



UNIVERSITI PUTRA MALAYSIA

***PREVALENCE AND ANTIBIOGRAM OF *Staphylococcus aureus*
ISOLATED FROM A RESEARCH LABORATORY, ITS TRANSMISSION
AND CROSS CONTAMINATION TO FRUITS AND KITCHEN UTENSILS***

VITALIS RONALD EDEN

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By

VITALIS RONALD EDEN

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

June 2018

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DEDICATION

**Dedicated to my beloved parents, family members, and friends for their
endless love and support**



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

PREVALENCE AND ANTIBIOGRAM OF *Staphylococcus aureus* ISOLATED FROM A RESEARCH LABORATORY, ITS TRANSMISSION AND CROSS CONTAMINATION TO FRUITS AND KITCHEN UTENSILS

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June 2018

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Staphylococcus aureus is an important foodborne pathogen found on the skin and in the nose of a healthy person as normal flora. *S. aureus* can be acquired and transmitted from contaminated hands to fruits and kitchen utensils. The objectives of this research were to determine the prevalence and antibiogram of *S. aureus* in microbiology research laboratory, transmission and its cross contamination to fruits and kitchen utensils. A total of 320 isolates from nose=80; hands=80; door knobs=80 and table surface=80 were taken from microbiology research laboratory in Petaling Jaya, Selangor were examined for the presence of *S. aureus* using Most Probable Number method. The strain of *S. aureus* obtained were used in simulation test using cutting board=15; knife=15; fork=15; table cloth=15; and watermelon=15. The prevalence of *S. aureus* estimated in microbiology research laboratory was 81.56% whereby 75% before work and 88.12% after work ranged from 3.6 MPN/mL to 140 MPN/mL. In simulation test, it was identified that *S. aureus* can be transmitted via contaminated hands to fruits and kitchen utensils during food preparation whereby about 65.3% of *S. aureus* obtained from 75 isolates and watermelon recorded the highest load of *S. aureus* about 86.6%. Meanwhile in antibiogram test, *S. aureus* were found resistant to oxacillin (85.4%) and gentamicin (74.3%). The Actor Network Theory applied in this study identified that the strains of *S. aureus* obtained in microbiology research laboratory were able to contaminate the fruits and kitchen utensils during food preparation. Furthermore, Risk Ranger estimated that the risk of acquiring *S. aureus* were moderate with 4 illnesses predicted per annum scoring about 83/100. Hence, contemporary healthcare strategies must be taken immediately to prevent the transmission of *S. aureus* in microbiology research laboratory and its cross contamination to fruits and kitchen utensils

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

**PREVALEN DAN KECENDERONGAN ANTIBIOTIK *Staphylococcus aureus*
DALAM MAKMAL, PENYEBARAN DAN PENCEMARAN SILANG KEPADA
BUAH-BUAHAN DAN PERALATAN DAPUR**

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Staphylococcus aureus adalah sejenis patogen yang terdapat pada kulit dan hidung manusia yang sihat seperti flora biasa. *S. aureus* boleh diperoleh dan disebarkan dari tangan yang tercemar ke buah-buahan dan peralatan dapur. Objektif kajian ini adalah untuk menentukan kelaziman dan antibiogram *S. aureus* dalam makmal penyelidikan mikrobiologi, penghantaran dan pencemaran silang kepada buah-buahan dan perkakas dapur. Sejumlah 320 isolat dari hidung=80; tangan=80; tombol pintu=80 dan permukaan meja=80 diambil dari makmal penyelidikan mikrobiologi di Petaling Jaya, Selangor diperiksa untuk mengetahui kehadiran *S. aureus* menggunakan kaedah Most Probable Number. Ketegangan *S. aureus* yang diperolehi digunakan dalam ujian simulasi menggunakan papan pemotong=15; pisau=15; garfu=15; kain meja=15; dan tembikai=15. Lazimnya, *S. aureus* yang dianggarkan dalam makmal penyelidikan mikrobiologi adalah 81.56% di mana 75% diperolehi sebelum berkerja dan 88.12% selepas berkerja adalah dari 3.6 MPN/mL hingga 140 MPN/mL. Dalam ujian simulasi, *S. aureus* boleh dihantar melalui tangan yang tercemar ke buah-buahan dan perkakas dapur semasa penyediaan makanan di mana kira-kira 65.3% *S. aureus* yang diperolehi dari 75 isolat manakala buah tembikai mencatatkan beban tertinggi mengandungi *S. aureus* kira-kira 86.6%. Sementara itu dalam ujian antibiogram, *S. aureus* didapati tahan terhadap oxacillin (85.4%) dan gentamicin (74.3%). Actor Network Theory yang digunakan dalam kajian ini mengenal pasti bahawa ketegangan *S. aureus* yang diperolehi dalam makmal penyelidikan mikrobiologi dapat mencemari buah-buahan dan perkakas dapur semasa penyediaan makanan. Tambahan pula, Risk Ranger menganggarkan bahawa risiko memperoleh *S. aureus* adalah sederhana dengan 4 penyakit yang diramalkan setiap tahun yang mencatat skor kira-kira 83/100. Oleh itu, strategi penjagaan kesihatan kontemporari perlu diambil dengan serta-merta untuk

mencegah pencemaran *S. aureus* dalam makmal penyelidikan mikrobiologi dan pencemaran silang ke buah-buahan dan perkakas dapur.



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

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- the research conducted and the writing of this thesis was under our supervision;
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LIST OF ABBREVIATIONS

ANT	Actor Network Theory
AST	Antimicrobial susceptibility testing
ATCC	American Type Culture Collection
BAM	Bacteriological Analytical Manual
BHI	Brain-heart Infusion Broth
CDC	Centre for Disease Control and Prevention
CFSAN	Centre for Food Safety and Applied Nutrition
CFU	Colony forming unit
CLSI	Clinical and Laboratory Standard Institute
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
EPS	Extracellular Polymeric Substance
FAO	Food and Agriculture Organization of the United Nations
FDA	Food and Drug Administration
FRAT	Falls Risk Assessment Tool
FSIS	Food Safety and Inspection Service
H ₂ O ₂	Hydrogen Peroxide
h	hour
HACCP	Hazard Analysis Critical Control Points
MAR	Multiple antibiotic resistance
MH	Mueller Hinton
min	minute
MPN	Most probable number
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
MSA	Mannitol Salt Agar
NCCLS	National Committee for Clinical Laboratory Standards
NCRA	National Committee on Risk Analysis
PBP2A	Penicillin Binding Protein 2A
PRR	Probabilistic Risk Ranger
RNA	Ribonucleic acid
RR	Risk Ranger
SCCmec	<i>Staphylococcal</i> Cassette Chromosome Mec
SFD	<i>Staphylococcal</i> Foodborne Disease
TSA	Trypticase soy agar
TSB	Tryptic soy broth
U	Unit
UPM	Universiti Putra Malaysia
USA	United States of America
USDA	United States Department of Agriculture
UV	Ultraviolet
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 Background

Staphylococcus aureus (*S. aureus*) is one of the leading causes of bacterial infection in community and hospital setting producing mortality rate between 65-70% in the pre-antibiotic era and about 20-40% currently (Melzer *et al.*, 2013). Approximately, one-third of the human population become long-term carrier of *S. aureus* as it can live on waterless area in an extended period (Tadayuki *et al.*, 2010). Meanwhile, it is estimated that in 600 million peoples, 1 in 10 suffers from foodborne disease caused by contaminated food with *S. aureus* and about 420,000 dies every year (World Health Organization., 2015). *S. aureus* is described as a bacterium that capable of producing heat-resistant enterotoxin such as enterotoxin A, enterotoxin B, enterotoxin C1, enterotoxin C2, enterotoxin C3, and enterotoxin D affecting the intestine leading to food poisoning (Reiser *et al.*, 1984; Shimizu *et al.*, 2000).

S. aureus can cause a various type of illnesses ranging from minor to life-threatening including endocarditis, bacteraemia, osteomyelitis, pneumonia, meningitis, sepsis and toxic shock syndrome (Devapriya *et al.*, 2013). Moreover, it is found that the cross contamination of *S. aureus* occurs in a workplace whereby food handlers are identified to be one of the contributors through unhygienic practices including not washing hands properly, exposing food items at room temperature in extended period and neglecting the cleanliness of working area (Mutalib *et al.*, 2012). Furthermore, it is stated that lack of self-hygiene, unclean water supply and dirty environment contributing to the *Staphylococcal* food poisoning as it is easily transferred between workers due to poor hand washing techniques (Carboneau *et al.*, 2010). On the other hand, the emergence of antibiotics has resulted in major clinical cases and signification worldwide problem. Evidence of this, *S. aureus* is identified to cause resistant to many commonly used antibiotics therefore it is used as the key selective force driving to the resistance (Golzari *et al.*, 2013). In this case, β -lactam penicillinase is the best-known mechanism of bacterial resistance which may be plasmid-mediated or chromosomally and be constitutive to protects *S. aureus* against antibiotic group called Penicillins (Ali *et al.*, 2004).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one example of antibiotic resistance which is infrequently identified from patients in the community whereby it is reported that community-acquired Methicillin-resistant *Staphylococcus aureus* has increased leading to a change in epidemiology (Ghojazadeh *et al.*, 2014). The cases of foodborne illness among industrialized food production continue to prevail as people often blame the nature rather

than exploring the connection between humans and non-humans which classified as actors involved in their surroundings. Therefore, the framework of Actor Network Theory (ANT) is developed to enable researchers to explore the connection between human and non-human entities which involved in a vast quantity of food preparation (Stuart *et al.*, 2011).

Correspondingly, researchers identified *S. aureus* as one of the leading bacteria which cause food intoxication therefore prevention steps should be taken to combat its occurrence. In the same study, it is mentioned that the emergence of MRSA could be from various reasons which can be referred to the practice of infection control, antimicrobial pressures and host factors (Naghavi-Behzad *et al.*, 2015). Various factors contribute to foodborne illness such as population growth and agriculture, socioeconomic status, and culture. Hence, these might lead to improper food handling practices and insanitary procedures of a certain population leading to foodborne illness (Kau *et al.*, 2012). Meanwhile, a person with low immune system are vulnerable to the infection of *S. aureus* regardless of their age. Therefore, it is suggested that the law regarding self hygiene of food handlers in preparation of food should be tightened including closing down the dirty food premises (Gorman *et al.*, 2005). Different types of risk factors lead to *Staphylococcal* food poisoning such as the interaction between *S. aureus* and the food as well as between other microorganism contaminating the food, humidity, the composition of gas and different storage temperature (Abdul-Mutalib *et al.*, 2015).

1.2 Problem Statement

A study conducted at Liberty University in United States of America found that *S. aureus* and Methicillin resistant *Staphylococcus aureus* (MRSA) strain can be isolated from students and healthcare workers in microbiology laboratory (Gillen *et al.*, 2014). This indicated that human can be the carrier of *S. aureus* and MRSA therefore, the chances of acquiring *S. aureus* infection and transmission to food is possible. Furthermore, the Ministry of Health Malaysia (MOH) state that, the rate of *Staphylococcal* food poisoning cases has increased from 2005-2008 and death cases have also been reported even though numerous control strategies have been implemented (Ministry of Health Malaysia., 2009).

Several studies reported that *S. aureus* found in healthcare workers which constitute an important reservoir and estimated that the rate of colonization of *S. aureus* is high among the healthcare workers especially in laboratory (Kluytmans *et al.*, 1997). Unfortunately, the major reservoir for *S. aureus* are humans where it often colonizes the anterior nares and known to be a potentially lethal opportunistic pathogen. Apart from human, *S. aureus* can survive on inanimate objects and various dry surfaces such as door knob, mattress, blanket, and garments (Brown *et al.*, 2013). The occurrence of

widespread of foodborne illness persists to highlight problems with industrialized food production and the consumption of contaminated food product with *S. aureus* or non-infectious agents like toxin and chemicals may produce symptoms like nausea and vomiting leading to diarrhoea product contaminated (Linscott *et al.*, 2011).

Foodborne illness not only affecting the economic massively in healthcare cost, but also quality of life of a person and responsible for loss of productivity (Scharff *et al.*, 2012). Most of foodborne illness cases Malaysia is associated with unhygienic food handling practices including not washing hands properly, using unclean kitchen utensils and working in dirty working surfaces. Thus, the burden of foodborne illness identified in Malaysia is lower compared as most of the cases are not well-defined compare to other countries (Soon *et al.*, (2011). Prior to that, a suitable condition and temperature for bacterial growth contributes in increase of foodborne illness cases (Bezirtzoglou *et al.*, 2011). Thus, the factors affecting food contamination from work place to domestic kitchen can be the location, water supply, pest control, ventilation and temperature of food storage, self-hygiene of food handlers including clothing and their health condition while preparing food (Ministry of Health, 2009). *S. aureus* found to be the highest contributing agent of cross contamination in households compare to other pathogenic bacteria whereby poor self and environmental hygiene is the contributing factors of cross contamination of *S. aureus* in the meals prepared by consumers in domestic kitchen (Gorman *et al.*, (2005).

1.3 Objectives

The objectives of this study are:

1. To determine the prevalence and antibiogram of *Staphylococcus aureus* in laboratory.
2. To determine the transmission of *Staphylococcus aureus* by using Actor Network Theory.
3. To determine the cross contamination of *Staphylococcus aureus* by using simulation test.
4. To estimate the risk of acquiring *Staphylococcus aureus* by using Risk Ranger.

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