UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND DISTRIBUTION OF STAPHYLOCOCCAL CASSETTE CHROMOSOME mec AMONG METHICillin-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI

HUDA BINTI SABER ABU BAKR SALEH

FPSK(M) 2018 30
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By

HUDA BINTI SABER ABU BAKR SALEH

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

April 2018
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DEDICATION

I would like to specially dedicate this work to my beloved late father, my mother, husband and the other family members that have been motivating and supporting me from the beginning till the end of this project. I would not be this successful without their supportive souls.
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the Degree of Master of Science

ANTIMICROBIAL SUSCEPTIBILITY PATTERN AND DISTRIBUTION OF STAPHYLOCOCCAL CASSETTE CHROMOSOME _mec_ AMONG METHICILLIN-RESISTANT COAGULASE-NEGATIVE STAPHYLOCOCCI

By

HUDA BINTI SABER ABU BAKR SALEH

April 2018

Chair : Rosni binti Ibrahim, MD, MPath
Faculty : Medicine and Health Sciences

Coagulase-negative staphylococci (CoNS) are notorious in causing nosocomial infections. _Staphylococcus epidermidis_ is deemed the most significant species infecting human, apart from _Staphylococcus haemolyticus_ and _Staphylococcus chromogenes_. In Malaysia, there is an increasing trend of antimicrobial resistance among CoNS whereby more than 50% has been reported as methicillin-resistant coagulase-negative staphylococci (MR-CoNS) which these organisms harbour _mecA_ gene which is acquired by a mobile genetic element in staphylococci called staphylococcal cassette chromosome _mec_ (SCC _mec_). This study aims to investigate species distribution among 100 MR-CoNS, to determine antimicrobial susceptibility pattern among the species and to detect their SCC _mec_ types.

Coagulase-negative staphylococci (CoNS) isolated from blood cultures were collected from Microbiology laboratory, Hospital Serdang in year 2016 and proceeded to phenotypic identification by gram-staining, catalase and coagulase test. Species identification was done by using API® Staph kit. Antimicrobial susceptibility testing (AST) was performed by using Kirby-Bauer method with nine antibiotic discs and was interpreted following Clinical and Laboratory Standards Institute (CLSI) 2016. Detection of SCC _mec_ was performed by using multiplex polymerase chain reaction (PCR). _Staphylococcus epidermidis_ (n=56, 56%) was the most common species isolated in this recent study, followed by _S. haemolyticus_ (n=19, 19%), _S. chromogenes_ (n=12, 12%), _Staphylococcus xylosus_ (n=6, 6%), _Staphylococcus hominis_ (n=5, 5%), _Staphylococcus capitis_ (n=1, 1%) and _Staphylococcus cohnii_ (n=1, 1%). All isolates were resistant to cefoxitin (n=100, 100%) and penicillin (n=100, 100%). More than 80% of the isolates were resistant to erythromycin and 70% were resistant to fucidic acid. All isolates were sensitive to vancomycin. A total of 54 (54%) isolates harboured SCC _mec_ type IVa (n=32, 32%) in which was widely distributed in _S. epidermidis_ (n=27, 48.2%). Fifteen (15%) isolates showed combination types which the most
common was type I & IVa (n=9, 9%) and another 31 strains (31%) were non-typeable. Type IVa was observed to have multiple antibiotic resistance with high rates of resistance towards erythromycin (n=32, 100%) followed by fucidic acid (n=25, 78.1%) and clindamycin (n=24, 75%).

In conclusion, *S. epidermidis* was the most common isolated species. Apart from penicillin, high percentages of resistance towards erythromycin and fucidic acid were observed in this recent study. This is probably due to the high usage of these antibiotics in outpatient clinical setting. Type IVa was the most detected SCCmec with multiple antibiotic resistance harbouring.

Keywords: Antimicrobial susceptibility pattern, *mec*A, MR-CoNS, SCCmec
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Master Sains

CORAK KERINTANGAN ANTIMIKROB DAN TABURAN KASET KROMOSOM STAFILOKOKUS mec DI KALANGAN STAFILOKOKUS KOAGULASE-NEGATIF BERINTANGAN TERHADAP METISILIN

Oleh

HUDA BINTI SABER ABU BAKR SALEH

April 2018

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Fakulti : Perubatan dan Sains Kesihatan

Stafilokokus koagulase-negatif (CoNS) terkenal dalam menyebabkan jangkitan nosokomial. Di samping Staphylococcus haemolyticus dan Staphylococcus chromogenes, Staphylococcus epidermidis dikatakan sebagai spesies paling signifikan yang menjangkiti manusia, Di Malaysia, terdapat peningkatan kecenderungan kerintangan antimikrob dalam kalangan CoNS dimana lebih daripada 50% telah dilaporkan sebagai CoNS yang mempunyai kerintangan terhadap metisilin (MR-CoNS) yang mengandung gen mecA yang mengekod ‘penicillin-binding protein 2a’ (PBP2a) yang mempunyai pengikatan pertalian yang rendah kepada semua antibiotik β-lactam.

Gen tersebut diperolehi oleh elemen genetik bergerak dalam stafilokokus yang dipanggil kaset kromosom stafilokokus (SCCmec). Tujuan kajian ini adalah untuk menyiapkan taburan spesies dikalangan 100 MR-CoNS, menentukan corak kerintangan antimikrob spesies dan jenis-jenis SCCmec.

Stafilokokus koagulase-negatif (CoNS) yang telah diisolasi daripada kultur-kultur darah dikumpulkan dari makmal Mikrobiologi, Hospital Serdang dalam tahun 2016 dan diteruskan kepada pengenalpastian fenotipik menggunakan pewarnaan ‘gram’, ujian katalase dan koagulase. Pengenalpastian spesies dilakukan dengan menggunakan kit API® Staph. Ujian kerintangan antimikrob (AST) telah dijalankan menggunakan kaedah ‘Kirby-Bauer’ berserta sembilan disk antibiotik dan ditafsirkan mengikut ‘Clinical and Laboratory Standards Institute’ (CLSI) 2016. Pengesanan SCCmec dijalankan dengan menggunakan ‘multiplex polymerase chain reaction (PCR)’.

Staphylococcus epidermidis (n=56, 56%) merupakan spesies yang paling banyak diisolasi dalam kajian baru-baru ini, diikuti oleh S. haemolyticus (n=19, 19%), S. chromogenes (n=12, 12%), Staphylococcus xylosus (n=6, 6%), Staphylococcus hominis (n=5, 5%), Staphylococcus capitis (n=1, 1%) dan Staphylococcus cohnii (n=1, 1%). Semua isolat rintang kepada sefoksitin (n=100, 100%) dan penisilin (n=100, 100%). Lebih daripada 80% isolat rintang kepada eritromisin (n=87, 87%) dan 70% rintang
kepada asid fusidik. Semua isolat sensitif kepada vankomisin. Sebanyak 54 (54%) isolat mengandungi SCCmec jenis IVa (n=32, 32%) dimana ianya merupakan jenis yang paling banyak dituburkan dalam S. epidermidis (n=27, 48.2%). Lima belas (15%) isolat menunjukkan jenis kombinasi dimana jenis yang mendominasi adalah jenis I & IVa (n=9, 9%) dan 31 (31%) ‘strain’ yang lain tidak dapat dijeniskan. Jenis IVa diperhatikan mempunyai kerintangan terhadap pelbagai antibiotik dengan kadar peratusan kerintangan yang tinggi terhadap eritromisin (n=32, 100%), diikuti oleh asid fusidik (n=25, 78.1%) dan klindamisin (n=24, 75%).


Kata kunci: Corak kerintangan antibiotik, meca, MR-CoNS, SCCmec
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I certify that a Thesis Examination Committee has met on 17 April 2018 to conduct the final examination of Huda binti Saber Abu Bakr Saleh on her thesis entitled "Antimicrobial Susceptibility Pattern and Distribution of Staphylococcal Cassette Chromosome mec among Methicillin-Resistant Coagulase-Negative Staphylococci" 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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Date: 30 July 2018
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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Signature: _________________________________________
Name of Member of Supervisory Committee: Dr. Tengku Zetty Maztura binti Tengku Jamaluddin
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<tr>
<td>ADH</td>
<td>Arginine DiHydrolase</td>
</tr>
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<td>AST</td>
<td>Antimicrobial Susceptibility Testing</td>
</tr>
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<td>ATCC</td>
<td>American Type Culture Collection</td>
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<td>BA</td>
<td>Blood agar</td>
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<td>bp</td>
<td>Basepair</td>
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<td>DNA Binding Buffer</td>
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<td>CAUTIs</td>
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<td>ccr</td>
<td>Cassette Chromosome Recombinase</td>
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<td>CDC</td>
<td>Centre for Disease Control and Prevention</td>
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<td>CLI</td>
<td>Clindamycin</td>
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<td>CLSI</td>
<td>Clinical Laboratory Standards Institute</td>
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<td>CoNS</td>
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<td>CRBSIs</td>
<td>Catheter-related Bloodstream Infections</td>
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<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<td>CVC</td>
<td>Central Nervous Catheter</td>
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<td>dH2O</td>
<td>Distilled water</td>
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<td>DNA</td>
<td>Deoxyribonucleic acid</td>
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<td>dNTP</td>
<td>Deoxyribonucleotide triphosphate</td>
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<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
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<td>Abbreviation</td>
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<td>ERM</td>
<td>Erythromycin</td>
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<td>HCW</td>
<td>Health Care Workers</td>
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<td>H₂O</td>
<td>Water</td>
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<td>HVS</td>
<td>High Vaginal Swab</td>
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<td>ica</td>
<td>Intercellular Adhesion</td>
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<td>ICUs</td>
<td>Intensive Care Units</td>
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<td>International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements</td>
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<td>Kilobase</td>
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<td>kDa</td>
<td>Kilodalton</td>
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<td>L</td>
<td>Litre</td>
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<td>MDR</td>
<td>Multidrug resistance</td>
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<td>mecA</td>
<td>Methicillin resistance</td>
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<td>mg</td>
<td>Miligram</td>
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<tr>
<td>mg/ml</td>
<td>Milligram per millilitre</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimum Inhibitory Concentration</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>mL</td>
<td>Mililitre</td>
</tr>
<tr>
<td>MLST</td>
<td>Multi Locus Sequence Typing</td>
</tr>
<tr>
<td>mM</td>
<td>Milimolar</td>
</tr>
<tr>
<td>MR-CoNS</td>
<td>Methicillin-resistant coagulase-negative staphylococci</td>
</tr>
<tr>
<td>MREC</td>
<td>Medical Research and Ethics Committee</td>
</tr>
<tr>
<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MRSE</td>
<td>Methicillin-resistant <em>Staphylococcus epidermidis</em></td>
</tr>
<tr>
<td>MRSH</td>
<td>Methicillin-resistant <em>Staphylococcus haemolyticus</em></td>
</tr>
<tr>
<td>msrA</td>
<td>Methionine Sulfoxide Reductase A</td>
</tr>
<tr>
<td>NaCL</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>NC</td>
<td>Negative control</td>
</tr>
<tr>
<td>NCBI</td>
<td>National Center of Biotechnology Information</td>
</tr>
<tr>
<td>ng</td>
<td>Nanogram</td>
</tr>
<tr>
<td>NHSN</td>
<td>National Healthcare Safety Network</td>
</tr>
<tr>
<td>NICUs</td>
<td>Neonatal Intensive Care Units</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>NIT</td>
<td>Nitrate</td>
</tr>
<tr>
<td>ORFs</td>
<td>Open reading frames</td>
</tr>
<tr>
<td>PAL</td>
<td>Alkaline Phosphatase</td>
</tr>
<tr>
<td>PBP</td>
<td>Penicillin binding Protein</td>
</tr>
<tr>
<td>PBP2a</td>
<td>Penicillin-binding Protein 2a</td>
</tr>
<tr>
<td>PC</td>
<td>Positive control</td>
</tr>
<tr>
<td>PCN</td>
<td>Penicillin</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulse Field Gel Electrophoresis</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>pH</td>
<td>Potential of hydrogen</td>
</tr>
<tr>
<td>PIA</td>
<td>Polysaccharide Intercellular Adhesion</td>
</tr>
<tr>
<td>PJIs</td>
<td>Prosthetic Joint-associated Infections</td>
</tr>
<tr>
<td>PYR</td>
<td>Pyrrolidonyl Arylamidase</td>
</tr>
<tr>
<td>RA</td>
<td>Rifampin</td>
</tr>
<tr>
<td>rcf</td>
<td>Relative centrifugal force</td>
</tr>
<tr>
<td>rpm</td>
<td>Revolutions per minute</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal ribonucleic acid</td>
</tr>
<tr>
<td>SCCmec</td>
<td>Staphylococcal cassette chromosome mec</td>
</tr>
<tr>
<td>SPSS</td>
<td>Statistical Package for the Social Sciences</td>
</tr>
<tr>
<td>SXT</td>
<td>Trimethoprim/sulfamethoxazole</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-Borate-EDTA</td>
</tr>
<tr>
<td>TSB</td>
<td>Trypticase soy broth</td>
</tr>
<tr>
<td>URE</td>
<td>Urease</td>
</tr>
<tr>
<td>UTIs</td>
<td>Urinary Tract Infections</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>V</td>
<td>Volts</td>
</tr>
<tr>
<td>VAN</td>
<td>Vancomycin</td>
</tr>
<tr>
<td>VP</td>
<td>Voges Proskauer</td>
</tr>
<tr>
<td>µg</td>
<td>Microgram</td>
</tr>
<tr>
<td>µg/mL</td>
<td>Microgram per millilitre</td>
</tr>
<tr>
<td>µL</td>
<td>Microlitre</td>
</tr>
<tr>
<td>µm</td>
<td>Micrometer</td>
</tr>
<tr>
<td>%</td>
<td>Percent</td>
</tr>
<tr>
<td>ºC</td>
<td>Degree Celcius</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Study Background

Staphylococci which are members of Micrococccae family, are gram-positive bacteria with single or grape-like cluster arrangements, possessing catalase-positive characteristics. Besides being normally isolated from mucous membranes and skin of humans and animals, staphylococci can also be found in environment, water and food (Widerström, 2010). Categorized into coagulase-positive staphylococci (CoPS) and coagulase-negative staphylococci (CoNS) groups, Staphylococcus aureus is the significant CoPS species while Staphylococcus epidermidis is the most significant CoNS (Kloos & Bannerman, 1994; Widerström, 2010). Since CoNS are major in colonizing skin and mucous membranes of mammals, they frequently contaminate blood cultures and cause uncertainty in determining their significance (Al-Mazroea, 2009; Elzi et al., 2012).

Presently, CoNS has been existing as one of the major nosocomial pathogens (Becker et al., 2014). They have the capability to cause infections include foreign body-related infections (FBRIs), preterm newborns infections and endocarditis (Becker et al., 2014). This is because CoNS possess a virulence factor called biofilm that assist them to adhere to medical devices in hospitals (Fredheim et al., 2009). Accumulation of biofilm is caused by ica genes which involve in the biosynthesis of polysaccharide intercellular adhesion (PIA) molecules (Namvar et. al., 2013). Biofilm-producing CoNS have been observed to become resistant to multiple antibiotics classes such as lincosamides and macrolides (Otto, 2008; Fredheim et al., 2009). Serious nosocomial infections can occur if the hospital environment is colonized by multidrug-resistance biofilm-forming CoNS (Wojtyczka et al., 2014). Major concern among clinicians is towards the increasing numbers of methicillin and multidrug-resistant strains (Becker et al., 2014). Widely spread in hospitals, most commonly isolated methicillin-resistant coagulase-negative staphylococci (MR-CoNS) species include S. epidermidis, Staphylococcus haemolyticus, Staphylococcus saprophyticus and several more (Mehdinejad et al., 2008). These organisms harbour mecA gene that encodes penicillin-binding protein 2a (PBP2a) which contributes to low binding to all β-lactam antibiotics (Hartman & Tomasz, 1984; Becker et al., 2014). The gene is acquired by staphylococcal cassette chromosome mec (SCCmec) (Wielders et al., 2002). This mobile genetic element possesses two important components which are mec gene and cassette chromosome recombinase (ccr) gene complexes; mec gene complex consisting of mecA with classes of A, B, C1, C2, D and E, the regulatory genes and associated insertion sequences express methicillin resistance function whereas ccr and a few surrounding genes assist SCCmec integrate into and out from the chromosome (IWG-SCC, 2009; Zong et al., 2011). In addition, there are also J regions (for SCCmec subtypes determination) and several non-essential components which may carry additional antimicrobial resistance determinants (IWG-SCC, 2009). Specific combinations of mec gene and ccr gene complexes produce different types of SCCmec which include types I-XI (Zong et al.,
Type III, IV and V were prevalently found in MR-CoNS and an isolate may possess more than one type (Zong et al., 2011). According to Barbier et al. (2010), SCCmec displays more polymorphous structure in MR-CoNS in terms of ccr-mec combinations compared to methicillin-resistant \textit{Staphylococcus aureus} (MRSA).

In Malaysia, data related to these organisms is limited. Thus, this study aims to determine the distribution of MR-CoNS species isolated from clinical blood cultures and their SCCmec types as well as to observe the antimicrobial susceptibility pattern and its relatedness with the identified SCCmec types. The outcomes from this research particularly the SCCmec genes findings, can be a set of preliminary data that can be used for further research.

1.2 Problem Statement

In Malaysia, there is an increasing trend of antimicrobial resistance among CoNS whereby 50% have been reported as MR-CoNS (Sani et al., 2011). Causing more severe infections until today besides being resistant to various antibiotics, it is understood that CoNS and especially MR-CoNS have appeared to be important nosocomial pathogens. Considering that the data related to these organisms are limited in Malaysia, particularly on SCCmec among MR-CoNS in hospitals, more studies should be conducted as the findings could contribute in providing local data as well as assisting clinicians in managing MR-CoNS infections.

1.3 Objectives

1.3.1 General Objective

This study attempted to determine antimicrobial susceptibility pattern and SCCmec type distribution among MR-CoNS species isolated from blood cultures in Hospital Serdang.

1.3.2 Specific Objectives

1. To determine the distribution of MR-CoNS species from blood culture isolates
2. To determine the antimicrobial susceptibility pattern among the isolated MR-CoNS species
3. To detect the SCCmec types among the isolated MR-CoNS species
4. To study the antimicrobial susceptibility pattern among the SCCmec types
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Mitsan, O., & Oladeinde, B. (2016). Staphylococcal cassette chromosome mec (sccmec) typing of methicillin-resistant staphylococci obtained from clinical samples in south-south, Nigeria.


