CHARACTERIZATION OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CATS AND DOGS

By

JACKLYN NG WEI SZE

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

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Specially dedicated to my beloved family,
my late grandmother, Han Guang,
Cardio & Lucky

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in partial fulfilment of the requirement for the degree of Master of Science

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Supervisor : Zunita Zakaria, PhD

Faculty : Veterinary Medicine

Globalization has entailed a massive increase in trade and human mobility facilitating the rapid spread of infectious agents, including those that are drug resistant. A particularly serious threat to human health is posed by methicillin-resistant staphylococcal strains which have acquired molecular mechanisms to evade the β-lactam antibiotics (BLAs). The number of methicillin-resistant *Staphylococcus aureus* (MRSA) infection in humans is increasing, thus it seems likely that MRSA infection in animals is becoming more frequent too. This study addressed the significance of MRSA among cats and dogs, animal species that are in close proximity to humans. The first part of the study was designed to characterize the microbiological and biochemical properties of *S. aureus* and MRSA using both conventional and molecular methods. Samples comprising nasal and skin swabs collected from hospitalized and homeless cats and dogs at the University Veterinary Hospital, Universiti Putra Malaysia, (UVH-UPM) and animal shelter operated by the Society for the Prevention of Cruelty to Animals (SPCA) respectively between May to October 2004. The isolation and identification of MRSA using both the conventional and molecular methods in this study were found to be consistent and

reliable. Conventional methods such as differential staining, biochemical tests, Slidex Staph Plus Kit (latex agglutination test) and selective media Mannitol Salt Agar (MSA) and Oxacillin Resistance Screening Agar Base (ORSAB) were used to detect the MRSA from cats and dogs. A total of seven (1.92%) MRSA out of 364 samples were successfully isolated from the animal subjects. These MRSA isolates were also found to be resistant to at least four antimicrobial agents. The second part of the study investigated the microbial resistance of the MRSA using molecular techniques. Polymerase Chain Reaction using specific primers amplified a 533-bp fragment of the *mec A* gene. The partial *mec A* gene sequences exhibited >97% amino acid similarities among the seven local MRSA isolates and the amino acid were highly conserved, and categorized in five different groups. Plasmid analysis indicated the presence of at least three plasmid bands in the seven MRSA isolates. In the last part of the study, the bacterial acid methyl esters (BAMEs) profile of the *S. aureus* and MRSA was characterized in order to detect probable differences between isolates and across host specificities. The branched chain 3-hydroxy dodecanoic, stearic and eicosaenoic acids were the principal fatty acids in both *S. aureus* and MRSA. However, lauric acid was unique to *S. aureus* only, whereas nonanoic acids were found more frequently among MRSA. Gradual changes in the fatty acid composition from *S. aureus* to animal MRSA and finally to human MRSA further reaffirmed the possibility of animal-human MRSA cross-transmissions. To our knowledge, this is the first report on the isolation of MRSA in animals and the use of molecular techniques to confirm the identity of the *mec A* gene from animals in Malaysia. The findings of the study would enable the formulation of suitable measures to control MRSA.

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

PENCIRIAN *STAPHYLOCOCCUS AUREUS* TAHAN METISILIN YANG DIASINGKAN DARIPADA KUCING DAN ANJING

Oleh

JACKLYN NG WEI SZE

Januari 2006

Pengerusi : Zunita Zakaria, PhD

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Globalisasi telah menyebabkan suatu gejala yang luas dan pergerakan manusia mempermudahkan lagi sebaran penyakit yang berjangkit secara mendadak, termasuklah sebaran agen jangkitan yang berentan terhadap ubat-ubatan tertentu. Suatu ancaman tertentu yang serius, terhadap kesihatan manusia adalah strain *Staphylococcus* yang berentan terhadap metisilin yang memerlukan mekanisme molekular untuk menembusi antibiotik β-lactam (BLAs). Bilangan jangkitan "methicillin-resistant *Staphylococcus aureus*" (MRSA) terhadap manusia kian meningkat, maka, ianya amat besar kemungkinan bahawa jangkitan MRSA pada haiwan juga akan menjadi kian kerap. Kajian ini menumpu kepada keertian MRSA di antara kucing dan anjing disebabkan haiwan ini berhubungan rapat dengan manusia. Bahagian pertama dalam kajian ini adalah menggunakan kaedah-kaedah secara konvensional dan molekular untuk menciri *Staphylococcus aureus* dan MRSA. Sampel diambil dari bahagian rongga hidung dan kulit daripada kucing dan anjing di Hospital Veterinar Universiti, UVH dan tempat teduhan haiwan SPCA, dari Mei hingga Oktober 2004. Pemencilan dan pengenalpastian MRSA yang menggunakan kaedah konvensional dan molekular adalah konsisten dan boleh

diharap. Kaedah konvensional seperti pewarnaan gram, ujian-ujian biokimia, Slidex Staph Plus Kit (ujian agglutinasi lateks), serta penggunaan media yang selektif seperti Mannitol Salt Agar (MSA) and Oxacillin Resistance Screening Agar Base (ORSAB) digunakan untuk menentukan MRSA. Tujuh (1.92%) MRSA daripada 364 sampel berjaya dipencilkan daripada kucing dan anjing. MRSA ini juga didapati berentan terhadap sekurang-kurangnya empat agen antimikrobial. Bahagian kedua dalam kajian ini mengkaji kerentanan mikrobial MRSA dengan menggunakan kaedah-kaedah molekular. Amplifikasi PCR dengan menggunakan primer spesifik telah menghasilkan frakmen 533-bp dari gen *mec A*. Sekuen sebahagian gen tersebut menunjukkan >97% persamaan asid amino antara kesemua isolat MRSA, dan asid amino didapati sangat terpelihara serta dikategorikan dalam lima kumpulan. Analisis plasmid membuktikan kehadiran sekurang-kurangnya tiga jalur untuk tujuh isolat MRSA. Bahagian terakhir kajian ini menggunakan asid methyl ester bakteria (BAMEs) untuk menentukan perbezaan antara isolat dan hos secara spesifik *S. aureus* dan MRSA. Rantai bercabang asid 3-hidroksi dodekanoik, asid stearik dan asid eikosaenoik merupakan asid lemak major untuk *S. aureus* dan MRSA. Namun begitu, asid laurik hanya didapati dan adalah unik untuk *S. aureus* sahaja, manakala asid nonanoik didapati lebih banyak untuk MRSA. Perubahan secara gradual dalam komposisi asid lemak *S. aureus* kepada MRSA dari haiwan dan seterusnya kepada manusia mengesahkan sebaran penyakit yang berkemungkinan berlaku antara haiwan dengan manusia. Untuk pengetahuan kita, ini merupakan berita pertama tentang pencirian MRSA daripada haiwan dan penggunaan kaedah molekular untuk mengenal pasti identiti gen *mec A* di Malaysia. Keputusan yang diperolehi daripada kajian ini akan memudahkan cara pencegahan MRSA.

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I certify that an Examination Committee has met on the 3rd of April 2006 to conduct the final examination of Jacklyn Ng Wei Sze on her Master of Science thesis entitled "Characterization of Methicillin-Resistant *Staphylococcus aureus* Isolated From Cats and Dogs" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

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JACKLYN NG WEI SZE

Date: 7th January 2006

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