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MOLECULAR EPIDEMIOLOGY OF Staphylococcus aureus AMONG FOOD HANDLERS AND IN COOKED FOOD IN THE KLANG VALLEY, MALAYSIA

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By

SEOW WEN LI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

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MOLECULAR EPIDEMIOLOGY OF *Staphylococcus aureus* AMONG FOOD HANDLERS AND IN COOKED FOOD IN THE KLANG VALLEY, MALAYSIA

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June 2020

Chairman: Noor Azira Abdul Mutalib, PhD
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World Health Organization (WHO) stated that the burden of foodborne disease achieves 600 million cases each year with one of ten people in the world falls ill after consuming contaminated food. In Malaysia along, food poisoning cases were occupied 15,023 cases in 2018. The foodborne illness is associated with poor handling of cooked food by the food handlers rather than cross-contamination from raw food in food premises. This study aimed to (i) determine the prevalence of both *S. aureus* hand carriers and methicillin resistant *S. aureus* (MRSA) strains among food handlers and in cooked food, (ii) identify the correlation between the socio-demographic characteristics profile of the food handlers with the prevalence and multidrug resistant (MDR) *S. aureus* strains among food handlers, and (iii) evaluate the epidemiological relationship among *S. aureus* strains obtained from food handlers' hands and cooked food samples. Two hundreds hand swab samples with 100 cooked food samples were collected from Grade A, B, and C food premises throughout Klang Valley which socio-demographic information of food handlers was recorded. Several analyses were performed to detect the presence of *S. aureus* and MRSA, such as mannitol salt agar test, biochemical tests, and antimicrobial susceptibility test. DNA extraction and polymerase chain reaction (PCR) analysis for phylogenetic sequence analysis were further performed. Statistical analysis was done by using the software IBM SPSS Statistic 22. The results showed that the prevalence of *S. aureus* among food handlers and cooked food were 95.0% and 50.0%, respectively. None of the food handlers inhered MRSA strains with four (8.0%) cooked food samples were detected with MRSA strains. Further Fisher-Freeman Halton analysis revealed that there was no significant correlation between food handlers' socio-demographic profile and the prevalence of *S. aureus*. The occupational information and lifestyle habits of food handlers, however, were correlated to the prevalence of *S. aureus*. The grading system authorized by local authorities ($P = 0.000$), grades of food premises ($P = 0.010$), years of employment ($P = 0.049$) and working responsibility ($P = 0.026$) of food handlers were significantly correlated with the prevalence of *S. aureus*. Nonetheless, no correlation was found between MDR *S. aureus* with the socio-demography profile, occupational information and lifestyle habits of food handlers. On the other hand, PCR molecular

analysis identified that the overall prevalence of *S. aureus* and MRSA among food handlers was 25.0% and 1.0%, respectively. For cooked food samples, the prevalence of *S. aureus* was 9.0%. The further phylogenetic tree sequencing analysis showed that most of the cooked food samples had different similarities with the food handlers' *S. aureus* strains. However, there was epidemiological relationship between food handler (HL11) and cooked food (F11) which the current study assumed that cross contamination from food handler to food was occurred during food preparation. This urges for the improvements in constructive and sustainable food safety practises among food handlers to reduce the risk of foodborne illness outbreaks.

Keywords: multidrug resistant Staphylococcus aureus; methicillin resistant Staphylococcus aureus; food handler; prevalence; molecular epidemiology

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

EPIDEMIOLOGI MOLEKUL *Staphylococcus aureus* DALAM KALANGAN PENGENDALI MAKANAN DAN MAKANAN YANG DIMASAK DI LEMBAH KLANG, MALAYSIA

Oleh

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Pertubuhan Kesihatan Sedunia (WHO) menyatakan bahawa beban penyakit bawaan makanan mencapai 600 juta kes setiap tahun dengan satu daripada sepuluh orang di dunia jatuh sakit setelah termakan makanan tercemar. Di Malaysia, kes-kes keracunan makanan mencapai sebanyak 15,023 kes pada tahun 2018. Penyakit bawaan makanan dikaitkan dengan pengendalian makanan masak oleh pengendali makanan, bukannya daripada pencemaran silang dari makanan mentah di premis makanan. Kajian ini bertujuan untuk mengetahui prevalensi pembawa tangan *S. aureus* dan strain *S. aureus* yang tahan methicillin (MRSA) dalam kalangan pengendali makanan dan makanan yang dimasak; untuk mengenal pasti korelasi antara profil ciri sosio-demografi pengendali makanan dengan strain *S. aureus* dan rintangan multidrug (MDR) *S. aureus* dalam kalangan pengendali makanan; dan untuk menilai hubungan epidemiologi antara strain *S. aureus* yang diperolehi dari tangan pengendali makanan dan sampel makanan masak. Dua ratus sampel swab tangan dengan 100 sampel makanan masak telah dikumpulkan dari premis makanan Gred A, B dan C di Lembah Klang di mana maklumat sosio-demografi pengendali makanan telah direkodkan. Beberapa analisis dilakukan untuk mengesan kehadiran *S. aureus* dan MRSA, seperti ujian *mannitol salt agar*, ujian biokimia, dan ujian kerentanan antimikrob. Analisis reaksi DNA dan reaksi rantai polimerase (PCR) untuk analisis pokok filogenetik telah dijalankan. Analisis statistik dilakukan dengan menggunakan perisian IBM SPSS Statistic 22. Hasil kajian menunjukkan bahawa prevalensi *S. aureus* dalam kalangan pengendali makanan dan makanan masak adalah 95.0% dan 50.0%. Tiada seorang pun daripada pengendali makanan yang mempunyai strain MRSA dengan empat (8.0%) sampel makanan masak dikesan dengan strain MRSA. Analisis Fisher-Freeman Halton selanjutnya mendedahkan bahawa tidak terdapat hubungan yang signifikan antara profil sosio-demografer makanan dan prevalensi *S. aureus*. Walau bagaimanapun, maklumat pekerjaan dan gaya hidup pengendali makanan dikaitkan dengan prevalensi *S. aureus*. Sistem penggredan yang diberi kuasa oleh pihak berkuasa tempatan (P = 0.000), gred premis makanan (P = 0.010), tahun pekerjaan (P = 0.049) dan tanggungjawab kerja (P = 0.026) dikaitkan dengan prevalensi *S. aureus*. Walau bagaimanapun, tiada korelasi

ditemui di antara MDR *S. aureus* dengan profil sosio-demografi, maklumat pekerjaan dan gaya hidup pengendali makanan. Sebaliknya, analisis molekul PCR mengenal pasti bahawa prevalensi keseluruhan *S. aureus* dan MRSA dalam kalangan pengendali makanan adalah 25.0% dan 1.0%. Untuk sampel makanan masak, prevalensi *S. aureus* adalah 9.0%. Analisis penjujukan pokok prevalensi selanjutnya menunjukkan bahawa kebanyakan sampel makanan masak mempunyai persamaan yang berbeza dengan strain *S. aureus* pengendali makanan. Walau bagaimanapun, terdapat hubungan epidemiologi antara pengendali makanan (HL11) dan makanan yang dimasak (F11) yang mengandaikan bahawa pencemaran silang daripada pengendali makanan kepada makanan berlaku semasa penyediaan makanan. Ini menggalakkan penambahbaikan dalam amalan keselamatan makanan yang dapat dipupuk dalam kalangan pengendali makanan untuk mengurangkan risiko wabak penyakit bawaan makanan.

Kata kunci: tahan multidrug Staphylococcus aureus; tahan methicillin Staphylococcus aureus; pengendali makanan; prevalensi; epidemiologi molekul

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LIST OF ABBREVIATIONS

%	Percentage
<	Less than
>	More than
a_w	Water activity
CFU/g	Colony forming unit per gram
℃	Degree Celsius
H_2O_2	Hydrogen peroxide
MSA	Mannitol salt agar
PCA	Plate count agar
APC	Aerobic plate count
MHA	Mueller Hinton agar
PCR	Polymerase Chain Reaction
MDR	Multidrug resistant
TSB	Tryticase soy broth
µg	Microgram
µL	Microliter

CHAPTER 1

INTRODUCTION

1.1 Background

World Health Organization (WHO) stated that the burden of foodborne disease achieves as many as 600 million cases each year with 1 of 10 people in the world falls ill after consuming contaminated food, and the foodborne disease cases in the world were caused by 31 agents, such as microorganism, chemicals, radioactivity or even physical agents (WHO, 2015). Additionally, out of 600 million cases, 420,000 cases cause death with 30% in children who are less than five years old (WHO, 2015). Food premises are contemplated as the most vital platform which responsible for the foodborne illness outbreaks (Mun, 2020).

Nowadays, no doubt, habit of dining out has been a trend for people who want to save time and who work away from home. According to Department of Statistic Malaysia (2017), Malaysian food and beverage industry showed steady growth from year 2010 to year 2015 with growth rate of 5.1%, from 130,570 establishments to 167,490 establishments respectively. Number of individuals involved in food and beverage services achieved 6.7% of annual growth with 891,616 people in 2015 compared to 645,743 people in 2010. The manpower in food and beverage industry recorded a total of 569,632 people which included 63.9% of paid full-time employees, 254,364 people (28.5%) of working proprietor's & unpaid family workers and paid part-time employees with 67,620 people or 7.6% per cent. The value of gross output of industry generated an increase of 11.2 per cent annually posted at RM 28.0 billion in year 2015. Steady growth of established food premises gives rise to the concern of hygiene and sanitation levels of the food handlers and the premises. This results in increasing the risk of foodborne diseases occurrence in Malaysia.

A multiracial country, Malaysia, is a food heaven country which variety of fantastic food are the staple food for the people (Yusoff et al., 2013). Surprisingly, foodborne illnesses are frequently occurred in Malaysia. However, not all of the foodborne outbreaks are reported. This may due to the infected people do not consult to the appropriate treatment in local hospitals, especially when the gastrointestinal symptoms such as diarrhoea and vomiting are common to them (Abdul-Mutalib et al., 2015). Consumption of contaminated food and water has emerged as the crucial root that causes five types of food and water borne diseases, such as food poisoning, dysentery, typhoid, cholera and viral hepatitis A. In 2015, the incidence rate (IR) of foodborne poisoning was the highest at 47.3 per 100,000 population with 14,433 per 15,346 cases of food and vector borne diseases in Malaysia (Department of Statistic, Malaysia, 2016).

In 2014, incidence rate of food and water borne diseases for cholera, dysentery, Hepatitis A, Typhoid and food poisoning were 0.45, 0.27, 0.21, 0.70 and 58.65, respectively, per 100,000 population. Surprisingly, food poisoning was not only counted 58.65 for

incidence rate, it was also responsible for mortality rate of 0.01 per 100,000 population (Ministry of Health Health Fact 2015, 2016). Between 2015 and 2016, incidence and mortality rates for cholera, dysentery, hepatitis A and typhoid have decreased, and yet the country recorded an increasing of 7.87 food poisoning incidences and 0.01 per 100,000 populations of mortality rates (MoHM, 2017, as cited in Eleanor et al., 2019).

Until April 2016, there were 60 food poisoning outbreaks occurred in educational institutes that involved 2,325 cases in the country with 47 (78.3%) of outbreaks involved 1,900 school students under Ministry of Education Malaysia (Abdullah, 2016). In 14th March 2017, 11 students had symptoms of vomit and nausea at elementary school in *Batu Pahat, Johor* after consuming packaged pudding snack (Abdullah, 2017). In 2018, a food poisoning case created an uproar throughout the nation which food poisoning outbreak of *Salmonella enterica* involved 83 people with two death cases in *Baling, Kedah* after eating contaminated *Laksa*, a signature dish of herbal fish spicy soup noodles (Abdullah, 2018). One of the significant epidemiological investigation was done in 1983 which there was a study mentioned that a food poisoning outbreak due to S. aureus was happened in Kapar, Kelang with 48 primary school students being treated in Main Health Center, Kapar after eating fried *meehoon*. The main symptoms were related to the upper gastro-intestinal tract, nausea and vomiting (Lekhraj Rampal, 1983).

1.2 Problem statement and significant of the study

The contamination of food which leads to foodborne disease are easily transmitted in the community as nowadays people tend to eating out and travel internationally. Food contamination might be originally from human strains which it is associated with poor handling of cooked food, such as cooked meat rather than cross-contamination from raw meat (Young et al., 2014). Human, as one of disease transmission routes, may affect the hygiene and sanitation levels of foodservice industry. There is a relative few of empirical researches mentioned that food handlers may be the potential source of foodborne disease (Dagnew et al., 2012). Argud ń et al. (2010) reported that food poisoning disease carried by *S. aureus* is related to improper handling of food and subsequent storage of food at elevated temperatures. It has been demonstrated that 27.2% of the food handlers' hands were found to have at least one type of pathogenic bacteria, and a total of 12% showed mixed pattern of colonization by two or more pathogenic bacteria with 36.1% of food handlers' hands were contaminated with *S. aureus* (Bassyouni et al., 2012).

The previous researches which have focused on the study of prevalence and molecular epidemiology of pathogenic *S. aureus* among food handlers in Malaysia is paucity. Very few published studies have identified the prevalence of *S. aureus* among food handlers and cooked food in different regions of Malaysia. One of the significant studies done by Tan et al. (2014) showed that the prevalence of *S. aureus* from food handlers' hands at primary school canteens in Selangor was very high, indicating from 65.88% to 74.12% with 5.41% of MRSA. Noor-Azira et al. (2012) identified that 23.4% of the food premises food handlers were tested positive to *S. aureus*.

The result of the current study can be representing the prevalence study of *S. aureus* in Klang Valley food handlers' population. This may further utilize by the policy makers to review and to evaluate proposed changes of food premises grading system. In addition, by tracing the source of bacteria origin, the Ministry of Health (MOH) can enhance the policy regarding safe food handling practices among food handlers and increase the hygiene and sanitation levels in various types of food premises. By creating the awareness among foodservice operators, proper training designed for the food handlers can be initiated in order to prevent contamination of food from food handlers (Vatansever et al., 2016).

1.3 Objectives

1.3.1 Research objectives

The main objective of this research project has therefore been to determine the molecular epidemiology of *S. aureus* among food handlers and in cooked food in the Klang Valley, Malaysia. Specifically, the objective seeks

1. To determine the prevalence of both *S. aureus* hand carriers and methicillin resistant *S. aureus* (MRSA) strains among food handlers and in cooked food
2. To identify the correlation between the socio-demographic characteristics profile of the food handlers with the prevalence and multidrug resistance *S. aureus* strains among food handlers
3. To evaluate the epidemiological relationship among *S. aureus* strains obtained from food handlers' hands and cooked food samples.

1.4 Research hypothesis

The key hypothesis of this research include:

1. There is a high prevalence of *S. aureus* with few carriers of MRSA strains among food handlers and in cooked food.
2. There is a correlation between the socio-demographic characteristics profile of the food handlers and the prevalence of *S. aureus*.
3. There is a correlation between the socio-demographic characteristics profile of the food handlers with the multidrug resistance strains of *S. aureus*.
4. There is an epidemiological correlation between *S. aureus* from food handlers' hands and food samples.

1.5 Research flow

The research flow of molecular epidemiology of *S. aureus* among food handlers and in cooked food in the Klang Valley, Malaysian is presented in Figure 1.1.

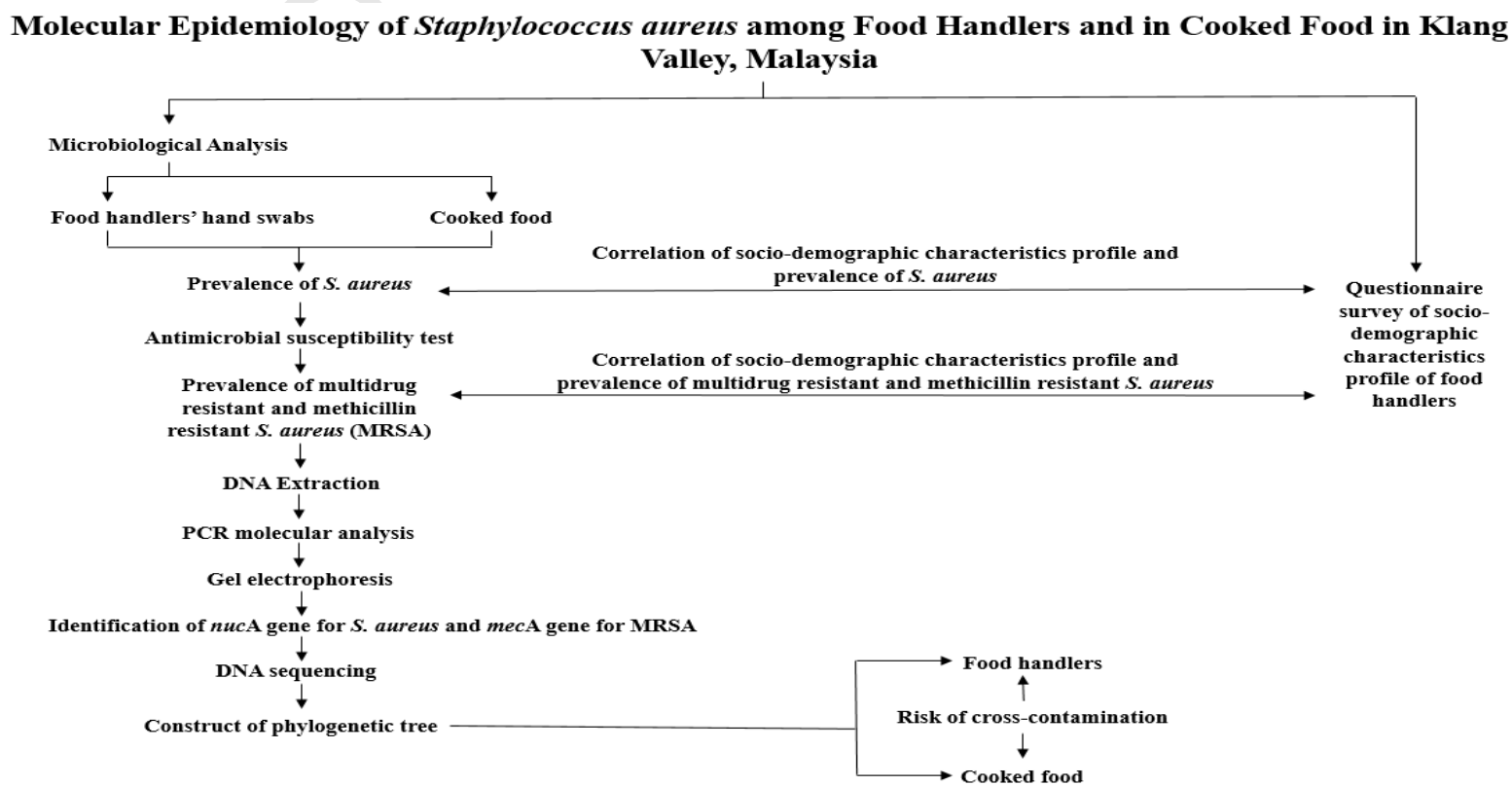

Figure 1.1: The research flow of whole study

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