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INITIAL ASSESSMENT OF ENVIRONMENTAL MICROBIAL HAZARDS IN DOHA RESTAURANTS

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ABSTRACT

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Title: Initial Assessment of Environmental Microbial Hazards in Doha Restaurants

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This study was carried out to assess hygiene conditions, food handling practices, food safety knowledge of food service providers (FSPs), and the microbial quality of food served in different food service establishments in Doha. Fifty-three FSPs were randomly selected among 200 FSPs. Face-to-face interviews with the food safety managers at each participating FSP were conducted using a survey consisting of 40-questions (demographic data on workers, HACCP training, knowledge on personal hygiene, and safe-food handling practices) in October-December 2015. In addition to survey questionnaire, a checklist was used to determine the implementation of international food safety standards by observing actual practices applied at each FSP. The microbial quality of food samples (n=105) served and swabs collected from food preparation surfaces (n=58) were also assessed using select media (APC, MCA, XLT4, and LSA). The identification of positive samples was carried out using VITEK-2 system.

The survey results indicated that average service years of FSPs was 11, the average age of food safety managers interviewed was 33, most managers (66%) had college degree, and 68% of them were trained on HACCP. It was demonstrated that casual-sit-in and fine-dine-in restaurants are the only FSP types which consistently kept records (100%), followed by fast-food (36%), and catering (14%) FSPs. The microbial analysis indicated that the average APC in food samples collected from all FSPs met the international standards, while the APC counts of swab samples were considered unsatisfactory since the

levels were above 10^6 Log_{10} CFU/cm^2. The highest bacterial count was reported in swab samples (7.26 Log_{10} CFU/cm^2) collected from preparation area in takeaway restaurants. Concerning the target organisms (*Escherichia coli, Salmonella* spp., and *Listeria monocytogenes*), among 105 food samples and 58 swab samples collected, 13 samples (8%) exhibited positive results for possible target pathogens. Positive samples were identified as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa,* and *Pantoea* spp.

Results obtained in this study might help food safety managers in these select FSPs to better understand the need for implementing effective control measures in order to prevent contamination and eventually protect the public health.

DEDICATION

I would like to dedicate this study to the residents of Qatar and those who need this knowledge.

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CHAPTER I: LITERATURE REVIEW

1.1 Environmental Health and Food Safety:

Food safety is a specific field of environmental health dealing with preparation, handling, and storage of food materials to be able to reduce/eliminate foodborne illnesses that can result in serious public health issues. Food safety deals with foodborne illness outbreaks, keeping records of foodborne illness, investigating and testing the food (ready to eat / fresh food) for any harmful containments (Tauxe *et al*., 2010). These containments can be from chemical to any pathogenic bacteria. Basically, food safety handles all the associated risks which may arise during the pre- and post-harvest stages of food preparation. Food safety is regulated by cooperation of food industries and agencies at government or state level. Foodborne illness caused by consuming food contaminated with pathogenic microorganism or physical or chemical agents can cause burden of disease on the country. Biological hazards include bacteria, viruses, and parasites that are major challenges for food safety since these organisms cannot be seen with naked eye. Chemical hazards can be allergens; toxins synthesized by molds, mushrooms and marine species, including puffer fish and shellfish; pesticides; polychlorinated biphenyls (PCB); cleaning agents; and metals such as lead and mercury. Ingestion of food contaminated by microbes causes foodborne infections resulting in symptoms like diarrhea, vomiting, and stomach pain etc. While foodborne diseases usually caused by chemical hazards are called intoxications (Frumkin, 2016). Physical hazards can occur due to poor handling of food during processing and at food retails in the establishment. Pieces of glass, stones, dust particles, jewelry, and bone

fragments can all be included in physical hazards class. Every foodborne disease has an onset time of foodborne illness which depends on the type of poison, amount of poison, exposed person's age, weight, and his health status (Frumkin, 2016).

Environmental health services have major concern about food safety in developing countries as it is not handled carefully it can take a serious toll on public health creating an economic burden on the society since the treatment of foodborne diseases is very costly. Common symptoms after consuming contaminated food include gastrointestinal problems, vomiting, diarrhea, but it can also be more severe as chronic, immunological, neurological, gynecological, multi-organ failure, and death (WHO, 2007). Disability adjusted life year (DALY) can be used as a framework to assess or monitor the health problems caused by foodborne diseases. Disability adjusted life year is to evaluate the disease burden (which in this case is foodborne disease) causing health problems, disability or early death over the period of number of years (Haagsma *et al*., 2013). Assessment of the magnitude of health diseases caused by foodborne pathogens will help in allocating how much medical resources needed to withstand the foodborne outbreak and also will help in what possible interventions are needed in the food chain industry of a country (Havelaar *et al*., 2007). To achieve this, there is a need of extensive epidemiological studies on health effects caused by foodborne outbreaks that occurred in a given country (Havelaar *et al*., 2012). A recent study carried out by Cassini *et al*. (2016) in Europe reported the incidence verses prevalence, risk of health complication and death included in the modeling to assess the burden of diseases output and DALYs. It was concluded that this model can provide comprehensive comparison between risks and hazards which can be important tool in food safety at national,

regional, and global level.

1.2 Food Safety Issues:

Foodborne outbreaks (FBO) are major concerns for public health officials all over the world. Duty of public health officials is to investigate the source of food product related to the outbreak of that foodborne diseases and remove those products from the establishment (Marvin *et al*., 2009). Outbreaks from the food served in restaurants or institutions like school, nursing homes etc. account for about 40% of all outbreaks (Angulo & Jones, 2006). When two or more cases of the same foodborne pathogen is recorded after the consumption of the same type of food, it is declared by clinicians as an outbreak (Rocourt *et al*., 2003). Through surveys asking questions about history of persons becoming ill and the record from where the contaminated food has been bought can accelerate the foodborne outbreak investigation (Hu *et al*., 2016). Foodborne disease is an issue that can be globally recognized. Incident rate of about 2,100 cases in France, 2,600 in the United Kingdom, 48 cases in Malaysia, and 25,000 cases in Australia and the United States are recorded with respect to population of 100,000 inhabitants, respectively (Abdul-Mutalib *et al*., 2015). According to the United States (U.S.) Center for Disease Control and Prevention (CDC) online database, about 358,391 people became sick, 13,715 were hospitalized, and 318 people died between 1998 and 2014 due to foodborne illnesses (CDC, 2014). Globally, it is estimated that about 600 million people become ill every year after consuming contaminated food (WHO, 2015).

Table 1.1. *The total Foodborne DALYs in the Different regions of the World from 2005-2015* (WHO, 2015).

Region	Foodborne Illnesses	Foodborne Disability-Adjusted Life Years	Illnesses	Deaths
North American	2,537,838	69,160	4,060,384	1,129
Middle Eastern	17,371,237	397,759	47,182,976	12,719
European	3,367,514	102,780	4,641,359	1,677
Australian	1,623,277	30,674	2,401,319	482

1.3 Foodborne Outbreaks in Food Service Operations:

Foodborne outbreaks (FBOs) in Food Service Operations (FSO) such as restaurants and food industries can be defined as occurrence of more than 2 cases of illness from the consumption of the same type of food, or eating food from the same FSO or eating in the same facility (Wu *et al*., 2014). According to previous studies carried out between 1986-2004, about 9,040 FOBs were recorded by the CDC, 52% of which (4,675) were linked to restaurants including cafeterias (Angulo& Jones, 2006). Another study conducted in China concluded that 39% of the FBOs occurred in restaurants, 30% from workplace and school cafeterias, and 15% of reported outbreaks occurred at residential kitchens (Wu *et al*., 2014). Studies in Austria concluded that about 31% of the outbreaks occur in restaurants, hotels, pubs, cafes, and bars and 17% was linked to school catering and workplace canteens (Pichler *et al*., 2014).

Table 1.2. *Number of outbreaks related to restaurant, catering and takeaway establishments in the USA between 2006-2015* (CDC, 2016).

Pathogens	Outbreaks	Illnesses	Hospitalizations	Deaths
Listeria spp.	2	17	3	2
E. coli	46	997	158	0
Salmonella spp.	189	8317	750	1

According to the Health Ministry of Saudi Arabia, 1,647 and 2,066 cases of foodborne outbreaks were recorded in 2010 and 2011, respectively (Health Ministry of Saudi Arabia, 2013). When investigated further in 2010, 1,029 out of 1,647 cases (62%) were related to foods purchased from commercial restaurants. In 2013, Dubai has confirmed 518 cases of foodborne illnesses related to the foods purchased from commercial kitchens (Khaleej Times, 2014).

Qatar food safety authorities have been active in recent years, the Ministry of Municipality and Environment (MME) conducted 26,055 unexpected inspections at various restaurants, eateries, juice stalls, and food stores in Doha in 2015 (Doha News, 2016). Among these inspected food services, 161 were temporarily closed due to violating food safety rules. Similarly, a total of 175 cafe and food establishments were shut down in 2013 (Doha News, 2014). Under the Ministry of Public Health (MoPH), a special department was introduced to handle all food safety violation cases in restaurants, which was previously handled by two organizations, Ministry of Municipality and Urban Planning and MoPH. Also, about 250 inspectors were trained

on how to identify chemical, physical, and microbial risks in food items and how these hazards can be prevented, also how to manage a food business in Qatar according to international standards (Gulf Times, 2014).

As part of their duty, the inspectors collected 800 samples from different restaurants to determine microbial and chemical quality, 745 out of 800 samples were identified safe for human consumption, 44 samples violated the standards, and 11 samples were determined to be unfit for human consumption (Doha News, 2016).

1.4 Major Foodborne Pathogens:

Food contamination can be caused by air, water, soil, food handlers, packaging materials, animals (rodents and insects), food contact surfaces, and ingredients used in food preparation (Frumkin, 2016). Bacterial contamination with pathogenic bacteria is different from spoilage bacteria because change in food texture can be observed due to oxidation and color changes when spoilage bacteria are present in foods (Gram *et al.*, 2002). While contamination with pathogenic bacteria may not change the texture of food and does not look, smell or taste any different from safe to eat food (WHO, 2015).

For food safety, it is necessary for potentially hazardous foods to be processed at certain temperature and time to control the growth of pathogenic organisms and their toxins, usually secreted during their growth stages. There are two kinds of bacteria which can cause foodborne illness: those are spore-forming bacteria and non-spore-forming bacteria. Spore forming bacteria exist as vegetative cells in which some rod-shaped bacteria form spores (Ray & Bhunia, 2007). Spores are inactive state of bacteria which will grow when suitable pH, temperature, and humidity or food is present (Ray

& Bhunia, 2007). Spore-forming bacteria can survive many months. Vegetable and spices are major sources for the spore-forming bacteria as they naturally grow in soil. *Clostridium perfringens* and *Clostridium botulinum* are examples of spore-forming bacteria that are known to be foodborne pathogens (Frumkin, 2016). Non-spore-forming bacteria also exist as vegetative cells but do not form spores, thus cannot sustain themselves at high temperatures. These bacteria can be destroyed by heat during food preparation stage of cooking and pasteurization. *Eshereichia coli, Listeria, Salmonella,* and *Staphylococcus* are examples of non-spore-forming pathogenic bacteria (Ray & Bhunia, 2007).

Common pathogens causing foodborne illness are *Escherichia coli O157:H7*, *Salmonella enterica*, *Listeria monocytogenes, Campylobacter jejuni* as incidents are gathered and reported to European Centre for Disease Prevention and Control (ECDC, 2013).

E. coli generally found in the human gut normal flora but some pathogenic strains of *E. coli* like Serotype *E. coli* O157:H7 cause diarrhea, vomiting, abdominal cramps (Balakrishnan *et al.*, 2016). Serotype *E. coli* O157:H7 is an enterohemorrhagic *E. coli* (EHEC), which is also known to produce Shiga toxin (Stx). *E. coli* O157:H7 has been known to be associated with foods like ground beef, raw vegetables, unpasteurized milk and cheese made from it, as well as contaminated water (untreated water) and food animals, especially from cows, sheep, and goats (van Schothorst, 1997). Cross contamination can occur if hands are not washed properly after touching animals and their environment. As cattle are host for pathogens like *E. coli*, during slaughtering and cutting of cattle's body parts, these pathogens can contaminate portions of beef (Kundu

et al., 2014). *E. coli* O157:H7 with the infectious dose of between 1 to 100 CFU will be effective in causing infectious disease, as it is very resistant to low pH (Rybarczyk *et al.,* 2017). If *E. coli* O157:H7 contaminated food is consumed, it can lead to hemolytic uremic syndrome (HUS) and diarrhea which can be as severe as bloody diarrhea and hemorrhagic colitis (Rosser *et al.,* 2008). The transmission of *E. coli* O157:H7 is usually through fecal contamination or cross contamination (Nataro & Kaper, 1998). *E. coli* O157:H7 outbreaks due to ground beef have been reported many times, though much efforts have been made by meat processors to control the contamination, thus improper handling of ground beef can lead to cross contamination of foods which do not have to be thermally processed (Ready to Eat salads) (Zhou *et al*., 2016). In 1982, *E. coli* O157:H7 was first recognized as a human pathogen with 26 outbreak cases, 19 of which were hospitalized (Riley *et al*., 1983). There is an increased prevalence of *E. coli* O157:H7 in food products since the discovery of *E. coli* O157:H7 (Nyachuba, 2010). Each year about 75,000 cases of illness due to *E. coli* O157:H7 were recorded in the United States (Ho *et al*., 2013). Studies conducted in 7 different regions of China concluded the prevalence of *Listeria monocytogenes*, *Salmonella spp., Staphylococcus aureus* and diarrheagenic *Escherichia coli* in Ready to Eat meat products (Yang *et al*., 2016). In the United States, about 512 outbreaks were recorded for *Escherichia coli,* including 1,900 hospitalizations and 34 deaths from 1998 to 2014 (CDC, 2014).

Listeria monocytogenes is a Gram-positive and facultative anaerobic bacterium. The source of *L. monocytogenes* is usually the ready to eat meat and refrigerated patties and meat spreads, unpasteurized dairy products and milks, smoked and refrigerated

seafood (Muhterem-Uyara *et al*., 2015). *L. monocytogenes* is able to survive in food having low pH, high salt concentration (14%), water activity of minimum 0.9 (a_w), and is able to grow in temperature ranging from -4° to 45°C (Iannetti *et al*., 2016 & Osimani *et al*., 2016). Listerosis is often involved in outbreaks associated with the consumption of Ready to Eat (RTE) food products which do not require further cooking and are stored in the refrigerator for a long period with specific humidity and pH (Iannetti *et al*., 2016). This pathogen can be found in various kinds of food matrix including raw milk and raw meat. At risk are mostly individuals who are immune compromised, pregnant, had organ transplant, and adults over 65 years old (Chan and Wiedmann, 2009). There are only three types of pathogenic strains of *L. monocytogenes*, namely serotype 1/2a, 1/2b, and 4b which cause 90% of the human Listeriosis cases (Ward *et al*., 2004). The tolerance rate for the *L. monocytogenes* varies for different regions: EU has a limit of 25g of RTE food not to have been contaminated with *L. monocytogenes* and the USA has zero tolerance for *L. monocytogenes* (European Commission, 2005; USFDA, 2006). For all the major outbreaks in Europe and North America since 1980's, *L. monocytogenes* serotype 4b has been responsible for 30 to 55% of sporadic human cases for all major foodborne outbreaks, respectively (Ward *et al*., 2004). In 2012, about 1,642 listeriosis cases were recorded in the European Union (EU) (EFSA, 2014). Latest data shows alarming incline in listeriosis cases in Europe, showing increase of 8.6% from 2012 to 2013 and 30% from 2013 to 2014, 2,100 estimated cases of human listeriosis were reported yearly in the EU (Iannetti *et al*., 2016). In the United States, 58 outbreaks of Listeriosis were recorded from 1998 to 2014, 521 were hospitalized and 116 faced death (CDC, 2014).

Salmonella enterica is a Gram-negative, rod shape, facultative anaerobic bacterium belonging to a genus Salmonella. Food source for Salmonella can be from unpasteurized milk or juice, contaminated eggs, poultry, meat, and raw vegetables (sprouts and melons) (Park *et al*., 2015; McCabe *et al*., 2011; Threlfall, 2002). Also, food products made from raw eggs, sprouted seeds, beef, nuts, unpasteurized fruit juices may be contaminated with *S. enterica* (Abdelhaseib *et al*., 2016). Salmonella originates from the feces of almost all animals and can find its way to the food if kitchen is not kept hygienic (Barker *et al*., 2003). In recent years, most of the recorded foodborne illnesses is due to Salmonellosis (Kotzekidou, 2013). *S. enterica* serotype Entritidis is one of the most common causes of human salmonellosis (Deng *et al*., 2014). In the United States, 1,491 cases of *S. enterica* and its serotypes were recorded during 1998-2008, 403 of which were implicated with food products of single commodity (defined as aquatic animal derived, animal derived and plant derived foods) (Jackson *et al*., 2013). *Salmonella* has been associated with 2,273 foodborne outbreaks, 6,952 hospitalizations, and 79 deaths in the US from 1998 to 2014 (CDC, 2014). The annual incident rate for laboratory confirmed salmonellosis cases per 100,000 population were recorded in Qatar through 2004 to 2012 as 12.3, 23.0, 30.3, 19.4, 15.3, 18.0, 22.7, 18.5, and 18.1, respectively (Farag *et al*., 2016). Higher incident rates for salmonellosis were usually recorded during summer (between May and September) months and low incident rate was reported during winter (between January and April) months (Farag *et al*., 2016). Most encountered salmonellosis cases were serotype b, then serotype d, and least were serotype c1 with 41.9%, 26.9%, and 12.2%, respectively (Farag *et al*., 2016).

Studies have been conducted to detect *Salmonella spp*., *Listeria monocytogenes*,

Escherichia coli and *E. coli* 0157:H7 on food sold by street vendors in Souq Waqif, Doha. It was determined that *E. coli* was the major contaminated isolated from two food samples (Elobeid *et al.*, 2014). A recent study completed in Doha concluded that *E. coli* is the major gastroenteritis-causing pathogen in majority of patients visiting hospitals in Doha (Weam *et al.*, 2016). Mohammed *et al*. (2012) reported that there is a threat to food safety at the preharvest levels of the food supply chain in Doha. Four pathogens were detected, namely non-O157:H7 Shiga-toxin producing *E. coli* which were detected in retail food and animal products in higher rate, while *E. coli* 0157:H7 were detected at the rate of 6% in food and animal products. A recent study also emphasized the need to improve the cooking practices for animal related food products since serotypes non-O157 STEC *E. coli* were found to be the major threat to the food supply system in Qatar (Mohammed *et al.*, 2015).

Peters *et al.* (2014) studied the risk of foodborne pathogens in retail foods sold in the USA. They recorded high prevalence of Shiga toxin-producing *E. coli* (STEC) with the rate of 16.6% and STEC serotype O45 with the rate of 20.1% and concluded that there is a need to properly cook the meat products to eliminate or control these pathogens.

Table 1.3. *The list of foodborne illnesses and deaths and associated foodborne pathogens between 2005-2015* (WHO, 2015).

Region		Campylobacter spp.	Enteropathogenic *E. coli*	Shiga toxin-producing *E. coli*	Non-typhoidal *S. enterica*	Total
North American	Foodborne Illnesses	1,254,852	35,716	30,099	1,072,185	2,537,838
	Foodborne Deaths	182	0	5	543	761
Middle East	Foodborne Illnesses	2,809,845	623,139	108,410	2,620,360	17,371,237
	Foodborne Deaths	1,334	933	2	1,007	4,509
European	Foodborne Illnesses	2,326,017	39,304	145,103	797,668	3,367,514
	Foodborne Deaths	245	0	23	886	1,188
Australian	Foodborne Illnesses	1,149,438	15,399	36,483	395,362	1,623,277
	Foodborne Deaths	72	0	6	235	326

1.5 Controlling Foodborne Pathogens in Food Service Establishments:

People choose dining option based on the hygiene of the premises but not keeping in mind the food safety level of the establishment (Lee *et al*., 2012). There should be a system of food safety management, specifically concentrating on the hygiene practices used in the food processing stages in any given establishment (Djekic *et al*., 2016). The rate of foodborne illnesses related to the food service establishments is estimated to be about 48.7% in Europe (EFSA, 2010). The difference in numbers was observed between chain restaurants and non-chain restaurants (Jones *et al*., 2004; Roberts *et al*., 2008). These studies also confirmed that major role was played by food safety inspections on site and the location of restaurant itself (Murphy *et al*., 2011). The

number of inspection was important as they keep checking the follow-up training and valid certifications for the compliance purposes. A checklist can be used by food safety inspectors to summarize the local issue of the restaurants and keep in checking the safety of food by concentrating on environmental contamination sources. As for an example, Harris *et al*. (2014) used a checklist which helped the research team to determine 55 violations in select restaurants in the state of Florida. The checklist was prepared with the help of local staff considering the local laws of the state of Florida. According to the U.S. Food and Drug Administration (USDA, 2009) "*local, state, tribal, and federal regulators use the FDA Food Code as a model to develop or update their own food safety rules and to be consistent with national food regulatory policy.*" Studies showed that contributing factors to foodborne disease outbreaks in restaurants were the use of bare-hands and the major factor was the food handled by infected person. Additionally, the difference between the non-outbreak and outbreak restaurants was the presence of Certified Kitchen Manager (CKM), as most CKMs are trained on food safety management system and are familiar with safe food handling practices (Hedberg *et al*., 2006). It is suggested that there are three major criteria which play an important role in foodborne outbreaks, namely knowledge, attitude, and practice by the food handlers (Sharif & Al-Malki, 2010). According to a survey conducted by the Qatar Statistics Authority (QSA), the number of workers involved in food service activities in Qatar is about 30,269 (QSA, 2011). Many of these workers have little or no knowledge on food safety. As it is known that the transmission of foodborne diseases is mainly due to the lack of food safety knowledge of food handlers (Osaili *et al*., 2013) and cross contamination of pathogens transmitted from raw-meat, cutting boards, and knifes to the ready to eat (RTE) foods (Ravishankar *et al.*, 2010). It is essential that

food operators implement international standards, such as Hazard Analysis and Critical Control Points (HACCP), to reduce foodborne illnesses by monitoring the food processes.

The concept of HACCP was formed in 1971 by the Pillsbury Company in collaboration with the National Aeronautics and Space Administration (NASA) (Sperber & Stier, 2009). Once the success of this system was proven, it has been used by many food processors and food retail establishments all over the world. For the implementation of HACCP, seven steps are used; from identification of hazards, evaluation of safety procedures, to control of food safety hazards (USFDA, 1997).

Qatar being a developing country has international chain restaurants and hotels. These international chain restaurants (majority of which are fine dine-in, casual sit-in and some fast food restaurants) have standards including HACCP and internal training of their employees for the better implementation of HACCP. One of the example is Banana Island Resort Doha by Anantara which implements the standards for the reputation and status of their brand (AMEinfo, 2015). On the other hand, local restaurants mostly include catering, takeaways, fast-food, and cafeteria which do not have restriction to have HACCP certification and implementation. However, Ministry of Public Health (MoPH) and Ministry of Municipality and Environment (MME) have strict guidelines for food safety in food establishment premises. Even though HACCP is not mandatory in Qatar, training of food safety inspectors and guidelines are based on similar methodology used in HACCP plans (Faour-Klingbeil *et al*, 2016).

1.6 Steps in Implementation of HACCP in Food Establishments:

1.6.1 Preliminary steps for the implementation of HACCP:

- HACCP trained person and assembly of HACCP team:

For the implementation of HACCP, it is necessary to assemble the team and team head who is trained on HACCP, it is not necessary that person should be the employee of an establishment, but should be available at the time of developing the plan and at the time of reassessing the HACCP plan.

- Describing the method of production and distribution of food also identifying the use and consumer of that food product:

This step helps to focus on the food product and its specification according to its composition, the end user, packaging material used, its shelf life, and storage and special labelling requirements.

- Flow Diagram:

The flow diagram should be prepared to get a schematic diagram of the process that is included in the plan while preparing the food product. It can be a simple schematic diagram which includes all processing steps. To make the flow diagram accurate, there is a need for a HACCP team to walkthrough the food establishment and see if all the steps are included in the diagram for the preparation of that food product. These are the same steps followed by food and health inspectors during the site inspection.

- Grouping the food products having the same processing steps:

Categorizing the foods in groups having the same processing steps is helpful to avoid the extra HACCP paper work, in a way that single HACCP plan can be used for products which are prepared in a similar manner. These preliminary steps are used in the preparation and implementation of HACCP plan which consists of seven principals:

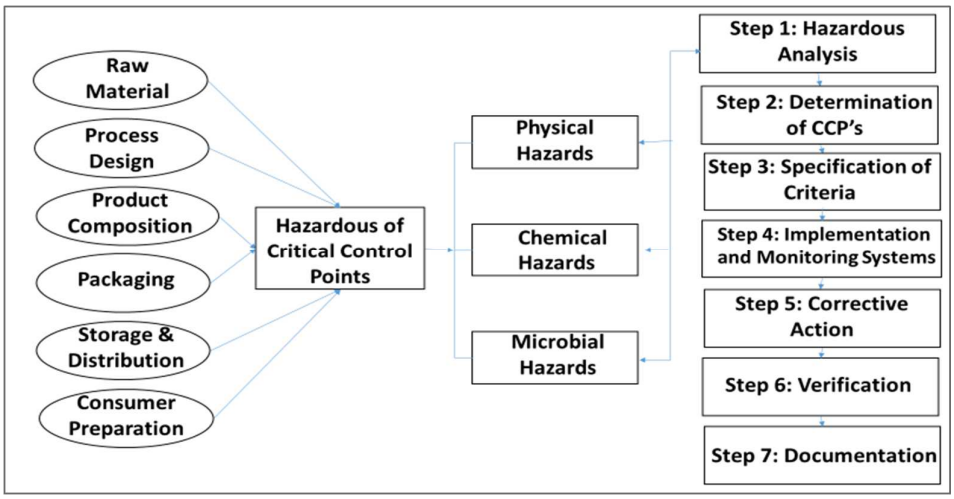

Figure 1.1. Steps used in the implementation of HACCP (adapted from Corlett, 1998).

1.6.2 Seven Steps of HACCP plan:

- Step 1: Identify Hazards:

Hazards can be introduced in the food processing plants in the form of physical, chemical, and biological hazards. These types of hazards can be introduced by the lack of worker hygiene, lack of knowledge or due to the natural contamination from food itself or by facility equipment. This step will identify that such hazard can affect the production of safe food; thus, it should be introduced in the HACCP plan to be monitored and carefully planned in such a manner to control these hazards.

- Step 2: Identify the Critical Control Points (CCPs):

Critical control points are the points which require the application of control measures to limit or eliminate the hazards. These points can be implemented at different stages, like receiving area, storage, preparation area, cooking area, or serving area. These are some few examples of CCPs. There can be much more CCPs depending on the nature of the food process. Sometimes different CCPs are established for the same kind of food in different food service operations.

- Step 3: Specification of CCPs:

This step includes the establishment of the limits at each CCP to control hazards, such as chemical, physical, and biological. The acceptable limit at each stage of establishment will be determined by the HACCP team. These critical limits are created based on the scientific literature or legislation imposed by the local governments.

- Step 4: Establish an Implementation and Monitoring System:

This step requires the routine checkup at given CCPs, either through employee or mechanical means and it helps in creating a record for future reference. These monitoring procedures in a restaurant may include checking the products arriving in the

receiving area of the establishment, temperature at the receiving area and storage area, record of routine cleanup of the premises, checking the temperature for cooling facilities, record of routine cleanliness of equipment used for making food products, checking the cooking temperature at the time of cooking the food with a thermometer, time period at which food is kept at room temperature or in serving area and restoring of food after serving. Frequency of monitoring procedures may depend on each CCP.

- Step 5: Corrective Actions:

This step is very important to HACCP plan as the results obtained from this step help implement an effective safety plan to provide safe to consume food products. So, during the monitoring and implementation procedure, if a deviation from the critical limits of the CCPs is determined, then corrective actions will be urgently needed. HACCP team should develop the necessary corrective action plan for each CCP if their limits are breached. Thus, there is a need to apply a corrective action plan for each CCP when deciding the acceptable limits for each one of them.

- Step 6: Verification:

In this step, the HACCP team verifies that the HACCP plan made for the certain establishment is working and safety of food is being acquired. Verification process includes the verification of hazards (physical, chemical, and biological) to be tested and shown to control these hazards in the premise. There can be chemical and microbiological studies to test and verify if the HACCP plan is working and acceptable food product is being produced. Though physical and chemical test results are quicker and can be obtained easily, the verification of HACCP plan should not be ignored while

testing.

Routine verification is carried out by observing the HACCP records, calibration of the instruments, evaluating the monitoring system, and if corrective actions are being implemented properly. Records of HACCP plan should be reviewed to see if they are maintained and fitted to the requirement of the HACCP plan.

- Step 7: Documentation:

Record keeping from all the steps of HACCP, such as CCPs limits, monitoring of the CCPs and from the verification step will help the HACCP team to observe the overall working plan of HACCP. These procedures or forms should be very simple, and employees should be trained on how to take measures and accurately document the process.

HACCP is strengthened by some prerequisite programs, namely Good Manufacturing Practices (GMP), Good Handling Procedures (GHP), Standard Operating Procedures (SOP), and Sanitation Standard Operating Procedure (SSOP) (Baş, Ersun & Kıvanç, 2006).

Good Manufacturing Practices (GMP) and Good Handling Procedures (GHP) can be the first step in the implementation of HACCP. Good manufacturing practices ensures food safety by describing the practices and necessary process, packing or storage of food. For the GMP implementation, it is necessary to train employees on personal hygiene, if they are ill they should not be working in the production premises, should be provided with hairnets, gloves, clean uniform, and shoes. The area of the food manufacturing premises should also be cleaned regularly, well drained, and the dates

of cleaning activities should be documented. GMP also includes the design of the premises in such a manner that cross contamination is prevented. Additionally, the utensils and equipment used to prepare food in the premises should be regularly cleaned and documented. All these GMP regulations can be prepared and documented, explaining what procedure and policies should be followed by each employee. While, GHP covers the sanitary and hygienic practices for food processors. These prerequisite programs like SOP and SSOP should be well documented, reviewed regularly and objectives can be added if there is a need to ensure food safety (Baş, Ersun & Kıvanç, 2006).

As GMP and GHP provide instructions for the plant/restaurant to not to cause adulteration of food, while SOP and SSOP provide specific instructions on how the process should be carried out for preparing the food to enhance food safety. SOP and SSOP also include the training of employees, but every employee's responsibility is well described in these programs. Under SOP, employee is assigned to a routine specific procedure, example of which might include what to do to complete the specific task and the steps for the completion of the procedure to finalize the food process without adulteration (Jeng & Fang, 2003). SSOP includes pre-operating procedures like cleaning the equipment before using and checking the food temperature. It also includes operating procedures like product handling during raw and cooked food, monitoring the process of food production. There should be a specified employee that is responsible to handle the monitoring process of food, considering chemicals, physical, and biological hazards. Moreover, it includes the corrective actions which should be pre-determined. If any divergence occurs during this process, corrective actions should be

implemented with the record of all the activities applied to ensure the safety of food.

These prerequisite programs are important for the establishments (restaurants), and provide a plan for controlling the low risk hazard to its limit and prevent it from becoming a food safety hazard. These programs also help create the basic environment and operating conditions for preparing the food safely. HACCP is involved in the prevention of hazards rather than hazard detection, the microbial analysis can become helpful in the implementation and evaluation of methods used in the HACCP plan (Hamaq, 2005). HACCP is implemented in different industries such as food retailers including fresh produce markets and food establishment, School Food Caterers and Canteens (USDA, 2005) meat and poultry retailers (USDA, 1999), fish and fish product retailers (USFDA, 2014), and juice and nectar retailers (USFDA, 2009).

1.7 Factor Affecting Food Safety within Restaurant Premises:

There are many factors which can affect the food safety within the premises of the establishment, such as food safety education and training of the workers, personal hygiene, hygiene of the contact surfaces, and controlling time and temperature.

1.7.1 Food Safety Education and Training

As food handlers play an important role in the safety of food, it is necessary that their knowledge and attitude towards food safety are appropriate. Employees working in the food establishments should be trained frequently for the efficient implementation of food safety and minimization of the food safety hazards (Al-Shabib *et al*., 2016). According to a study, about 97% of all foodborne illnesses are due to improper handling of the employees (Egan *et al*., 2007). The unsanitary conditions of workers during the preparation of food are the major factors that contribute to 98% of the foodborne

outbreaks from restaurants in the USA (Shinbaum *et al*., 2016). The U.S. Food and Drug Administration (USFDA) reported that the major factor in recalling food products is the ineffective employee training based on the data recorded from 1999 to 2003 to analyze the current good manufacturing practices in restaurants (Shinbaum *et al*., 2016; USFDA 2013).

Studies have also shown significant results before and after the training of food handlers on temperature control and hygiene of food preparation surfaces as temperature control and hygiene criteria are important components of the HACCP plan (Garayoa *et al*., 2014). In recent studies, it has been observed that after the introduction of new legislation in Siberia which makes it mandatory to implement the HACCP, there was a significant difference in the hygiene of food contact surfaces and cooling facilities before and after the implementation of HACCP (Djekic *et al*, 2016). In another study, it was indicated that implementation of HACCP decreases the total microbial count in meat retail facilities and plants (Tomasevic, 2016). After the implementation of HACCP, 1.0 $\log_{10}$ CFU/cm^2 decrease was observed in food contact surfaces while 2.0 $\log_{10}$ CFU/cm^2 decrease was determined in cooling facilities (Tomasevic, 2016). There was also a decrease in the level of Enterobacteriaceae and *Staphylococcus* in the meat samples after the implementation of HACCP in meat retailers and processing companies (Tomasevic, 2016).

Many studies suggest that implementation of HACCP not only decreases the microbial count but also increases the shelf life of the product, reduces product wastes,

decreases production price, increases sales as the consumer confidence is improved (Maldonado *et al*., 2005 & Macheka *et al*., 2013). In a recent study conducted on food handlers working in restaurants in Saudi Arabia, it was determined that employees' food safety knowledge, attitude, and practices were at satisfactory level but still there were weaknesses in maintaining hygiene and controlling temperature while cooking (Al-Shabib *et al*., 2016). In another study, people residing in Al Ain, United Arab Emirates, were surveyed on their knowledge of food safety. It was concluded that there is a need of awareness on food safety even for highly educated residents of Al Ain who have good attitude on washing hands (Afifi & Abushelaibi, 2012).

1.7.2 Personal Hygiene

When handling food in a restaurant, personal hygiene is an important factor to prevent food contamination. Workers may carry pathogens on unclean hands, skin or hair (Todd *et al*., 2008). Understanding the basic food protection practices and maintaining a high level of personal hygiene and a good sanitation practice will decrease the likelihoods of contamination in food products. An effective personal hygiene program can be practiced by wearing clean uniform, washing hands before touching food, wearing gloves and changing the gloves frequently, and wearing hair- and beard-nets (Todd *et al*., 2010 & Padilla-Zakour, 2009). Washing hands after touching bare body part, smoking, coughing, sneezing, eating, drinking, after handling garbage, and touching animal or aquatic animal is essential to prevent contamination. Creating awareness of personal hygiene and designing the regular inspections and motivating the employees to follow personal hygiene will also decrease the count of food contamination within the premises as it may stop cross contamination (Annor &

Baiden, 2011).

1.7.3 Hygiene of the Contact Surfaces

During the food handling within restaurant premises, food goes through different processes such as; cutting, chopping, garnishing, mixing etc.; all of which need a clean environment including the utensils used and food contact surfaces for safe food processing (Losito *et al*., 2017). SOPs require separation of ready to eat food from raw food and GHPs include personal hygiene and cleaning & sanitization of utensils, which are essential for preventing cross contamination. As there are types of food which may not go through cooking processes at a high temperature (e.g., salads, sandwiches, and fruits), it is likely that poor hygienic conditions of contact surfaces and utensils may cause cross contamination and increase the possibility of pathogenic bacterial contamination in these foods (Saad *et al*., 2013). It has been reported that knives, preparation tables, and mixers were the most often contaminated utensils and surfaces with spoilage bacteria, such as *Pseudomonas* and *Enterobacteriaceae* (Gounadaki *et al*., 2008). Moreover, poor hygiene and not using disinfectant for the cleaning of food contact surfaces, not only decrease the shelf life of the food product but also increase the presence of pathogenic bacteria (Moore *et al*., 2001).

1.7.4 Controlling Time and Temperature

Besides hygiene of the premises, temperature control is important factor in eliminating microbial contamination. During the establishment of the critical control points with respect to temperature, contamination of food with pathogenic bacteria should be considered and controlled (Byrd-Bredbenner *et al*., 2013). It is known that growth of bacteria is dependent on temperature, time, pH level, and water activity (A_w)

of the food product (USFDA, 2001). Temperatures between >5°C to <60°C are ideal for the growth of spoilage and pathogenic bacteria (USDA, 2016). To limit and eliminate the growth of spoilage and pathogenic bacteria in animal-based food products, holding temperature should be kept at >68°C and >73°C for meat and poultry, respectively (Saucier, 2016 & USDA, 2016). Cooling of the hot food should be done using chillers to decrease the temperature to <5°C within 2 hours to limit the growth of microorganisms. Also, defrosting should be done while keeping the food product in the fridge or in a microwave only if food is to be cooked immediately (FSA, 2007).

1.8 Food Service Industry in Qatar

Qatar as a developing country, setting its eyes on the vision of sustainable Qatar 2030 and preparing itself for the major events, such as Football World Cup 2022, has no shortage of restaurants and hotels. According to a recent report published by the Ministry of Development Planning and Statistics (MDPS), there are 1,323 registered restaurants with a total revenue of $1,465,072 for restaurants and $1,477,596 for hotel restaurants, which adds up to revenue of $2,942,668 from food service establishments (MDPS, 2014). An estimated amount of $814,749 for goods used by restaurants and hotels and $407,308 for services was reported in 2014 (MDPS, 2014). Total of 52,595 employees are employed in these establishments, having compensation value of $583,550 in 2014 (includes establishments with 10 employees and establishments less than 10 employees) (MDPS, 2014). According to a more recent MDPS report (2015), the value of consumer price index (CPI) has increased for restaurants and hotels from 3.7 to 6.1 between 2007 and 2013, an increase of 2.4 which indicates that the consumers are spending more money in restaurants and hotels, showing the changing trend in

Qatari residents' eating habits, dining in restaurants rather than eating at home which helps the Qatar's food and hospitality industries.

1.9 Food Safety Rules and Regulations in Qatar

The food safety laws implemented in Qatar are Law No. 8 (1990) which is now updated to Law No.4 of 2014 by Ministry of Municipality and Environment (MME), focuses on food control regarding the safe process of food and if the food operations do not meet the requirement, they may face penalties such as temporary closure of the establishment, fine, and even jail time (MME,2014). Law No.17 (2005) focuses on the cleanliness of the premises, maintenance and cleanliness of the equipment used for storing food safely. In addition to these laws, there is also a law on licencing (Law No. 3 of 1975). The implementation of these laws is controlled by joint efforts of the Ministry of Municipality and Environment (MME) in collaboration with the Ministry of Public Health (MoPH). Most restaurants are inspected by the MME and cafeterias and catering services at local schools and universities are inspected by the MoPH.

Routine or unexpected inspections/audits can be done, but the most effective way to improve food safety in food establishments is training of the managers or employees, at least annually. Despite the increasing number and diversity of the food service establishments, there is no systematic food surveillance system in the country, which creates a gap in terms of determining the food safety attitudes and practices applied in the food industry. This is an area on which both ministries are working, especially in establishing a Food Safety Authority to oversee all food safety issues, inspection, and regulations. Also, more labs are built to conduct microbial and chemical analyses of food products consumed in the country.

1.10 Current Food Safety Situation in Qatar

The rapid economic growth, urbanization, and import of foods have impacted the eating habits of Qatari and non-Qatari people. The food consumption trend in hotels and restaurants (food prepared away from home) increased with the increase in urbanization and income (Dong and Hu, 2010). The total foodborne diseases recorded between years 2008-2011 was 11,420 in Qatar which was about 5.4% out of total communicable diseases according to the National Health Strategy (NHS, 2013). Also, Qatar as being not an agriculture-based country most of its food products (about 92%) are imported from other agriculture-based countries. According to Qatar Statistics Authority (QSA), about 1.4 billion kg of food was imported in 2012 and 7.5% of which was contaminated due to harmful microbes or chemicals (QSA, 2013). Organizations like the Ministry of Public Health (MoPH) are all emphasizing to have a system to monitor food within Qatar and making the Qatar as a food secure country by improving public health and having regular and standard food inspections as stated in the NHS (NHS, 2013). The Health Control Section of the Ministry of Municipality and Environment in collaboration with the MoPH conducts inspection tours to various restaurants and hotels, issues warrant against the violators, and disposes confiscated food in Qatar (Law No. (8) Of 1990). It has also been seen that employees selling unfit food were fined, jailed and deported (FSN, 2015).

1.11 Rationale for the Study

As there is a change in the eating habits of Qataris and non-Qataris, it is expected

that the number of foodborne illness will increase in the near future. Qatar is preparing itself to host a world-class event in 2020 (Football World-cup) and the Qatar National Vision 2030 aims at having a sustainable development; therefore, it is an utmost necessity to meet the international food safety standards. Up to now, there has not been any research carried out to determine the current food safety situation in different types of restaurants in Qatar. This initial assessment in Doha restaurants can help in understanding the food service providers' (FSPs) level of knowledge on GHP, GMP, HACCP and providing the baseline data on environmentally hazardous microbes present in their premises, which will help the Ministry of Public Health to introduce policies to minimize the outbreak of foodborne illness in Qatar. By this way, the number of restaurants serving safe food may increase in coming years.

1.12 The specific objectives of this research were to:

1) Conduct a survey to evaluate the level of awareness on possible environmental hazards in food service settings,

2) Determine the type of microbial contaminants at different stages of food preparation, and

3) Identify the major microbial contaminants using VITEK technique.

It is hypothesized that employees working in the food service operations (FSO) are trained on HACCP and have knowledge about the food safety practices within the

food establishments. Every FSO is expected to implement HACCP and document if there are any corrective measures have been taken. Therefore, it is assumed that food prepared in majority of FSOs are safe for human consumption.

1.13 Approach

As education of the employees play an important role in the production of safe food prepared and served in restaurants, it is important to conduct a baseline survey to determine the food handlers' level of knowledge on food safety (Al-Shabib *et al*., 2016).

Also, the implementation of HACCP has been helpful in reducing the overall microbial count in food (Tomasevic, 2016). Thus, microbial analysis is an essential step to be included in the entire assessment process to determine the safety level of food with respect to different stages of preparation (receiving area, storing area, preparation area, cooking area, and serving area) in different types of restaurants (fine dine-in, casual dine-in, takeaway, catering, and fast food). This step is helpful for identifying the critical points at which hazards must be controlled.

CHAPTER II: METHODOLGY

2.1 Survey

A baseline survey was conducted to assess the participating food service providers' (FSP) level of understanding on food safety in terms of Good Handling Practices (GHPs), Good Manufacturing Practices (GMP), and Standard Operating Procedures (SOP), and Hazard Analysis Critical Control Points (HACCP). Out of more than 200 FSPs registered for inspection under MoPH, 53 FSPs, (all located in Doha municipality), accepted to participate in this study. The participating restaurants were categorized based on the food services they provided, namely fine dine-in, casual sit-in, fast food, catering, and takeaway. Fast-food restaurants are chain of restaurants where food is prepared in minutes and orders are given not from the table but over front counters and seating can be done by own choice. Take-away restaurants are types of restaurants which have very limited number of seats and mostly customers buy food and eat else where. Catering restaurants always provide food service on remote site such as workplaces, weddings, outdoor events, etc. Casual dine-in restaurants are mostly chain restaurants where prices of food sold are higher than average fast food restaurants and a casual environment with full table service is provided. Fine dine-in restaurants have very formal environment, sometimes having dress codes and formal sitting arrangements, and provide food of highest quality and fancier menu.

During the initial contacts with FSPs, the goal of the study was explained. The surveys were conducted with the help of food inspectors from the Ministry of Public Health (MoPH) during the months of October and December 2015. Face-to-face

interviews with the food safety managers at each participating FSP were conducted using a 40-questions survey questionnaire. The questionnaire consisted of questions on the establishment date and the number of workers, demographic data on the managers and workers (education level, gender, age, length of employment), specific food safety training received, knowledge on food hygiene and safety, and safe-food handling practices. A written informed consent was obtained from FSPs at the time of each visit. Each survey took about 15-20 minutes to complete by a food safety manager at each participating FSP. An ethical approval for the study was obtained from the Institutional Review Board of Qatar University (QU IRB #340-E/14).

In addition to survey questionnaire, an audit checklist developed by the research team with the help of inspectors from MoPH was used to determine the implementation of principles of international food safety regulations and guidelines by observing actual practices applied at each FSP. The audit checklist included questions on a) employee hygiene (health surveillance records; hygiene practices (e.g., hand washing; use of gloves, aprons, hairnets, jewelry; hand-washing facilities; washrooms, etc.), b) cleaning and sanitation practices applied at the facility (chemicals used, cleaning records, cleanliness of working areas (cutting boards, food preparation surfaces, etc.), kitchen hygiene), c) conditions in receiving area, cold storage area (temperature records, cleanliness of shelves), cooking area, and food transportation area, and d) employee training records. The data collected using the checklist were based on yes/no questions.

2.2 Microbial Quality Assessment of Foods and Food Contact Surfaces in select FSPs

2.2.1 Food Sampling and Type of Food Samples Collected

All FSPs who took part in the survey were invited to participate in the microbial quality assessment study. Out of 53 establishments, 10 FSPs (2 fine-dine-in, 2 casual sit-in, 2 catering, 2 fast-food, and 2 takeaway) accepted to provide food and swab samples from their entities. At the time of each visit to select FSPs, various menu items (food cooked in a short time, ready-to-eat foods, vegetables, dairy-based deserts, sandwiches, and raw seafood, e.g. oysters) were sampled in duplicate (based on the daily menu prepared at the time of sampling) at different food preparation stages (receiving, food storage, food preparation, holding/cooking, and serving). The purpose of this step was to identify the food safety hazards that might be present in the food given the food preparation process, the facility, and general characteristics of the food itself with the field data or general information of the ingredients used, each activity conducted in the process, the equipment used, sanitation practices, the final product, and its method of storage and distribution using the international hazard categorization (Table 2.1).

Table 2.1 *Categorization of food items based on the associated biological hazard* (Gilbert *et al*., 2000).

Food Group	Product	Category
Meat	- Beef burgers	1
	- kebabs	2
	- meat meals (shepherds/cottage	2
	- poultry (unsliced)	2
	- salami and fermented meat products	4
	- sausages (smoked)	4
	- sausage roll	1
	- scotch egg	1
Seafood	- Seafood crustaceans (crab, lobster, prawns)	3
	- other fish (cooked)	3
	- seafood meals	3
	- molluscs and other shellfish (cooked)	4
Dessert	- Dessert cakes, pastries, slices, and desserts - with dairy cream	3
	- cakes, pastries, slices, and desserts without dairy cream	2
Savory	- Vegetable Curry (onion, spinach, vegetable)	1
	- cheese-based bakery products	2
	- fermented foods	4
	- humus and other dips	4
	- mayonnaise/dressings	2
	- pâté (meat, seafood, or vegetable)	3
Vegetable	- Vegetable coleslaw	3
	- fruit and vegetables (fresh)	4
	- prepared mixed salads	4
	- rice	3
	- vegetables and vegetable meals (cooked)	2
Dairy	- Dairy cheese	4
	- ice cream, milk shakes	2
Ready to Eat Meals	- Ready-to-eat pasta/pizza	2
	- Sandwiches with salad and filled without salad	4
	- rolls with cheese	4

Food samples (~100 g) were collected using sterile utensils aseptically, kept in sterile plastic bags, and placed in an icebox to be transported to the Qatar University Microbiology laboratory. Two-three food samples at different food preparation stages were collected, giving a total of 105 food samples from 10 restaurants during the entire period of the study. The conditions, such as storage/cooking temperature and time of food samples at each stage were reported. All samples were kept refrigerated (0-4°C) until further analysis.

2.2.2 Food Contact Surface Sampling

Three different working surfaces having high food preparation activity were examined, including cutting boards, working tables, and serving tables. The surfaces (10 x 10 cm) were swabbed using sterile polypropylene swabs before handlers started working at each time of sampling. A total of 58 swab samples were collected during the entire study period and immersed in 2 mL of brain heart infusion broth (BHI, VWR Chemicals, Geldenaksebaan, Germany). The samples were transported to the QU Microbiology lab on ice and kept in a refrigerator until further analysis.

2.2.3 Microbial Analysis of food samples:

All microbial determinations were carried out by using the standard methodologies with slight modifications, namely aerobic plate count method (Bacteriological Analytical Manuals, BAM, 2001), detection of *E. coli* and coliforms in foods (BAM, 2002), *Salmonella* spp. enumeration and detection (BAM, 2007), and detection and enumeration of *Listeria* spp. in foods (BAM, 2016). Briefly, ten grams of each food sample was placed in a sterile plastic bag containing 90 mL of buffered

peptone water (BPW), homogenized for 2 min using a homogenizer, and serially diluted in BPW. The following microbial analyses were performed on food samples: total aerobic mesophilic count, total coliforms, *E. coli*, *Salmonella* spp., and *Listeria* spp.

For enumeration of bacteria, 1 mL of each serially diluted food sample was spread on plate count agar (PCA, VWR Chemicals, Geldenaksebaan, Germany), MacConkey Agar (MCA, VWR Chemicals, Geldenaksebaan, Germany), Xylose Lactose Tergitol™ 4 (XLT4, VWR Chemicals, Geldenaksebaan, Germany), and Listeria Selective agar (LSA, VWR Chemicals, Geldenaksebaan, Germany). The plates were incubated for 48 hours at 37°C, a separate set of MCA plates were incubated at 25°C for the determination of total coliforms. After 48 hrs, the bacterial colonies were counted and recorded as Log colony forming unit per gram of sample analyzed (Log_{10} CFU/g).

2.2.4 Microbial Analysis of Swabs:

Food contact surface swab samples were dipped in 2 mL of BHI and were incubated for 18 hours for enrichment. After 18 hrs, swab samples were vortexed and serially diluted in BHI. The diluted samples were spread plated on PCA, MCA, XLT4, and LSA which were then incubated at 37°C for 48 hrs. The results were expressed as Log_{10} CFU/cm^2.

2.3 Confirmation of Presumptive Target Colonies using VITEK system:

The presumptive target colonies (*Salmonella enteritis, Listeria monocytogenes* and *E. coli* O157:H7) grown on selective media were identified by their morphology and by using VITEK (BioMe´rieux, Marcy l'Etoile, Microbiology Lab, Hamad Hospital). On MacConkey agar, red pinkish lactose-positive colonies surrounded by

precipitation zones were considered presumptive *E. coli*. These colonies were isolated and grown as pure culture which were then used for molecular identification. Colonies exhibiting black or dark grayish with a black centre on XLT4 plates were considered to be positive *Salmonella* colonies. Although there was no positive Listeria growth on LSA plates since no black colonies were observed, abundant colonies grown on the plates were still sub-cultured and used for further identification.

VITEK® 2 Compact System was used for the identification of presumptive colonies by inserting colorimetric reagent cards specifically designed to detect Gram-positive and Gram-negative bacteria. The inoculum was prepared for presumptive colonies by using sterile swabs and transferring them into plastic tube of 12x75 mm, which contained 3 ml saline solution. Suspension turbidity was checked by using a turbidity meter (DensiChek™, France). These test tubes were placed in special VITEK rack and reagent cards put in the neighbouring slots while dipping the transferring tube into the test tube. The comparison of raw data with the threshold reaction was carried out by a program within the VITEK for the determination of target pathogens as positive (+) or negative (-) (Pincus, 2006).

2.4 Statistical Analyses

The survey data were analyzed using STATA software. The Pearson's chi-square test was used to determine significance of factors (education level of food safety managers, the implementation of food safety practices, food safety knowledge, etc.) and their interdependence. Additionally, Pairwise Correlation Matrix was used to test the inter-variable correlation at $P<0.05$.

The means and standard errors for different microbial counts were calculated. The microbial count data was analyzed by using Generalized Linear Model (GLM) considering independent variables as food preparation stages and different type of restaurants and their interaction at $P \leq 0.05$ was determined (Statistical Analysis Software, SAS/STAT®, SAS Institute Inc., NC, USA). Tukey Kramer post-ANOVA test was used to determine the significant differences between individual group means across restaurants and food preparation stages at $P \leq 0.05$.

CHAPTER III: RESULTS & DISCUSSION

3.1 Survey

A total of 53 FSPs participated in the study, among those 21%, 21%, 23%, 13%, and 22% of them were fast food, take-away, casual dine-in, catering, and fine dine-in restaurants, respectively (Figure 3.1).

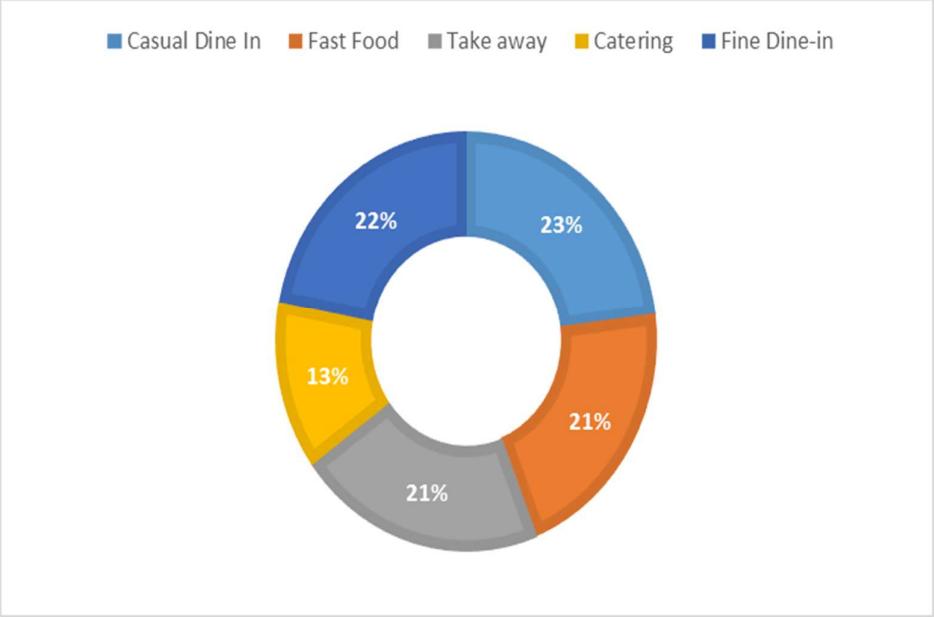

Figure 3.1. Percent distribution of type of FSPs surveyed.

The average service years of FSPs was 11, with the oldest being established in 1982 and the newest was established in 2015 (Table 3.1). The large number (50%) of new establishments (2010-2015) indicates that the food industry in the country is

developing at a very rapid rate. It also proves that there is direct relationship among the economic growth in the country, increasing income level as well as the wide diversity of its residents which eventually creates a high demand for new and various types of restaurants (Dong and Hu, 2010).

Table 3.1. *The average establishment years of Food Service Providers.*

Year of Establishment	**Percentage**
1982-1999	12%
2000-2009	38%
2010-2015	50%

In the first part of the survey, the managers were asked questions related to demographics. Based on their answers, it was found that the average age of food safety managers interviewed was 33 (ranging from 26 to 60), where higher percentage of managers (50%) were between the age of 30-39 (Figure 3.2).

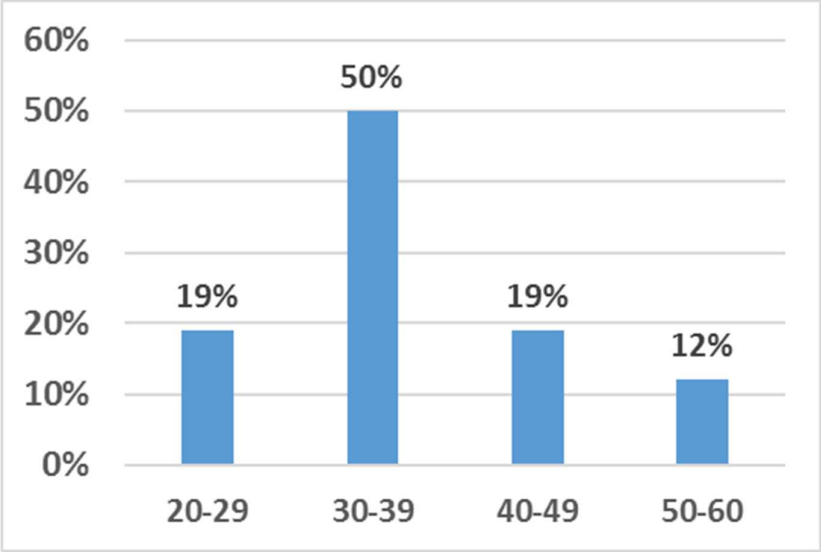

Figure 3.2. Average age of FSP managers.

The results also indicated that 36% and 17% of managers and employees had a graduate school degree, respectively (Figure 3.3). It is reported that workers' education has a direct impact on their behaviour and implementation of food safety in food establishments (Clayton *et al*., 2002; Kunadu *et al*., 2016). It is expected that the higher the education level is, the better the implementation of food safety practises will be. It has been determined that there was a strong correlation (correlation matrix = 0.890) between the education level of managers and employees and their attitudes towards food safety. The majority of managers were Egyptian (22%) and Indian (18%) origin; however, some of them were also from Philippines (12%), Lebanon (10%), Syria (9%), Turkey (8%), Sri Lanka (6%), France (4%), Palestine (4%), and Jordan, South Africa, Qatar, Malaysia, Mauritius, Sudan, and Morocco (1%) (Figure 3.4).

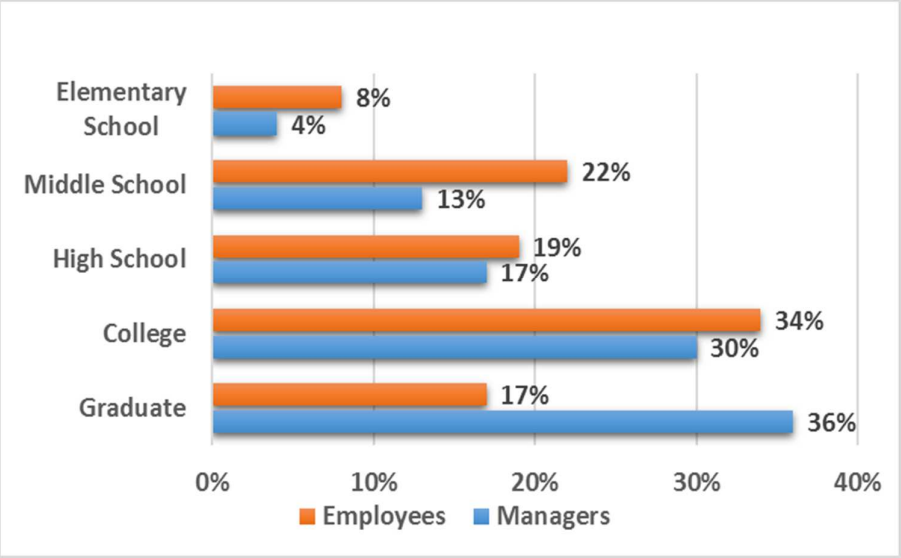

Figure 3.3. Education level of managers and employees working in FSPs surveyed.

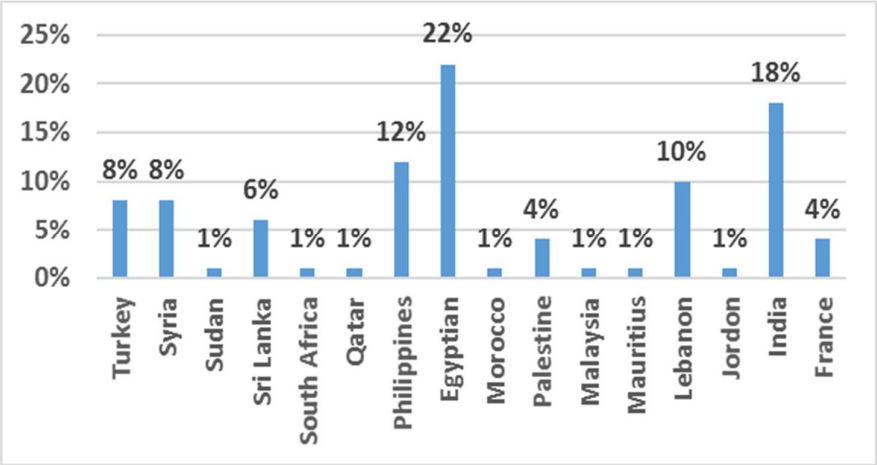

Figure 3.4. Nationality percentage of FSP managers.

In terms of years of experience, there was a large variation. Overall, the mean average for the length of employment in FSP business was 7, ranging from 1 to 35 years of experience (Figure 3.5). Most managers (68%) were trained on food safety management system (specifically on HACCP). Our results suggest that FSPs managers' training and education level are highly important variables that affect the

probability of employees' having food safety training as well. It was interesting to report that managers with elementary, middle, and high school education level had no formal training on food safety. Similarly, employees' food safety training is positively affected by their education level. As the employee gets more educated, the probability of being trained on HACCP became higher.

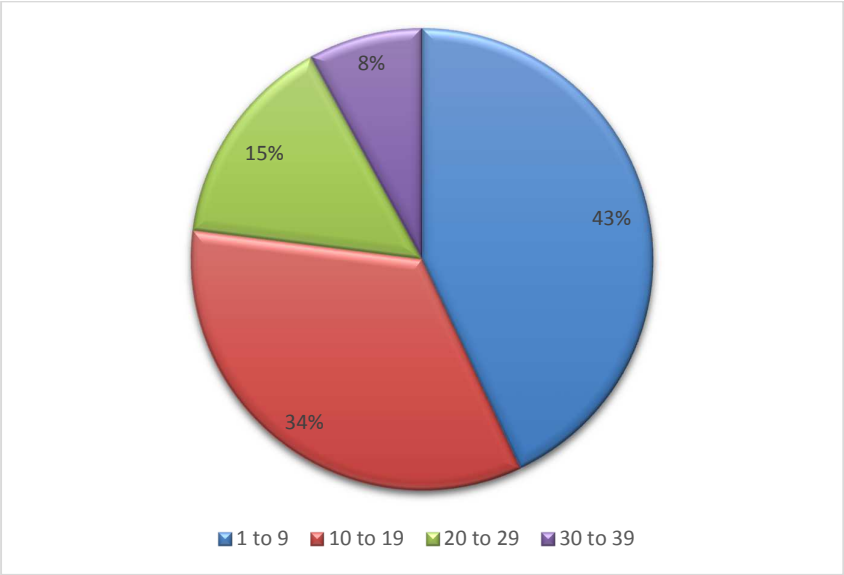

Figure 3.5. Managers' Years of experience in the food industry.

In the second part of the survey, the managers were asked to answer questions on documentations and records as part of the Standard Operating Procedures (SOP). When implementing HACCP, critical control points are considered to be important criteria to be continuously recorded to control/eliminate the growth of microorganisms. Figure 4.6 shows the percentage of restaurants keeping records on CCPs. Fine dine-in and casual sit-in restaurants exhibited the best practice in keeping records on CCPs at a rate of 100%, while catering and takeaway restaurants failed to keep records on CCPs (0% and 18%, respectively). Studies have shown that after the implementation of

HACCP which includes documentation of CCPs, there were lower incidents of pathogenic bacteria contamination within the restaurant premises (Soriano *et al*., 2002). These results suggest that catering and take-away restaurants are in need of food safety training, emphasizing the importance of documenting critical control points, such as holding, cooking, and storage temperature at each food preparation stage.

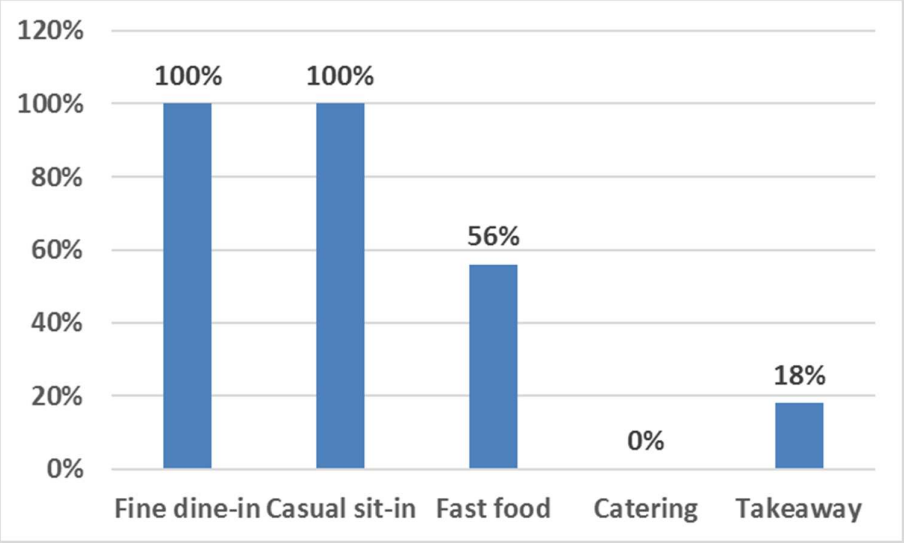

Figure 3.6. The percent distribution of FSPs applying correct practices on keeping CCPs records.

As discussed previously, hygiene condition of food contact surfaces and food preparation areas is important in controlling the microbial hazards (Losito *et al*., 2017). When the managers were asked if they keep records on cleaning and sanitization in their facilities, it was demonstrated that casual sit-in and fine dine-in restaurants are the only FSP types which consistently kept records (100%), followed by fast food FSPs (36%), and catering FSPs (14%) (Figure 4.7). These results are highly correlated with

the FSP managers' training. Since managers working in fine dine-in and casual sit-in FSPs were trained on HACCP, they presented the best practices by keeping records at 100% as noted in this study. If the managers are not trained on food safety management system which was the case for most catering, take-away, and fast-food FSPs, they were not familiar with the entire food safety management system.

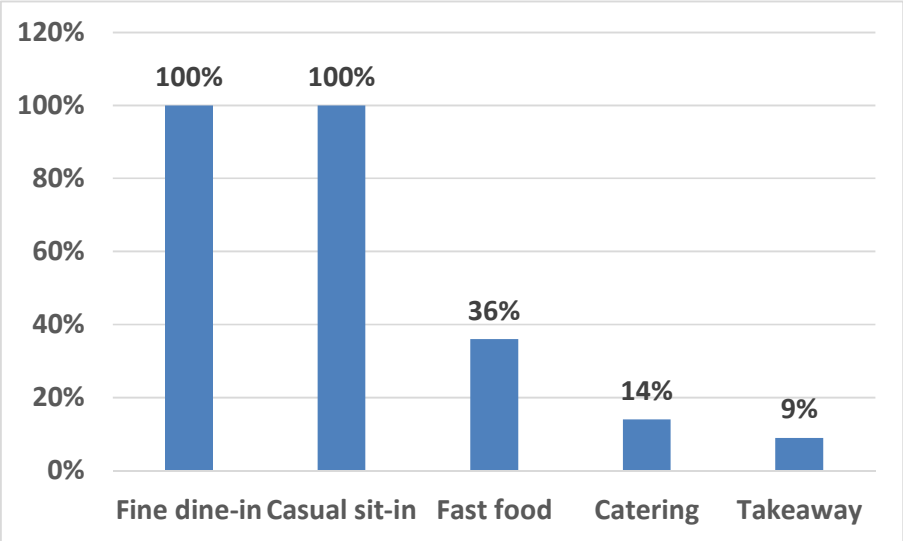

Figure 3.7. The percent distribution of FSP type and keeping the records on cleaning and sanitation.

In the implementation of food safety management system (e.g. HACCP), the type of CCPs included are specific, such as holding temperature, specific set of temperatures for cooking, chilling and storing to control the spoilage and pathogenic bacteria to over-grow the satisfactory limit of CFU (Byrd-Bredbenner *et al*., 2013). Figure 3.8 presents the results on the correlation between the type of FSP and keeping records on time and temperature while the food is prepared. As seen in the figure below, casual sit-in and fine dine-in restaurants topped the list (100%) since they were required

to keep these records as part of their food safety management system. It was highly surprising to report that catering FSPs had no records on this crucial step and many of them did not own a thermometer on site (as observed during our walk-though audit), indicating poor food handling practices.

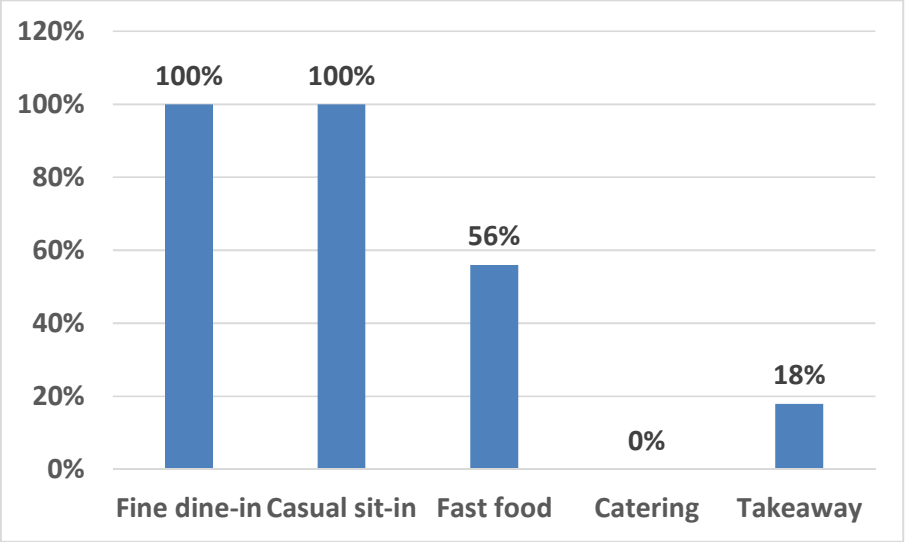

Figure 3.8. The percent distribution of FSP type and keeping records on time and temperature of food at different preparation stage.

It has been suggested by Ismail *et al*. (2016) that personal hygiene and attitudes play an important role in keeping the process of food safe within the premises. Daily documentation of personal hygiene will help in regular practice of SOP's. In 53 FSPs visited, catering FSPs had the worst scoring in terms of keeping records on employee personal hygiene (Figure 3.9). They had no employee hygiene records and majority of catering establishments had no washroom facilities on site (as observed during our walk-though audit). Several employees in these establishments were also working in the kitchen with their normal daily clothes without wearing aprons, gloves, hair net, etc.

These types of practices should be avoided to meet the requirements of food safety management system.

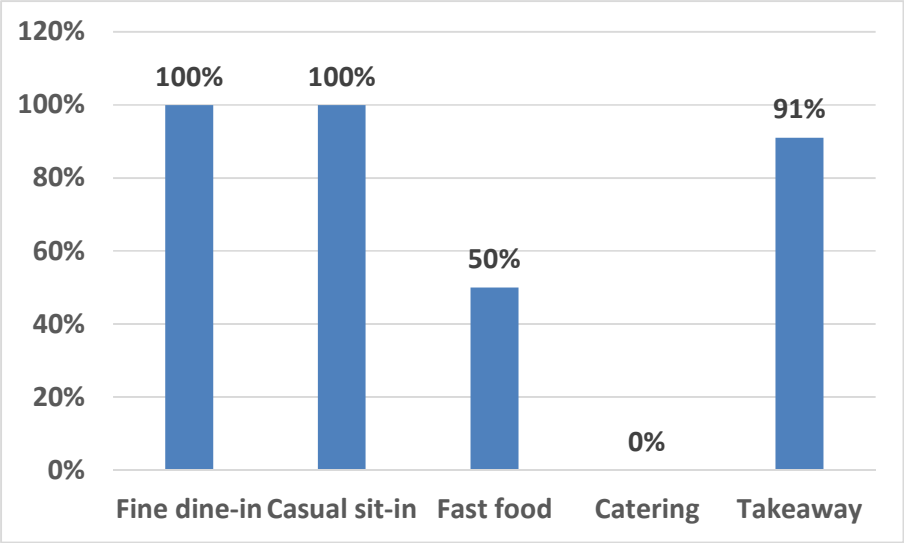

Figure 3.9. The percent distribution of FSP type and the Records on Employee Personal Hygiene.

According to food safety management system, the separation of raw food from Ready to Eat food (RTE), cleaning of the food contact surfaces, and personal hygiene are important aspects to prevent cross contamination (FSA, 2015). Documentation of cross contamination through different stages of food process in these specific areas such as separation and cleaning and sanitation is vital. As observed in the previous figures, the type of FSPs and the education level of and training received by the food safety managers greatly affect the practices on keeping records for preventing cross contamination. Since the managers working in FSP types, such as casual sit-in and fine dine-in received training on food safety management system, they exhibited better practices in keeping records on preventing cross contamination methods consistently

(Figure 4.10).

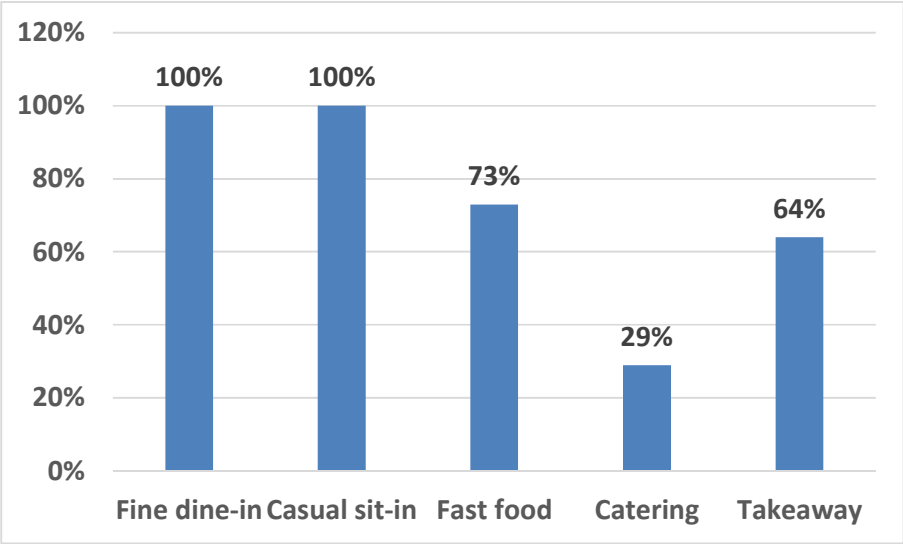

Figure 3.10. The percent distribution of FSP type and having records on preventing cross contamination.

In the table 3.2, it can be seen that *p* values for Chi square tests are rejecting the null hypothesis and presenting the results that record keeping practices are not applied at the same level for each FSP type. In other words, those practices vary significantly by type of FSPs. For example, causal sit-in and fine dine-in FSPs kept records on all safe food handling practices measures, while catering did not follow the same (Table 3.2).

When all factors were combined and compared to each other using Pairwise Correlation, it was noted that manager's training is the most significant factor in influencing the food safety practices applied at each FSP (Table 3.3). Additionally, employees' education and training on food safety positively impact the implementation of food safety practices (Al-Shabib *et al*., 2016).

Table 3.2. *Summary of all the record keeping compliance by FSP on select food safety practices with their significance.*

FSP Type Record Keeping Practices on	Fast Food	Take Away	Casual Sit-in	Catering	Fine dine-in	▪ 2	p Value
Cleaning & Sanitation	36%	9%	100%	14%	100%	35.44	<0.0001
Time& Temperature Control	56%	18%	100%	0%	100%	34.76	<0.0001
Employee Personal Hygiene	50%	91%	100%	0%	100%	38.03	<0.0001
Preventing Cross Contamination	73%	64%	100%	29%	100%	22.66	<0.0001
Critical Control Points	56%	18%	100%	0%	100%	16.75	<0.002
Checking the integrity of received items	73%	36%	100%	71%	100%	22.66	<0.0001

Table 3.3. *Estimated results of the Pairwise comparison between managers and employee's education and their food safety training.*

Variables	Estimates	SE	*P*
FSP type	-0.621	0.606	0.063
Manager's training	1.683***	0.573	0.000
Manager's education	-0.249	0.756	0.243
Employees' training	0.890***	0.310	0.000
Employees' education	-2.537***	0.944	0.000

These results suggest that food safety managers' food safety training, employee's education level, and employees' training are key important factors which directly impact the food safety in any given FSPs. If the managers have food safety training in food safety, especially on HACCP, the probability is much higher for employees' to be trained and apply the safe food handling practices (Rebouças *et al.,* 2017).

The walk-through audits were conducted at the time of surveying the select FSPs. According to the audit results, it has been observed that there was a conflict between the observed and actual practices (Table 3.4). Although majority of managers declared to keep records on important items, such as employee hygiene, cleaning & sanitation, cooking and storage temperature, etc., the observational study results prove the opposite. The audit showed the lowest compliance results in employees' training records (39%) followed by cleaning and sanitation records (49%) (Table 3.4). Records were personally checked and were asked to be shown during walk through audit. Establishment cleanliness condition, which includes general premises cleaning, cleanliness of receiving area, cold storage area, and cooking area, were observed by the experts from the Ministry of Public Health. It was noticed that managers had the knowledge on the importance of keeping the premises clean, but they were not able to apply this knowledge into practice at a satisfactory level. When comparing the estimated results on important factors such as education level and training of managers and employees (Table 3.3) to the results obtained from the walk-through audit (Table 3.4), it was clearly observed that audits are necessary for confirming if the HACCP plan implemented in any given FSP is effective or not. The internal or external audits should be conducted often to make sure that the training and education of employees

and managers are properly implemented.

Table 3.4. *Results of the Walk-through Audit*

Records on	Compliance (%) n= 53 FSPs
Employee Hygiene Policy	54% (29 FSPs meet the criteria)
Cleaning & Sanitation	49% (26 FSPs meet the criteria)
Cleanliness of Receiving Area	54% (29 FSPs meet the criteria)
Cold Storage Area Condition	60% (32 FSPs meet the criteria)
Cleanliness of Cooking Area	58% (31 FSPs meet the criteria)
Safe Transportation of Food	64% (34 FSPs meet the criteria)
Employees Training	39% (21 FSPs meet the criteria)

3.2 Microbial Analyses of Food Samples:

Microbiological counts, such as aerobic colony count and *Enterobacteriaceae* counts, are used to evaluate the hygiene conditions of establishments and are also used to asses if there is high risk in terms of the presence of pathogenic species such as *Listeria* spp. or *Salmonella* spp. (Ghafir *et al.,* 2008). Hygiene indicator organisms in this case are used to suggest the hygiene condition of the premises, personal hygiene and hygiene of the utensils used.

The presence of *Escherichia coli* suggests that GMPs are not applied appropriately during processing the food, tracing the contamination of food by fecal matter directly or indirectly. *Enterobacteriaceae* presence indicates temperature-abuse conditions while storing, processing or cooking the food (Mohamedin *et al.*,2015). Poor hygiene, cross contamination, and poor handling during processing of food also contribute to high levels of *Enterobacteriaceae* in food samples (Gilbert *et al*., 2000). Foods like salad and fresh fruits or food containing these products may contain high levels of normal micro-flora of *Enterobacteriaceae* (Centre for Food Safety Hong Kong, 2014).

The standards with which our microbial results were compared are extracted from the Public Health Laboratory Services of United Kingdom (Gilbert *et al*., 2000), Food Standards Australia and New Zealand and Centre for Food Safety Hong Kong. These standards were simplified in a way for the food inspector to easily interpret the microbiological results for different kinds of food samples and to determine satisfactory (s), borderline (b) and unsatisfactory (u) levels (Table 3.5). These standards prepared by these agencies of different countries were mainly based on the Codex Alimentarius Commission standards. The standard plate counts or aerobic plate counts (APC) results are divided into 4 different levels to cover different food types. *Enterobacteriaceae* can also be known as total coliform count (TC). The criteria for satisfactory *Salmonella* spp. and *Listeria* spp. states that these organisms should not be detected in food samples of any type.

Table 3.5. *Microbiological Quality Standards of organisms as Hygiene indicators and Pathogens in Food Processing Stages.* (Gilbert *et al.*, 2000; Food Standards Australia and New Zealand, 2001; Centre for Food Safety Hong Kong, 2014).

Criterion as Indicators	**Satisfactory**	**Borderline**	**Unsatisfactory/ Hazardous**
Standard Plate Count Levels			
(1) RTE meals	3 Log_{10} CFU/g	3-≤4Log_{10}CFU/g	>4 Log_{10} CFU/g
(2) RTE pizza & pasta, bakery products without dairy, meat products and cooked vegetables	<4Log_{10}CFU/g	4-≤5Log_{10}CFU/g)	>5 Log_{10} CFU/g
(3) Seafood, Coleslaw, Dessert dairy cream, rice and pate	<5Log_{10}CFU/g	5-≤6Log_{10}CFU/g	>6 Log_{10} CFU/g
(4) Salad, fruits, Sandwiches with salad	<6Log_{10}CFU/g	6-≤7Log_{10}CFU/g	≥7 Log_{10} CFU/g
Enterobacteriaceae	<2Log_{10}CFU/g	2-<4Log_{10}CFU/g	≥4 Log_{10}CFU/g
Pathogens			
Salmonella spp.	not detected in 25g sample		detected in 25g sample
Listeria monocytogenes	not detected in 25g sample		detected in 25g sample
E. coli O157 & other VTEC	not detected in 25g sample		detected in 25g sample

In this study, food samples were collected from six (6) different food preparation stages which included receiving, storing, cutting/preparation, cooking, serving and restoring. The microbial counts of food samples were compared with the international standards and percentages of satisfactory, borderline and unsatisfactory microbial counts were obtained for each type of FSP (Table 3.6).

The mean microbial counts (total aerobic, total coliform, total *Salmonella spp.* and total *Listeria spp.* counts) for food samples collected from fast food restaurants were significantly lower ($P<0.05$) compared to the samples collected from other restaurants (Table 3.6). The mean total aerobic, total coliform, *Salmonella* spp. and *Listeria* spp. levels were 2.35, 1.01, 0.99, and 0.94 Log_{10} CFU/g, respectively. These results show that microbial counts of food samples collected from fast food restaurants are within satisfactory levels according to the international standards. This might mean that fast food restaurants with significantly low mean microbial counts implement the HACCP plan effectively at different stages of the food preparation. When the survey results and the food samples' microbial counts are compared, there is a contradiction as fast-food FSPs did not practice keeping records consistently, especially on cleaning and sanitation records (36% compliance, Table 3.2).

On the other hand, the total coliform counts of food samples collected from fine dine-in FSPs, which kept records at 100% rate based on the survey results, had the highest unsatisfactory levels 62.4% (15/24 samples), followed by casual sit-in 54.2% (13/24 samples), indicating poor hygiene practices and sanitary conditions (Gilbert *et al.,* 2000). The reason for such high coliform counts might be that fine dine-in restaurants usually prepare their foods in bulk quantity which usually go through several processes, such as preparing, cooking, and cooling. At any of these stages, such large volumes might get contaminated since the time to prepare, cool, and reheating might require longer duration at which microorganisms might multiply easily (Faour-Klingbeil *et al.,* 2016). During the walk-through audit, such factors (preparation of bulk density food) were also observed and it was noticed that there is a need to further improve the hygiene practices in these types of FSPs, especially on

employees' and surface hygiene practices. The audit team also reported that many of food items prepared were restored to be served later, which can also contribute to the high microbial counts (Osimani *et al.,* 2013).

The total aerobic counts of food samples collected from catering and takeaway FSPs were not significantly high; therefore, they obtained the highest satisfactory levels 100% (18/18 samples) and 93.4% (14/15 samples), respectively, followed by fast food FSP samples 100% (24/24 samples). Additionally, the food samples of these FSPs reportedly had lower percentage of unsatisfactory levels, 22.3% (4/18 samples) and 26.7% (4/15 samples), respectively, for total coliform counts compared to those of fine dine-in (62.4%, 15/24 samples) and casual sit-in (54.2%, 13/24 samples) FSPs.

These microbial results contradict with the results of record keeping compliance for especially catering and takeaway FSPs (Table 3.2). Catering establishments tend to keep (0%) no records on time & temperature control, employee personal hygiene, and critical control points, while the microbial counts data indicated that the food samples they serve met the satisfactory level at 86% (Table 3.6). These findings can be explained by the fact that the food prepared at catering FSPs are usually small quantities which are prepared based on the clients' demand and delivered to the clients immediately after preparation. The short period from storage to serving, safe delivery and no restoring of served food contribute to lower microbial counts in food samples collected from catering establishments. Similarly, for takeaway establishments no restoring, short period of food preparation might also limit the microbial growth in food samples collected from their premises. These results were in agreement with the findings of Mohamedin *et al.* (2015).

The levels for *Salmonella* spp. counts were significantly lower for fast food (0.99 Log_{10} CFU/g) when compared to results of fine dine-in (3.41 Log_{10} CFU/g). Similarly, the level of *Listeria* spp. count was determined to be significantly lower for fast food (0.99 Log_{10} CFU/g) when compared to those results obtained from food samples collected from casual sit-in (2.32 Log_{10} CFU/g) and catering establishments (2.36 Log_{10} CFU/g). However, it is important to note that none of the colonies isolated from XLT4 and LSA plates were positive for *Salmonella* spp. and *Listeria* spp. based on the VITEK analyses. Therefore, all food samples meet satisfactory levels (100%) for the presence of these pathogens according to the international standards.

Table 3.6. *Mean microbial counts* of food samples collected from different FSPs.*

Establishment (n=Total number of samples collected)	Total Aerobic*	Min-Max*	S%	B%	U%
Fine dine-in. (n=24)	4.19±0.4	<1.00-6.31	87.5	8.4	4.2
Casual sit-in. (n=24)	4±0.2	2.02-5.36	87.5	12.5	0
Fast Food (n=24)	2.35±0.3[a]	<1.00-4.84	100	0	0
Catering (n=18)	3.7±0.3	1.4-5.55	100	0	0
Takeaway (n=15)	3.92±0.2	2.72-5.03	93.4	6.6	0
Establishment	Total Coliform	Min-Max	S%	B%	U%
Fine dine-in. (n=24)	3.63±0.5	<1.00-6.58	33.4	4.16	62.5
Casual sit-in. (n=24)	3.31±0.4	<1.00-5.48	25	20.8	54.2
Fast Food (n=24)	1.01±0.3[b]	<1.00-5.34	70.8	20.8	8.4
Catering (n=18)	2.67±0.4	<1.00-5.63	27.8	50	22.3
Takeaway (n=15)	2.81±0.4	<1.00-4.61	13.4	60	26.7
Establishment	Total Salmonella spp.	Min-Max	S%	B%	U%
Fine dine-in. (n=24)	3.41±0.5	<1.00-6.44	100	0	0
Casual sit-in. (n=24)	2.16±0.4	<1.00-5.41	100	0	0
Fast Food (n=24)	0.99±0.3[c]	<1.00-4.85	100	0	0
Catering (n=18)	2.6±0.4	<1.00-4.65	100	0	0
Takeaway (n=15)	2.63±0.3	<1.00-4.36	100	0	0
Establishment	Total Listeria spp.	Min-Max	S%	B%	U%
Fine dine-in. (n=24)	1.26±0.4	<1.00-4.85	100	0	0
Casual sit-in. (n=24)	2.32±0.3	<1.00-5.08	100	0	0
Fast Food (n=24)	0.94±0.3[d]	<1.00-3.45	100	0	0
Catering (n=18)	2.36±0.4	<1.00-5.56	100	0	0
Takeaway (n=15)	2.37±0.3	<1.00-3.74	100	0	0

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level based on the international standards as listed in Table 4.5.
*: mean Log_{10} CFU/g ± standard deviation.
[a]: For Total Aerobic counts, fast food restaurants have significantly different results ($P<0.05$) when compared to fine dine-in, casual sit-in, catering and takeaway restaurants.
[b]: For coliform count, fast food restaurants have significantly different results ($P<0.05$) when compared to fine dine-in and casual sit-in.
[c]: For *Salmonella spp.* count, fast food restaurants have significantly different results ($P<0.05$) when compared to fine dine-in.
[d]: For *Listeria spp.*, fast food restaurants have significantly different results ($P<0.05$) when compared to casual sit-in and catering.

Food and swab samples were collected from each restaurant based on their availability. At the time of each sampling, the research team did not have the privilege to collect the same type of food samples from each of these premises. Only available food samples at the time of the visit were collected from the participating FSP. This created a challenge since each FSP had different food menu item at the time of the visit, limiting our sampling to collect only whatever the food was prepared and served on that specific day. Thus, collection of the same food samples from every FSP was not possible. However, it is also important to note that the focus of this study was to study the effect of different factors on the microbial quality of food served in these select restaurants and if these levels are considered satisfactory or not based on the international standards. The microbial counts of each sample collected from different FSP and their comparison to international standards are provided in tables 3.7, 3.8, 3.9, 3.10 and 3.11.

It can be clearly observed that the food samples collected from fine dine-in restaurants had satisfactory levels for most aerobic counts, but coliform counts are considered to be unsatisfactory (Table 3.7 A and B). This may be due to temperature effect, which was not kept at the required level to maintain hygiene conditions during

the processing and cutting of vegetables, leading to the growth of *Enterobacteriaceae* (Garayoa *et al.*, 2014). Another reason could be that restoring of the food products after serving in fine dine-in restaurants might contribute to the high counts of *Enterobacteriaceae* (Osimani *et al.*, 2013). Although VITEK analyses of presumptive isolated colonies show no signs of target pathogens, these results demonstrate that there is a need for improvement in storing, cutting, cooking, and serving areas in such premises (Faour-Klingbeil *et al.*, 2016)

Table 4.7. *Log_{10} CFU/g of select food samples collected from Fine Dine-in establishments (A and B).*

(A) Log_{10} CFU/g for 1st Fine Dine-in establishment

Sampling Area	Samples	Total Aerobic Count*	Total Coliform*	Total Salmonella spp.*	Total Listeria spp.*
Receiving	Oyster	0±0(S)	0±0(S)	0±0(S)	.
	Arabic Cheese	0±0(S)	0±0(S)	0±0(S)	.
Storing	Fruit Tart	6.25±0.6 (U)	5.55±1.3 (U)	6.18±0.6(S)	.
	Lobster Salad	6.11±0.2 (B)	6.39±0.1 (U)	6.01±0.1(S)	.
Cutting/Preparation	Salad	4.78±0.9 (S)	4.78±0.8 (U)	6.44±0.1(S)	.
	Fruit Salad	4.4±0.1 (S)	5.79±1.1 (U)	6.25±1.1(S)	.
Cooking	Salad (Parsley)	5.6±0.5 (S)	6.32±0.7 (U)	6.34±0.4(S)	.
	Solomon Salad	6.31±0.6 (B)	6.58±1.2 (U)	6.2±1.1(S)	.
Serving	Sea Food Salad	4.85±0.3 (S)	6.37±1.5 (U)	6.41±1.9(S)	.
	Cole slaw	5±0.1(S)	4.65±0.2(U)	6.32±1.7(S)	.
Restoring	Custard Cake	5.34±0.3 (S)	0±0(S)	0±0(S)	.
	Pistachio cake	5.16±0.3 (S)	0±0 (S)	0±0(S)	.

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

(B) Log_{10} CFU/g for 2nd Fine dine-in establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp. ***	**Total Listeria spp. ***
Receiving	Tomatoes	2.8±0.1 (S)	2.54±0.5 (S)	2.64±0(S)	2.74±0.2 (S)
	Lettuce	5.2±0.3 (S)	5.08±0.4 (U)	4.18±0.4 (S)	2.74±0.2 (S)
Storing	Cucumber	5.34±0.3 (S)	5.52±0.4 (U)	4.62±0.4 (S)	4.85±0.1 (S)
	Zucchini	3.69±0.2 (S)	5.35±0.5 (U)	4±0.3(S)	4.68±0.1 (S)
Cutting/Preparation	Salad lettuce	5.28±0.5 (S)	4.94±0.5 (U)	4.04±0.4 (S)	2.68±0.1 (S)
	Salad with anion and Lettuce	5.15±0.3 (S)	5.39±0.4 (U)	4.1±0.4(S)	1.78±0(S)
Cooking	Chicken Stake	2.59±0 (S)	0±0(S)	0±0(S)	0±0(S)
	Beef Stake	2.84±0.2 (S)	0±0(S)	0±0(S)	0±0(S)
Serving	Salad with Tuna	4.33±0.5 (S)	4.64±0.4 (U)	3.99±0.4 (S)	3.57±0.1 (S)
	Salad with Beef	4.34±0.5 (S)	4.61±0.4 (U)	4.08±0.4 (S)	4.54±0.3 (S)
Restoring	Pastry Chocolate	2.51±0.5 (S)	0±0(S)	0±0(S)	0±0(S)
	Pastry with White Chocolate coffee	2.69±0.2 (S)	2.65±0.4 (B)	0±0(S)	2.54±0.2 (S)

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

Casual sit-in restaurants also show satisfactory levels in aerobic counts, except for the total coliform counts of food samples collected from Casual sit in restaurant #1 (Table 3.8 A). These results indicate that storage temperature was not maintained at the levels required to control the microbial growth or the environment where the food samples were prepared was not clean enough to reduce the microbial counts. Similarly, the coliform counts of food samples collected from casual sit in restaurant

#2 were also considered to be unsatisfactory (Table 3.8 B). These results are in contradiction with the survey results that show 100% compliance for record keeping measures though unsatisfactory coliform counts shows otherwise. The high coliform counts suggest that employees do not follow hygiene practices (e.g., hand washing) regularly (Lambrechts *et al.,* 2014). This was confirmed as well during the walkthrough audit as the handwashing basins were not located at appropriate places in casual sit-in establishments. Other reasons for such high counts could be due to improper implementation of GMP's, as recorded in walk through audit.

Table 4.8. *Log_{10} CFU/g of select food samples collected from Casual Sit-in establishments (A and B).*

(A) Log_{10} CFU/g for 1st Casual Sit-in establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Red Chilies	3.48±0(S)	2.45±0.5(B)	0±0(S)	0±0(S)
	Red Capsicum	2.02±1.4(S)	1.78±1.2(S)	0±0(S)	1.18±0.8(S)
Storing	Tomatoes	2.95±0.1(S)	2.47±0.2(B)	0±0(S)	0.7±0.4(S)
	Mushrooms	5.1±0.1(S)	5.62±0.2(U)	4.6±0.2(S)	5.08±0.1(S)
Cutting/Preparation	Green Onion Chopped	3.69±0(S)	5.18±0.4(U)	5.09±0.5(S)	4.28±0.4(S)
	Garlic peeled	2.61±1.8(S)	0±0(S)	0±0(S)	2.28±0.3(S)
Cooking	Chicken Pasta	2.89±0.3(S)	0±0(S)	0±0(S)	0±0(S)
	Rice Boiled	2.96±0.2(S)	1.3±0.9(S)	0±0(S)	2.29±0.4(S)
Serving	Meat already cooked	2.02±0.1(S)	0±0(S)	5.41±0.1(S)	1.88±0.1(S)
	Chicken Already cooked	2.85±0.8(S)	2.52±0.2(B)	2.28±0(S)	0±0(S)
Restoring	Salad	2.69±0(S)	2.84±0.1(U)	0±0(S)	2.85±0(S)
	Shrimps	3.73±0.1(S)	0±0(S)	0±0(S)	2.57±0.4(S)

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

(B) Log_{10} CFU/g for 2nd Casual Sit-in establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Tomatoes	5.06±0.3(S)	3.96±0.5 (U)	4.1±0.4 (S)	2.33±0(S)
	Lettuce	5.07±0.3(S)	4.63±0.6 (U)	4.14±0.3 (S)	2.18±0(S)
Storing	Mint Leaves	5.15±0.4(S)	5.21±0.3 (U)	4.12±0.3 (S)	4.79±0.4(S)
	Zucchini	5.36±0.5(S)	5.42±0.5 (U)	4.11±0.5 (S)	4.78±0.4(S)
Cutting/Preparation	Bread Cheese Lettuce	4.83±0.4(S)	4.83±0.3 (U)	3.97±0.5 (S)	2.59±0.1(S)
	Parsley	4.06±0.2(S)	5.18±0.4 (U)	4.13±0.4 (S)	3.76±0.3(S)
Cooking	Rice	4.56±0.2(S)	2.82±0.4 (B)	2.43±0.3 (S)	1.78±0(S)
	Potato	4.64±0.3(S)	2.93±0.4 (B)	2.19±0(S)	2.42±0.1(S)
Serving	Biryani Mutton	4.92±0.3(S)	4.78±0.5 (U)	0±0(S)	0±0(S)
	Fried Chicken Breast	5.15±0.5(B)	4.6±0.4 (U)	0±0(S)	0±0(S)
Restoring	Custard Chocolate milk	5.18±0.4(B)	5.45±0.5 (U)	2.77±0.1 (S)	3.93±0.3(S)
	Water Melon Cubes	5.12±0.4(B)	5.48±0.5 (U)	2.52±0(S)	3.93±0.4(S)

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

Fast food restaurants showed mostly satisfactory and less borderline results for the total aerobic, coliform, and *Salmonella* spp. counts (Table 3.9 A and B). These results prove the fact that majority of fast food restaurants apply their own internal food safety standards as required by their parent companies. These standards applied by chain restaurants are effective in reducing the occurrences of microbial organisms under satisfactory levels (Harris *et al*., 2014). There were no presumptive colonies detected in any select media used for sampling select food items collected from fast food restaurants.

It is also noteworthy to mention that most fast food establishments in Qatar are international chain restaurants. As a result, they receive already chopped vegetables and frozen raw products from their suppliers who had already processed the select food items using proper SOP procedures; deliver such food items to the establishments using optimum storage conditions as well. At the time of preparation, these ready to eat vegetables are served or used by simply opening the packages and serving them within the premises, thus minimizing the cross-contamination issue.

At the time of sampling, it was also observed that prepared or cooked food was sold immediately after preparation in fast food restaurants. Thus, reflecting the low aerobic and coliform results which are mostly at satisfactory level. While in other types of restaurants, food prepared and served, if not consumed immediately, will be stored back in the refrigerators, making it more vulnerable to contamination as reported by Osimani *et al.* (2013).

Table 4.9. *Log_{10} CFU/g of select food samples collected from fast food establishments. (A and B).*

(A) Log_{10} CFU/g for 1st Fast food establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Chicken Nuggets Raw	3.93±0.2 (S)	0±0(S)	3.37±0.1 (S)	2.02±0 (S)
	Raw Kofta	3.61±0.5 (S)	2.78±0.3 (B)	2.96±0.2 (S)	2.7±0 (S)
Storing	Tomato	2.89±0.2 (S)	0±0(S)	0±0(S)	0±0(S)
	Pickle	3.02±0.3 (S)	0±0(S)	0±0(S)	0±0(S)
Cutting/Preparation	Salad (Olive, Lettuce, tomatoes)	2.8±0.4 (S)	2.58±0 (B)	2.18±0.3 (S)	2±0(S)
	Coleslaw	3.69±0.1 (S)	3.41±0 (U)	2.95±0.2 (S)	2.34±0.1 (S)
Cooking	Chicken cooked	2.83±0.1 (S)	0±0(S)	0±0(S)	0±0(S)
	Cooked Kabab	2.69±0(S)	0±0(S)	0±0(S)	0±0(S)
Serving	Rice	2.55±0.2 (S)	0±0(S)	0±0(S)	0±0(S)
	Khobos (Bread)	2.7±0.1(S)	0±0(S)	0±0(S)	0±0(S)
Restoring	Chopped Tomatoes	2.96±0.1 (S)	0±0(S)	0±0(S)	0±0(S)
	Sauce (Mayonnaise chili)	2.9±0.2(S)	0±0(S)	0±0(S)	0±0(S)

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

(B) Log_{10} CFU/g for 2nd Fast food establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Lattice	4.84 ±0.1(S)	5.34±0.2 (U)	4.85±0.5 (U)	3.45±0.3 (S)
	Cheese	3.49±0.2 (S)	3.48±0.1 (B)	2.56±0.6 (B)	3.04±0.5 (S)
Storing	Mayonnaise Dressing	2.52±0 (S)	2.98±0.5 (B)	0±0(S)	2.78±0.4 (S)
	Meat beef	2.45±0 (S)	0±0(S)	0±0(S)	0±0(S)
Cutting/Preparation	Chicken Pieces	1.93±0 (S)	0±0(S)	1.81±0(S)	1.3±0 (S)
	Buns	0±0(S)	0±0(S)	0±0(S)	0±0(S)
Cooking	Chicken nuggets	0±0(S)	0±0(S)	0±0(S)	0±0(S)
	Chicken salad	4.54±0.3 (S)	3.71±0.4 (B)	3.08±0.1 (B)	2.98±0 (S)
Serving	Meat	0±0(S)	0±0(S)	0±0(S)	0±0(S)
	Fries	0±0(S)	0±0(S)	0±0(S)	0±0(S)
Restoring	Ice Cream	0±0(S)	0±0(S)	0±0(S)	0±0(S)
	Apple Pie	0± 0(S)	0± 0(S)	0± 0(S)	0± 0(S)

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

The overall microbial counts of food samples collected from catering restaurants (Table 3.10 A & B) showed satisfactory levels in aerobic counts, but coliform counts were found to be unsatisfactory at the storing, preparation, and serving stages. These results are similar to the results obtained from casual sit-in restaurants, indicating that employee's hygiene is an important factor contributing to these high levels of coliform counts (Tan *et al.,* 2013). The relatively high coliform counts of samples collected at the preparation stage can be due to improper sanitization of vegetables and temperature-abuse conditions.

In a study carried out by Mohamedin *et al.* (2015), it was suggested that short holding time during cutting/preparation and serving decreases the risk of bacterial growth above limits. In our study, it has been noticed that catering restaurants have 0% record keeping compliance with respect to time and temperature control, employee's hygiene, and CCP's (Table 3.2). Hence, not implementing SOP's and GMP's. During walk through audit, it has been observed that catering facilities exhibited good cleaning and sanitization practices at their premises, but had limited access to hand washing facilities equipped with hand soap and paper towels. All of these factors might be the reasons for cross contamination and poor coliform counts at different food preparation stages in the catering establishments (Lambrechts *et al.,* 2014). These results highlight the need to establish appropriate measures to improve hygiene practices applied by the employees as well as the cleaning of the premises in catering FSPs.

Table 4.10. *Log_{10} CFU/g of select food samples collected from Catering establishments. (A and B).*

(A) Log_{10} CFU/g for 1st Catering establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Lettuce	3.79±0.4 (S) .	2.98±0.1 (B) .	3.54±0.1 (S) .	3.91±0.5 (S) .
Storing	Spinach	4.86±0 (S) .	4.67±0.9 (U) .	4.65±0(S) .	2.88±0.6 (S) .
Cutting/Preparation	Salad Carrot Cabbage	1.4±0.2 (S) .	0±0 (S) .	0± 0(S) .	1.02±0.1 (S) .
Cooking	Vegetable Curry	2.18±0.8 (S) .	0±0 (S) .	0±0 (S) .	0±0(S) .
Serving	Chicken Curry	3.15±0.1 (S) .	2.17±1.7 (B) .	2.52±0.4 (S) .	2.4±0.5 (S) .
Restoring	Tomatoes (Already chopped) Lettuce	4.25±0.1 (S) .	3.63±0.1 (B) .	3.7±0.1 (S) .	3.55±0(S) .

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

(B) Log_{10} CFU/g for 2nd Catering establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Tomatoes	4.05±0.1 (S)	2.9±0.1(B)	2.85±0(S)	2.92±0.1 (S)
	Cucumber	4.08±0.1 (S)	3.88±0.5 (B)	3.62±0.1 (S)	3.08±0 (S)
Storing	Salami	2.15±0.2 (S)	1±0(S)	1.93±0(S)	0±0(S)
	Spanish	4.55±0.1 (S)	5.44±0.3 (U)	3.4±0(S)	5.53±0.4 (S)
Cutting/Preparation	Parsley	5.55±0.2 (S)	5.63±0.4 (U)	4.45±0.4 (S)	5.56±0.3 (S)
	Chopped Tomatoes	3.41±0 (S)	2.54±0.4 (B)	2.6±0.4(S)	2.61±0.5 (S)
Cooking	Cookies	2.54±0.4 (S)	0±0(S)	0±0(S)	0±0(S)
	Brownies	2.42±0 (S)	0±0(S)	0±0(S)	0±0(S)
Serving	Chicken	5.1±0(S)	4.62±0.1 (U)	4.5±0.4(S)	4.87±0.4 (S)
	Tuna Salad	3.1± 0(S)	2.75±0(B)	2.9±0.2(S)	0±0(S)
Restoring	Rice	5.4±0.3 (S)	3.12±0.1 (B)	3±0.3(S)	1.7±0(S)
	Grape Leaves with rice	4.61±0.5 (S)	2.72±0.2 (B)	3.08±0.2 (S)	2.55±0 (S)

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

Based on the survey results, it was found that takeaway establishments do not implement HACCP and do not keep records on employee hygiene. These practices might eventually lead to unsatisfactory levels of coliform counts as observed at all stages of food preparation in these restaurants (Table 4.11 A and

B). It has also been demonstrated during the walkthrough audit that there were limited number of hand washing facilities within these types of premises which can be a causative factor for high borderline (60%) and unsatisfactory (26.7%) levels of coliform counts. Since the aerobic bacterial counts are used as hygiene indicator in any premises, the total APC counts in food samples collected from take away establishments were considered to be satisfactory, contrary to the coliform counts.

Although none of the presumptive coliform colonies turned out to be positive pathogenic *E. coli*, unsatisfactory levels of coliform counts might be directly linked to inadequate employee hygiene issues in these FSPs, which might cause a public health concern (Tan *et al.,* 2013). With regard to employee hygiene and safe food handling practices, a microbial reduction of 3 Log_{10} CFU/g for coliforms was observed when adequate food safety procedures were applied (Pereira *et al.,* 2013).

Table 4.11. *Log_{10} CFU/g of select food samples collected from Takeaway establishments. (A and B).*

(A) Log_{10} CFU/g for 1st Takeaway establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Refrigerated Meat	3.04±0 (S) .	2.24±0 (B) .	1.48±0.1 (S) .	2.77±0.1 (S) .
Storing	Refrigerated Chicken	4.36±0.4 (S) .	2.16±0.3 (B) .	1.65±0(S) .	3.7±0.3 (S) .
Cutting/Preparation	Tomatoes (Already chopped)	4.080.8 (S) .	3.82±0 (B) .	2.84±0.1 (S) .	3.55±0.1 (S) .
Cooking	Potatoes Green Peas	3.31±0.3 (S) .	0±0(S) .	0±0(S) .	1.29±0.2 (S) .
Serving	Falafel Sandwich	3.8±0.5 (S) .	3±0(B) .	2.85±0.3 (S) .	3.91± 0.8(S) .
Restoring	Holumi Sandwich Meat	2.72±0.1 (S) .	2.1±1.4 (B) .	2.35±1.6 (S) .	2.19±0.1 (S) .

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

(B) Log_{10} CFU/g for 2nd Takeaway establishment					
Sampling Area	**Samples**	**Total Aerobic Count***	**Total Coliform***	**Total Salmonella spp.***	**Total Listeria spp.***
Receiving	Tomatoes	4.73±0.7 (S) .	4.04±0.5 (U) .	4.09±0.3 (S) .	2.99±0.1 (S) .
Storing	Sausage Chopped	3.93±0.4 (S) .	4.19±0(U) .	3.76±0(S) .	3.07±0.2 (S) .
Cutting/Preparation	Flour Dough	6.05±0.6 (B)	3.04±0.2 (B)	2.77±0.1 (S)	2.26±0 (S)
	Chopped Tomatoes	5.03±0.2 (S)	4.48±0 (U)	4.36±0.1 (S)	2.7±0.5 (S)
Cooking	Sausage Boiled	3.53±0 (S) .	2.27±1.1 (B) .	3.4±0.1(S) .	2.27±0.4 (S) .
Serving	Tahini with Tomatoes	3.91±0 (S)	4.61±1.1 (U)	2.71±0.1 (S)	1.04±0.4 (S)
	Fatayer	3.52±0.1 (S)	3.45±0(B)	2.97±0.2 (S)	0±0 (S)
Restoring	Chilies Sauce	3.14±0.5 (S)	0±0(S)	0.7±0(S)	0±0(S)
	Shrimps	3.71±0.5 (S)	2.74±0.3 (B)	3.53±0.2 (S)	3.74±0.2 (S)

Note: S=Satisfactory level, B=Borderline level, U=Unsatisfactory level.

*mean Log_{10} CFU/g ± standard error.

The survey data obtained from fine dine-in, casual sit-in, and fast food restaurants revealed that these establishments were using color-coded cutting boards. The importance of using color-coded cutting boards in reducing cross-contamination problem has been reported by several authors (Faour-Klingbeil *et al.*, 2016; Pichler *et al.,* 2014). On the other hand, catering and takeaway restaurants were not strictly using color-coded cutting board system. However, managers claimed that they use different white cutting boards for each raw product. Additionally, use of sanitizing tablets has been found to prevent cross contamination as studied by Tambekar *et al.* (2006), before chopping and

preparation of vegetables. Vegetable sanitization process was observed mostly in fine dine-in, casual sit-in, and fast food establishments but not used as a common practice by catering and takeaway establishments where vegetables were washed with tap water (Ali *et al.,* 2015).

3.3 Microbial Analyses of Swab Samples:

Studies have suggested that cleanliness of the premises and food contact surfaces, as well as the use of different utensils during the preparation of food are important factors to be considered as part of GMPs (Abdul-Mutalib *et al.,* 2015). Thus, swabs collected from cutting boards can elaborate if the GMPs are followed or not in any given FSPs. Additionally, swabs from preparation and serving areas may indicate the overall cleanliness of the establishment premises and if there is a need to implement proper food safety measures (Djekic *et al*., 2016). Table 3.12 lists the international standards used for microbial counts for the swabbed surfaces. There is zero tolerance for *Listeria spp.* and *Salmonella spp*. in surface swabs.

Table 3.12. *Standard guidelines for aerobic colony count from surface swabs.* (Sagoo *et al.*, 2003; NSW Food Authority 2012; Willis *et al.*, 2015, Henroid *et al.*, 2004 and Sneed *et al.*, 2004).

	Satisfactory	**Borderline**	**Unsatisfactory**
Total Coliform	<1.0 $Log_{10}/CFU/cm^2$	-	≥1.0 $Log_{10}/CFU/cm^2$
Aerobic Colony Count	<1.9 $Log_{10}/CFU/cm^2$	1.9-3 $Log_{10}/CFU/cm^2$	>3 $Log_{10}/CFU/cm^2$

The aerobic microbial counts of food contact surfaces demonstrated that surface swab samples collected from fine dine-in establishments had the overall lowest APC counts (4.41±0.4 Log_{10} CFU/cm^2), while the highest levels were obtained from swab samples collected from causal sit-in and catering FSPs (5.54±0.3 and 5.52±0 Log_{10} CFU/cm^2), respectively (Table 3.13), though results were not significantly different ($P>0.05$). Based on the standards as listed in Table 3.12, all swab samples collected from most FSPs received unsatisfactory remarks since the APC levels were above the set standards (Sagoo *et al*., 2003; NSW Food Authority 2012; Willis *et al*., 2015), indicating inadequate level of cleaning practices applied on food contact surfaces (Losito *et al.*, 2017). The minimum and maximum APC counts were detected in takeaway establishments (<1.00-7.26 Log_{10} CFU/m^2). It has been reported that the presence of high aerobic microorganism counts indicates improper sanitation of the surfaces of cutting board, preparation area, and serving area (Alia *et al.,* 2016). Some of these food contact surfaces can also become a source of pathogenic bacteria if the surfaces are not cleaned properly (Abdul-Mutalib *et al.,* 2015). Studies have proved that regardless of grade and status of FSP, there is a likelihood of transformation of foodborne bacteria from cutting boards (Alia *et al.,* 2016). Therefore, proper cleaning and disinfection of cutting boards and food contact surfaces should be one of the top priorities of any given FSPs to avoid the growth of pathogens.

The total coliform and *Salmonella* spp. counts (5.59 and 4.8 Log_{10} CFU/m^2, respectively) of swab samples collected from catering FSPs were significantly different ($P<0.05$) from those of others. These findings are in alignment with the

survey results which demonstrated that record keeping compliance for catering restaurants was poor. The lowest range of total coliform counts of surface swabs were recorded in fine dine-in, fast food and takeaway establishments (<1.00 Log_{10} CFU/m^2), while the highest levels were recorded in takeaway FSP (7.26 Log_{10} CFU/m^2). Similarly, the total *Salmonella* spp. count was recorded at low levels in all establishments (<1.00 Log_{10} CFU/m^2), except in catering restaurants (3.53 Log_{10} CFU/m^2) which had significantly higher counts (P>0.05) comparing to the other FSP's. These results are in agreement with the survey results demonstrating that hygiene practices and sanitation of the utensils and surfaces need to be improved in catering FSPs in order to avoid food contamination. Garayoa *et al*. (2017) stated that record keeping measures on personal hygiene and temperature control help track the wrong hygiene and food processing practices. However, it is important to note that the presumptive *Salmonella* colonies isolated from XLT4 plates were all negative based on the VITEK analysis.

In terms of total *Listeria* spp. counts of contact surfaces, some differences were observed between FSPs (Table 3.13). Similar to the results of total *Salmonella* counts, takeaway FSPs topped the list with having the highest total *Listeria* spp. counts (4.14+0.7 Log_{10} CFU/cm^2). Although none of the presumptive *Listeria* colony isolated from swab samples collected from any FSPs turned out to be positive for *Listeria monocytogenes*, relatively high *Listeria* spp. counts suggest that there might be a need of reanalyzing the food safety management plan in takeaway restaurants to determine the gaps at which sanitation issues should be first addressed as suggested by Balzaretti *et al*. (2013).

Table 3.13. *Means of microbial counts* of swab samples collected from different FSPs.*

Establishment	Total Aerobic	Min-Max	U	S
Fine dine-in (n=12)	4.41±0.4(u)	2.21-5.95	100%	0%
Casual sit-in (n=12)	5.54±0.3(u)	4.56-6.83	100%	0%
Fast Food (n=12)	5.12±0.1(u)	4.51-5.62	100%	0%
Catering (n=12)	5.52±0(u)	5.27-5.69	100%	0%
Takeaway (n=10)	5.16±0.7(u)	2.00-7.26	100%	0%
Establishments	Total Coliform	Min-Max	U	S
Fine dine-in (n=12)	2.85±0.9	<1.00-5.81	50%	50%
Casual sit-in (n=12)	5.21±0.3	3.55-6.61	100%	0%
Fast Food (n=12)	3±0.7	<1.00-5.49	66.6%	33.4%
Catering (n=12)	5.59[c]±0.1	5.03-6.03	100%	0%
Takeaway (n=10)	4.1±0.9	1.00-7.26	100%	0%
Establishments	*Salmonella spp.*	Min-Max	U	S
Fine dine-in (n=12)	2.74±0.8	<1.00-5.55	0%	100%
Casual sit-in (n=12)	2.8±0.6	<1.00-5.65	0%	100%
Fast Food (n=12)	0.92±0.3	<1.00-2.66	0%	100%
Catering (n=12)	4.8[b]±0.2	3.53-5.62	0%	100%
Takeaway (n=10)	1.25±0.8	<1.00-6.24	0%	100%
Establishments	*Listeria spp.*	Min-Max	U	S
Fine dine-in (n=12)	0.89±0.6	<1.00-2.75	0%	100%
Casual sit-in (n=12)	5.18[a]±0.4	3.55-6.63	0%	100%
Fast Food (n=12)	2.15±0.7	<1.00-4.74	0%	100%
Catering (n=12)	3.55±0.6	<1.00-5.01	0%	100%
Takeaway (n=10)	4.14[d]±0.7	<1.00-6.29	0%	100%

Note: S=Satisfactory level and U=Unsatisfactory level based on the international standards as listed in Table 4.7.

* mean Log_{10} CFU/m^2 ± standard error

[a] For Listeria spp. counts, Casual Sit-in restaurants have significantly different results ($P \leq 0.05$) when compared to fine dine-in and fast food restaurants.

[b] For *Salmonella spp.* counts, catering restaurants have significantly different results ($P \leq 0.05$) when compared to takeaway and fast food restaurants.

[c] For coliform counts, catering restaurants have significantly different results ($P \leq 0.05$) when compared to fast food and fine dine-in restaurants.

[d] For *Listeria spp.* counts, take away restaurants have significantly different results ($P \leq 0.05$) when compared to fine dine-in restaurants.

3.4 VITEK Analyses of Presumptive Colonies isolated from food and swab samples:

Out of 163 samples collected (105 food samples + 58 swab samples) only 13 samples were identified to have presumptive target colonies (8% of all samples). The molecular analysis of presumptive target colonies isolated from food and swab samples is listed in Table 3.19. The VITEK results demonstrated that none of the isolated colonies was positive for *Listeria monocytogenes*, *E. coli O157:H7* or *Salmonella enteritidis*.

Table 3.14. *Presumptive colonies isolated and their percentages of positive identification with respect to samples collected from each FSP.*

Establishments (n=food samples+swab samples)	*E. coli* spp. (%Positive Identification)	*Salmonella* spp. (%Positive Identification)	*Listeria* spp. (%Positive Identification)
Fine Dine-in (n=24+12)	2 (0%)	0	0
Casual Sit-in (n=24+12)	3 (0%)	1 (0%)	0
Fast food (n=24+12)	0	0	0
Catering (n=18+12)	4 (0%)	0	0
Takeaway (n=15+10)	2 (0%)	1 (0%)	0

The VITEK test results revealed that *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Pseudomonas aeruginosa,* and *Pantoea* spp. were the most prominent organisms in food and surface swab samples (Table 3.19). The presence of *Klebsiella* spp.in food samples might be the indication of poor employee hygiene since this bacterium is commonly isolated from people with bronchitis, urinary tract infections, and/or pneumonia (Gautam *et al.*, 2015 & Tan *et al.,* 2013).

Similar results were also observed in a study conducted in Algeria which analyzed the microbial quality of RTE sandwiches (Yaici *et al.*, 2017). As reported previously, finding *Staphylococcus* spp. in food samples is directly associated with poor hygiene practices of food handlers (Ray & Bhunia, 2007; Yang *et al.,* 2016; Tomasevic, 2016). The occurrence of *Pseudomonas* spp. in food processing contact surfaces has been reviewed by Meliani *et al.* (2015) who stated that food processing environment is favorable for the formation of *Pseudomonas spp*. biofilms as this organism can obtain sufficient nutrients from food contact surfaces and moisture. *Pseudomonas* spp. are commonly found in vegetables such as sprouts, lettuce, and spinach, as well as other raw material and can grow in food preparation premises which are difficult to clean such as walls, floors, drains, basins and pipes. Since sanitation and cleaning of such areas is difficult, the situation becomes favorable for this organism to form biofilms (Bower *et al.*, 1996; Fett *et al.*, 2000). Haleem *et al*. (2013) suggested that presence of *Klebsiella pneumoniae, Pseudomonas spp.* and *E. coli* indicates the lack of hand cleaning facilities in the premises or employees not following proper hand washing behavior.

Table 3.15. *Presumptive colonies on selective media.*

Samples	MacConkey	XLT4	Listeria Selective
Cucumber	*Pseudomonas aeruginosa*	-	-
Cucumber (Chopped)	*Pseudomonas aeruginosa*	-	-
Tomatoes (Chopped)	*Pantoea spp.*	-	-
Tomatoes	*Pseudomonas aeruginosa*	-	-
Tomatoes	*Klebsiella oxytoca*	-	-
Tomatoes	*Pantoea spp.*	-	-
Falafel Sandwich	*Pseudomonas aeruginosa*	-	-
Green Onion (Chopped)	*Klebsiella pneumoniae*	-	-
Tuna Salad	*Pantoea spp.*	-	-
Lettuce	*Pantoea spp.*	-	-
Swab Cutting board	*Klebsiella pneumoniae*	-	-

These findings demonstrate the need to improve conditions especially on sanitation of food contact surfaces, personal hygiene, and cleanliness of FSP premises in order to enhance microbial quality of foods prepared and served in the FSPs assessed in this study. Furthermore, the risk of cross-contamination might also be controlled by implementing safer food handling practices.

CHAPTER IV: CONCLUSIONS

To the best of our knowledge, this is a first study reporting the food safety knowledge and attitudes of food handlers at select restaurants in Doha and the microbial quality of foods that they prepare. It was determined that majority of managers and food handlers participated in the survey were trained on food safety management system. However, applying their knowledge into practice was not observed during the walkthrough audits, indicating a need to implement sound approaches to food safety management. Overall, fine dine-in and casual sit-in restaurants were in compliance at a rate of 100% for keeping records on important factors (e.g., temperature and time control) followed by fast food. While takeaway and catering restaurants had poor compliance with record keeping practices which are essential parts of HACCP. The results obtained from walkthrough audit showed that only 39% (21 out of 53 restaurants) keep records on employee training. The results provide sufficient information to recommend that managers and food handlers should be provided with periodic training on food safety to make them better prepared to do their jobs safely. Important training topics should specifically include monitoring and recording temperature of food prepared, cleaning and sanitization of the food preparation areas, use of color-coded system to separate raw food from ready to eat food

items, and cleanliness of storage and serving areas. Additionally, monitoring of the effectiveness of cleaning and sanitization activities should be conducted periodically to ensure food safety in such food establishments.

The microbial counts (total APC, *Salmonella* spp., and *Listeria* spp. counts and total coliforms) of food samples was significant ($P<0.05$) for fast food restaurants. The microbial quality of food samples prepared at fast food restaurants were of 100% satisfactory quality in terms of total APC, *Salmonella* spp., and *Listeria* spp. counts and for total coliforms the satisfaction level was at 71%. It has been recorded that fine dine-in and casual sit-in restaurants have 100% record keeping compliance while the percentage of total coliform counts was determined to be at unsatisfactory level 62.5% and 54.2%, respectively. These results indicate that managers have adequate training on food safety but there is a lack of implementation. The high coliform counts in any given food premise closely correlates with lack of personal hygiene practice, GMP's and SOP's, as observed especially in catering and takeaway restaurants.

The swab sample results showed no significant differences among all FSP types when it comes to total aerobic count since all FSPs had 100% unsatisfactory level of APC according to the international standards. The mean coliform (5.59 Log_{10} CFU/m^2) and *Salmonella* spp. (4.8 Log_{10} CFU/m^2)

counts were significantly high for catering restaurants compared to those of others. The presence of *Salmonella* and coliforms in especially surfaces might be a concern since these organisms were directly linked to a number of recently reported global foodborne outbreaks. The high microbial load of food contact surfaces in select restaurants highlight the need to promote awareness on the cleanliness of the equipment and surfaces used to prepare food items.

The VITEK analyses for isolates of presumptive colonies revealed that none of the samples was contaminated with target pathogens, but *Klebsiella spp., Staphylococcus spp., Pseudomonas aeruginosa*, *and Pantoea spp.* were identified as contaminant source in food and swab samples. These pathogenic bacteria can also be hazardous for human health and should be taken into consideration while planning SOPs in any given food service operation.

Finally, the research findings underscored the need to take immediate actions to improve sanitary and good hygiene practices to reduce or eliminate contamination and cross-contamination sources that might create a public health risk during food preparation stages. Therefore, it is necessary that monitoring of critical control points (CCPs) should be conducted on a regular basis by internal or external auditing committee to prevent contamination of food with any environmental microbial hazards. Assessing the effectiveness of

implementation of training and record keeping procedures at different stages of food premises is also necessary to maintain good hygiene levels. It has been observed in this study that restaurants implementing HACCP have shown unsatisfactory coliform counts which can only be controlled by adopting regular monitoring and surveying practices to mitigate food safety risk factors.

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