



**UNIVERSITI PUTRA MALAYSIA**

**MOLECULAR CHARACTERISATION OF EXTENDED-SPECTRUM  
BETALACTAMASE-PRODUCING *ESCHERICHIA COLI* IN SELAYANG  
HOSPITAL, MALAYSIA**

**RUSMAH BINTI YUSOF**

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**MOLECULAR CHARACTERISATION OF EXTENDED-SPECTRUM BETA-LACTAMASE- PRODUCING *ESCHERICHIA COLI* IN SELAYANG HOSPITAL, MALAYSIA**



**By  
RUSMAH BINTI YUSOF**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra  
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Science**

**August 2010**



***To my husband, Appri Beyan; my daughters, Afrina and Rifqah; and my sons, Irfan, Akid and Iman for their love, understanding and encouragements***

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

**MOLECULAR CHARACTERISATION OF EXTENDED SPECTRUM BETA-LACTAMASE-PRODUCING *ESCHERICHIA COLI* IN SELAYANG HOSPITAL, MALAYSIA**

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**August 2010**

**Chairman: Associate Professor Zamberi Sekawi, MD**

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Infection caused by extended spectrum beta-lactamases *E. coli* (ESBLs-*E. coli*) strains, which are resistant to many classes of antibiotics, is an important nosocomial infection and one of the public health concerns in this country. The molecular characteristics of these organisms in the local setting were insufficiently documented. The recent increase in diversity of the ESBL types and the prevalence of emerging CTX-M types worldwide in the hospital setting and also in the community, warrants a study to improve detection protocols. Although most CTX-M types preferentially hydrolyse cefotaxime and are therefore resistant to cefotaxime rather than ceftazidime, currently the appearance of CTX-M types capable of hydrolysing both ceftazidime as well as cefotaxime at a high level of efficiency has made phenotypic recognition difficult. Confusion arises when some CTX-M types are also capable of hydrolysing ceftazidime instead of cefotaxime. Therefore, this study was conducted to portray a preliminary genetic characterisation as well

as the clonal relatedness of ESBL-producing *E. coli* from a tertiary hospital in Malaysia, and to resolve the difficulty in the identification of ESBL types based on phenotypic traits. The alternative approach using molecular methods to specifically detect CTX-M types was investigated.

ESBL types, from a total of sixteen isolates obtained over the months of January to December 2006 from Hospital Selayang, were characterized by PCR and DNA sequencing based on genes encoding for *bla<sub>tem</sub>*, *bla<sub>shv</sub>* and *bla<sub>CTX-M</sub>*, respectively. Clonal relatedness of the isolates was determined by MLST and plasmid profiling. The CTX-M  $\beta$ -lactamase gene produced by *E. coli* was detected by a membrane assay based on an optimised dot blot hybridisation technique.

All strains that were phenotypically determined to be ESBL were found to carry at least one of the ESBL genes. The prevalence of *bla<sub>CTX-M</sub>* and *bla<sub>TEM</sub>* genes was 81.3% and 75%, respectively, while 12.5% carried the *bla<sub>SHV</sub>* gene. This study demonstrated that CTX-M types are dominant strains. This is parallel to the worldwide trend where CTX-M types have overtaken the global dominance of traditional TEM- and SHV-types. In view of the confusion surrounding *bla<sub>CTX-M</sub>* detection based on phenotypic hydrolysing of ceftazidime and cefotaxime, the molecular characterisation of CTX-M was further elucidated. Further determination of CTX-M nucleotide and deduced protein sequences showed that 46% were found to be CTX-M-15, 31% were CTX-M-14, 15% were CTX-M-69 and 8% were CTX-M-3. The emergence of CTX-M-15, a particularly common type, was also revealed in this study. This

study also illustrated MLST typing as the first molecular surveillance tool developed for local ESBL *E. coli*. The finding revealed the presence of several new ESBL *E. coli* strains with new strain type (ST) numbers assigned from the global MLST database published on the Web. A dendrogram which was developed based on the MLST STs revealed clustering of strains isolated from a local hospital setting based on allelic profiles.

A rapid genotypic-based method for detection of CTX-M illustrated that a CTX-M biotin-labelled probe, designed from consensus DNA sequences of local CTX-M variants of *E. coli*, were sensitive and specific for the respective *E. coli* target genes. The developed probe was able to detect local CTX-M variants as well as a number of CTX-M control strains. The optimised membrane assay, using the colorimetric detection technique, was capable of completing the assignment within one working day without specific molecular equipments. This finding suggests that with further independent validation, the detection of CTX-M types by dot blot assay can be potentially used in Diagnostic Clinical Microbiology Laboratories to replace current phenotypic detection methods.

In essence, the confusion surrounding the phenotypic detection of CTX-M warrants resolution since these strains are more dominant than *bla*<sub>TEM</sub> or *bla*<sub>Shv</sub>. The rapid genotypic-based method investigated in the study consistently detected the CTX-M gene in all phenotypic-positive CTX-M strains. In addition, the differentiation of CTX-M types was also elucidated through molecular approaches. The establishment of the MLST-typing

method is another highlight of this research since this is the first MLST-typing study for local *E. coli* ESBL stains. The rapid CTX-M genotypic detection assay is very noteworthy since the assay provided an easy, fast and reliable method of detecting various CTX-M genes from local ESBL-producing *E. coli* as well as control strains from Canada and France.



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**KARAKTERISASI MOLEKUL SPEKTRUM LANJUTAN BETA-  
LAKTAMASE-MEMPRODUKSI *ESCHERICHIA COLI* DI HOSPITAL  
SELAYANG, MALAYSIA**

Oleh

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**Ogos 2010**

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Jangkitan yang disebabkan oleh strain-strain spektrum lanjutan *E. coli* beta-laktamase (ESBL- *E. coli*) yang rintang terhadap pelbagai kelas antibiotik merupakan jangkitan nosokomial yang penting dan menjadi suatu perkara yang membimbangkan kesihatan awam di negara ini. Karakteristik molekul organisma-organisma ini di arena tempatan kurang didokumentasikan. Kebelakangan ini, terdapat peningkatan kepelbagaian jenis ESBL serta prevalen jenis CTX-M yang muncul dan tersebar luas di seluruh dunia samada di hospital juga di dalam komuniti. Oleh yang demikian, Kajian untuk menambahbaikkan protokol pengesanan organisma jenis ESBL dan CTX-M ini adalah sangat perlu. Kebanyakan jenis CTX-M menghidrolisis cefotaxime secara memilih dan oleh itu rintang kepada cefotaxime dan bukan ceftazidime, walaubagaimana pun kini dengan adanya kemunculan jenis-jenis CTX-M yang mampu menghidrolisis kedua-dua ceftazidime dan cefotaxime pada tahap kecekapan yang tinggi telah menyukarkan pengecaman fenotifik organisma tersebut. Kekeliruan timbul apabila

beberapa jenis CTX-M juga mampu menghidrolisis ceftazidime dan bukan cefotaxime. Maka kajian ini dilaksanakan untuk memberikan gambaran awal karakteristik genetik dan pertalian klon *E. coli* yang menghasilkan ESBL dari hospital tertuari di Malaysia. Kajian ini juga dijalankan untuk mengatasi kesulitan dalam pengecaman jenis-jenis ESBL yang berdasarkan kepada sifat-sifat fenotipik. Pendekatan alternatif menggunakan kaedah molekul untuk mengesan jenis CTX-M secara spesifik juga telah disiasat.

Jenis-jenis ESBL dari sejumlah enam belas isolat yang diperolehi dari Hospital Selayang antara bulan Januari hingga Disember 2006 telah dikarakterisasikan menggunakan PCR dan turutan DNA yang berdasarkan gen-gen mengekodkan untuk *bla<sub>tem</sub>*, *bla<sub>shv</sub>* dan *bla<sub>CTX-M</sub>*. Pertalian klonal isolat-isolat telah ditentukan dengan MLST dan pemprofilan plasmid. Gen  $\beta$ -lactamase CTX-M yang dihasilkan oleh *E. coli* telah dikesan melalui satu ujian membran berdasarkan teknik “dot blot hybridisation” yang telah dioptimumkan.

Semua jenis *E. coli* yang telah ditentukan sebagai ESBL secara fenotipik didapati membawa sekurang-kurangnya satu gen ESBL. Prevalen gen-gen *bla<sub>CTX-M</sub>* dan *bla<sub>TEM</sub>* masing-masing adalah 81.3% dan 75%, manakala 12.5% membawa gen *bla<sub>SHV</sub>*. Kajian ini menunjukkan bahawa jenis-jenis CTX-M adalah jenis paling dominan. Ini adalah sejajar dengan tren di seluruh dunia di mana jenis-jenis CTX-M telah menawan kedominanan global jenis-jenis tradisional TEM dan SHV. Karakterisasi molekul CTX-M telah diperjelaskan untuk mengatasi kekeliruan dalam pengesanan *bla<sub>CTX-M</sub>* yang berdasarkan

hidrolisis fenotipik ceftazidime dan cefotaxime. Penentuan lanjutan nukleotida dan kesimpulan jujukan-jujukan protein CTX-M menunjukkan bahawa 46% terdiri daripada CTX-M-15, 31% adalah CTX-M-14, 15% adalah CTX-M-69 dan 8% adalah CTX-M-3. Kemunculan CTX-M-15, iaitu jenis CTX-M yang sangat biasa diasingkan, juga telah didedahkan dalam kajian ini. Kajian ini telah dapat menggambarkan penggunaan "MLST-typing" sebagai alat surveilan molekul pertama yang dibangunkan untuk *E. coli* ESBL tempatan. Penemuan ini juga telah menunjukkan adanya beberapa jenis *E. coli* ESBL baru. *E. coli* ESBL tempatan yang baru ini ditunjukkan dengan nombor-nombor jenis strain baru (ST) yang diberikan daripada pangkalan data MLST global yang diterbitkan di dalam jaring. Dendrogram yang dibangunkan berdasarkan ST-ST MLST tersebut telah menunjukkan strain yang diisolasi dari hospital tempatan dikelompokkan mengikut profil alelik.

Satu kaedah genotipik yang cepat untuk pengesanan CTX-M telah menunjukkan bahawa prob CTX-M berlabel biotin yang direka daripada jujukan-jujukan DNA dari sepersetujuan varian-varian *E. coli* CTX-M tempatan adalah peka dan spesifik untuk gen-gen sasaran *E. coli* berkenaan. Prob yang telah direka itu mampu mengesan varian-varian CTX-M tempatan, termasuk beberapa jenis CTX-M kawalan. Ujian membran yang telah dioptimumkan dan menggunakan teknik pengesanan kolorimetrik mampu menyelesaikan ujian ini dalam satu hari bekerja tanpa peralatan-peralatan molekul yang spesifik. Penemuan ini mencadangkan dengan validasi lanjut yang berasingan, pengesanan jenis-jenis CTX-M melalui ujian "dot blot" ini mempunyai potensi untuk dijalankan di Makmal Mikrobiologi

Diagnostik Klinikal sebagai menggantikan kaedah-kaedah pengesanan fenotipik yang digunakan masa kini.

Kesimpulannya, kekeliruan yang dihadapi dalam pengesanan fenotipik CTX-M perlu diselesaikan kerana kedominanan jenis-jenis tersebut adalah lebih tinggi berbanding dengan *bla*<sub>TEM</sub> atau *bla*<sub>shv</sub>. Kaedah yang cepat berdasarkan genotip yang telah disiasat dalam kajian ini berupaya mengesan gen CTX-M dalam semua jenis CTX-M fenotipik positif secara konsisten. Pembezaan jenis-jenis CTX-M juga telah diperjelaskan melalui pendekatan molekul. Satu lagi kemuncak penyelidikan ini adalah kaedah "MLST-typing", kerana ini merupakan kajian "MLST-typing" yang pertama untuk jenis-jenis *E. coli* ESBL tempatan. Kaedah pengesanan CTX-M yang cepat berdasarkan genotip merupakan satu kaedah yang sangat signifikan dimana ia merupakan satu kaedah yang mudah, segera dan boleh dipercayai untuk mengesan pelbagai gen CTX-M daripada *E. coli* tempatan yang menghasilkan ESBL dan juga strain kawalan dari Kanada dan Perancis.

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I certify that a Thesis Examination Committee has met on 24 August 2010 to conduct the final examination of Rusmah Binti Yusof on her Master thesis entitled “Molecular Characterisation of Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Selayang Hospital, Malaysia” in accordance with Universities and University College Act 1971 and the constitution of 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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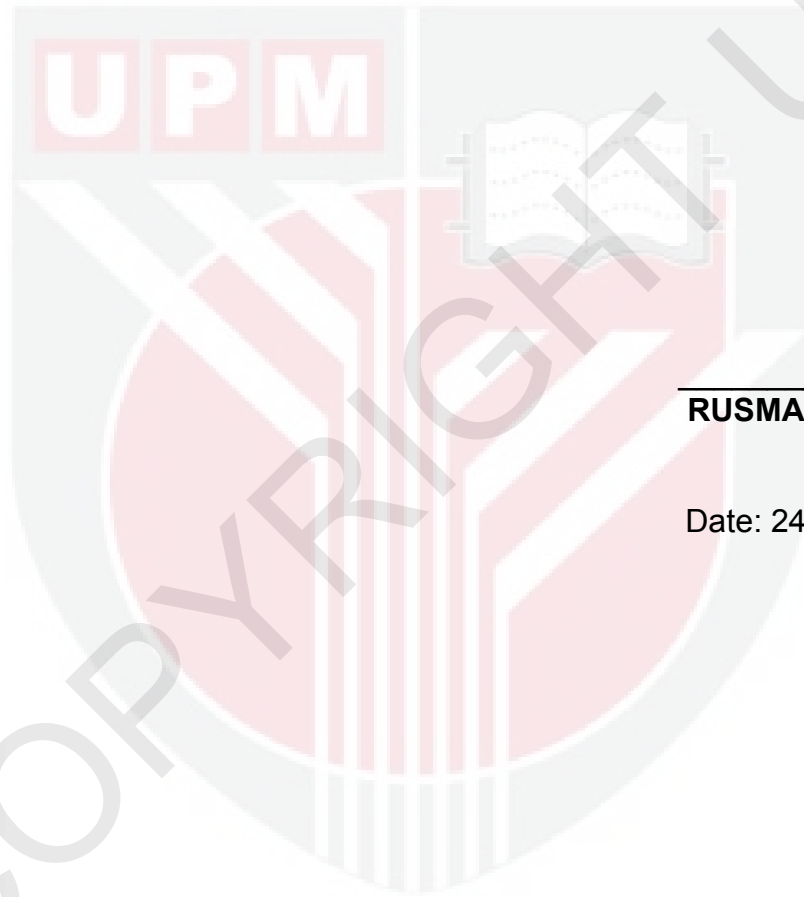
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## DECLARATION

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



**RUSMAH BINTI YUSOF**

Date: 24 August 2010

## TABLE OF CONTENTS

|   | Page  |
|---|-------|
| <b>DEDICATION</b>                                       | ii    |
| <b>ABSTRACT</b>   | iii   |
| <b>ABSTRAK</b>  | vii   |
| <b>ACKNOWLEDGEMENTS</b>                                 | xi    |
| <b>APPROVAL</b>   | xiii  |
| <b>DECLARATION</b>                                      | xv    |
| <b>LIST OF TABLES</b>                                   | xix   |
| <b>LIST OF FIGURES</b>                                  | xx    |
| <b>LIST OF ABBREVIATION</b>                             | xxiii |
| <b>CHAPTER</b>  |       |
| <b>1 INTRODUCTION</b>                                   | 1     |
| <b>2 LITERATURE REVIEW</b>                              |       |
| 2.1 <i>Escherichia coli</i>                             | 7     |
| 2.2 Antimicrobial resistance                            | 8     |
| 2.3 Nosocomial and Community Acquired Infections        | 9     |
| 2.4 Extended-Spectrum $\beta$ -lactamases (ESBLs)       | 11    |
| 2.5 TEM, SHV, and CTX-M types of $\beta$ lactamases     | 14    |
| 2.6 Infections due to ESBL-producing <i>E. coli</i>     | 17    |
| 2.7 Detection Methods of ESBLs                          | 22    |
| 2.7.1 Phenotypic Confirmatory Test for ESBL production. | 23    |
| 2.7.2 ESBLs detection methods by commercial device      | 24    |
| 2.7.3 ESBLs detection via molecular methods             | 25    |
| 2.8 Multilocus Sequence Typing                          | 26    |
| 2.9 Application of MLST                                 | 28    |
| 2.10 Plasmid Profile Analysis                           | 30    |
| 2.11 Polymerase Chain Reaction                          | 30    |
| 2.12 Oligonucleotide Probe                              | 31    |
| 2.13 Dot Blot Hybridisation                             | 33    |
| 2.14 DNA Sequencing                                     | 34    |
| <b>3 MATERIAL AND METHODS</b>                           |       |
| 3.1 Bacterial Isolates                                  | 36    |
| 3.1.1 Bacterial Collection                              | 36    |
| 3.1.2 Phenotypic identification                         | 36    |
| 3.1.3 Storage and Maintenance of Bacterial Isolates     | 37    |
| 3.1.4 Control Strains                                   | 37    |
| 3.1.5 Antimicrobial Susceptibility Testing              | 37    |
| 3.2 DNA Preparation                                     | 38    |
| 3.2.1 Genomic DNA Extraction                            | 38    |

|          |   |    |
|----------|---|----|
| 3.2.1    | Analysis of Chromosomal and plasmid DNA by Gel Electrophoresis  | 42 |
| 3.2.2    | Chromosomal and Plasmid DNA Quantitation  | 43 |
| 3.2      | Multilocus Sequence Typing (MLST)   | 43 |
| 3.3.1    | Choice of loci  | 44 |
| 3.2.2    | MLST Polymerase Chain Reaction (PCR) amplification  | 45 |
| 3.3.3    | Analysis of PCR product by Gel Electrophoresis  | 55 |
| 3.3.4    | PCR purification  | 55 |
| 3.3.5    | Nucleotide sequence determination   | 56 |
| 3.3.6    | MLST data analysis  | 56 |
| 3.3.7    | Dendrogram  | 57 |
| 3.4      | Determination of Plasmid Profile  | 58 |
| 3.5      | $\beta$ - lactamase genes identification  | 58 |
| 3.5.1    | Evaluation of Genomic DNA extraction Protocol   | 58 |
| 3.5.2    | Primers and PCR amplification protocol  | 59 |
| 3.5.3    | Nucleotide Sequence Analysis for Detecting ESBL type  | 59 |
| 3.6      | Development of a probe based on CTX-M fragment sequences for the detection of CTX-M types of ESBL producing <i>E. coli</i>                      | 60 |
| 3.6.1    | Designing an oligonucleotide biotin-labelled Probe  | 60 |
| 3.6.2    | Development of a membrane assay by dot blot technique using biotin-labelled probe   | 61 |
| 3.6.3    | The Sensitivity of Oligonucleotide Biotin-Labelled Probe.   | 65 |
| 3.5.4    | The specificity of Oligoneucleotide Biotin-Labeled Probe  | 65 |
| 3.7      | Molecular Marker to differentiate NIs and NNIs CTX-M-types producing <i>E. coli</i> based on local strains CTX-M-types producing <i>E. coli</i> | 66 |
| <b>4</b> | <b>RESULTS</b>  | 69 |
| 4.1      | Bacterial Isolates  | 69 |
| 4.1.1    | Antibiotic Sensitivity Profile  | 70 |
| 4.2      | DNA Preparation   | 70 |
| 4.2.1    | Rapid chromosomal DNA preparation by boiling method   | 70 |
| 4.2.2    | Analysis of chromosomal DNA preparation by commercial DNA extraction kit.   | 71 |
| 4.2.3    | Plasmid DNA Extraction  | 71 |
| 4.3      | Multilocus Sequence Typing (MLST) Data Analysis   | 72 |
| 4.3.1    | MLST Polymerase Chain Reaction (PCR) amplification  | 72 |

|          |   |     |
|----------|---|-----|
| 4.3.2    | MLST Sequence Types Analysis  | 81  |
| 4.3.3    | MLST Sequence Types Analysis by manual method   | 86  |
| 4.3.4    | MLST Dendrogram   | 89  |
| 4.4      | $\beta$ -lactamase Genes Identification   | 94  |
| 4.4.1    | Evaluation of Genomic DNA extraction in PCR amplification of <i>bla</i> <sub>tem</sub>  | 94  |
| 4.5      | Plasmid Profile   | 98  |
| 4.6      | Development of a probe based on CTX-M fragment sequences for the detection of CTX-M types of ESBL producing <i>E. coli</i>                                | 100 |
| 4.6.1    | Designing of an oligonucleotide biotin-labelled probe   | 100 |
| 4.6.2    | Membrane assay by dot blot technique using biotin-labelled probe  | 101 |
| 4.6.3    | Sensitivity of oligonucleotide biotin-labelled CTX-M probe for detection of CTX-M-types producing <i>E. coli</i> .  | 103 |
| 4.6.4    | Specificity of oligonucleotide biotin labelled CTX-M probe for detection of CTX-M-types producing <i>E. coli</i>  | 104 |
| 4.7      | Molecular Marker to differentiate NIs and NNIs CTX-M-types producing <i>E. coli</i> based on local strains of CTX-M-types producing <i>E. coli</i>        | 105 |
| <b>5</b> | <b>DISCUSSION</b>   |     |
| 5.1      | ESBL-producing <i>E. coli</i> in tertiary hospital in Malaysia  | 107 |
| 5.2      | Optimisation of genotypic method enables successfully amplification of several genes  | 108 |
| 5.3      | MLST  | 110 |
| 5.3.1    | MLST analysed by <i>E. coli</i> MLST public domain  | 111 |
| 5.3.2    | Comparisons of MLST public domain, In-house MLST and plasmid profile  | 117 |
| 5.4      | Characteristics of local ESBL-producing <i>E. coli</i> based on $\beta$ -lactamase genes (SHV, TEM and CTX-M)   | 120 |
| 5.5      | CTX-M biotin-labelled probe for detection and identification of local CTX-M – types ESBLs-producing <i>E. coli</i> by use of dot blot hybridisation assay | 122 |
| 5.6      | Molecular Marker to differentiate NIs and NNIs CTX-M-types producing <i>E. coli</i> based on local strains CTX-M-types producing <i>E. coli</i>           | 126 |
| <b>6</b> | <b>CONCLUSION</b>   | 129 |
|          | <b>REFERENCES</b>   | 134 |
|          | <b>APPENDICES</b>   | 150 |
|          | <b>BIODATA OF THE STUDENT</b>   | 172 |