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Microbiological Quality of Food Contact Surfaces (Spoons) at Selected Restaurants in Klang Valley, Malaysia

(Kualiti Mikrobiologi Permukaan Sentuh Makanan (Sudu) di Restoran Perkhidmatan Makanan Terpilih di Lembah Klang, Malaysia)

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ABSTRACT

Food borne diseases increase worldwide and contamination of food contact surfaces serves as one of the reasons for their occurrence. The aim of this study was to determine the microbiological quality of spoons at selected restaurants in Klang Valley, Malaysia. Five restaurants were selected therein for the study. They were respectively labelled A, B, C, D and E. A total of 150 cleaned spoons (30 spoons from each restaurants) that were ready to be used by customers at the restaurants were examined. Total plate counts (CFU/cm^2) of the spoons were determined; the presumptive and confirmatory tests for the presence of Escherichia coli *on the spoons were also conducted. The samples were collected by surface swabbing. The result showed that restaurant C and B had the highest and lowest total plate counts (TPC), respectively. Samples from 3 of the 5 selected restaurants (restaurants C, D and E) showed positive results for the presence of* E. coli *mainly due to poor dishware cleansing. On the contrary, negative results for the presence of* E. coli *at restaurant A and B were associated with the advance cleaning procedure, which used more hygienic method with dishwashers.*

Keywords: E. coli*; food safety; food service; total plate count*

ABSTRAK

Keracunan makanan semakin meningkat di seluruh dunia dan pencemaran pada permukaan sentuh makanan adalah salah satu sebab kejadian ini berlaku. Tujuan kajian ini adalah untuk menentukan kualiti mikrobiologi sudu yang digunakan di restoran perkhidmatan makanan di Lembah Klang, Malaysia. Lima restoran telah terpilih untuk kajian ini. Setiap restoran dilabel sebagai restoran A, B, C, D dan E. Sejumlah 150 sudu yang bersih (30 sudu daripada setiap restoran) dipilih sebelum digunakan oleh para pelanggan restoran tersebut. Jumlah hitungan plat mikrob (CFU/cm2) sudu telah ditentukan dengan menggunakan ujian andaian dan ujian pengesahan kehadiran Escherichia coli *pada sudu tersebut. Sampel telah dikumpulkan menggunakan penyapuan permukaan. Jumlah kiraan mikrob plat sudu menunjukkan bahawa restoran C dan B mempunyai hasil tertinggi dan terendah. Daripada 5 restoran tersebut, 3 restoran iaitu C, D dan E telah menunjukkan keputusan yang positif bagi kehadiran* E. coli *disebabkan oleh tahap pencucian yang rendah. Manakala restoran A dan B menunjukkan keputusan negatif bagi kehadiran* E. coli, *disebabkan oleh tahap pencucian yang lebih bagus, dengan penggunaan mesin basuh pinggan mangkuk yang lebih bersih.*

Kata kunci: E. coli*; jumlah hitungan plat; keselamatan makanan; perkhidmatan makanan*

INTRODUCTION

Foodborne diseases remain a global challenge with higher rates of incidence in the developing nations. In 2010, the World Health Organization's Foodborne Disease Burden Epidemiology Reference Group had an estimate of 582 million cases of foodborne infections with 351,000 deaths globally (WHO 2015). It has also been estimated by the WHO that annually, about 2.2 million deaths occurred as a result of foodborne and waterborne diseases combined. Little has been known about the global economic burden due to foodborne disease, however, United Kingdom alone has suffered £1.8 billion out of the treatment cost (Food Standards Agency 2013).

Some of the main causes of food borne infections are inadequate cooking and improper food storage as well as poor hygienic conditions. Food contact surfaces may serve as a potential health hazard if not properly cleaned and sanitized. Moreover, to prevent food borne disease outbreaks, it is necessary that the food hygiene practices be strengthened and the HACCP system should be complied (Lund & O'Brien 2009). Many Malaysians nowadays find it more preferable eating at restaurants than at their respective homes. This is owing to the fact that the people are busy with activities, hence the preference to dine out. Considering this, the cleanliness of restaurants is very important toward alleviating or totally eliminating the incidence of food borne outbreaks. Environmental sanitation of food premises is equally imperative in avoiding the possibility of food borne ailments. Food borne outbreaks traced to a restaurant can certainly create for such a restaurant a bad reputation. Additionally, issues of food safety and hygiene are especially critical for restaurant

managers and owners because customers' perceptions for poor sanitation might lead consumers patronise another restaurant consequently resulting in a low income generation.

An impression that a restaurant does not comply with food safety ethics likely causes people patronising another restaurant when dining out (Knight et al. 2007). In fact, a study found out that cleanliness is the most important determinant for consumers' perceptions of restaurant food safety (Henson et al. 2006). Cleanliness is, however, a relative concept, what is acceptable as being clean in one situation may be unacceptable in another (Moore & Griffith 2002). Consumers are likely to judge the cleanliness and safety of restaurants based on visual perceptions. Although health inspectors use an inspection manual and the food code to inspect food service restaurants, their judgments rely heavily on visual assessment and perceptions, as reported by Lee et al. (2009), which stated that health inspectors did show variations in regards to their opinions of cleanliness of restaurants. Kitchen surface and equipment may have sparkling clean look, they may however have the presence of bacteria in large number (Julie 2007). Since traditional microbiological analysis takes up to 48 h after the sample collection, microbiological assessment of restaurants is generally not done as part of the inspection process. Furthermore, the equipment that provides a real-time microbiological analysis is expensive. However, this has become an issue, as bacterial and viral contaminations are not detectable by visual assessment (Choi et al. 2011). In fact, the results of using hygiene swabs and agar contact plates have shown that visual inspection is a poor indicator of cleaning and hygiene of the restaurants as well as the safety of foods (Griffith et al. 2000; Moore & Griffith 2002). In addition, consistent cleaning of certain food contact surfaces outside the kitchen may not be done in all restaurants (Choi et al. 2011). There are indications that foods could be contaminated to unsafe levels at the points of consumption with air flora and other microorganisms from handlers, equipment, utensils and raw food materials, though epidemiological evidences on outbreak of food borne disease are scarce (Edema et al. 2008). To ensure acceptable levels of contamination and avoid adverse human health consequences of food borne illnesses, effective hygiene control through bacteriological testing is vital (Moyo & Baudi 2004).

The presence of *E. coli* is considered as an indicator of faecal contamination, which in addition, might contain pathogenic parasites, bacteria or viruses (Sneed 2004). *E. coli* lives in the enteric tract of humans and other warm-blooded animals. Food borne diseases ranging from bloody diarrhoea to haemorrhagic colitis and haemolytic uremic syndrome are caused by *E. coli* strains (Nataro & Kaper 1998). Slight contamination of surfaces may cause serious infections. Presence of *E. coli* has been shown to survive for extended periods on stainless steel surfaces and domestic plastic cutting boards (Wilks et al. 2005).

MATERIALS AND METHODS

SAMPLE COLLECTION

Five restaurants around Klang Valley area were selected and labelled as A, B, C, D and E for this study, from February until November 2012. The selected food contact surfaces were spoons. A total of 150 cleaned spoons (n=150) from five selected restaurants (30 spoons from each restaurants) were examined. The 150 spoons from which 150 samples obtained were cleaned spoons that were ready to be used by customers at the restaurants. The spoons were swabbed for microbial sample collection. Sterilized cotton sticks were used to swab the ellipse shape of the front and back of the spoon area. The sterilized cotton stick was dipped in 10 mL of peptone water (Merck, Germany), prior to swabbing. After the swabbing process, the swabbed cotton sticks were returned to the universal bottles containing the 10 mL peptone water. The samples were immediately labelled and transported to the laboratory for further analysis.

MICROBIOLOGICAL ANALYSIS

The techniques of total plate count (TPC) and *E. coli* detection involving presumptive and confirmatory tests were employed for the microbiological analysis of the samples. Each sample in the 10 mL peptone water was evenly mixed using vortex (VTX-3000L, Tokyo, Japan) for 10-30 s.

TOTAL PLATE COUNT (TPC)

Plate count agar (Merck, Germany) was used for this procedure to enumerate the aerobic mesophilic bacteria in the sample. The amount of bacteria in a sample is a key indicator of the overall microbiological quality and safety. For the total plate count method, ten-fold serial dilution was prepared to a dilution factor of 10^{-3} and 1 mL of each dilution was pour plated against the 15 mL of the PCA (Merck, Germany) in labelled Petri dishes in triplicates. The labelled Petri dishes were incubated at 30ºC for 72 ± h. After the incubation, plates with 30-300 colonies were counted on a standard colony counter (Galaxy 230, Rocker).

PRESUMPTIVE DETECTION AND ENUMERATION OF *E .COLI*

The presumptive test was conducted using Most Probable Number (MPN) technique with a three tubes (3 sets of 3 test tubes) system. In this study, the MPN method was used to estimate the concentration of viable microorganisms in the sample by means of replicate broth growth in ten-fold serial dilutions until dilution factor 10^{-3}. One mL of sample was added to each tube (triplicate) of single strength 9 mL Lauryl Sulfate Tryptose broth (Oxoid, United Kingdom), containing inverted Durham tubes. The suspensions were then incubated at 37ºC and the presence of gas was observed after 24 h or 48 h of incubation. The test tubes, within which there is the presence of gas trapped in the

inverted Durham tubes after 24 h of incubation, signified the positive tubes. Further incubation was conducted until 48 h after which if there was no gas production, the tube was considered negative. The positive samples from Lauryl Sulfate Tryptose broth (Oxoid, United Kingdom) were further used for enumeration. About 1 mL suspension of positive dilutions was transferred into 9 mL EC medium (Difco™, USA) in test tube, also containing inverted Durham tubes. The tubes were incubated at 44ºC for 24 h to observe the presence of visible gas. Further incubation until 48 h was done if the gas was not detected after 24 h. The gas positive tubes of EC were then recorded.

CONFIRMATORY TEST FOR THE PRESENCE OF *E. COLI*

Following positive presumptive test, the confirmatory test of *E. coli* was conducted using Eosin Methylene Blue (EMB) agar (Merck, Germany). This was enhanced using streak plate method. A loop full of the suspension from presumptive test positive tube of EC medium was transferred and streaked onto the EMB agar plate and incubated. The production of dark-centred colonies with a greenish metallic sheen confirmed the presence of *E. coli*.

DATA ANALYSIS

The results of total plate count were tabulated and analysed using Microsoft Excel (Microsoft Corp, Redmond, WA). The MPN values for the detection and enumeration of presumptive *E. coli* were generated from the MPN table of three tubes system. Moreover, the results for the *E .coli* confirmatory tests were also recorded and tabulated.

RESULTS

TOTAL PLATE COUNT (CFU/CM²)

The total plate count (TPC) data was manually recorded in an excel spreadsheet (Microsoft Corp, Redmond, WA) and the raw data was converted into CFU/cm². The total plate count was used to indicate the bacterial population on a plate. The detection of coliforms is widely used as a means of measuring the effectiveness of sanitation programs. The comparison of total plate count for spoons at five different restaurants (Figure 1) shows that restaurant C had the highest number (4.03±0.94 log CFU/cm²) followed by restaurant D (3.14±1.71 log CFU/cm²), A (2.83±1.59 log CFU/cm²), E (2.30±0.74 log CFU/cm²) and lastly restaurant B (0.68±0.47 log CFU/cm²) (Figure 1).

CONFIRMATORY TEST FOR *E. COLI*

Both restaurants A and B were negative for the confirmatory test of *E. coli,* while restaurants C, D and E showed positive results in more than 30% of their spoon samples affected; with restaurant C had the highest number of affected samples (46.67%) followed by restaurant D (43.33%) and lastly restaurant E (33.33%) (Figure 2).

DISCUSSION

It could be deduced based on the results of this study that there exists a correlation between TPC and the presence of *E. coli* in all the restaurants with regard to the sanitary consideration of the spoons under analysis. In this study, the evaluation for microbiological quality of each restaurant using TPC and *E. coli* detection analysis showed that Restaurant B had the lowest value for TPC and was also negative for the presence of *E. coli*. This is similar to the work of Nik Rosmawati et al. (2014) in which microbiological analysis of food handlers' hands, chopping board, apron and kitchen utensils (spoons inclusive) was conducted. However, restaurant C recorded the highest value for TPC as well as for the presence of *E. coli*.

The presence of *E. coli* considers as an indicator for faecal contamination. This may consequently results in the presence of other indicator organisms, other bacteria and viruses (Lues & Tonder 2007; Sneed 2004). Some *E. coli* strains cause food borne diseases ranging from bloody diarrhoea to haemorrhagic colitis and haemolytic uremic syndrome (Nataro & Kaper 1998). Based on the results, the presence of *E. coli* in restaurants C, D and E indicates that customers patronising these restaurants are liable to be infected with *E. coli* and food borne diseases. There is no real treatment for *E. coli* infections, other than monitoring

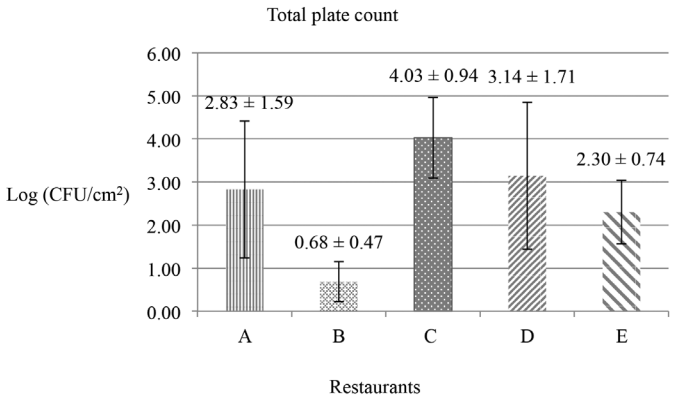

FIGURE 1. The total plate count for the five different restaurants

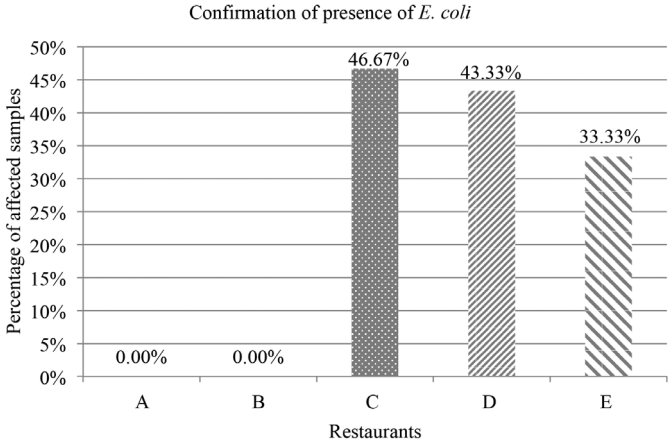

FIGURE 2. Percentage positive for spoons with *E. coli* for the five restaurants

the illness, providing comfort and preventing dehydration through proper hydration and nutrition (Health Canada 2012).

From the observation, it is interesting to note that, restaurants using dishwashers, such as restaurant A and restaurant B did not have any presence of *E. coli*; analogous to the work of Sahai et al. (2015) who certainly did not find microbial existence in the course of assessing utensils cleaned by domestic dishwashers in Ontario, Canada. While restaurants C, D and E which used manual dish washing method with normal temperature of the tap water had shown the presence of *E. coli*.

E. coli dies at temperature of 70ºC (Health Canada 2012). Therefore, dishwashers such as the one used in restaurant A and B killed *E. coli* on spoons as they warm up the cutleries to the temperature of 80ºC. Commercial dish washers use an internal heater to heat water to 80ºC in order to kill germs and effectively remove grease from dishes (McGuire 2010).

Restaurants C, D and E were positive for the presence of *E. coli* because these restaurants had used normal temperature of tap water. Furthermore, these restaurants did not follow the standard method of manual dish washing, which requires the use of hot water with three sinks compartment. Based on Maricopa.gov (2011), manual method of washing the dishes should be conducted in five steps. The first is the pre-scrape step by removing and throwing away the leftover food and grease from the dishes. Second is washing the dishes thoroughly with detergent and hot water in the first sink. Third, rinsing the dishes in clean hot water to remove the soap in the second sink (mixing detergent with sanitizer can prevent the disinfectant from killing the bacteria and viruses). Fourth is sanitizing the dishes in warm water in the third sink. The sanitizer shall be prepared in accordance with the manufacturer's specification. The dishes should remain completely submerged in the solution for at least 30 s. Lastly, placing all dishes and utensils on the drain board or rack and let them air-dry. Towels should not be used to dry the dishes as they might contaminate the clean dishes.

A study conducted by Mattick et al. (2002), demonstrated that the commercial kitchen that use hot water, had lower bacterial counts. This implies that if restaurant C has adopted such a system, there wouldn't have been the highest amount for TPC and the presence of *E. coli* as they engaged only in the usage of tap water for washing-up the cutleries and dishes. Running water implies that the flushing action of the water is sufficient to rinse food particles down the drain with volumes of water adequate to reduce concentrations of bacteria to safe levels (Schmidt & Rodrick 2003).

Restaurant C with the highest amount for TPC and the presence of *E.coli* did not have proper dish washing area. The staff usually put detergent and water barrels on the floor near the drain. The detergent and water might be cross contaminated through fluid from the drain or the surrounding air. In addition, cats and pests are allowed to pass around the dish washing area thereby cross contaminating the detergent, water and the washed utensils. According to FSA (2010), the sources of pathogens are people, raw foods, pests, pets, air, dust, dirt and food waste. Indirect contamination can occur, when the sources transfer pathogens on to something which may later come into contact with food (FSA 2010).

In this study, restaurant D has the second highest amount for TPC and the presence of *E. coli* after restaurant C. The reason may be as a result of sharing the same area for food preparation and dishwashing. The cleaned dishes and cutleries might cross contaminate the raw chicken and vice versa while food is being prepared. A study reported by Gorman et al. (2001), has found that 80% of the raw chickens contain one or more intestinal disease microorganisms. These microorganisms were found to cross-contaminate 12% of dishcloths, 24% of persons' hands, 4% refrigerator door handles, 20% oven door handles, 24% counter-tops and 32% draining boards (Gorman et al. 2001).

Restaurant E has the third highest amount of affected samples with *E. coli*. Dish washing area for restaurant E is located at the back of the premise near the drainage system which is an open air area. Cross contamination of the washed utensils could occur with the contaminated air-borne pathogens from the large drain. According to Gorman et al. (2001), pathogenic microorganisms could be introduced via

people, food, pets, insects, contaminated water supplies and the air.

CONCLUSION

Spoons in all restaurants showed the presence of TPC with the highest value at 4.03 log CFU/cm^2 in restaurant C. Spoons at 3 restaurants (C, D and E) showed the presence of *E. coli,* with restaurant C recorded the highest number of affected spoons (46.67%). It can be summarized that Restaurant C had the highest values for both the TPC and percentage of spoons contaminated with *E. coli*. In this study, it shows the use of dishwasher eliminate the *E. coli* on spoons in restaurants A and B.

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