PREVALENCE, IDENTIFICATION, SURVIVAL CURVE AND RISK ASSESSMENT OF Staphylococcus aureus IN RAW FOOD IN SELANGOR, MALAYSIA

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FSTM 2019 29

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By

RAMZI OTHMAN SAEED BAHUMAISH

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

September 2019

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DEDICATION

Dedicated to my beloved parents, wife, siblings and friends for their love and support

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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September 2019

Chairman : Professor Son Radu, PhD
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Staphylococcus aureus is an opportunistic pathogen that colonizes the nares and skin surfaces of several animal species, including human. *S. aureus* can cause a wide variety of infections ranging from superficial soft tissue and skin infections to severe and deadly systemic infections. Habitually *S. aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) have been associated with hospitals, but during the past decades, MRSA has emerged in the community and now a new branch of MRSA has been found in association with livestock (LA-MRSA). Antimicrobial-resistant *S. aureus*, especially methicillin-resistant *S. aureus* (MRSA), has been emerged and considered as a major public health concern. There is an increasing risk of food production animals serving as a reservoir and transmitting *S. aureus* and MRSA in community environments. Due to the increased food safety risk posed by MRSA and its multidrug resistance, the aim of this study was to investigate the transmission distribution of the MRSA in food and the associated risk. In this study, a total of 262 samples were collected by random sampling at wet-markets and supermarkets. The combination of the method of most portable number-duplex and multiplex polymerase chain reaction (MPN-dPCR and mPCR) was used to detect and determine pathogens of interest. The findings of this study revealed that *S aureus* and MRSA were more frequently detected in samples from the wet market compared to the supermarket at the prevalence range of 15-100% and 8.33-66.70%, respectively. In addition, the isolated strains obtained from different samples were examined based on their antibiotic resistance profile. The majority of the isolates recorded multiple resistances to at least three out of seventeen antibiotics, and the majority of the MRSA isolates (96.7%) showed Multiple Antibiotics Resistance index > 0.18. Also, the results showed that the heat treatment (grilling) resulted in a significant log reduction ($P<0.05$) for chicken burger, beef burger and fish burger when compared to uncooked positive controls for each of the three grilled meat burgers. Furthermore, a step-wise risk assessment was preformed to evaluate the

potential risk of acquiring staphylococcal foodborne posed by methicillin resistant *Staphylococcus aureus* (MRSA) from the consumption of food in Malaysia. The results for that analysis showed that the risk estimate of acquiring staphylococcal for whole Malaysian population were 19 cases, 3.43×10^{-2} cases, and 1.09×10^{-1} cases per 100,000 population. Additionally, this study gave a glimpse of the scenario in Malaysia that chicken, shellfish, and milk can act as possible vehicles for the occurrence of staphylococcal foodborne. Therefore, high-risk groups are recommended to apply appropriate handling practices and sufficient cooking times prior to consumption of high-risk foods such as meats and milk products. Findings of this study suggested that raw food could provide a diverse reservoir of MRSA. Also, the presence of MRSA in raw food might pose potential threat of infection to individuals who handle the food. Moreover, the public education on safe raw food handling and cooking practices should be continued as well as education of food handlers until there is a better understanding of the infectious dose of MRSA in raw food.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

TABURAN, IDENTIFIKASI , LEKUK HIDUP DAN PENILAIAN RISIKO UNTUK *Staphylococcus aureus* DALAM MAKANAN MENTAH DI SELANGOR, MALAYSIA

Oleh

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Staphylococcus aureus adalah patogen oportunistik yang menetap di permukaan hidung dan kulit beberapa spesies haiwan, termasuk manusia. *S. aureus* boleh menyebabkan pelbagai jangkitan dari jangkitan tisu lembut cetek dan kulit hingga jangkitan sistemik yang teruk, dan boleh membawa maut. Biasanya, *S. aureus* dan *Staphylococcus aureus* yang tahan methicillin (MRSA) telah dikaitkan dengan hospital, tetapi sejak beberapa dekad yang lalu, MRSA telah muncul dalam masyarakat, dan kini suatu cabang MRSA telah ditemui dalam ternakan (LA-MRSA). *S.aureus*, yang tahan antimikrob, terutamanya *S. aureus* yang tahan methicillin (MRSA), telah muncul dan dianggap sebagai masalah kesihatan awam utama. Terdapat peningkatan dalam risiko terhadap haiwan pengeluaran makanan yang berfungsi sebagai sumber dan penyebaran *S.aureus* dan MRSA dalam persekitaran masyarakat. Disebabkan oleh peningkatan risiko keselamatan makanan yang ditimbulkan oleh MRSA dan tahanannya terhadap pelbagai ubat,tujuan kajian ini adalah untuk menyiasat penyebaran MRSA dalam makanan dan risiko yang berkaitan. Dalam kajian ini, sebanyak 262 sampel telah dikumpulkan melalui persampelan rawak di pasar basah dan pasar raya. Gabungan kaedah reaksi rangkaian nombor dupleks dan multipleks mudah alih (MPN-dPCR and mPCR) digunakan untuk mengesan dan menentukan patogen yang menarik. Hasil kajian ini mendedahkan bahawa *S aureus* dan MRSA lebih kerap dikesan dalam sampel dari pasar basah berbanding dengan pasar raya pada kadar prevalens 15-100% dan 8.33-66.70%, masing-masing. Di samping itu, strain terpencil yang diperolehi daripada sampel yang berbeza telah diperiksa berdasarkan profil tahanan antibiotik mereka. Kebanyakan isolat mencatatkan pelbagai tahanan terhadap sekurang-kurangnya tiga dari tujuh belas antibiotik, dan majoriti isolat MRSA (96.7%) menunjukkan indeks Tahanan Pelbagai Antibiotik > 0.18. Selain itu, keputusan menunjukkan bahawa rawatan haba (panggang) menghasilkan pengurangan log yang ketara ($P<0.05$) untuk burger ayam, burger daging lembu dan burger ikan jika dibandingkan dengan

kawalan positif mentah bagi setiap tiga burger daging panggang tersebut. Tambahan pula, penilaian risiko secara berperingkat dibuat untuk menilai potensi risiko mengidap jangkitan staphylococcal yang dibawa oleh makanan yang mengandungi *Staphylococcus aureus* di Malaysia. Keputusan analisis tersebut menunjukkan bahawa anggaran risiko mengidap jangkitan staphylococcal untuk seluruh penduduk Malaysia adalah 19 kes, 3.43×10^{-2} kes, dan 1.09×10^{-1} kes bagi setiap 100,000 penduduk. Selain itu, kajian ini memberi gambaran tentang senario di Malaysia bahawa ayam, kerang, dan susu mingkin boleh bertindak sebagai kenderaan untuk penyebaran jangkitan staphylococcal melalui makanan. Oleh itu, kumpulan berisiko tinggi disyorkan untuk menggunakan amalan pengendalian yang sesuai dan masa memasak yang mencukupi sebelum memakan makanan berisiko tinggi seperti daging dan produk susu. Penemuan kajian ini mencadangkan bahawa makanan mentah dapat menyediakan pelbagai sumber untuk MRSA. Di samping itu, kehadiran MRSA dalam makanan mentah mungkin menimbulkan potensi ancaman terhadap jangkitan kepada individu yang mengendalikan makanan. Tambahan pula, pendidikan awam mengenai pengendalian makanan mentah dan amalan masakan yang selamat serta pendidikan untuk pengendali makanan harus diteruskan sehingga wujud pemahaman yang lebih baik tentang dos MRSA dalam makanan mentah yang boleh menghasilkan jangkitan.

ACKNOWLEDGEMENTS

First and foremost, I would like to extend my heartiest thanks and gratitude to my main supervisor, Prof. Dr. Son Radu for his patience in guiding and encouragement throughout my study. My warmest gratitude also goes to my co-supervisors, Prof. Dr. Cheah Yoke Kqueen and Prof. Dr. Abdulkarim Sabu Mohammed for their valuable advices, help and support.

Besides, I would also like to take this opportunity to thank all my laboratory members of Bacteriology Food Safety Laboratory, Dr.Raymond, Tan Chia wanq, Dr.Thung, Dr.Weisan, and Dr.Vivian, for their kind assistance and for sharing their experiences and knowledge.

Most importantly, a special thank goes to my family for their sacrifices in terms of time, financial support, endless love and encouragement which had brought me one step closer to my goals in life. A sincere apology to my parents for my misbehaved and all the worries I had brought to them. Thank you to keep believing in me and made me who I am today.

Also, I want to give special thanks and obligedness to Aunt Rabeaa Algenaee, Aunt Badreiah Almutaweaah, and Aunt Norriah Alomeari for their sensory and moral support in whatever way they could during this challenging period

I would also like to present a token of appreciation to everyone who I had met, especially, Dr.Ahmed Dr.Belal and everyone from the Division of Postgraduate, Research and Innovation, Kak Tinie, as well as to the personnel in the School of Graduate Studies during my life as a Ph.D student for your kind help, advices and memories to be remembered.

First and last, I would like to thank Allah for the blessing and strength given to me, assisting me to sail through all the hardship that I had encountered in every single part of my journey. Without Your blessing, I would not able to go this far.

Be cherished and thank you!

This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

16*S rRNA*	16 subunit ribosomal ribose nucleic acid
µg	microgram
µl	microliter
ºC	Degree Celsius
ASEAN	Association of Southeast Asian Nations
ATCC	American Type Culture Collection
bp	Base pair
CAC	Codex Alimentarius Commission
CDC	Centres for Disease Control and Prevention
CFU/g	Colony forming unit per gram
CFU/ml	Colony forming per milliliter
CLSI	Clinical and Laboratory Standard Institute
cm	Centimetre
coa	Coagulase
COM	Commission of the European Communities
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DSM	Department of Statistics Malaysia
D-value	Decimal reduction time (D-value) the time needed to destroy 90% of microorganisms (to reduce their numbers by a factor of 10)
DVFA	Danish Veterinary and Food Administration
ECDC	European Centre for Disease Prevention and Control
EFSA	European Food Safety Authority
EMEA	European Medicines Agency

eqn	Equation
EPA	Environmental Protection Agency
EU	European Union
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FSIS	Food Safety and Inspection Service
GMP	Good Manufacturing Practices
h	Hours
HACCP	Hazard Analysis Critical Control Points
IDSA	The Infectious Diseases Society of America
IPH	Institute of Public Health of Malaysia
MAR	Multiple Antibiotic Resistance
mecA	Gene found in bacterial cells which allows a bacterium to be resistant to antibiotics such as methicillin, penicillin and other penicillin-like antibiotics
$MgCl_2$	Magnesium chloride
ml	millilitre
mM	miliMolar
MOA	Ministry of Agriculture and Agro-based Industry Malaysia
MOH	Ministry of Health Malaysia
MPN	Most probable number
nuc	Nuclease
OIE	World Organization of Animal Health.
PCR	Polymerase chain reaction
s	Seconds
sea	*Staphylococcus aureus* Enterotoxin A
Taq	*Thermus aquaticus* DNA (polymerase)

TBE	Tris-Borate EDTA electrophoresis buffer
TE	Tris-EDTA buffer
TSB	Tryptic soya broth
US	United States
USDA	United States Department of Agriculture
V	Volt
WHO	World Health Organization
Z-value	the number of degrees Celsius required to bring about a 10-fold change in decimal reduction time of microorganisms

CHAPTER 1

INTRODUCTION

1.1 Background

Based on the report about foodborne diseases, published by the World Health Organization (WHO), entitled "Safer Food Saves Lives", foodborne diseases are the top causes of morbidity and mortality and they hinder the development of the socio-economic aspects of the country. More specifically, one of the top causes of foodborne diseases all over the globe as evidenced in prior studies by Kadariya, Smith and Thapaliya (2014) and Fetsch (2017), is *staphylococcus aureaus.*

Diseases caused by contaminate food and water are a great significant concern for all countries around the world. Globally, much unsafe food appearing illness cause different unpleasant and human suffering. According to WHO systematic monitoring in the last decades, more than one third of humanity health endangered by biological contaminated food (Hefnawy, 2011). According to statistics, foodborne illnesses affect 48 million individuals a year, cause 128,000 to be hospitalized, and are the causes behind the deaths of 3000 people. There are many germs, which can contaminate foods whereas many researchers identified more than 250 foodborne diseases. *S aureus* is one of the top germs that cause illnesses from food eaten in the United States (CDC, 2017a).

S. aureus is a foodborne pathogen that can cause infections in humans due to having contaminated food (Rodriguez et al., 1996; Kadariya et al., 2014). *S. aureus*, recognized in several saleable food products, was tangled in numerous foodborne outbreaks. From antimicrobial resistance properties, methicillin-resistant *S. aureus* (MRSA) is the main concern. Current studies in many countries reveal the detection of MRSA in commercial food (Motarjemi, 2014).

Despite the fact that toxin production is the main cause behind foodborne illness from S. *aureus*, there are other factors that contribute to the pathogenicity, which includes biofilm formation and antibiotic resistance. In fact, the major public health concern lies in drug-resistant staphylococci due to the bacteria's spread throughout the food chain. The past 2 decades indicated that methicillin-resistant S. *aureus* (MRSA) infection has proliferated all over the globe. Consequently, multidrug-resistant S. *aureus* strains have been consistently alienated from food handlers and foodstuff (Al-Shabib et al., 2017). However, owing to several antibiotics resistance, attention has increased over the finite choice of antimicrobial agents used to treat life-threatening diseases, as a result of which, the hospital stay and cost of treatment is increased (Akpaka et al., 2006; Udo et al., 2006).

Most animals can become colonized with *S. aureus* (de Neeling et al., 2007; Moon et al.,2007; Lewis et al., 2008; van Belkum et al., 2008; Guardabassi et al., 2009; Persoons et al.,2009), and contamination of carcasses may occur during slaughtering (de Boer et al., 2009). Recently, MRSA strains have been isolated from several food-producing animals (de Neeling et al., 2007; Moon et al., 2007; Lewis et al., 2008; van Belkum et al., 2008; Guardabassi et al.,2009; Persoons et al., 2009); and from retail meat worldwide (de Boer et al., 2009; Pu et al.,2009; Lim et al., 2010; Weese et al., 2010; Bhargava et al., 2011; Hanson et al., 2011), representing a potential risk for its transmission to humans.

Malaysia was recorded MRSA by early of 1970 (Lim & Zulkifli, 1987) and from the total *S aureus* isolated from all clinical samples in 2016, 18% were MRSA. This showed a slight decrease in MRSA rates when compared to 19.3% in 2015 (MOH, 2016). Recently, MRSA becomes widespread and cause common severe infection in health facilities and community (WHO, 2106a). A death rate of 64% with people infected by MRSA is more likely with others infected with a non-resistant form (WHO, 2016b). The occurrence and growing rates of MRSA in hospitals are becoming a global problem in many parts of the world including Malaysia (Alreshidi et al., 2017). In 2014, the Ministry of Health documented 49.79 cases of food poisoning per 100,000 population (MOH, 2014a). The trend of healthcare associated with MRSA incidence from 2015-2016 around 0.17-0.15 per 100 admissions. The majority of food poisoning cases around 50% or more due to abuse of food handling by handlers (MOH, 2007). The outbreaks recorded in academia are around 43% of the total food poisoning in Malaysia (MOH, 2014b).

Molecular techniques concerns as confirmatory tools for the detected pathogens as well as in disease control programs. Nevertheless, it can detect the DNA sequences of pathogens that do not indicate that pathogens are applicable in the cell host and the infection is recognized (Aranguren & Figueras, 2016). Approaches used for rapid detection are categorized into nucleic-acid, sequence-based methods like polymerase chain reaction, multiplex-PCR, real-time PCR, loop-mediated isothermal amplification, nucleic acid sequence-based amplification as well as DNA microarrays, Some other employed detection methods are; biosensor-based technology in the form of optical biosensors, electrochemical biosensors, mass-based bio-sensors and biochemical sensors, as well as immunological-based methods such as, lateral flow immunoassay and enzyme-linked immunosorbent assay (ELISA). The above methods are characterized as being sensitive, efficient, timely, specific and dependable compared to their conventional counterparts (Hameed, Xie, & Ying, 2018).

On a global scale, interest on the methodological developments and rules pertaining to the decrease of foodborne disease and the food safety progress has notably increased. Moreover, quantitative microbial risk assessments (QMRAs) are described as robust probabilistic modeling mechanisms that are invaluable for risk managers, public health officials, government entities, and procedures to identify and quantify the illness probability, related with specific food and food pathogen.

Specifically, the microbial risk assessment is utilized to conduct an evaluation of the bacterial risk in the hopes of averting foodborne disease and recognizing environmental factors that influence the growth of the microbe (EPA, 2012). Such assessment should encompass identification of hazard, assessment of exposure, characterization of the hazard and characterization of the risk (CAC, 1999).

Malaysia as a member of the Association of Southeast Asian Nations (ASEAN) played and continues to play the leading role in health development agenda in improving healthy lifestyles, reacting to risks and appearing threats, fortifying health systems and access to care, and assuring food safety (WHO, 2017a). The national and international organizations strongly advise the application of risk assessment methods to food safety issues (WHO/FAO, 1999) and these methods have been reviewed by various researchers (van Gerwen et al., 2000; Robertson et al., 2005; Kuan et al., 2015). Hence, in this study, the prevalence data obtained were used to estimate the microbial risk of different food consumption by the consumers.

World Health Organization (WHO) global strategy for the containment of antimicrobial resistance revealed that the rate of emergence of antimicrobial resistance strain is expected to be increased by misuse of antibacterial substances (WHO, 2001). Moreover, the resistant microorganisms present in food products originating from the animal source may cause human infections that are difficult to cure. In addition, WHO (2014) reported that antimicrobial resistance is a growing public health threat and has been designated by the WHO as an emerging public health problem and economic burden. Therefore, this study has examined strains that isolated from different types of food to investigate the transmission distribution of the MRSA and the associated risk.

1.2 Problem statements

According to Dinges, Orwin and Schlievert (2000) and Pereira et al. (2009), common foodborne pathogens, like S. *aureus* are not the direct causes of illness but the produced enterotoxin from the bacteria is what causes results in food poisoning under specific food contamination circumstances. S. *aureus*, according to prior studies (e.g., Kitai et al., 2005; Normanno et al., 2005; Pu, Han & Ge, 2009), is characterized as being ubiquitous and commonly found in domestic animals, contaminated food with pathogen. In fact, S. *aureus* contaminates different food products like meat products, eggs, dairy products, vegetables and processed foods like sandwich fillings or even chocolate éclairs (Normanno et al., 2005). S. *aureus* may also be introduced to food through inappropriate food handling during preparation and temperature abuse following food preparation. Aside from the food handlers, S. *aureus* contamination may also occur through equipments and environment surfaces. More specifically, food poisoning is prone to happen if the contaminated food is kept at the wrong temperature range, the right temperature of which is below 7.2°C or higher than 60°C as mentioned in the studies by Almonacid-Merino, Thomas and Torres (1993) and Altekruse et al. (1996). Additionally, being in contact with food products that are contaminated or a careless

food handler may also up the risk of different infections caused by S. *aureus* (Kadariya, Smith & Thapaliya, 2014).

S. *aureus* is capable of colonizing various sites of animal foods (like poultry, pig or cow) in an asymptomatic manner and thus, such animals will function as a transmission vehicle of the bacteria and MRSA. Moreover, according to Vanderhaeghen et al. (2010), food products that are obtained from the animals may also be contaminated by both during the slaughtering and processing phases. Several countries have succeeded in isolating MRSA from meat or dairy products, with the inclusion of Netherlands, Australia, the U.S., Italy and Japan (Kitai et al., 2005; Normanno et al., 2005; Pereira et al., 2009; Pu, Han & Ge, 2009). However, the role of meat products as potential MRSA source and the possible connection between meat contamination and MRSA infections have yet to be examined by studies.

It is worthy to note that the antibiotics that are utilized on animals heighten the possibility of the transferring MRSA to humans and to colonize the same. This is compounded by the different methods of raising animals that are common practice that makes it challenging to determine the true source of the risks, which leads to the question as to whether colonized animals are transferring MRSA to humans or if it's the other way around.

1.3 Hypothesis

This study hypothesizes that methicillin-resistant *S aureus* (MRSA) strains exists in meat-producing animals, retail raw meat, chicken, milk, and seafood. In addition, humans might be exposed to MRSA from food animals via handling of foods of animal origin.

1.4 Objectives

The main objective of this study is to analyse the transmission distribution of the MRSA in food and the associated risk.

The specific objectives are as follows:

1) To quantify and determine the prevalence of *S aureus* and MRSA in food which is purchased from wet market and supermarkets.
2) To examine the antibiotics resistance profile of MRSA, which is isolated from samples, collected from wet market and supermarkets in Malaysia.
3) To determine the survival rate of MRSA in burger at different grilling temperature.
4) To develop a quantitative risk assessment model to determine the risk of acquiring MRSA from consumption food in Malaysia.

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