

MOLECULAR IDENTIFICATION OF ANTIBIOTIC RESISTANCE AND VIRULENCE GENES OF STAPHYLOCOCCUS AUREUS FROM NASAL ISOLATES AMONG MEDICAL LABORATORY TECHNOLOGY (MLT) STUDENTS IN UITM PUNCAK ALAM

 $\mathbf{B}\mathbf{y}$

RUFAIDA BINTI MUHAMMAD

Thesis Submitted in Partial Fulfillment of the Requirements for Bachelor of Medical Laboratory Technology (Hons),

Faculty of Health Sciences, Universiti Teknologi Mara

ACKNOWLEDGEMENT

First and foremost, I am so grateful to Allah S.W.T because of His blessings and merciful allowing me to complete my thesis successfully on the time given. This final year project is possible made successfully through the help and support from everyone including lecturers, laboratory staff, family, friends, and all the Medical Laboratory Technology (MLT) students in UiTM Puncak Alam.

I would like to give my special appreciation to my research supervisors, Mr Mohd Fahmi bin Mastuki for his guidance and support in every step throughout the process of completing this project. I am feeling so lucky as have understanding and dedicated supervisor that always helped me when there are problem regarding this project and he always responded to my questions punctually. Without his assistance and dedicated involvement in every step throughout the process, this paper would have never been accomplished.

Next, very special thanks to my co-supervisor Dr Siti Nazrina Binti Camalxaman for her supportive, patient and enthusiastic in guiding me from the beginning until the end of this project. In addition, special thanks to all the laboratory staff particularly to Mrs Aziyana, Mrs Norzila, Mrs Iadah, Mrs Masmadianty, Mrs Dina, Mr Nazzihan, Mr Zainuddin, Mr Nornizam and all of laboratory staff for their cooperation and assistance in this study progress by providing me with the laboratory equipment and apparatus in order to perform the laboratory works in Pathogen laboratory and Molecular laboratory.

I wish to express my thankfulness to all MLT students that participated in this study because volunteered become participants for nasal swab collection. Moreover, my appreciation to my laboratory mates Iman Binti Abdul Aziz, Nur Zarith Fatihah Binti Johari, Nur Anisah Binti Noor Habibullah and Ain Syakirah Binti Mat Zanggi for their help regarding laboratory works, motivating and all enjoyable moment over these past few months. Without them, this project cannot be completed. I would also like to thanks to Research Ethics Committee UiTM Selangor because proved my project and this project cannot be performed without their permission.

Finally, I would like to say a lot of thanks to my beloved parents, family and friends for their continued encouragement and prayers, helping, ideas, suggestions and comments in the preparation and accomplishment of this study, may Allah blesses and rewards them.

TABLE OF CONTENTS

AUTHOR'S DECLARATION INTELLECTUAL PROPERTIES ACKNOWLEDGEMENT TABLE OF CONTENTS LIST OF TABLES LIST OF FIGURES LIST OF ABBREVIATIONS AND GLOSSARY ABSTRACT		ii vi vii x xi xii			
			CHAPTER 1: INTRODUCTION		1
			1.1	Introduction	1
			1.2	Problem Statement	5
			1.3	Objectives	6
			1.4	Hypothesis of study	6
			CHAPTER 2: LITERATURE REVIEW		7
			2.1	Staphylococcus aureus	7
2.2	Nasal carriage of S.aureus	8			
2.3	Antibiotic resistance gene of Staphylococcus aureus (mecA gene)	9			
2.4	Virulence gene of Staphylococcus aureus (PVL gene)	9			
2.5	Association of mecA and PVL gene with MRSA	10			
2.6	Possible risk factor associated with nasal carrier of MRSA	10			
CHAPTER 3: MATERIAL AND METHODOLOGY		12			
3.1	Material and equipment	12			

ABSTRACT

Staphylococcus aureus is gram positive bacterium that known as normal flora in human skin and nasal passages but it can be infectious as the presence of antibiotic resistance gene (mecA) and virulence gene (PVL) which contribute to Methicillin Resistance Staphylococcus aureus (MRSA). About 4.4% students that exposed to hospital environment during their clinical practice become nasal carrier of MRSA (Baliga et al., 2008). As Medical Laboratory Technology (MLT) students will be exposed to hospital environment, they might become potential nasal carrier of MRSA. Hence, this study was conducted to identify the presence antibiotic resistance (mecA) and virulence (PVL) gene of S.aureus from nasal isolates among MLT students in UiTM Puncak Alam as well as to evaluate the association of possible risk factor with MRSA carrier. The nasal swab samples were taken from 144 students comprising 70 clinical and 74 pre-clinical students and questionnaire was given before nasal swabs collected. Cultural characteristic and biochemical test were identified S.aureus isolates and then proceeding to molecular analysis by using real time PCR as to determine the presence of mecA and PVL gene. Association of possible risk factor with MRSA were evaluated by statistical analysis of a questionnaire. Out of 144 nasal swab sample, cultural characteristics and biochemical reaction showed only 18 (12.5%) were S.aureus carrier. However, molecular analysis by using real time showed no amplification curve seen for mecA and PVL gene in any 18 isolates nasal carriage of S.aureus. Therefore, this study revealed that none of them were carrier of for MRSA and the association possible risk factor with MRSA carrier could not be determined.

Keywords: *Staphylococcus aureus*, antibiotic resistance gene, virulence gene, MRSA, real time-PCR

CHAPTER 1: INTRODUCTION

1.1 Introduction

Staphylococcus aureus is a bacterium that known as gram positive bacteria which one of the member of the Micrococcaceae family and also considered as a member of Macrococcus genus (Lowy F 1998). S.aureus is a gram positive bacterium which shows cocci in cluster as grape-like cluster on microscopical examination. S. aureus is an aerobic and non-motile organism that grows readily on Sheep Blood Agar (SBA) with white-golden color colonies which surrounded by clear zones of beta hemolysis. White-golden color colonies in SBA are produced by carotinoid pigments and it responsible for the species name aureus which meaning "golden" in Latin. Moreover, the staphylococcal golden pigment also causes to destroy polymorphonuclear granulocyte and then promotes virulence through its antioxidant activity. Besides that, S.aureus also shows golden yellow pigmented colonies on Nutrient Agar (NA) with circular and convex shape. However, S. aureus can be easily differentiating from other staphyloccal species by positive result of coagulase, catalase, deoxyribonuclease (DNAse) test. In addition, mannitol fermentation also the most efficient and clear cut identification test to differentiate S.aureus from other staphylococcal species by showing yellow colonies which surrounded by yellow zones or halo on the Mannitol Salt Agar (MSA) as it ferments mannitol by producing acid production (Tang, Y. W., & Stratton, C. W., 2010).

Staphylococcus aureus is a gram positive bacterium which known as one of the most common pathogen that may cause wide range of infection. This bacterium is a normal flora that frequently colonizes in human skin and nasal passage. Moreover, Lowy F (1998) also stated that about 30% of human population carries S.aureus in the nostrils. However, S.aureus colonization is quietly common in the anterior nares of nostrils because moist squamous epithelium of the nares become its primary habitat (Santhosh DV et al., 2007). It also supported and agreement with Kaplan (2005) that about 40% S.aureus colonize in the anterior nares. According to