

**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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LAI YUN MEI

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THE DEGREE OF DOCTOR OF PHILOSOPHY**

**FACULTY OF MEDICINE AND HEALTH
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JUDUL: **DETECTION AND CHARACTERIZATION OF UROPATHOGENIC *ESCHERICHIA COLI* SEQUENCE TYPE 131 IN SABAH, MALAYSIA**

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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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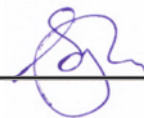
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Lai Yun Mei
29 Aug 2018



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-lineages (ST131H30, ST131H30R and ST131H30Rx), 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 ST131H30Rx 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 ST131H30Rx was the common sub-lineage. Duplex MAMA-PCR was successfully developed as the rapid



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



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 menyebarkan 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*



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 <i>E. coli</i>
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



D	Aspartic Acid
dfr	dihydrofolate reductase
dH₂O	double distilled Water
DHFR	dihydrofolate reductase
DHPS	dihydropteroate synthetase
DNA	Deoxyribonucleic acid
dNTP	Deoxy-Ribonucleoside Triphosphate
E	Glutamic Acid
<i>E. coli</i>	<i>Escherichia coli</i>
EHEC	Enterohemorrhagic <i>Escherichia coli</i>
EMB	Eosin Methylene Blue agar
ESBL	Extended-spectrum beta lactamases
ExPEC	Extra intestinal pathogenic <i>Escherichia coli</i>
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	<i>Hospital Wanita dan Kanak-Kanak Sabah</i>
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	<i>Klebsiella pneumonia</i>
kpsmII	group 2 capsule synthesis
L	Leucine
malX	pathogenicity island marker

