UNIVERSITI TEKNOLOGI MARA

MOLECULAR CHARACTERIZATION OF COAGULASE-NEGATIVE *Staphylococcus* AND BIOFILM-ASSOCIATED GENES IN *S. capitis*

AZIYAH BT ABDUL AZIZ

Thesis submitted in fulfillment
of the requirements for the degree of
Doctor of Philosophy

Faculty of Applied Sciences

July 2015

AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations for Post Graduate, Universiti Teknologi MARA, regulating the conduct of my study and research.

Name of Student : Aziyah bt Abdul Aziz

Student I.D. No. : 2010674894

Programme : Doctor of Philosophy

Faculty : Applied Sciences

Thesis Title : Molecular characterization of coagulase-negative *Staphylococcus* and biofilm-associated genes in *S. capitis*

Signature of student : .....

Date : July 2015

ABSTRACT

The coagulase-negative *Staphylococcus* (CoNS) is a group of bacteria that are gaining prominence as emerging pathogens of hospital-acquired infections. One such species is *S. capitis*, which is now the major cause of bloodstream infection especially in neonatal intensive units. The major virulence factor of *S. capitis* appears to be its ability to form a biofilm structure. A total of 200 local clinical isolates of CoNS was obtained from the Hospital Tuanku Ampuan Rahimah, Klang in between December 2010 to May 2011. Nine species of CoNS were identified with *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. capitis* being the most prevalent strains. Identification of the isolates by biochemical tests using the Microgen Staph ID kit was less than 50% accurate while identification via the *sodA* gene sequence provided better discrimination and accuracy. The ERIC-PCR fingerprinting was then used to genotype the CoNS strains and the Discriminative Index (*D*) was calculated. At $D = 0.949$, ERIC-PCR can be used with confidence to discriminate between the *S. hominis* strains. However, low discriminative power ($D < 0.9$) was observed for *S. capitis, S. epidermidis* and *S. haemolyticus* implying that ERIC-PCR fingerprinting is not sufficient to genotype these strains. A multiplex PCR method was successfully developed to probe for the presence of *icaABCD* operon in a majority of the bacterial strains. At 88%, *S. capitis* showed the highest ability to form biofilm with a large percentage of these forming dense biofilm structures while the *icaABCD* operon was found to be present in all of the strains. Biofilm formation was however less frequent in other species, e.g. 39.2% in *S. epidermidis*, 16.7% in *S. hominis* and 3.3% in *S. haemolyticus*. Antimicrobial susceptibility test showed that for *S. capitis*, the formation of biofilm significantly increased the resistance of the biofilm cells to six types of antibiotics, similar to that reported for *S. epidermidis*. However, except for the case of ciprofloxacin, the thickness of biofilm did not appear to have any effect on the antibiotic resistance of the cells. Strain *S. capitis* B102 was selected for screening of novel biofilm-associated genes due to its ability to consistently form a very thick biofilm. Attempts to generate biofilm-defective mutants by transposon-mediated mutagenesis using the *bursa aurealis* system was however unsuccessful. Comparative genomics of B102 and three other *S. capitis* strains P27 (a non-biofilm former), B63 (moderate biofilm) and B145 (very strong biofilm) revealed that the *S. capitis* genome was dynamically shaped by horizontal gene transfer (HGT) via prophages, Staphylococcal Chromosome Cassettes (SCC) and plasmids. Some mobile genetic elements (MGE) present only in B102 and B145 are found to carry genes implicated in biofilm formation e.g. the *Atl* autolysin. By comparing the SNP profiles in strains with different biofilm phenotype, a list of seven candidate biofilm-associated genes was obtained. The ability of *S. capitis* to acquire additional genetic elements via HGT, and its propensity to form robust biofilm which enhances its antibiotic resistance, points to the possibility of this organism evolving into a significant pathogen.

ACKNOWLEDGEMENT

'Believe you can and you're halfway there'.

Theodore Roosevelt

This journey *would not* have been possible *without* the tremendous *support* and kindness showered by the people around me. I am deeply grateful to my supervisor, Associate Prof Dr Sharifah Aminah for her ideas, guidance and discussions throughout the study. It has been a pleasure working along with her and I am especially thankful for her understanding and assistance. I would also like to extend my thankfulness to my co-supervisor Associate Prof Dr Zaini Mohd Zain for her support during my study.

And not forgetting my colleagues, the technical staff and other post-graduate students who have lend me a helping hand in a way or another throughout my study, thank you so much

How time flies and my four wonderful children, Siddiq, Faruq, Nadhirah and Muaz, have grown up together with this long challenging journey. Thank you to all of you for your love and support.

To my husband Faiz who has been the pillar of my strength during this journey, I can't thank you enough for your love, encouragement and support. Thank you for sharing my ups and downs, my smile and happiness during the good days and tolerating me during my bad days. Thank you again and again for just being there, for me.

CHAPTER ONE
INTRODUCTION

1.1 BACKGROUND OF STUDY

Coagulase-negative staphylococci or CoNS is a group of *Staphylococcus* species which is distinguished from the more virulent *Staphylococcus aureus* by their inability to coagulate plasma. To date, there are more than 50 species of CoNS that have been identified including the most recent addition called *S. jettensis* (De Bel *et al.* 2013) and *S. argenteus* (Tong *et al.* 2015). CoNS were once considered relatively avirulent and have long been dismissed as culture contaminants since they are normal inhabitants of human skin and mucous membranes. The potential pathogenicity of CoNS in human medicine was first reported in 1958 but only in the 1970s that these organisms have become increasingly recognized as agents of clinically significant infections especially hospital-acquired opportunistic infections or nosocomial infections (Becker *et al.* 2014; Piette & Verschraegen 2009). CoNS are also known to be a leading cause of infections in infants. In the USA, data collected through The National Institute of Child Health and Human Development Neonatal Research Network revealed that CoNS accounted for 48% of late-onset sepsis in very low birth weight or VLBW infants (Stoll *et al.* 2002). In UK, the Neonatal infection surveillance networks reported that CoNS accounted for 54% of similar incidence in infants (Vergnano *et al.* 2011)

Among the CoNS species frequently isolated from clinical samples include *S. epidermidis, S. haemolyticus*, *S. hominis* and *S. capitis* (Gatermann *et al.* 2007; Koksal *et al.* 2009). *S. epidermidis* and *S. haemolyticus* are recognised nosocomial agents, and represent a large proportion of clinical CoNS isolates. Both are well studied due to their prevalence. In recent years, however, *S. capitis* has become a major concern as an infectious agent especially in neonatal patients. A large scale study of late-onset sepsis in a neonatal intensive care unit indicates that methicillin resistant, vancomycin-heteroresistant *S. capitis* could emerge as a significant pathogen in these settings (Rasigade *et al.* 2012). In addition, *S. capitis* strains which are multi-resistant towards commonly used antiseptics have also been reported (Lepainteur *et al.* 2013).