



UNIVERSITI PUTRA MALAYSIA

***ASSESSING *Staphylococcus aureus* IN READY-TO-EAT FOOD AND
SANITATION LEVEL OF FOOD PREMISES
IN PUTRAJAYA, MALAYSIA***

SHAFIZI BIN ABDUL WAHAB

FSTM 2016 9



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IN PUTRAJAYA, MALAYSIA**

By

SHAFIZI BIN ABDUL WAHAB

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

May 2016

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DEDICATIONS

IN THE NAME OF ALLAH S.W.T

THE MOST GRACIOUS AND THE MOST MERCIFUL

AND

ALLAH'S PEACE BE UPON PROPHET MUHAMMAD

A DEDICATION TO...

MY MUM & DAD

MY LOVELY WIFE

NUHA & MUHAMMAD

COURSEMATES

LABMATES

FAMILY

FRIENDS



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia as fulfillment of the requirement for the degree of Master of Science.

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SHAFIZI BIN ABDUL WAHAB

May 2016

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Faculty : Food Science and Technology

Food and waterborne diseases especially food poisoning cases are still a problem in public health. The incidence happened due to unsanitary food premise and unhygienic food handlers practice during food handling. Therefore, the information of food safety and sanitation of food premises is crucial in planning strategies to prevent or reduce the incidence of food poisoning cases. A cross sectional study was conducted with the objective of the study were to determine the presence of *Staphylococcus aureus* in ready-to-eat (RTE) food, assessing the level of sanitation of food premises and determining the association between the presence of *S. aureus* in RTE food and the level of sanitation of food premises in Putrajaya, Malaysia. Method of most probable number (MPN) and polymerase chain reaction (PCR) were combined in this study to determine the number of *S. aureus* in RTE food. A total of 106 samples of RTE food were purchased from 53 food premises have been analyzed and the results confirmed by 56 (53%) samples contained *S. aureus* meanwhile the rest 50 (47%) not detected presence of *S. aureus*. Analyzed also show RTE food cooked detected *S. aureus* by 50 (45%) of the samples compared to RTE food that is still raw with the number 56 (55%). As for assessment, the assessment form has nine (9) main parameters consisting of 40 variables as a basis, was conducted on 53 of food premises to determine the level of sanitation of the premises. The pre-test to the assessment form was implemented to 10 food premises in Kuala Lumpur beforehand and the result was a practical and consistent for use in research. The results of the assessment carried out revealed a total of 49 (92%) were in a state of sanitary premises, and the remaining 4 (8%) were categorized as unsanitary premises. In terms of risk level, a total of 4 (8%) were high risk premises, 26 (49%) moderate risk, and the remaining 23 (43%) were at low risk. The study also found no significant association between the presence of *S. aureus* in RTE food with the level of sanitation of food premises in Putrajaya.

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

**PENILAIAN *Staphylococcus aureus* DALAM MAKANAN SEDIA DIMAKAN
DAN TAHAP SANITASI PREMIS MAKANAN DI PUTRAJAYA,
MALAYSIA**

Oleh

SHAFIZI BIN ABDUL WAHAB

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Penyakit bawaan air dan makanan terutamanya kes keracunan makanan masih menjadi masalah dalam kesihatan awam. Kejadian ini berpunca daripada premis makanan tidak sanitari dan pengendali makanan yang tidak mengamalkan kebersihan diri semasa mengendalikan makanan. Justeru itu, maklumat berkenaan keselamatan makanan dan tahap sanitasi premis makanan adalah penting dalam merancang strategi untuk mencegah atau mengurangkan kejadian kes keracunan makanan. Satu kajian keratan rentas telah dijalankan dengan objektif kajian adalah untuk menentukan kehadiran *Staphylococcus aureus* dalam makanan sedia dimakan, menilai tahap sanitasi premis makanan dan menentukan hubungan di antara kehadiran *S. aureus* dalam makanan sedia dimakan dengan tahap sanitasi premis makanan di Putrajaya, Malaysia. Kaedah jumlah paling mungkin (MPN) dan tindakbalas rantaian polimerase (PCR) telah digabungkan dalam kajian ini untuk menentukan kehadiran *S. aureus* dalam makanan sedia dimakan. Bagi penentuan kehadiran *S. aureus*, sejumlah 106 sampel makanan sedia dimakan yang diperolehi daripada 53 premis makanan telah dianalisa dan hasil ujian mengesahkan sebanyak 56 (53%) sampel mengandungi *S. aureus* manakala bakinya 50 (47%) tidak dikesan kehadiran *S. aureus*. Analisa juga menunjukkan makanan yang telah dimasak, dikesan dengan kehadiran *S. aureus* sebanyak 50 (45%) sampel berbanding makanan sedia dimakan yang masih mentah dengan jumlah 56 (55%). Manakala bagi penilaian premis makanan, satu borang penilaian mengandungi sembilan (9) parameter utama yang terdiri daripada 40 pembolehubah sebagai asas penilaian, telah dijalankan ke atas 53 premis makanan yang menjual makanan sedia dimakan untuk menentukan tahap sanitasi premis tersebut. Sebelum itu, ujian pra terhadap borang telah diimplentasikan kepada 10 premis makanan di Kuala Lumpur dan hasil menunjukkan borang penilaian adalah praktikal dan konsisten untuk kegunaan kajian. Hasil penilaian yang dijalankan mendapati sebanyak 49 (92%) premis berada dalam keadaan sanitari, manakala bakinya 4 (8%) premis dikategorikan sebagai tidak sanitari. Dari segi tahap risiko pula, sebanyak 4 (8%) premis adalah berisiko tinggi, 26 (49%) berisiko sederhana dan bakinya 23 (43%) berada pada tahap risiko rendah. Hasil kajian juga mendapati tiada hubungan yang bererti di antara kehadiran *S.*

aureus dalam makanan sedia dimakan dengan tahap sanitasi premis makanan di Putrajaya.



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I certify that a Thesis Examination Committee has met on 24 May 2016 to conduct the final examination of Shafizi Bin Abdul Wahab on his thesis entitled “Assessing *Staphylococcus aureus* in Ready-To-Eat Food and Sanitation Level of Food Premises in Putrajaya, Malaysia” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The committee recommends that the student be awarded the Degree of Master of Science.

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LIST OF ABBREVIATIONS

%	percent
°C	degree Celsius
µg	microgram
µl	microliter
CA-MRSA	community-acquired MRSA
CDCIS	Communicable Disease Control Information System
CoNS	coagulase negative staphylococci
DNA	Deoxyribonucleic Acid
Eh	potential of oxidation-reduction
ET	exfoliative toxins
FPAR	Food Premises Assessment Report
FSC	Food Standards Code
g	gram
HACCP	Hazard Analysis Critical Control Point
kDa	kilodalton
kg	kilogram
KLIA	Kuala Lumpur International Airport
km	kilometer
MgCl ₂	magnesium chloride
ml	milliliter
mM	miliMolar
MOH	Ministry of Health
MPN	Most Probable Number
MRSA	Methicillin Resistant <i>Staphylococcus aureus</i>
MSC	Multimedia Super Corridor
NaCl	sodium chloride
ng	nanogram
PBP2a	penicillin-binding protein 2a
PCR	Polymerase Chain Reaction
pH	potential of Hydrogen
PPE	personal protective equipment
RAPD	randomly amplified polymorphic DNA
RTE	ready-to-eat

SEs	staphylococcal enterotoxins
SFP	staphylococcal food poisoning
SOP	Standard Operating Procedures
spp.	species
SPSS	statistical package for the social sciences
ST38	sequence type 398
<i>Taq</i>	<i>Thermus aquaticus</i> DNA (polymerase)
TBE	tris-borate EDTA electrophoresis buffer
TSB	trypticase soy broth
TSST-1	toxic shock syndrome toxin 1
UHLG	Ministry of Urban Wellbeing, Housing and Local Government
UPM	Universiti Putra Malaysia
V	Volt
w/v	weight per volume
WHO	World Health Organization
<i>x g</i>	unit gravity
μM	micromolar

CHAPTER 1

INTRODUCTION

1.1 Introduction

This study aims to find the association between *Staphylococcus aureus* presence in ready-to-eat (RTE) food and the level of food premises sanitation in Putrajaya. In this chapter, the background of the study leading to the problem statement is will be elaborated upon. The objectives, significance and the scope of the study is also contained in this chapter, together with the operational definition of terms and organization of the study.

1.2 Background of the Study

The incident of foodborne diseases especially food poisoning is on the rise worldwide and becomes an important issue in public health because of its fast contagiousness plus lethality (Meldrum et al., 2006; Nguz, 2007). As a developing country, this problem is one of the main public health problems in Malaysia (Satcher, 2000), and this occurs due to the altered decision making process that involves food production, consumption and storage. These are caused by the globalization and liberation of food trade and the importance of food (Abdelgadir et al., 2009). An outbreak of foodborne disease is defined as more than two case incidences of similar illnesses from an investigation of the consumption of the same food source (Olsen et al., 2000).

S. aureus is widely known worldwide as a major disease agent for foodborne disease in humans (Loir et al., 2003; EFSA, 2011). The lethal enterotoxin produced by *S. aureus* commonly causes food poisoning (Dinges et al., 2000), pneumonia, wound infections and nosomial bacteraemia (Tiemersma et al., 2004). *S. aureus* produces staphylococcal enterotoxin (SEs) in compromised food, and ingestion said food induces severe symptoms which include emesis and pyrexia with or without nausea and diarrhea, which onset is less than 8 hours (usually between 3 and 4 hours) (Jett et al., 2001). Staphylococcal food poisoning (SFP) is an intoxication resulting from the ingestion of food or beverages containing preformed SEs usually produced by *S. aureus* (Su and Wong, 1997). SFP is limited to one individual and is resolved within 24 - 48 hours post onset. Most cases are unreported making the actual incidence of SFP to probably be much higher than reported (Lawrynowicz-Paciorek et al., 2007; Smyth et al., 2005).

S. aureus are gram positive spherical bacteria, facultative anaerobic and forms a golden yellow colony in agar (Todar, 2008). As a major human pathogen, *S. aureus* produces various toxic proteins, for examples are toxic shock syndrome toxin 1

(TSST-1), exfoliative toxins (ET) and enterotoxins which causes food poisoning (Boynukara et al., 2008). The classification of SEs are divided to nine groups based on the antigenic type (SEA, SEB, SEC, SED, SEE, SEF, SEG, SEH, SEI, and SEJ, thus far) (Akineden et al., 2001; Iandolo, 1989).

Host of *S. aureus* are food handlers (Cogan et al., 2002), especially in the nasal area and the hands which functions as a carriage of enterotoxigenic *S. aureus*. The food handlers themselves are important source of food contamination by staphylococci in eateries (Colombari et al., 2007). According to Taylor et al., (2000), there is evidence has shown that pathogens are transferred onto hands by the handling of food and through unsatisfactory personal hygiene among food handlers. The presence of *S. aureus* or its enterotoxins in processed food is generally an indication of poor sanitation (Bennett and Lancette, 2001).

1.3 Problem Statement

Foodborne diseases caused by microbiological agents are still major problems faced by developing countries such as Malaysia (Satcher, 2000). *S. aureus* has been singled out as the pathogen in many outbreaks of food poisoning (Bennett and Lancette, 2001). SEs derived from *S. aureus* are resistant to proteolysis and are heat stable (Omoe et al., 2005). Methicillin resistant *Staphylococcus aureus* (MRSA) elicit countless of morbidity and mortality worldwide (Ardic et al., 2006; Pesavento et al., 2007; Ho et al., 2008). In 2011, the Ministry of Health (MOH) reported 1629 cases of food poisoning and zero fatalities (KKM, 2012), in which statistically Wilayah Persekutuan Kuala Lumpur & Putrajaya recorded 114 cases (PKP, 2012). The Health Office of Putrajaya has received 12 reports and complaints about poor sanitation premises and 10 premises were closed (PKP, 2012). Then base on statistics on Women, Family and Community 2011, number of population in Putrajaya was estimated around 76,400 people and the number was increased followed by year (KPWK, 2014). However there is very limited study or survey in Malaysia to investigate and to link the hygiene status in food premises to the detection of *S. aureus* in the RTE food served to consumers.

1.4 Objectives

The purposes of this study are:

- 1.4.1 To determine the presence of *S. aureus* in RTE food at food premises in Putrajaya.
- 1.4.2 To assess the sanitation level of food premises in Putrajaya.
- 1.4.3 To determine the association between the presences of *S. aureus* in RTE food and the sanitation level of food premises in Putrajaya.

1.5 Significance of Study

In most countries, foodborne diseases remain a public health predicament in spite of the improvement in hygiene standards, improved food processing practices, education of food handlers and consumer awareness (Dominguez et al., 2002). This study will provide the new baseline data for the Food Safety & Quality Programme, MOH in Putrajaya, which will build and extend the index of *S. aureus* food contamination and relieve the insufficient data on microbiological hazards (Kim et al., 2011). Meanwhile the data on risk factors for foodborne diseases indicate that the majority of outbreaks result from faulty food handling practices (Clayton et al., 2002). The data can be used to assist the Health Office of Putrajaya and local authority to establish the implementation of control measures as well as to improve the sanitation level of food premises and specially the food handlers' practices. This was conducted to survey on whether the food premises which sell the RTE food complied with the legal requirements (Meldrum et al., 2009). Putrajaya is the new federal government administrative centre and its food sanitation standards should be maintained to uphold its reputable image as Malaysia's governing capital as well as the new home to all civil servants and their families (Sarah Moser, 2010).

1.6 Scope of the Study

This study focused on the association of *S. aureus* presence in RTE food and the sanitation level of food premises. The selection criterion for the focus of a RTE food was narrowed to the cooked chicken and meat products and also raw vegetables as known as 'ulam'. The study was carried out at the food premises in Putrajaya.

1.7 Operational Definition of Terms

The following terms were operationally defined for the purpose of this study:

1.7.1 Food Premise

The term refers to the premises used for or in connection with the preparation, preservation, packaging, storage, conveyance, distribution or sale of any food, or the relabeling, reprocessing or reconditioning of any food (FA, 1983).

1.7.2 Sanitation

The term refers to the state of being free from any condition or circumstances affecting health, and includes which may lead to, or cause contamination of food

with microbiological contaminants or toxic substances which can render the food hazardous to health (FHR, 2009).

1.7.3 Ready-To-Eat Food

The term is defined as the status of the food being ready for immediate consumption at the point of sale. It can be either raw or cooked, hot or chilled, and can be consumed without further heat treatment including reheating (FEHD, 2001).

1.7.4 Food Handler

The term includes any person who is directly involved in the preparation of food; comes into contact with food or food contact surfaces; and handles packaged or unpackaged food, or appliances, in any food premises (FHR, 2009).

1.7.5 Most Probable Number Test

This concept was developed for estimation of the number of organisms based on the probability (Cochran, 1950).

1.7.6 Polymerase Chain Reaction Test

Is an enzyme catalysed reaction for the replication or amplification of nucleic acids (Bertram-Drogatz, et al., 2000).

1.8 Organization of the Study

The dissertation is divided into five chapters. The first chapter highlights the important of *S. aureus* in incidence of the food poisoning as stated in the objectives and significance of the study. Chapter 2 reviews the extant literature in order to provide a background for the research framework and proposed hypotheses. Chapter 3 will delineate the research approaches and methods used for this study. Chapter 4 presents the empirical findings about the presence of *S. aureus* in RTE food and sanitation's level of food premises. With the findings, the study is able to identify the association between the presence of *S. aureus* in RTE food and the sanitation level of food premises in Putrajaya. Then also explains and discusses the evidence gained from the outcome in perspective to the study objectives. The final chapter summarize all chapters and make conclusion section wraps up the study as well as giving recommendations and implications for the responsible parties and food premises owners and handlers in Putrajaya. There are also state on a limitations and provide an improvement for further studies.

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