# DETECTION AND CHARACTERIZATION OF UROPATHOGENIC *ESCHERICHIA COLI* SEQUENCE TYPE 131 IN SABAH, MALAYSIA

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**FACULTY OF MEDICINE AND HEALTH SCIENCES**
**UNIVERSITI MALAYSIA SABAH**
**2018**

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# DETECTION AND CHARACTERIZATION OF UROPATHOGENIC *ESCHERICHIA COLI* SEQUENCE TYPE 131 IN SABAH, MALAYSIA

**LAI YUN MEI**

**THESIS SUBMITTED IN FULFILMENT FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

**FACULTY OF MEDICINE AND HEALTH SCIENCES**
**UNIVERSITI MALAYSIA SABAH**
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## DECLARATION

The materials in this thesis are original except for quotations, accepts, summaries and references, which have been duly acknowledged.

22 FEBRUARY 2018

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**CERTIFICATION**

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VIVA DATE : **09 JULY 2018**

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## ABSTRACT

*Escherichia coli* (*E. coli*) Sequence type 131 (ST131) is the multi-drug resistant pathogen causing urinary tract infection (UTI) as well as other extra-intestinal infections and intestinal infections in human. It is the pandemic pathogen and spreading worldwide. Mega-plasmids carried by ST131 contain multiple drug resistance gene and virulence encoding genes together. MLST was used to identify this pathogen by sequencing seven housekeeping genes for each sample. The heavy workload and time-consuming procedure of MLST ST131 need to be overcome for the detection of ST131. In Sabah, the presence of ST131, ST131 clade C sub-linages (ST131*H30*, ST131*H30R* and ST131*H30Rx*), drug resistance genes profile, the virulence factor profile and type of replicon in ST131 remained unknown. In this study, Uropathogenic *E. coli* (UPEC) 294 samples were isolated from UTI patients from Hospital Queen Elizabeth I and *Hospital Wanita Dan Kanak-Kanak Sabah*. From these samples, multi-drug resistant isolate was detected by antibiotic susceptibility tests. ST131 isolates were investigated among these ciprofloxacin resistant samples by Achtman Scheme. Duplex mismatch amplification mutation assay - Polymerase Chain Reaction (MAMA-PCR) was developed with the identified ST131 isolates. Clade C sub-lineage was identified among the ST131 isolates. Common virulence encoding genes, drug resistance genes and types of replicon were identified from the ST131 isolates. Seventeen ST131 isolates were identified among the UPEC isolates. Duplex MAMA-PCR was applied as the rapid detection method for detection of ST131. Among the ST131 isolates, nine isolates were ST131*H30Rx* sub-lineage. Only one ST131 isolate carried eight common drug resistance genes. In this study, *usp*, *hlyA* and *cnf-1* were absent in ST131 isolates. *OmpT*, *fyuA*, *iutA*, *kpsmII* and *fimH* were 100% present among the ST131 isolates. F1B and $F_{rep}$ were the common replicon types found among the ST131 isolates. This is the pioneer study on ST131 isolates in Malaysia as well as in South East Asia region. This study showed ST131 are frequently presence in gentamicin resistant UPEC isolates rather than ciprofloxacin resistant UPEC isolates. Different drug resistance gene profile and virulence factor profile showed the unique characteristics of ST131 isolates. Replicon types F1B and $F_{rep}$ were common and co-exist, a finding which was similar to the previous study. Study showed the presence of ST131 in the collected isolates and ST131*H30Rx* was the common sub-lineage. Duplex MAMA-PCR was successfully developed as the rapid




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method for ST131 detection. Molecular characteristics observed in this study will provide important information for the future study.



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## *ABSTRAK*

### *PENGESANAN DAN PENCIRIAN UROPATHOGENIC ESCHERICHIA COLI JENIS JUJUKAN 131 DI SABAH, MALAYSIA*

*Escherichia coli (E. coli) Jenis jujukan 131 (ST131) adalah patogen yang tahan pelbagai ubat menyebabkan jangkitan saluran kencing (UTI) serta jangkitan luar usus dan jangkitan usus dalam manusia. Ia adalah patogen pandemik dan menyebar di seluruh dunia. Mega-plasmid yang dibawa oleh ST131 mengandungi pelbagai gen rintangan drug dan gen pengekodan virulen. MLST digunakan untuk mengenal pasti patogen ini dengan penjujukan tujuh gen penyelenggara untuk setiap sampel. Masalah seperti beban kerja berat dan prosedur memakan masa perlu diatasi untuk menjalani MLST bagi mengesan ST131. Di Sabah, kehadiran ST131, ST131 clade C sub-keturunan (ST131H30, ST131H30R dan ST131H30Rx), profil gen rintangan dadah, profil faktor kevirulenan dan jenis replikon di ST131 masih belum diketahui. Dalam kajian ini, 294 pencilan Uropathogenic E. coli (UPEC) telah diasingkan daripada pesakit UTI dari Hospital Queen Elizabeth I dan Hospital Wanita Dan Kanak-Kanak Sabah. Dari sampel ini, pencilan tahan pelbagai ubat dikesan oleh ujian kerentanan antibiotik. ST131 dikaji di kalangan sampel tahan Ciprofloxacin dengan Skim Achtman. Asai dupleks amplifikasi mutasi salah padan - tindak balas berantai polimerase (MAMA-PCR) dibangunkan dengan pencilan ST131 yang telah dikenalpasti. Keturunan Clade C dikenal pasti di kalangan pencilan ST131. Gen pengekodan virulens am, gen rintangan drug dan jenis replikon telah dikenal pasti daripada pencilan ST131. Tujuh belas pencilan ST131 dikenalpasti di kalangan pencilan UPEC. Duplex MAMA-PCR telah digunakan sebagai kaedah pengesanan pantas untuk mengesan ST131. Di antara pencilan ST131, sembilan pencilan adalah sub-keturunan ST131H30Rx. Hanya satu pencilan ST131 yang membawa lapan jenis rintangan drug. Dalam kajian ini, usp, hlyA dan cnf-1 tidak dikesan dalam pencilan ST131. OmpT, fyuA, iutA, kpsmII dan fimH hadir dengan 100% di kalangan pencilan ST131. F1B dan $F_{rep}$ adalah jenis replikon am yang ditemui di kalangan pencilan ST131. Ini adalah kajian perintis mengenai pencilan ST131 di Malaysia dan di rantau Asia Tenggara. Kajian ini menunjukkan ST131 lebih kerap wujud di UPEC yang rintang gentamicin dan bukannya pencilan UPEC rintang Ciprofloxacin. Profil gen*




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*rintangan drug dan profil faktor virulen yang berbeza menunjukkan ciri-ciri unik pencilan ST131. Jenis replikon F1B dan $F_{rep}$ adalah jenis umum dan wujud bersama, penemuan ini serupa dengan kajian terdahulu. Kajian menunjukkan kehadiran ST131 dalam UPEC yang dikumpulkan dan ST131H30Rx adalah sub-keturunan umum. Duplex MAMA-PCR berjaya dibangunkan sebagai kaedah pantas untuk mengesan ST131. Ciri-ciri molekul yang diperhatikan dalam kajian ini akan menjadi maklumat penting untuk kajian masa depan.*



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## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| **˚C** | Degree Celcius |
| **%** | Percentage |
| **Min** | minute |
| **mL** | Mililiter |
| **µL** | Microliter |
| **g** | Gram |
| **mg** | Miligram |
| **ng** | Nanogram |
| **µg** | Microgram |
| **Sec** | second |
| **-** | Negative |
| **+** | Positive |
| **10X** | 10 times |
| **1X** | 1 time |
| **3GC** | third-generation cephalosporins |
| **A** | Alanine |
| **ADH** | adenine dinucleotide |
| **ADP** | adenosine diphosphate |
| **AMP** | adenosine monophosphate |
| **AMP** | Ampicillin |
| **ApE** | A Plasmid Editor |
| **APEC** | avian pathogenic *E. coli* |
| **AST** | Antibiotic susceptibility testing |
| **ATCC** | American Type Culture Collection |
| **ATP** | adenosine triphosphate |
| **bp** | Base pair |
| **C** | Cysteine |
| **CAZ** | Ceftazidime |
| **CIP** | Ciprofloxacin |
| **CIP-R** | Ciprofloxacin resistant |
| **CLSI** | Clinical and Laboratory Standards Institute |
| **Cnf-1** | Cytotoxic Necrotizing Factor-1 |
| **CTX** | Cefotaxime |



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| | |
|---|---|
| **D** | Aspartic Acid |
| **dfr** | dihydrofolate reductase |
| **$dH_2O$** | double distilled Water |
| **DHFR** | dihydrofolate reductase |
| **DHPS** | dihydropteroate synthetase |
| **DNA** | Deoxyribonucleic acid |
| **dNTP** | Deoxy-Ribonucleoside Triphosphate |
| **E** | Glutamic Acid |
| ***E. coli*** | *Escherichia coli* |
| **EHEC** | Enterohemorrhagic *Escherichia coli* |
| **EMB** | Eosin Methylene Blue agar |
| **ESBL** | Extended-spectrum beta lactamases |
| **ExPEC** | Extra intestinal pathogenic Escherichia coli |
| **fyuA** | yersiniabactin receptor |
| **G** | Glycine |
| **GEN** | Gentamicin |
| **GEN-R** | Gentamicin resistant |
| **HCA** | healthcare associated |
| **HGT** | horizontal gene transfer |
| **HlyA** | α-Hemolysin |
| **HQE1** | Hospital Queen Elizabeth I |
| **HWKSS** | *Hospital Wanita dan Kanak-Kanak Sabah* |
| **I** | Intermediate (Chapter 2) |
| **I** | Isoleucine (Chapter 4) |
| **iha** | adhesion siderophore receptor |
| **IMP** | inosine monophosphate |
| **Inc** | incompatibility |
| **iutA** | aerobactin receptor |
| **K** | Lysine |
| **kBs** | kilobases |
| **KP** | *Klebsiella pneumonia* |
| **kpsmII** | group 2 capsule synthesis |
| **L** | Leucine |
| **malX** | pathogenicity island marker |



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