UNIVERSITI PUTRA MALAYSIA

ANTIMICROBIAL RESISTANCE AND MOLECULAR TYPING OF *STENOTROPHOMONAS MALTOPHILIA* CLINICAL ISOLATES FROM HOSPITAL KUALA LUMPUR

SEYEDEH ZAHRA ROUHANI RANKOUHI

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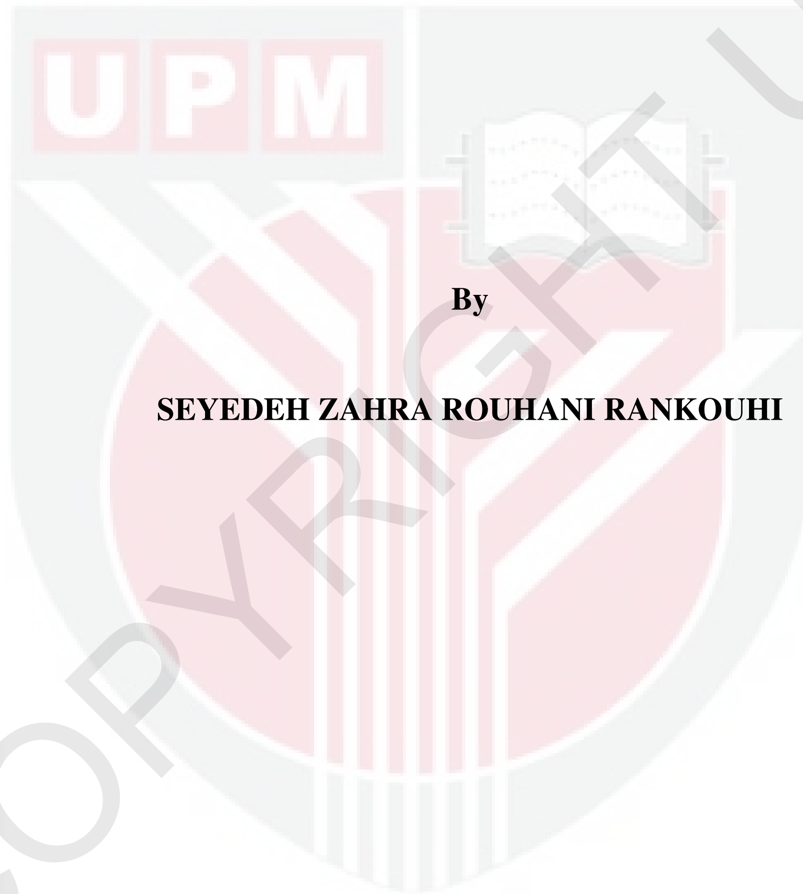

By

SEYEDEH ZAHRA ROUHANI RANKOUHI

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

October 2011

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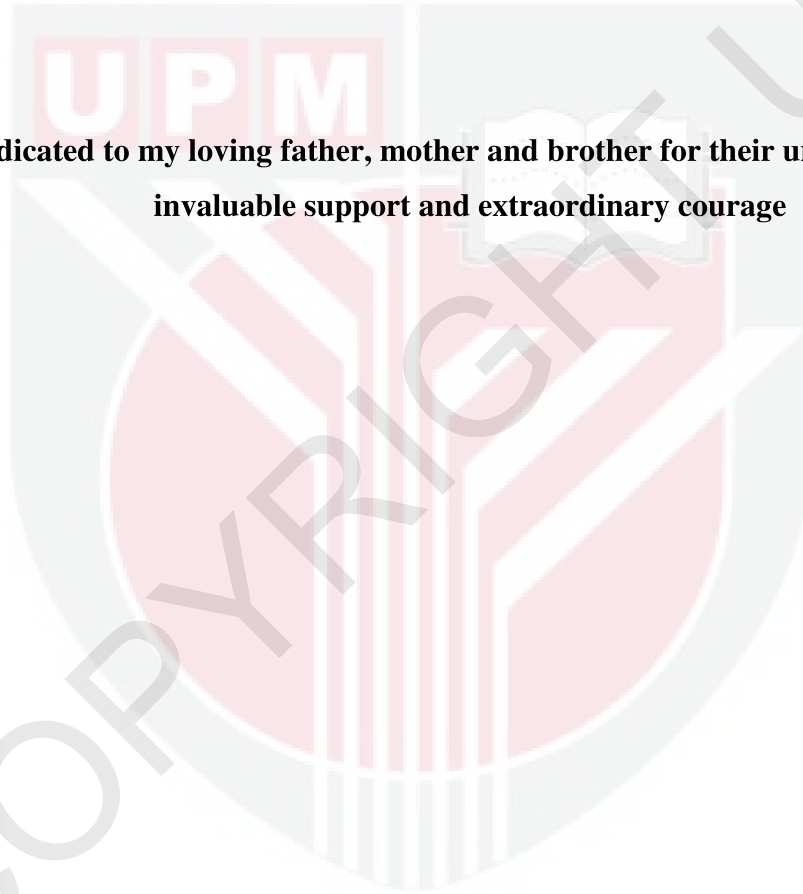
In the Name of God the Compassionate the Merciful



Dedicated to my loving father, mother and brother for their unending love, invaluable support and extraordinary courage

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements of the degree of Master of Science

ANTIMICROBIAL RESISTANCE AND MOLECULAR TYPING OF *STENOTROPHOMONAS MALTOPHILIA* CLINICAL ISOLATES FROM HOSPITAL KUALA LUMPUR

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October 2011

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Stenotrophomonas maltophilia (*S. maltophilia*) is an emerging pathogen, is now recognized as an important cause of hospital acquired infection, causing significant morbidity and mortality especially among immunocompromised patients. *S. maltophilia* cause a wide variety of diseases such as cystic fibrosis, bacteremia, pneumonia, urinary tract infection, surgical site infection, and peritonitis. The lack of proper laboratory diagnosis system and its intrinsic resistance to a plethora of antimicrobial agents that severely limit commonly used empiric standard antimicrobial therapies makes *S. maltophilia* a great challenge in the clinical setting.

In Malaysia, the rate of *S. maltophilia* isolation has increased over the recent years with more than 100 cases in Hospital Kuala Lumpur (HKL) since the year 2003. Therefore, the present study was aimed at developing a selective culture media for the fast and

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accurate isolation of *S. maltophilia* that can be routinely used in diagnostic laboratory and to determine the antibiotic resistance mechanism and molecular epidemiology of local clinical isolates.

In this study, a total of hundred *S. maltophilia* isolates were collected from HKL from January to December 2008, of which only 64 were available for the study, since most of the remaining isolates either failed to grow or were contaminated or were not confirmed as *S. maltophilia*.

In order to develop a selective culture media for *S. maltophilia* isolation, media such as modified mannitol agar and MacConkey agar was prepared with the addition of antibiotics such as vancomycin, mereopenem and amphoterecin. In addition modified DNase toluidine-blue agar (mDTBO) which is a chromogenic medium that specifically selects *S. maltophilia* with an additional property of DNase production was formulated in the present study. All these methods were compared with a previously mVIA mthod. Evaluation of the mDTBO with 64 *S. maltophilia* isolates and a panel of gram negative and gram positive bacteria showed 100% specificity and 75% sensitivity. The reason for reduced sensitivity when queried through DNase tube test, it was found that, some strains produced DNase after 48 hours and few did not produce DNase. From this study, it is found that mDTBO is a fast and accurate isolation media for *S. maltophilia*, however, isolates that show negative result on agar plate but having a positive clinical implication need to be confirmed by DNase tube test. Compared to mDTBO, mVIA is more selective with 98% sensitivity. Among the four methods compared for the isolation

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of *S. maltophilia* mVIA agar gave maximum sensitivity compared to blood agar, MacConkey agar and mDTBO agar.

Resistance is usually mediated by *sul1*, *sul2* genes carried by integrons or plasmids. The antibiotic susceptibility pattern and the genes coding for *sul1*, *sul2*, and class I and class II integrons are investigated in the Malaysian isolates. Of the 64 isolates tested, one isolate (1.56%) showed resistance (MIC > 32 mg/L) to TMP/SMX which possessed *sul1* gene and carried the class I integron. None of the isolates carried *sul2* or class 2 integron. All isolates were 100% resistant to meropenem, imipenem and piperacillin/tazobactam. All *S. maltophilia* isolates were susceptible to Minocycline (MH). This study presents the first report on TMP/SMX resistance in *S. maltophilia* in Malaysia. Since TMP/SMX is the mainstay therapy for *S. maltophilia* infection, monitoring its resistance is important, as it has the potential to increase by means of mobile elements. As from the current study, the antibiotic minocycline is found to be promising with 100% susceptibility *towards S. maltophilia,* it is recommended for consideration.

To determine the epidemiology of *S. maltophilia* in the largest tertiary care hospital in Malaysia (HKL), 63 isolates were genotyped by Pulsed Field Gel Electrophoresis (PFGE) technique using the *Spe*I restriction endonuclease enzyme. Analysis of the PFGE banding patterns by BioNumerics 6.1 software yielded 58 distinct patterns, including 5 clusters of patterns with 100% and 3 clusters with over 80% similarity. Some isolates that showed close relatedness were isolated from the same patient (isolates 2 and 3, 6 and 7), but in some cases, isolates were from different patients,

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sources and wards (55 and 12, 1 and 50, 41 and 46). In some cases, strains isolated from the same patients were genetically distinct. The results from PFGE analysis indicate that *S. maltophilia* isolates are genetically diverse and polyclonal emergence is possible, but person to person transmission of strains across the ward is not common.

In conclusion, this study has developed an mDTBO media for the fast and correct isolation of *S. maltophilia* however mVIA with better sensitivity is more suitable for routine use in diagnostic labs. Current study reports the first TMP/SMX resistant *S. maltophilia* in Malaysia. The epidemiological investigation has shown that *S. maltophilia* strains are genetically diverse with possible polyclonal emergence. Prompt diagnosis, early treatment with appropriate drugs and epidemiological monitoring across the country is crucial for the effective management of this rapidly emerging nosocomial pathogen *S. maltophilia.*

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

KERINTANGAN TERHADAP ANTIMIKROB SERTA PENJENISAN MOLEKUL ISOLAT KLINIKAL *STENOTROPHOMONAS MALTOPHILIA* DARI HOSPITAL KUALA LUMPUR

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Stenotrophomonas maltophilia merupakan patogen yang semakin menonjol dan dikenali sebagai salah satu penyebab penting jangkitan dari hospital yang menyebabkan kadar morbiditi dan kematian ketara terutama di kalangan pesakit yang imunnya terjejas. *S. maltophilia* boleh menyebabkan pelbagai jenis penyakit seperti cystic fibrosis, bacteremia, radang paru-paru, jangkitan sistem urin, jangkitan luka pembedahan, dan peritonitis. Kekurangan dalam sistem diagnosis yang sesuai serta daya tahan bakteria ini terhadap pelbagai agen antimikrob membantutkan terapi antimikrob piawai yang sering digunakan dan menjadikan *S. maltophilia* cabaran besar dalam persekitaran klinikal.

Di Malaysia, kadar pengasingan *S. maltophilia* meningkat sejak kebelakangan ini dengan lebih daripada 100 kes didapati daripada Hospital Kuala Lumpur sejak tahun 2003. Oleh itu, kajian ini bertujuan untuk menghasilkan media kultur selektif bagi

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mengasingkan *S. maltophilia* dengan pantas dan tepat yang boleh digunakan di makmal diagnostik serta mengenalpasti mekanisma rintang antibiotik dan epidimiologi molekul isolat klinikal tempatan.

Dalam kajian ini, sebanyak satu ratus isolat *S. maltophilia* diperolehi daripada HKL dari Januari hingga Disember 2008. Namun hanya 64 isolat sahaja dapat digunakan oleh kerana isolat selebihnya sama ada tidak hidup, tercemar, atau tidak dapat disahkan sebagai *S. maltophilia*.

Untuk menghasilkan media kultur selektif bagi mengasingkan *S. maltophilia*, media seperti agar mannitol dan MacConkey yang diubahsuai telah disediakan dengan menambahkan antibiotik seperti vancomycin, mereopenem dan amphoterecin. Tambahan pula, agar DNase toluidune-blue terubahsuai (mDTBA) yang merupakan medium kromogen yang membolehkan pemilihan *S. maltophilia* secara spesifik dengan kelebihan menghasilkan DNase telah dihasilkan dalam kajian ini. Kesemua kaedah tersebut telah dibandingkan menggunakan kaedah mVIA terdahulu. Penilaian penggunaan mDTBA dengan 64 isolat *S. maltophilia* bersama sepanel bakteria gram positif dan negatif menunjukkan ianya 100% spesifik dan 75% sensitif. Tahap sensitif yg berkurangan apabila melalui ujian tiub DNase adalah kerana beberapa stren menghasilkan DNase selepas 48 jam manakala beberapa stren lain tidak. Dari kajian ini, didapati bahawa mDTBA adalah media pengasingan yang cepat dan tepat bagi *S. maltophilia* namun, isolat yang didapati negatif di atas agar tetapi mempunyai implikasi klinikal yang positif perlu disahkan melalui ujian tiub DNase. Berbanding dengan mDTBO, mVIA adalah lebih sensitif dengan kadar 98% sensitif. Di antara kaedah-

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kaedah yang dibandingkan bagi pemencilan *S. maltophilia*, agar mVIA memberikan kadar sensitif maksimum berbanding dengan agar darah, MacConkey, dan mDTBO.

Kerintangan lazimnya dibantu oleh gen *sul1* dan *sul2* yang dibawa oleh integron atau plasmid. Corak kerentanan antibiotik dan gen yang mengkod untuk *sul1*, *sul2*, dan integron kelas I dan II dikaji di kalangan isolat Malaysia. Daripada 64 isolat yang diuji, satu (1.56%) menunjukkan rintangan (MIC > 32 mg/L) terhadap TMP/SMX yang mempunyai gen *sul1* dan membawa integron kelas 1. Tiada isolat yang memnbawa *sul2* atau integron kelas 2. Kesemua isolat adalah 100% rintang terhadap meropenem, imipenem, dan piperacillin/tazobactam. Kesemua isolat *S. maltophilia* adalah rentan terhadap Minocycline (MH). Kajian ini adalah yang pertama melaporkan kerintangan terhadap TMP/SMX oleh *S. maltophilia* di Malaysia. Memandangkan TMP/SMX merupakan terapi andalan bagi jangkitan *S. maltophilia,* pemantauan kerintangan adalah penting kerana kadar kerintangan ini berpotensi untuk meningkat melalui unsur mudahalih. Dari kajian ini, antibiotik minocycline memberikan harapan dengan kerentanan 100% oleh *S. maltophilia* dan disyor untuk dipertimbangkan.

Bagi menentukan epidimiologi *S. maltophilia* dalam hospital jagaan tertier terbesar di Malaysia (HKL), penjenisan gen dilakukan ke atas 63 isolat melalui teknik Pulsed Field Gel Electrophoresis (PFGE) menggunakan enzim 'restriction endonuclease' *Spe*I. Analisis corak penjaluran PFGE oleh perisisan komputer BioNumerics 6.1 menghasilkan 59 corak berlainan, termasuklah 5 gugusan corak dengan 100% persamaan serta 3 gugusan dengan lebih 80% persamaan. Beberapa isolat yang menunjukkan hubungkait rapat telah diasingkan daripada pesakit-pesakit yang sama

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(isolat 2 dan 3, 6 dan 7), tapi dalam beberapa kes, isolat diasingkan daripada pesakit, sumber, dan wad yang berlainan (55 dan 12, 1 dan 50, 41 dan 46). Dalam beberapa kes, stren yang diasingkan daripada pesakit yang sama menunjukkan hubungan genetik yang berlainan. Keputusan daripada analisis PFGE menunjukkan yang isolat *S. maltophilia* adalah pelbagai dari segi genetik dan kemunculan poliklonal adalah mungkin, tetapi pemindahan stren dari seseorang ke seseorang yang lain dalam wad adalah tidak lazim.

Kesimpulannya, kajian ini telah menghasilkan media mDTBA untuk pengasingan *S. maltophilia* yang cepat dan tepat, namun kaedah mVIA yang lebih sensitif adalah lebih sesuai untik penggunaan lazim dalam makmal diagnostik. Kajian ini melaporkan *S. maltophilia* rintang terhadap TMP/SMX yang pertama di Malaysia. Siasatan epidimiologi menunjukkan bahawa stren *S. maltophilia* adalah berbeza dari segi genetik dengan kemungkinan kemunculan poliklonal. Diagnosis yang segera, rawatan awal dengan ubat yang sesuai, serta pemantauan epidimiologi seluruh negara adalah penting untuk pengurusan yang berkesan dalam menangani *S. maltophilia*, patogen hospital yang kian muncul ini.

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I certify that a Thesis Examination Committee has met on 12 October 2011 to conduct the final examination of Seyedeh Zahra Rouhani Rankouhi on her thesis entitled "Antimicrobial Resistance and Molecular Typing of *Stenotrophomonas maltophilia* Clinical Isolates from Hospital Kuala Lumpur" in accordance with the Universities and University College 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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DECLARATION

I declare that the thesis is my original work except for quotations and citation which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for other degree at Universiti Putra Malaysia or other institutions.

SEYEDEH ZAHRA ROUHANI RANKOUHI

Date: October 2011

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