risk assessment

Control of microbial contamination of food mainly depends on the knowledge of the (1) types and modes of food hazards, (2) genetic factors and the virulence traits of the food-contaminating microorganisms, (3) toxins associated with food deterioration, (4) clear idea on the “food safety system” constitutes producers, processors, shippers, retailers, food preparers; (5) the legislative bodies including governmental or non-governmental organizations (NGOs), (6) sufficient research support on food microbiology; and finally, (7) setting the food standards to protect the public from threats to food safety, fraud and adulteration (Noor & Feroz, 2017; Noor & Feroz, 2015; Institute of Medicine and National Research Council Committee, 1998; Ghosh, 2014; Lawrence et al., 2019). Prominent examples of the regulatory authorities for controlling food quality and food safety include the World Health Organization (WHO), Food and Agricultural Organization (FAO), Center for Science in the Public Interest (CSPI), Centers for Disease Control and Prevention (CDC), FoodNet, Food and Drug Administration (FDA), Department of Health and Human Services (DHHS); European Food Safety Authority (EFSA). Food Safety and Inspection Service (FSIS) of the US Department of Agriculture (USDA); the Environmental Protection Agency (EPA), Food Standards Australia New Zealand (FSANZ), Food Standards Program (FSP), International Commission on Microbiological Specifications for Foods (ICMSF), dynamic risk management system (DRMS), etc. (Institute of Medicine and National Research Council Committee, 1998; Ghosh 2014; Lawrence et al., 2019). The Joint FAO/WHO Food Standards Program is regulated by the Codex Alimentarius Commission (Codex), which aims to ensure harmonized global food standards, guidelines for maintaining food quality, instructs the codes of practice, and provides the scope for handling public health problems (FSANZ, 2022, Lawrence et al., 2019).

Risk analysis for food constitutes of (1) risk assessment; i.e., estimation of the risk to a given target microorganism, hazard identification and characterization; microbial contaminant exposure assessment and characterization of the risk posed; (2) risk management; i.e., use of surveillance and gathering of data to accurately measure the global prevalence and consequences of FBD, detection of the prevalence of microbial pathogens in food, environmental exposure, consumer perspective and attitude as well as the development of policies to prevent foodborne diseases. and finally, (3) the risk communication with the public which may arise from the contamination of foods or it may be a consequence of the occurrence of a food crisis following a food safety incident in response to chronic food safety issues (Galbiati et al., 2022; CAC, 2015; Dowell et al., 2016).

Such risk communication is needed for any accidental changes in the food supply chain (associated with food hygiene practices), for optimizing consumer protection regarding food consumption, and for increasing confidence in the organizations responsible for assessing and managing food risks. The requirement for effective risk communication is also based on the application of specific food-processing technologies/ agricultural practices which may exhibit any societal concern (Frewer et al., 2016). Another aspect of risk communication is that the food industries are continually trying inventive development and production of food items that employ new techniques are also being employed as stated above. However, these changes may lead to differences in the microbial pathogens encountered. This results in a better understanding of the prevalence pattern of pathogens in food products based on their origins. Thus, microbiological risk

assessment (MRA) serves as a systematic tool to take actual decisions to reduce the influence of foodborne pathogens as well as to maintain a sustainable public health situation (Voysey & Brown, 2000). Indeed, the need for a risk-based approach for the production of safe food is based on the hazard analysis: critical control point (HACCP), and the other risk management metrics involving Food Safety Objectives (FSO) and Performance Objectives (PO), proposed by the International Commission on Microbiological Specifications for Foods (ICMSF) and adopted by the Codex Alimentarius Commission (Institute of Medicine and National Research Council, 2003).

This is again to state that the consumption of leafy vegetables, salads, and fruits has been increasing considering their health benefits. However, as mentioned earlier, such an increased consumption of fresh produces is also linked to a substantial number of foodborne outbreaks which pose a threat to public health and the country's economy. As food safety starts on the agricultural/manufacturing farm and ensues over the supply chain, food producers should adhere must follow specific procedures to avoid food hazards along the supply chain. Thus, to provide safe products, (Macieira et al., 2021).

Conclusion

A flawless perception of the mechanism of food-spoiling microbial pathogenesis contributes to identifying and resolving foodborne complications. Technological advancement for the detection of spoiling microorganisms and the associated toxins/ other virulence factors are also possibly derived from the knowledge gained from the extensive study of food microbiology. Detection of drug-resistant, as well as MDR pathogens in food, is of huge significance to ensure food safety. Eventually, food safety management systems augment the risk analysis posed by foods that can be contaminated by different species of microorganisms.

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