Research Article

Emergence of ST131 H30-Rx Subclones among Uropathogenic *Escherichia* coli Isolates from Two Large Hospitals of Kota Kinabalu, Sabah, Malaysia

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ABSTRACT

***Escherichia coli* sequence type 131 (ST131) carries multiple drug resistance (MDR) genes as well as virulence genes. Drug resistant characteristics give a management problem to health care personnel. Four MDR *Escherichia coli* ST131 H30-Rx subclones were identified among 80 Uropathogenic *E. coli* (UPEC) isolates by using 4 allelic-specific Polymerase Chain Reactions (PCR) in two hospitals of Kota Kinabalu, Sabah, Malaysia. There is emergence of multidrug resistant *E. coli* in Kota Kinabalu.**

Keywords: Uropathogenic *E. coli*, ST131 H30-Rx subclones, multiple drug resistance, hospitals of Kota Kinabalu

INTRODUCTION

E. coli ST131 is a worldwide pandemic clone of *E. coli*, causing multiple antimicrobial-resistant infection.[1] Multi-locus sequence typing (MLST) examines the nucleotide sequences of several (i.e. 6 – 10) housekeeping genes for the molecular epidemiological typing of bacterial pathogens.[2, 3] MLST identifies the pandemic spread of CTX-M-15 extended-spectrum beta-lactamase (ESBL)-producing *E. coli* from three continents in 2008. Previous studies have confirmed the worldwide prevalence of ST131 and it carried a broad range of virulence and resistance genes on a transferable plasmid.[1] Regarding *fim*H gene responsible for adhesion of *E.coli* to host cells, three types of *fim*H alleles are well known for ST131 clones. H30 is associated with O25b serotypes while H41 is associated with O16 serotypes and H22 is sensitive to ciprofloxacin and the serotype is O25b.[4] The term H30-R is used for ciprofloxacin resistant isolates.[5]

Hundred per cent of $bla_{CTXM-15}$ producer *E.coli* isolates were included in the H30Rx subclones and these can give rise to healthcare associated (HCA) episodes.[6] In this $bla_{CTX-M\ 15}$ nomenclature: '*bla*' is the beta-lactamase gene, 'CTX-M' is the sub-family and cefotaximase from Munich and '15'is the specific variant.[7] H30-Rx is resistant to at least third generation cephalosporin (3GC) and ciprofloxacin.

Allelic-specific PCR is the method applied for selected amplification of one allele among many alleles of one gene. Although there are seven house-keeping genes to be amplified in MLST to identify each ST, the two genes (*mdh* and *gyr*B) have 2 or 3 single nucleotide polymorphisms (SNPs) in ST131. Using these SNPs, forward primers and reverse primers are designed so that ST131 can be identified by adjusting the PCR conditions.[8] The same concept can be usable in H30 allele and CTX-M15 to be amplified out of various fimH alleles and CTX-M alleles respectively.

In this study, we tried to detect ST131, H30-Rx subclones by the principle of allelic-specific PCR so that the physicians of hospitals of Kota Kinabalu were aware of these highly pathogenic bacteria.

MATERIALS AND METHODS

Samples

Eighty isolates of UPEC from two main hospitals of Kota Kinabalu, Sabah, Malaysia namely Hospital Queen Elizabeth (47 isolates) and Hospital for Women and Children (33 isolates) were included in this study. Sample collection

was done from the period of 1 January to 30 April of 2016.

Antibiotic Susceptibility Tests

The isolates were studied for the antibiotic susceptibility tests by disc-diffusion method with 10 antibiotics discs namely ciprofloxacin, cefotaxime, cotrimethoxazole, gentamicin, cefuroxime, imipenem, ceftazidime, cephalothin, piperacillin and ampicillin. Antibiotic susceptibility test was performed as mentioned in the Clinical and Laboratory Standards Institute guidelines.[9]

Allelic-specific PCRs for Detection of ST131

Those isolates with drug resistant patterns to at least two antibiotics ciprofloxacin and third generation cephalosporin (3GC) were investigated for *mdh* and *gyrB* allelic-specific PCR. The primer sequences for forward and reverse primers and expected amplicon size together with PCR conditions were mentioned in Table 1.[8] The bacterial DNA from bacterial suspension in L-broth incubated overnight at 37°C was denatured by a boiling method for 10 min in boiling water bath, 5 ***µl*** of template DNA was added to PCR reaction mixture containing 2 ***µl*** of 100 pmol each primer, 1 ***µl*** of dNTPs 20 mmol, 2.5 ***µl*** of 10× buffer, 1.25 unit of Taq polymerase (Takara Bio Inc, Shiga, Japan) and PCR was done in a thermocycler (Applied Biosystems, Foster City, USA). The size of PCR product was checked by gel documentation apparatus Alpha Imager® HP System after it was run in 1.5 % agarose gel and stained by florosafe.

Table 1 Primer sequences, PCR conditions, Amplicon sizes of four allelic specific PCR for investigations of ST131 H30-Rx

Target gene	Primer sequences (References)	PCR conditions	Amplicon size
mdh	F:5'– GTT TAA CGT TAA CGC CGG T-3' R:5'– GGT AAC ACC AGA GTG ACC A-3'[9]	94°C – 5m 30×: 94°C – 30s, 58°C – 30s 72°C – 10m, 4°C –∞	275 bp
***gyr*B**	F:5'– CGC GAT AAG CGC GAC -3' R:5'– ACC GTC TTT TTC GGT GGA A -3'[9]	94°C – 5m 30×: 94°C – 30s, 58°C – 30s 72°C – 10m, 4°C –∞	132 bp
fim*H *30	F:5'– CCG CCA ATG GTA CCG CTA TT -3' R:5'– CAG CTT TAA TCG CCA CCC CA -3'[11]	95°C – 8m 37×: 94°C - 20s, 65°C – 45s 72°C – 5m, 4°C –∞	354 bp
***bla*$_{\text{CTX-M 15}}$**	F:5'– ATA AAA CCG GCA GCG GTG G -3' R:5'– GAA TTT TGA CGA TCG GGG -3'[7]	95°C – 8m 37×: 94°C –30s, 65°C –45s 72°C –5m, 4°C –∞	483 bp

Confirmation by MLST using Achtman Scheme

Confirmation of ST131 was done for positive isolates by Achtman Scheme using PCR of seven house-keeping genes *adk*, *fum*C, *gyr*B, *icd*, *mdh*, *pur*A and *rec*A and sequencing of these amplicon as described in Wirth et al.[10]

Allelic-specific PCR for *fim*H30 Allele

The procedure and ingredients of PCR were same as above. The primer sequences, PCR conditions and expected amplicon sizes for *fim*H30 allele and $bla_{CTX\text{-}M15}$ allele were mentioned in Table 1.[7, 11]

Allelic-specific PCR for $bla_{CTX\text{-}M\ 15}$

For the H30-Rx subclones, $bla_{CTX\text{-}M15}$ was investigated by fourth allelic specific PCR. Ingredients of PCR were the same as mentioned in *mdh* and *gyr*B PCR.

RESULTS

Four isolates were positive for both alleles (*mdh* and *gyr*B) in the identification of ST131, *fim*H30 and $bla_{CTX\text{-}M15}$ allelic specific PCRs. The characteristics of four isolates which were observed to be ST131 H30Rx including antibiotic resistance patterns, results of four allelic specific PCR, zone of inhibition in ciprofloxacin resistance were shown in Table 2. The gel electrophoresis picture of two allelic-specific PCRs for *fim*H30 and $bla_{CTX\text{-}M15}$ is shown in Figure 1 and Figure 2 respectively.

The significant observation in this study was that all the ST131 H30Rx positive cases were female patients and age of over 60. Of four positive isolates, three were from Hospital for Women and Children where the number of old women patients hospitalized in this institution is more than Hospital Queen Elizabeth.

Table 2 Characteristics of four UPEC isolates observed to be ST131 H30-Rx in this study

Isolate No.	Resistant Antibiotics	*mdh*	*gyr*B	H30 allele	H30R (determined by ciprofloxacin resistant)	H30-Rx allele
Q5	*CIP, CTX TMP-STX, GM, CXM, CAZ, KF, PRL, AMP	+	+	+	Inhibition zone size in ciprofloxacin susceptibility test = 6 mm -	+
W19	CIP, CTX TMP-STX, CXM,CAZ, KF, PRL, AMP	+	+	+	ll -	+
W43	*CIP, CTX TMP-STX, CXM, CAZ, KF, PRL, AMP	+	+	+	ll	+
W47	*CIP, CTX TMP-STX, CXM, CAZ, KF, PRL, AMP	+	+	+	ll	+

*Short terms of antibiotics studied in the disc-diffusion method - ciprofloxacin = CIP, cefotaxime = CTX, cotrimethoxazole = TMP-STX, gentamicin = GM, cefuroxime = CXM, imipenem = IMI, ceftazidime = CAZ, cephalothin = KF, piperacillin = PRL and ampicillin = AMP

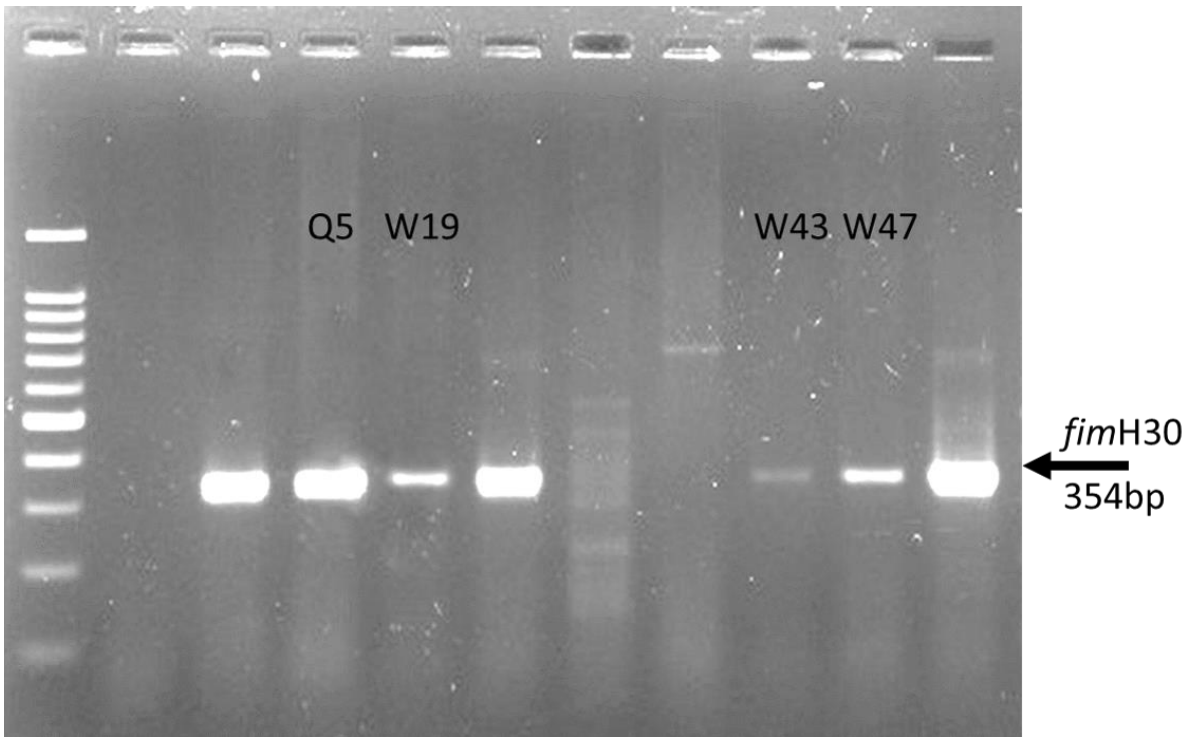

Figure 1 The gel electrophoresis picture of allelic-specific PCR for *fim*H30
Seven isolates including Q5, W19, W43, W47 were positive for PCR product showing 354bp band. The molecular marker used in the study was 100 bp ladder.

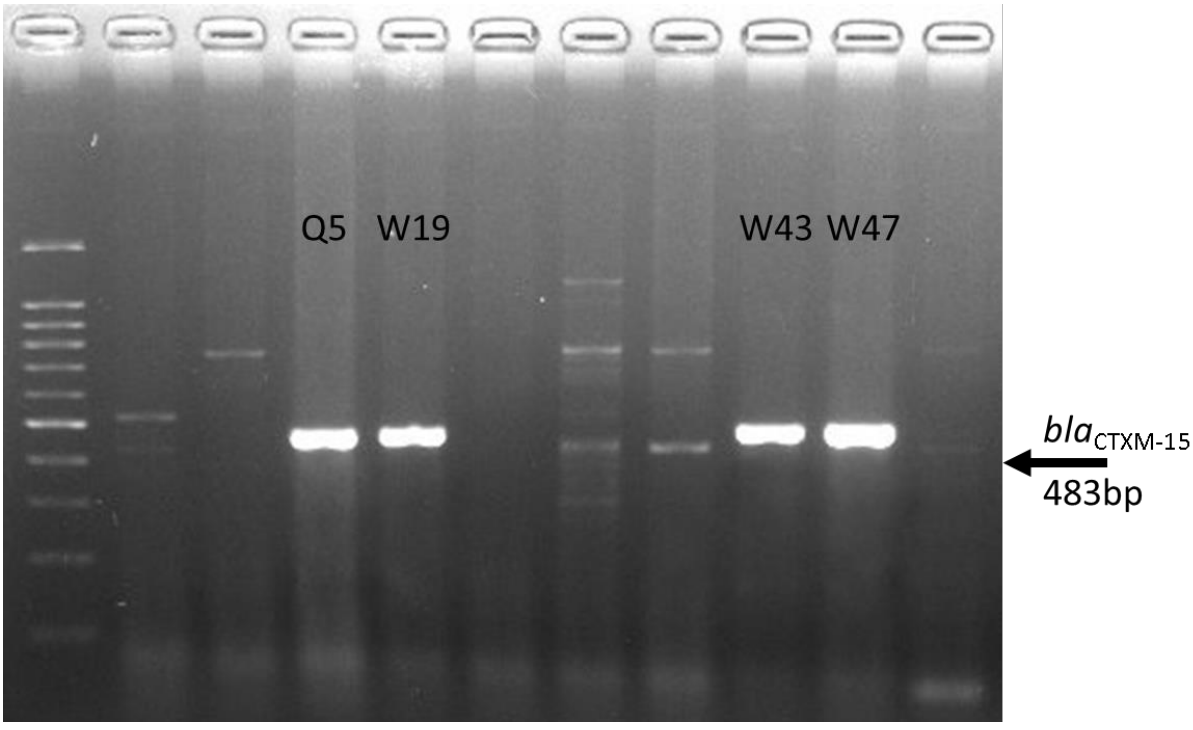

Figure 2 The gel electrophoresis picture of allelic-specific PCR for $bla_{\text{CTX-M 15}}$
Isolates no.Q5, W19, W43, W47 were positive for PCR product showing 483bp band. The molecular marker used in the study was 100 bp ladder.

DISCUSSION

Pulsed field gel electrophoresis (PFGE) is considered the gold standard for the outbreak investigations of the *E. coli* pathotypes.[12,13] This method is based on principle of restriction fragment length polymorphisms after digestion of whole genome by restriction enzymes. PFGE is excellent for molecular typing of *E. coli* responsible for recent outbreaks. However, it is time consuming, labour-intensive and requires technical experts.[13] In addition, there is a problem of comparison of data generated in different laboratories. MLST is an ideal method for the study of molecular epidemiology of antimicrobial resistant bacteria including *E. coli* pathotypes. Sequence types (STs) can indicate common ancestry lineages among bacteria. It is possible to compare data generated in different laboratories.[2, 3] However, MLST, according to Achtman Scheme, needs to do seven PCR and seven DNA sequencing for testing each isolate.[10] Due to this technical workload, researchers tried to find the allelic specific primers after comparison of DNA sequences specific to ST131 and other STs. The results pointed out *mdh* gene and *gyr* B gene were consistently specific in SNPs and consequently allelic specific PCR of these two genes were recently applied for detection of ST131 clones.[9]

Information on ST131's geographical distribution is incomplete. Analysis on previous studies has shown it has distributed as a human infection in Europe, North America, Canada, Japan and Korea. There was limited data from Asia, the Middle East and Africa[1]. The clone is also detected in companion animals, non-companion animals and foods. Urinary tract infection was predominant in human infections and life threatening sepsis was a serious complication[1]. Phenotypic detection of the ST131 clone is not possible and Genotyping was done by DNA-based techniques including MLST and PCR to identify known single nucleotide polymorphisms, which are the method of choices[1]. Whole genome sequencing is the latest method for the detection of this pathogen.

In Malaysia including Sabah state, there was no previous study on this globally disseminated pathogen, *E. coli* ST131 multi-drug resistant strains although there were two studies from University of Malaya which studied on samples from Pune, India.[14, 15] The other study was done on Malaysian isolates but the researchers have investigated extended-spectrum beta-lactamase (ESBL) producing *E.coli* and not multi-drug resistant strains including H30-R.[16] The current study is the first attempt to detect highly resistant H30-Rx subclones of ST131.

This kind of molecular epidemiological study is necessary to be done in the future as a timely manner because spread of ST131 is increasing year by year. The physicians should be aware of this pathogen which causes treatment failure, prolonged stay in hospital, bacteraemia of urinary origin and fatality due to disseminating sepsis.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

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