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A MICROBIOLOGICAL RISK ASSESSMENT AT THE FRESH PRODUCE WHOLESALE MARKET IN DOHA

BY

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ABSTRACT

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Food contamination is a challenging problem in many developing countries, like Qatar where the majority of food is imported. The wholesale fresh produce market (WSFPM) in Doha is considered as the major produce market from which the supermarkets, restaurants, and people obtain their produce. The WSFPM is an open-air market located in close proximity to the fish market, slaughterhouse, and industrial area. At the moment, there is no information available on the prevalence of microorganism is in and the impact of the surrounding markets on the microbial quality of produce sold at this market. Therefore, this study was carried out to 1) determine the source of the microbiological hazards associated with sanitary conditions at the WSFPM; 2) evaluate the different sources contributing to the microbial hazards; 3) assess the workers' level of food safety knowledge and their behavior in handling produce; and 4) conduct a microbial risk assessment to determine the potential risks associated with fresh produce consumption.

Different produce (e.g. cucumber, green onion, lettuce, tomato, and parsley), soil, air, and surface swabs samples were collected from WSFPM and the surrounding areas between July 2016 and June 2017. Selective media were used to determine the presence of pathogenic *E. coli*, *Listeria* spp., *Staphylococcus* spp., and *Salmonella* spp. The presumptive colonies were isolated and identified using MALDI-TOF and molecular techniques. The workers who were in direct contact with the produce were

examined in the winter of 2015. During the survey application, hand-swab samples were also collected. The risk of getting sick through consumption of green onion, cucumber, tomato, green pepper, and romaine lettuce contaminated with select microorganisms was calculated using the sQMRA model.

The microbial counts of produce samples determined, indicated that the samples were heavily contaminated (> 4 Log_{10} CFU/g) with *Bacillus*, *Enterococcus faecium*, *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas*, *Enterobacter*, *Alternaria*, *Aspergillus*, and *Penicillium* species. There were no pathogenic bacteria identified in any of the sample tested in this study. The same trend was also observed in soil, air, and surface samples. It was surprising to find out that none of the workers had any training on safe produce handling practices. Almost 70% of them claimed to wash their hands 4-5 times per day. However, the hand-swab samples' results verified that the hygiene practices applied by the handlers are not sufficient since pathogens such as *Klebsiella* and *Staphylococcus* were identified. These results demonstrated that there was an urgent need to organize an educational training to familiarize the workers on food safety and hygiene practices.

The mean annual probability of getting sick after consuming the raw produce contaminated by coliforms ranged between 0.27 and 0.46, indicating that attention needs to be paid specifically on 1) improving the sanitary conditions at this target market, 2) educating the produce handlers and consumers on safe food handling practices, and 3) applying appropriate mitigation measures, such as periodical monitoring and adopting a risk-oriented approach in order to avoid any costly produce outbreak.

DEDICATION

I dedicate this dissertation to my father's soul and my mother for their endless love, support, and encouragement. To my sisters & brothers, to my beloved husband, Dr. Mohamed Salman, and to my dear daughter, Mirna.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

Food is one of the most important resources for sustaining and enjoying life. Fresh produce (fruits and vegetables) is considered as good source for nutrients like vitamins, fibers, minerals, and other compounds having antioxidant and antimicrobial properties (Sharma and Kumar, 2013). In recent years, the demand for fresh produce has increased worldwide. The open nature of the fresh produce cultivation process enhances the contamination of such commodities with different pathogens at several stages during the production chain (Nuesch-Inderbinen and Stephan, 2016). Therefore, fresh produce can play an important role in carrying hazards and causing diseases by being a good vehicle and medium for many microorganisms like viruses, bacteria, fungi, and protozoans. It was assumed that washing fresh produce is sufficient to remove most of the field contaminants (Feliziani *et al.*, 2016). However, several research studies provided evidence that the post-harvest washing step has limited efficiency to decontaminate the fresh produce and can sometimes lead to cross-contamination (Gombas *et al.*, 2017). Francis *et al.* (2012) mentioned that sometimes applying inadequate agricultural and handling practices could introduce human pathogens into the fresh produce during the production chain. Hence ensuring the food safety for fresh produce, consumers are concentrating on minimizing the contamination in both pre- and post-harvest stages (Murray *et al.*, 2017).

In the last decades, the increase in fresh produce consumption resulted in a higher number of foodborne illnesses all over the world (Warriner *et al.*, 2009). The outbreaks related to fresh produce consumption have become too common recently and fresh produce is considered as a leading cause (CDC, 2017). Notable among these are *E. coli* O157:H7 in sprouts (Buchholz *et al.* 2011), *Listeria monocytogenes* in cantaloupes (McCollum *et al.*, 2013), *Salmonella* Poona in imported cucumbers in the

USA (CDC, 2016 b), *Listeria monocytogenes* in Australia's rock melon (Flynn, 2016), and *E. coli* in romaine lettuce (Jeff , 2018).

Foodborne outbreaks are considered one of the most important public health issues worldwide. The main causes related to foodborne outbreaks are due to the poor food handling practices especially for food prepared at home, unsanitary food services used during catering such as street food selling operations, cross-contamination, and temperature abuse (WHO, 2008). For example, in Jordan where more than a hundred bacterial strains were isolated from Shawarma sandwiches mainly made of ground beef or chicken (Nimri *et al*., 2014). *E. coli* O157:H7 was the most dominant foodborne pathogen (28.3%) isolated from chicken sandwiches, followed by *Salmonella* spp. (25.5%). The authors also identified several species of *Salmonella* such as *S. paratyphi* A, *S. cholerasuis,* and *S. pullorum*, while *S. paratyphi* A was the most common among the identified species (51.4%). Most of these isolates were resistant to different antibiotics such as tetracycline and streptomycin. Additionally, *Citrobacter freundii* and *Staphylococcus aureus* were also isolated at a percentage rate of 15.9% and 8.3%, respectively. These microbes have the ability to produce a heat resistant toxin, which leads to food poisoning. The investigators concluded that the risk in such sandwiches could be reduced by improving hygiene conditions at all stages of sandwich preparation, such as cooking and serving. The same situation was witnessed in Cairo (Egypt) in 2013 when 160 university students got sick after consuming a meal served in their dorm. Although the pathogen causing this outbreak was not identified, the Ministry of Health linked the spoiled tuna meal as the source of poisoning for this case (Ministry of Health – Egypt 2013). Moreover, the microbial quality of dairy products sold in Ramallah and Al-Bireh district, Palestine was investigated during 2001-2004 (Khatib and Al Mitwalli, 2009). Several dairy

products, such as yogurt, pasteurized milk, raw milk, “labneh” (concentrated yoghurt), cooked cheese, and salted cheese were collected from restaurants, grocery shops, dairy factories, sweet shops, and vendors. Majority of samples tested were contaminated with coliforms, fecal coliforms, *Staphylococcus aureus*, molds, yeasts, and *E. coli* at a rate of 21.0% 15.2%, 1.0%, 10.3%, 2.3% and 14.3%, respectively. The authors noticed the following issues during their sampling process: there was no systematic food surveillance system in place; the food handlers were not following hygiene practices; such as wearing uniform, gloves, and head covers; and the conditions of milk collected from animals were not matching the milk hygiene measures. Similar results were also reported by Al-Tahiri (2005) who tested yoghurt, raw milk, labnah, and white soft cheese samples in Jordan.

Recently, Huang *et al*. (2019) evaluated the effect of temperature abuse and nutrients availability on the pathogens colonization. They tested *Salmonella enterica*, and *Listeria monocytogenes* under different temperatures with variation of food matrices such as extracted juice and fresh-cut produce using watermelon, cantaloupe, honeydew, pineapple, and radish as hosts. They determined that the inoculated pathogens had a potential growth in the juice extracts compared to cut produce. In addition, the recorded population of *S. enterica* reached up to 5.28 Log_{10} CFU/g, while *L. monocytogenes* reached 7.77 Log_{10} CFU/g after 7 days incubation at 8-12 °C.

Unfortunately, it is a difficult task to estimate the global incidences of foodborne diseases since many countries do not have an efficient food surveillance system. Preventing food contamination from harmful causes such as bacteria, viruses, and toxic chemicals, which are considered as main sources of foodborne illness is problematic in many developing countries, such as Qatar. The majority of food (90%) consumed in Qatar comes mainly from Lebanon, Jordan, Egypt, Syria, Holland,

Denmark, Spain, India, China, Pakistan, Sri Lanka, Iran, Turkey, the USA, and Australia (Qatar Statistics Authority, (QSA, 2012). In 2015, Ministry of Public Health (MoPH - Qatar) reported that 2.59 billion tons of food items imported to the country, of which about 2.2 million tons were returned to the origin country because they did not comply with the local food safety standards and 2.9 million tons were destroyed because they were classified as unfit for human consumption. The rejection was normally based on microbial, chemical and physical food safety features of these food products (Gulf Times, 2016). For example, during 2016, around 901.5 million tons of foodstuff passed through the food inspection services at the seaport in Qatar. Among which just 897.6 million tons were approved to pass, while 3.9 million tons were rejected. Through the land border "Abu Samra", about 898.4 million tons of food were allowed to enter the country, 445.8 million tones were destroyed and 704 million tones were re-exported to the original country for non-compliance. Through air cargo, about 738 million tons of foods were imported into the country of which 24.8 million tons were destroyed at the customs (Gulf Times, 2016).

Qatar experienced one of the fastest economic growths over the last decades in the world because of expansion of the oil and gas industries. This expansion had many positive impacts on Qataris, such as increased income levels, which ultimately resulted in changing food consumption habits, especially among young Qataris. Since fresh produce is considered a healthy food choice, in recent years many young Qataris have been including more fruits and vegetables in their diet. According to Alpen Capital (2017), the consumption rate of fruits and vegetables in Qatar was increased by 6% between 2012 and 2017 and it is considered as the highest growth rate among the GCC countries.

The wholesale produce market is the major market from which most of the

consumers/individuals get their fresh produce (fruits and vegetables) either daily or weekly. In addition, the local restaurants, industrial food services, grocery stores, and supermarkets obtain their fresh produce to prepare different types of food products in their establishments or to resell these produce to the consumers as a retail product. The market offers domestic and imported fresh produce entering Qatar from the surrounding countries, such as Iran, Jordan, Kuwait, Lebanon, Oman, etc. at reasonable prices. Once these produce are delivere to the market, they do not receive any additional cleaning, processing, or packaging. The wholesale produce market is the only major vegetables and fruits market in Doha, which is an open-air market located in an uncultivated area (mainly desert area), without any air-conditioning where the average temperature is above 35°C throughout the year. The market is open daily for the public from 6:00 am to 1:00 pm, except on Friday. Additionally, the market has no refrigerators at the display area, while there are refrigerated-rooms located about 500 meters away from the market to store the unsold produce. The market is fully managed by Doha Municipality Authority, which regularly inspects the produce for the presence of pesticides, but no periodical microbial inspection is conducted unless a complaint is received from the consumers. The wholesale produce market is also located in close proximity to other surrounding markets, such as the fish market, Doha slaughterhouse, poultry, large animal markets, and industrial area, which may all contribute to increased risk of microbial contamination in fresh produce purchased from this market.

At present, there is no data available about the relation between the consumption of fresh produce and the number of foodborne diseases occurring in Qatar. Therefore, there is an urgent need to assess the association between the microbial quality of fresh produce sold in Qatar, specifically at the wholesale

produce market located in Abu-Hamour in Doha, and the risk of infection due to consuming the produce purchased from the target market.

Therefore, this study was carried out to:

1) Investigate the microbiological hazard(s) such as bacteria and fungi associated with select fresh produce sold at the WSFPM.

2) Determine the source of the microbiological hazards associated with sanitary conditions at the wholesale market.

3) Evaluate to which extent the different sources contribute to the microbial hazards expected to be present at the market.

4) Conduct a preliminary microbial risk assessment (MRA) for target pathogens identified as the source of contamination in fresh produce-related outbreaks in Qatar, and propose appropriate guidelines to control the growth of pathogens, thereby reduce the number of fresh produce-related foodborne illnesses in Qatar.

1. LITERATURE REVIEW

1.1 Environmental Health and Food Safety

Humans are in direct contact with their environment. This interaction affects the quality of life and the number of years lived as healthy. It is important to improve the public health via enhancing environmental health (WHO, 2006a). According to WHO (2004), environmental health are "*those aspects of human health, including quality of life, that are determined by physical, chemical, biological, social and psychosocial factors in the environment. It also refers to the theory and practice of assessing, correcting, controlling, and preventing those factors in the environment that can potentially affect adversely the health of present and future generations.*"

1.2 Environmental Related Diseases

Most of the environmental health researchers are explaining how the environment is connected with the etiology of several diseases (Frumkin, 2016). From the total world population, about 23% of the death and 22% of total Disability Adjusted Life Years (DALYs) around the world are caused because of environmental exposures to toxins and pathogens (Prüss-Üstün *et al*., 2016). This percentage can be reduced by preventing the environmental risk via eliminating the environmental exposures, hence saving public lives, especially in the developing countries. The WHO report (published by Prüss-Üstün *et al*., 2016) listed the diseases of the largest total annual health burden related to the environmental factors in term of DALYs per year. These diseases are; a) diarrheal diseases from unsafe food, water, and poor hygiene (58 million), b) respiratory infections from air pollution (37 million), c) malaria from poor water (19 million), d) accidental injuries from industrial workplace (21 million), e) traffic injuries related to poor urban/environmental design (15 million), and f) other diseases related to exposure to workplace dust and air pollution (12 million).

Food safety is one of the important components of environmental health, which protects the food supply in each production step from farm to table and reduces the possibility of getting sick. Environmental health experts have the responsibility of improving the quality of life by reducing all means of food contamination and providing high standards to have safer food.

Environmental health plays a role in enhancing food safety and hygienic practices during each step of the food chain e.g. production, handling, processing, distribution, and storage. Environmental health ensures the quality of food consumed by a human through enhancing the food inspection system, handling practices, and certifying the suitable food production establishments. Environmental health ensures the hygienic conditions in places and prevents food contamination. In addition, the environmental health practitioners take a role in educating the food handlers about the best handling practices by training them and ensuring their health condition to prevent the diseases transmitted during food handling. They also enforce the food safety laws and regulations to maintain the food safety standards (Musoke *et al*., 2016). Furthermore, environmental health plays a role in enhancing the surveillance system and investigating food contamination before the outbreak and providing a good food management system to reduce food hazards and mitigate the foodborne outbreaks.

1.3 Food Safety and Foodborne Diseases

Foodborne diseases are one of the most important public health problems around the world. The incidence of foodborne diseases and foodborne outbreaks has increased worldwide, in both developed and developing countries. According to the most recent WHO report (WHO, 2015), eating contaminated or uncooked food such as egg, meat, fresh produce, fish, and dairy products can lead to diarrhea, which is the main cause of the burden of foodborne diseases. The report estimated that

approximately 550 million people suffer from foodborne diseases worldwide per year with nearly 420,000 annual deaths. Moreover, children under 5 years old are at high risk and 125,000 of them die every year due to foodborne diseases. The main causes of these diseases are pathogenic bacteria (e.g. *Salmonella, Campylobacter, and Enterohaemorrhagic Escherichia coli, Listeria, and Vibrio* cholera), viruses (Noroviruses and Hepatitis A), parasites (e.g. *Echinococcus spp, Taenia solium, Ascaris, Cryptosporidium, Entamoeba histolytica,* and *Giardia*), toxins (e.g. aflatoxins), and chemicals (e.g. pesticides, dioxins, and heavy metals).

The symptoms of foodborne diseases can range from severe bloody diarrhea to serious infections, such as meningitis, while chemical contaminations may lead to poisoning which might lead to chronic diseases and cancer. In rare cases, foodborne diseases may lead to lifelong disability and death (WHO, 2015). Table 1 presents examples of foodborne pathogens and their medical and social impacts.

The main global challenges of the foodborne diseases can be summarized as a) the identification of emerging foodborne pathogens, b) the increase in the proportion of the foodborne illnesses susceptible groups (e.g. children, elderly, and pregnant women, and people with immunocompromised diseases) in the developed countries, c) the increase of food globalization via food trading, which spread the pathogens all over the world, d) the increase of the tourism activity between countries, which may spread some new foodborne pathogens and put select people at risk, e) the introduction of new pathogens into a new environment, which might affect the sensitivities of these pathogens to antibiotics; thereby, producing resistance to antibiotic, and f) the increasing rate of consuming food outside the home, which enhances the risk related to poor handling practices of some food establishments (WHO, 2008).

Table 1. Common foodborne pathogens and their medical and economic impacts (adopted from Fung et al., 2018).

Foodborne hazards	Common Infectious or toxic agents	The incidence of foodborne illness	Death due to foodborne illness	Total DALYs*
Bacteria	*Brucella, Campylobacter, E. coli, Listeria, Salmonella, Shigella, Vibrio*	359,747,420	272,554	20,188,792
Virus	Norovirus, Hepatitis A	138,513,782	120,814	3,849,845
Protozoa	*Entamoeba, Giardia, Cryptococcus, Toxoplasma*	77,462,734	6242	1,311,435
Worms	Cestodes (tapeworms), Helminths (parasites), Nematodes (round worms), Trematodes (flatworms)	26,063,664	90,261	11,599,735
Toxins/Chemicals	Aflatoxins, Cyanogenics, Dioxins, Heavy Metals	217,632	19,712	908,356

* Disability-Adjusted Life Years

Safe food can play a role in supporting the national economy, nutrition security, tourism, trade, and even sustainable development. Changes in the consumers' food habits, globalization of food items, the introduction of international food cuisine, urbanization, increase in travel activities, and eating publicly outside the home have resulted in the complex food chain. In addition, the fast-growing population and climate change enhanced food production industrial activities, such as agricultural and animal production. All these factors impact food safety where the food is modified, the temperature is changed, and new means are introduced in food production, storage, and distribution. These factors put great pressure on food producers and handlers to keep food safe. The global food trading increased the chances of spreading a foodborne outbreak from being a local issue to an international issue (WHO, 2015). Two of the most recent significant outbreaks in this regard are: the contamination of fenugreek sprouts that took place in Germany 2011 killing 47 people (Olaimat and Holley, 2012), and the contamination of baby formula in China 2008 that resulted in 300,000 babies being effected in the death of six of them (El-Nezamy *et al.*, 2013). Recently, chemical food contamination became a serious problem because of environmental pollution, which is due mainly to industrial development (Song *et al.*, 2017).

Foods can be contaminated by chemicals from several sources such as; environmental contaminants (e.g. air, soil, water, and disinfectants); processing contaminants, which are introduced during baking, canning, fermentation, heating, roasting, and hydrolysis; and packaging contaminants (Schrenk, 2004; Mastovska, 2013; Rather *et al.*, 2017). The longtime taking in food preparation and processing enhances the potential of chemical residues. Some food additives, which are added to increase the shelf life, can also lead to chemical contamination (Gorman *et al.*, 2002) or the accidental chemicals leaking from animal feed or antibiotics into the food chain

(Martin and Beutin, 2011). Furthermore, food transportation under poor sanitary conditions may increase the risk of food contamination with chemicals (Unnevehr, 2000).

Food contamination by chemicals has serious negative effects on human health (Robertson *et al.*, 2014). In 2013, the CDC in the USA confirmed about 11,000 cases of foodborne diseases caused by biological agents and other agents such as toxins, metals and other chemical pollutions (Salter, 2014; Callejón *et al.*, 2015). Foodborne illness symptoms caused by chemical contamination can range from minor gastroenteritis to advanced hepatic and kidney failure, and neurological diseases. In addition, food contaminated with heavy metals, pesticides, and other chemical pollutants can cause abdominal infections (Song *et al.*, 2017). Exposure to heavy metals by consuming contaminated food with heavy metals, such as cadmium and lead, can deplete specific nutrients in the human body and reduce the efficiency of the immune system (Khan *et al.*, 2008). It can also increase the rates of gastrointestinal diseases and cancer (Khan *et al.*, 2008). In addition, the presence of pesticides, poly chlorinated biphenyls (PCBs), and heavy metals in food can result in adverse human health effects, such as kidney damage, congenital disabilities, fertility problems, susceptibility to cancer, and other metabolic complications (Bassil *et al.*, 2007; Androutsopoulos *et al.*, 2013; Grandjean and Landrigan, 2006).

One of the well-known incidents about chemical contamination of food is the melamine-contaminated baby-milk formula happened in China in 2008. In this outbreak, more than 300,000 babies were affected, of which 52,000 were hospitalized and six babies died (Branigan, 2008; El-Nezamy *et al.*, 2013). A review of the number and examples of chemical food contamination issues was published by McKay and Scharman (2015).

1.4 Global Facts on Food Safety

Food safety is an essential element of environmental health that protects our food supply chain. According to the Codex Alimentarius Commission (CAC, 2003), food safety is defined as "*The assurance that food will not cause harm to the consumer when it is prepared or eaten according to its intended use.*" Every year, about 220 million children suffer from diarrhea and 96,000 of them die because of consuming contaminated foods (WHO, 2015). Food safety is an important public health issue around the world and needs to be enhanced with the involvement of governmental authorities along with public health centers. Food safety policies usually involve a collaborative effort between the food industry and regulatory organizations. The main objectives of these policies are to 1) ensure that the suppliers follow the food safety standards and 2) reduce the frequency of foodborne diseases. Food safety management systems have to adopt several protocols and combine them together to ensure the safety of food products, such as Good Manufacturing Practices (GMP), Hazard Analysis and Critical Control Point (HACCP) system, and sanitation standard operating procedures (SSOP) (de Oliveira *et al.*, 2016).

Improving food safety in a nation can enhance sustainable development. Therefore, governments should put food safety as one of their top public health priorities. This can be achieved by developing effective and appropriate policies and regulations and implementing an active food safety system to ensure that consumers receive safe food. Food producers also play a major role in food safety since the majority of food contamination issues occur as a result of improper preparation and mishandling of food (WHO, 2015). During production, food can become contaminated with pathogens such as bacteria, viruses, or parasites, and other chemical agents or toxins at any stage of the food chain (WHO, 2015). These contaminants are usually

introduced into the food when; a) the agricultural practices are inappropriate, b) using chemical materials incorrectly, c) poor hygiene practices are applied at any stage in the food chain, d) using contaminated raw materials, e) storing food inappropriately, and f) preventive control system during food processing and preparation are not effective (WHO/FAO, 2003).

1.5 Types of Food Associated with Foodborne Outbreaks

The US CDC (2015) defined a foodborne disease-causing outbreak as "*The occurrence of more than two cases of a similar illness resulting from ingestion of a common food."* Food can be a source of illness and unsafe to consume especially when it is contaminated by pathogens. These pathogens might enter the food chain during harvesting or handling and preparation. In all cases, food may look fine but could be harmful and dangerous to consume, especially for the susceptible groups of people (e.g. children, elderly, and pregnant women). Some foods have the ability to be classified under the category of risky or hazardous food because pathogens can grow rapidly on these foods. These risky foods can be raw or undercooked: meat, poultry, fish, shellfish, milk or milk products, some soft cheeses, eggs and egg products. In addition, raw sprouts and unwashed fresh produce or unpasteurized fresh juices can be classified as risky food. Most of these products have to be cooked well and stored at suitable temperatures to keep them safe until consumption.

1.5.1 Meat and Poultry

The most serious meat safety issues resulting in consumer health problems and products recall of contaminated meat products are associated with microbial contamination, especially bacterial pathogens. Bacteria such as *E. coli* O157:H7 and *L. monocytogenes* are highly associated with foodborne diseases linked with meats in the United States (Sofos, 2008). These organisms mainly grow during the processing

stage of the product, e.g., slicing, packaging or sometimes storage under temperature abuse conditions (WHO, 2008). While the presence of viral agents, such as Norovirus, in the food service industry is the major cause of foodborne disease in the United States and around the world (CDC, 2013).

There are many foodborne pathogens associated with meat, seafood, and poultry products, such as *Vibrio cholera nonO1, Vibrio vulnificus, Cryptosporidium parvum, Cyclospora cayetanensis, Enterobacter sakazakii, prions, E. coli, Campylobacter, Shigella,* and *Listeria*. Some other unknown pathogens associated with animal health epidemics include avian influenza (AI) and foot-and-mouth disease (FMD) viruses (Whitley and Monto, 2006; Grubman and Baxt, 2004). In addition, there are other significant pathogens linked to meat products, such as *Mycobacterium avium* sub spp., *Paratuberculosis, Escherichia albertii,* and *Clostridium difficile* (Oliver *et al.*, 2005; Manabe *et al.*, 1995).

On the other hand, studying the ecology of these pathogens and improving the methodology used in detecting these pathogens is highly required before enrolling the pathogens in the safety of meat and other food products. Animal health threats with possible human health associations, for example, Avian influenza and foot-and-mouth disease will endure real difficulties in the future and may lead to major epidemics or disasters of global concern (Sofos, 2008).

Microbial hazards and related issues will keep being significant difficulties to meat safety for the upcoming future. It is essential to understand that assessment of meat safety risk should focus on coordinated action and approach that applies to all areas or subdivisions, from the manufacturer through the processor, provider, packer, retailer, food facility staff members, and consumers. Mishandling of foods is one of the major factors in foodborne diseases. Pathogens associated with animals can easily

be introduced into the environment and lead to diseases associated with drinking water or feeding on some food types. So that, improving meat quality and safety can take place if the consumers are educated. The environmental contamination issues should be the main goals for any efforts to strengthen food animal safety (Sofos, 2008).

Omer *et al.* (2018) listed the foodborne outbreaks associated with meat products between 1980 and 2015. The research was based on the confirmed identification of the causal agent of these outbreaks. The authors found that the main reason of most outbreaks is consuming undercooked meat products and it is related to bacterial contamination especially verotoxin-producing *E. coli* (VTEC) and *Salmonella*, while other pathogens such as *Bacillus cereus, Staphylococcus aureus, Clostridium perfringens, Clostridium botulinum*, and *L. monocytogenes* were less frequent in causing outbreaks. Most of the recent reports listed the fermented meat products as the main source of meat outbreaks (Moore, 2004; Omer *et al*., 2018) due mainly to either the reduction of salt concentration in the processed meat products or the type of meat product.

In the Arab countries, several outbreaks were reported after consuming contaminated meat products. For example, a foodborne outbreak was reported in 2006 (Al-Joudi, 2007) sickening 50 soldiers in Mina (Kingdom of Saudi Arabia - KSA). The soldiers complained about diarrhea and abdominal cramps after ingesting a meal containing rice mixed with meat. Although the pathogen was not identified, it was expected that *Bacillus cereus* was the causative agent. Other reported cases of foodborne disease outbreaks date back to the 2003 Hajj season when 400 Iranian pilgrims were hospitalized after consuming rice and chicken meal served in their hotel located in Al-Madinah (KSA) (Al-Maghderi and Al-Mazroa, 2003). Patients' stool cultures indicated that *Staphylococcus aureus* was the main pathogen associated with

this outbreak. In the Qassim region of KSA, 31 foodborne outbreaks with more than 250 cases were reported in the months of June and August of 2006 (Al-Goblan and Jahan, 2010). Of the total number of cases, 68.1% were adults, with *Salmonella* spp. and *Staphylococcus aureus* as the main causative agents. The main foods linked to these outbreaks were "Shawarma" sandwiches and chicken/mutton with rice (Al-Goblan and Jahan 2010).

The risk of various pathogenic bacteria in different retail food products sold in Qatar was studied by Peters *et al.* (2017). The researchers collected swabs from pre-cooked retail food products (especially meats) to screen for the prevalence of pathogenic bacteria. They reported a high prevalence (16.6%) of Shiga toxin-producing *E. coli* (STEC) in the tested food samples (n=287). The percentage of STEC serotype O45 was about 20%. The authors stressed the importance of cooking food properly to eliminate the risk of these pathogens, especially in meat products.

1.5.2 Dairy Products

Dairy products can have a variety of microorganisms and can serve as an important source for pathogens and foodborne diseases. The main source of pathogens in dairy products is coming from the environment, which is directly attached to the milk source in the farm such as an infected animal or contaminated soil (Oliver *et al.*, 2005).

The number of people consuming dairy products, especially the unpasteurized milk, increased because of the nutritional characteristics and health benefit of milk (Oliver *et al.*, 2009). On the other hand, the scientific data shows that a variety of milk-borne diseases can be associated with consuming raw milk leading to outbreaks. Unpasteurized dairy products can be easily contaminated with a wide range of microorganisms, such as *Campylobacter* spp., Shiga toxin–producing *E. coli*, *Listeria. monocytogenes,* and *Salmonella* (Oliver *et al.*, 2009).

Langer and his co-researchers (2012) compared the hospitalization rate after consuming pasteurized and unpasteurized dairy products. They found that the hospitalization rate of the outbreaks caused by unpasteurized dairy products was 13% compared with the pasteurized dairy products which were just 1% of the total dairy products consumed e.g. fluid milk and cheese. In another study, the outbreaks associated with unpasteurized milk for the period between 2007–2012 in the USA was investigated by Mungai *et al.* (2015). It was found that there were about 81 outbreaks associated with consuming raw milk. These outbreaks caused about 979 diseases and 73 hospitalizations with no death. Intensive analysis of the listed outbreaks provided the following data; *Campylobacter* spp. was the main causative agent, followed by Shiga toxin–producing *E. coli, Salmonella enterica* serotype *Typhimurium*, and *Coxiella burnetii* (Mungai *et al.*, 2015).

Dairy products are highly consumed by the people of the Middle East, especially in the Arabian Gulf region. It was reported that the raw camel milk samples sold in Saudi Arabia were commonly contaminated with *Staphylococcus aureus* (70%) and *Salmonella* (24%) (El-Ziney and Al-Turki, 2007). These results emphasize the link between the consumption of raw camel milk and possible foodborne disease and outbreaks. An important microorganism that is responsible for the milk-borne disease is *Brucella* spp. The region witnessed approximately 100 cases of human brucellosis per 100,000 persons/year. The highest incidence rates of brucellosis occur in Iraq, Saudi Arabia, and Jordan (Rubach *et al.*, 2013). People have been infected with *Brucella* due to the consumption of raw milk and unpasteurized dairy products, eating meat from infected animals and through direct contact with them (Rahil *et al.*, 2014). Guanche and his coworkers (2016) stated that brucellosis is the most common zoonotic disease in Qatar and stressed the fact that brucellosis comes from direct contact with

infected camels or from drinking unpasteurized camel milk. The authors documented the most recent 14 confirmed cases of brucellosis (caused by *Brucella abortus* and *Brucella melitensis*), which occurred in February 2015, all of which were from the same family living in a rural area in Al Shahaniya (Qatar).

In another study, Mohammed *et al.* (2015) studied the presence of various pathogens in dairy products collected from animal production units and processing plants in Qatar. The prevalence of non-O157:H7 Shiga-toxin producing *E. coli* and *E. coli* O157:H7 in raw milk was 58% and 6%, respectively. The authors concluded that there is an urgent need for innovative intervention strategies to mitigate the risk associated with the consumption of raw milk. The same authors (Mohammed *et al.*, 2014) conducted a health risk assessment study to establish the link between the risk of consuming raw camel milk contaminated with *Campylobacter* spp. (*C. jejuni* or *C. coli*) and health outcomes. It was determined that the chance of getting sick as a result of drinking raw camel milk contaminated with *Campylobacter jejuni* is relatively high (5–13 times), indicating the need to implement an efficient pasteurization process in order to protect public health.

1.5.3 Seafood

Seafood is also classified under a healthful diet and can carry some risk for the consumers. Seafood can become contaminated with different pathogens like bacteria, viruses, and parasites. There are specific factors associated with seafood contamination. Such factors can be the environment they come from, the type of feeding, the season of harvesting, the techniques used in packaging, preparing, and serving this type of food (Iwamoto *et al.*, 2010).

The consumption of raw fish or uncooked fish is a vital cause of the foodborne illness outbreaks associated with fish. The causes of diseases are mainly due to bacterial

pathogens like *Salmonella*, *E. coli*, *Staphylococcus* spp., *Shigella* spp., *Vibrio cholera*, *Clostridium botulinum*, viruses, and parasites (CDC, 2014 b). In the case of unsuitable treatment, storage, and processing, the fish will possibly be contaminated with these microbes. The safety of fish can be achieved by applying suitable transportation, handling, storage, and processing techniques. In addition, maintaining the quality of the water, and applying GMP and HACCP systems will prevent and control the contamination of seafood (Elbashir *et al.*, 2018).

The contaminated environments, such as waterbody or sediment, play a role in contaminating seafood by different microbes like *Vibrio* spp., *Aeromonas*, and enteric bacteria, which result in risky seafood. In addition, seafood can be contaminated during harvesting, handling, transportation, preparation, processing, and storage. (Lee and Rangdale, 2008). One of the microbial concern in seafood outbreaks is Norovirus, where 48% of Norovirus outbreaks occurred due to the consumption of raw of contaminated shellfish (Alfano-Sosbey *et al*., 2012; Woods *et al*., 2016).

Iwamoto and his coworkers (2010) investigated the foodborne outbreaks associated with seafood between 1973 and 2006. There were about 188 outbreaks resulting in 4020 sicknesses, 161 hospitalizations, and 11 deaths in the USA. Bacteria was the major source for these outbreaks (76%) followed by viruses (21.3%) and then parasites (2.6%). This research emphasized that bacteria and particularly *Vibrio* caused further outbreaks during the warm seasons, while the Norovirus was dominant in the cold seasons.

People in the Arabian Gulf region consume a high amount of seafood, especially fish since many of the countries are surrounded by the sea. The average consumption rate has increased in recent years, reaching 14.4 kg/year (Towers, 2014). People in the UAE and Oman are the top seafood consumers in Middle East with an estimated rate

of 28.6 kg/capita/year, while Qatar's consumption rate was 24.5 kg/capita/year (Towers, 2014). In addition, the average consumption of meat and meat products is higher in this region (66.1 kg/capita) compared to that of the entire world (42.8 kg/capita) (Pandey, 2017). In a review article, Jami *et al.* (2014) reported a high prevalence of *L. monocytogenes* in lightly preserved and ready-to-eat (RTE) seafood samples collected from several aquatic ecosystems including those in the ME region (e.g., fish and shellfish from Egypt, fresh fish, shrimp, and crustaceans from Iran, and anchovies and mussels from Turkey). In addition to biological contaminants, various seafood products easily become contaminated with chemicals, such as heavy metals, polychlorinated biphenyls, pesticides, etc.

In Kuwait, Husain *et al*, (2017) measured the concentration of Arsenic (As) in 578 fish/seafood samples. They did a risk assessment for (As) exposure for Kuwaiti population. Their results revealed that the intake of As was 0.058 µg/kg/day, which is not exceeding the incremental lifetime cancer risk (ILCR) at 1×10^{-4}, but the Kuwaiti children (up to 12 years) were under risk since the mean level of intake exceeded this limit by 0.10 µg/kg/day.

In addition, the level of heavy metals (e.g. copper, zinc, lead, and mercury) and fecal contamination in fish caught from the Qatari coast were investigated by Al-Jedah and Robinson (2001). The results indicated that the concentrations of heavy metals in the tested fish did not reach toxic levels and were considered to be within the acceptable range (i.e., 0.24 mg/kg was the maximum detectable level of mercury, while the lead level was well below the detection level in most of the samples). The number (CFU/g) of fecal pathogens counted in uncooked fish ranged between 1.0×10^3 and 8.0×10^5, meaning that the locally caught fish were safe to eat after cooking (Al-Jedah and Robinson, 2001). Al-Qaradawi *et al*. (2015) studied the radioactivity levels (^{137}Cs, ^{40}K,

^{226}Ra, ^{228}Ra, and ^{238}U) in the marine environment in Qatar. They tested several samples from deep sediments, algae, fish, starfish, sponge, oyster, crabs, shrimp, seashell, dugong, and mangrove. The results showed that the levels obtained in this study were in agreement with other studies conducted in different Gulf region countries and worldwide, concluding that there is no significant negative impact on human health after consuming seafood in Qatar.

Al-Ansari and his coworkers (2017) recorded the concentration of total mercury (THg) in different parts of demersal fish caught from different coastal locations in Qatar. The concentration of Hg ranged between 0.016 to 0.855 ppm (mg/kg w/w) in various fish samples. When a risk assessment was conducted, it was determined that there is no health concern associated with the consumption of demersal fish caught in this region.

1.5.4 Fresh Produce

Fresh produce is one of the main components in a healthy diet and as mentioned before consuming fresh produce can protect human health from many diseases, like cardiovascular diseases and cancer. According to the Global Burden of Disease study (GBD, 2017), diets low in vegetables were accountable for about 1.8 million DALY, while diets low in fruit accounted for approximately 3.4 million DALYs. In addition, diet rich in fruits and vegetables could help in protecting people from chronic diseases, type 2 diabetes, cancer, and obesity; besides providing our bodies with vitamins, fibers, minerals, and bioactive compounds, which lower the risk of having certain cancers and cardiovascular diseases (World Cancer Research Fund, 2008; Blanck *et al.*, 2008; CDC, 2018 a). In addition, consuming fresh produce, which is characterized as a low energy mass, can have a good benefit in weight management (Rolls *et al.*, 2004).

Internationally, fresh produce consumption increased by about 4.5% per year

between 1990 and 2004 (Olaimat and Holley, 2012). The FAO/WHO published a report in 2004 entitled "Fruit and Vegetables for Health." They recommended eating about 400 g of fresh produce daily (eliminating potatoes) to protect human health from chronic diseases, like heart disease, cancer, diabetes, and obesity. In addition, fresh produce can prevent and mitigate numerous micronutrient deficiencies, especially in poor countries.

In the USA, the rate of fresh produce import doubled to reach $12.7 billion in the period between 1994-2004 (Aruscavage *et al.*, 2006) and by 2005, the cut produce touched 6 million packages per day (Jongen, 2005). The high demand for fresh produce in the last 20 years encouraged the organizations to have most of the fresh produce available for consumers the year around (Warriner *et al.*, 2009). Canada is following the same trend, from 1963 to 2010, the yearly consumption of fresh produce increased by 56% and 26%, respectively (Olaimat and Holley, 2012). However, just 12.2% and 9.3% of American adults meet the required daily intake of fruits and vegetables as recommended by the WHO, respectively. Most of the children do not even meet national recommendations for fruit and vegetable portions (Lee-Kwan *et al.* 2017; Moore *et al.*, 2017)

The mainstream of European people, following a healthy diet full of fruit and vegetable, believe that this type of lifestyle protects them against different types of diseases. According to the European Food Information Council (EUFIC, 2012), the reported amount of fruit and vegetables consumed per country in Europe reached more than 400 g per person per day as recommended by WHO. Based on the most recent WHO (2016) report from 2013-2014, about 50% of European children do not eat fruit and vegetables every day.

The majority of Arab countries consume less amount of fruits and vegetables

than the recommended level (about 400 g/day) (Hwalla *et al.*, 2015), and the lowest intake of fruits and vegetables was reported in Libya (about 60 g/day) (Hwalla *et al.*, 2015). This was also confirmed previously by Kamleh *et al.* (2012) who reported that the amount of fresh produce consumed in the Arab countries was below the levels of consumption in Europe, except in Lebanon, Tunisia, and Egypt. The intake of fruits and vegetables is significantly low among children about 4 years old (<200 g/day) and it is normally 330 g/day for children from 5-14 years old (Musaiger, 2011).

Several types of fresh produce act as vehicles for foodborne pathogens like *Salmonella*, *L. monocytigenes, E. coli* O157:H7, and Norovirus (Wadamori *et al.*, 2017; Alegbeleye *et al.*, 2018; Murray *et al.*, 2017; CDC 2016a, 2017, and 2018b). Table 2 lists some of the recent fresh produce outbreaks around the world.

The major source of foodborne pathogens is fecal contamination of water used in crop irrigation, or water used to treat and handle fresh produce (Shuval *et al.*, 1997). Most of the fresh produce, such as fruits and green vegetables, are consumed without cooking or slight cooking, such as soups (Pierangeli *et al.*, 2014). This makes the customers highly vulnerable to pathogenic infection if the fresh produce they consume is contaminated. In many countries, the fresh produce industry applies several risk management practices mainly designed to reduce the probability of contamination.

More than 400 fresh produce outbreaks were recorded since 1990, while leafy greens, cantaloupes, tomatoes, and soft fruits were the most susceptible produce for infection (Murray *et al*., 2017). Sharma *et al*. (2017) reported that all the fresh produce have the potential for contamination and causing foodborne diseases. This appeared from the diversity of microbes associated with outbreaks. For example, before 2012 cucumbers were rarely reported to be associated with Salmonella outbreaks and the same issue happened with papaya fruits (Sharma *et al*., 2017). In contrast, tomato contaminated with Salmonella was used to be rare before 2011 (Sreedharan *et al*., 2014).

Table 2. The most common foodborne outbreaks related to fresh produce in the last twenty years (data extracted from Wadamori *et al.*, 2017; Alegbeleye *et al.*, 2018; Murray *et al.*, 2017; CDC 2016a, 2017, and 2018b).

Food type	Country	Pathogen	No. of cases	No. of hospitali-zations	Deaths	Year
Strawberries (frozen)	Australia	Hepatitis A	19	0	0	2015
Rockmelon	Australia	*S. Hvittingfoss*	97	ND	ND	2016
Sprouts (Alfalfa)	Australia	*Salmonella*	100	ND	ND	2016
Lettuce	Australia	*S. Anatum*	144	ND	ND	2016
Spinach	Canada	*Shigella sonnei*	31	ND	ND	2001
Cilantro	Canada	*C. cayetanensis*	11	ND	ND	2003
Cucumbers	Canada	*S. Brandenberg*	12	ND	ND	2004
Basil	Canada	*C. cayetanensis*	200	ND	ND	2005
Baby carrots	Canada	*Shigella sonnei*	4	ND	ND	2007
Romaine lettuce	Canada	*E. coli* O157:H7	29	ND	ND	2008
Spanish onion	Canada	*E. coli* O157:H7	235	ND	ND	2008
Onion sprouts	Canada	*S. Cubana*	20	ND	ND	2009
Lettuce	Canada	*E. coli*	34	ND	ND	2017
Frozen raspberries	Denmark	Norovirus	561	0	0	2005
Lettuce	Denmark	Norovirus	260	0	0	2010
Frozen raspberries	Finland	*Calicivirus*	509	0	0	2009
Strawberries	Germany	Norovirus	~11	ND	ND	2012
Raspberries (from China)	Sweden	Norovirus	433	0	0	2001
Fresh vegetables	New Zealand	*Yersinia pseudotuberculosis*	334	65	ND	2014
Snow peas	Sweden	*Shigella*	35	0	0	2010
Basil	UK	*Salmonella*	32	ND	ND	2007
Salads	UK	*S. Singapore*	4	ND	ND	2014
Lettuce, cucumber	UK	*E. coli* 096	50	ND	ND	2014
Imported salad	UK	*E. coli* O157:H7	161	60	2	2016
Green onion	USA	Hepatitis A	43	ND	ND	1998

ND = Not determined

Table 2 Cont. The most common foodborne outbreaks related to fresh produce in the last 20 years (data extracted from Wadamori *et al*., 2017; Alegbeleye *et al*., 2018; Murray *et al*., 2017; CDC 2016a, 2017, and 2018b).

Food type	Country	Pathogen	No. of cases	No. of hospitalization	Deaths	Year
Tomatoes/peppers	USA	*Salmonella*	1442	ND	ND	2010
Mangoes	USA	*Salmonella*	127	ND	ND	2012
Pomegranates	USA	Hepatitis A	165	69	ND	2013
Mixed salad	USA	*Cyclospora cayetanensis*	631	50	0	2013
Imported Cucumbers	USA	*Salmonella enteritidis*	84	17	6	2013
Coriander	USA	*Cyclospora cayetanensis*	304	7	0	2014
Caramel apples	USA	*L. monocytogenes*	35	31	7	2014
Cucumbers	USA	*Salmonella* spp.	991	221	6	2015
Packaged salads	USA	*L. monocytogenes*	19	19	1	2016
Lettuce	USA	*E. coli*	84	45	0	2016
Frozen	USA	Hepatitis A	134	ND	ND	2016
Spinach and spring	USA	*E. coli*	33	13	0	2017
Pine nuts	USA	*Salmonella* spp.	43	2	0	2017
Sprouts	USA	*E. coli*	59	17	0	2017
Tomatoes	USA	*Salmonella* spp.	111	22	0	2017
Fresh spinach	USA	*E. coli*	199	102	3	2017
Cantaloupe	USA	*Salmonella* spp.	332	113	3	2017
Sprouts	USA	*Salmonella* spp.	506	65	0	2017
Raw Jalepenos	USA	*Salmonella* spp.	1442	286	2	2017
Del-Monte Fresh	USA	*Cyclospora* sp.	250	8	0	2017
Romaine Lettuce	USA	*E. coli* O157:H7	210	96	5	2018
Raw Sprouts	USA	*Salmonella* spp.	10	0	0	2018
Shredded Coconut	USA	*Salmonella* spp.	27	0	0	2018

ND = Not determined

On the other hand, there were specific produce commonly associated with foodborne outbreaks, such as lettuce, spinach, parsley, basil, green onions, sprouted seeds, melons, cantaloupe, and berries (Olaimat and Holley, 2012; Dennis *et al*., 2016; Alegbeleye *et al*., 2018). The CDC (2014b) reported vegetables as one of the most common sources for 16 multistate outbreaks out of 68 outbreaks occurred between 2006 and 2014.

There are different examples of outbreaks on *Salmonella enterica* associated with ready-to-eat vegetable salads, e.g. outbreaks occurred in the UK and Scandinavia because of the consumption of contaminated rocket leaves (Nygard *et al.,* 2008). In addition, different outbreaks, related to imported basil, affected the UK, the USA, Denmark, and the Netherlands (Pezzoli *et al.*, 2008; Pakalniskiene *et al.*, 2009). Many outbreaks caused by consuming contaminated seeds are frequently spread over different geographic regions (Erickson and Doyle, 2007; Emberland *et al.*, 2007; Werner *et al.*, 2007). In the US, foodborne diseases associated with fresh produce were more than those associated with meat, seafood, poultry, and eggs. By looking at the average number of patients per outbreak in the period between 1998 and 2007, 39.1% of cases were caused by consuming fresh produce (CSPI, 2009). DeWaal and Bhuiya (2009) reported that 47.8 % of outbreaks were associated with produce between 1990 and 2005. In an outbreak took place in Canada and the USA in 2006, spinach was the major source carrying *E. coli* O157:H7, which led to 199 cases with three deaths (Wendel *et al.*, 2009). In a 2010 outbreak occurred in the USA, *E. coli* O145 was found to be the pathogen contaminating the shredded romaine lettuce and resulting 26 confirmed cases (CDC, 2010). In 2011, a large outbreak occurred in Germany, which was caused by *E. coli* O104:H4 contaminating the fenugreek seed sprouts. This outbreak resulted in 47 deaths out of 3911 cases and 777 cases developed hemolytic uremic syndrome (HUS)

(Olaimat and Holley, 2012). In the same year, another outbreak was linked to cantaloupe contaminated with *L. monocytogenes* causing 146 sicknesses with 33 deaths in the USA (CDC, 2017). In addition, a multistate outbreak of *L. monocytogenes* contaminating ready to eat salad took place in the USA in 2016, resulting in 19 hospitalizations and one death (CDC, 2016 a). In another outbreak in 2015, cucumber was found to be the source for *Salmonella* Poona contamination, leading to more than 900 cases with 204 hospitalizations and 6 deaths (USFDA, 2016).

Consuming fresh juice especially apple cider was the cause of several outbreaks because of *Salmonella* and *E. coli* O157 contaminations (Vojdani *et al.*, 2008; Jain *et al.*, 2009). Outbreaks associated with *E. coli* O157 are normally related to eating green-leafy vegetables (Wendel *et al.*, 2009). In 1996, one of the largest outbreaks took place in Sakai City, Osaka, Japan, after consuming white radish sprouts contaminated with *E. coli* O157 (Michino *et al.*, 1999). Table 2 summarizes some significant fresh produce-related outbreaks in the last 20 years.

Other pathogens can play a role in fresh produce contamination and led to significant outbreaks, such as the New Zealand outbreak occurred in 2014 caused by *Yersinia pseudotuberculosis*, and the USA outbreaks in 2013 and 2014 caused by *Cyclospora cayetanensis*. Among all, the two major pathogenic bacteria, associated with fresh produce having high public health concern, are *Salmonella* and *Listeria monocytogenes* (Wadamori *et al*., 2017).

The number of outbreaks associated with fresh produce expanded lately due to the increase in the consumption of fresh produce (Warriner *et al.*, 2009). The higher number of fresh produce associated outbreaks are the result of the changes in individual consumption, presence of livestock markets in close proximity to the produce market, accessibility of the produce around the world e.g. some products come from nations

with dubious hygienic practices, and the high statistics of people having an impaired immune system (Beuchat, 2002). Different varieties of fresh produce now come from countries where the hygiene conditions do not meet the required standards (e.g. India, Jordan, Lebanon, Turkey,…etc.). Although fresh produce provides a good source of nutrients, they are also considered as an excellent medium for microbial growth, especially the pathogenic microorganism.

Many microorganisms are often connected with diseases and identified from the patient after the consumption of the fresh produce. These microbes can be viruses such as Hepatitis A and Norovirus; protozoans like, *Cyclospora cayetanensis* and *Cryptosporidium parvum*; or bacteria such as: *Bacillus cereus, Clostridium* spp., *Aeromonas hydrophila, E. coli* O157:H7, *L. monocytogenes, Vibrio cholera, Shigella* spp., *Campylobacter* spp, *Salmonella* spp., and *Yersinia enterocolitica* (Olaimat and Holly, 2012). Table 3 summarizes the most commonly involved etiological agents in fresh produce borne illnesses. *E. coli* O157:H7 and *Salmonella* spp. are the major cause of foodborne diseases associated with fresh produce, which led to large outbreaks (Warriner *et al.*, 2009; USFDA, 1998; Buck *et al.*, 2003). Just *E. coli* O157:H7 and *Salmonella* are responsible for about 7% and 71% of produce outbreaks, respectively (CSPI, 2009).

As discussed in the above mentioned paragraphs, hundreds of research papers have been published in the last decade documenting the foodborne outbreaks associated with fresh produce all over the world. However, studies on fresh produce, either on microbiological risk assessment, or on outbreaks are very limited in developing and under developing countries.

Table 3. The most commonly implicated etiological agents in fresh produce-borne illnesses (Adopted from Alegbeleye *et al.*, 2018).

Bacteria	*Salmonella spp., Escherichia coli, Campylobacter* spp., *Vibrio* spp., *Listeria monocytogenes, Bacillus cereus, Shigella* spp., *Clostridium* spp., *Yersinia* spp., *Pseudomonas* spp., *Aeromonas* sp., *Staphylococcus* spp.
Viruses	Hepatitis A virus, Rotavirus, Norovirus, Norwalk and Norwalk-like, Sapovirus, Calicivirus
Parasites	*Cyclospora, Cryptosporidium parvum, Giardia* sp., *Trichinella* spp., *Ascaris* spp., *Trichuris trichiuria, Toxoplasma gondii*
Fungi	*Alternaria* spp., *Fusarium* spp., *Aspergillus niger*

1.6 Contributing Factors for Fresh Produce Contamination

1.6.1 Risk Factors Present at the Pre-harvest Stage

The environment surrounding the fresh produce could be a source of contamination. The contamination of fruits and vegetables can take place before or after harvesting (Beuchat, 2002). Before harvesting, contamination might occur due to the use of contaminated irrigation water, animal feces, soil, application of fungicides and insecticides, ineffectively composted manure, insects, dust, animals (wild or domestic), and insufficient means of human handling (Beuchat, 2002; Alegbeleye *et al.* 2018). After harvesting, human handling can play an important role in addition to insects, dust, rinse water beside using contaminated harvesting and/or processing equipment and containers used to transport fresh produce (Beuchat, 2002; Alegbeleye *et al.* 2018; de Freitas *et al.*, 2019).

At the agriculture farms, the source of the irrigation water is an important issue. If the water comes from an unsecured source, it can be easily contaminated (Faour-Klingbeil *et al*., 2016). Water used in irrigation and transport via canals can interact with soil sediments and algae, while water transported by pipes could be interacting with the biofilms in the inner surfaces of the pipes (Pachepsky *et al*., 2014). In addition, irrigation water stored in tanks or pools have a high probability to be contaminated with animals or birds' droppings (Higgins *et al*., 2009). Furthermore, the studies proved the fact that using water spray systems instead of a drip irrigation system can lead to fresh produce contamination (Mitra *et al.*, 2009; Pachepsky *et al*., 2014).

There are other environmental factors, which play a major role in spreading foodborne pathogens, such as pH, temperature, and water activity (a_w). Lanciotti and his coworkers (2001) studied the effect of these factors on the growth of different pathogens like *Salmonella enteritidis*, *Staphylococcus aureus,* and *Bacillus cereus* in model system, and reported that low temperature, as an example, can limit the microbial growth especially for the mesophilic bacteria, e.g. *Staphylococcus aureus.* They concluded to use these factors to limit the growth of foodborne pathogens as a preventive method.

Soil serves as a media for different contaminants such as plant debris, roots, and seeds. This contaminates could have some pathogenic microorganisms. Pathogens can infect the soil and contaminate the environment after mixing the soil with imperfectly composted manure or sewage, polluted irrigation water, or municipal solid wastes (Santamaria and Toranzos, 2003, Sant'Ana *et al.,* 2014). The soil characteristics (pH, salinity, water content, nutrients) are also important factors for the survival of pathogens in the soil (Alegbeleye *et al*., 2018). To sustain the fresh produce safety, it is important to understand the entry mechanisms of the pathogens, their endpoint, their

transport mechanisms, and their potential to grow and survive in soil, water, or manure. Therefore, intensive studies conducted to explain the behavior of the pathogen in the host, their association, and interaction (Alegbeleye *et al*., 2018).

Other factors which might affect the presence of pathogens and infect the fresh produce is the animals, birds, and insects in the farm, which may carry several pathogens and act as a vector for these pathogens, such as *E. coli*, *Salmonella* and *Campylobacter* (Wani *et al*., 2004). Transferring pathogens through insects is one type of produce contamination as proven in studies led by Sela *et al.* (2005) and Talley *et al.* (2009). Different insects belonged to the *Muscidae* and *Calliphoridae* families are involved in attaching to cattle and carrying *E. coli* O157:H7 (Talley *et al.*, 2009). Furthermore, the use of some fallen fruits in juice making has been also proven to enhance the contamination of the final product (Vojdani *et al.*, 2008).

Contact surfaces also play a role in the fresh produce environment. The surfaces, which are not in direct contact with the fresh produce like drains, floors, and ceilings, normally contaminate the produce indirectly. Nevertheless, these can be a pool for the pathogens, which can be aerosolized during cleaning (Tebbutt, 2007).

Contamination of produce by airborne microbes is rarely taking place but it has been considered as a factor (Drudy *et al.*, 2006). During the outbreaks, if the techniques used in cleaning the fresh produce are highly effective, then air sampling could be considered as a possible contamination factor (Gudbjornsdottir *et al.*, 2004).

1.6.2 Risk Factors Present at the Post-harvest Stage

1.6.2.1. Roles of Food handlers

One of the main causes of food contamination is the improper implementation of hygiene practices during food handling. The unsanitary conditions of workers at the food preparation stage is a major risk factor that contributes to 98% of the foodborne

outbreaks linked to restaurants (Shinbaum *et al.*, 2016). In fact, coliforms presented in contaminated produce are normally used as indicators for the presence of either animal or human feces (Johnston *et al.*, 2005). In addition, infected workers by viruses and *Shigella* are considered as main sources of foodborne diseases (Berger *et al.*, 2010; Warriner *et al.*, 2009). Berger *et al.* (2010) published a comprehensive review explaining the role of fresh produce in transmitting human pathogens and how improper food handling plays an important role in food contamination. Shaw *et al.* (2015) reported the importance of monitoring the handlers' personal hygiene and health to reduce the risk of fruit contamination such as strawberries. Globally, there are general guidelines to reduce the risk of cross-contamination, such as training food handlers on hand washing practices and the proper use of restroom facilities, prohibiting eating and smoking in the handling area, and asking for appropriate clothing during the work hours (Kroupitski *et al.*, 2009; Mann, 2011; WHO, 2009; Jacxsens *et al.*, 2010; USFDA 2013). The literature is replete with studies conducted to evaluate food safety knowledge and practices among food handlers in different parts of the world. However, these types of studies are limited in the Middle East region especially in Qatar.

In a recent study, Eltai *et al.* (2018) examined the prevalence of antibiotic resistant *E. coli* isolated from stool samples of food handlers working in Qatar. Authors reported that 60% of *E. coli* (n = 78 positive *E. coli* isolates from 456 stool samples tested) were resistant to one antibiotic; whereas, 27% were multi-drug resistant (MDR) organisms. It was concluded that the antibiotic resistant *E. coli* were quite common in the food handlers' stool samples, which may negatively impact the public health in Qatar. In Al Madinah region of KSA, the prevalence of intestinal parasites was examined in 2,732 stool samples collected from farmers, food handlers, housemaids, and drivers (Taha *et al.*, 2013). Out of the 2,732 samples, 14.9% were positive for

intestinal parasites, mostly infecting (18.5%) 20–29-year-old workers. Among all nationalities, Pakistani workers had the highest prevalence (23.2%) of intestinal parasites, followed by Filipinos (22.2%) and then Sudanese (18.7%) workers. The most common parasite identified in stool samples was *Entamoeba histolytica* (66.6%). It is important to emphasize that the spread of these parasites among the workers in Al-Madinah could lead to a serious health problem affecting not only the residents but also more importantly, the visitors during the pilgrimage (Hajj) season. Most of these studies recommended addressing the issue of hygiene practices of food handlers urgently by the responsible agencies before serious health issues arise. Faour-Klingbeil and coworkers (2016) conducted a study to understand and identify the microbial hazards and the critical areas in the food chain of vegetables cultivated in Bekaa Valley and sold in the central vegetable market in Lebanon. The researchers examined the presence of specific pathogens during pre- and post-harvest stages in lettuce, parsley, and radish. The results revealed a high prevalence of *E. coli*, total coliforms, *Staphylococcus aureus*. The authors emphasized the need to enhance control measures and mitigate the risks through applying Good Agricultural Practices (GAPs), Good Hygiene Practices (GHPs) at each step of the food chain.

Food handlers who are trained in GHP represent the first defense against contamination of food across the supply chain. According to the U.S. Food and Drug Administration (USFDA, 2017), the spread of pathogenic microorganisms can be reduced by applying some basic hygiene practices such as appropriate hand washing practices. The hand palm of the food handlers can carry several types of microorganisms, such as *E. coli* O157:H7, *Shigella* spp., *Salmonella* Typhi, non-typhoidal *Salmonella*, Norovirus, and Hepatitis A virus, all of which come from human fecal residue and the environment. In addition, touching raw food materials can lead to

the colonization of hands with bacteria, such as *Salmonella* spp. and *E. coli* O157:H7 (USFDA, 2017). These pathogens can be easily transferred from food handlers' palms to food products during handling or preparation.

The produce handlers' personal hygiene practices are usually tested by detecting the presence of indicator microorganisms, such as *Staphylococcus* spp., coliforms, etc. These types of personal indicators used to determine the contamination factor directly linked to the food handlers during food preparation (Jacxsens *et al*., 2009; Balzaretti & Marzano, 2013).

1.6.2.2. Viral hazards

Globally, the most frequent virus related outbreaks associated with fresh produce consumption are Norovirus (48.7%), Hepatitis A (46.1%) and other viruses (5.2%) (Chatziprodromidou *et al*., 2018). The viruses can survive in a wide range of fresh produce for long period and sometimes exceeding the shelf life of the packed produce. This depends on the moisture content, temperature, and pH (USFDA, 2013). These viruses can be transmitted to the produce at the pre-harvest stage via polluted irrigation water or by using contaminated manure (Wei *et al*., 2010). In the post-harvesting stage, the contamination of produce by Norovirus or Hepatitis A, normally comes from the workers who are already infected with these pathogens and working in picking the produce, in the market, or in place near to the market, but the mode of transmission is different (Baert *et al*. 2009). In addition, using contaminated water in the processing and preparation stage may lead to outbreaks (Baert *et al*. 2009).

The techniques used in the identification of these pathogens were improved and helped in disease diagnosis; this improvement listed the Norovirus as a main agent for many outbreaks (Chatziprodromidou *et al.,* 2018). Several outbreaks of Norovirus (gastroenteritis) related to infected raspberries have been reported, for exclusively in

Europe (Cotterelle *et al.*, 2005; Hjertqvist *et al.*, 2006). Likewise, different outbreaks caused by viral infection of Hepatitis A have been linked to raspberries, strawberries, green onions, and lettuce (USFDA, 2013).

According to CDC (2018c), about 58% of the foodborne diseases in the USA are related to Norovirus, which cost about $2 billion due to the loss of productivity and health care expenses. These outbreaks lead to 2 million cases, 71 thousands hospitalizations of children under 5 years or elderly people, and 800 deaths yearly. Table 2 shows some of the foodborne outbreaks related to viral infection (Norovirus and Hepatitis A) associated with fresh produce consumption.

1.6.2.3. Parasitic hazards

The global estimate of loss regarding foodborne parasitic diseases is about 12 million DALYs (Torgerson *et al*., 2015). The health authorities started giving an attention to foodborne parasites because; a) investigating parasites is little bit difficult since most of the parasites have complex lifecycle, b) the incubation period for parasite is long thus make discovering of the source difficult (FAO/WHO, 2014), c) the laboratory standards used to identify parasite are poor or not found (Robertson, 2014), and d) physicians rarely recognize the parasite etiology as a foodborne disease (Tefera *et al.*, 2018).

FAO/WHO report (2014) listed the potential parasites which infected the fresh produce in decreasing order: *Taenia solium*, *Echinococ cusgranulosus*, *Echinococcus multilocularis*, *Toxoplasma gondii*, *Cryptosporidium* spp., *Entamoeba histolytica*, *Trichinella spiralis*, *Opisthorchiidae*, *Ascaris* spp., *Trypanosoma cruzi*, *Giardia duodenalis*, *Fasciola* spp., *Cyclospora cayetanensis*, *Paragonimus* spp., and *Trichuris trichiura*. Whereas, other studies listed different parasites as the most dominant infectins in fresh produce (Bouwknegt *et al*., 2018; Tefera *et al*., 2018). The major

problem with parasites infecting fresh produce is that many parasites can survive longer period under humid and cold condition (produce storage temperature) compared to the survival time in the environment (Utaaker *et al*., 2017).

Several outbreaks associated with consuming berries contaminated by different parasites were reported recently by Tefera *et al*. (2018). The USFDA (2013) reported the highest contamination of fresh produce with *Cryptosporidium oocysts,* which normally occurs in the rainy seasons and humid atmosphere. Although there is a rare documentation of the parasitic outbreaks in the fresh produce and leafy green vegetables, there are some documented outbreaks linked to berries contaminated with intestinal protozoan, *Cyclospora cayetanensis* (Palumbo *et al*., 2013) and *Trypanosoma cruzi* transmitted via eating berries or drinking berries juice (de Noya *et al*., 2015).

Transfer of parasites from food handlers into food is one of the most commonly identified sources in an outbreak. Abu-Madi *et al.* (2008) evaluated the prevalence of intestinal parasites in newly arrived food handlers in Qatar. Several species of parasites were isolated from stool samples of male and female food workers, such as the protozoans *Blastocystis hominis*, *Entamoeba histolytica/dispar*, *Giardia lamblia,* non-pathogenic *Entamoeba,* and the nematodes *Ascaris lumbricoides*, hookworms, and *T. trichiura*. In a follow-up study by the same authors (Abu-Madi *et al.*, 2011), it was observed that after a period of residency in Qatar (ranging between 1 and 4 years), the workers still had the intestinal parasitic infection, but at a lower percentage compared to their initial arrival time. This percentage varied by sex, a region of origin, a period of residency, and place of work; especially among females working as housemaids, who lived in better accommodations with the host families. The authors also reported that Nepalese workers still had a high prevalence of infection by intestinal helminths, mainly *Giardia duodenalis* and hookworms, even after staying in Qatar for a long

period of time (up to 4 years), compared to other nationalities coming from West and East Asia, or North and Northeast Africa. The authors concluded that this issue needs urgent attention from the health authorities to protect consumers.

1.6.2.4. Fungal hazards

Fungi have the ability to produce mycotoxins at the end of the exponential growth phase as secondary metabolic products, which can be harmful to human if they consume food contaminated with fungi (Jay, 2012). The symptoms of mycotoxin contamination can range from diarrhea, abdominal pain, gastrointestinal complication, or it may be developed to severe cases causing cancer (Adams and Moss, 2000). The human susceptibility to mycotoxins can be affected by exposure time and type, sex, age, and health status (Forsythe, 2010).

Fresh produce is infrequently contaminated with foodborne fungi; however, there is an absence of effectiveness of disinfectants for eliminating these pathogens (Beuchat, 1998). This can be partially recognized to complications in providing sterilizers applied on the surface of produce in which fungal growth may be stopped (Burnett and Beuchat, 2001). Treatment with chemical solutions can occasionally leave residual moisture on fruits and vegetables, which can support the growth of molds. Different gases like ClO_2 can be used to prevent the contamination and stop the spreading of molds on fruits. There are different types of molds and yeasts that can be classified as plant pathogens and they can easily grow on the plant surface and fruits, such as *Eurotium, Penicillium, Candida,* and *Saccharomyces cerevisiae* (Han *et al.*, 1999).

Fungi can contaminate the fresh produce at the pre-harvest stage via air, soil, or seeds (Forsythe, 2010). In fresh produce, the low pH of some fruits can reduce bacterial contamination and enhance the fungal growth, while the opposite occurs in vegetables

with relatively high pH (Moss, 2008). Yeni and his coworkers (2015) published a full review of the most common mycotoxins in fresh produce, such as aflatoxin, citrinin, ochratoxin A, and patulin and the outbreaks connected with these contaminations.

Several studies were conducted to measure the presence of yeast and mold on fresh produce surfaces. For example, Mritunjay and Kumar (2017) measured the microbial load of bacteria and fungi on the surface of some fruits and vegetables consumed raw. They found that the range of yeasts and molds was between 0.3 and 5.5 Log_{10} CFU/g produce, but they did not identify the yeasts and molds. In addition, they recommended applying some washing methods to reduce the amount of microbial load, since there is an adverse effect associated with produce contaminated with molds, which linked to mycotoxin production. On the other hand, Badosa *et al.* (2008)'s study revealed a higher range on molds on fresh produce, which reached up to 8 Log_{10} CFU/g. Tournas (2005) not only measured the fungal load on fresh produce, but also identified the most dominant fungi on ready-to-eat salads and sprouts to determine the potential toxigenicity of these fungi. The most dominant strains were *Alternaria*, *Cladosporium*, *Penicillium*, and *Phoma*, while *Fusarium*, *Rhizopus*, *Mucor*, and *Geotrichum* were less often presented in the samples tested.

1.6.2.5. Bacterial hazards

The predominant soil microorganisms which may cause foodbornr outbreaks are *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, and *Listeria monocytogenes* (Whipps *et al.*, 2008). In addition, *E. coli* O157:H7 and *Salmonella* may live in soil from 7 to 25 weeks, depending on the physical conditions of the soil e.g. temperature, moisture, pH, and soil type (Erickson *et al.*, 2010). It was reported that *E. coli* O157, *Listeria monocytogenes*, *Salmonella*, and *Campylobacter jejuni* could survive in soil from 45 to 100 days (Nicholson *et al.*, 2005). Holley *et al.* (2006)

indicated that *Salmonella* and other zoonotic pathogens could live longer in moist clay-based soils mixed with manure at low temperatures. It is confirmed in many studies that the presence of manure in any soil can enhance the growth of enteric bacteria, which have the ability to live in the soil for several years (Doyle and Erickson, 2008). Himathongkham and his coworkers (1999) reported that feces contain between 10^2 and 10^5 CFU/g of *E. coli* and between 10^2 and 10^7 CFU/g of *Salmonella* spp., while manure contains between 10^2 and 10^7 CFU/g of *Salmonella* spp. (Pell, 1997). In addition, both sewage and ruminants' manure is considered as the main sources of *Salmonella*, *E. coli* O157:H7, and *Campylobacter jejuni* being the normal microflora of the gastrointestinal tract of poultry, pigs, and cattle (Warriner *et al.*, 2009).

The condition of the soil and the situations of the location are considered as major factors that impact the microbial safety of fresh produce. For example, the temperature, water content, soil type, and the surrounding environment can increase the availability of pathogens (Erickson *et al.*, 2010). The bacterial species, like *Shigella*, caused 43% of the outbreaks and 35% of the illnesses, while *Salmonella* caused 27% outbreaks and 37% cases, all of which were associated with fresh produce (Scharff, 2010). Sivapalasingam (2004) published a review explaining how bacteria especially *Salmonella* was the major cause of most of the foodborne diseases associated with fresh produce leading to about 50% of the outbreaks in the USA between 1973 and 1997. In the USA, CDC (2017) reported a multistate outbreak of *Salmonella* Urbana linked to papayas consumption imported from Mexico, which led to 7 cases and 4 hospitalizations. Moreover, *Salmonella* Montevideo multistate outbreak reported in February 2018 linked to raw sprouts consumption and led to 10 cases. In July 2018, the consumption of pre-cut melon contaminated with *Salmonella* Adelaide resulted in 77 cases and 36 hospitalizations (CDC, 2018b).

E. coli O157:H7 was responsible for about 21% of the outbreaks between 1982-2002 (Rangel *et al.*, 2005). In the USA, the CDC (2018) recently reported several multistate outbreaks related to a) *E. coli* O121 infection linked to raw clover sprouts consumption and led to 19 cases, b) *E. coli* O157 linked to alfalfa sprouts consumption with 11 cases, and c) *E. coli* O157:H7 associated with leafy greens consumption resulting in 25 cases (Table 5). However, the newly reported outbreak (January 2019) related to infected romaine lettuce with Shiga toxin-producing *E. coli* O157:H7 cuased 62 illness-cases and 25 hospitalizations (CDC, 2017).

Another example of the foodborne pathogen is *Listeria*, which is found normally in the soil, water, and animal feces. One of the listed multistate *Listeria* outbreaks reported in the USA was in Dec 2014 (Table 4). This outbreak of *Listeria monocytogenes* was linked to the consumption of prepackaged caramel apples, which led to 32 cases, 31 hospitalizations, and 6 deaths (Garner and Kathariou, 2016). Moreover, CDC (2016 b) listed several outbreaks related to *Listeria*, such as multistate outbreak linked to frozen vegetables with reported 9 cases, 9 hospitalizations, and 3 deaths; another one was associated with packaged salads causing 9 cases, 9 hospitalizations and one death. *Listeria* remains to be a major problem in the fresh produce industry due to its ability to grow at the refrigeration temperature (Fresh Plaza, 2018).

Table 4. Examples of fresh produce and juice contaminated with bacterial pathogens (data extracted from Buck *et al.*, 2003; Murray *et al*., 2017; Alegbeleye *et al*., 2018).

Pathogen	Product
Aeromonas	alfalfa sprouts, asparagus, broccoli, cauliflower, celery, lettuce, pepper, spinach
Bacillus cereus	alfalfa sprouts, cress sprouts, cucumbers, mustard sprouts, soybean sprouts
Campylobacter jejuni	green onions, lettuce, mushroom, potato, parsley, pepper, spinach
Clostridium botulinum	cabbage, mushrooms, pepper
E. coli O157:H7	alfalfa sprouts, apple juice, cabbage, celery, cilantro, coriander, cress sprouts, lettuce, tomato, spinach, watermelon, cantaloupe, mango, bell pepper, imported salad
Listeria monocytogenes	bean sprouts, cabbage, chicory, cucumber, eggplant, lettuce, mushrooms, potatoes, radish, salad vegetables, tomato, frozen vegetables, caramel apples, cantaloupe, spinach.
Salmonella	alfalfa sprouts, clover, artichokes, beet leaves, celery, cabbage, cantaloupe, cauliflower, chili, cilantro, eggplant, endive, fennel, green onions, lettuce, mung bean sprouts, mustard cress, orange juice, parsley, pepper, salad greens, spinach, strawberries, tomato, watermelon, cucumber, mango, papaya, Jalapenos pepper, cantaloupe, tomato, green salad, fruit salad, spinach
Shigella	celery, cantaloupe, lettuce, parsley, scallions
Staphylococcus	alfalfa sprouts, carrot, lettuce, onions sprouts, parsley, radish
Vibrio cholera	cabbage, coconut milk, lettuce

1.7 Mitigation Methods Commonly Used in the Produce Industry

To minimize the risk of pathogen contamination, the USFDA (1998) published a "Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables" which listed the main sources of pathogen contamination and how to control these pathogens associated with fresh produce based on the following principles: (1) Prevention of fresh produce contamination by microbes is better than depending on the corrective actions; (2) Using a good agricultural and management practices is necessary to accomplish by growers, packers, or shippers; (3) Fresh produce can be contaminated by microbes at any stage in the farm-to-table chain and the main source of contamination is the human/animal feces; (4) Controlling the contamination of the fresh produce coming from water during irrigation or any other point is necessary; (5) A special restriction has to be taken into account during using the animal manure with fresh produce to minimize the microbial contamination; (6) Worker hygiene and sanitation practices during all stages of food production play a critical role in fresh produce safety; (7) It is important to follow all the laws and regulations for agricultural practices, and (8) The responsibility and answerability, during all stages of agriculture, are important to have safe food (USFDA 1998).

Besides all of these principles, the presence of qualified staff and an active monitoring system have to be in place to ensure that all elements of the program function correctly. The main and traditionally used method to reduce the microbial load on fresh produce is washing them by tap water and sometimes adding some salt or vinegar to the tap water or using chlorinated water (Garcia *et al.*, 2003). These methods are not efficient especially with spore-forming pathogens and the remaining chlorine residue could be harmful to a human in a long-term or change the taste of fresh produce (Akbas and Olmez, 2007; Hassenberg *et al.*, 2008). Additionally, there are some other

factors limiting the effectiveness washing, such as internalization of pathogens within plant tissue, the bacterial formation of biofilm, and the plant surfaces hydrophobicity (Whipps *et al.*, 2008).

There are other methods to control the contamination of fresh produce. These methods include irradiation (Gomes *et al.*, 2009; Mahmoud, 2010); ozone treatment (Najafi and Khodaparast, 2009; Selma *et al.*, 2007); using bacteriophages which can be applied on the fresh produce (Abuladze *et al.*, 2008; Kocharunchitt *et al.*, 2009; Sharma *et al.*, 2009; Boyacioglu *et al.*, 2013 and 2016); by means of using antagonistic bacteria (Cooley *et al.*, 2006; Scolari and Vescovo, 2004; Trias *et al.*, 2008); and by using a combination of antagonistic bacteria with bacteriophages (Ye *et al.*, 2009). Also, chemical sanitizers could be used as a mean of prevention methods. There is a wide variety of chemical sanitizers and some of them are used as household chemicals, such as Sodium hypochlorite, which is used under some precautions with low concentration in fresh produce sanitization (University of Rhode Island, 2015).

Ozone treatment can be used for the harvested fresh produce before or after storage by adding it in the air, water or even atmosphere during transport or storage stage (Palou *et al.*, 2002). It is important to understand the mode of action of ozone, the best working conditions, and optimal ozone concentration for each type of fruits and vegetable (Horvitz and Cantalejo, 2014). The type of produce even within the species have to be taken into consideration before the application of ozone to avoid the produce damage, and the concentration has to be measured for each particular product (Horvitz and Cantalejo, 2014). The food industry has to consider that a high concentration of ozone might negatively impact the quality of produce and workers' health (Horvitz and Cantalejo, 2014).

One of the novel approach to reduce the microbial contamination on fresh

produce is the application of edible-coated antimicrobial agents extracted from natural sources (Qadri *et al.*, 2015). Several materials can be used in this regard, while chitosan is the popular one, which has the ability to inhibit several pathogens (Romanazzi, 2002). Sometimes multilayers can be used during the antimicrobial coating agents and have a significant effect on increasing the shelf life of the produce, especially the fresh-cut produce (Sipahi *et al.*, 2013). Qadri *et al.* (2015) demonstrated that the antimicrobial coating agents had significant effects on the microbial loads on several fresh-cut produce by reducing or inhibiting the microbial growth for psychrotrophs, coliforms, yeasts, and molds.

The selection of the method for mitigation depends on several factors, such as type of the produce, surface nature of the produce, water quality used in sanitization, contact time during mitigation, time and amount needed for each techniques, and the acceptance of the technique by public (Parish *et al.*, 2003).

1.8 Economic Impacts of Foodborne Outbreaks

The foodborne outbreaks have a negative impact on the economy, especially on the companies and on persons who are infected by pathogens. Just one case of a foodborne outbreak can cause unexpected economic losses. The CDC estimates that the yearly-sickened people in the USA from foodborne illness is about 48 million, while most of these cases are not reported and only 9.4 million cases are with identified causal pathogens (Hoffmann *et al*., 2015). About 95% of these foodborne illnesses cost about $15.5 billion yearly, and *Salmonella*, *Toxoplasma gondii*, *Listeria monocytogenes*, *Norovirus*, and *Campylobacter* are responsible for 90% of this economic burden (Hoffmann *et al.*, 2015).

de Noordhout and his coworkers (2014) estimated the annual global burden for listeriosis to be about 23,000 illnesses with 5500 deaths in 2010. In Canada, 24 deaths

were related to listeriosis occurred in 2008 after consuming contaminated processed meat. The total estimated cost for medical, nonmedical, productivity loss, the meat processing plant and federal agencies responding to this outbreak was about $242 million (Canadian dollars, CAD) with an estimated cost per case about CAD $2.8 million including death (Thomas *et al.*, 2015). The long-term consequences of listeriosis such as psychological and neurological situations are not included in the final estimates since no sufficient data are available to support these estimates (de Noordhout *et al.*, 2014)

The food companies have to improve the safety and quality of food for all local and export markets to avoid any food safety alerts. Outbreaks can affect the economy seriously leading to closure of the food companies and thus affecting a part of the food industry**.** Consequently, it is important for any food company to; 1) follow the international food safety standards, 2) screen changing business conditions, 3) think through the impact of shipping of foods, 4) stimulate food safety by working with governmental organizations and professional associations, and 5) improve food safety awareness. In the US, the predictable annual cost of foodborne outbreaks is about $7 billion (Hussain and Dawson, 2013). This amount is used to be spent on alerting customers, getting rid of contaminated food from shelves, and paying legal fees to those who are affected. The real problem behind these issues is losing public confidence, which might be difficult to overcome (Hussain and Dawson, 2013). Table 5 lists the expensive foodborne outbreaks around the world.

Table 5. Examples of some expensive foodborne outbreaks and recalls around the world (Adopted from Hussain and Dawson, 2013).

Year	Contamination/Food Product	Estimated Economic Loss	Region/Country
2013	*Clostridium botulinum*/ Whey concentrate	Unknown	New Zealand
2011	*E. coli* O104/ fenugreek sprouts	$304 million	Germany
2009	*Salmonella*/Peanut products	$70 million	USA
2008	*Salmonella*/Tomatoes	$250 million	USA
2007	*Salmonella*/Peanut butter	$133 million	USA
2006	*E. coli* O157:H7/Spinach	$350 million	USA
1992	*E. coli*/Hamburgers	$160 million	USA

This part of literature review described under this section is extracted from El-Nemr, I; Alali W; Goktepe, I. A review on Foodborne Outbreaks and Current Food Control System in the Middle East with focus on GCC Countries. Submitted to Food Science and Technology International. January 2019.

1.9 Food Industry in the GCC countries, particularly in Qatar

The Gulf Cooperation Council (GCC) countries, including Bahrain, Kuwait, Oman, Qatar, KSA, and UAE (Figure 1), have an estimated population of about 51.5 million living in an area of 2,572,991 km^2 (World Bank, 2016). These GCC countries share the same cultural, social, linguistic, and environmental conditions, and depend mostly on oil and gas revenue as their main source of income. They are also

characterized by having poor soil, infrequent cultivation activities, a harsh climate, and limited water resources (Business Intelligence Middle East, 2006). Some crops are cultivated, such as tomatoes, cucumber, and leafy greens; however, food production quantities and varieties are low to meet the demands of the growing population.

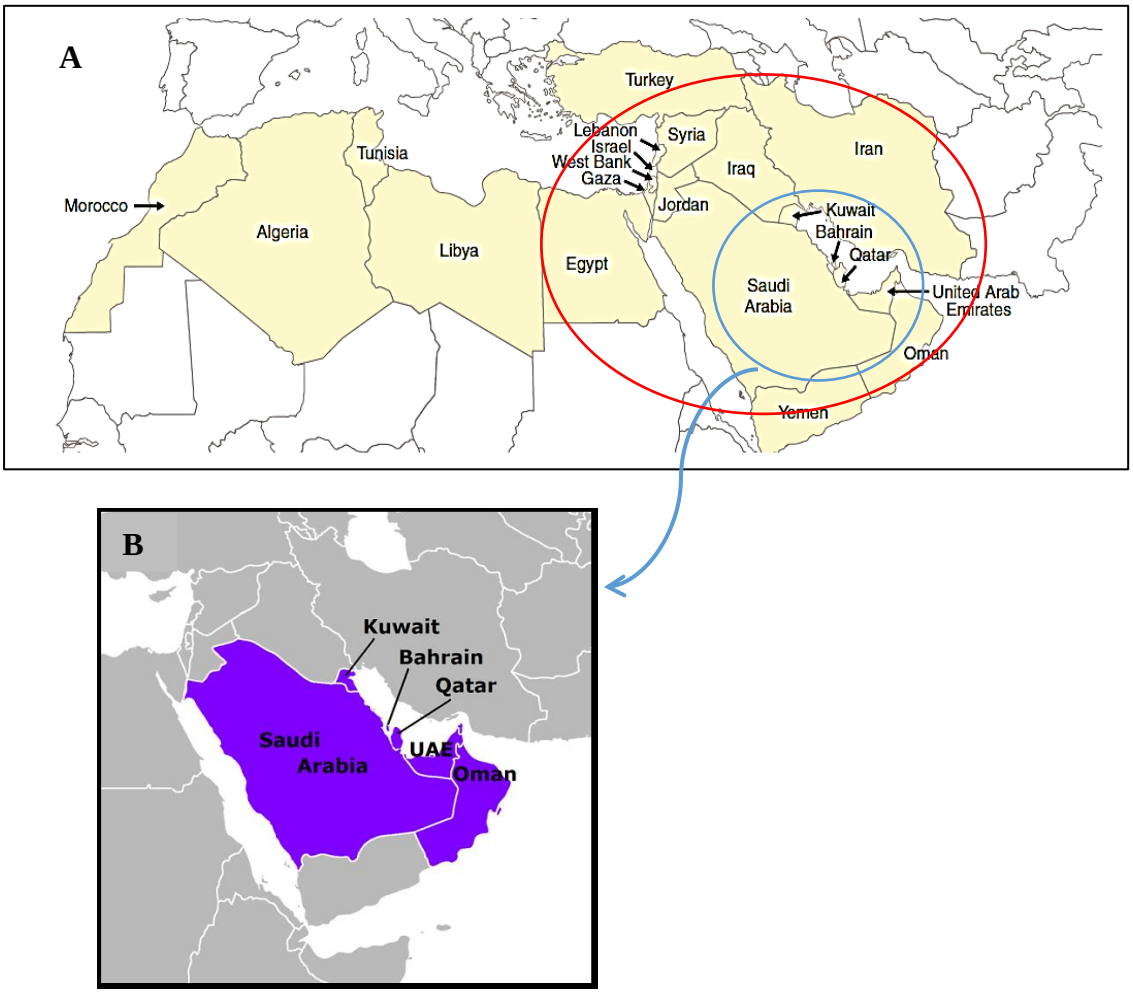


Figure 1. A. Map of the Middle East Countries (USDA, 2015). (Red circle), B. Map of the GCC (Gulf Map. 2016).

The majority of GCC countries are classified as high-income countries, and they recruit many experts, workers, and laborers from different countries to work with non-

taxed salaries. These foreigners make up, on average, more than one-third of the total population of GCC countries, but this number may reach up to 80% and 90% in Qatar and the UAE, respectively (Al-Kandari and Jukes, 2009). As people move from one place to another for work, they tend to bring their food culture with them. As a result, the number of food establishments serving diverse cultural foods has been increasing in the region. For instance; there are more than 10,000, 9000, and 7000 food establishments in Kuwait, Dubai (UAE), and Qatar, respectively, with the majority serving Indian or Middle Eastern cultural foods (Alomirah *et al.*, 2010; Dubai Statistics Center, 2017; The Peninsula Qatar, 2015).

The total food consumption in the GCC countries was 48.1 million metric tons (MT) in 2016 (Alpen Capital, 2017). Saudi Arabia is the most populated country in the GCC region with the highest food consumption rate among the GCC countries. The projected rate of food consumption in Saudi Arabia is 37.7 million MT by 2021 with an annual increase of 4.2% since 2016 (Alpen Capital, 2017). Qatar and the UAE are also expected to have increased food consumption rates, with the Compound Annual Growth Rate (CAGR) values of 5.8% and 4.4%, respectively (Alpen Capital, 2017). Additionally, the Qatari government has initiated an infrastructure-spending plan totaling $50 billion after winning the bid to host the FIFA 2022 World Cup (Alpen Capital, 2017). This initiative is likely to have a tremendous effect on the population growth, which in turn will boost food demand. This will lead to high consumption of food per capita by value, thus benefiting the food industry. The GCC countries import approximately 5 million tons of fresh produce per year, which costs about US$3 billion.

Qatar's population has been growing at a relatively high rate over the past 15 years. The population was 0.48 million in 1990 and now it reached 2,757,437 million showing a 5.5 folds growth over the past 28 years (Qatar Statistics Authority, QSA,

2018). This fast population growth is driven by the massive discovery of oil and gas in Qatar and the technology developed to import this vital commodity all over the world. This exponential growth put the country at the top of the list of high income earners for having the world highest GDP per capita ($65,000) in 2018 (QSA, 2018).

Qatar is highly dependent on imported food to meet the consumers' requirements. Most of this food was used to be imported from KSA and UAE, or even from other surrounding countries such as Kuwait, Lebanon, Syria, and Jordan via the land border between Qatar and KSA "Abu-Samrah". After the blockade (5th of June 2017), thousands of trucks carrying food supplies could not pass this over land border eventually and stuck on the KSA side. This created some difficulties for the Qatari government to meet the food requirements for the consumers and they started to switch their food supplies by importing food via air or sea from Turkey, Iran, Kuwait, and Pakistan at most and other Middle East countries besides Europe (Saul and El Dahan, 2017). On the other hand, the Qatari government started to enhance the local food production and industry. The local production of vegetables used to not to exceed 15% of the domestic requirement of vegetables before the blockade, while between 2017 and 2018 the production increased about 300% from the Qatari farms (Castelier, 2018). In addition, the government started supporting the local vegetable production by giving people about 1 million m^2 of agricultural land, since they are targeting to get about 20,000 to 30,000 tons of vegetables per year from these local farms (Castelier, 2018).

1.10 Foodborne Outbreaks in the GCC Countries

According to the FAO/WHO report (2005), the data on the number of reported cases of foodborne illness is limited to certain countries in the GCC region. This limitation has forced many researchers to depend on the media, especially local newspapers that generally circulate the news on foodborne outbreaks based on hospital visits. In most cases, the news articles do not provide detailed information about the causative agent, a number of cases and the source of contamination to describe the full

situation. Most of the published works in the region have focused heavily on the food quality in local markets and the food safety practices and knowledge of the food handlers in such markets.

In a recent review article, Todd (2017) mentioned that sharing food during public gatherings, feasts, labor camps, schools, and military camps in the GCC region were the major pathways of exposure to foodborne pathogens, such as *Shigella* spp., *Salmonella* spp., *Campylobacter coli, C. jejuni*, Hepatitis A virus, Rotavirus, and parasites. Table 6 summarizes the list of foodborne outbreaks have been reported during the last 10 years in the GCC. It is important to note that the majority of outbreaks are usually reported as news articles in the region.

The KSA hosts millions of Muslim pilgrims every year during the Hajj season. Therefore, the KSA government has implemented very stringent infection control measures to prevent the incidence of foodborne disease outbreaks. Although the food safety rules are very strict in the country, there have been several major outbreaks reported in the last decade, especially during the annual Hajj pilgrimage season (Mohamed *et al.*, 2017). The major pathogens associated with these reported outbreaks were *Bacillus cereus*, *Clostridium perfringens*, and *Salmonella enteritidis*. The major issue identified as the cause of such outbreaks is the application of inappropriate handling practices due to the massive number of consumers (Mohamed *et al.*, 2017). In addition, one of the most memorable foodborne outbreaks was reported by the International Society for Infectious Diseases in the KSA during the 2011 Hajj period, when 81 Bangladeshi pilgrims were hospitalized after consuming a meal prepared by an unlicensed catering company in Al-Madinah (ProMED, 2011). An earlier foodborne outbreak was also reported in 2006 (Al-Joudi, 2007), which sickened 50 soldiers in Mina, KSA. The soldiers complained about diarrhea and abdominal cramps after ingesting a meal containing rice mixed with meat. Although the pathogen was not identified, it was suspected that *Bacillus cereus* was the causative agent.

Table 6. The list of reported foodborne outbreaks during the last ten years in the Gulf Cooperation Countries (GCC).

Causative agent	Country	Year	Source	Number of cases	Reference
Salmonella spp.	Bahrain	2014	A franchise restaurant	ND	Barf Blog (2014)
ND	Bahrain	2018	School Canteen	Several students	The Daily News of Bahrain (2018)
ND	Ras Al-Khaimah (UAE)	2008	Chinese restaurant	14 Employees	Gulf News (2014)
ND	Dubai (UAE)	2009	Chinese restaurant	2 Deaths	Gulf News (2014)
ND	Dubai (UAE)	2014	Cake supplied by hotel	12 Hospitalization	Gulf News (2014)
ND	Al Ain (UAE)	2018	School	30 Students	Sebugwaawo 2018
ND	KSA	2014	Salads at restaurants	150 Customers	Al-Hamid 2014
Salmonella spp.	KSA	2015	Food Handlers	12 Customers	Al-Sulami 2015
ND	KSA	2016	Restaurant	33 Customers	Arab News (2016)
ND	Riyadh (KSA)	2017	Shawarma Sandwiches	150 Customers	Medical press (2017)

Table 6 Cont. The list of reported foodborne outbreaks during the last ten years in the Gulf Cooperation Countries (GCC).

Causative agent	Country	Year	Source	Number of cases	Reference
ND	KSA	2018	Hotel restaurant	>100 Hospitalization	Ahram Online (2018)
Salmonella spp.	Kuwait	2018	Soldiers camp	5 Soldiers	Myers, 2018
ND	Kuwait	2018	Laborers camp	45 Workers	Arab times (2018)
Salmonella spp.	Kuwait	2018	Falafel sandwich	287 Cases	Al-Shurafa 2018
ND	Oman	2013	ND	360 Customers	Y of Oman (2015)
ND	Oman	2014	Restaurants food	60 Customers	Y of Oman (2015)
ND	Oman	2015	Restaurants food	20 Customers	Y of Oman (2015)
ND	Oman	2017	Wedding party	30 Customers	Times Of Oman (2017)
ND	Oman	2017	School canteen	55 Students	Muscat Daily (2017)

A summary of foodborne disease cases occurred in Abu Dhabi (UAE) was reported by Malek (2012). There were several foodborne disease cases detected between 2010 and 2012 in Abu Dhabi including, 561 cases reported in 2010, 667 in 2011, and 627 cases in the first six months of 2012. More than half of the cases were caused by Rotavirus, followed by typhoid and non-typhoid *Salmonella*. In Dubai (UAE), the number of foodborne diseases slightly declined from 1,663 cases in 2011 to 518 cases in 2013, which was partly due to better practices applied in the food industry (Saseendran, 2017). In one of the most recent outbreaks that occurred in July 2018 in Hawally, Kuwait, about 287 people suffered from foodborne disease symptoms after consuming falafel sandwiches from a local restaurant (Al-Shurafa, 2018). The people were hospitalized in more than five public hospitals, as well as other private hospitals. This case prompted the Ministry of Health in Kuwait to activate an emergency plan, since the number of people suffering from foodborne disease was too high. One action that was immediately taken by the Public Authority for Food and Nutrition was to shut down the restaurant. Although the food sample tests provided positive results for *Salmonella* spp. contamination in the falafels, no authorized data about the causative pathogen of the outbreak was released (Al-Shurafa, 2018).

In the GCC, workers in labor camps are regularly served with meals prepared by catering companies. Although reports are very limited, one outbreak was reported in November of 2010 with more than 300 laborers becoming sick after eating macaroni salad mixed with mayonnaise, that was made with raw eggs at the Ras Laffan Industrial City in Qatar. In this outbreak, *Salmonella* group D was identified as the causative agent (Nazzal *et al.*, 2012). In Muscat (Oman), more than 300 cases were reported among employees in an oil company camp in 2013. Some of these employees were hospitalized, but no information was released on the agent, nor the food that caused this outbreak (Vaidya, 2013).

1.11 Food Safety Management and Inspection System in the GCC

There are different approaches used in regard to food safety management. The approach used in most GCC countries (e.g. Kuwait, Oman, Qatar and Dubai in UAE) depends on the presence of a direct exclusive unit like municipalities. There is another approach used in Saudi Arabia, and Abu Dhabi in UAE, that depends on having a "national food safety agency" in charge of the control and management of the food chain and risk analysis (Al-Kandari and Jukes, 2009). The advantage of the second approach is; more efficient and ensures the follow-up steps. Lately, the GCC countries updated their laws to be in compliance by obeying the international regulations. They started applying Codex Alimentarius standards, (Al-Kandari and Jukes, 2009). In Qatar, the food safety management is observed by municipalities and their responsibilities include food sanitation, hygienic practices, and foodborne disease surveillance.

In general, the GCC countries face many challenges especially in implementing a comprehensive monitoring program to sustain food quality, while their food safety regulations are still being updated and upgraded to adapt high international regulations standards such as Codex Alimentarius, which follows the new standards in labeling the produce, the expiry dates beside the health claims, and the nutritional value of the food (Kamleh *et al.*, 2012).

As a result of not having a unified food safety management system in this region, the following recommendations are proposed: (1) Identify governmental and non-governmental entities that will lead the effort for all food safety stakeholders, develop and implement policies and strategies, and respond to food safety concerns (2) Applying risk analysis for proper risk assessment, management, and communication by implementing the following: (a) Develop and upgrade foodborne surveillance,

monitoring and inspection systems at all levels of the food chain (farm to fork). This is so important for identifying specific microbiological hazards in foods, (b) Develop the capabilities of food inspectors through continuing on-the-job training and education, (c) Establish a certified education program to ensure all workers have passed a certain level in food safety and hygiene standards, (d) Upgrade and certify food testing laboratories, (e) Upgrade food safety legislation with emphasis on traceability and recall programs, (f) Implement good agricultural practices and management such as GMPs, GHPs, and HACCP in all local food markets to increase the quality of food products.

One of the major factors showing the significances of food safety quality control measures is applying food inspection. In general, it is rare to find a monitoring program based on risk analysis in Arab countries, and the real situation is quite messy (Kamleh *et al.*, 2012). For example, in the GCC countries, the public health inspectors are mainly untrained in food science and safety, and they are not fulltime employees in food inspection and have other duties. The inspection of food shops is limited. These shops follow low hygiene standards and also, the way of food samples collected is not normally representative of the actual situation. Additionally, the analytical labs are limited to carry out such issues tests and most of them are not accredited except the ones in the UAE. All these increase the challenges in these countries to have an efficient effort to apply quality control actions (Al-Kandari and Jukes, 2009; Kamleh *et al.*, 2012).

In Kuwait, the food quality information, training, and communication actions are still not significant (Alomirah *et al.*, 2010). The Saudi Food and Drug Administration and Abu Dhabi Food Control Authority have websites providing information on food safety and food handling and are conducting different workshops

to apply up-to-date food safety systems such as GMP, GHP, and HACCP (Kamleh *et al.*, 2012).

Food safety in the GCC region is one of the main challenges and it is an economical load and public health risk factor. Many steps have to be taken in this respect, starting from developing strategies to respond actively to the food safety issues-approving risk analysis system for good risk assessment, management, and communication-implementing several steps and following recommended steps applied in the highly developed nations.

1.12 Microbial Risk Assessment

Food safety issues have increased lately because of the high incidence of foodborne diseases associated with both imported and domestic food products. Fears from different diseases caused by pathogens like avian flu, *Salmonella, Listeria*, *Campylobacter*, *E. coli* O157:47 causing disease, or by some toxic chemicals, like acrylamide, dioxins, antibiotic and pesticide residues, and exposure to radiation, enforced the food authorities and organizations to create standard approaches or techniques to reduce the risk and enhance the trust of the public in food safety regulations.

The risk is a measure of the probability of a hazard causing harm and the adverse effect of this harm after the exposure to this hazard (EPA, 2012). All activities related to food production, processing, and handling involve some hazards. It is important to determine the level of risk and know how to reduce or eliminate food hazards. This will help to set up food safety controls' measures, lower the risks for consumers, and take actions as an important part in risk analysis (EPA, 2012).

Risk analysis is an essential policy in developing food safety levels and standards. According to Codex Alimentarius; risk management defined as "*The process*

of weighing policy alternatives in the light of the results of risk assessment and implementing appropriate control options." (FAO, 2007). WHO (2009 b) has recognized a risk-based approach as an obligatory approach to be applied during food production, processing, and distribution stages around the world. This risk-based approach helps to reduce human exposure to food contamination such as foodborne pathogens by preventing, reducing, or eliminating these hazards. The organizations have to improve the disease surveillance system and the detection methods used in the microbial analysis for both patients and food. Countries must take an action to reduce the disease spreading and have to offer safe food for the consumers. One of the most effective tools in this regard is the risk analysis, which consists of risk assessment, risk management, and risk communication (Figure 2). FAO (2005) identified the main stages in any risk analysis process as coated below:

1. "*Risk assessment: an assessment is made of the human health risk associated with a particular foodborne hazard.*
2. *Risk management: decisions are made according to the acceptable level of risk and methods for control of the risk.*
3. *Risk communication: information about the risk and chosen methods of control are communicated with all involved parties*."

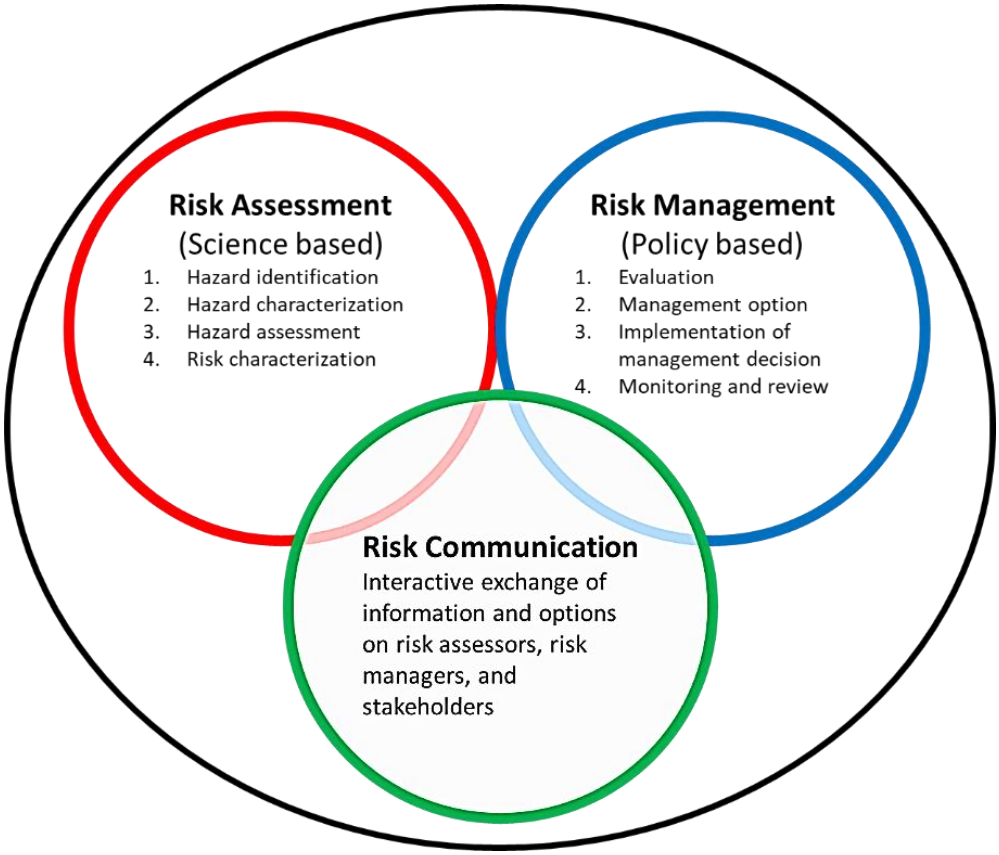


Figure 2. Risk Assessment Framework and its Relationship with Other Components of Risk Analysis (e.g., risk assessment, risk management, risk communication) (Adapted from Channaiah, 2014).

In 2008, FAO/WHO defined the Microbiological Risk Assessment (MRA) as *"a tool used in the management of the risks posed by foodborne pathogens, including the elaboration of standards for food in international trade*". It helps in creating food safety standards for the nation, while the risk management helps the decision makers to evaluate the risk related to public health. In addition, the risk communication helps to ensure the outcomes, logic, and limitations of risk assessment done by risk assessors, risk manager, and other stakeholders (Channaiah, 2014).

The main objective of MRA is documenting all source of hazards at different stages of the food preparation. Risk assessment needs to clarify the unknowns about the

risk in a logical way and document this risk in a good manner to be easily indicated by anyone involved in the development of risk assessment. This assessment can be descriptive, qualitative or quantitative or mix of them as a semi-quantitative method. The qualitative MRA is the type of assessment that needs collecting, joining, and giving evidence to explain the risk strongly. Having figures, data, and analysis normally goes under qualitative risk characterization. On the other hand, the quantitative risk assessment needs a mathematical data and analysis for this data. It can be deterministic or probabilistic, e.g. food additive safety assessment vs. microbial risk assessment (FAO, 2005).

Nowadays, most of the food safety management organizations are adapting risk-based management to apply high food safety standards and improve the quality of food provided to the public. MRA approach helps food safety system in evaluating the risk, implementing food safety programs, preventing the outbreaks or recalling, enhance the public health, and ensuring the transparency of decision-making.

The Codex Alimentarius (which is a collection of food standards, guidelines, and practices) defines microbial risk assessment as a scientifically based approach consists of four steps (FAO, 2005):

A-Hazard identification

It is the first qualitative step in a risk assessment approach. The microbial pathogens, which normally have an adverse effect on human health, need to be identified. Information regarding food pathogens such as their existence and the related food in concern must be collected at this stage. This can be done with the help of a literature review, the foodborne database from the public health authorities, epidemiological reports, and clinical studies. In addition, laboratory studies, microbial characteristics and their interaction with the environment are needed in this stage (Soller, 2006; WHO, 1999).

B- Hazard characterization

This step can be a quantitative or qualitative step. The severity and the duration of the hazard and its adverse effect after ingestion of the contaminated food have to described here, which means a full explanation of the relation between the pathogen and its adverse effects (EPA, 2012).

C- Exposure assessment

At this step, a quantitative or qualitative evaluation of the tested pathogen presented in the contaminated food at the time of ingestion needs to be estimated. Likewise, the probability of the presence of the tested pathogens from other sources such as water or even air atmosphere needs to be estimated. In addition, evaluating the interactive characteristics of the source, the exposure mechanisms, pathogen prevalence, and the frequency of the exposure have to be evaluated (Channaiah, 2014).

Predictive models and Monte Carlo simulation

During the exposure assessment stage, it is needed to establish the certainty of the presence of the pathogen in food during consumption. It is the likelihood of individual (or public) to be exposed to a specific amount of microbial hazard during the food production stages (Lammerding and Fazil, 2000). Therefore, having a tool to quantify the prediction of the interested pathogens will be helpful and facilitate the process. These tools include microbiology predictive models and Monte Carlo simulation model (Collado *et al.*, 2011). Microbiology predictive model may be used to combine microbiology, mathematics, and statistics to predict microbial behavior under specific conditions (Collado *et al.*, 2011). The model can explain the dynamics of the pathogen during food processing stages. Different studies established quantitative dose-response models, explaining the relationships of microorganisms and making it possible to establish the risk of the infected people after ingestion of some

dose of pathogens. Two of the most used models for most pathogens in this regard are the exponential and beta-Poisson model, originated by Haas (Haas, 1983; Haas *et al.*, 1999).

The exponential models are based on the following assumptions:

1. The microbial distribution is randomly distributed following the Poisson distribution,
2. At least, one pathogen needs to be present in the host to cause infectious disease, and
3. There is a constant probability for the organism infection per ingestion.

The mathematical probability for the exponential model of the infection is denoted as P (d) and expressed by the following equation (Haas, 1983; Haas *et al.*, 1999):

$$P(d) = 1 - e^{-rd}$$

Where P (d) is the probability of infection at dose (d) of the interested pathogen, and "d" unit is in Colony Forming Units (CFU), while "r" is the specific parameter for each pathogen in the dose-response function, which refers to the probability of one pathogenic cell to survive in host and initiating illness. On the other hand, the beta-Poisson model is following only the first two exponential model assumptions, while the third beta-Poisson assumption requires the probability of infection per ingested pathogen to be varied with the population. Therefore, the model "r" is not constant as the exponential model, but it is beta distributed by two parameters (α and β). The mathematical equation of the beta-Poisson model is expressed as (Haas, 1983; Haas *et al.*, 1999):

$$P(d) = 1 - (1 + d/\beta)^{-\alpha}$$

Where P (d) is the probability of infection at a dose (d) of the interested pathogen, "d" is the dose (CFU), β and α, are parameters of the beta distribution that

describe the host-pathogen interaction. The beta-Poisson is usually linear at low doses, but when α increases, it approached the exponential model (Haas *et al.*, 1999). The beta Poisson model is commonly used for bacteria and some viruses (Soller, 2006).

Monte Carlo simulation model is also used to assess several food safety-related problems. Using mathematical models with exposure assessment to assess dose-response can help in explaining the presence of pathogens in food, their proliferation, elimination processes, viable microbes count in the ingested in food and the consequence of these pathogens on the public health of consumers. In addition, the probability distribution can be characterized by the variability and the uncertainty related to the input variables for the model. Monte Carlo simulation model includes the probability distributions, which are used to produce the parameters estimates involved in the model and provides a human sickness estimate with the uncertainty associated with that estimate. Computer Software is available for this process. Example of these models is Microsoft Excel @Risk.

D- Risk characterization

The data collected from the three steps explained above need to be combined together to get a quantitative (or qualitative) risk estimate and the probability of the severity of the adverse health effect occurring after the exposure to identified pathogens via consuming the contaminated food. At this step, the uncertainties associated with measured estimate need to be identified to have a full quantitative microbial risk assessment (QMRA) (Dennis *et al.*, 2002). This estimate is affected by the uncertainty, variability, the data availability, and the assumption put in the previous steps (WHO, 1999). It is necessary to know the amount of uncertainty, which is associated with the given estimates. Monte Carlo analysis is one of the simulation models, which can be used to give a factor for the variables associated with food processing steps until the final risk

estimate measured (Buchanan *et al.*, 2000). According to Dennis and his coworkers (2002), the risk characterization is a combination between exposure assessment and hazard characterization to get a dose-response assessment, which is normally expressed mathematically to get the probability of the tested effect on public health. All these steps have to be explained carefully by the risk assessor to give a logical full image to the decision makers (Dennis *et al.*, 2002).

1.12.1 The use of Quantitative Microbial Risk Assessment in Predicting Microbial Hazards in Fresh Produce

Nowadays, several mathematical models can be used to predict microbial activities and behavior in the food environment. This creates a new area in research regarding predicting microbial hazards in food. The use of computer software and intensive information about food microbiology, which is introduced in these models, enhanced the microbial risk assessment field in the last decades (Pérez-Rodríguez and Valero, 2013). Example of the information to be used in the model can be microbial growth pattern, the interaction between microbes, the inactivation methods, and the probability of microbial growth under several environmental conditions. Currently, the predictive microbial models are considered as an important tool in the food safety field to provide a quick decision regarding the food safety problem (Pérez-Rodríguez and Valero, 2013). Most of the developed countries incorporate the risk-based programs in their food safety policies by using QMRA studies supported by several applications of predictive models to enhance the food quality. For example, Koseki and Isobe (2005) estimated the microbial safety of lettuce by using predictive models to study the growth pattern of *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes* under different temperatures during the distribution chain. Delaquis *et al.* (2007) used predictive models to understand the behavior of *E. coli* O157:H7 in lettuce and spinach from

production to storage step. They identified remarkable uncertainties for each stage of the produce industry and the fate of the contaminant under low temperature during the storage stage, which helped in developing an effective control measure for the pathogen. In addition, Pang and his coworkers (2017) used different QMRA models to study the potential risk associated with consuming fresh-cut lettuce contaminated by *E. coli* O157:H7. The study provided sufficient information to set effective decontamination strategies in reducing the public health risks. Based on the research findings, the storage temperature at home and retail were the most important factors that affected the growth patterns of *E. coli* O157:H7 in fresh-cut lettuce.

1.13 Risk Management

Risk management can be defined as "*the process, distinct from risk assessment, of weighing policy alternatives, in consultation with all interested parties, considering risk assessment and other factors relevant for the health protection of consumers and for the promotion of fair trade practices, and, if needed, selecting appropriate prevention and control options"(Codex) (*FAO, 2005). Risk management is an important element of any risk analysis process. Thus, risk management is considered as the main player at the beginning of any risk analysis process, especially to identify the problems related to food safety and determining the best ways to manage this problem. Protecting the public health by controlling the probable risk and applying the suitable corrective measures is the main goal for risk management (USFDA, 1997). Therefore, the risk management process is a repetitive, frequent process and not a linear one, like risk assessment. The risk management models have to be flexible, so it can be reviewed, repeated, and adapted based on the needs of each activity during the assessment.

Literature is rich in listing experiences gained from the application of different

models in risk assessment and management strategies by different countries (WHO, 1997; Trienekens and Zuurbier, 2008). Some of these strategies are Good Manufacturing Practice (GMP), Good Hygiene Practice (GHP), and Hazard Analysis and Critical Control Point (HACCP) systems. The use of these models led to reduce the risk or prevent foodborne illness associated with consuming different types of food (Huss *et al.*, 2000). For example, applying GMP, GHP and HACCP led to growth reduction of *Listeria monocytogenes* in fish products (Huss *et al.*, 2000). Luning and his coworkers (2006) pointed out, how applying these systems enhanced customer satisfaction and improved the system of preventing diseases.

1.14 Risk Communication

Covello (1992) defined risk communication as the "*process of exchanging information among interested parties about the nature, magnitude, significance, or control of a risk*". There is a special need for dialogue between correspondents and sponsors (Palenchar, 2005). Risk communication has to be wisely planned, applied achieved, and managed to guarantee the results. Risk communication is the process of exchanging information regarding the risk and talking about the illness and the severity and what is the action to reduce this risk. Risk communication includes two major purposes: 1) Notify the managers about the risk, to help them make a decision and 2) Notify the public about the risk, so they can understand the nature of the risk and then they can act accordingly (EPA, 2012). The good risk communication results in useful and dynamic conversations, which can combine problem-solving by legitimate participants and the government. Populations, in general, need a system to communicate about current issues, developing, emerging, and evolving risks. There is a broad-spectrum harmony that risk communication is a two-way process between the communicator(s) and the recipients of the messages, there are many definitions regularly containing exclusive variables and identifications.

1.15 Research Justification

According to WHO/EMRO (2017), it was estimated that the number of food poisoning cases in Qatar exceed 2,000 per year. These cases were caused by commonly known pathogens, such as *Salmonella* spp., *Campylobacter* spp., and *E. coli* O157:H7 (WHO/EMRO, 2017). The impact of foodborne diseases associated with not only human health, but also economic cost. The literature is replete with studies conducted to evaluate microbial quality of fresh produce and associated risk factors in different parts of the world (Castro-Ibáñez *et al*. 2015; Pang *et al*, 2017; Kundu *et al*., 2018; Franz, 2018; Miranda *et al*.,2018). However, these types of studies are limited in Qatar, mainly now that Qatar is turning into an international hub for big sports' events and international conferences, such as the FIFA World Cup 2022, at which millions of people will be visiting the country and consuming local foods.

Another important fact is that most of the food is imported from countries where hygiene practices are not considered sufficient. Additionally, food handlers hired in Qatar come from Bangladesh, Egypt, India, Indonesia, Sri-Lanka, Pakistan, and the Philippines. As they come, they bring their traditions and habits with them, which eventually impact the safety of food sold in the country. Moreover, the current regulations, monitoring, and inspection systems are not sufficient to control food contamination risks at the food production system, especially in the wholesale fresh produce market in Doha. The wholesale fresh produce market (WSFPM) is an open-air market located in the uncultivated area (mainly desert area) of Abu Hamour in Doha, without any air-conditioning where the average temperature is above 35°C throughout the year. It is the major produce market, from which most of the consumers/individuals obtain their fruits and vegetables often. Additionally, the local restaurants, industrial food services, grocery stores, and supermarkets purchase the needed fresh produce,

either to prepare meals in their establishments or to resell these produce in their retail stores.

On the other hand, there are domestically grown or imported fresh produce sold at reasonable prices. The produce available at the market are not usually treated with any additional process. The market is open daily to the public from 6:00 am to 1:00 pm, except on Friday. The market is fully managed by Doha Municipality, which regularly inspects the produce for the presence of pesticides, but no periodical microbial inspection is conducted unless a complaint is received from the consumers. The target market is located in close proximity to food-animal markets, such as the fish market, poultry market, and large animal markets, which may all contribute to increased risk of microbial contamination in fresh produce purchased from this market. Therefore, the objectives of the percent study were to 1) Assess the microbial quality of select fresh produce sold at the wholesale market in Doha; 2) Determine the source of the microbiological hazards associated with sanitary conditions at the wholesale market; 3) Evaluate to which extent the different sources contribute to the microbial hazards expected to be present at the market; and 4) Conduct a Microbial Risk Assessment (MRA) to determine potential health risks associated with fresh produce-related outbreaks in Qatar.

CHAPTER 2: MATERIALS AND METHODS

Objective 1. Assess the microbial quality of select fresh produce sold at the wholesale market in Doha

Task 1. Carry out a comprehensive study to determine microbiological hazard(s) (e.g., common foodborne pathogens and fungi) in select produce sold in the wholesale market

Sample collection

Depending on our communication with the wholesale market manager, Doha Municipality, and Hamad Medical Corporation (HMC), the most commonly consumed raw fresh produce associated with foodborne diseases/outbreaks in Qatar were collected between June 2016 and July 2017 at the wholesale fresh produce market (WSFPM) located in Abu-Hamour, Doha (Figure 3). The fresh produce samples selected in this study were : Romaine lettuce (*Lactuca sativa L. var. longifolia*), tomato (*Lycopersicon esculentum*), parsley (*Petroselinum crispum*), cucumber (*Cucumis sativus*), and green onion/scallions (*Allium cepa*) purchased from the WSFPM monthly and usually collected on the first Sunday of each month to determine their initial microbial quality. The select produce samples tested in this study were mainly imported from the Kingdom of Saudi Arabia and Jordan, and none of them were cultivated locally in Qatar.

Triplicate samples of each produce were collected from three different produce vendors at the market. All samples were placed in individual sterile polyethylene plastic zipper bag and kept on ice in an icebox. The samples were then delivered to the Microbiology Laboratory at Qatar University (QU), which is about 20 km far away and the transit time needed to reach the lab was about 30 min.

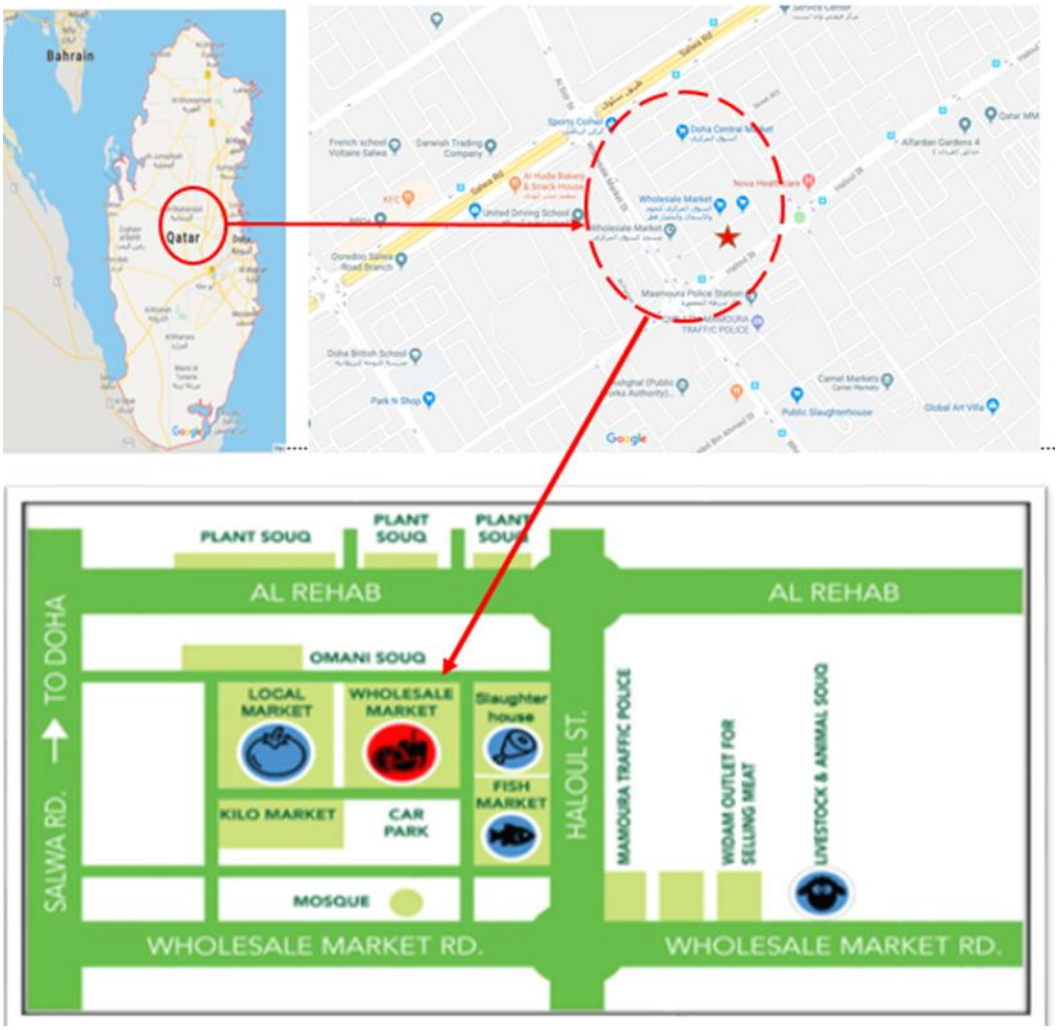


Figure 3. Location of the wholesale fresh produce market (WSFPM) in Doha and the surrounding markets.

Microbial Analysis of Produce Samples

The methodologies described under this section are extracted from El-Nemr, I, Mushtaha, M., Sundaraju, S., Fontejon, C., Suleiman, M., Tang, P., Goktepe, I., Hasan, M.R. 2019. Application of MALDI Biotyper System for rapid identification of bacteria isolated from fresh produce market. Published in Current Microbiology, 76(3):290-296 (DOI.org/10.1007/s00284-018-01624-1).

A- Microbiological enumeration and isolation of select pathogens

The total viable count technique and selective media were used to determine the presence of various microorganisms. The produce samples were analyzed immediately on the same day of collection by weighing 25 g of each produce sample in a sterile zipper bag. An amount of 225 mL of 0.1% sterile peptone buffer water (BPW, Oxoid-UK) was added to each sample to make serial 1:10 dilution. The samples were mixed and homogenized for 2-3 min using medium speed stomacher (Model 400 Circulator, Seward, UK). Then, the produce suspension was processed for microbial analysis according to the Bacteriological Analytical Manual (BAM) (USFDA, 2001).

About 5 ml of each homogenized mixture were added to the same volume of sterile Brain Heart Infusion broth and incubated at 37°C with 150 rpm continues shaking for about 3 to 6 hrs to enrich the microorganisms. The enrichment mixtures were used to prepare tenfold serial dilutions (from 10^{-1} to 10^{-7}) for microbial analysis. Exactly 0.1 ml of each diluted sample was spread in duplicate on the following selective media: Plate-Count Agar (PCA) used for total aerobic bacteria, Baird-Parker Agar (BPA) supplemented with egg yolk for *Staphylococcus* spp., Eosin Methylene Blue Agar (EMBA) for total coliforms, *Listeria* Selective Agar (LSA) base, MacConkey Agar (MCA) to determine generic *E. coli*, Xylose Lactose Tergitol 4 agar (XLT4) for generic *Salmonella* spp., Potato Dextrose Agar (PDA) for total fungi, and Rose Bengal Chloramphenicol Agar (RBCA) for yeasts and molds enumeration. The BPA and LSA media were prepared following the manufacturer's instructions mentioned on each bottle and the supplements were added after autoclaving and cooling down the media to 55°C. While for XLT4 media, only boiling step was used without autoclaving. All media used in this study were purchased from Oxoid Ltd. Hampshire, UK.

After inoculation, the plates were sealed and incubated at 37°C for 24-48 hrs,

except PDA and RBCA, which were incubated at 25°C for 5-7 days. Then the total and individual strains were counted, taking the dilution factor into consideration, to calculate the number of colony forming unit of bacteria per gram of sample (CFU/g). Later, all total CFU counts were converted and presented as Log_{10} CFU/g. The identification of presumptive colonies was based on the colony morphology as described by the manufacturer (Oxoid Ltd.) and listed in Table 7.

Table 7. The morphological key used for microbial identification.

Target organisms	Selective media	Morphological key for identification
Total coliform	Eosin Methylene Blue Agar (EMBA)	Tiny dark pink colony, and colony with green sheen for *E. coli*
Enterobacteriaceae and *E. coli.*	MacConkey Agar (MCA)	Dark pink colony
Listeria monocytogenes	*Listeria* Selective Agar + Listeria supplement	Brown colony with clear halo.
Salmonella spp.	Xylose Lactose Tergitol 4 agar (XLT4)	Black-centered colony with yellowish pink periphery
Staphylococcus spp.	Baird Parker Agar (BPA) + egg yolk	Small shiny black colony with halo.

Representative strains for each presumptive colony were isolated from the same medium, purified and coded by a special number for culture collection and kept in a fridge for further identification by using molecular analysis.

The same microbial analysis technique was used to isolate the fungal species,

but by using Potato Dextrose Agar (PDA) medium and incubating the plates at 25ºC for 5-7 days. The fungal strains were primarily identified by consulting an expert in this field at QU and by using specific systematic manuals for fungal identification. All isolated strains were also purified on PDA and coded for culture collection and kept at 4°C for further examination using molecular analysis.

B- Identification of target microorganisms (including pathogens) using MALDI-TOF MS

Bacterial strains isolated from fresh produce samples collected from the WSFPM were subcultured on LB agar (Thermo Fisher Scientific, UK), which were incubated for 24-48 h at 37ºC, and identified using a Bruker MALDI-TOF MS Biotyper System (Matrix Assisted Laser Desorption/Ionization Time Of Flight Mass Spectroscopy, Microflex LT, Bruker) according to manufacturer's instructions. The calibration of the mass spectrometer was performed by an automatic calibration procedure using the Bruker Bacterial Test Standard (BTS), which is a preparation of *Escherichia coli* DH5α, spiked with two additional proteins to enable calibration over a mass range of 4 to 17 kDa. A sum spectrum is automatically obtained from different positions on the BTS control after 6X40 laser shots were applied. The minimum number of peaks was defaulted to 7 during the calibration process.

An individual colony from a fresh culture of each isolate was picked by using a sterile toothpick and smeared on the designated spots on a standard MALDI target plate. After loading all test organisms, the plate was left to air dry for approximately 5 minutes at room temperature (25°C). One µl of HCCA Matrix (saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, 2.5% trifluoroacetic acid) was then added on the top of each spot no later than 10 minutes after drying the samples. The sample spots were then air dried again at room temperature for approximately 5 minutes before

loading the plate into the mass spectrometer. Each bacterial colony was tested in duplicate. Data acquisition was performed by MALDI Biotyper Realtime Classification (MBT-RTC) software (Bruker). MBT-RTC compares spectral peaks from unknown sample with reference peaks in a database (MBT Compass 4.1, Bruker), and generates a log (score) between 0.000 and 3.000 using a statistical algorithm. An identification score of ≥2.00 was considered valid for species-level identification, while identification scores ranging from 1.70 to 1.99 were considered for genus level-identification (Bruker, 2016).

For isolates that were unidentifiable by direct transfer of colonies to the MALDI target plates, bacterial proteins were extracted by an ethanol/formic acid extraction method described by the manufacturer. Briefly, a loop-full of bacteria was transferred to and resuspended in 0.3 ml deionized water using a 10 µL inoculation loop, followed by the addition of 0.9 ml 100% ethanol, thorough mixing and centrifugation at 16,000 X g for 2 minutes. The bacterial pellets were briefly dried, sequentially mixed with 50 µL (20 µL for small pellets) of 70% formic acid and 50 µL (20 µL for small pellets) of acetonitrile, and centrifuged at 16,000 X g for 2 minutes. One µL supernatant was then pipetted onto a MALDI target plate sample spot, dried, and overlaid with 1 µL HCCA matrix solution before being analyzed by the Bruker MALDI Biotyper System (Bruker, 2011).

C- Identification of target pathogens using 16S rRNA gene sequencing

For bacterial identification by 16S rRNA gene sequencing, the V1-V3 region of the 16S rRNA gene was amplified using the following primers: 16S-8F (AGAGTTTGATCATGGCTCAG) and 16S-519R (GWATTACCGCGGCKGCTG). One colony of a pure bacterial culture in LB agar (Thermo Fisher Scientific, UK) was suspended in 1 ml of sterile nuclease free water. Five (5) µl of this suspension was

subjected to PCR using Platinum™ PCR SuperMix High Fidelity (Thermofisher Scientific) with an initial denaturation step for 2 min at 94ºC, followed by 30 cycles of denaturation for 30 s at 94ºC, annealing for 30 s at 55ºC, and extension for 1 min at 68ºC and a final extension step for 10 min at 72ºC. The PCR products were analyzed by electrophoresis on 1.5% (w/v) agarose gel. PCR was repeated for samples that showed no amplification using 5 µl of extracted DNA from the same bacterial suspension. DNA extraction was performed from 0.2 ml bacterial suspension on a Nuclisens EasyMAG (bioMérieux) according to instruction manual from the manufacturer. All PCR products were purified using the Qiagen QIAquick PCR Purification Kit (Qiagen) and DNA concentration was measured in Nanodrop – 8000 Spectrophotometer (Thermofisher Scientific). The cycle sequencing reactions were performed in a GeneAmp PCR System 9700 (Thermofisher Scientific) using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermofisher Scientific) and reaction products were purified using the BigDye XTerminator purification kit (Thermofisher Scientific) according to manufacturer's instructions. Sequencing was performed on a 3500xL Genetic Analyzer (Thermofisher Scientific) at the Pathology Genetics Laboratory of Sidra Medicine in Qatar. Forward and reverse sequences were assembled using an online DNA sequence assembler PRABI-Doua (Huang and Madan, 1999) and forward and reverse primer sequences were trimmed. The sequences were then analyzed by Basic Local Alignment Search Tool (BLAST) against the GenBank 16S ribosomal RNA database (Altschul *et al*., 1997) (sequences from type material only) and the top ten closest matches according to nucleotide identity were recorded. Results were interpreted according to Clinical and Laboratory Standards Institute guidelines (CLSI) (2008). Correlation between the results from MALDI-TOF MS and 16S rRNA gene sequencing was determined by Cohen's kappa test.

For fungal strains, as mentioned before, a preliminary identification of the most dominant fungal strains was carried out using systematic manual. To confirm the species type of each isolated fungal strain, the total DNA was extracted using Invirtogene PureLink™ Plant DNA purification kit (Thermo Fisher Scientific, UK) following the manufacturer's protocol. The extracted DNA was amplified using PCR techniques with the 18S rRNA and ITS regions primers were used; ITS1F (F) (TCCGTAGGTGAACCTGCGG) and ITS4 (R) (TCCTCCGCTTATTGATATGC) (White *et al.*, 1990) as a universal DNA marker for fungal identification. The PCR reaction mix (50 µl) contained 2 µl DNA, 0.15 mM of each primer, 1X of PCR buffer with dNTPs and Taq-polymerase enzyme (Platinum PCR Supermix; Thermo Fisher, CA, USA). The PCR reactions' tubes were carried out using thermal cycler (GeneAmp PCR System 9700, Applied Biosystems, CA, USA). The PCR amplifications were performed with an initial denaturation step for 2 min at 94ºC, followed by 35 cycles of denaturation for 30 sec at 94ºC annealing for 30 sec at 52ºC. Finally, the cycles were completed with a final extension for 1 min at 72ºC. The PCR products were checked by electrophoresis on 1% (w/v) agarose gel. The PCR products were purified using the PureLink® PCR Purification Kit and kept at -20˚C. The PCR amplicons were sequenced using 20 µL sequencing reactions with the primers mentioned for each region and Big Dye™ Terminator v. 3.0 cycle sequencing premix kit (Applied Biosystems, Foster City, CA, United States) under default conditions at Molecular Infectious Diseases Laboratory in Sidra Medicine, Qatar. The sequences were assembled using Geneious (Biomatters Ltd., Auckland, New Zealand). The sequenced DNA was then subjected to Basic Local Alignment Search Tool (BLAST) using GenBank database as a reference to get the closed identification for each fungal strain.

Objective 2. Determine the source of the microbiological hazards associated with sanitary conditions at the wholesale produce market

The methodologies described under this section have already been published in El-Nemr, I, Mushtaha, M., Irungu, P, Asim, H. and Goktepe, I. 2019. Assessment of Food Safety Knowledge, Self-Reported Practices, and Microbiological Hand Hygiene Levels of Produce Handlers in Qatar. Published in Journal of Food Protection. 82 (4), 561-569.

Task 1. Evaluate the food safety knowledge level of workers at the WSFPM

Out of one hundred fifty produce handlers, one hundred twenty, who are in direct contact with fresh produce, (normally consumed raw such as cucumber, green onion, and leafy green vegetables, sold at the wholesale fresh produce market (WSFPM) in Doha), were surveyed from December 2015 to February 2016 using a structured questionnaire (Appendix 1). The questionnaire contained 25 multiple-choice questions; four of these questions were related to participants' demographic characteristics, while the remaining questions were used to collect data on the good handling practices (GHP), food safety knowledge and attitude among produce handlers, the managerial practices regarding food safety applications, hygiene level of the bathrooms, and the sanitary conditions of the WSFPM. The survey was administered in two languages (Arabic and English) and lasted for 20-25 minutes in each instance and each participant was compensated with $5. Since the workers were mostly from Bangladesh, India, and Pakistan, the survey questions were also translated orally to Urdu, Hindi, and Bengali, and explained in detail for each participant by the research team members originating from the same countries. A written informed consent was obtained from all produce handlers participated in this study at the time of surveying.

The survey questionnaire was approved by the Qatar University's Institutional Review Board (QU-IRB) committee (No. QU-IRB 509-E/15).

Task 2. Assessment of Hand Hygiene of Produce Handlers

At the time of surveying, hand swab samples were collected from each participant by rubbing a sterile wet swab (MEUS S.r.L., Italy) on their hand palms (approximately 10 cm x 10 cm) and between fingers. Each swab was directly placed in a sterile screw cap tube containing sterile semi-solid peptone water. All hand swab samples were collected in the early morning when the produce handlers were handling, cutting, and removing the spoiled parts of the produce. All swabs in tubes were kept on ice and transferred to a Microbiology laboratory at Qatar University.

It is important to emphasize here that since the produce handlers work on hourly rate salary, it was difficult to collect several swab samples from their hands during the day due to their busy work schedule. Furthermore, due to the market being managed and operated by Doha Municipality, a permission to conduct the survey and collect hand swab samples from the produce handlers was required. The time allocated to collect hand-swab samples from all handlers was around 9:00 am on those select days as permitted by the municipality.

Tubes containing swabs were vortexed vigorously for 30 seconds using a Vortex-Genie 2T (USA Scientific) to enhance the release of microorganisms into the peptone water. A series of dilutions was prepared using sterile peptone water and spread-plated in duplicate on Plate Count Agar (PCA) for total aerobic bacterial determination. The same technique was applied by using other selective media, such as MacConkey Agar (MCA) for determination of total *Enterobacteriaceae*, Eosin Methylene Blue Agar (EMBA) for total coliforms, Xylose Lactose Tergitol 4 agar (XLT4) for generic *Salmonella* spp., and Baird-Parker Agar (BPA) for *Staphylococcus*

spp. All media used in this study were purchased from Oxoid Ltd. Hampshire, UK. The presumptive target colonies (*Salmonella* Typhi, *E. coli* O157:H7, *S. aureus*) grown on selective media were identified by their color and morphology. These target organisms were selected for their prevalence and their associated in recent outbreaks witnessed in Qatar. All plates were incubated at 37-42ºC for 24-48 hr. After incubation, the total and individual strains were counted and the number of colony-forming units were expressed as Log_{10} CFU/cm^2. All bacterial colonies identified as presumptive pathogens were subjected to additional analysis as detailed above. The identification of presumptive target colonies was based on initially morphological analysis. Once the target organisms were morphologically confirmed, molecular identification techniques as described above in objective 1 were used for genus and species identification. In addition, clothing photos (uniform and shoes) of workers were collected using digital camera to assess the handlers outfit hygienic level and evaluate the standards followed by produce handlers.

Task 3. Conduct a walk-through audit as an observational study to determine the implementation of international food safety standards at the target market

During each visit to the market, a food safety assessment was conducted by the research team members (5 members) by simply observing the regular practices applied at the market and determining the implementation of international food safety standards at the target market. This walk-through audit assessed the cleanliness of the market (e.g. toilets, display area, and entrance of the market), food safety training provided to inspectors, and food safety training provided to the workers by using a checklist (Appendix 2).

Objective 3. Evaluate to which extent the different sources contribute to the microbial hazards expected to be present at the market.

While collecting fresh produce samples during each visit to the market, soil, air, and surface samples were also collected to determine the microbial hazards associated with produce samples. In addition, environmental parameters, such as temperature, wind speed, humidity and precipitation rate were obtained from the Qatar Meteorology Department (QMD).

Task 1. Collect soil and air samples to determine microbial hazards

Soil samples were collected monthly from four different sites in close proximity to the wholesale market at the time of fresh produce sample collection. These samples were collected from the following sites: a) soil between tiles at the display area of the fresh produce market, b) soil from the customers' car parking area at the fresh produce market, c) soil from the trucks' parking area at the fresh produce market, and d) soil close to the livestock market. The soil samples were collected from the upper soil surface (2 to 3 cm depth) by using sterile spatula and sterile polyethylene plastic bags, then they were kept in icebox and delivered to the Microbiology Lab at QU.

Ten grams of each soil sample was mixed vigorously with 90 ml sterile peptone water. The mixture left to settle down for 5 min, then dilution and inoculation techniques were applied using appropriate media and incubation conditions as mentioned above (Objective 1).

Air samples were analyzed using a culture settling plate method from seven different sites surrounding the WSFPM. The air samples were collected from a) periphery of the display area of the fresh produce market, b) at the center of the display area at the fresh produce market, c) in the middle of the area used for local produce

(locally grown produce), d) in the corridor where they refrigerate the produce, e) in the middle of the fish market, f) in the middle of the slaughterhouse, and g) same site where the soil samples were collected at the livestock market.

Triplicate culture plates of each media listed above were placed with lids open at the test sites for 5 min. The plates were then closed, sealed, kept in icebox, and transferred to the Microbiology Lab at QU for incubation under the proper conditions. The second technique used for air sample collected was carried out using Deployable Particle Samplers (DPS). On the same day of sample collection, the DPS samplers were placed on the roof of the WSFPM (about 7 m elevation) for overnight. The DPS is a 24-hour Li-ion battery-charged system that is easy to operate and portable. Each DPS was equipped with a compact internal impactor containing a PTFE filter (SKC Omega Specialty Division, 2.0 µm pore size, 47 mm diameter).

After each collection period, the filters were removed and placed in a tube containing 10 ml of sterile peptone water. The tubes were vortexed vigorously for 30 seconds using a Vortex-Genie 2T (Fisher Scientific, UK) to enhance the release of microorganisms into the peptone water. Then the microbial load was determined for each filter (monthly) using serial dilution technique and standard plate count method as described above (Objective 1). It is important to note that this technique was determined to be not effective in collecting air samples. Therefore, after 6 months of sampling, its use was abandoned.

Task 2. Conduct surface sampling study to determine microbial contamination levels on truck surfaces, produce containers, and trolleys

In addition to soil and air samples, surface swabs from produce containers, trolleys, and trucks' floor were collected in duplicate by wiping a sterile wet swab

(MEUS S.r.L., Italy) over each surface (10cm X 10cm). The swabs were then inserted directly in a sterile tube containing semi-solid peptone water. All swabs were kept on ice and delivered to the Microbiology Lab at QU for microbial analysis. Tubes containing swabs were treated using the same methodology as described above under objective 1.

After counting plates, the results were expressed as Log_{10} CFU/g for soil samples, Log_{10} CFU/5 min for air samples, and Log_{10} CFU/cm^2 for surface swab samples. The presumptive colonies detected on select media were sub-cultured to obtain pure cultures for identification purposes and coded by a special number for culture collection and kept in a fridge for further identification. The bacterial and fungal cultures were identified using MALDI-TOF MS and 16S rRNA techniques as mentioned before under objective 1 methodology.

Furthermore, several meteorological parameters such as temperature, wind speed, humidity, and precipitation rate were recorded during the full year study. These data were collected from Qatar Meteorology Department (QMD) for Abu-Hamour station to study the effect of environmental factors on the prevalence of different microorganisms.

Objective 4. Conduct a Microbial Risk Assessment (MRA) to determine potential health risks associated with fresh produce-related outbreaks in Qatar.

At the moment, there is no data available on the quality of fresh produce imported from overseas. Additionally, there is no microbial data available on the produce (remain unsold) at the end of each day. However, it is assumed that the unsold produce is kept in refrigerated rooms located in the market. Each of these rooms contains a temperature controlled cooling system monitored daily.

Task 1. Obtain data on food safety from MoPH regarding foodborne illnesses and outbreaks in Qatar.

A communication with the experts from the Ministry of Public Health (MoPH) and Hamad Medical Corporation (HMC) was initiated to obtain a permission to have an access to the data regarding food safety and foodborne outbreaks in Qatar. A summarized data was obtained from a workshop entitled "*Salmonella: Epidemiological Profile, clinical features, biotechnologies and challenges*" and organized by Hamad Medical Corporation (HMC) regarding the foodborne outbreaks in Qatar on Feb 13, 2017.

Task 2. Conduct a preliminary microbial risk assessment for target pathogens identified as source of contamination in fresh produce related outbreaks in Qatar, and propose appropriate guidelines to control the growth of pathogens and, thereby reduce the number of fresh produce related foodborne illnesses in Qatar.

To conduct a preliminary microbial risk assessment (MRA), the sequential steps presented in Figure 4 were followed.

A survey consisting of 22 multiple-choice questions was administered to customers at the target market mainly to: 1) determine if there is any risk associated with consuming the produce purchased from this market, 2) test their knowledge regarding the food safety practices, and 3) evaluate their satisfaction about the sanitary condition at the market. About 230 customers, who were visiting the market at the time of produce sample collection during the months of January 2016 to November 2016, participated in the survey. Throughout the administration, the research team helped the survey volunteers by reading and clarifying the questions to save time. A copy of the

questionnaire is presented in Appendix 3. The customer survey was administered in two languages (Arabic and English) and took 10-15 min. A written informed consent was obtained from all customers participated in this study. The questionnaire was approved by the Qatar University's Institutional Review Board (QU-IRB) committee (No. QU-IRB 509-E/15). The data obtained from this survey were also used in building the exposure assessment (model step 3).

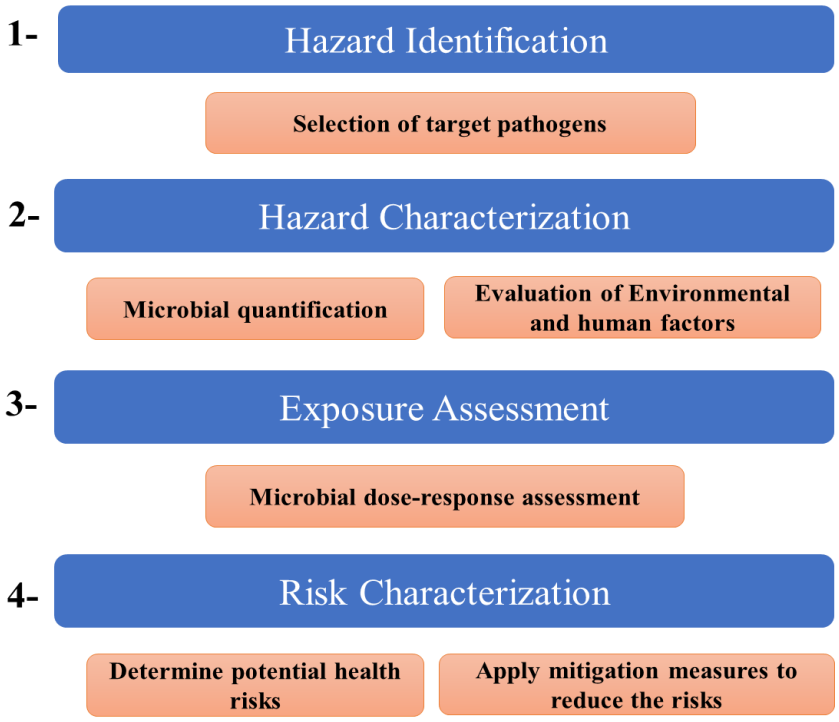


Figure 4. Schematic framework of steps of microbial risk assessment (MRA) used to conduct at the wholesale market (WSFPM) (EPA, 2012).

Step 1: Identification of the hazards and selection of the target pathogens

Based on the literature review related to the pathogens associated with fresh

produce, the following bacteria were selected as target pathogens; *Salmonella* spp., *Listeria monocytogenes*, *Staphylococcus aureus*, generic and pathogenic *E. coli*. In addition, *Alternaria*, *Aspergillus*, *Penicillium*, and *Fusarium* species were chosen as target fungal pathogens. These species were selected based on their common occurrence on fresh produce (Olaimat and Holley 2012; Qadri *et al*., 2015).

Step 2: Hazard Characterization

All the data collected from microbial analyses of produce, air, soil, surface, and hand swab samples were analyzed to identify the target pathogens. Based on molecular identification methods, none of the target pathogens was detected in any of the sample tested in this study. However, the total aerobic and coliform counts were determined to be significantly higher than the acceptable levels (> 6.7 Log_{10} CFU/g for Aerobic microbial counts and > 4 Log CFU/g for Enterobacteriaceae, and > 2 Log_{10} CFU/g for generic *E. coli*). Therefore, hazards were characterized as "total coliforms" to conduct the microbial risk assessment. The fresh produce samples tested in this study were products carrying the hazards through ingestion route.

Step 3: Exposure Assessment

The main objective of the exposure assessment is to find the probability of having pathogens in the fresh produce at the time of consumption or to estimate the dose (concentration) of the target pathogens and their effect on the population who are exposed to these pathogens. In this study, the risk of getting sick through consumption of 5 different produce (green onion, cucumber, tomato, green pepper, and romaine lettuce) was calculated using the improved swift quantitative microbial risk assessment (sQMRA) model implemented in @RISK 5.7 (Palisade) using 10,000 iterations (Chardon and Evers, 2017). sQMRA is "*a tool implemented in Microsoft Excel used to*

calculate the relative public health risk to pathogens in a food product in several points along the food chain. In addition to attribution to storage and preparation method. The model starts with consumption data and prevalence, and the concentration data at the retail level. After storage of the food at the consumers' home, it is prepared, where heating and cross-contamination are considered. Contaminated portions are the input of a dose-response relation, which is used to calculate the number of cases of illness" (Chardon and Evers, 2017).

The parameters used to build the model are listed in Table 8. The exposure assessment was conducted using the concentration of the target pathogen (coliforms) in raw vegetables (Log_{10} CFU/g). The information on the amount of consumed produce per month and the frequency of exposure was collected through applying a questionnaire conducted on customers visiting the target market (Objective 4, Task 2). In addition, the uncertainty and variability factors were identified using the same questionnaire to 1) determine if there is any risk associated with consuming the produce purchased from the target market, 2) test the food safety knowledge and practices of customers visiting the market, and 3) evaluate the customers' satisfaction with the sanitary conditions of the market. To have a good estimate for exposure assessment, the food handlers' contribution in the exposure, the environmental conditions, the exposure during processing and storage stages, and the sanitary conditions applied by consumers, beside including the duration and frequency of exposure were considered as important parameters (WHO, 1999, Dennis *et al*., 2002). However, some of these factors, such as the environmental effect and the food handler's contribution in the exposure were not inclusive factors in the sQMRA model, all of which increased the uncertainty during the risk assessment.

Table 8. The input parameters used to build the model.

Pathogen		Coliform		
Food product		*(filled using each produce alone)		
Population specification		Qatar		
Population size (POP)		2500000 (QSA, 2018)		
Consumption period in days		**(Filled depending on the produce		
Question		Symbol	Value	Unit
1	Portions consumed			
	Point estimation: portions/person/month	Npppm	**	/pppm
2	Pathogen prevalence in retail			
	Point estimation of prevalence	Cont	1	---
3	Portion size			
	Portion size: mean	Mmean	**	g
	Portion size: st dev	Mstdev	**	g
4	Pathogen concentration			
	Concentration: mean	log10Cmean	*	log10 (cfu/g)
	Concentration: st dev	log10Cstdev	*	log10 (cfu/g)
5	Endpoint dose-response model	no parameter	Illness	---
6	Dose-response parameters			
	Beta-binomial parameter: alpha	A	0.155	---
	Beta-binomial parameter: beta	B	2.4E4	---
7	DALY per case	DALY case	1000	Daly/case

* Data collected from the microbial counts (objective 1, Table 9)
** Data collected from the consumer survey (objective 4) and QMD
*** Data collected from QMD
All other parameters are the default values as built in the model (sQMRA by Chardon and Evers, 2017)

Step 4: Risk Characterization

Depending on the results obtained from the experimental study and the beta-Poisson model (risk exposure), the total effect of the hazard was considered as the potential of the target pathogen/microorganisms to be present in tested produce and causing disease. The relative exposure of a population to target microorganisms was determined by identifying the severity of disease associated with consuming fresh produce purchased from the target market. The risk was characterized by using the data collected from this study, literature review, Qatar Statistics Authority (QSA), Qatar Meteorology Department (QMD), and the default probabilities built in sQMRA model.

To characterize the risk associated with consuming the produce contaminated with coliform, the following data were used:

- The total Qatar population for people above 4 years old, which were considered as the population at risk.
- The consumption rate in gram for each produce/person/month collected from QSA (2018).
- The mean size of the produce $\pm$ standard deviation, obtained from this study. The probability of portion size was computed by using Gamma distribution with shape k and scale θ.

$$\Gamma(k, \theta),\ k, \theta > 0,\quad k = \mu^2/\sigma^2,\ \theta = \sigma^2/\mu \quad \text{(Built in the model)}$$

Where:

μ = The mean size of the produce

σ = The standard deviation of the produce size

- The mean coliform counts as expressed Log_{10} CFU/g $\pm$ standard deviation for each produce. The Log_{10} CFU/g probability function followed the normal distribution:

  $N(\mu, \sigma^2)$

  Where:

  μ = The mean of the distribution

  σ = The standard deviation

  σ^2 = The variance. Log_{10} CFU portion followed the Poisson distribution

  $Pois(\lambda)$

  Where:

  λ = portion size x Log_{10} CFU/g

- The percentage of the contaminated sample with coliform count more than the acceptable level ($\geq$4 Log_{10} CFU/g) as set by PHLS of UK guidelines (Gilbert *et al.*, 2000). In this study, all produce samples surveyed were considered as positive, so that the Binomial probability of consuming contaminated produce was "1" for all models tested.
- Dose-response measured by using Beta-binomial distribution for *E. coli* at α = 0.16, and β = 2.4e4 (Haas *et al.*, 1999).

All other probability distributions for the raw food at retail were already built in the model and used as default probability functions. The exposure data were combined with the dose-response to characterize the risk for illness probability per year. The Beta stimulation was used with 10,000 iterations in Excel @Risk model. Then the estimated risk of getting sick was assessed for consuming each produce by using dose-response model built for *E.coli* since there is no default model for coliforms.

Statistical Analysis

All data were analyzed using SPSS version 24.0 for Windows (SPSS Inc., Chicago, IL Statistical software) to obtain a 95% confidence intervals for pathogen dominance; including geometric means, standard deviations, and ranges. A multivariate linear regression analysis was conducted to study the effect of environmental factors (temperature and humidity) on the microbial counts for all samples (e.g. fresh produce, soil, air, and environmental surfaces' swabs samples). In addition, a mixed ANOVA analysis was used to compare the differences between the mean of microbial counts of all samples and the mean of all environmental data collected at different sites. A difference was considered statistically significant if *P* was less than 0.05.

Furthermore, descriptive analysis, such as means, percentage, and frequencies, was carried out on the survey data and the significance of variations was determined using ANOVA and Chi-square test (χ^2). The questions on food safety practices of handlers and handlers' opinion regarding the hygiene conditions of the market were grouped into subsets for which responses were converted into average scores. The ANOVA test was used to evaluate the significance of the demographic characteristics' of handlers on means of subsets using Bonferroni Post Hoc test. Scores associated with this "subsets" were computed by taking the mean of responses to each listed question in Tables 13 and 14. Pearson correlation was also used to estimate the significance of associations between demographics of the handlers and microbial loads of their hand palms. In addition, Microsoft Excel sQMRA model implemented in @RISK 5.7 (Palisade) was used to characterize the risk.

CHAPTER 3: RESULTS AND DISCUSSION

3.1 Assessment of the microbial quality of select fresh produce sold at the wholesale market in Doha

3.1.1 Determination of microbiological hazard(s) in select produce sold at the wholesale market

The microbial analyses conducted by using different media revealed that the leafy green produce, especially parsley and green onion, were highly contaminated with different microbiota (except for the TAC counts) compared to the fruity produce, such as tomato (Table 9). The microbial count shows that there is no significant differences in microbial quality of produce collected from different vendors, but there was a significant difference in their mean counts between the leafy green produce and fruity produce ($P< 0.05$). The high microbial load on leafy green vegetables may be due to the fact that these produce are grown close to the soil, while fruity vegetables (e.g. tomato and cucumber) grow in vines. During the sample collection, it was noticed that most green onion samples had soil particles attached to their roots, which might have increased the microbial load of this produce. These findings were also supported by many stduies (Johnston *et al.* 2005 and 2006; Aycicek *et al*., 2006; Seow *et al*., 2012; Büyükünal *et al*., 2015; Cardamone *et al.*, 2015; and Ssemanda *et al.*, 2017). Moreover, the surfaces of the leafy green produce is not smooth like the fruity vegetables, hence, the microorganisms might have a better chance to attach to the curvy areas on the surface.

Globally, various research studies were conducted to evaluate the presence of the indicator microorganisms on the fresh produce, but in Qatar, this study is considered as the first in this field. Therefore, the data collected in this study were compared to

international standards, such as HACCP-TQM technical guidelines (HACCP-TQM,1998) Public Health Laboratory Services (PHLS) of UK guidelines, and Health Protection Agency – London (HPA-UK, 2009) guidelines for ready to eat food, since there is no specific guidelines established to monitor the quality of fresh produce in Qatar.

In this study, the mean total aerobic bacteria (TAB) counts ranged between 5.39 and 6.68 Log_{10} CFU/g, which are slightly high compared to similar studies (Seow *et al*., 2012; Büyükünal *et al*., 2015; Cardamone *et al.*, 2015) that tested the same type of produce (e.g. tomato, lettuce, parsley, and cucumber). Furthermore, Johnston and his coworkers (2006) evaluated the TAB counts of the imported fresh produce coming from Mexico to the USA. The authors found that the TAB counts did not exceed 6.64 Log_{10} CFU/g for all produce samples tested, but there were no significant differences between the domestic and the imported produce. In this study, the highest mean average count was 6.68 Log_{10} CFU/g for parsley, which is normally imported from KSA. Johnston and his coworkers (2006) reported that the average microbial load, such as *E. coli*, could increase up to 2 Log CFU/g during the processing and transportation stages of the produce chain. Other studies also reported that the microbial load on fresh produce increased during the produce chain starting at the farm and finishing at the wholesale markets (Ssemanda *et al*., 2017, Faour-Klingbeil *et al*., 2016). This might indicate the use of poor or unsanitary practices at the transportation or handling stages or the effect of environmental factors (e.g. temperature) impacting the quality of produce (Gil *et al*., 2015; Faour-Klingbeil *et al*., 2016; Ssemanda *et al*., 2017).

Table 9. The mean counts (Log_{10} CFU/g) of various microorganisms in different fresh produce collected for a whole year from WSFPM.

Microbial Indicator	Sample type				
	Cucumber n = 108	Green onion n= 108	Lettuce n = 108	Parsley n = 108	Tomato n = 108
Total aerobic bacteria (TAB)	6.02 ± 0.06 (4.54-7.22)	6.53 ± 0.06 (5.29-7.78)	6.0 ± 0.11 (4.93-7.19)	6.68 ± 0.04 (5.76-7.47)	5.39 ± 0.02 (4.42-6.77)
Total *E. coli*	5.71 ± 0.05 (4.52-6.89)	5.83 ± 0.02 (4.9-6.29)	5.55 ± 0.03 (4.29-6.32)	6.18 ± 0.16 (4.7-7.07)	4.88 ± 0.03 (2.96-6.3)
Total coliform	5.63 ± 0.05 (4.42-6.64)	5.68 ± 0.02 (3.91-6.8)	5.51 ± 0.14 (4.76-6.37)	6.04 ± 0.05 (4.12-7.12)	5.02 ± 0.11 (4.4-6.57)
Total *Listeria* spp.	5.72 ± 0.05 (3.65-5.8)	4.53 ± 0.27 (2.7-6.20)	4.60 ± 0.03 (3.64-5.56)	5.15 ± 0.05 (3.31-7.02)	3.67 ± 0.42 (2.6-6.25)
Total *Salmonella* spp.	5.74 ± 0.14 (4.42-6.52)	5.83 ± 0.09 (4.76-7.02)	5.5 ± 0.17 (4.7-6.45)	6.15 ± 0.55 (4.72-7.08)	4.78 ± 0.07 (3.0-6.15)
Total Staphylococcus spp.	4.31 ± 0.05 (2.65-5.46)	4.73 ± 0.03 (3.17-6.09)	3.82 ± 0.07 (2.15-5.24)	4.67 ± 0.05 (3.59-5.93)	4.23 ± 0.24 (3.6 - 5.68)
Total Yeast/Molds (on PDA)	4.3 ± 0.09 (2.73-6.01)	5.08 ± 0.05 (3.82-6.28)	4.3 ± 0.08 (3.17-5.7)	4.48 ± 0.10 (3.27-7.25)	4.10 ± 0.21 (1.38-5.94)
Total Yeast/Molds(on RBCA)	3.39 ± 0.04 (2.88-3.92)	4.00 ±0.04 (2.0-5.26)	3.32 ± 0.05 (2.05-4.45)	3.75 ± 0.03 (2.52-4.93)	2.41 ± 0.16 (1.67-3.81)

The results are expressed as mean ± SD of all samples collected. The fresh produce samples collected in triplicate from three different vendors over a year.

It is important to mention here that determination of microbial contamination occurring at each step of produce chain was impossible in this study. Therefore, a full-year comprehensive study was carried out to evaluate the effect of the location and the environmental condition of the WSFPM.

According to the HACCP-TQM technical guidelines (HACCP-TQM, 1998), raw foods containing TAB of <4 Log_{10} CFU/g is considered as "*good*", TAB ranging between 4 and 6.7 Log_{10} CFU/g receives "*average*" rating, TAB from 6.7 to 7.7 Log_{10} CFU/g are rated as "*poor*", and TAB above 7.7 Log_{10} CFU/g points the food as "*spoiled food.*" Based on these ratings, the quality of the produce samples collected from WSFPM can be considered as "*average,*" depending on the time of the year. In certain months, the quality of produce can be rated as "*poor*" since all the TAB counts were between 6.7 and 7.7 Log_{10} CFU/g. This high reading could be normal for the root vegetables based on the United Kingdom guidelines for ready to eat foods (HPA-UK, 2009). However, these guidelines were not set as a limit for the total aerobic counts in fresh produce, because most of these microbes are considered as normal soil microflora. In addition, several studies demonstrated that fresh produce samples could still be rated as "good" even with the level of TAB reaching up to 10 Logs (Korir *et al*., 2016; Mritunjay and Kumar, 2017; Ssemanda *et al*., 2017).

The total aerobic count is normally included to test the quality of fresh produce, which gives an indication of the exposure of produce to contamination sources and how this contamination spreads and proliferates (Aycicek *et al*., 2006; Ssemanda *et al*., 2017). According to Pianetti *et al*. (2008), the total aerobic count does not connect to the foodborne diseases, but it is an indicator of the produce quality and their shelf life. Beuchat (1992) estimated that 30% of the fresh produce is lost because of the microbial spoilage from farm to the table.

The ubiquity of the pathogens in foods usually occurs in low numbers, and they normally appear periodically while most of the time they are absent (Buchanan and Oni, 2012). This makes the use of indicator microorganisms' helpful in providing more information about the microbial load of the samples and giving an idea for applying appropriate preventive measures (Buchanan and Oni, 2012). The most recommended indicator microorganisms listed in the literature are *E. coli*, total coliform, *Salmonella* spp., and *Listeria* spp., which were all used as target species in this study.

Based on the research findings, the mean Log_{10} CFU/g for generic *E. coli*. among the fresh produce analyzed ranged between 4.88 and 6.18 Log_{10} CFU/g, while the total coliform ranged from a mean of 5.02 and 6.04 Log_{10} CFU/g (Table 9). These findings were higher than the reported values in other studies testing the same fresh produce samples (Johnston *et al*., 2006; Oliveira *et al*., 2010; Seow *et al*., 2012; Büyükünal *et al*., 2015; Cardamone *et al*., 2015; Shenge *et al*., 2015; and Korir *et al*., 2016). Based on the PHLS of UK guidelines (Gilbert *et al*., 2000), these results could be considered as *"Unsatisfactory"* for all produce samples tested, since the *E. coli* count is more than 2 Log_{10} CFU/g and the coliform count is $\geq$ 4 Log_{10} CFU/g according the UK guidelines. When our results were compared to those of other studies published in KSA, India, and Rwanda, the average counts of all target organisms were relatively lower (Al-Holy *et al*. 2013; Mritunjay & Kumar, 2017, Ssemanda *et al*., 2017), except for the total coliform counts in lettuce and parsley which were at the same levels (Aycicek *et al*., 2006). The coliform counts and other microbial counts can be affected by several factors, such as produce type, environmental conditions, the added fertilizer or manure during cultivation, and the sampling location (Tango *et al*., 2018). This study mainly concentrated on the impact of the environmental conditions and the location of the market on the microbial quality of imported produce sold at the WSFPM.

The black-centered colony with yellowish-pink periphery on XLT4 agar plates was used to determine the presence of *Salmonella* spp. Moreover, a pinkish colony on MCA plates and black colony surrounded by clear zone on BPA plates were used for determination of *E. coli* O157 and *S. aureus*, respectively. The mean *Staphylococcus* spp. count for all produce tested in this study was 4.35 Log_{10} CFU/g and ranged between 3.82 and 4.73 Log_{10} CFU/g. None of the isolated *Staphylococcus* spp. led to the confirmation of *S. aureus* as a target pathogen after using molecular identification. This does not mean that the risk of *Staphylococcus* spp. is low since several species having the ability to produce high heat-stable toxins (Bhunia, 2018). The mean *Listeria* spp. (3.67 - 5.72 Log_{10} CFU/g) and *Salmonella* spp. (4.78 and 6.15 Log_{10} CFU/g) counts followed the same trend by having high levels similar to those of other studies.

Internationally, several studies reported the presence of *Salmonella* spp. in fresh produce (e.g. Jerngklinchan *et al.*, 1993; Nygard *et al*., 2008; Jain *et al*., 2009, Kroupitski *et al*. 2009; Mahmoud, 2010; Büyükünal *et al*., 2015; Sharma *et al*., 2017). In this study, the mean Log_{10} count for *Salmonella* spp. for most tested produce was more than 4 Log_{10} CFU/g. This value mainly is considered as "*Unacceptable/Potentially hazardous"* once it was detected in 25 grams of analyzed fresh produce (Gilbert *et al*., 2000). However, no black-centered isolates led to the identification of *Salmonella* spp. (Table 10), this may due to the fact that most of these strains sub-cultured and kept it in the fridge for more than six months before they were sent for identification and this action may have played a role in losing the violent characteristics of these isolates.

On the other hand, the mean count for *Listeria* spp. for most produce tested was more than 4 Log_{10} CFU/g, which is considered high when compared to the results of similar studies published in Japan, New Zealand, Spain, and UK (Kaneko *et al*., 1999;

Zhu, 2015; Selma *et al*., 2007; and Little and Mitchell, 2004). It is important to note that all presumptive *Listeria* spp. colonies (brown colonies with aesculin hydrolysis) detected in this study did not lead to the confirmation of *L. monocytogenes*. In general, *Listeria* spp. are normally associated with food production and agriculture environments (Chapin *et al*., 2014). Moreover, Angelidis and his co-workers (2015) reported that many of the presumptive *Listeria* spp. might not be accurately *Listeria* once it gives the same morphology on the selective agar plate. These findings are in agreement with the results obtained in this study since none of the presumptive colonies isolated from *Listeria* selective agar was identified as *Listeria* spp. after using 16S rRNA gene sequencing technique (Table 10). In many countries, the tolerance level for *Listeria monocytogenes* in produce and other food products is considered as "0" due to the risk of listeriosis after consuming raw vegetables, especially for the sensitive groups (e.g., children, pregnant women, elder people, and immuno-compromised individuals) (Food Standards Australia and New Zealand, 2018).

Furthermore, several fungal species were isolated from the surveyed produce. The mean fungal counts on PDA was 4.45 Log_{10} CFU/g, with min and max counts of 4.1 to 5.08 Log_{10} CFU/g (Table 9). To enhance the isolation and identification of yeasts/molds, Rose Bengal Chloramphenicol Agar (RBCA) media was also used as recommended by Mossel *et al*. (1962) for enumeration and isolation of yeasts and molds from food samples. It is clear in Table 9 that the total yeasts/molds counts on RBCA were lower than the counts determined on the PDA plates, which enhanced the isolation of the target colonies for molecular identification. The most important issue regarding the presence of fungi on fresh produce is that these fungi have the ability to grow and survive at low temperature and moisture condition increased during storage. This enhances the production of the mycotoxins and increases the potential risk of

infection toxicoinfection (Tournas, 2005).

These high TAB microbial loads on fresh produce demonstrated the unhygienic conditions applied at the target market. The produce contamination might come not only from the poor conditions at the market but also from poor conditions applied during cultivation, transportation, distribution, storage, and handling. To reduce the microbial loads, a washing step is commonly applied to remove the soil participles, which usually carry a large number of microorganisms, followed by disinfecting step to remove the remaining contaminants or inactivate some of them. Using just chlorinated water in the washing step can reduce the microbial load up to 2 Log (WHO, 1998). Corato *et al*. (2019) recently published a critical review on how to enhance the shelf life of the fresh produce by using traditional and novel preservation technologies. Based on the existing literature, it could be suggested that strict rules should be applied at the wholesale produce market in order to reduce microbial contamination levels. Specific suggestions could include: Examining the microbial quality of the imported fresh produce at the arrival point to the country (mainly ports); Applying preventive measures and decontamination techniques depending on the suitability of produce samples; Improving the sanitary conditions at the market; and lastly training the produce workers on safe produce handling practices.

The following section was extracted from El-Nemr, I, Mushtaha, M., Sundaraju, S., Fontejon, C., Suleiman, M., Tang, P., Goktepe, I., Hasan, M.R. 2019. Application of MALDI Biotyper System for rapid identification of bacteria isolated from fresh produce market. Published in Current Microbiology, 76(3):290-296 (early online print: DOI.org/10.1007/s00284-018-01624-1).

3.1.2 Identification of target pathogens using MALDI-TOF MS and 16S rRNA gene sequencing

All produce samples analyzed in this study were examined for the presence of *E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* using molecular techniques. After isolating the presumptive colonies from select agar plates, MALDI-TOF MS and 16S rRNA gene sequencing techniques were carried out to determine the target organisms at the genus and species level. The results indicated that none of the target pathogens was identified as a positive target pathogen in any of the fresh produce sample tested in this study.

The results of 16S rRNA sequencing (Table 10) revealed that the majority of the identified strains isolated from fresh produce belonged to *Enterobacteriaceae* family, *Acinetobacter*, *Aeromonas*, *Bacillus* spp., *Pseudomonas* spp., *Sanguibacter baumanni*, *Staphylococcus*, and *Stenotrophomona*. The presence of *E. coli* in the fresh produce samples indicated fecal contamination, which might come from the human intestinal tract, using poor quality of manure, or irrigating produce with contaminated water (Aycicek *et al*., 2006).

In addition, other normal soils, water, and produce microflora with no public health concern were isolated from the produce samples, such as *Citrobacter* spp., *Enterobacter* spp., and *Klebsiella* spp. These non-fecal coliform are normally isolated from fresh produce, especially lettuce (Johannessen *et al*., 2002). However, the presence of *Klebsiella pneumoniae* in the produce samples might be a public health concern since it might cause pneumonia for susceptible consumers, such as children, pregnant women, elderly people, and patients with immune system problem (Food Standards Australia & New Zealand, 2018).

Most of the strains listed in Table 10 were consistent with normal fresh produce

microbiota as identified before in parsley, lettuce, green onion, and coriander (Al-Holy *et al.*, 2013). Falomir and his coworkers (2010) isolated several coliform bacteria from fresh produce collected from cultivation fields, supermarkets, and salads. They also isolated some strains that were also identified in produce samples tested in this study, such as *Klebsiella*, *Enterobacter*, *Serratia*, *Citrobacter*, and *Pantoea*. Al-Kharousi *et al*. (2016) investigated the prevalence of pathogens in local and imported fresh produce sold in Oman. The authors reported that *Enterobacteriaceae* (60%) were the most prevalent species in fruits and vegetables tested. In addition, *E. coli* (22%) and *S. aureus* (7%) were dominant pathogens in vegetables analyzed in their study.

One of the most common microorganisms was identified as *Pseudomonas aeruginosa*, which is considered as an opportunistic disease causing bacteria for human, as well as animals, and plants. This pathogen is also resistant to different antibiotics, increasing its severity when it is associated with fresh produce that are normally consumed raw (Falagas *et al*., 2006).

In terms of the identification of fungal species from produce samples analyzed in this study, it was found that the most dominant fungi were *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., *Fusarium* spp., and *Saccharomyces* spp. (not confirmed by molecular analysis, because of lack of the DNA primers), all of which are common species in soil and the environment. Some of the identified fungi are considered as plant pathogens, such as *Alternaria alternate*, which is normally isolated from infected plant leaves. This organism *Aspergillus niger* can infect the upper respiratory tract in humans causing asthma especially for the immuno-compromised people (Barac *et al*., 2018). The occurrence of fungi on produce enhanced the potential risk factor since most of these fungal strains produce mycotoxins and result in serious long-term health effects (Moss, 2008; Trucksess and Scott, 2008). As mentioned before, most of molds have the ability to grow at low storage temperature and produce mycotoxins, which increase the potential risk of food intoxication (Tournas, 2005).

Table 10. Identification of the bacterial/fungal species isolated from fresh produce using 16S/18S rRNA gene sequencing.

Microbial strain	Identity	Prevalence (%)*
Acinetobacter radioresistens [4]	99.0	16
Aeromonas sobria [1], [6]	100	17
Bacillus licheniformis [2]	99.0	19
Bacillus pumilus [2]	99.0	10
Bacillus subtilis [2]	100	13
Citrobacter freundii [1]	100	17
Enterobacter cloacae [1, 6]	99.0	16
Enterobacter hormaechei [1]	100	9
Enterobacter xiangfangensis [1]	99.0	6
Enterococcus faecium [4]	100	30
Enterococcus gallinarum [4]	100	13
Erwinia endophytica [1]	98.0	9
Escherichia coli [1]	99.0	11
Escherichia coli [4]	99.0	13
Klebsiella pneumonia [4]	100	20
Kosakonia cowanii [1]	99.0	3
Lelliottia amnigena [6]	99.0	14
Pantoea agglomerans [4]	98.57	8
Proteus mirabilis [1]	99.0	7

*Prevalence (%) depending on average monthly counts of microorganisms analyzed for 12 months

[1] isolated from EMBA, [2] isolated from PCA, [3] isolated from BPA, [4] isolated from MCA, [5] isolated from LSA, [6] isolated from XLT4, and [7] isolated from PDA

Table 10 Cont. Identification of the bacterial/fungal species isolated from fresh produce using 16S/18S rRNA gene sequencing.

Microbial strain	Identity	Prevalence (%)*
Pseudomonas aeruginosa 2	99.0	17
Pseudomonas azotoformans [2]	100	10
Pseudomonas putida [2]	99.0	9
Sanguibacter suarezii [5]	100	34
Serratia plymuthica [1]	94.0	5
Staphylococcus cohnii [3]	99.0	40
Staphylococcus sciuri [3]	100	53
Stenotrophomonas maltophilia [6]	99	42
Alternaria alternate [7]	99	13
Alternaria destruens [7]	99	5
Aspergillus niger [7]	100	13
Aspergillus oryzae [7]	99	5
Fusarium oxysporum [7]	99	10
Penicillium aurantiocandidum [7]	99	20
Penicillium expansum [7]	100	20
Penicillium spinulosum [7]	99	9
Trichoderma citriviride [7]	98	5

*Prevalence (%) depending on average monthly counts of microorganisms analyzed for 12 months

[1] isolated from EMBA, [2] isolated from PCA, [3] isolated from BPA, [4] isolated from MCA, [5] isolated from LSA, [6] isolated from XLT4, and [7] isolated from PDA

3.2. Determination of the source of the microbiological hazards associated with sanitary conditions of the wholesale produce market.

This section was extracted from El-Nemr, I, Mushtaha, M., Irungu, P, Asim, H. and Goktepe, I. (2019), entitled Assessment of Food Safety Knowledge, Self-Reported Practices, and Microbiological Hand Hygiene Levels of Produce Handlers in Qatar. Published in Journal of Food Protection. 82 (4), 561-569.

3.2.1 Evaluation of the hygiene condition of workers and their level of food safety knowledge

The survey results revealed that most of the produce handlers were Bangladeshi (50.0%) and Indian (42.5%), while 7.5% came from other nations (e.g., Nepal and Pakistan). The median age interval of the workers was 31-40 years and 65.0% of the workers had worked at the market for more than 5 years. In terms of education, 57.5% of them had lower than high school attainment. Table 11 shows the demographic data for the participating produce handlers.

The preliminary assessment of food safety knowledge and hygiene level of produce handlers working at the wholesale produce market in Doha, Qatar, revealed that none of the produce handlers received an official training on good handling practices (GHP) nor on food safety (Table 12). All produce handlers were required to have an annual health check-up for typhoid, tuberculosis, and viral diseases such as Hepatitis A and B to renew their work permit. Wearing of gloves, head coverings or closed shoes was not enforced. Although the workers were supposed to wear a specific green colored uniform for easier recognition by customers and inspectors, there were no guidelines about the cleanliness of their uniforms. When the participants were asked about the frequency of changing their uniforms, 90.0% claimed to change their uniforms daily, which is a good indicator of personal hygiene.

Table 11. Demographic characteristics of surveyed produce handlers (n=120).

Parameter	Characteristic	Number (%)
Country of origin	Bangladesh	60 (50)
	India	51 (42.5)
	Others (Nepal/Pakistan)	9 (7.5)
Age	≤ 20 years old	0 (0.0)
	21-30 years old	27 (22.5)
	31-40 years old	44 (36.7)
	41-50 years old	28 (23.3)
	51- 60 years old	19 (15.8)
	> 60 years old	2 (1.7)
Education level	No formal education	3 (2.5)
	Elementary	42 (35.0)
	Middle school	24 (20.0)
	High school	45 (37.5)
	College	4 (3.3)
	Graduate	2 (1.7)
Work experience	< 1 year	3.0 (2.5)
	1-3 years	15.0 (12.5)
	>3-5 years	24.0 (20.0)
	> 5 years	78.0 (65.0)

It was observed that 92.0% of produce handlers did not wear gloves during the fresh produce handling process, which is a recommended practice in any food establishment according to WHO guidelines for hand hygiene (WHO, 2012). Wearing gloves can reduce the risk of food contamination coming from food handlers; however, this does not affect or replace the importance of washing hands (Berger *et al*., 2010; Monaghan & Hutchison, 2016). In a summary of factors leading to foodborne outbreaks in the USA between 1961 and 1982, about 18.0% of the outbreaks occurred because of inappropriate practices applied by food handlers, leading to contamination with pathogenic microorganisms (Bryan, 1988; Olaimat and Holley, 2012). To address this risk from food handlers, Todd *et al*. (2010) advised the use of physical and chemical barriers such as gloves, head and shoe covers and sanitizers to reduce the risk of microbial contamination of food. One interesting finding of this study was that none of the produce handlers reported taking sick leave during any illnesses since they were paid daily and did not want to lose their income (Table 12).

All participants indicated on using city water (municipal water) to wash the display area and 91.0% of them used municipal water to clean the fresh produce plastic containers. About 9.0% of participants did not wash the containers at all since they were using disposable containers, and none of them mentioned the use of detergent or disinfectant to clean these plastic containers (Table 12).

Almost 98.0% of the surveyed workers claimed to wash their hands after using the bathroom (Table 13). In fact, more than 76.0% of produce handlers participated in the study indicated to wash their hands four or more times daily with soap. However, these results (Tables 13 and 14) were not supported by our observations during the walk-through audit for the bathrooms where no soap was available, and some of the bathroom sink faucets were broken which created a long queue to wash hands. The

WHO and CDC advise that proper hand washing involves rubbing wet hands together using soap for about 20-30 seconds, followed by rinsing with clean water and drying (CDC, 2015; WHO, 2009 & 2012).

Table 12. Self-reported hygiene practices and food safety knowledge of produce handlers participated in the survey (n=120).

Questions (Did/Do you…)	Yes (%)	No (%)
Receive training before working?	0	100
Have the compulsory annual health check-up?	100	0
Have a systematic hygiene check (e.g. hair and nails)?	100	0
Wear a uniform?	100	0
Take sick leave when ill?	0	100
Use the first aid kit located in the market?	43	56
Visit the closest clinic if injured?	50	50
Keep unsold produce in a refrigerator?	100	0
Clean the display area daily using municipal water?	100	0
Clean the plastic containers daily using tap water, detergents or other disinfectants?	91	9*

* The respondents mentioned that they are using disposable containers.

Table 13. Self-reported hygiene practices applied by surveyed produce handlers (n=120).

Questions	Multiple choice answers	Frequency (%)
How do you wash your hands after using the bathroom?		
	No washing at all	1 (0.8)
	Just tap water	55 (45.8)
	Using liquid soap	63 (52.5)
	Using liquid soap and hand sanitizer	1 (0.8)
How often do you wash your hands during the workday?		
	None	1 (0.8)
	2-3 times	27 (22.5)
	4-5 times	76 (63.3)
	More than 5 times	16 (13.3)
Do you wear gloves during work?		
	No	110 (91.7)
	Yes, Working gloves	1 (0.8)
	Yes, Disposable gloves	9 (7.5)
How many days do you wear the same uniform?		
	More than 5 days	2 (1.7)
	4-5 days	1 (0.8)
	2-3 days	9 (7.5)
	1 day	108 (90.0)

Table 14. Produce handlers' opinion regarding the sanitary conditions of the bathrooms at the WSFPM and their daily practice on visiting the surrounding markets.

Questions	Multiple choice answers	Frequency (%)
How do you rate the hygiene level of the bathrooms?	Poor	44 (36.7)
	Needs some improvement	5.0 (4.2)
	Good	43 (35.8)
	Very Good	8 (6.7)
	Excellent	20 (16.7)
How do you rate the sanitary conditions of the WSFPM?	Good	39 (32.5)
	Needs some improvement	71 (59.2)
	Don't know	10 (8.3)
Do you visit the surrounding markets? (e.g. fish market, poultry market & slaughterhouse)	Rarely	11 (9.2)
	Sometimes	36 (30.0)
	Most of the time	13 (10.8)
	No	60 (50.0)

The produce handlers were asked to answer other detailed questions regarding their safe produce handling practices, hygiene practices, and their opinions on the cleanliness of the market and surrounding areas. Surprisingly, the handlers considered the sanitary conditions of the bathrooms located in the market as "good or excellent" (Table 14). In addition, 59.0% of the produce handlers felt that the sanitary conditions of the wholesale produce market, in general, need some improvement. The chi-square test (χ^2 test) showed no statistically significant association between the age of produce handlers and any of the hygiene practices (P=0.4, data not shown). Similarly, the

Bonferroni post hoc test exhibited no significant relationship with age or work experience against the "food safety practices subset score" ($P > 0.05$). These results are in disagreement with other studies (Al-Sakkaf, 2013; Brennan *et al*.,2007; Nesbitt *et al*., 2009; Unusan, 2007) in which it was demonstrated that age is an important factor associated with understanding the application of safe food handling practices. However, the level of education attained had a significant association with handlers' self-reported handwashing practices and the frequency of hand washing during work hours (Fisher exact test, $P<0.05$ and Bonferroni test, $P< 0.001$), especially with those who had elementary school degree vs. high school or more level of education. The produce handlers' attitudes and practices reported in this study (Tables 14 and 15) can be compared with the results of studies conducted in Ghana (Annor and Baiden, 2011) and Brazil (Soares *et al*., 2012), demonstrating that age plays no role in food safety knowledge for food handlers. Furthermore, Bonferroni post hoc test detected a significant difference between the "food safety practices subset score" of handlers classified under "Other nationalities" compared with "Indians" and "Bangladeshis." This was expected since the "Other nationalities" group had very few members (7.5%) when compared to "Indians" (42.5%) and "Bangladeshis" (50.0%) groups. As the education level of the handlers increased, their rating on the sanitary conditions of the market decreased (Table 11). The results also revealed a positive correlation ($P< 0.001$) between the "food safety practices subset score" and handlers' opinion. Which mean handlers with higher handling practice score were higher in their opinion to be close to the actual situation of the sanitary conditions of the market. Clayton *et al*. (2002) explained the need to understand the food hygiene behavior of food handlers before designing any training course because, besides personal beliefs and attitudes, environmental and social behavior play a major role in food safety and handling

practices. Similarly, the produce handlers' opinion regarding the hygiene level of the wholesale produce market and the condition of the bathrooms were significantly related at $P<0.05$. As no food safety training was provided to the produce handlers, this finding suggests that education level may have been the most important factor contributing to personal hygiene and the application of a moderate degree of appropriate food safety practices at the market. Hamuel *et al*. (2014) and Abdul-Mutalib *et al*. (2012) reported the importance of providing food handlers with specific training to enhance food safety practices to match acceptable standards. Additionally, management support and infrastructure are important in the transfer of food safety knowledge (Seaman and Eves, 2010) as well as in the application of appropriate hand hygiene practices.

Another interesting result obtained in this study was the frequency of the produce handlers' visits to the surrounding markets (e.g., fish market, Doha slaughterhouse, poultry, large animal markets). Among 120 produce handlers that participated in this study, 36% of them claimed that they visited some of these markets daily, as they help customers carry the purchased produce from the wholesale produce market area to other areas (Table 14). This preponderance might enhance the acquisition of livestock-associated pathogens, such as *Salmonella* spp. and *E. coli* O157:H7, which are easily colonized and transferred either by shoes or by touching the fresh produce in the absence of proper cleansing (USDA, 2017).

Table 15 presents the average microbial counts of hand swab samples collected from the 120 participants. The ANOVA analysis revealed highly significant differences among the nationality groups (at 5% level of significance) for the total aerobic microbial counts ($P<0.001$). The main differences were observed between Bangladeshi and "Other nationalities" and between Indian and "Other nationalities" ($P<0.05$) groups, but no statistical difference was recorded between Bangladeshi and Indian

workers (Table 14). This result may be attributed to the low sample number of "Other nationalities" compared to Indian and Bangladeshi groups. On the other hand, the total staphylococcal, total coliform and generic *E. coli*, and enteric bacterial counts exhibited no statistical differences across different nationalities ($P>0.05$). Furthermore, the Bonferroni Post hoc test did not show any significant differences between the microbial loads of produce handlers' palms tested against the demographical characteristic ($P>0.05$). Additionally, the Pearson Correlation analysis revealed that the "food safety practices subset scores" were not correlated to the "microbial loads" of the food handlers' palms.

Table 15. Microbiological assessment of produce handlers' hand hygiene levels (Log_{10} CFU/cm^2).

Microbial Indicator	Produce Handlers' Nationality			
	Bangladeshi n = 60	Indian n= 51	Other n = 9	All Handlers n = 120
Total aerobic bacteria	6.42 ^{a}± 1.26	6.39^{a} ± 0.96	6.00^{b} ± 0.72	6.3 ± 1.13
Generic *E. coli*	5.54 ± 1.47	5.08 ± 1.41	5.46 ± 1.14	5.37 ± 1.44
Total coliform	5.62 ± 1.30	5.78 ± 0.89	5.9 ± 0.36	5.74 ± 1.13
Salmonella spp.	5.35^{a} ± 1.23	5.15^{a} ± 1.14	6.5^{b} ± 0.08	5.45 ± 1.20
Staphylococcus spp.	4.42 ± 1.17	4.30 ± 1.14	4.8 ± 0.28	4.47 ± 1.14

The results are expressed as mean ± SD of all samples collected at the same time from each group of food handlers.

^{a,b,c} Values in the same row not sharing the same superscript are significantly different at $P<0.05$.

Bacteriological assessment of produce handlers hand hygiene (Table 15) revealed that the average bacterial counts of total aerobes, total coliform, total enteric bacteria, and *Staphylococcus* spp. exceeded the allowable limit of <2 Log_{10} CFU/cm^2 as suggested for food handlers in general (Jacxsens *et al*., 2010; de Quadros Rodrigues *et al*., 2014). It is important to note here that the produce handlers' were constantly handling wholesale produce starting from 6 am until the time of swabbing. Therefore, the high microbial loads of handlers' palms could also be due to handling produce that has not been washed or processed. This plausible reasoning needs further investigation to prove the fact that the workers had some theoretical knowledge of ideal food safety practices, but failed to apply this knowledge into practice or the results were directly related to the natural sources (unwashed produce). The main goal of handwashing is to remove bacteria from hands, palms, fingers, and fingertips (WHO, 2009 a, b). Additionally, the major purpose of using antimicrobial soap is to kill microorganisms (WHO, 2009 a, b). In a study to explain the importance of training food handlers on proper hand washing practices to enhance the safety of food, it was reported (Jevšnik *et al*., 2008) that proper hand washing led to a microbial reduction in hands, especially in *E. coli* and *Staphylococcus aureus* counts.

The molecular identification of the most dominant bacterial strains (isolated from produce handlers' using 16S rRNA gene sequencing) revealed that the following organisms were the most prevalent strains: *Bacillus circulans* (40%), *Staphylococcus sciuri* (25%), *Brachybacterium conglomeratum* (17%), *Enterococcus faecium* (17%), *Klebsiella pneumonia* (17%), *Alcaligenes faecalis* (14%), and *Stenotrophomonas maltophilia* (14%). In addition, *Brevibacterium antiquum, Pantoea agglomerans, Corynebacterium callunae, Pseudomonas mucidolens, Planococcus halocryophilus, Cronobacter zurichensis, Pseudomonas geniculata,* and *Kocuria flava* were also

detected but with prevalence rates of 10% or lower (data not shown).

It is important to emphasize that no pathogenic bacteria, such as *S. aureus, E. coli* O157:H7, and *Salmonella spp.* were detected from the hand swab samples collected from produce handlers participated in this study. Some of the microorganisms isolated from the hands of produce handlers were identified as normal skin microflora, such as *Brevibacterium* spp., *Corynebacterium* spp., and *Staphylococcus sciuri*. However, the identification of several opportunistic pathogens, such as *Enterococcus faecium, Klebsiella pneumonia,* and *Pantoea agglomerans* might indicate either poor hand hygiene practices applied by produce handlers or handling raw produce contaminated with a range of bacteria. The results could also be related to the frequent movement of produce handlers between the wholesale fresh produce market and surrounding markets, i.e., fish market and poultry slaughterhouse. This free movement between markets might have encouraged the transfer of some of these microorganisms of different origins via hands, shoes or shared equipment such as trolleys.

It is well known that when food handlers work with dirty hands or carry infectious pathogens, they can contaminate food and transfer pathogens, such as *Klebsiella pneumoniae, Salmonella* spp., *Shigella* spp., *Pseudomonas* spp., *Stenotrophomonas* spp. and *Proteus* spp. to food items, which can cause foodborne diseases and/or outbreaks (Feng *et al*., 2002; Jay, 2012). This is because *E. coli*, *Salmonella*, *Shigella*, etc. are normally found in the intestinal tract of humans and animals (Feng *et al*., 2002; Jay, 2012). On the other hand, human palms and mucous membranes could also have *S. aureus* as a normal skin flora (Noble, 1998). It was also demonstrated (Tan *et al*., 2014) that many microorganisms isolated from food handlers, such as *E. coli* and *S. aureus*, can be highly resistant to multiple antibiotics, thus further increasing the public health risk. Hence, food handlers should maintain appropriate

hand hygiene and sanitary conditions by applying good hygiene practices.

The uniform and shoes photos of workers were also collected to assess the handlers outfit hygiene level and evaluate the standards followed by produce handlers (Figure 5). The photo analysis revealed that all produce handlers were wearing a special uniform with specific color (dark green) as recommended by the Doha Municipality. This outfit makes the handlers easily recognized by the market customers, while the vendors have yellow color of uniform. About 45% of the workers did not follow the proper standards set for their uniforms, e.g. uniform cleanness, long sleeves shirt, and closed toe shoes. Figure 5 provides examples of some photos of the workers taken at the time of the hand swab collection. These photos confirm the fact that there is no systematic inspection regarding handlers' personal hygiene and their outfits. Based on a conversation with the market supervisor, it was reported that the Municipality provides each handler with 4 suitable uniforms (2 for winter and 2 for summer) in addition to proper shoes, since all the handlers are considered as a very low income workers who cannot afford to buy the uniforms.

3.2.2 Assessment of the implementation of international food safety standards at the target market by conducting a walk-through audit

During each visit, a food safety assessment was conducted by simply observing the regular practices, which are applied at the market. Although the director of the wholesale produce market claims that international food safety standards (such as HACCP) are applied at the wholesale market, no indication of such application was observed during the walk-through audit (Table 16).

Figure 5. Photos of workers exhibiting the improper uniform codes (e.g., pulled sleeves, open-toe shoes, and not clean uniforms).

It is important to note that no systematic produce sampling is regularly carried out to quantitatively analyze the microbial quality of produce sold at this target market. This is a usual practice applied as part of the HACCP standards. It should be emphasized here that the Municipality conducts certain tests including 1) chemical analysis to determine the presence and levels of pesticide residues in randomly selected produce and 2) sensory analysis (a smell test) to identify if the produce coming from overseas or local farms meets the sensory guidelines. The produce sampling for chemical

analysis and smell test are usually carried out by a Doha municipality inspector at the time of produce truck arrival. The produce samples collected for pesticide analysis are then sent to the food analysis lab under the jurisdiction of the Ministry of Public Health. A microbial analysis for select produce takes place only when there is a customer complaint. It is clear that the HACCP standards are not closely followed based on the stated international guidelines. The HACCP standards require periodic sampling,

Table 16. The results of Walk-through Audit determining the implementation of food safety standards at the WSFPM.

Observation	Compliance (%)*
Systematic inspection conducted	0%
Food safety training provided to inspectors	100%
Food safety training provided to workers	0%
Produce storage place	70%
Cleanliness of the market:	
o Entrance	50%
o Garbage disposal and storage	50%
o Toilets	10%
o Display area	40%
o Surrounding place	30%
o Presents of insects/animals (e.g. flies, mosquito, and cats)	40%

*Compliance (%) is the average taken from 5 research team members evaluating the situation during several visits to the WSFPM during the sampling step.

which means the usual procedure should include sampling, verifying, and validating steps to identify the common microbial hazards and then apply corrective actions to control these hazards. At the time of walk-through audit, it was noticed that the food safety knowledge of the inspectors was found to be very limited to no existence.

3.3 Evaluation of the effect of different sources contributing to the microbial hazards determined at the market.

3.3.1 Effect of environmental factors on the produce quality

To study the effect of environmental conditions on the produce quality sold at the WSFPM, a detailed comprehensive microbial count for each produce using several selective media was plotted with the environmental parameters such as; temperature and relative humidity recorded during the 12 months of the study (Figure 6. A, B, C, D, E, F and G). A multivariate linear regression analysis was conducted to study the effect of environmental factors on the microbial counts and their associations with produce type as a control factor. This test revealed that there is a significant ($P < 0.05$) effect of temperature, humidity, and wind speed (not presented in Figure 6) on the microbial counts of all samples analyzed in this study, except for *E. coli*. The results showed that an increase in temperature and humidity by 1 unit led to a decrease in the microbial load by 0.01-0.02 unit ($P < 0.05$), while an increase of wind speed by 1 unit resulted in an increase by 0.2 to 0.4 unit in microbial load ($P < 0.05$). This may explain the impact of the surrounding environment on the microbial quality of fresh produce sold at the WSFPM, which is located in an open uncultivated environment. The wind may carry various microorganisms some of which could be pathogens. Since the WSFPM is in close proximity to the livestock market, fish market, slaughterhouse, and industrial area, the high microbial loads in fresh produce samples could be attributable to the

environmental factors. In this study, it was impossible to collect the produce samples from the trucks at the port (Abu-Samra borders); therefore, the study was not able to assess the microbial quality of the produce at the time of arrival to the country. The results obtained for the effect of environmental factors (e.g., temperature, humidity, and wind speed) on the microbial quality of fresh produce samples are in consistent with previous research findings in which it was proven that the environmental factors significantly impact the produce quality (Marvasi *et al*., 2013; Gil *et al*., 2015; Ssemanda *et al*., 2017). Choi *et al*. (2011) listed several environmental parameters, such as temperature, humidity, and ultraviolet light, affecting the viability of pathogens on plant leaves. The plant surface microbiota can be influenced by these environmental factors, which may impact positively or negatively the presence of pathogens by promoting or preventing their growth (Cooley *et al*., 2006; Lopez-Velasco *et al*., 2011).

Furthermore, the microbial identification of the most dominant species (Section 3.1.2) showed that *Bacillus*, *Enterococcus*, and *Klebsiella* were the most dominant strains in summer months (45°C daytime and 35°C nighttime), while *Pseudomona*s and *Enterobacter* were the most abundant species during the winter months (24°C daytime, 12°C nighttime). It is clear that environmental conditions, such as temperature and humidity may play a role on the growth of different microorganisms in fresh produce. Chapin and his co-workers (2014) reported that the prevalence of *Listeria* spp. isolated from vegetables in both agricultural and retail environment can be affected by meteorological factors; also the geographical location can play a role in *Listeria* spp. isolation. Additionally, Marvasi *et al*. (2013) reported that the proliferation rate of *Salmonella* spp. on tomato fruits can be affected by seasonal variation and the most favorable condition for *Salmonella* spp. growth was during low relative humidity and precipitation conditions. It should be emphasized here that the precipitation rate in Qatar is too low since the state is located in an arid area. During the period of sample

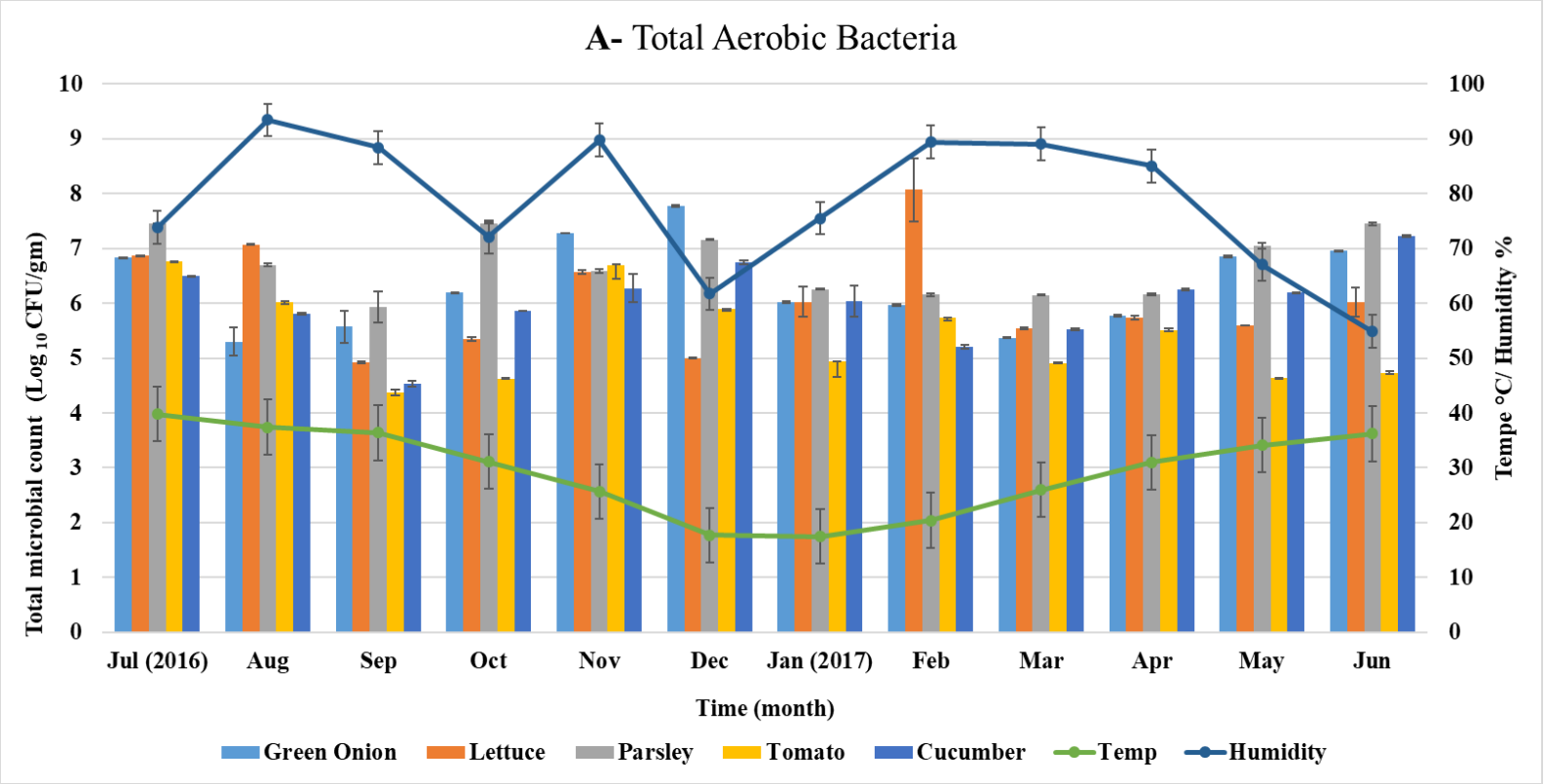


Figure 6. Total microbial counts (Log_{10} CFU/g) of select produce surveyed during the period of July 2016 - June 2017. Monthly averages of the A-Total Aerobic Bacteria on PCA, B- Total *E. coli* on MCA, C- Total Coliform on EMBA, D- Total *Listeria* spp. on LSA, E- Total *Salmonella* spp. on XLT4 agar, F- Total *Staphylococcus* spp. on BPA, and G- Total Yeats/Molds on PDA.

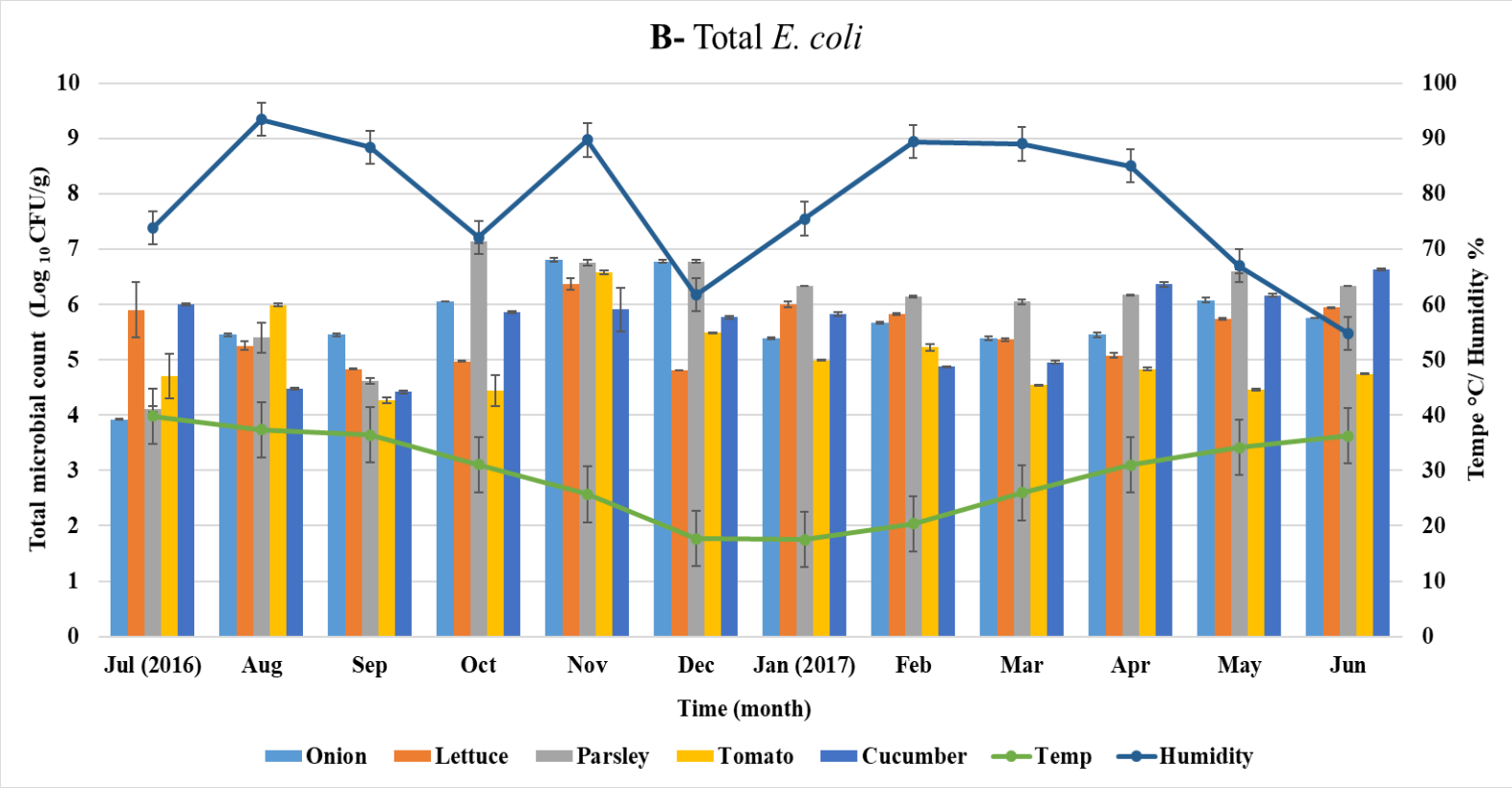
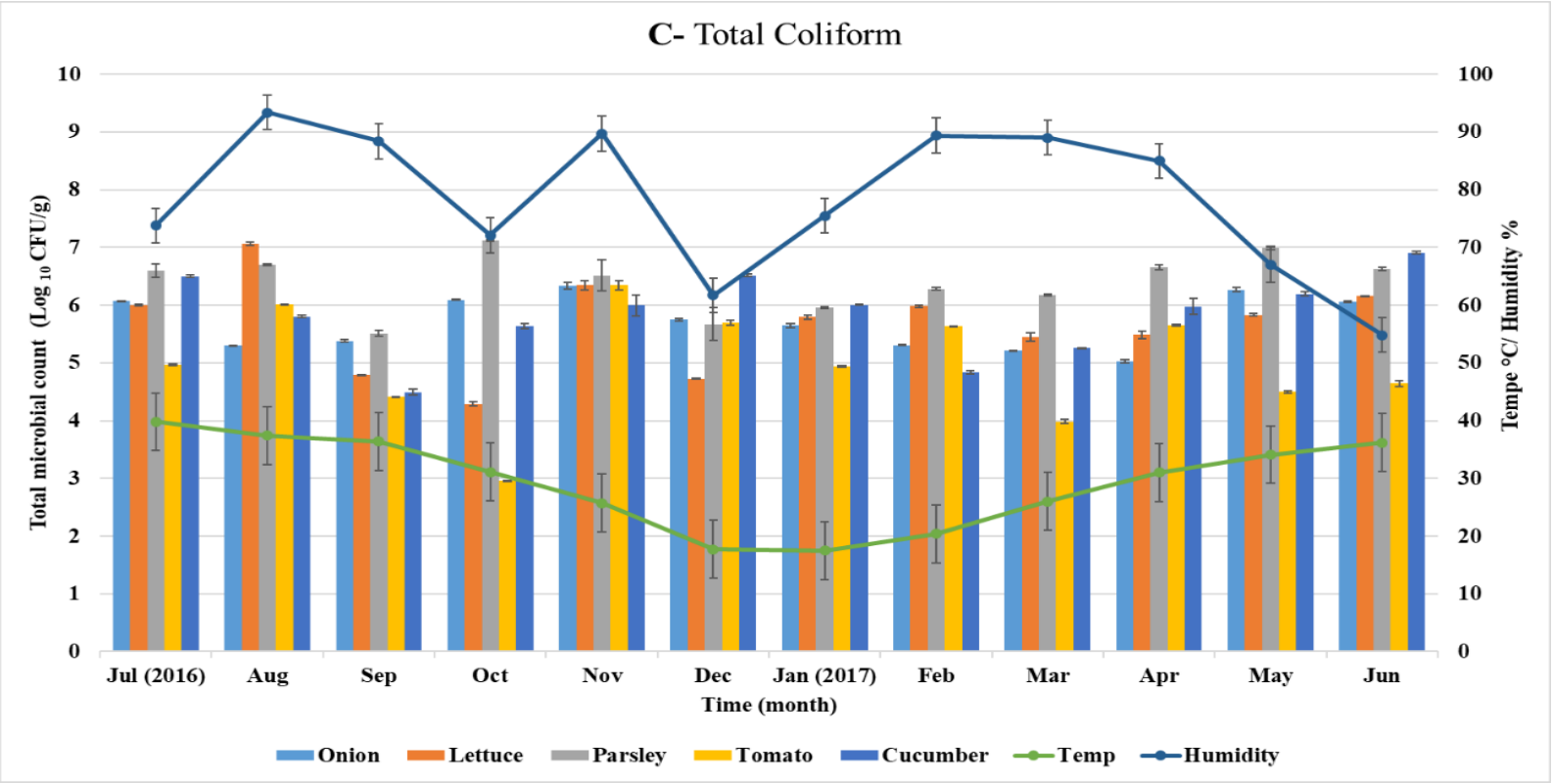


Figure 6 Cont. Total microbial counts (Log_{10} CFU/g) of select produce surveyed during the period of July 2016 - June 2017. Monthly averages of the A-Total Aerobic Bacteria on PCA, B- Total *E.coli* on MCA, C- Total Coliform on EMBA, D-Total *Listeria* spp. on LSA, E- Total *Salmonella* spp. on XLT4 agar, F- Total *Staphylococcus* spp. on BPA, and G- Total Yeats/Molds on PDA.



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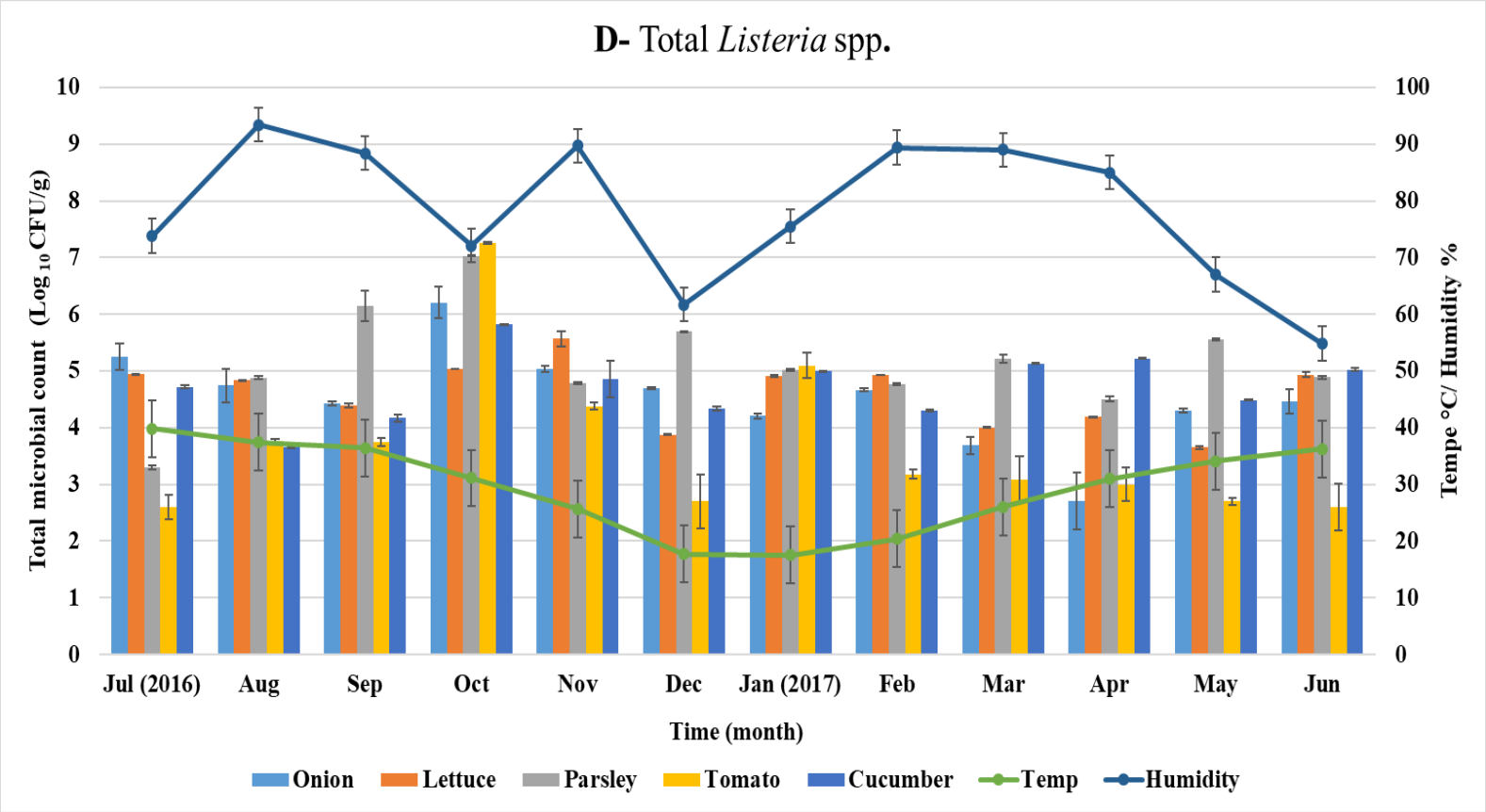

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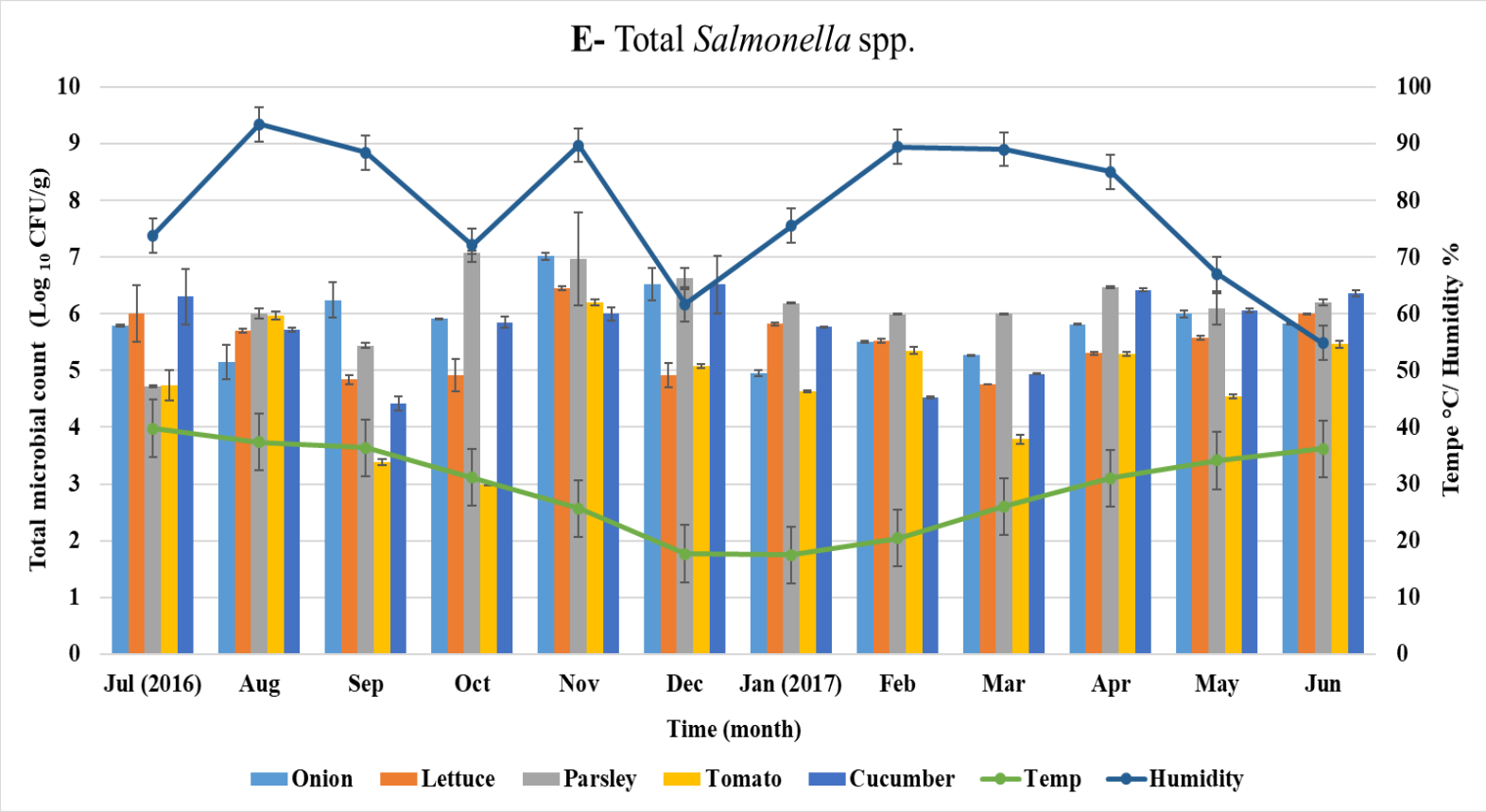

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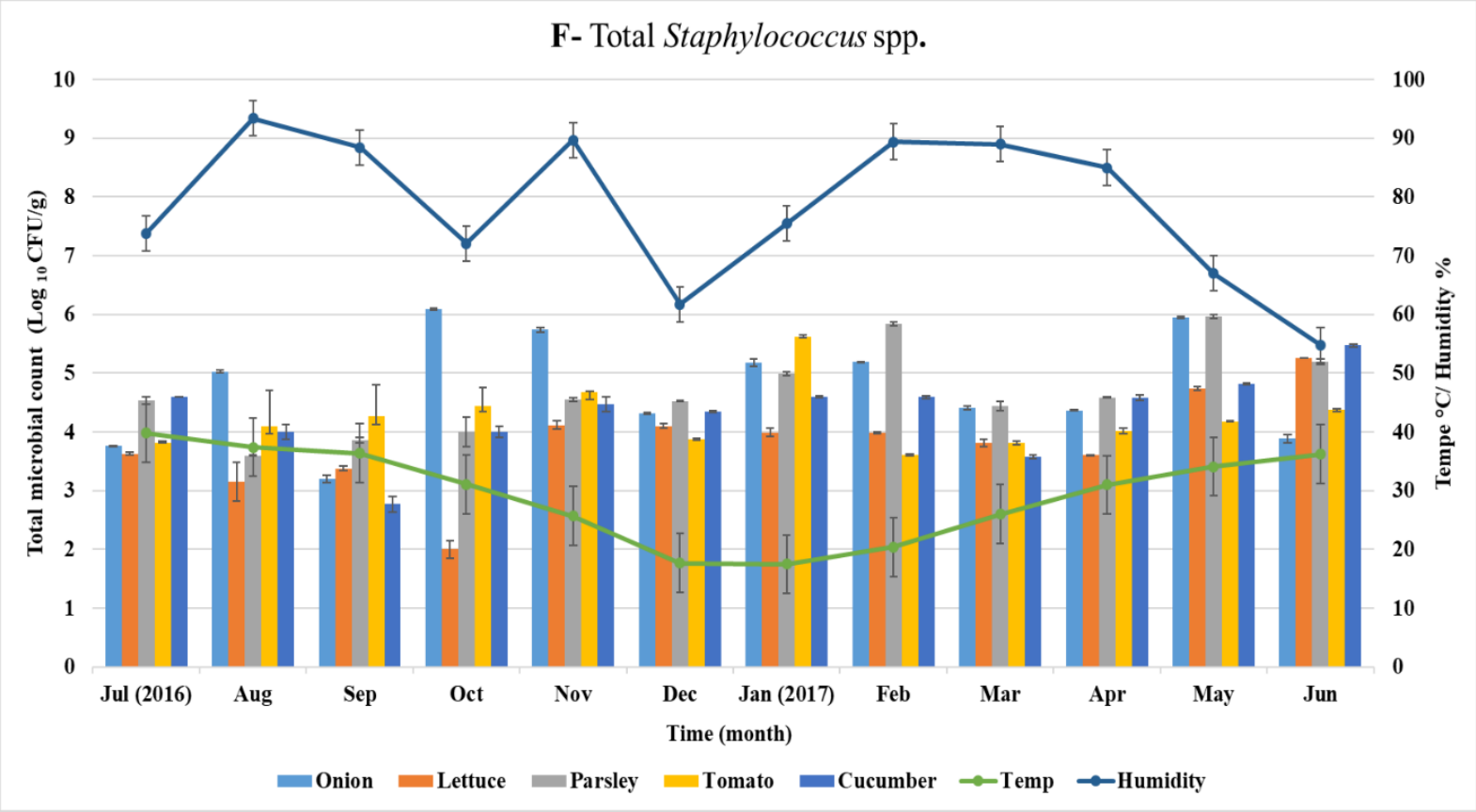

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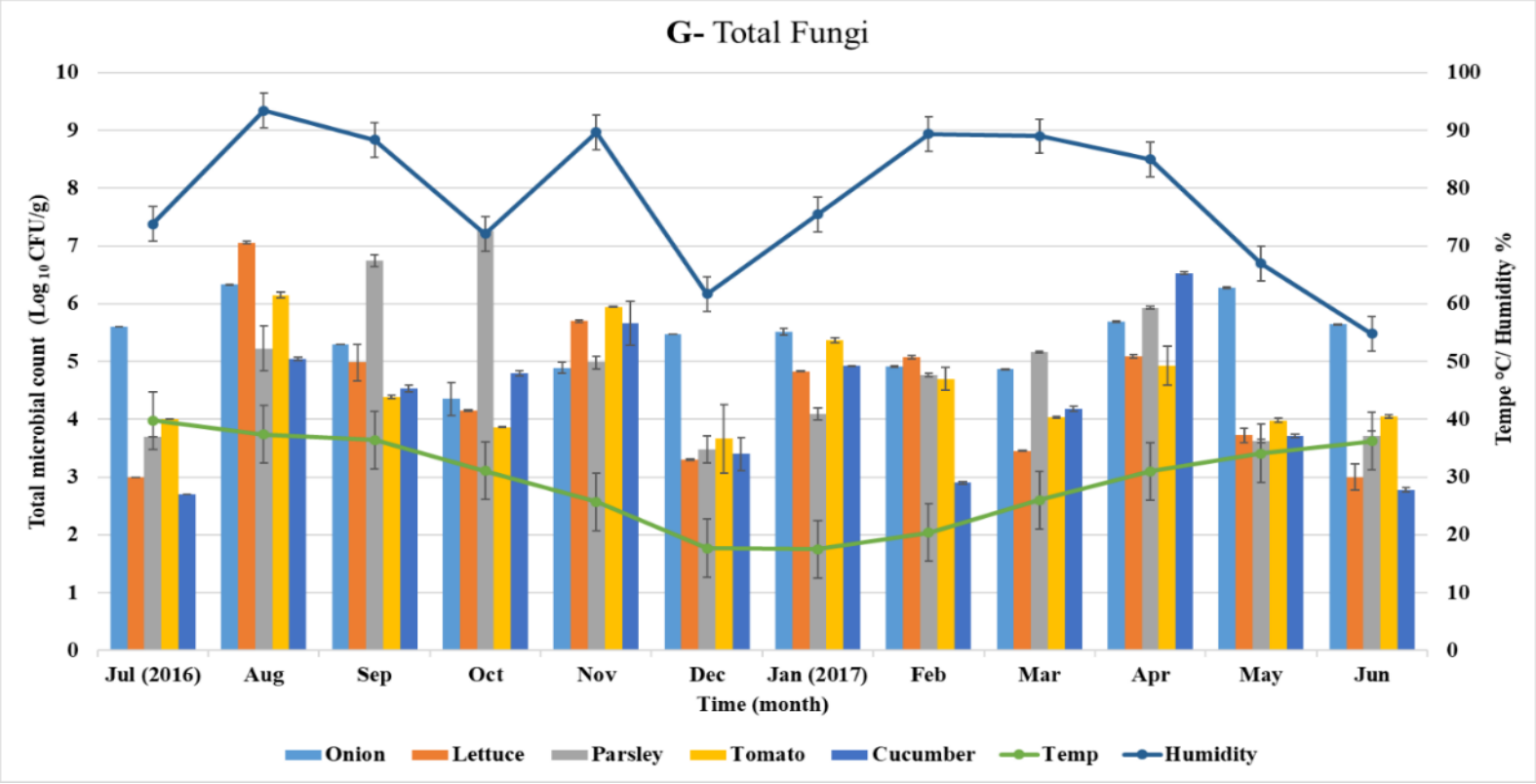

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collection, the rainfall was recorded just during the months of February and March and ranged from 0.1 to 14 mm/day. This low precipitation rate during these two months of 2017 did not affect the microbial counts significantly compared to other months, but it increased the relative humidity, which in turn impacted the diversity of the dominant strains isolated during these months (February and March).

3.3.2 Microbial hazards associated with soil and air samples collected from the surrounding area of the WSFPM and the impact of environmental conditions on their microbiota.

The four different soil samples collected from the surrounding area of the produce display area were used to study the effect of environmental conditions on microbial counts. The average counts (Log_{10} CFU/g) for each microbial group was plotted against the environmental parameters, such as temperature, relative humidity, and wind speed recorded over 12 months and presented in Figure 7 (A, B, C, D, E, F and G). To understand the relationship between the environmental factors and total microbial counts, a linear regression analysis was applied to study the effect of environmental factors on the soil, using the soil location as a control factor. This test revealed that there is a significant effect of temperature, humidity, and wind speed (not presented in Figure 7) on the microbial counts of all microbial groups studied ($P < 0.05$).

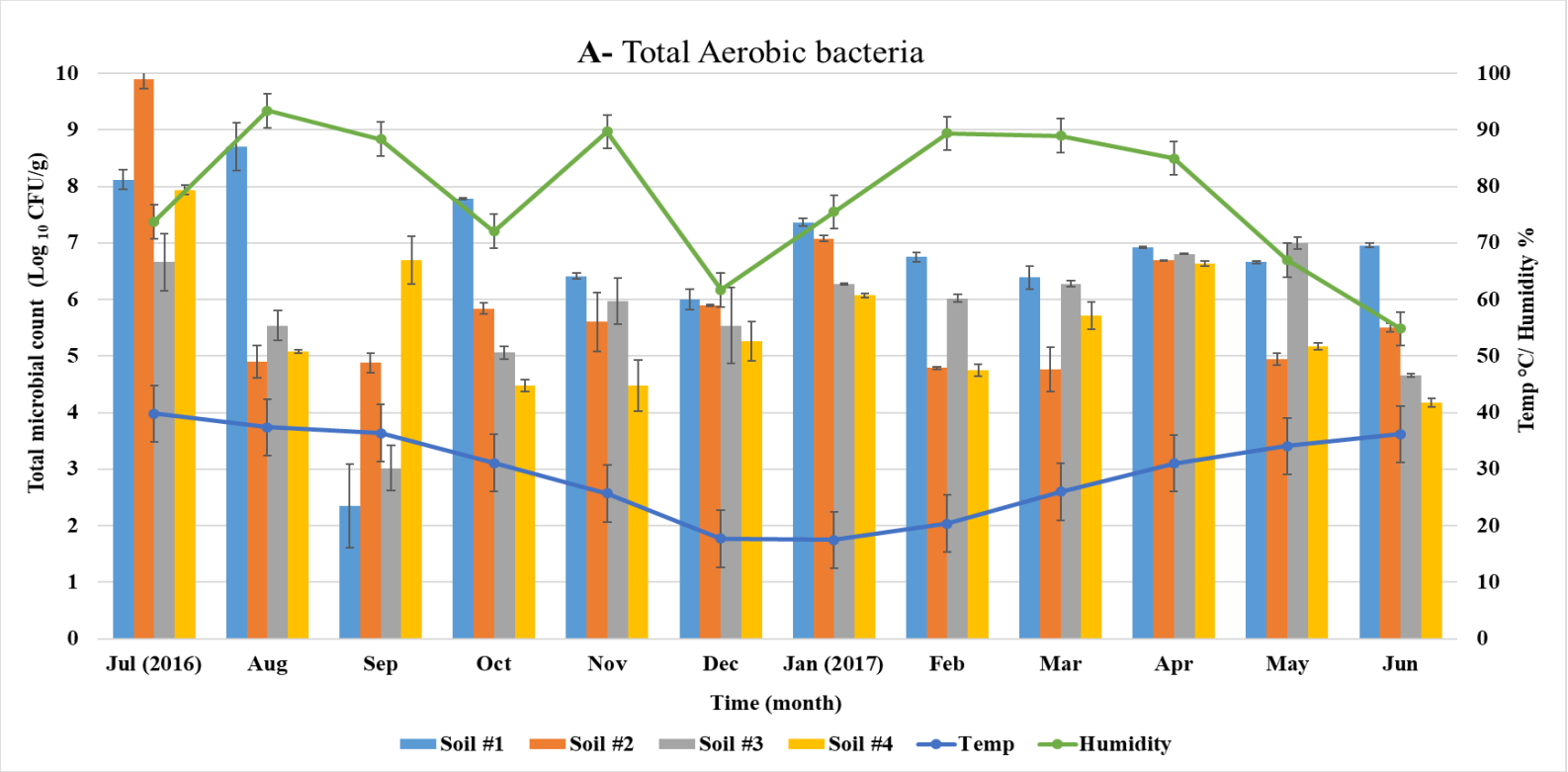

Figure 7. Total microbial counts (Log10 CFU/g) of soil samples surveyed during July 2016-June 2017

Soil # 1 Soil between tiles at the display area of the fresh produce market,

Soil # 2 Soil from the customers' car parking area at the fresh produce market,

Soil # 3 Soil from the trucks' parking area at the fresh produce market,

Soil # 4 Soil close to the livestock market.

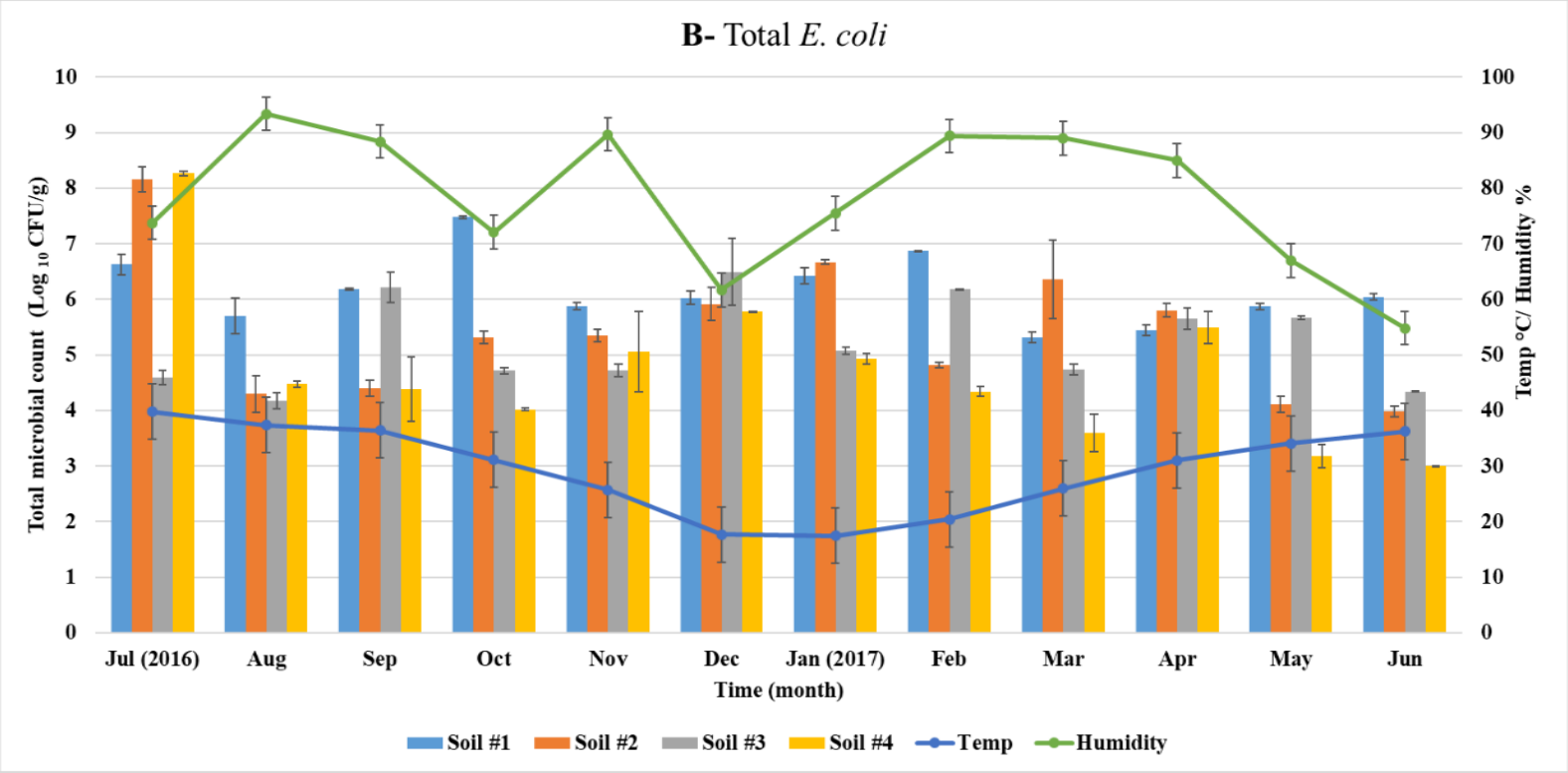

Figure 7 Cont. Total microbial counts (Log10 CFU/g) of soil samples surveyed during July 2016-June 2017

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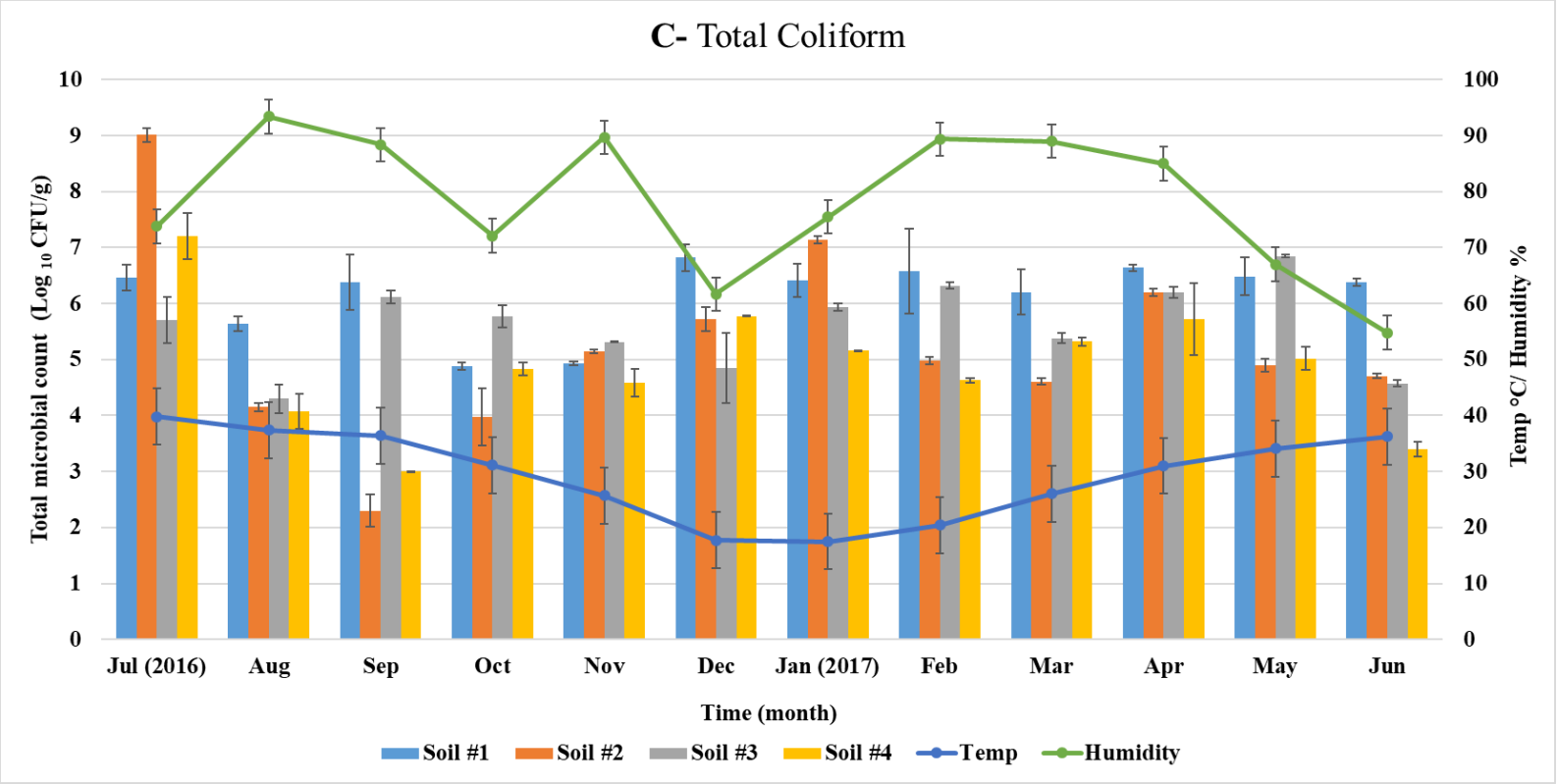

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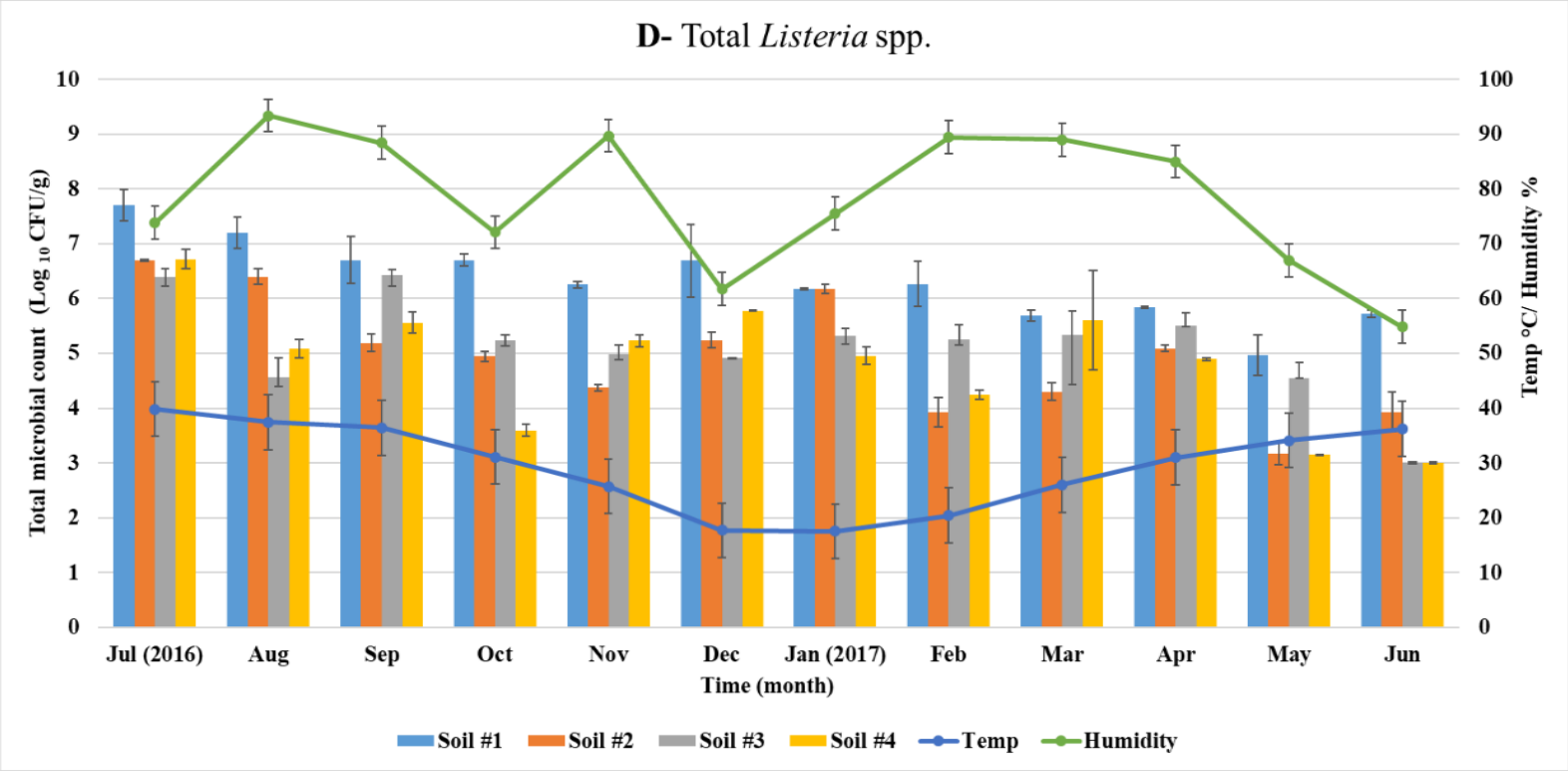
Soil # 4 Soil close to the livestock market.



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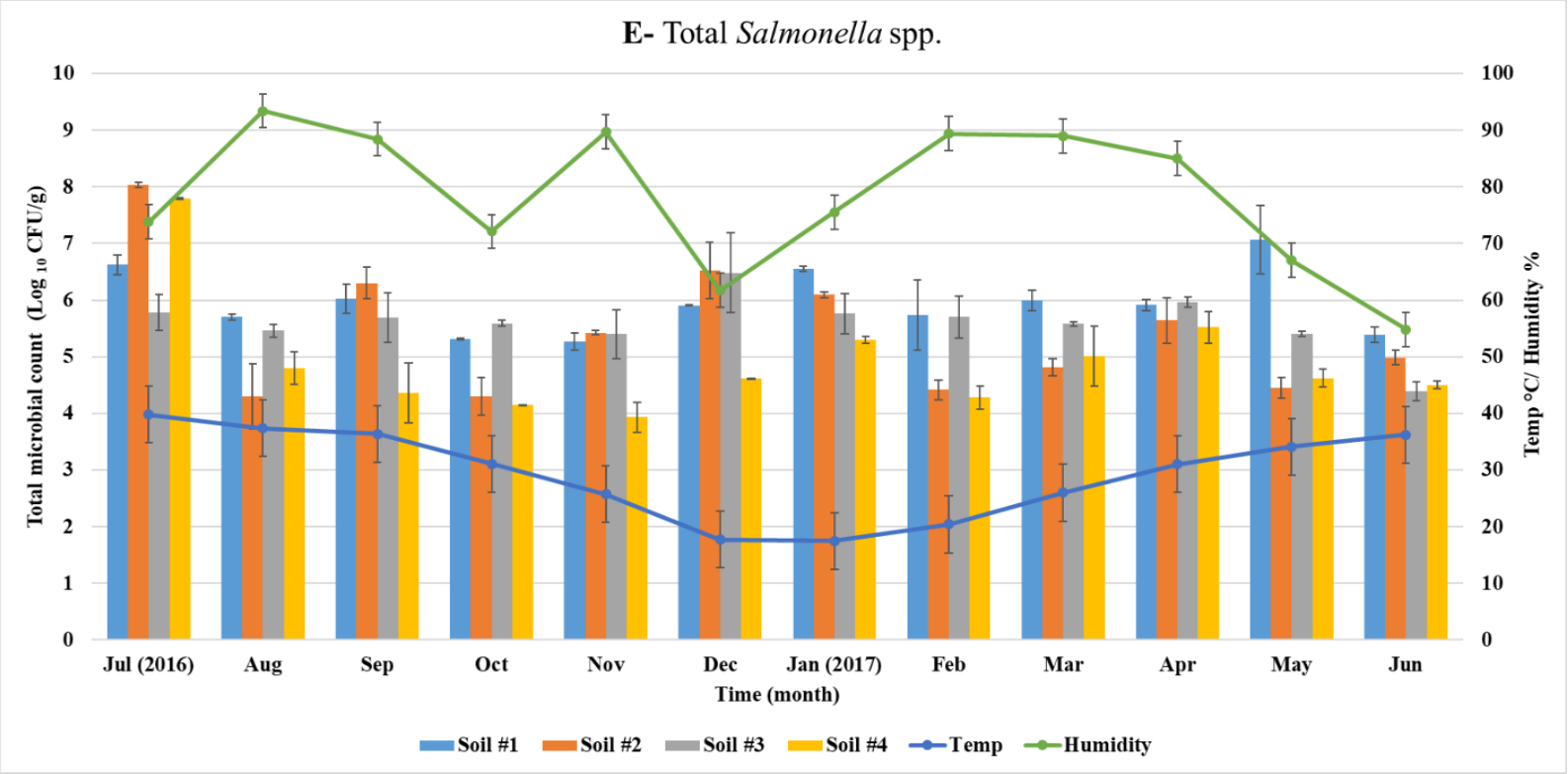

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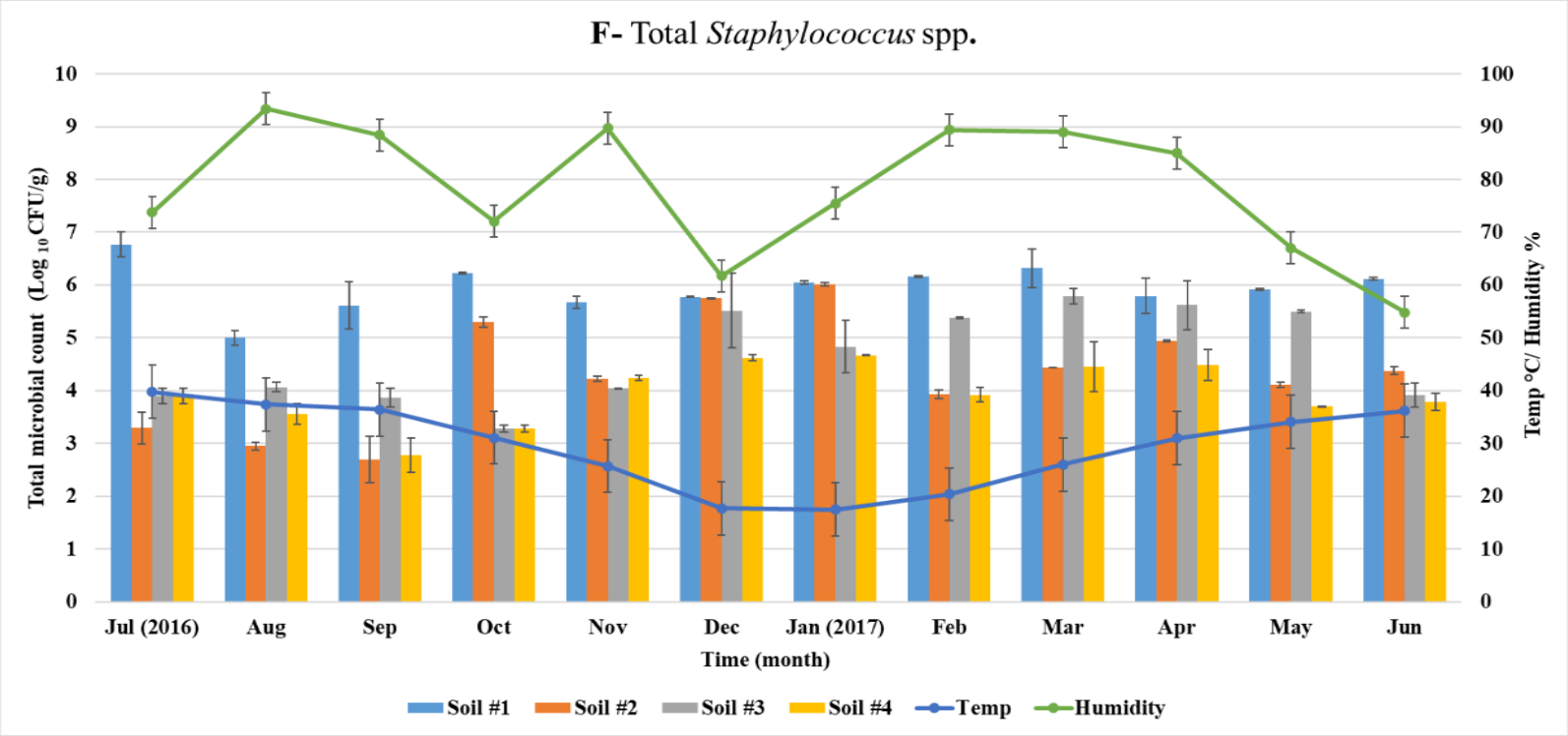

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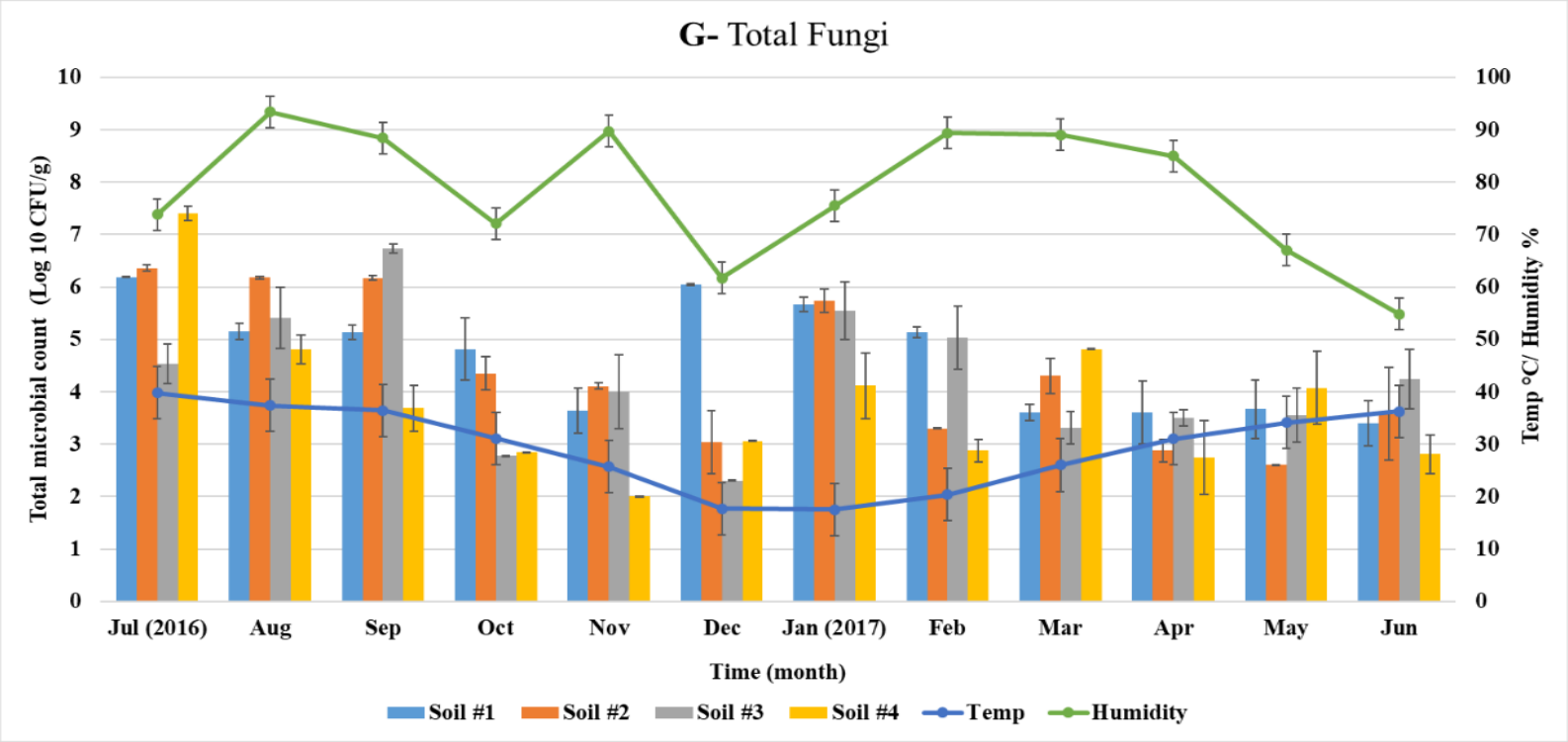

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Soil # 3 Soil from the trucks' parking area at the fresh produce market,

Soil # 4 Soil close to the livestock market.

In addition, the annual mean count (Log_{10} CFU/g) of each microbial group for all soil samples was also evaluated and presented in Table 17. A mixed ANOVA analysis was carried out to compare the mean differences between the soil samples using time as a co-factor within subjects. The test results indicated that there is a significant difference ($P< 0.05$) between the means of soil microbial counts, which changed from month to month significantly, and temperature, humidity, and wind speed. The differences in microbial counts between the soil samples tested depended on the amount of nutrient available in each soil sample. For example, the soil collected between the tiles on the floor of the WSFPM (soil # 1) was full of produce debris, compared to the those collected from the truck parking, while the soil collected beside the livestock market was loaded with animal manure. There is no standard limit regarding the microbial count in soils, since soil naturally contains various microorganisms. The main concern was the risk presented from these microorganisms found in soil samples. The presence of some specific microbial groups, such as coliform, indicates that there is a source of contamination coming from the food debris, animal/human feces, or fish debris. Table 18 illustrates the identification of the most dominant colonies isolated from different soil sample using 16S/18S rRNA sequencing. It was indicated that the soil samples collected during the summer months (June, July and August) were heavily loaded with Gram positive bacteria, which can easily adapt to high temperature (more than 40°C) compared to colder temperatures of winter months. It is interesting to note that *Bacillus circulans* was also isolated from the hands of produce handlers, indicating that this bacterium is easily transferred from soil to surfaces and surfaces to the hands. Furthermore, the free movement of the customers, handlers, and market workers' between these closed markets can increase the possibility of pathogenic contamination, which is colonized on the produce handlers' shoes or trolleys' wheels and transferred easily to the produce.

Table 17. Total microbial counts (Log_{10} CFU/g) of soil samples collected from different locations during the months of July 2016-June 2017.

Microbial Indicator	Sample type			
	Soil # 1 n = 36	Soil # 2 n= 36	Soil # 3 n = 36	Soil # 4 n = 36
Total aerobic bacteria (TAB)	6.70± 0.17	5.88± 0.16	5.71± 0.22	5.52± 0.17
Total *E. coli*	6.15± 0.1	5.29± 0.35	5.16± 0.18	4.68± 0.2
Total coliform	6.11± 0.26	5.22± 0.14	5.60± 0.17	4.87± 0.18
Total *Listeria* spp.	6.29± 0.23	4.93± 0.15	5.10± 0.19	4.78± 0.16
Total *Salmonella*	5.39± 0.25	5.40± 0.25	5.52± 0.32	4.82± 0.2
Total Staphylococcus	5.94± 0.15	4.33± 0.1	4.59± 0.21	3.93± 0.16
Total Yeast/Molds (on PDA)	4.59± 0.31	4.31± 0.26	4.14± 0.41	3.53± 0.32
Total Yeast/Molds (on RBCA)	4.15± 0.26	3.10± 0.29	3.02± 0.17	2.94± 0.14

The results are expressed as mean ± SD.

The soil samples were collected in triplicates from four different sites surrounding the WSFPM for a full year.

Soil # 1 Soil between tiles at the display area of the fresh produce market.

Soil # 2 Soil from the customers' car parking area at the fresh produce market.

Soil # 3 Soil from the trucks' parking area at the fresh produce market.

Soil # 4 Soil collected from the area close to the livestock market.

The air quality in the WSFPM was also surveyed by measuring microbial air counts (Log CFU/min) using different media (Table 19). The mean for all microbial groups was about 2 Log CFU/min. In general, these values are considered as low compared to a study conducted in Philippine (Vital *et al.*, 2014), where the microbial counts of air reached up to 4 Log CFU/min in an open-air market.

Several studies reported the negative impact of chemical pollutants on air on the produce quality sold in open-air markets (Brust, 2013; Pathak *et al*., 2017), but studies are rare for determining the microbial air pollution in open-air markets (like WSFPM). Moreover, the effect of airborne microbes on contaminating fresh produce is rarely studied, but still it could be considered as a factor for produce contamination (Drudy *et al*., 2006).

Table 18. Identification of the bacterial and fungal species isolated from different soil samples (n=144) using 16S rRNA gene sequencing and 18S rRNA (ITA region), respectively.

Identification	Prevalence (%)*
Bacillus circulans	11
Bacillus subtilis	21
Enterobacter cloacae	7
Enterococcus faecium	11
Enterococcus hirae	5
Micrococcus caseolyticus	4
Pantoea agglomerans	8
Pseudomonas libanensis	7
Pseudomonas fluorescens	13
Staphylococcus sciuri	7
Stacollected lugdunensis	3
Stenotrophomonas maltophilia	3
Alternaria alternate	15
Alternaria destruens	12
Aspergillus niger	11
Aspergillus oryzae	5
Cladosporium sphaerospermum	6
Fusarium fujikuroi	6
Mucar spp.	10
Penicillium aurantiocandidum	15
Penicillium expansum	12
Penicillium spinulosum	8

* The prevalence (%) = the percentage of each strain over the total number of the most dominant species identified, which measured separately for bacteria and fungi.

Table 19. Total microbial counts (Log CFU/min) of air samples collected from the WSFPM during 2016-2017.

Microbial Indicator	Sample type						
	Site # 1 n = 36	Site # 2 n= 36	Site # 3 n = 36	Site # 4 n = 36	Site # 5 n = 36	Site # 6 n = 36	Site # 7 n = 36
Total aerobic bacteria (TAB)	1.97 ± 0.09	1.91 ± 0.2	2.12 ± 0.15	1.19 ± 0.18	1.32 ± 0.2	1.65 ± 0.12	1.88 ± 0.07
Total *E. coli*	1.37 ± 0.16	1.47 ± 0.17	1.59 ± 0.13	0.46 ± 0.13	0.75 ± 0. 14	0.89 ± 0.16	1.04 ± 0.13
Total coliform	1.3 ± 0.11	1.72 ± 0.15	1.6 ± 0.08	1.0 ± 0.17	0.85 ± 0.19	0.9 ± 0.08	1.34 ± 0.16
Total *Listeria* spp.	1.81 ± 0.11	1.65 ± 0.12	2.16 ± 0.15	0.99 ± 0.13	1.01 ± 0.22	1.44 ± 0.11	1.93 ± 0.1
Total *Salmonella*	1.39 ± 0.18	1.5 ± 0.12	1.72 ± 0.09	0.76 ± 0.08	0.84 ± 0.15	0.98 ± 0.24	1.53 ± 0.13
Total Staphylococcus	1.81 ± 0.1	1.85 ± 0.02	2.21 ± 0.07	1.37 ± 0.19	0.93 ± 0.14	1.35 ± 0.62	1.76 ± 0.13
Total Yeast/Molds (on PDA)	1.2 ± 0.11	1.49 ± 0.19	1.26 ± 0.2	1.05 ± 0.12	0.7 ± 0.17	0.8 ± 0.17	1.02 ± 0.17
Total Yeast/Molds (on RBCA)	1.48 ± 0.11	1.57 ± 0.14	1.53 ± 0.12	1.08 ± 0.14	0.81 ± 0.1	0.9 ± 0.11	1.2 ± 0.09

The results are expressed as mean ± SD.

The air samples collected in triplicates from 7 sites.

Values between brackets show the range of each count in Log CFU/min.

Site # 1 Periphery of the display area of the fresh produce market, Site # 2 At the center of the display area at the fresh produce market, Site # 3 In the middle of the area used for local produce (locally grown produce), Site # 4, In the middle of the storage area, Site # 5 In the middle of the fish market, Site # 6 In the middle of the slaughterhouse

Site # 7 Same site where the soil samples were collected at the livestock market.

The environmental conditions (e.g. temperature and relative humidity) were used to evaluate the air quality at the WSFPM and the surrounding markets. The mean count for each site were plotted for the full year and presented in Figure 8 A, B, C, D, E, F and G. The statistical analysis using linear regression analysis revealed that there is no significant effect of temperature, humidity, and wind speed on the air quality ($P > 0.05$) when using location as a control factor. On the other hand, there was a significant difference between the average means of air counts collected from different sites ($P < 0.05$) using mixed ANOVA analysis. This may explain how the surrounding environment could influence the microbiota of the fresh produce, especially in the dusty/humid days.

It is important to mention here that Deployable Particle Samplers did not provide a reasonable result regarding the microbial air quality, since this instrument is mainly used to measure the air particles not the microorganisms in the air.

The location of the WSFPM was found to negatively impact the air quality at the market. Specifically, samples collected near the livestock market, fish market, and slaughterhouse increased the risk of contamination of produce with several microorganisms. Some examples of microorganisms identified in air samples, which might be considered as public health risks factors, include *P. aeruginosa*, *K. pneumonia*, *Enterococcus faecium*, *Staphylococcus* spp. and *Aspergillus* spp. (Table 20).

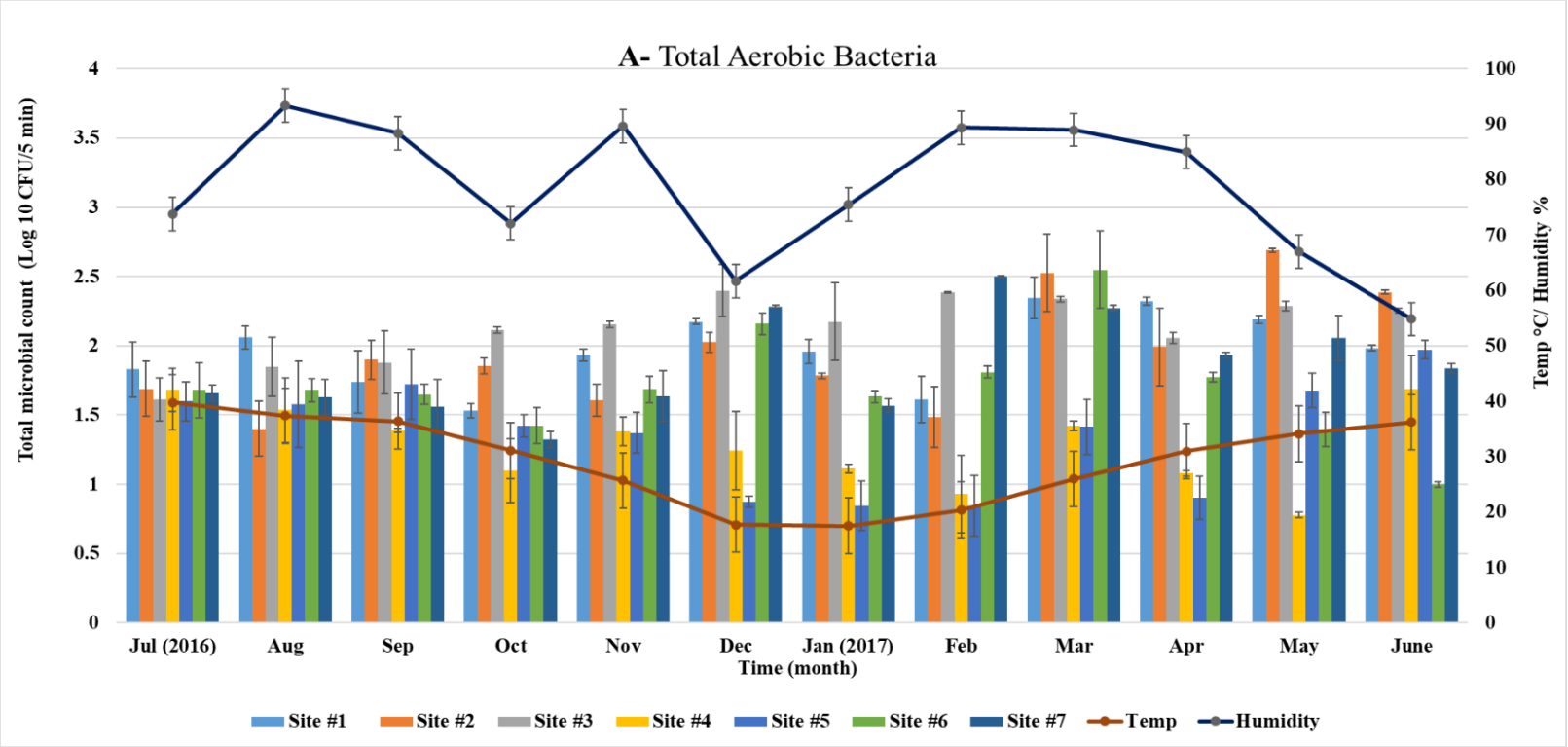

Figure 8. Total microbial counts (Log_{10} CFU/min) of air samples collected during the period of July 2016 June 2017. Monthly averages of the A- Total Aerobic Bacteria on PCA, B- Total *E. coli* on MCA, C- Total Coliform on EMBA, D- Total *Listeria* spp. on LSA, E- Total *Salmonella* spp. on XLT4 agar, F- Total *Staphylococcus* spp. on BPA, and G- Total Fungi on PDA.

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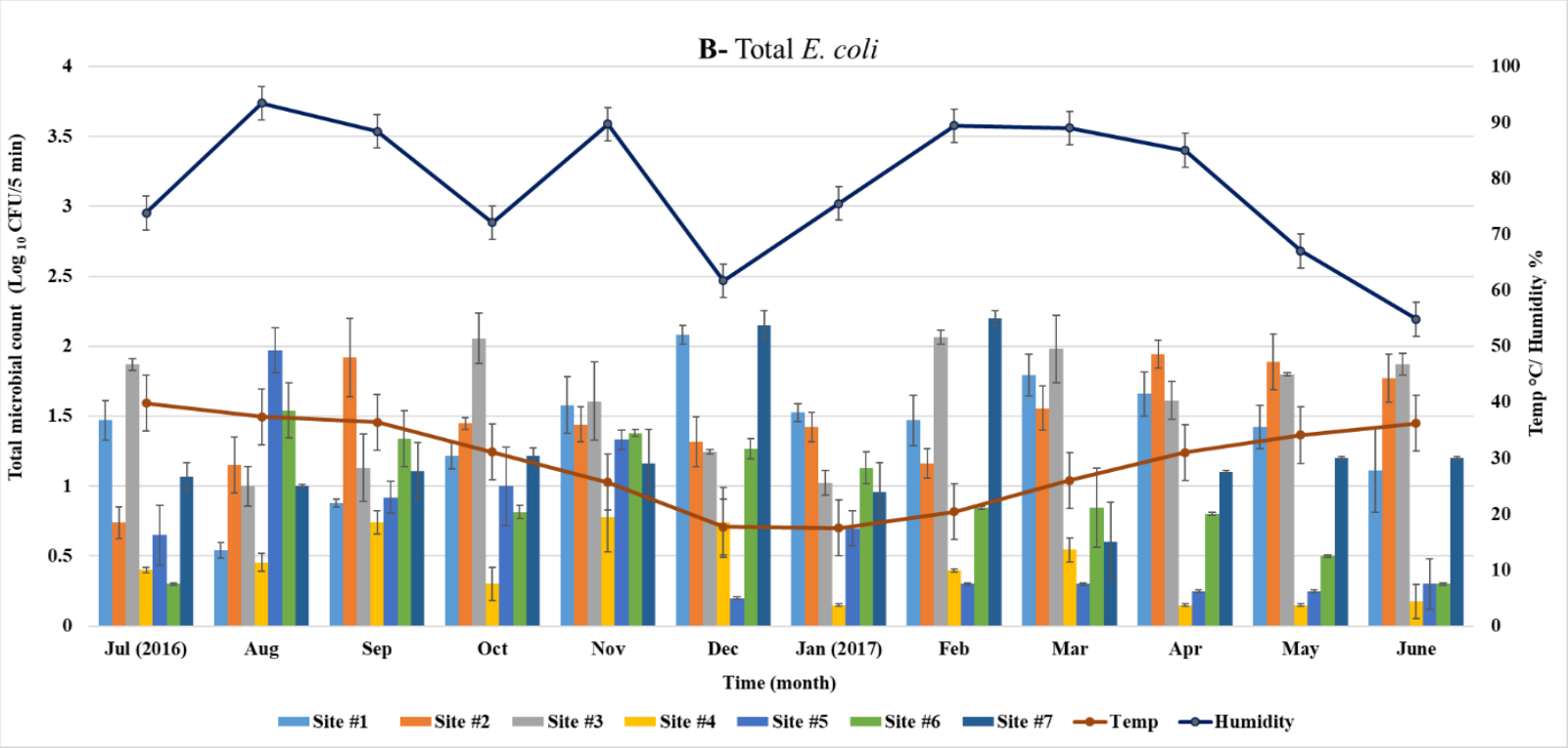
Site # 3 In the middle of the area used for local produce (locally grown produce), Site # 4 In the middle of the storage area,
Site # 5 In the middle of the fish market, Site # 6 In the middle of the slaughterhouse, and
Site # 7 Same site where the soil samples were collected at the livestock market.



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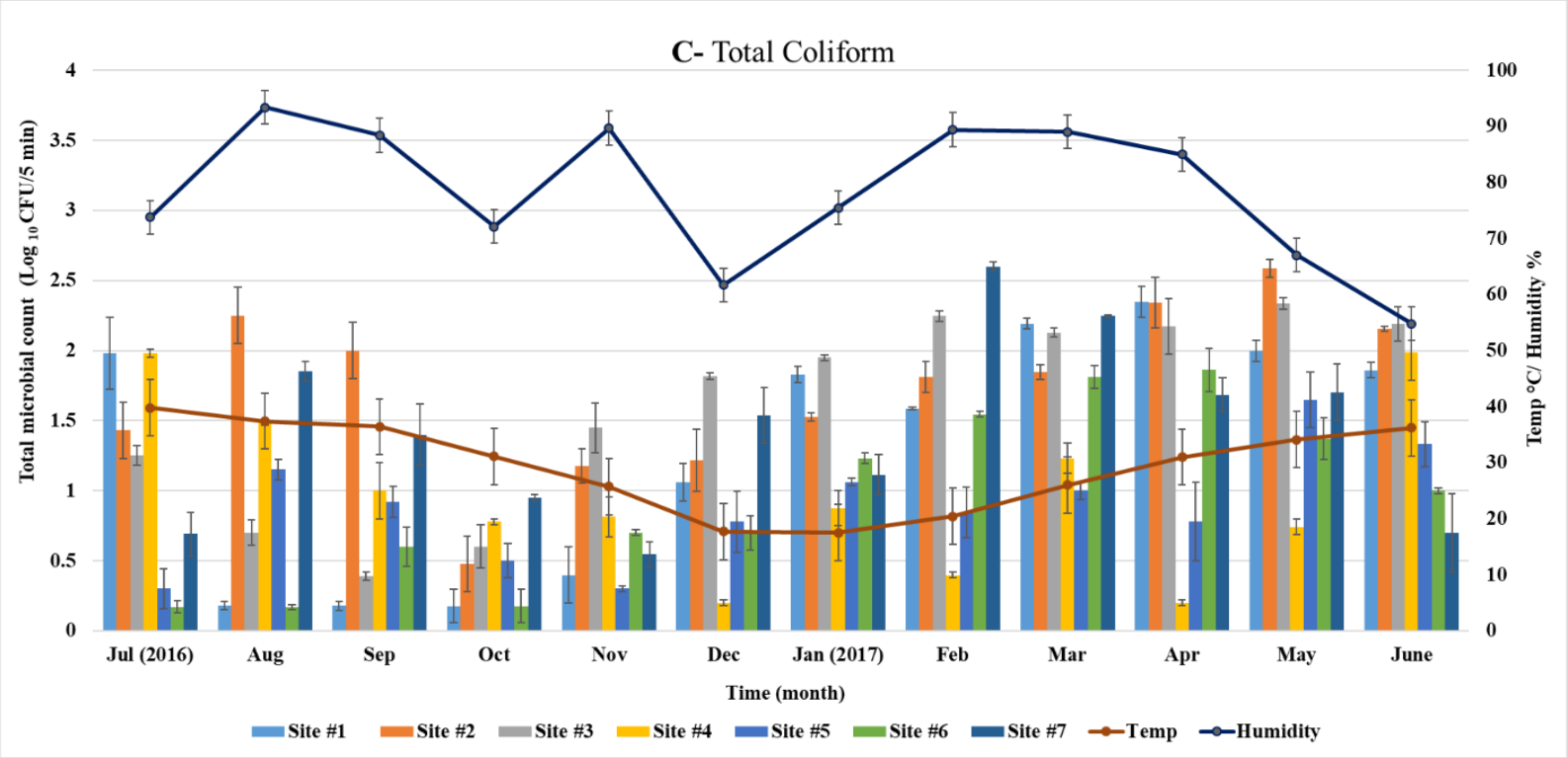

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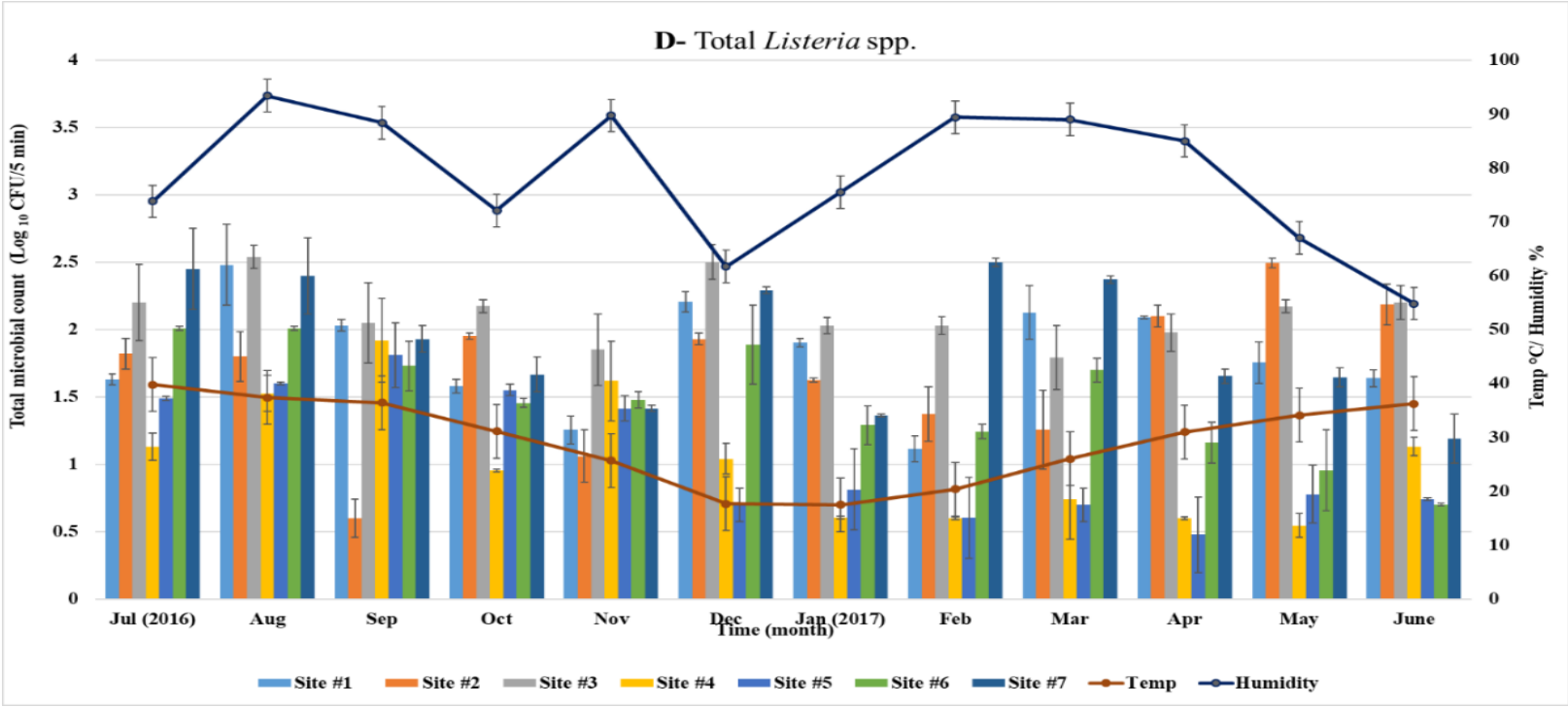

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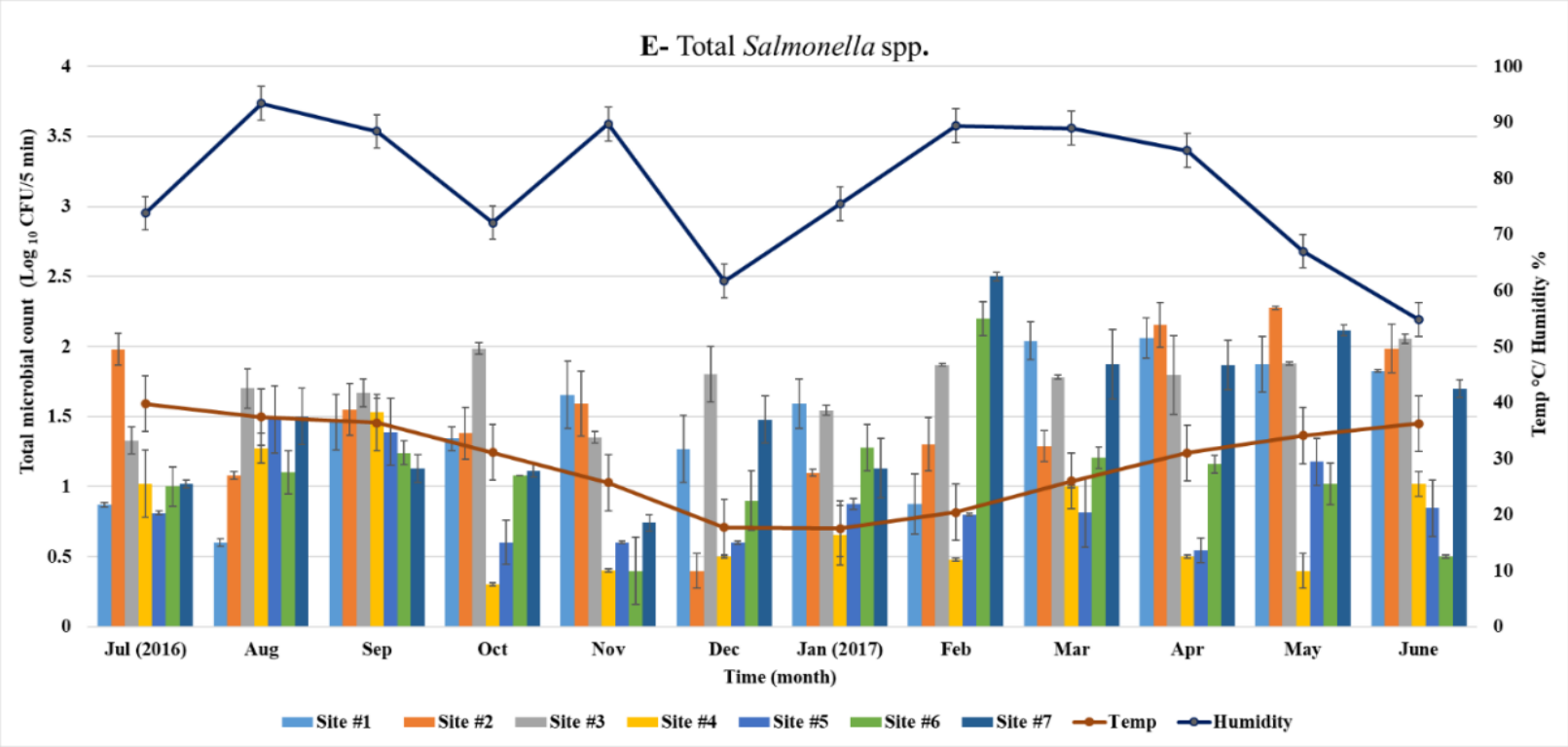

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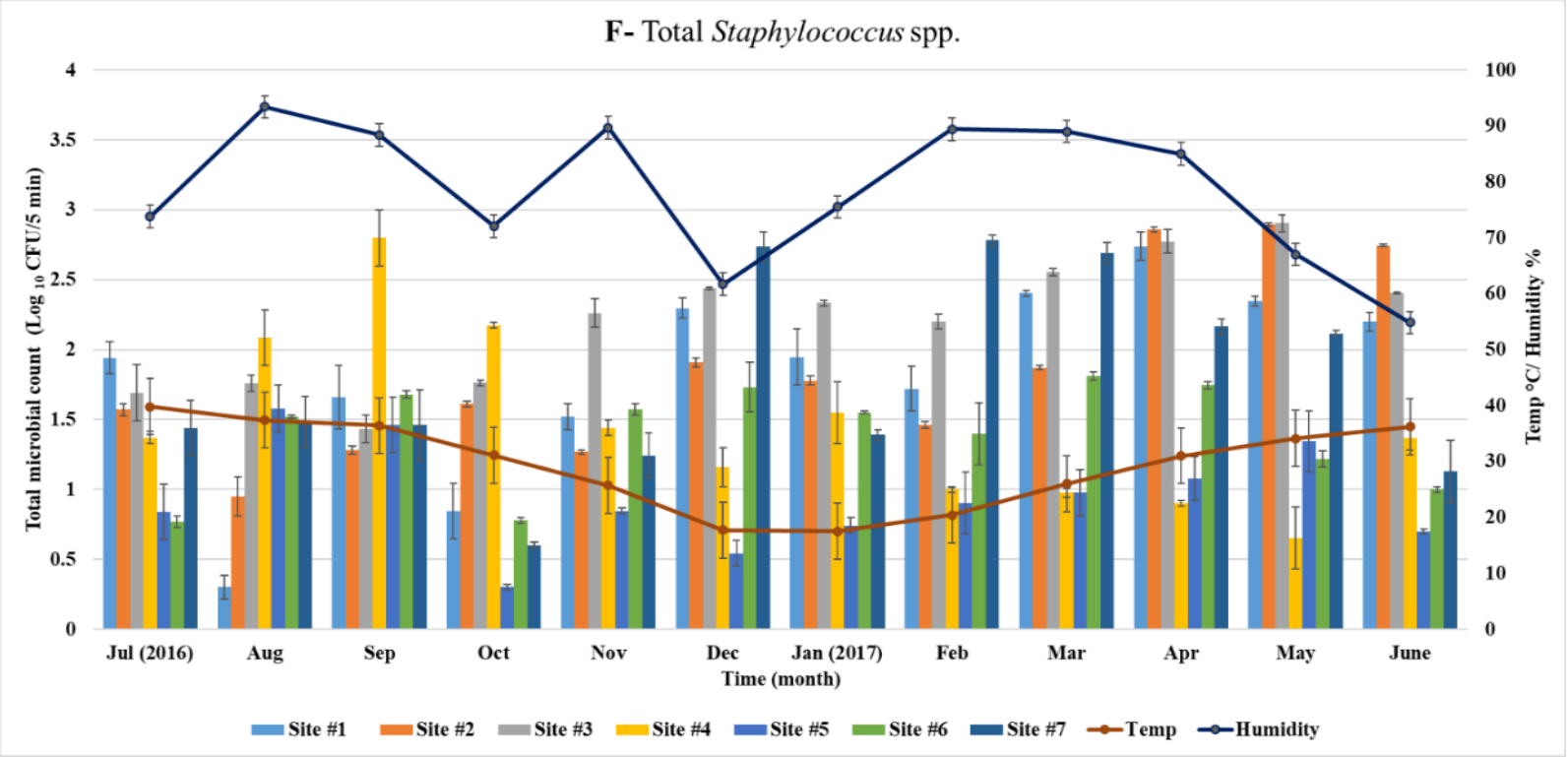

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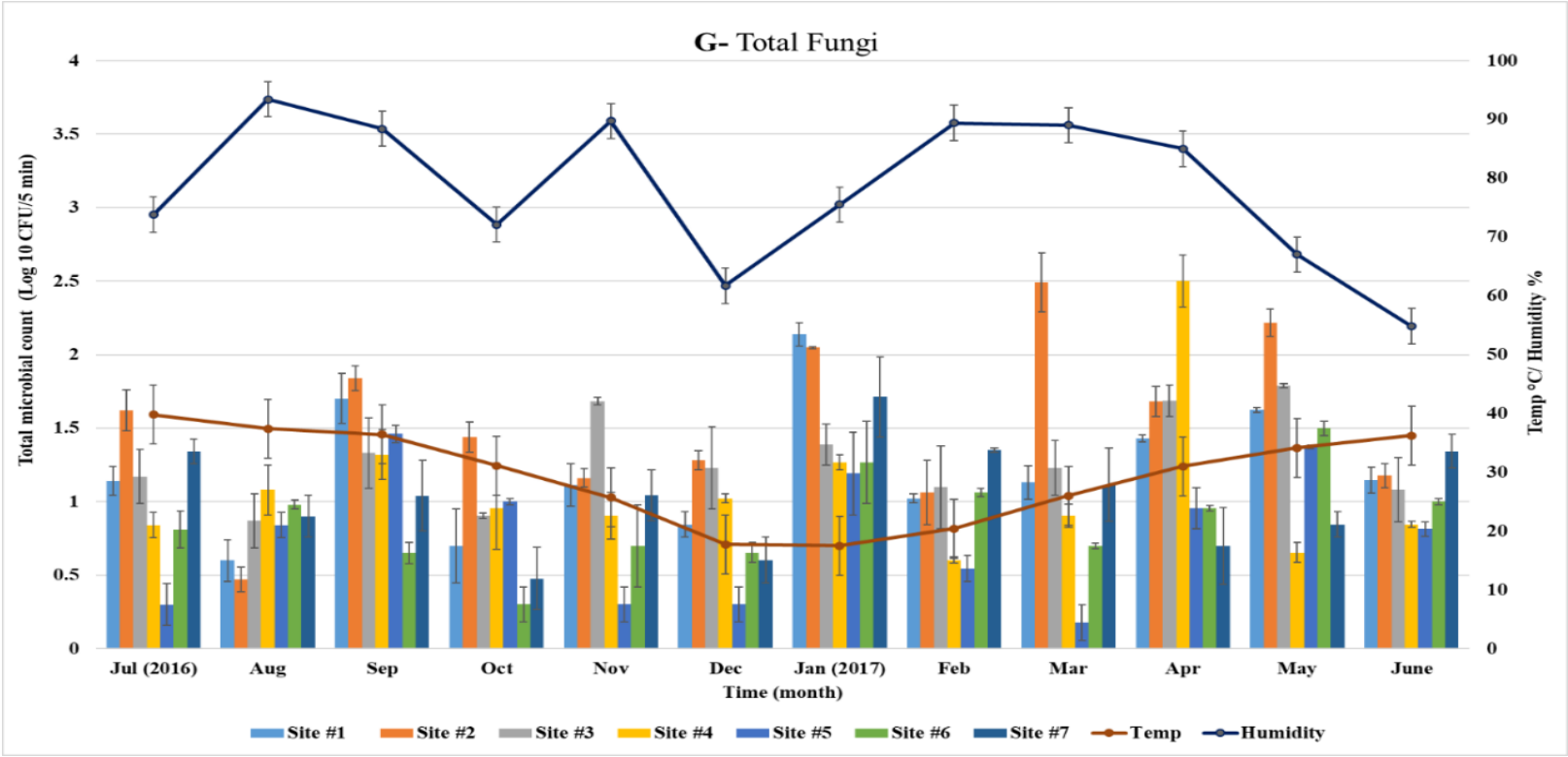

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Site # 7 Same site where the soil samples were collected at the livestock market.

Table 20. Identification of the bacterial and fungal species isolated from different air samples (n=252) using 16S rRNA gene sequencing and 18S rRNA (ITA region), respectively.

Bacterial/ Fungal strains	Prevalence (%)
Bacillus subtilis	20
Brevibacterium spp.	4
Enterobacter cloacae	12
Enterococcus faecium	12
Klebsiella pneumonia	9
Pseudomonas aeruginosa	9
Pseudomonas azotoformans	5
Pseudomonas putida	4
Staphylococcus arlettae	7
Staphylococcus warneri	9
Staphylococcus cohnii	9
Aspergillus niger	30
Cladosporium sphaerospermum	4
Fusarium fujikuroi	19
Penicillium aurantiocandidum	16
Penicillium digitatum	17
Penicillium expansum	14

* The prevalence (%) of each strain computer related to the total isolates separately for bacteria and fungi.

The results (Table 20) demonstrated that the air samples were carrying different microorganisms, some of which originated from soil, plants, animals, or human. Most of the fungal species identified could be easily transferred to fresh produce, since their spores can fly and settle on the produce. Many of the species identified are also common soil fungal species, which can also grow on plant surface and rotten

vegetables. The major concern from the food safety point is that several species of these fungi (e.g. *Aspergillus niger*) are toxin-producing species, which are known to cause adverse health effects on humans. In addition, *Klebsiella* spp, *Enterococcus faecium*, and *Staphylococcus* sp. were identified as pathogens in air, soil, and fresh produce samples, which indicate the negative impact of the surrounding environment on the produce quality. These findings emphasize the urgent need to improve hygiene conditions at the WSFPM, and the implementation of more stringent rules to avoid any pathogens transferring to fresh produce.

3.3.3 Assessment of the microbial contamination sources coming from truck surfaces, produce containers, and trolleys.

To evaluate the contamination level associated with surfaces, which are in direct contact with the fresh produce sold in the WSFPM, several swab samples were collected from different surfaces, such as containers, trolleys and the produce trucks' floor. The microbial load of the examined surfaces was measured and expressed as Log_{10} CFU/cm^2 (Table 21). The statistical analysis indicated a significant differences between the mean microbial counts of different surfaces ($P< 0.05$). The results showed that the surfaces were contaminated at different levels; in most cases the counts exceeded 4 Log_{10} CFU/cm^2. The international guidelines recommended 2 Log/cm^2 as the maximum limit for TAB count on any food surfaces, while 1 Log/cm^2 as the highest limit to be accepted for total coliforms (Johnston *et al*., 2006).

Studies are limited on the microbial contamination of the surfaces where the produce samples are displayed and/or sold in open-air markets or farmer markets. Johnston *et al*. (2006) conducted a study to examine the environmental swab samples collected from several surfaces. The authors found that the Log_{10} CFU/cm^2 of boxes used to store leafy greens produce did not exceed 1 Log_{10} CFU/cm^2 for total *E.coli* and

coliform, while the TAB counts reached 3 Log_{10} CFU/cm^2. In this study, the TAB and coliform counts were ranged between 5.7 to 6.5 Log_{10} CFU/cm^2, showing that the quality of environmental swabs collected from the WSFPM were not meeting the set standards. These findings might be due to remaining produce debris in the boxes, trolley, and truck floors. The results might also indicate that these surfaces were not washed properly or disinfected using suitable cleaners to clean food surfaces. In addition, these surfaces were covered with soil particles released from different produce carried in the containers, which could be a source of contamination. Some of the trolleys analyzed were used to carry food from other surrounding markets, such as fish market and slaughterhouse, which might explain the presence of several bacteria of animal origin.

The results of the microbial counts of surfaces collected during the study period is presented in Figure 9. It is clear that the surfaces surveyed were heavily loaded with different microorganisms. The same statistical analysis (ANOVA and multivariate regression) applied for soil and air samples' analysis were used to determine the relationship between surfaces and fresh produce quality. The tests revealed that the microbial load on the surfaces differs from surface to surface significantly ($P < 0.05$). As mentioned above, the availability of nutrients coming from produce debris or soil particles may play a role in influencing the microbial load on the surfaces surveyed.

Table 21. Mean (Log_{10} CFU/cm^2) of microbial counts of surface samples collected from trolleys, containers, and trucks' floor during 2016-2017.

Microbial Indicator	Sample type					
	Surface # 1 n = 36	Surface # 2 n= 36	Surface # 3 n = 36	Surface # 4 n = 36	Surface # 5 n = 36	Surface # 6 n = 36
Total aerobic bacteria (TAB)	6.56± 0.66	6.54± 0.54	6.47± 0.31	5.76± 0.74	6.65± 0.39	6.34± 0.58
Total *E. coli*	6.48± 0.88	6.40± 0.5	6.49± 0.47	5.80± 0.6	6.74± 0.39	6.18± 0.69
Total coliform	6.15± 1.02	6.19± 0.39	6.27± 0.49	5.73± 0.66	6.56± 0.28	6.06± 0.71
Total *Listeria* spp.	4.35± 0.7	4.3± 0.53	4.14± 0.67	4.04± 0.89	4.37± 0.83	3.99± 0.99
Total *Salmonella*	5.98± 0.82	5.73± 0.81	5.85± 0.54	5.29± 0.76	6.0± 0.73	5.59± 0.85
Total Staphylococcus	4.22± 0.41	4.0± 0.76	3.81± 0.5	3.71± 0.6	4.45± 0.72	4.04± 0.83
Total Yeast/Molds (on PDA)	4.77± 0.84	5.09± 0.78	5.22± 0.4	4.29± 0.62	5.08± 0.87	4.97± 1.04
Total Yeast/Molds (on RBCA)	4.13± 0.74	4.12± 0.89	4.25± 0.93	3.53± 0.42	4.57± 0.78	4.51± 0.67

The results are expressed as mean ± SD.

The swab samples collected in triplicates.

Values between brackets show the range of each count as Log_{10} CFU/cm^2.

Surface #1 = L-Shaped Trolley, Surface #2 = Green wheel barrow trolley,

Surface #3 = White foam container, Surface #4 Plastic net boxes,

Surface #5 Floor of the produce truck (J), and Surface #6 =Floor of the produce truck (S).

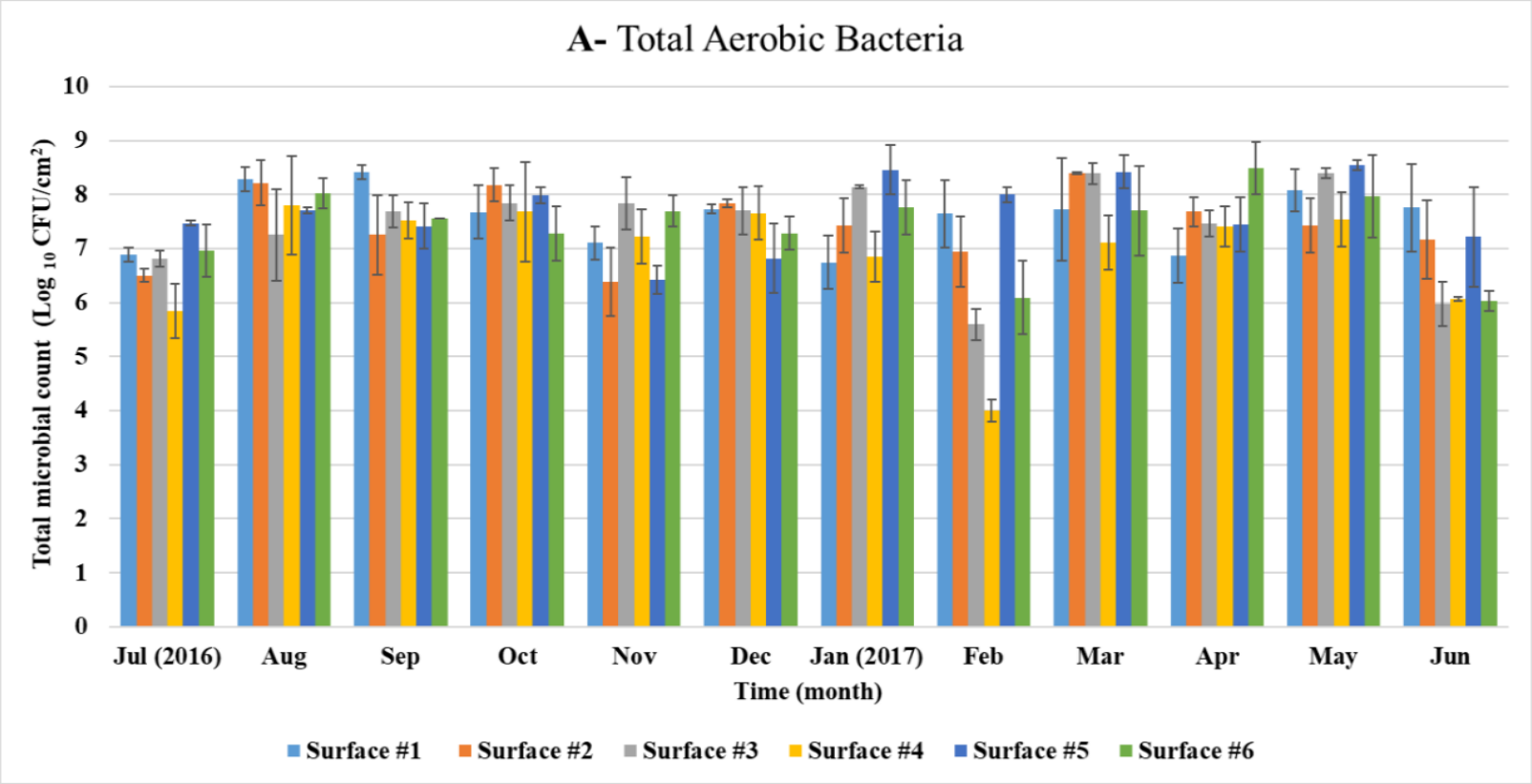

Figure 9. Total microbial counts (Log_{10} CFU/cm^2) of environment surface samples surveyed from July 2016-June 2017.

Surface #1 = L-Shaped Trolley, Surface #2 = Green wheel barrow trolley,

Surface #3 = White foam container, Surface #4 Plastic net Boxes Black boxes,

Surface #5 Floor of the produce truck (Jordan), and Surface #6 =Floor of the produce truck (KSA).

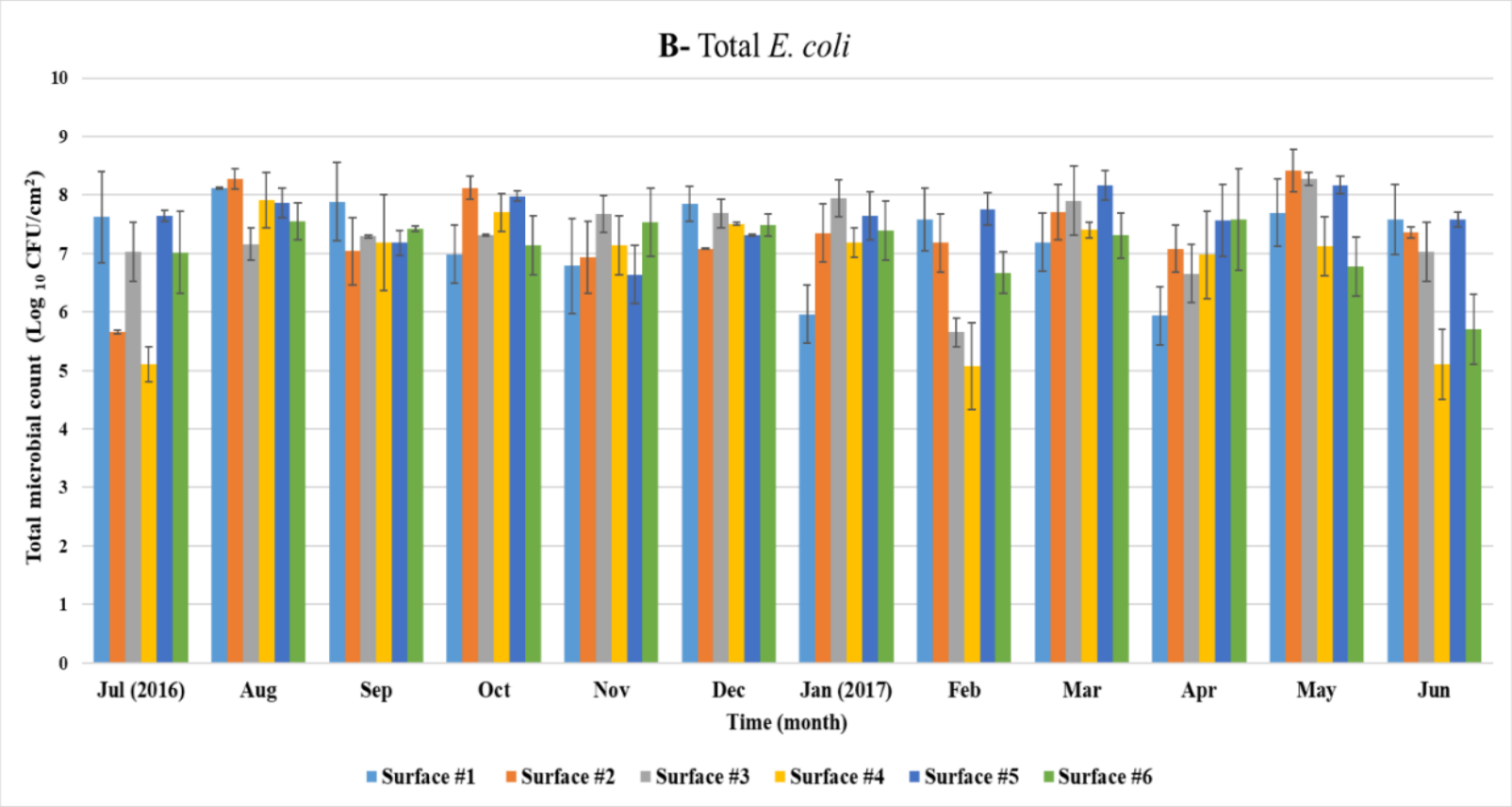

Figure 9 Cont. Total microbial counts (Log_{10} CFU/cm^2) of environment surface samples surveyed from July 2016-June 2017.

Surface #1 = L-Shaped Trolley, Surface #2 = Green wheel barrow trolley,

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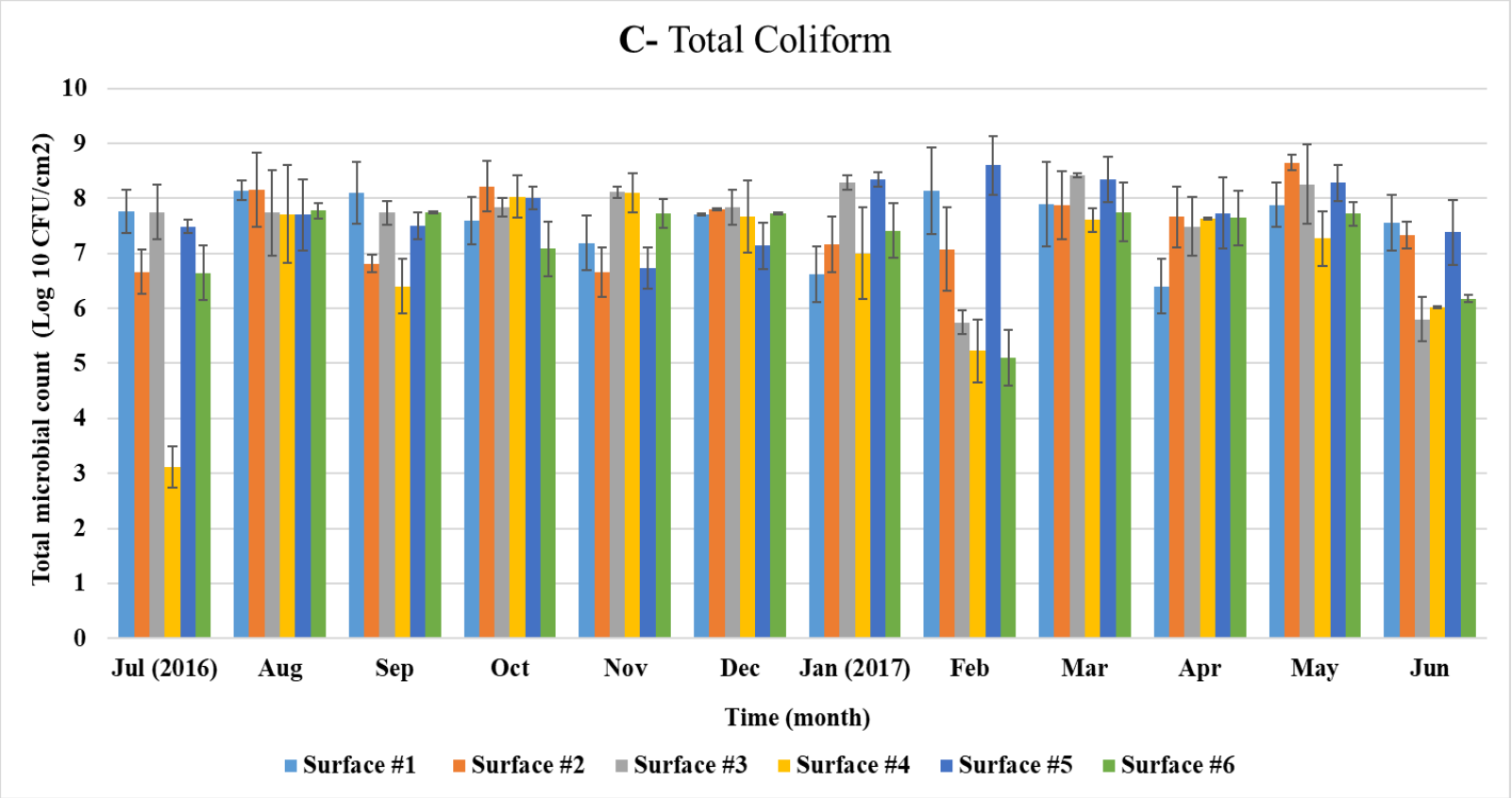

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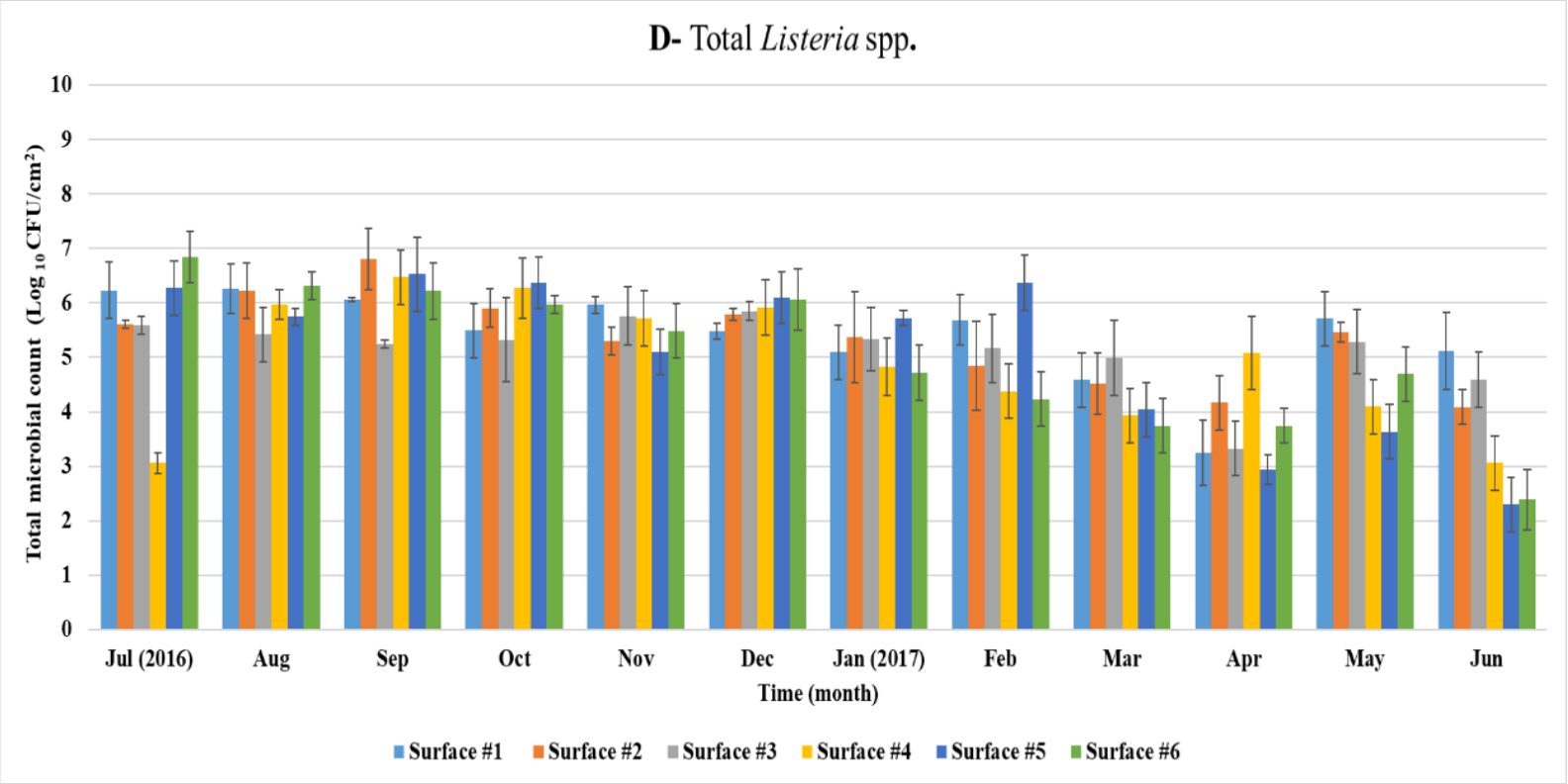

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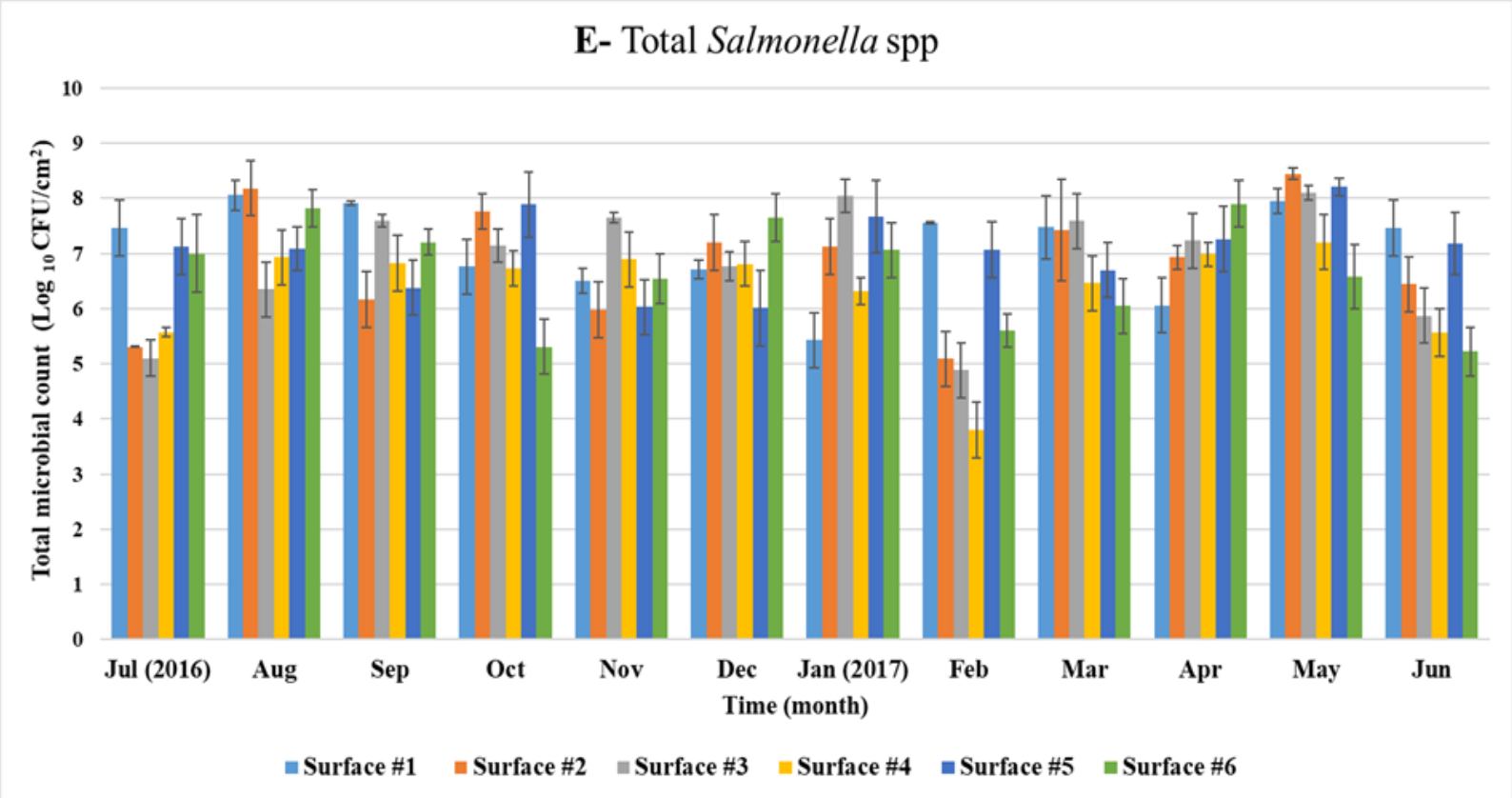

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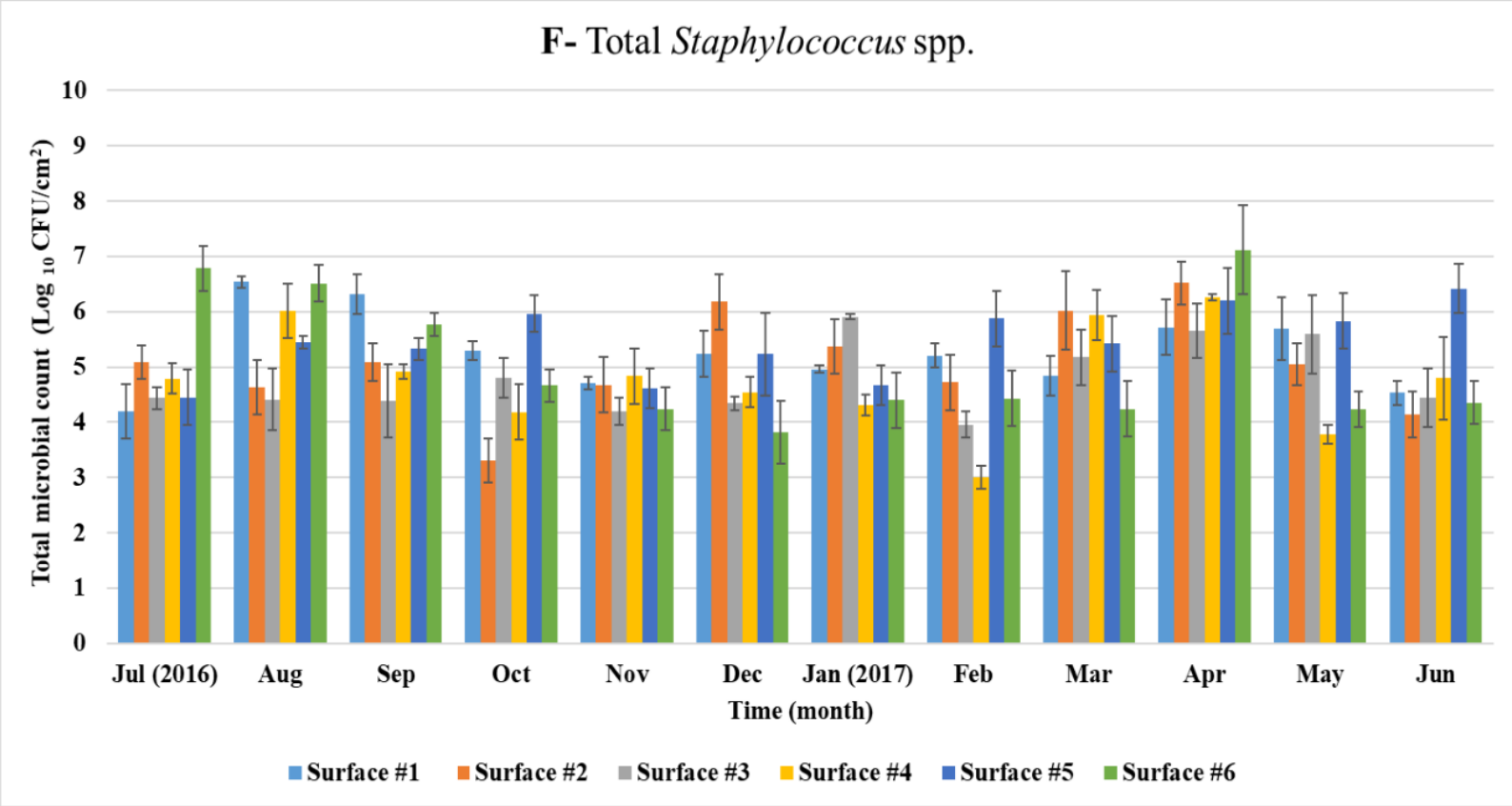

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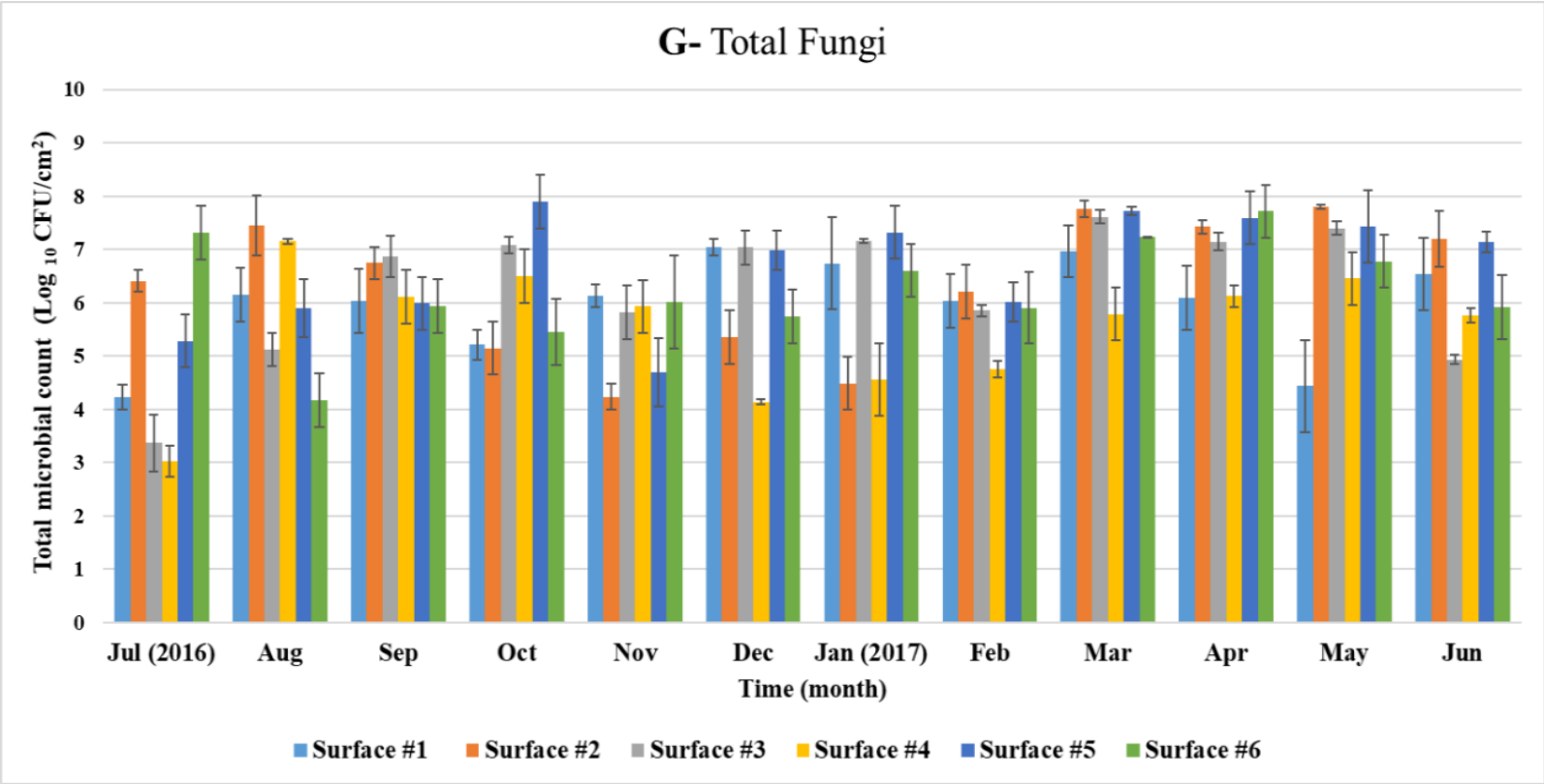

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The most dominant species were identified as *B. subtilis*, *P. aerugonosa*, *C. testudinoris*, and *E. faecium* with prevalence rates of 20%, 17%, 13%, and 12%, respectively, in addition to fungal strains e.g. *Penicillium* spp., *Alternaria* spp., and *Aspergillus* spp. (32%, 24%, and 18% respectively) (Table 22). These microbes mostly originated from soil, e.g. *Bacillus* spp. and *Pseudomonas* spp. On the other hand, the presence of pathogenic microbes e.g. *Enterococcus faecium*, which is normally found in animal and human intestine, meaning that the trucks or trolleys may probably be used to transfer other materials like manure or other type of food rather than just produce. This contamination source might be transferred to the produce, especially if these produce having damaged parts, which might potentially create a public health risk.

It is clear that the surface swabs collected were heavily contaminated with various microorganisms. However, it is noteworthy to emphasize here that none of the target pathogen was identified from any of the surfaces. Based on the evaluation of microbial hazards of environmental origin present at the market, the following points can be summarized as:

a) The environment in the WSFPM lacks proper hygiene. The air quality of the target market is considered low since the air contained examples of pathogenic bacteria not targeted in this study (such as, *Klebsiella pneumonia, Enterococcus faecium,* and *Pseudomonas aeruginosa*).
b) The presence of pathogenic microbes, which are normally found in animal and human intestine (e.g. *Enterococcus faecium),* on surfaces (trucks or trolleys) indicate the possible use of the same trolleys and trucks for other purposes, such as carrying manure or food animals. This eventually might result in contamination of produce samples if they are in direct contact with these contaminated surfaces.

Therefore, it is recommended that the Municipality should increase their inspection frequency at the target market in order to avoid any costly produce related outbreak.

Table 22. Identification of the bacterial and fungal species isolated from different environmental swabs (surfaces) (n= 216) using 16S rRNA gene sequencing and 18S rRNA (ITA region), respectively

Bacterial/ Fungal strains	Prevalence (%)*
Bacillus subtilis	20
Enterobacter cloacae	12
Citrobacter braakii	7
Citrobacter koseri	3
Corynebacterium testudinoris	13
Enterococcus faecium	12
Georgenia spp.	4
Pseudomonas aeruginosa	17
Pseudomonas azotoformans	3
Staphylococcus warneri	9
Alternaria alternate	12
Alternaria destruens	12
Aspergillus niger	18
Fusarium oxysporum	10
Mucar hiemalis	6
Penicillium aurantiocandidum	16
Penicillium expansum	18
Penicillium spinulosum	8

* The prevalence (%) of strains computer related to the total isolates separately for bacteria and fungi.

3.4 Conduct a Microbial Risk Assessment (MRA) to determine potential health risks associated with fresh produce-related outbreaks in Qatar

3.4.1 Outcomes of the Customer Survey

The number of male and female customers visiting the market was almost the same (51.7 % male and 48.3 % female). Most of the WSFPM customers were of Arab origin (46.5%), followed by Asian descendants (33.9%). The majority of the customers were married (74.8%), with 66.1% of them having family members ranging between 3 and 6 persons, while 23.0% had more than 7 persons in their families. The median age interval for the customers was 31-40 years old, and more than 82% had a college or graduate degree. In terms of family income, 80% of the customers earned 30,000 QAR or less per month. Table 23 summarizes the demographic data for the customers participated in the survey questionnaire.

Another set of questions, listed in Table 24, were used to determine the frequency of visiting the market and food safety knowledge levels of customers. These questions indicated that 75.5% of customers visit WSFPM 1 to 3 times per month. About 7.4 % of the customers answered the question on "if they get sick after consuming fresh produce purchased from the market" positively, among whom 85% did not visit the health clinic after getting sick, meaning that most of the food poisoning cases were mild and did not require medical assistance. In addition, 69.5% of the customers preferred to buy the produce imported from the surrounding region such as GCC countries, Jordan, and Lebanon (data not presented in the table). Regarding the produce quality, 76.5% of the customers found damaged produce with different frequency, indicating the need for implementation of more rigorous inspection. The regular inspection is usually carried out by a smell or appearance test at the market. It

is a known factor that any type of damage on fresh produce might enhance the probability of pathogen contamination, which in turn might increase the potential risk factor for public health.

Table 23. Demographic characteristics of customers participated in the survey (n=230).

Parameter	Characteristic	Number (%)
Gender		
	Male	119 (51.7)
	Female	111 (48.3)
Marital status		
	Married	172 (74.8)
	Not Married	58 (25.2)
Country of origin		
	Qatari	41 (17.8)
	Arabs	107 (46.5)
	Asian	78 9(33.9)
	European/American	(1.74)
Age		
	Less than 30 years	62 (27.0)
	31-40 years old	84 (36.5)
	41-50 years old	55 (23.9)
	51 and more	29 (12.6)
Education		
	Elementary to middle school	14 (6.09)
	High school	27 (11.7)
	College	114 (49.6)
	Graduate school	75 (32.6)
Family size		
	1-2 members	25 (10.9)
	3-4 members	60 (26.1)
	5-6 members	92 (40.0)
	7 members or more	53 (23.0)
Family income		
	<10,000 QAR	57 (24.8)
	10,001 to 30,000 QAR	127 (55.2)
	30,001 to 50,000 QAR	20 (8.7)
	>50,001 QAR	16 (7.0)

Table 24. Questionaire used to survey WSFPM customers (n=230).

Questions	Multiple choice answers	Frequency (%)
Frequently shopping from WSFPM	1-2 times/ week	43 (18.7)
	3-4 times / week	14 (6.1)
	1-3 times/ month	173 (75.2)
Getting sick after consuming fresh produce		
	Yes	17 (7.4)
	No	173 (75.2)
	I don't remember	40 (17.3)
Visiting hospital after getting sick		
	Yes	25 (10.9)
	No	196 (85.0)
	I don't remember	9 (4.0)
Preferences for the produce origin		
	Yes	70 (30.4)
	No	160 (69.5)
Finding damaged/spoiled produce between the purchased produce		
	None	9 (4.0)
	Rarely	45 (19.6)
	Sometimes	143 (62.2)
	Most of the time	30 (13.0)
	Every Time	3 (1.3)

The customers were also asked about their knowledge and practices of food safety. The analysis demonstrated that 86.5% of them wash the produce with only water before consumption, while only 12.2 % used food sanitizers to decontaminate the produce before eating. The majority of the customers indicated that they also visited the surrounding markets at the time of their visit to the WSFPM. This was observed as something common since the other markets (fish, food animal, and poultry) are in close proximity to the WSFPM, the customers wanted to save their time. As they visit the other markets, 85% used the same trolley in which produce was carried, 15% of the customers preferred to carry their own purchase. Such practices applied by the majority of customers may increase the potential risk factor of getting sick and enhance the infection of their family members since most of the customers had more than 3 family members.

It is clear that food safety practices need to be improved by educating the families especially those who are in charge of preparing meals at home for the entire family in order to reduce the risk of infection and decrease cross contamination (Angelillo *et al.*, 2001; Ayaz *et al.*, 2018; Alqurashi *et al.*, 2019). When the customers were asked to rate the level of sanitary conditions of the market, most of them agreed that the hygiene situation needs to be improved and the market should be moved to a covered (indoor) area. The most surprising finding was related to the customers' awareness of the different sources of produce contamination, which may play a major role in contaminating the fresh produce sold at the market, such as soil, temperature, wind, workers, or surrounding markets (Table 25).

Table 25**.** Questionaire used to create the scores of food safety practices and customers' satisfaction regarding the sanitary conditions of the WSFPM.

Questions	Multiple choice answers	Frequency (%)
Washing fresh produce with water before eating		
	Rarely	2 (0.9)
	Sometime	3 (1.3)
	Most of the time	26 (11.3)
	Every time	199 (86.5)
	Don't know	0 (0)
Washing fresh produce with food sanitizers before eating		
	Rarely	120 (52.2)
	Sometimes	34 (14.8)
	Most of the time	15 (6.5)
	Every time	28 (12.2)
	Don't know	33 (14.3)
Visiting the surrounding markets at the time of your visit to the WSFPM		
	None	43 (18.7)
	Rarely	29 (12.6)
	Sometimes	111 (48.3)
	Most of the time	40 (17.4)
	Every Time	7 (3.0)
Asking the workers to carry your purchased produce to the car		
	None	34 (14.8)
	Rarely	40 (17.4)
	Sometimes	78 (33.9)
	Most of the time	45 (19.6)
	Every Time	33 (14.3)

Table 25 Cont. Multiple-choice questions used to create the scores of food safety practices and customers' satisfaction regarding the sanitary conditions of the WSFPM.

Questions	Multiple choice answers	Frequency (%)
Your opinion regarding the main source of contamination of fresh produce		
	Soil	25 (10.0)*
	Workers	49 (21.3)
	Surrounding area	92 (40.0)
	Environmental factors	122 (53.0)
	Don't know	25 (10.9)
Satisfaction regarding the sanitary conditions in the WSFPM		
	No	49 (21.3)
	Yes	20 (8.7)
	Needs some improvement	148 (64.3)
	Don't know	13 (5.7)
Satisfaction regarding the location of the WSFPM		
	No	68 (29.6)
	Yes	49 (21.3)
	Change location	98 (42.6)
	Don't know	15 (6.5)
Satisfaction regarding the application of hygienic standards by the workers at WSFPM		
	No	91 (39.6)
	Yes	10 (4.3)
	Needs some improvement	108 (47.0)
	Don't know	21 (9.1)

*This question does not show the % (out of 100), because many customers chose more than one answer.

4.4.2 Health risk associated with consuming fresh produce contaminated with coliforms

To assess the annual potential risk of getting sick after consuming raw produce purchased from the WSFPM; the risk estimate scores developed from sQMRA model implemented in @Risk were collected and presented in Table 26. The results indicated that the mean risk estimates for consuming the fresh produce contaminated with coliforms as surveyed in this study ranged between 0.27 and 0.46. The highest risk estimate was associated with consuming cucumber (0.46) compared to other produce tested. This high estimate could be due to several factors, such as the estimated portion of consuming cucumber per individual (person/month) was more than those of lettuce, parsley, and green onion. Although the estimated consumption portion for tomato (person/month) was higher than cucumber, the estimated risk was lower. This is mainly due to the fact that many consumers cook tomato, which reduces this risk even if the raw tomatoes were heavily loaded with coliforms.

Table 26. Annual probability of getting sick after consuming the surveyed raw fresh produce contaminated by coliforms

Produce	The probability of illness		
	2.5%	Mean	97.5%
Parsley	2.9×10^{-10}	0.37	1
Tomato	1×10^{-10}	0.31	1
Cucumber	2.3×10^{-9}	0.46	1
Lettuce	6.3×10^{-11}	0.27	1
Green onion	3.3×10^{-11}	0.27	1

In addition, the probability of illness after consuming the raw produce contaminated with coliforms for all produce surveyed in this study was estimated at 1.0 with 97.5^{th} percentile, meaning that there might be certain illness associated with consuming these produce. These estimates do not reflect the true risk of consuming produce, rather than indicating the high level of uncertainty related to the assumptions used in the preliminary risk assessment (Abong *et al.*, 2008). This exaggerated probability could be due to the high assumption of daily-consumed produce and the high count of coliform per gram, also the high risk associated with these produce comes from eating them raw and unpeeled e.g. cucumber (Hamilton *et al*., 2006). Moreover, the uncertainties were not taken into consideration in sQMRA model during the calculations, which usually increase the value of the relative risk estimates (Chardon and Evers, 2017). Furthermore, food culture, consumption rate, body mass, susceptibility to disease, age, storage temperature, distribution method, type of produce, proximity cultivation in ground, surface characteristic, treatment with antimicrobial agents, storage temperature and conditions, produce handlers' contribution, agriculture practices, and transportation conditions could be additional factors affecting the risk (FAO/WHO, 2008) and need to be implemented in the risk assessment model to obtain a more realistic risk estimates.

According to FAO/WHO (2008), coliforms are commonly isolated from fresh produce and their counts could reach up to 8 Log_{10} MPN/g. This count could be varied from one study to another, and it is affected by several factors such as sampling point and differences between plants surveyed. The fresh produce are usually characterized by having a high level of natural microflora, most of these genera are involved in the coliform group, for that FAO has not yet considered the coliforms as a significant issue

in food safety, but it is used as an indicator especially regarding the hygiene conditions of a processing facilities(FAO/WHO, 2008).

Having a reasonable and realistic risk assessment depends on the quality of data used to build the risk assessment. The lack of specific data may affect the sensitivity of the assessment and increases the degree of uncertainty (Boone *et al*., 2010). Measuring the variability and the uncertainty of the applied model will help to enhance the sensitivity of the risk assessment and getting risk estimate closed to the real situation. Normally, the range of the variability describes the diversity and the integral heterogeneity of the data during the assessment (EPA, 2012). The uncertainty range can be reduced by inputting more data in the model and improving the sampling step by covering several food production stages (Hamilton *et al*., 2006).

The sQMRA model measures the variability depending on the portion contaminated e.g. the pathogen concentration at the consumption level, retail, storage conditions, cross-contamination, and the survival during preparation (e.g. heating), besides the dose-response parameters. This study covered the contamination level at the retail and did not include the presence of risks at other stages, which could have impacted the variability (ranged between $3.4 \text{ X } 10^{+7}$ and $3.0 \text{ X } 10^{+8}$). It is important to emphasize here that the sQMRA does not consider the uncertainties in the calculation, which can be measured as a weak point of this model and the risk assessment. Identifying the uncertainties and considering them in the risk characterization, might reduce the risk estimates and provide a reasonable and more realistic risk estimate.

In general, this risk assessment study can be considered as a baseline for future studies. However, certain considerations need to be carefully taken into account when using these data. Since none of the target pathogens were identified in this study, the health risk assumptions were built on the default parameters as built in the sQMRA

model and the risk associated with produce contaminated with coliforms. This assumption was made mainly due to not being able to obtain real-life data on the number of foodborne illnesses and type of pathogens associated with produce outbreaks witnessed in Qatar. If these type of data become available, more realistic risk assessment study could be carried out.

The overall results of this study could assist the government agencies in adopting a risk-oriented approach to protect the public health from the negative effects of produce related outbreaks.

CHAPTER 4: CONCLUSION

This is the first of its kind study in the region, which could help in filling a knowledge gap in the literature and serve the public health in the region regarding the assessment of fresh produce quality sold in open-air markets, like the wholesale produce market in Doha. This knowledge gap may be basic in other regions where food safety has received ample attention, but it is highly important and needed in Qatar and GCC countries. Fresh produce has been associated with foodborne illness and outbreaks in an increasing rate in the last decades. This increase generated a growing interest in determining the risk factors at different stages of fresh produce marketing.

The result of this study indicated that the fresh produce surveyed were heavily loaded with different microorganisms compared to the international standards, especially for total aerobic counts and coliforms (both > 5 Log CFU/g), which are normally used to indicate the hygiene condition of any food establishment. None of the target pathogenic strains was identified from any of the surveyed produce during the whole study. The most dominant strains identified in summer months (47°C daytime and 35°C nighttime) were *Bacillus* spp., *Enterococcus* spp., and *Klebsiella* spp., while *Pseudomonas* spp. and *Enterobacter* spp. were the most abundant species during the winter months (24°C daytime, 12°C nighttime). In addition, *Penicillium* spp., *Aspergillus* spp., *Alternaria* spp., and *Fusarium* spp. were the most commonly isolated fungal species from the produce tested in this study. These results emphasize the urgent need to enhance the hygiene practices applied at the market. Examples of practices which can help improve the hygiene at the market include the application of HACCP by collecting and testing the microbial quality of produce samples periodically. The HACCP will help in recognizing the critical points at the transportation, storage, and handling steps. In return, this process might guide the regulatory agencies to apply

appropriate mitigation measures to prevent and control the contamination of fresh produce. Furthermore, the pathogens targeted in this study should be considered by the MoPH and municipalities and added to their inspection risk hazards list.

The results demonstrated that not only the market location contributed in the fresh produce contamination, but also the handling practices applied by food handlers, the local environmental, ecological conditions of the country, and the agriculture practices applied in the original country during produce cultivation. These factors can be used to predict the presence of some pathogens, which have to be controlled to reduce the relative risk factor associated with food pathogens. The municipalities in Qatar who are managing the produce markets have to redesign the produce markets to be far away from the expected source of contamination. Right before the completion of this study, the location of the target market was discussed at the ministry level and it was decided to relocate the fish market and the chicken slaughterhouse away from the WSFPM. The MoPH and Doha Municipality might need to work together in establishing guidelines and setting limits to control the microbial risk factors in fresh produce sold in the local markets. These guidelines have to be compatible with the international standards and local environmental factors, which negatively impact the produce quality.

The produce handlers' assessment revealed that the workers did not receive any training on food safety application, have no knowledge on basic food safety, and they lack the hygiene knowledge (appropriate handwashing; using gloves, bathrooms, and uniforms). In addition, they are moving freely between the WSFPM and surrounding markets; therefore, they carry organisms of different origins on their hand palm. Moreover, the walk-through audit results indicated an urgent need to improve the infrastructure of the market. These results might provide a basic information for the

public health agencies in Qatar to establish guidelines for compulsory on-site training for produce handlers in order to improve their knowledge on safe produce handling. In addition, families (especially mothers) and the person in charge of food preparation at home (e.g. the cook or housemaid) have to be involved in such trainings to improve their food safety knowledge, reduce the infection risk, and decrease the cross contamination.

The environmental sample (e.g. soil, air, and surfaces) analysis indicated that the location of the wholesale market influenced the fresh produce quality. The environmental conditions at the WSFPM, such as temperature and humidity, are the major factors affected the presence of different microorganisms and some pathogens, such as *Klebsiella pneumoniae* identified in the air and the produce samples as well.

The risk assessment estimates indicated that the annual mean risk estimate for getting sick after consuming raw fresh produce contaminated with coliform (as a significantly high count) was between 0.27 and 0.46. These results emphasize the need for improving the sanitary conditions at this major produce market. The results of this study could assist the government agencies in adopting a risk-oriented approach to protect the public health from the negative effects of produce related outbreaks.

Future studies should focus on conducting microbial risk assessment studies using more appropriate and local data in order to obtain reasonable risk estimates. In addition, it would be interesting to carry out a surveillance study to determine the frequency of diarrheal or other foodborne illnesses among produce handlers before and after educational training.

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APPENDIX

Appendix 1

Survey to Determine the Food Safety Knowledge of and Food Safety Practices applied by Workers at the Wholesale Produce Market in Doha

This is Israa El-Nemr, I'm a PhD. Student in the Dept. of Biological and Environmental Sciences at Qatar University. We are conducting this survey to determine the level of food safety knowledge of workers and food practices applied at the wholesale market in Doha. I appreciate your collaboration in taking about 10 minutes of your time in helping me to complete this survey. **Your answers and opinions will be kept strictly confidential.**

Part I

1. **Your age:**
   - [ ] less than 20 years old
   - [ ] 20-30 years old
   - [ ] 31-40 years old
   - [ ] 41-50 years old
   - [ ] 51-60 years old
   - [ ] Above 60 years old
2. **Your nationality:**
   - [ ] Indian
   - [ ] Bangladeshi
   - [ ] Pakistani
   - [ ] Nepali
   - [ ] Other (Please indicate the country _____)
3. **Your education level:**
   - [ ] Elementary
   - [ ] Middle School
   - [ ] High School
   - [ ] College
   - [ ] Graduate
   - [ ] None
4. **How long have you been working in this market?**
   - [ ] less than one year
   - [ ] 1-3 years
   - [ ] 3-5 years
   - [ ] More than 5 years

Part II

1- Did you take any training before you started working at this market?

[ ] Yes
[ ] No

2- Is there any annual injection (vaccination) you must take to be able to work at this market?

[ ] Yes
[ ] No

3- If you answer "yes", could you please mention what type of injection you take (e.g. Hepatitis A).

……

4- Is there any health checkup (inspection), you have to do as a worker in this market?

[ ] Yes, once a year
[ ] Yes, twice a year
[ ] No

5- Do you wash your hands after using the bathroom (restroom)?

[ ] Yes
[ ] No

6- How do you wash your hands after using the bathroom (restroom)?

[ ] Use liquid soap and hand sanitizer
[ ] Use liquid soap
[ ] Just tap water
[ ] Not wash at all

7- How often do you wash your hands during the work hours while handling fresh produce?

[ ] Once
[ ] 2-3 times
[ ] 4-5 times
[ ] More than 5 times
[ ] None

8- What do you think about the hygiene level of bathrooms at the work site?

[ ] Excellent
[ ] Very good
[ ] Good
[ ] Needs some improvement
[ ] Poor
[ ] Don't know

9- Do you have to wear gloves during your work?

[ ] Yes, working gloves
[ ] Yes, disposable gloves
[ ] No

10- How often do you visit the surrounding markets (fish market, slaughterhouse, livestock market) during your duty time at the wholesale market?

[ ] None
[ ] Rarely
[ ] Sometimes
[ ] Most of the time

11- Have you experienced any of the following symptoms in the last 3 months: nausea, diarrhea, vomiting, fever, and fatigue?

[ ] None
[ ] Rarely
[ ] Sometimes
[ ] Most of the time

12- Does the manager give you a sick leave when you get sick (e.g. having nausea, vomiting or diarrhea)?

[ ] Yes
[ ] No

13- Does the manager ask you to wear a uniform?

[ ] Yes
[ ] No

14- For how long do you keep wearing the same uniform?

[ ] 1 day
[ ] 2-3 days
[ ] 4-5 days
[ ] more than 5 days

15- Do you face any inspectors from the municipality checking your personal hygiene (e.g. hands, hair, nails…etc.)?

[ ] Yes
[ ] No

16- If you are injured during your work, is there any first aid kit available on site?

[ ] Yes
[ ] No

17- Do you clean the display benches every day?

[ ] Yes
[ ] No

18- How do you keep the produce not sold on the same day?

[ ] In the fridge
[ ] In the same presentation place
[ ] No keep at all, (throw or return extra produce)
[ ] Others (explain……..……)

19- What is the water source you use to clean the work area?

[ ] Own water tank
[ ] City water
[ ] Bucket of water collected from other place
[ ] Others ……..……..

20- How do you clean the plastic boxes you keep the produce?

[ ] Use detergent
[ ] Just tap water
[ ] Not wash at all

21- Do you think the sanitary conditions at the wholesale produce market are appropriate?

[ ] Yes
[ ] No
[ ] Needs some improvement
[ ] Don't know

Thank you for your time!

Appendix 2

Walk-through Audit Checklist for the Wholesale Market in Doha

Name of the research member…….…….Date ……

Observation	**Compliance (%)***	**Remarks**
Systematic inspection conducted		
Food safety training provided to inspectors		
Food safety training provided to workers		
Produce storage place		
Cleanliness of the market: o Entrance o Garbage disposal and storage o Toilets o Display area o Surrounding place o Presents of insects/animals (e.g. flies, mosquito, and cats)		

Appendix 3

Survey on Fresh Produce Safety at the Wholesale Market in Doha

This is Israa El-Nemr, I am a Ph.D. Student in the Dept. of Biological and Environmental Sciences at Qatar University. We are conducting this survey to analyze how the public are satisfied with the safety of fresh produce sold at the wholesale market in Doha. I appreciate your collaboration in taking about 10 minutes of your time in helping me to complete this survey. **Your visions and opinions will be kept confidential**.

Part I

1- Gender:
[ ] Female
[ ] Male

2- Your age:
[ ] Less than 20 years old
[ ] 21-30 years old
[ ] 31-40 years old
[ ] 41-50 years old
[ ] 51-60 years old
[ ] Above 60 years old

3- Your marital status?
[ ] Single
[ ] Married
[ ] Divorced
[ ] Widowed

4- Number of family members including yourself is:
[ ] 1-2 members
[ ] 3-4 members
[ ] 5-6 members
[ ] 7 and above members

5- Your nationality:
[ ] Qatari
[ ] Non Qatari (originally Arabs)
[ ] Non Qatari (originally Asian)
[ ] Non Qatari (originally European)
[ ] Non Qatari (originally American)
[ ] None of the above

6- Your education level:

[ ] Elementary
[ ] Middle School
[ ] High School
[ ] College
[ ] Graduate
[ ] None

7- Your family income per month:

[ ] < 10,000 QR
[ ] 10,001 - 30,000 QR
[ ] 30,001 - 50,000 QR
[ ] 50,001 - 70,000 QR
[ ] 70,001 - 100,000 QR
[ ] > 100,000 QR

Part II

8- How often do you buy fresh produce from the wholesale market?

[ ] 1-2 times a week
[ ] 3-4 times a week
[ ] 1-2 times a month
[ ] 3 or more times a month

9- How often do you wash fresh produce with water before eating?

[ ] Rarely
[ ] Sometimes
[ ] Most of the time
[ ] Every time
[ ] Don't know

10- How often do you wash fresh produce with food sanitizers or any other chemical before eating?

[ ] Rarely
[ ] Sometimes
[ ] Most of the time
[ ] Every time
[ ] Don't know

11- Did you ever get sick after consuming (vomiting, nausea, diarrhea, etc.) fresh produce (e.g. tomato, cucumber, lettuce, etc.) purchased from the wholesale market?

[ ] Yes (Answer question 5)
[ ] No (Skip question 5)
[ ] I don't remember

12- If you answer yes to Q #4, did you visit a hospital?

[ ] Yes
[ ] No
[ ] I don't remember

13- How often do you visit the surrounding markets (fish market, slaughterhouse, life stock market) at the time of your visit to the wholesale market?

[ ] None
[ ] Rarely
[ ] Sometimes
[ ] Most of the time
[ ] Every Time

14- How often do you ask the workers in the market to carry your purchased produce to the car?

[ ] None
[ ] Rarely
[ ] Sometimes
[ ] Most of the time
[ ] Every Time

15- How often do you purchase your produce from the open area of the market compared to the closed area?

[ ] None
[ ] Rarely
[ ] Sometimes
[ ] Most of the time
[ ] Every Time

16- Do you have any preferences for certain produce imported from specific countries?

[ ] No
[ ] Yes ……..

17- If you answer yes to Q#9, what is your preferred country when purchasing produce?

Name**:** ……..

18- How often do you find a damaged or spoiled produce at the time of your shopping?

[ ] None
[ ] Rarely
[ ] Sometimes
[ ] Most of the time
[ ] Every Time

19- In your opinion, what is the main source of contamination of the fresh produce sold at the wholesale market?

[ ] Soil
[ ] Workers
[ ] Surrounding area
[ ] Environmental factors
[ ] Don't know

20- Do you think that the sanitary situation in the wholesale market is satisfactory?

[ ] No
[ ] Yes
[ ] Needs some improvement
[ ] Don't know

21- Do you think that the location of the wholesale market is appropriate considering that it is located between the animal market and the fish market?

[ ] No
[ ] Yes
[ ] change location
[ ] Don't know

22- Do you think that the workers at the wholesale market apply hygiene standards?

[ ] No
[ ] Yes
[ ] Needs some improvement
[ ] Don't know

Thank you for your time!