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CHARACTERIZATION AND PRESENCE OF ica GENES IN COAGULASE NEGATIVE STAPHYLOCOCCI ISOLATES IN A PUBLIC HOSPITAL IN MALAYSIA

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CHARACTERIZATION AND PRESENCE OF *ica* GENES IN COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATES IN A PUBLIC HOSPITAL IN MALAYSIA

By

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CHARACTERIZATION AND PRESENCE OF *ica* GENES IN COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATES IN A PUBLIC HOSPITAL IN MALAYSIA

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October 2017

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Coagulase-negative staphylococci (CoNS) are often considered as contaminants or normal skin flora but in the past few decades, they had emerged as pathogenic bacteria causing serious infections especially in patients with prosthetic medical devices. Their ability to produce biofilm on polymer surfaces results in treatment failure as it increases the resistance to antibiotics and host defense. Biofilms formation are mediated by polysaccharide intercellular adhesin (PIA), encoded by intercellular adhesion (*ica*) genes and it has been postulated that the presence of *ica* genes in CoNS is associated with antimicrobial resistance and responsible for catheter and medical device-related sepsis. The aim of this study is to determine the species distribution, antibiotic susceptibility pattern and to detect the presence of *ica* genes (*ica*A, *ica*D, *ica*B, and *ica*C) among CoNS isolated from blood culture. This cross-sectional study was conducted between January 2015 and December 2015, with CoNS isolates obtained from the blood cultures of patients at Hospital Serdang. Coagulase negative staphylococci were identified by gram staining, catalase and coagulase test, followed by species identification by Analytical Profile Index (API) Staph identification strips. Antimicrobial susceptibility testing was performed using Kirby Bauer method interpreted according to the Clinical and Laboratory Standards Institute guidelines. The presence of *ica* genes were detected using multiplex polymerase chain reaction. *Staphylococcus epidermidis* dominated the total number of species isolated (n=64, 40.0%). A total of 160 CoNS, 72.5% were resistant to penicillin and 60% were methicillin-resistant (MR) CoNS. Majority of CoNS harbored *ica*D (59.3%) and in 4 (2.5%) strains, all *ica*ADBC genes were observed. Detection of *ica* genes indicates CoNS are able to produce biofilm that causes serious nosocomial infections and should not always be reported as colonizer or contaminants.

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

KARAKTERISASI DAN KEHADIRAN GENE *ica* DALAM ISOLASI COAGULASE-NEGATIF STAPHYLOCOCCI DARIPADA HOSPITAL AWAM DI MALAYSIA

Oleh

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Staphylococci-koagulase negatif (CoNS) sering dianggap sebagai kontaminasi atau flora kulit tetapi dalam beberapa dekad yang lalu, ia telah muncul sebagai bakteria patogenik yang menyebabkan jangkitan serius terutamanya pada pesakit yang menggunakan peranti perubatan palsu. Keupayaan mereka menghasilkan biofilem pada permukaan polimer menyebabkan kegagalan rawatan kerana ia meningkatkan daya tahan terhadap antibiotik dan imunisasi pesakit. Pembentukan biofilem dibantu oleh *polysaccharide intercellular adhesion* (PIA), yang dikodkan oleh gen perekatan (*ica*) antara sel dan telah dikenalpasti bahawa kehadiran gen *ica* pada CoNS dikaitkan dengan rintangan antimikrob dan bertanggungjawab untuk sepsis yang berkaitan dengan peranti perubatan. Tujuan kajian ini adalah untuk menentukan taburan spesies, corak kerintangan antibiotik dan untuk mengesan kehadiran gen *ica* (*ica*A, *ica*D, *ica*B, dan *ica*C) di kalangan CoNS dalam sampel darah. Kajian rentas keratan ini dijalankan antara Januari 2015 dan Disember 2015, dengan isolasi CoNS yang diperolehi dari kultur darah pesakit di Hospital Serdang. Staphylococci negatif koagulase telah dikenalpasti oleh ujian pewarnaan gram, catalase dan koagulase, diikuti dengan identifikasi spesies oleh Strip Pengenalan Staph Analisis Profil (API). Ujian kerintangan antimikrob dilakukan dengan menggunakan kaedah Kirby Bauer yang ditafsirkan mengikut garis panduan Institut Standarisasi Klinikal dan Makmal. Kehadiran gen *ica* dikesan menggunakan tindak balas rantaian polimerase multiplex. *Staphylococcus epidermidis* menguasai jumlah spesies yang terpencil (n = 64, 40.0%). Sebanyak 160 CoNS, 72.5% menunjukkan rintangan terhadap penicillin dan 60% adalah *methicillin-resistant* (MR) CoNS. Majoriti CoNS mempunyai *ica*D (59.3%) dan dalam 4 (2.5%) strain, semua gen *ica*ADBC dikenalpasti. Pengesanan gen *ica* memutuskan bahawa CoNS dapat menghasilkan biofilem yang menyebabkan jangkitan nosokomial yang serius dan tidak seharusnya dilaporkan sebagai kolonizer atau kontaminasi.

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I certify that a Thesis Examination Committee has met on 23 October 2017 to conduct the final examination of Subashini a/p Vnayakam on her thesis entitled "Characterization and Presence of *ica* Genes in Coagulase-Negative Staphylococci Isolates in a Public Hospital in 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 Master of Science.

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

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LIST OF ABBREVIATIONS

℃	degree Celcius
%	Percentage
α	Alpha
β	Beta
Aap	Accumulation of associated protein
API	Analytical Profile Index
ATCC	American Type Culture Collection
Bap	Biofilm-associated protein
BLAST	basic local alignment search tool
CAPD	Continuous ambulatory peritoneal dialysis
CDC	Centre for Disease Control
CLSI	Clinical and Laboratory Standards Institute
CoNS	coagulase negative *staphylococci*
CRBSI	catheter-related blood stream infection
CRF	coagulase-reacting factor
DNA	deoxyribonucleic acid
EPS	Extracellular Polymeric Substance
E-test	epsilometer test
H_2O_2	hydrogen peroxide
ica	intercellular adhesion
MIC	minimum inhibitory concentration
MR-CoNS	Methicillin-Resistant coagulase negative *Staphylococci*
NVE	Native valve endocarditis

PCR	polymerase chain reaction
PIA	polysaccharide intercellular adhesion
PNAG	Poly-N-acetylglucosamine
RNA	ribonucleic acid
UTI	urinary tract infection

CHAPTER 1

INTRODUCTION

1.1 Background of the study

The staphylococcus is an aerobic gram-positive spherical cell which are arranged in grapelike irregular clusters. The genus *Staphylococcus* is currently divided into 39 species (Euzeby, 2007). The staphylococci are the most frequently isolated pathogen in microbiology laboratory which causes a range of diseases in hospital-related infections. They are further classified generally into two major groups which are coagulase-positive staphylococci (e.g. *Staphylococcus aureus*) and coagulase-negative staphylococci (e.g. *Staphylococcus epidermidis*). Coagulase-negative staphylococci comprises of a variety of species and many different strains and among the predominant species isolated in these infections are *Staphylococcus epidermidis*, *Staphylococcus lugdunensis, Staphylococcus warneri, Staphylococcus hominis* and *Staphylococcus saprophyticus* (Garza-Gonza´lez *et al*., 2010).

For a long time, coagulase-negative staphylococci were considered as non-pathogenic as it is present on outer body surfaces as normal micro flora which naturally colonizes humans. Meanwhile, isolated CoNS were not further investigated as they were grown as a result of contamination (Majumder *et al*., 2014). Nowadays, they are often associated with chronic infections such as urinary tract infection, osteomyelitis, native and prosthetic valve endocarditis and intravenous catheter infection (Becker *et al*., 2014).

Coagulase-negative staphylococci emerged as pathogens due to rise in use of invasive procedures both temporarily inserted and permanently implanted such as implanted medical devices, joint prostheses, shunts and intravascular catheters especially in immunosuppressed patients, infants, transplant patients, intravenous drug abusers and geriatric patients (Al Wohoush *et al*., 2011). The use of indwelling medical devices is important in the treatment of critically and chronically ill patients, however bacterial colonization of implanted foreign material can cause major nosocomial infection (Mathur *et al*., 2006). Thus, biomaterial-associated infections (BAI) contribute to the increase of hospital-acquired infections.

Regardless of low virulence, CoNS are considered clinically significant due to their ability to adhere to and to form biofilms on the surface of medical devices (Los *et al*., 2010). In addition, production of biofilm serves as a protection of bacteria from the effects of antibiotics which results in antimicrobial resistance (Bozkurt *et al*., 2009).

In agreement with that, the capacity of certain strains to form a biofilm consists of multi-layered cell clusters or intercellular adhesion (*ica*) embedded in a matrix of extracellular

polysaccharide, which facilitates the adherence of these microbial to biomedical surfaces made it recognized as a potential pathogen (Piette and Verschraegen, 2009). This multi-step mechanism is one of the major factors involved in the pathogenicity of catheter-related infections caused by CoNS. Investigation of the second stage of biofilm formation has demonstrated that cell aggregation and biofilm accumulation are mediated by the products of the chromosomal *ica* gene locus, which comprises four intercellular adhesion genes (*ica*A, *ica*B, *ica*C and *ica*D) and one regulator gene (*ica*R) (Cafiso *et al*., 2004). These genes influence the production of polysaccharide intercellular adhesion (PIA), which acts as an intercellular adhesion on hydrophilic surfaces.

In the clinical laboratories, identification of coagulase-negative staphylococcus is often limited because distinction between pathogenic and contaminating isolates is difficult to perform (Huebner and Goldmann, 1999). The severity of infections differs in different species which emphasizes the need of species identification for a better choice of treatment. However, rapid screening tests could only distinguish *S.aureus*, while non *S.aureus* isolates are reported as CoNS (Hirotaki *et al*., 2011). Therapeutically, CoNS infections remains challenging to clinician due to the large proportion of isolates as limited clinical trials are available to determine optimal therapy.

1.2 Need and Significance

This study could be able to fill in the gap of knowledge in understanding the significance of CoNS which contribute to nosocomial infections especially in prosthetic devices and indwelling catheters.

1.3 Research Question

Does presence of intercellular adhesion (*ica*) genes determine the clinical significance of CoNS isolated from blood culture?

1.4 Objectives

1.4.1 General objective

To determine the characterization of coagulase-negative staphylococci isolated from blood culture.

1.4.2 Specific objectives

i. To determine the distribution of species of coagulase-negative staphylococci isolated from blood culture.

ii. To study the antibiotic susceptibility patterns of coagulase-negative staphylococci species.

iii. To detect the intercellular adhesion (*ica*) genes among the isolated coagulase-negative staphylococci species.

1.5 Hypothesis

There is presence of intercellular adhesion (*ica*) genes in coagulase-negative staphylococci which makes it clinically significant in nosocomial infections.

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