MICROBIAL PRESENCE ON THE FOOD HANDLERS' HANDS AND APRON SURFACES FROM SELECTED PRIMARY SCHOOL CANTEENS IN KOTA BHARU, KELANTAN

By

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CERTIFICATE

This is to certify that the dissertation entitled "Microbial Presence on the Food Handlers' Hands and Apron Surfaces from Selected Primary School Canteens in Kota Bharu, Kelantan" is the bonafide record of research work done by Miss Fatin Nabila Binti Mohd Saupe during the period from September 2013 to May 2014 under my supervision.

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LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMNS

-	To/Until
%	Percentage
/	Over to
x	Times
<	Less than
=	Equal to
≥	More than or equal to
µl	Microlitre
APC	Aerobic plate count
ATCC	American Type Culture Collection
BPA	Baird Parker agar
cfu	Colony forming unit
cm	Centimetre
E.coli	*Escherichia coli*
EMB	Eosin methylene blue

FSQCD	Food Safety and Quality Control Division
MAC	MacConkey
MKKM	Makmal Keselamatan dan Kualiti Makanan
ml	Millilitre
mm	Millimetre
MOH	Ministry of Health
NA	Nutrient agar
°C	Degree Celsius
PASW	Predictive Analytics Software
PW	Peptone water
S. aureus	*Staphylococcus aureus*
SPSS	Statistical Package for the Social Sciences
TCC	Total coliforms count
USM	Universiti Sains Malaysia
WHO	World Health Organization

ABSTRAK

KEHADIRAN MIKROB PADA PERMUKAAN TANGAN DAN APRON PENGENDALI MAKANAN DARIPADA KANTIN SEKOLAH RENDAH YANG TERPILIH DI KOTA BHARU, KELANTAN

Pengendali makanan di kebanyakan premis makanan didapati bertanggungjawab ke atas wabak penyakit bawaan makanan dan hasil laporan telah mengenalpasti bahawa tangan mereka merupakan sumber utama kepada patogen di dalam makanan yang berkaitan. Di Kelantan, majoriti episod keracunan makanan berlaku di sekolah rendah. Kajian ini bertujuan mengenalpasti kehadiran "kiraan plat aerobik" (KPA), "jumlah kiraan koliform" (JKK), *Escherichia coli* dan *Staphylococcus aureus* ke atas tangan dan apron pengendali makanan. Dalam kajian irisan lintang ini, sebanyak 135 sampel permukaan (tangan kanan, 45; tangan kiri, 45; dan apron, 45) diambil menggunakan persampelan mudah ke atas 45 pengendali makanan daripada 9 sekolah rendah di Kota Bharu. Pengambilan sampel swab dilakukan ke atas permukaan kedua-dua tangan dan apron pengendali makanan. Analisa mikrobiologi bagi KPA, JKK, *E. coli* dan *S. aureus* telah dijalankan dan ditentukan dalam cfu/cm^2. Daripada 45 pengendali makanan yang disampel, 28 (62%) daripada mereka mempunyai tahap kebersihan yang memuaskan dan 17 (38%) mempunyai tahap kebersihan yang tidak memuaskan. Bagi keseluruhan sampel swab, penemuan mendedahkan bahawa 60% dan 64% daripada sampel, masing-masing mempunyai tahap KPA ($<10^3$ cfu/cm^2) dan JKK ($<10^2$ cfu/cm^2) yang memuaskan. Walau bagaimanapun, 40% daripada apron, 42% daripada tangan kanan dan 38% daripada tangan kiri mempunyai tahap yang tidak memuaskan bagi KPA ($\geq 10^3$ cfu/cm^2). Begitu juga, 36% daripada apron, 38% daripada tangan kanan dan 33% daripada

tangan kiri mempunyai tahap yang tidak memuaskan ($\geq 10^2$ cfu/cm^2) bagi JKK. Bagi pengendali makanan yang mempunyai tahap kebersihan yang tidak memuaskan untuk kedua-dua tangan, mereka menunjukkan tahap KPA (77%) dan JKK (93%) yang sangat tinggi pada apron. Ini menunjukkan bahawa terdapat hubungan yang signifikan antara status kebersihan tangan dan kualiti mikrobiologi pada apron pengendali makanan. Walau bagaimanapun, *E. coli* dan *S. aureus* tidak dikesan dalam mana-mana sampel. Kesimpulannya, status kebersihan pengendali makanan masih kurang memuaskan dan ini mungkin akan berterusan menjadi faktor yang menyumbang kepada episod keracunan makanan di sekolah rendah.

ABSTRACT

MICROBIAL PRESENCE ON THE FOOD HANDLERS' HANDS AND APRON SURFACES FROM SELECTED PRIMARY SCHOOL CANTEENS IN KOTA BHARU, KELANTAN

Food handlers in many setting have been responsible for food borne disease outbreaks and reports have identified their hands as the major source of pathogens in the implicated food. In Kelantan, the majority of food poisoning episodes occurred in the primary school. This study aimed to determine the presence of aerobic plate count (APC), total coliforms count (TCC), *Escherichia coli,* and *Staphylococcus aureus* on food handlers' hands and their aprons. In this cross sectional study, a total of 135 surface samples (left hand, 45; right hand, 45; and apron, 45) were collected by convenience sampling from 45 food handlers at 9 primary school canteens in Kota Bharu. Sampling was performed by swabbing the surfaces of both hands and apron of the food handlers. The microbiological analyses for APC, TCC, *E. coli,* and *S. aureus* were carried out and determined in cfu/cm^2. Of the 45 food handlers sampled, 28 (62%) and 17 (38%) had satisfactory and unsatisfactory level of hygiene, respectively. Overall findings revealed that 60% and 64% of the swab samples had satisfactory APC ($<10^3$ cfu/cm^2) and TCC ($<10^2$ cfu/cm^2), respectively. However, 40 % of the aprons, 42% of the right hands and 38% of the left hands samples had unsatisfactory APC level ($\geq 10^3$ cfu/cm^2). Similarly, 36% of the aprons, 38% of the right hands and 33% of the left hands samples had unsatisfactory TCC ($\geq 10^2$ cfu/cm^2). Besides, food handlers with unsatisfactory hygiene level for both hands showed very high APC (77%) and TCC (93%) on the aprons. This indicates that there was a significant association between hygiene status of hands and the

microbiological quality on the apron of food handlers. However, *E. coli* and *S. aureus* were not detected in any of the samples. As a conclusion, the overall hygiene status of food handlers is still poor and may continually contribute to food poisoning episodes in primary schools.

CHAPTER 1: INTRODUCTION

1.1 Background of Study

Every year, millions of people fall ill and many have died from food borne diseases resulting from eating unsafe food. Food borne diseases are defined by the World Health Organization as diseases that are caused by the contamination of food with microorganisms or chemicals which can occur at any stage in the process from food production to ingestion (WHO, 2006). Food borne disease is known to contribute to both human morbidity and mortality as well as to health-care costs (Souza & Santos, 2009). It becomes a serious public health issue that has caused adverse impact on the populations' health around the world due to their widespread nature, especially to the children. Most food borne disease cases in Malaysia were caused by consuming food contaminated with microbiological hazards (Saad *et al.*, 2013) such as infectious viruses, bacteria and parasites (Tan *et al.*, 2013). Upset stomach, vomiting, abdominal cramps, diarrhoea, fever and dehydration are symptoms of food poisoning which occur within a short period of time after consuming contaminated foods (Saad *et al.*, 2013).

The school canteen is a common place for students having their meal and snack during schooling session. Food safety is essential and must meet the nutritional needs for childrens' healthy life. Therefore, all canteens have a common responsibility to provide safe food. Safe food means it is prepared, cooked, transported and served in such a way as to retain nutrients, and to minimize bacterial contamination and growth (NSW Department of Community Services, 2012). However, numerous food borne disease outbreaks in schools have been reported worldwide (Soares *et al.*, 2012). The Ministry of Health Malaysia (MOH, 2012) reported in 2008, the primary

schools were accounted for the majority of food poisoning episodes and Kelantan was listed at the second place from six states with the most cases of food poisoning. The ministry had inspected the hygiene status of the school canteen.

Consequently, 190 and 112 school canteens were ordered to close in 2011 and 2012, respectively under Section 11 of the Food Act 1983 due to poor hygiene condition (New Straits Times, 2013). On the other hand, from January until May 2013, 85 schools were ordered to close (Mohd Othman, 2013). These numbers show that the school children have been the foremost victim in food poisoning cases in Malaysia. Based on Figure 1.1, primary schools accounted for the majority of food poisoning episodes in 2008. Of the 420 food poisoning episodes in 2008, 263 episodes (62.6%) occurred in schools and 229 (88.1%) of these schools were under the administration of the Ministry of Education (MOH, 2012).

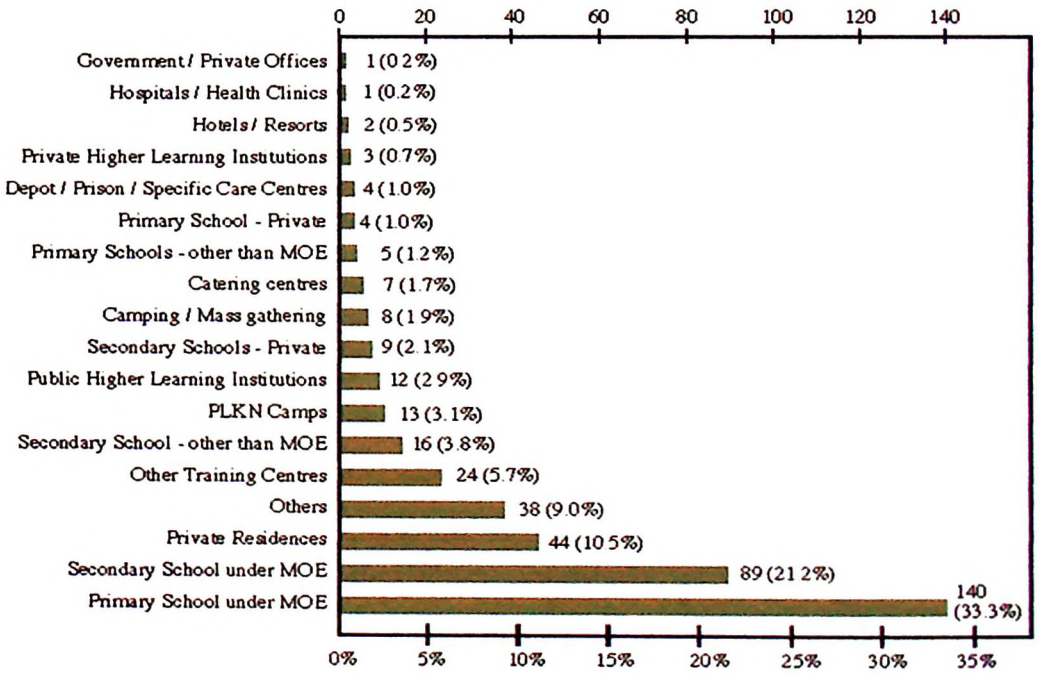

Figure 1.1 Food poisoning by place of occurrences (MOH, 2012).

According to Food Hygiene Regulation 2009 (FSQCD, 2013), a food handler is defined as "any person involved in food business who prepares, serves, touches, packages the food including drinks and handles kitchen and dining equipment, utensils, and plates". With regards to food handlers, there are three factors that play major role in the occurrence of food poisoning: knowledge, attitude, and practice (Soares *et al.*, 2012). Most outbreak and infectious diseases carried by foods were due to incorrect manipulation which associated with improper handling, preparing and storing of food, inadequate washing of hands and fingernails, poor personal hygiene habits and by transmission of microorganisms from the food handler to the food (Souza and Santos, 2009). Poor hygiene practices can act as a source of contamination which contributes to food spoilage and the spread of diseases and infections such as food poisoning (Saad *et al.*, 2013). Food handlers may become vehicles for microorganisms through their hands, cuts or sores, mouth, skin and hair when they do not practice proper personal hygiene or correct food preparation practices (Campos *et al.*, 2009).

Food handlers have the potency to transmit the pathogens to food from contaminated surface of another food, or from hands contaminated with organisms from their gastrointestinal tract (Souza and Santos, 2009). Food handlers with poor personal hygiene could be potential sources of infections from intestinal helminths, protozoa, and enteropathogenic bacteria (Andargie *et al.*, 2008). In hygiene practices, the associated indicator organisms include aerobic plate counts, total coliforms, *Escherichia coli* and *Staphylococcus aureus*, and members of the family *Enterobacteriaceae* (Lues and Van Tonder, 2007). Various bacteria associated with food borne illness such as *S. aureus*, *E. coli* and *Salmonella* spp. have the ability to survive on hands and surfaces for hours or even days after initial contact with the

microorganisms (Lues and Van Tonder, 2007). Therefore, the retention of bacteria on food contact surfaces increases the risk of cross contamination of these microorganisms to food.

1.2 Rationale of Study

Food poisoning is one of major health problems both in developed and developing countries (Aziz and Dahan, 2013). Among the state in Malaysia, Kelantan had the second most common cases of food poisoning (MOH, 2012). Despite many food handlers receive food hygiene training, many food poisoning outbreaks still occur. In addition, the spread of food borne diseases via food handlers are common and become a persistent problem worldwide (Dagnew *et al.*, 2012). Food handlers with poor personal hygiene can be the potential sources of infection of pathogenic organisms (Andargie *et al.*, 2008).

In fact, priority should be given to school canteen as there is an increase in the number of food borne outbreak episodes in schools and institutions over the years (Zain and Naing, 2002) and about 65.9% of food poisoning cases occur in primary and secondary schools (Jeyaletchumi *et al.*, 2006). Based on the report by MOH (2012), primary schools were accounted for most of the food poisoning episodes in 2008. Of the 420 food poisoning episodes in 2008, 62.6% cases occurred in schools and 88.1% of these schools were under the administration of the Ministry of Education.

School children become the foremost victim in many food poisoning cases in Malaysia. Meals prepared and distributed in schools should receive special attention

because the foods are intended for young children, a population with an increased risk for several diseases due to microbial pathogens in foods. Moreover, it is a concern as it affects vulnerable pupils which have more risk of getting sick because of weaker immune systems (Aziz and Dahan, 2013).

Therefore, it is important to study the microbial presence on the surfaces of apron and hands of food handlers in school canteen. Although there are previous reports in determining the food handlers' hand hygiene, however there was a few reported data on food handlers' apron hygiene. Therefore, the outcomes of this study are believed to give benefit, particularly to food handlers, schools and students in order to promote public health awareness by providing safe and healthy food and reduce the food poisoning cases.

1.3 Objectives of Study

The aim of this study was to investigate the microbiological quality of food handlers' hands and apron surfaces.

Specific objectives:

1. To determine the aerobic plate count (APC), total coliforms count (TCC), *E. coli* and *S. aureus* counts on the surfaces of apron and hands of food handlers.
2. To determine the association between hygiene status of the food handlers' hands and the microbiological quality of their aprons.

1.4 Scope of Study

Food handlers in many setting have been responsible for food poisoning and reports have identified their hands as the source of pathogens in the implicated food. This study aimed to screen for the presence of microorganisms and bacterial pathogens in the selected surfaces of samples on food handlers at primary school canteens in Kota Bharu. Therefore, a quick review was done before sampling to determine the types of samples from the canteen which highly reflected on the effectiveness of cleaning practices by food handlers of primary school canteen such as their hands and apron.

For each school, a few food handlers were randomly selected to collect samples from their hands and aprons. The spread plate technique was used to facilitate the enumeration of microbiological parameters. These samples were analyzed using standard plate count which includes several microbiological parameters such as APC, TCC, *E. coli*, and *S. aureus* counts. The microbiological methods used to screen and identify bacteria such as *E. coli* and *S. aureus* were based on the method recommended by Makmal Keselamatan dan Kualiti Makanan (MKKM).

APC is used as an indicator of bacterial populations and defined as the total number of bacteria which are able to grow in an oxygenated or aerobic environment (Morton, 2001). APC is a microbial method that uses colony formation of culture medium to approximate the levels of heterotrophic flora as a procedure for estimating the number of live heterotrophic bacteria (requiring organic compounds of

carbon and nitrogen for nourishment) (Jahed Khaniki *et al.*, 2010). Generally, it can give an estimate of the total number of recoverable bacteria present on the surfaces.

However, screening and detection of coliforms is used as an indicator of sanitary quality of water or as a general indicator of sanitary condition in the food processing environment (Feng e*t al.*, 2002). Particularly, coliforms and *E. coli* are traditional hygiene indicators of faecal contamination in water and other environmental samples (Saad *et al.*, 2013). Faecal coliforms or thermotolerant coliforms are a subset of total coliforms bacteria. However, only faecal origin will grow at higher incubation temperature of 44.5°C and is considered more likely to have come from the intestinal tract of a warm-blooded animal and human (Feng *et al.*, 2002).

There can also be possible contamination by food borne pathogens such as *S. aureus* and *E. coli*. *S. aureus* is an important cause of food intoxication throughout the world. This bacterium can contaminate several foods, including minimally processed ready to eat foods and processed products and produce several types of enterotoxins. Besides, *E. coli*, as enteric pathogens, is becoming increasingly important from the view point of public health, particularly strain *E. coli* O157:H7 which can grow minimally at 4–12°C causing hemorrhagic colitis (El-Hadedy and Abu El-Nour, 2012).

Therefore, the food handlers' practices was verified by environmental sampling based on a few guidelines published for an acceptable and unacceptable level of microbiological contamination (Little and Sagoo, 2009). The microbial counts obtained were interpreted by using the guidelines from previous studies such as from Willis *et al.*, (2012), Little and Sagoo, (2009) and Legnani *et al.*, (2004).

Outcomes for the microbiological analysis were reported as either satisfactory or unsatisfactory for each indicator used. Satisfactory hygienic standard has become one of the essential conditions for promoting and preserving health (Campos *et al.*, 2009). The research conceptual framework is shown in Figure 1.2.

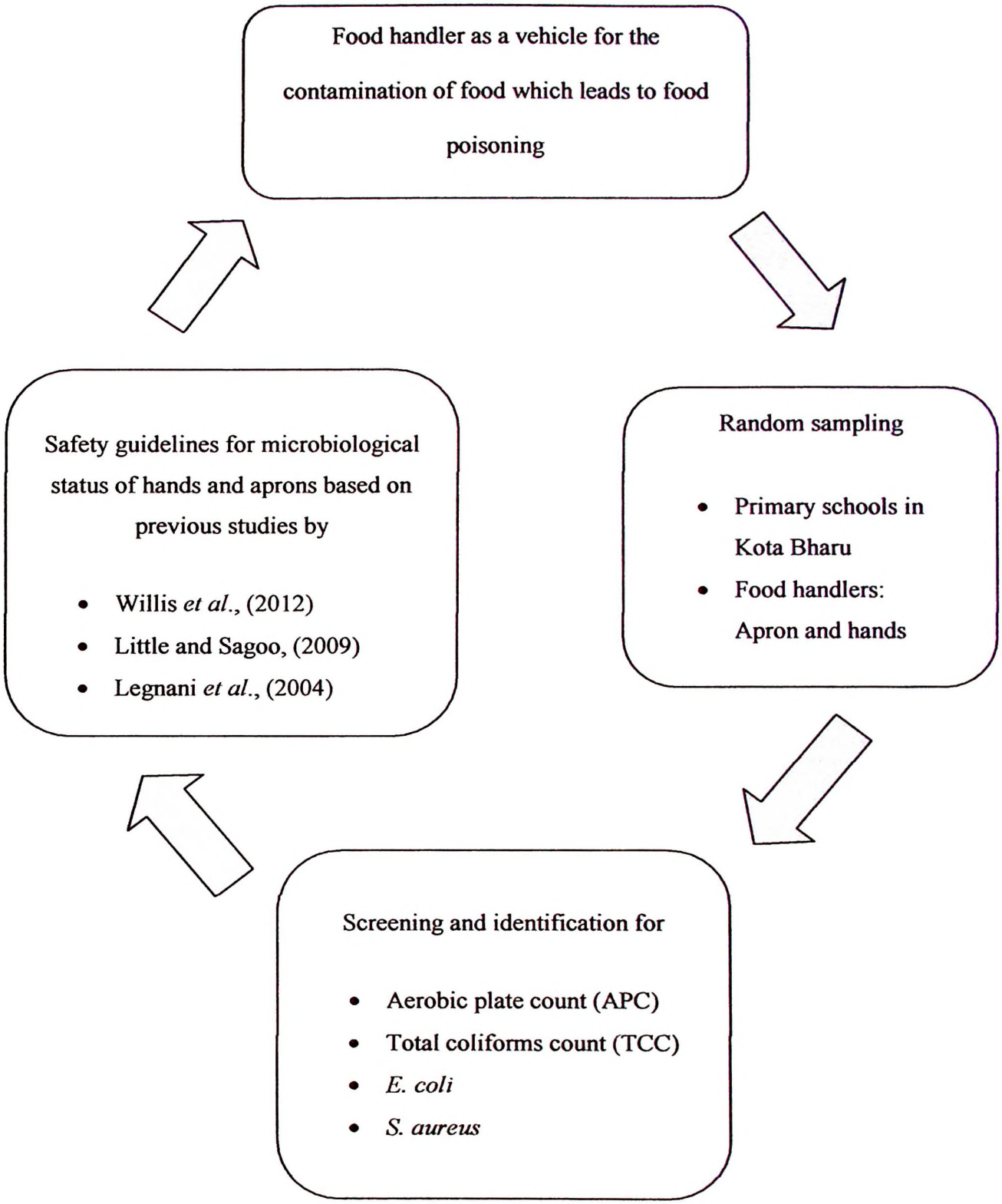

Figure 1.2 Research conceptual frameworks.

CHAPTER 2: LITERATURE REVIEW

2.1 Food Poisoning

Food is an important necessity for life, but diseases spread through foods are common and is a persistent problem which results in morbidity and occasionally mortality (Zain and Naing, 2002). Food borne diseases have been increasing in recent years, with greater impact on the health and economy of developing countries. The presentation can be mild to very serious, in particular for vulnerable groups. Food poisoning is caused mainly by the oral ingestion of viable microorganisms (infection) and/or of the toxins produced (intoxication) in sufficient amounts to develop pathology (Campos *et al.*, 2009).

Based on Robert *et al.*, (2008) report, the top five risk factors of food poisoning in food service operations include improper holding temperature, inadequate cooking, contaminated equipment, purchase and receipt of food from unsafe sources, and poor personal hygiene of food handler. Saad *et al.*, (2013) also reported that food poisoning has been implicated with poor hygiene practices and the microbial contamination of enteric pathogens which are transmitted through food, water, air, contaminated utensils and food contact surfaces.

Nowadays, the issue of food poisoning is very common and gradually increase in Malaysia (Jeyaletchumi *et al.*, 2006). In Malaysia, food poisoning has been recorded as the highest incidence rate of food and water borne diseases category (MOH, 2011). Ministry of Healths' statistics also showed that there was a three folds increased of food poisoning incidences reported within 10 years from 1993 to 2004 (Zaid & Jamal, 2011). Furthermore, of the 420 food poisoning episodes reported in

2008, the top six states with the highest episodes were, Sabah with 57 episodes (13.6%), followed by Kelantan with 49 episodes (11.7%), Johor with 46 episodes (11.0%), Perak 36 episodes (8.6%), Selangor 36 episodes (8.6%) and Pahang 32 episodes (7.6%) (MOH, 2012).

2.2 Food Poisoning in Schools

There are many cases of food borne diseases in schools reported worldwide (Soares *et al.*, 2012). The school age group is more affected by the disease than the general population. It is because students are captive customers who are usually incompetent to purchase food from external sources during school session and because of their weaker immune system (Aziz and Dahan, 2013). In the United States, numerous food borne diseases outbreaks in schools have been reported which resulted in 49 963 illnesses, 1514 hospitalizations and one death (Daniels *et al.*, 2002).

Furthermore, based on the report by MOH (2012), primary schools were accounted for the most food poisoning episodes in 2008. Of the 420 food poisoning episodes in 2008, 62.6% of cases occurred in schools and 88.1% of these schools were under the administration of the Ministry of Education (MOH, 2012). These numbers established that school children have been the foremost victim in many food poisoning cases in Malaysia.

2.3 Food Handler

Most of the food poisoning outbreaks resulted from faulty food handling practices. Food handlers are defined as individuals who have direct involvement in food handling processes, from the preparation to the ready-to-sell food (Azniza Ishak *et al.*, 2013). Occurrence of food poisoning can be attributed by many factors, and one of them is the handling process of food by food handlers (Lee *et al.*, 2012). Approximately, 10% to 20% of the food borne disease outbreaks were caused by contamination by the food handlers due to their personal hygiene and environmental sanitation (Zain and Naing, 2002; Saad *et al.*, 2013).

The lack of knowledge, attitudes, and practices towards food hygiene has been associated with the incidence of food poisoning (Ghazali *et al.*, 2012). In 2009, Little and Sagoo reported that those involved in food preparation and service, have a vital role in food borne disease prevention and their actions can be critical in preventing an outbreak of infection. Thus, a major concern for public health officers and inspectors in preventing food borne illness is the hygienic aspect of food handler.

Hands are the most convenient agent for spreading the bacteria to food and kitchen utensils because normally, hands will collect dirt and dust, including microorganisms (bacteria, viruses, and fungi). Hands can be a vector of dissemination of pathogens through cross contamination (Lee *et al.*, 2012). Food can become contaminated via dirty hands if there is a lack of proper hand hygiene among the food handlers as hands had been indicated as a potential vehicle for transferring food poisoning bacteria (Tan *et al.*, 2013). Lues and Van Tonder (2007) reported that, the food poisoning due to contact with hands or surfaces depends on both the level of contamination as well as the probability of transfer.

Besides poor hand and surface hygiene, lack of personal hygiene among food handlers was also one of the most commonly reported practices that can lead to food poisoning. Thus, food handlers who took serious note on the cleanliness of their hands, body, and clothing have helped in preventing incidence of cross contamination from occurring (Nee and Sani, 2011). Study by Pragle *et al.* (2007), found that the food workers' bad habits such as wiping nose and rubbing hands on apron were difficult to break. Furthermore, based on Roberts *et al.* (2008) report, there are many food handlers used their aprons to wipe and drying their wet hands which can cause the build up of food particles and bacteria on aprons.

In Malaysia, the training program of food handler was implemented starting in 1996 which was conducted by private training institutions accredited by the Food Safety and Quality Control Division (FSQCD) of the Ministry of Health (Zain & Naing, 2002). But, the effectiveness of such training in reducing the incidence of food borne disease is doubtful. Lee *et al.* (2012) stated that even though the number of food handlers which received the food hygiene education is high, but they still have a poor food preparation practices which can contribute to the occurrence of high food poisoning cases.

In 2008, a grading system also had been implemented. "Program Penggredan Premis Makanan" (PPPM) was introduced to motivate food handlers to achieve a better grade for food premises nationwide to ensure the cleanliness and food quality, to judge on their operating principles, quality and cleanliness of food, utensils as well as premises (Yuen Hui, 2008). The food premises were categorised into A, B, and C. Grade A for premises with high cleanliness, Grade B for moderate cleanliness, and Grade C is for low level of cleanliness (Ghazali *et al.*, 2012).

2.4 Microbial Contamination

E. coli, Salmonella, Shigella and *Staphylococcus* are the common infectious bacteria which cause food poisoning as these bacteria will release toxin that cause gastroenteritis (Tan *et al.*, 2013). Findings from Zaid and Jamal (2011) showed that APC was the major cause of microbiological contamination found on food samples followed by total coliforms, *S. aureus, E. coli, Salmonella* spp. and *Bacillus cereus.* Coliforms are microorganisms of the family *Enterobacteriaceae* that ferment lactose and produce gas at 37°C within 48 hours. However, the faecal coliforms subgroup is able to ferment lactose and producing gas at 44.5°C within 24 hours. The hygiene situation of the food handlers was further challenged by the isolation of several species of bacteria isolated from their hands and aprons (Table 2.1).

But it must be remembered that, there are two microbial populations recovered from hands that could be divided into resident flora and transient flora (contaminant) (Pittet, 2001). The resident flora is associated with the deeper layers of the skin such as the sebaceous glands and these organisms are inaccessible to hand hygiene preparations (WHO, 2009). The resident flora consists mainly of coagulase negative staphylococci, *Corynebacterium* spp. and anaerobes such as *Propionibacterium* spp., which rarely cause infections (Jumaa, 2005).

On the other hand, the transient flora colonise the superficial layers of the skin and are less adherent which are more easily removed by hand washing and may be transferred by direct hand contact between human skin and the inanimate environment such as work surfaces or food (Jumaa, 2005; WHO, 2009). The transient flora on hand include any bacteria that are deposited on the skin, such as *S.*

aureus, enterococci, Gram-negative bacilli such as *E. coli*, *Pseudomonas* spp., *Klebsiella* spp., and *Acinetobacter* spp. and which may be carried on the hands of food handlers (Jumaa, 2005).

Table 2.1 Type of bacteria isolated from apron and hands on food handlers.

Bacteria isolated	**Prevalence**	**References**
Hands of food handlers		
Total plate count	98%	Lues and Van Tonder, 2007
Coliforms	40%	Lues and Van Tonder, 2007
Faecal coliforms	55.6%	Campos *et al.*, 2009
E. coli	2%	Lues and Van Tonder, 2007
S. aureus	88%	Lues and Van Tonder, 2007
Enterobacteriaceae	44%	Lues and Van Tonder, 2007
Aprons of food handlers		
Total plate count	84%	Lues and Van Tonder, 2007
Coliforms	26%	Lues and Van Tonder, 2007
Enterobacteriaceae	16%	Lues and Van Tonder, 2007
E. coli	Not detected	Lues and Van Tonder, 2007
S. aureus	48%	Lues and Van Tonder, 2007

E. coli is becoming increasingly important from the view point of public health, particularly psychrotrophic strains *E. coli* O157:H7 (El-Hadedy and Abu El-Nour, 2012). *E. coli* is a bacteria found in the intestinal tract of humans and considered pathogenic to man (Campos *et al.*, 2009). *E. coli* also is a major species

of coliform in the faecal group and served as an indicator of faecal pollution (Jahed Khaniki *et al.*, 2010). The limit for *E. coli* on surfaces is 1cfu/cm^2 (Legnani *et al.*, 2004; Little and Sagoo, 2009; Lues and Van Tonder, 2007) because; *E. coli* is an acknowledged indicator for faecal contamination as well as for the possible presence of enteric pathogens (Lues and Van Tonder, 2007).

In many countries, *S. aureus* is considered as the second or third most common pathogen responsible for outbreaks of food poisoning (Veras *et al.*, 2008; Soares *et al.*, 2012) because of its capacity to produce enterotoxins and antimicrobial resistance (Soares *et al.*, 2012). *S. aureus* associated with food borne intoxication through production of enterotoxins (A, B, C_1, C_2, C_3, D, E, G, H, and I) can cause diarrhoea, vomiting, and dehydration (Souza and Santos, 2009). Based on a review by Le Loir *et al.* (2003), *S. aureus* contamination remains a major cause of food poisoning because it can contaminate food products during preparation and processing. *S. aureus* can be found in the nostrils, on the skin and hair of human and about 30% to 50% of the human population are carriers on their skin or in their mouth or nose (Milliorn, 2009).

CHAPTER 3: METHODOLOGY

This section of the dissertation describes the list of materials and instruments used in the study as well as the sampling method and the microbiological analyses. The work flow is summarized in Figure 3.4 at the end of the section.

3.1 Laboratory Requirements

Below is the list of laboratory apparatus, instruments, materials, microorganism that are required in this study.

Table 3.1 Equipments and instruments used.

No.	Items
1	10 cm x 10 cm plastic template
2	Autoclave machine
3	Beaker
4	Biological Safety Cabinet (BSC) Level II
5	Bunsen burner
6	Cool box
7	Electronic weighing balance
8	Forceps
9	Graph paper
10	Hockey stick
11	Incubator (Temperatures: 32°C, 37°C, and 44.5°C)

12	Marking pen
13	Pencil
14	Pipette
15	Scott Duran bottle
16	Thermometer
17	Universal bottle
18	Vortex
19	Wire loop

Table 3.2 Materials used.

No.	Material
1	70% alcohol swab
2	Baird Parker agar powder (Oxoid, Thermo Scientific)
3	Biohazard bag
4	Dropper
5	Eosin methylene blue agar powder (Oxoid, Thermo Scientific)
6	Glass slide
7	Gloves
8	Ice cube
9	Kovacs' reagent
10	MacConkey agar powder (Oxoid, Thermo Scientific)
11	Normal saline
12	Nutrient agar powder (Oxoid, Thermo Scientific)

13	Peptone water powder (Oxoid, Thermo Scientific)
14	Petri dish
15	Pipette tips (blue and yellow)
16	Plasma
17	Sterile cotton swabs
18	Sterile distilled water
19	Sterile toothpick
20	Tryptone water powder (Oxoid, Thermo Scientific)

Table 3.3 Microorganisms used.

No.	Microorganism
1	*E. coli* ATCC 25922
2	*Klebsiella pneumoniae* ATCC 33495
3	*S. aureus* ATCC 25923
4	*Staphylococcus epidermidis* ATCC 12228

3.2 Preparation of Culture Media

The preparation of the media used in this study is described below:

3.2.1 Preparation of 0.1% Peptone Water, Oxoid CM0009

Peptone water is used as an organic source of nitrogen, in the form that is readily available for bacterial growth and in microbiological culture media for cultivation of a wide variety of bacteria.

Procedure:

1 g of powder was dissolved in 1 litre of distilled water. The medium was mixed and distributed into final containers. Then, it was sterilized by autoclaving at 121°C for 15 minutes. Then, sterile solutions added after autoclaved; volume of water was reduced for reconstitution by an equal amount.

3.2.2 Preparation of Nutrient Agar, Oxoid CM0003

Nutrient agar is a general purpose and a basic culture medium used to subculture organisms for maintenance purposes or to check the purity of subcultures from isolation plates prior to biochemical or serological tests.

Procedure:

28 g of powder was dissolved in 1 litre of distilled water. The medium was mixed and distributed into final containers. It was sterilized by autoclaving at 121°C for 15 minutes. Then, sterile solutions added after autoclaved; volume of water was reduced for reconstitution by an equal amount.

3.2.3 Preparation of MacConkey Agar, Oxoid CM0115

MacConkey is a selective medium which giving excellent differentiation between coliforms and non-lactose fermenters with inhibition of Gram positive organisms.

Procedure:

51.5 g of powder was dissolved in 1 litre of distilled water. The medium was mixed and distributed into final containers. It was sterilized by autoclaving at 121°C for 15 minutes. Then, sterile solutions were added after autoclaved; volume of water was reduced for reconstitution by an equal amount.

3.2.4 Preparation of Eosin Methylene Blue Agar, Oxoid CM0069

Eosin methylene blue agar is selective for Gram negative bacteria against Gram positive bacteria. It is useful in isolation and differentiation of the various Gram negative bacilli and enteric bacilli, generally known as coliforms and faecal coliforms respectively.

Procedure:

37.5 g of powder was dissolved in 1 litre of distilled water. The medium was brought to boil to dissolve completely and distributed into final containers. It was sterilized by autoclaving at 121°C for 15 minutes. The medium was cooled at to 60°C and shaken in order to oxidize the methylene blue (restore its blue colour) and to suspend the precipitate which is an essential part of the medium.

3.2.5 Preparation of Baird Parker Agar, Oxoid CM0275

Baird Parker agar is a selective and diagnostic medium for the isolation and enumeration of *S. aureus* in foods. Contain sodium pyruvate, to protect damaged cells and aid their recovery and egg yolk emulsion as a diagnostic agent.

Procedure:

58 g of powder was suspended in 0.95 litre of distilled water by heating in a boiling water bath or in a current of steam; autoclaved (15 min, at 121°C); at 50 to 45°C and under sterile condition. 50 ml of egg yolk tellurite emulsion was added and if required 50 ml/l of sulphamethazine. Before poured into sterile Petri dishes, make sure it was mixed well.

3.2.6 Preparation of Tryptone Water, Oxoid CM0087

Tryptone water is a good substrate for the production of indole by microorganisms because of its high content of tryptophan. The ability of certain organisms to break down the amino acid tryptophan with formation of indole is an important property which is used for the classification and identification of bacteria.

Procedure:

15 g of powder was suspended in 1 litre of distilled water. The medium was mixed and distributed into final containers. It was sterilized by autoclaving at 121°C for 15 minutes.

3.3 Study Design

This was a cross sectional study on the microbiological quality assessment of food handlers' hands and aprons involving 45 randomly selected food handlers from nine primary schools in the vicinity of Kota Bharu.

3.4 Samples Collection

A total of 135 samples of right hand, left hand, and apron were collected during November 2013 through January 2014 at nine primary schools in the Kota Bharu. For each school, five food handlers were randomly selected. In this study, sampling was performed by swabbing the surfaces of food handlers' right hand, left hand and apron used. Related data such as food premises inspection and grading by the respective authorities was also recorded.

3.5 Sampling Technique

Swab procedures were performed on apron and hands in accordance with the guidelines from Kelantan Health Department which were adopted by MKKM, Peringat, Kota Bharu. In this study, the swab technique was done by using a sterile collection swab for each sample to obtain microorganisms from the sample surfaces. The procedures were done aseptically to minimize the risk of contamination. Swab containers or universal bottles were labelled clearly with the sample code numbers represent the school and the type of sample.

The sterile collection swab was removed from the pouch and moistened in a universal bottle containing sterile distilled water. The swabs were carefully moistened with distilled water allowing better collection of microorganisms from dry sample surface. Sampling was performed by swabbing the surfaces of each of the right hand and the left hand of the food handlers by rotating the swab between the thumb and forefinger by moving the swab back and forth across the surface with horizontal, vertical, and diagonal strokes (Tan *et al.,* 2013) covering the whole palm. The palm was illustrated on a graph paper in order to measure the sampling area. This was done after the sampling to avoid hand contamination.

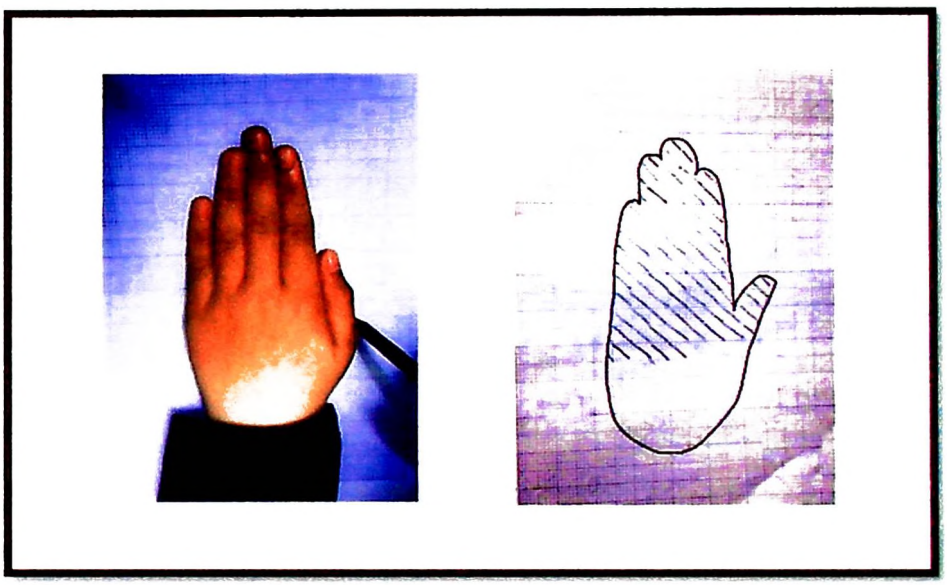

Figure 3.1 The swab area on hand was illustrated and measured on a graph paper.

For apron sampling, the area to be swabbed was identified. The area which usually exposed to contamination and considered as critical contact surface was selected for swabbing area as shown in Figure 3.2. A known area on an apron was swabbed by using a sterile template. For apron surfaces, templates help define the sample size and allow the determination of the number of organisms per cm^2. A 100