DETECTION OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT *Staphylococcus aureus* (CA-MRSA) TRANSMISSION IN THE LOCAL COMMUNITY USING MOLECULAR TYPING TECHNIQUES

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DECLARATION

I hereby declare that the content in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledged.

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ABSTRACT

Since the 1990s, Community-acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) has caused infections in population without healthcare-associated risk factors. Unlike nosocomial MRSA, CA-MRSA harbors *pvl* gene and SCC*mec*IV subtype, V, or untypable SCC*mec*. CA-MRSA is usually associated with suppurative infections among children. Children are more predisposed to be CA-MRSA carriers and transmit CA-MRSA to family members. This study aimed to elucidate CA-MRSA transmission in Sabah local community by molecular characterization of MRSA abscess isolates from pediatric patients in Hospital Likas and MRSA nasal isolates from participating family members within a one-year period. Thirty-seven MRSA abscess isolates of index patients which were identified from the cefoxitin antibiotic susceptibility tests were collected from Pathology Laboratory. Nineteen nasal swab samples were collected from family members and processed by the culture on blood agar, Gram staining, catalase test, coagulase test, and DNAse test for *S. aureus* identification. PCR of *mecA* gene were conducted on 37 MRSA abscess isolates and 14 *S. aureus* nasal isolates. PCR of *pvl* gene and PCR for SCC*mec* typing were conducted on MRSA isolates. PVL positive CA-MRSA isolates of index patients and family members which did not harbor similar SCC*mec* elements were tested for *spa* typing. Patients' age, ethnicity, and site of infection were recorded into patient information sheets. Research survey were given to participating family members. This study validated the methicillin resistance status of 37 MRSA abscess isolates and 14 nasal *S. aureus* based on the presence of *mec*A gene. This study supported previous studies where CA-MRSA predominated in abscess infections of pediatric patients. From 37 MRSA abscess isolates, 33 isolates were confirmed as CA-MRSA based on the presence of *pvl* gene and SCC*mec*IVa (n=12), IVc (n=1), V (n=1), or untypable SCC*mec* (n=19). From 37 index cases, only CA-MRSA isolates from 2 cases showed similarities of *spa* genes. CA-MRSA abscess isolates, A1167 (S2) and A5413 (S7), shared similar *spa* types with CA-MRSA isolates from family members, F1167 (S2.1) and F5413 (S7.1), respectively. From 37 CA-MRSA index cases, 18 patients had abscesses on scalp suggesting that infant locomotion development, skin-to-skin contact, and contaminated household surfaces leads to CA-MRSA transmission. This study has

extended the understanding of the CA-MRSA transmission. The data herein could be important in developing antidotes for CA-MRSA infections in Malaysia.

ABSTRAK

PENGESANAN PENYEBARAN STAPHYLOCOCCUS AUREUS RINTANG-METISILIN PEROLEHAN KOMUNITI DALAM KOMUNITI SETEMPAT MENGGUNAKAN TEKNIK-TEKNIK PENGKELASAN MOLEKULAR

Sejak tahun 1990-an, Staphylococcus aureus Rintang-Metisilin Perolehan-Komuniti (CA-MRSA) menyebabkan jangkitan-jangkitan dalam populasi yang tidak mempunyai faktor-faktor risiko berkaitan dengan pusat kesihatan. Berbeza daripada MRSA nosokomial, CA-MRSA mempunyai gen pvl dan SCCmec subtaip IV, V, atau SCCmec yang tidak dapat dikelaskan. CA-MRSA kebiasaannya dikaitkan dengan jangkitan kulit dan tisu lembut dalam kalangan kanak-kanak. Kanak-kanak lebih cenderung menjadi pembawa CA-MRSA dan menyebarkan CA-MRSA kepada ahli-ahli keluarga. Tujuan kajian ini adalah menentukan penyebaran CA-MRSA dalam masyarakat setempat Sabah melalui pencirian molekular isolat-isolat nanah MRSA daripada pesakit pediatrik di Hospital Likas dan isolat- isolat nasal MRSA daripada ahli-ahli keluarga yang menyertai kajian dalam tempoh setahun. Tiga puluh tujuh isolat nanah MRSA pesakit indeks yang dikenalpasti daripada ujian kerentanan antibiotik cakera cefoxitin dikumpul dari Makmal Patologi. Sembilan belas sampel nasal swab dikumpul daripada ahli keluarga dan diproses melalui kultur di atas agar darah, pewarnaan Gram, ujian catalase, ujian coagulase, dan ujian DNase untuk pengenalpastian S. aureus. PCR gen mecA dijalankan ke atas 37 isolat nanah MRSA dan 14 isolat nasal S. aureus. PCR gen pvl dan PCR untuk pengkelasan SCCmec dijalankan ke atas isolat-isolat MRSA. Isolat-isolat CA-MRSA PVL- positif daripada pesakit-pesakit indeks dan ahli-ahli keluarga yang tidak mempunyai SCCmec yang sama diuji untuk pengkelasan spa. Umur, etnik, dan tempat jangkitan pesakit indeks direkod dalam lampiran maklumat pesakit. Borang kaji selidik diberikan kepada ahli keluarga yang menyertai kajian. Kajian ini mengesahkan status kerintangan metisilin 37 isolat nanah MRSA dan 14 isolat nasal S. aureus berdasarkan kehadiran gen mecA. Kajian ini menyokong kajian-kajian lepas di mana CA-MRSA mendominasi dalam jangkitan nanah melibatkan kanak-kanak. Daripada 37 isolat nanah MRSA, 33 isolat disahkan sebagai CA-MRSA berdasarkan kehadiran gen pvl dan SCCmecIVa (n=12), IVc (n=1), V (n=1), atau

SCCmec yang tidak dapat dikelaskan (n=19). Daripada 37 kes indeks, hanya isolat-isolat CA-MRSA daripada 2 kes sahaja menunjukkan persamaan gen-gen spa. Isolat-isolat nanah CA-MRSA, A1167 (S2) dan A5413 (S7), mempunyai kelas spa yang sama dengan isolat-isolat CA-MRSA daripada ahli keluarga, F1167 (S2.1) dan F5413 (S7.1). Daripada 37 kes indeks CA-MRSA, 18 pesakit indeks CA-MRSA mempunyai jangkitan nanah di bahagian kulit kepala menunjukkan bahawa fasa pergerakan bayi, sentuhan kulit ke kulit, dan kontaminasi permukaan isi rumah menyebabkan penyebaran CA-MRSA. Kajian ini memberikan pemahaman yang lebih mendalam tentang penyebaran CA-MRSA. Kajian ini memberikan maklumat penting yang diperlukan untuk kajian lanjut tentang rawatan penyakit CA-MRSA di Malaysia.

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MRSA	-	Methicillin-Resistant *Staphylococcus aureus*
CA-MRSA	-	Community-acquired Methicillin-resistant *Staphylococcus aureus*
HA-MRSA	-	Hospital-acquired Methicillin-Resistant *Staphylococcus aureus*
SCC*mec*	-	Staphylococcus Cassette Chromosome *mec*
PVL/*pvl*	-	Panton-Valentine leukocidin
ST	-	Sequence Type
CC	-	Clonal Complex
MSSA	-	Methicillin-Susceptible *Staphylococcus aureus*
PBP	-	Penicillin-binding protein
PBP2a	-	Penicillin-binding protein 2a
PFGE	-	Pulsed-field gel electrophoresis
TLR2	-	Toll-like receptor 2
µg	-	microgram
µm	-	Micrometer
ml	-	milliliter
mm	-	millimeter
MIC	-	minimum inhibition concentration
MLST	-	Multilocus sequence typing
M-PCR	-	Multiplex PCR
V	-	Volt
CDC	-	Centers for Disease Control and Prevention

UV	-	Ultraviolet
PCR	-	Polymerase chain reaction
pmol	-	picomoles
µL	-	microliter
TBE	-	Tris/Borate/EDTA
mL	-	Mililiter
nm	-	Nanometer
g	-	gram
CFU	-	Colony forming unit
min	-	minute
hr	-	hour
LB	-	Luria-Bertani
s	-	second
rpm	-	revolutions per min
xg	-	G force
ASM	-	American Society for Microbiology
IWG-SCC	-	International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements

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CHAPTER 1

INTRODUCTION

1.1 Background

Staphylococcus aureus is a bacterium which usually inhabits human skin and anterior nares (Ridley, 1959; Wertheim *et al.*, 2006; Nouwen *et al.*, 2004; Tannock, 2011; Williams, 1946). *Staphylococcus aureus* is capable of causing localized suppurative infections and also responsible for deadly systemic infections (Lowy, 1998). Historically, in the 1960s', the introduction of methicillin to replace penicillin for staphylococci infections treatment led to the emergence of Methicillin-Resistance *S. aureus* (MRSA) in the nosocomial settings. MRSA emerged by the integration of a mobile genetic element, Staphylococcal Cassette Chromosome *mec* (SCC*mec*), into *S. aureus* chromosome (International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements (IWG-SCC), 2009). For several decades, MRSA has become a leading pathogen of nosocomial infections such as bacteremia, pneumonia, and urinary tract infections among hospitalized patients who had healthcare-associated risk factors throughout the world (Panlilio *et al.*, 1992; Rosenthal *et al.*, 2008).

However, in the 1990s', MRSA infections with no healthcare-associated risk factors have affected community settings (Udo *et al.*, 1993; Nguyen *et al.*, 2005). Unlike nosocomial MRSA isolates, community-associated MRSA clinical isolates are commonly identified from skin and soft tissue infections. Nosocomial MRSA isolates are multidrug resistant while community-associated clinical MRSA isolates are susceptible to other antibiotics than beta lactams (Herold *et al.*, 1998).

Community-associated MRSA clinical isolates possess different genetic features than nosocomial MRSA isolates (Hageman *et al.*, 2008). Centers for Disease Control and Prevention (CDC) then defined these distinct clinical entities as Community-acquired MRSA (MRSA) (Sowash et al., 2013).

Since the early CA-MRSA emergence, CA-MRSA infections involving young children have become a considerable healthcare concern. CA-MRSA can cause mild skin and soft tissue infections as well as severe diseases among children (Centers for Disease Control and Prevention (CDC), 1999; Adem *et al.*, 2005; Kravitz *et al.*, 2005). There are several factors which cause children to be affected with CA-MRSA infections and become the potential CA-MRSA reservoirs. According to Simon *et al.*, (2015), children have naive immune systems which make them easily exposed to infections. In addition, studies showed that children were more likely to be persistently colonized with *S. aureus* compared to the other age population (Larsson *et al.*, 2011; Armstrong-Esther *et al.*, 1976). *Staphylococcus aureus* persistent colonizers are usually heavily colonized with the bacteria which result in higher chance of disease manifestations and dispersal (Hu *et al.*, 1995). Based on the bacterial aspects, the ability of *S. aureus* to produce various virulence factors, comprising of proteins and enzymes, triggers mechanisms that lead to clinical presentations in host after prolonged colonization (Yamamoto *et al.*, 2012). Moreover, *S. aureus* is more likely to evolve into CA-MRSA because it preferably acquires the small-sized and transferrable SCC*mec* IV or V element compared to the larger-sized healthcare-associated SCC*mec* (Mongkolrattanothai *et al.*, 2003; Deurenberg *et al.*, 2007).

CA-MRSA is transmissible by skin closed contacts (Faden *et al.*, 2001; Knox *et al.*, 2012). As mentioned before, the predominant occurrence of CA-MRSA in children coupled with the tendency of children to frequently interaction with other children or their family members facilitates the CA-MRSA transmission. As a result, identical CA-MRSA strains can be found from the nasal colonization of children who went to the

same kindergartens or children' relatives who lived in the same households (Ho *et al.*, 2012; Biber *et al.*, 2012). CA-MRSA intrafamilial transmission cases which are associated with pediatric children is currently a critical issue since CA-MRSA can be transmitted from the pediatric patients to household members. Not only that, CA-MRSA intrafamilial transmission will lead to serious complications in the communities as the infected individuals in the households can disperse CA-MRSA to the workplace, school, and other areas within the same locality (Urth *et al.*, 2005).

1.2 Problem statement

CA-MRSA infection definition which is designated by Centers for Disease Control and Prevention (CDC) is restricted to community settings. However, recently, CA-MRSA has evolved and disseminated into the nosocomial environments. Currently, the nosocomial settings do not apply the molecular method to distinguish between CA-MRSA and nosocomial MRSA isolates. Thus, this study aims to apply molecular methods to resolve this problem. The source of transmission of CA-MRSA abscess infections which occur among young children without healthcare-associated risk factors is not known. Currently, the clinical management does not implement MRSA screening on family members. This study aims to determine the mode of CA-MRSA transmission by MRSA screening of participating family members and application of molecular methods on the MRSA isolates from pediatric patients and their family contacts.

1.3 Hypotheses

The hypothesis of this research:

In Sabah, CA-MRSA is transmitted via close familial contacts.

1.4 Objectives

i. To validate the methicillin resistance status of MRSA abscess isolates from CA-MRSA index cases and *S. aureus* nasal isolates from family members on the basis of *mecA* gene.

ii. To classify MRSA isolates which were identified from index cases and family members into CA-MRSA based on the presence of *pvl* gene and SCC*mec* types.

iii. To determine the intrafamilial CA-MRSA transmission based on the *spa* types of CA-MRSA isolates from index patients and family members as well as CA-MRSA transmission risk factors based on the patient case note and research survey.

1.5 Significance of Research

The outcome of the study will reveal the temporal distribution of CA-MRSA among pediatric patients in Sabah and help the clinical management to strategize the plans to limit the bacterial dispersal based on information on how the CA-MRSA dispersal occurs in the communities.

CHAPTER 2

LITERATURE REVIEW

This chapter focuses on the discussion of *S. aureus*, its virulence factors and key characteristics. Herein, the history of methicillin resistant *S. aureus* (MRSA), the disparities between the two different types (i.e., nosocomial MRSA and community associated MRSA, CA-MRSA), the global distribution and transmission of CA-MRSA strains and their associated risk factors have been also discussed. Also, molecular typing methods for *S. aureus* identification and classification has been discussed.

2.1 *Staphylococcus aureus*

Staphylococcus aureus is a Gram positive and coccus-shaped bacterium which forms grape-like cell arrangements (staph) under microscopic observation (Foster, 1996). *Staphylococcus aureus* appears to be purplish (Gram positive) because it retains the purple color crystal violet stain in the cytoplasm and cell wall. The washing of decolorizing agent after the penetration of crystal violet iodine complex in the *S. aureus* thick cell wall causes the cell wall to shrink followed by the closing of cell wall pores (Smith & Hussey, 2005). Additionally, *S. aureus* is known to be beta-hemolytic or able to perform complete lysis of red blood cells (American Society for Microbiology (ASM), 2017). *Staphylococcus aureus* is also a normal microflora of human skin (Tannock, 2011; Martin & Whitehead, 1949) and can be found abundantly in nares (Williams, 1946; Guinan, 1982). When the human skin barrier is breached, *S. aureus* become an opportunistic pathogen which invades through the human tissues and causes mild to threatening infections. *Staphylococcus aureus* is a major pathogen of mild skin infections such as carbuncles and furuncles. Severe infections which is