

Detection of Sequence Type 131 in Multi-drug Resistant Uropathogenic *Escherichia coli* Isolates from Two Hospitals of Sabah

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Abstract

Background: *Escherichia coli* sequence type 131 (ST131) has emerged among bacteria causing urinary tract infection (UTI) in the previous decade. This ST contains multiple drug resistant (MDR) genes together with genes encoding many virulence factors. As a result, this strain of uropathogenic *E. coli* (UPEC) gives rise to treatment failure with consequent prolonged stay in a hospital. Therefore, earlier identification of this strain in the hospital has advantage in combating severe type of UTI. **Objective:** To detect ST 131 strains in MDR UPEC isolates from two hospitals of Sabah. **Materials and Methods:** Antibiotic susceptibility tests were performed to detect MDR isolates. Two polymerase chain reactions (PCRs) including *mdh* and *gyrB* allelic-specific PCR were performed on these MDR isolates to detect ST131 strains. **Results:** The results showed four isolates were resistant to TMP-SMX, gentamycin, ciprofloxacin, and cefotaxime, and three isolates of these were investigated to be ST131 strains by two PCR reactions. **Conclusion:** There is the presence of ST131 strains in hospitals of Sabah. This information will be a guideline for the clinician in the management of UTI in the clinical settings.

Key words: Allelic-specific polymerase chain reaction, *Escherichia coli* sequence type 131, treatment failure, urinary tract infection

INTRODUCTION

Escherichia coli sequence type 131 (ST131) has recently emerged as a bacterium causing drug-resistant urinary tract infection (UTI). This *E. coli* strain is a pandemic multiple drug resistant strain causing community and hospital-acquired urinary tract and bloodstream infections. *E. coli* ST131 was observed to be a major strain which carried the CTX-M-15 extended-spectrum β -lactamase resistance in 2008.^[1,2] In the following years, researchers indicated *E. coli* ST131 has also been found to be resistant to fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole (TMP-SMX).^[3,4] Further treatment options are limited for this group because there were reports of carbapenem-resistant ST 131 strains.^[5]

Can *et al.* (2014) observed that resistance rates were above 25% against ciprofloxacin, TMP-SMX, and cefuroxime in their 294 isolates from acute cystitis cases. Treatment failure was three

times more common in the group infected with ST131 than other groups of *E. coli*. It was postulated by these scientists that the *E. coli* ST131 strain was a predictor of treatment failure in UTIs.^[6]

In the Sabah state of Malaysia, there were no previous studies concerned with this globally disseminated pathogen, *E. coli* ST131 multi-drug resistant (MDR) strains although there are two studies in West Malaysia.^[7,8] In this study, we tried to identify *E. coli* ST131 having MDR phenotype among isolates from two hospitals located around Kota Kinabalu, Sabah, Malaysia, namely, Hospital Papar and Hospital Queen Elizabeth.

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MATERIALS AND METHODS

Samples

About 162 UPEC isolates stocked in the Microbiology Laboratory of Faculty of Medicine and Health Sciences, Universiti Malaysia Sabah were included in this study. The samples for UPEC were collected between January and June 2013 from Hospital Papar and Hospital Queen Elizabeth.

Antibiotic susceptibility tests

Bacterial stocks already confirmed by bacteriological methods as *E. coli* were first subcultured on MacConkey agar, and these were investigated on Mueller-Hinton agar for antibiotic susceptibility test using disc diffusion method with 10 antibiotic discs, namely, TMP-STX, ciprofloxacin, gentamicin, cefotaxime, amikacin, ceftazidime, levofloxacin, tetracycline, chloramphenicol, and ampicillin. Confirmation was done by minimum inhibitory concentration determination using agar dilution method. Both disc diffusion test and agar dilution test were performed according to CLSI guidelines.^[9]

Allelic-specific polymerase chain reaction (PCR) for two candidate genes *mdh* and *gyrB*

The primer pairs used in this study for the PCR were mentioned in Table 1. Duplex PCR was used in this study for each candidate gene *mdh* or *gyrB* and *E. coli* specific 16S rRNA gene.

About 1 ml of culture was centrifuged, and the pellet was suspended in 50 µl sterile water while supernatant was discarded. The bacterial DNA was denatured by a boiling method for 10 min in boiling water bath. 5 µl of template DNA was added to PCR reaction mixture 20 µl containing 2 µl of 50 pmol each primer, 1 µl of dNTPs 10 mmol, 2.5 µl of 10x buffer, 1 unit of Taq polymerase (Takara Bio Inc, Shiga, Japan) and PCR was done in a thermocycler (Applied Biosystems, Foster City, USA). The PCR conditions were initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing and extension at 58°C for 30 s, and final extension at 72°C for 10 min. The size of PCR product was checked by 1.5 % agarose gel, which was stained by florosafe and recorded by gel documentation apparatus Alpha Imager® HP System. The molecular size

marker used in this study was 100 bp DNA ladder (1st BASE Singapore Ltd., Singapore).

RESULTS

Antibiotic susceptibility tests

Antibiotic susceptibility patterns of 162 UPEC isolates were shown in Figure 1. 42, 10, 6, and 4 isolates were resistant to TMP-SMX, gentamycin, ciprofloxacin, and cefotaxime, respectively. Four isolates EC067, EC070, EC257, and EC272 were consistently resistant to all of four antibiotics mentioned.

Allelic-specific PCR for two candidate genes *mdh* and *gyrB*

These four isolates were subjected to two PCR reactions, and three of four isolates were positive in both of two PCR reactions [Figure 2]. One isolate EC257 having intermediate resistant to ciprofloxacin was included in these four isolates, and the isolate was negative for both *mdr* and *gyrB* allelic-specific PCR in this study. Duplex PCR is positive for both 16S rRNA gene which is specific for *E. coli* species and candidate *mdr* gene in strain EC067, EC070, and EC272. The same isolates were positive for candidate gene *gyrB* duplex PCR.

DISCUSSION

Multilocus sequence typing is one of the molecular epidemiological methods for characterization of strains of bacterial pathogen within same species including UPEC. Ancestry lineages common in each bacterial species can be studied. Well-standardized scheme is used in this technology so that sequence types observed in various laboratories of the world can be compared and easily characterized.^[12] According to Achtman Scheme, it is essential to do seven PCR and consequent seven DNA sequencing reactions. Because of this technical burden, bacteriologists performed allelic-specific PCR using primers including single nucleotide polymorphisms present within two house-keeping gene *mdh* and *gyrB*. SNPs in *mdh* gene are C288T and C525T, whereas SNPs in *gyrB* gene are C621T, C729T, and T735C. The two genes have many alleles so that allele-specific for ST131

Table 1: Primers used in this study

Name of gene	Sequence of primers	Size of DNA fragment	Reference
<i>mdh</i>	F: 5'-GTT TAA CGT TAA CGC CGG T-3' R: 5'-GGT AAC ACC AGA GTG ACC A-3'	275 bp	[10]
<i>gyrB</i>	F: 5'-CGC GAT AAG CGC GAC-3' R: 5'-ACC GTC TTT TTC GGT GGA A-3'	132 bp	[10]
16S rRNA	F: 5'-CAG AAG AAG CAC CGG CTA AC-3' R: 5'-GGC AGT CTC CTT TGA GTT CC-3'	671 bp	[11]

TOTAL OF 162 ISOLATES	1	1	1	1	1	1	3	1	6	4	3	2	2	8	1	1	2	1	1	1	2	1	4	9	6	3	76	17	
TMP-STX	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
CIP	R	R	R	I	R	S	S	S	S	I	S	S	S	I	S	R	I	S	S	S	S	I	S	S	S	S	I	S	S
GM	R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S
CTX	R	R	R	R	S	S	R	S	S	S	S	S	S	S	S	R	R	R	S	S	S	S	S	S	S	S	S	S	S
AK	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S	R	S	R	R	S	R	R	R	S	S	S	S	S	S
CAZ	R	R	S	R	S	S	S	S	R	S	S	S	S	S	S	R	S	R	S	R	S	S	S	R	S	S	S	S	S
LVX	R	S	S	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
TE	R	R	R	R	R	S	R	R	R	R	R	S	R	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	S
CAM	S	S	S	S	S	S	R	R	S	S	S	R	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	S	S
AMP	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	R	S	R	R	S	R	R	S	R	R	S	R	R	S

Figure 1: Susceptibility patterns of different numbers of uropathogenic *Escherichia coli* isolates to 10 antibiotics. The long term for antibiotics in this figure is: TMP-STX: Trimethoprim-sulfamethoxazole, CIP: Ciprofloxacin, GM: Gentamycin, CTX: Cefotaxime, AK: Amikacin, CAZ: Ceftazidime, LVX: Levofloxacin, TE: Tetracycline, CAM: Chloramphenicol, AMP: Ampicillin. R: Resistant, I: Intermediate, S: Sensitive

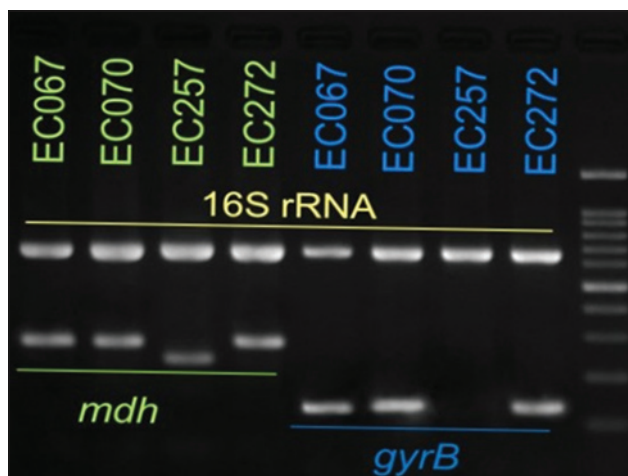


Figure 2: Gel electrophoresis picture of two duplex polymerase chain reaction (PCR) products for detection of *mdh* gene and *gyrB* gene in four isolates of multiple drug resistant uropathogenic *Escherichia coli* isolates. ECO67, ECO70, and EC272 in green are isolated positive for *mdh* gene showing 275 bp PCR product, and EC257 is the negative isolate. ECO67, ECO70, and EC272 in blue are isolated positive for *gyrB* gene showing 132 bp PCR product, whereas EC257 is negative isolate. The most right lane is 100 bp DNA molecular marker. The upper bands in all the lanes except the molecular marker are PCR product of 16s rRNA gene which are 671 bp in size

were tried to be detected in that study. With the primers pairs constructed with these SNPs, both *mdh* and *gyrB* allelic-specific PCRs were consistently successful with 34 ST131 control strains.^[10]

Adhesin of *E. coli*, which binds to host cells, is encoded by the *fimH* gene. There are three types of *fimH* alleles which are

commonly associated with ST131 strains. Of these, H30 is associated with O25b serotypes and highly prevalent in ST31 strains.^[13]

Ciprofloxacin-resistant isolates have SNPs in *gyrA* and *parC* genes in the chromosomal DNA of *E. coli* and other enteric bacteria.^[14] These ciprofloxacin resistant isolates associated with ST131 H30 allele are termed H30-R.^[15]

Highly drug-resistant CTX-M-15-producing ST131 isolates are named H30-Rx, and these isolates are more extensively drug-resistant having resistance to at least two antibiotics: Third generation cephalosporin (3GC) and ciprofloxacin.^[15,16] As a further recommendation, the highly pathogenic ST131 H30-Rx needs to be investigated in the hospitals of Sabah.

CONCLUSIONS

There is the presence of ST131 strains in hospitals of Sabah. A large number of *E. coli* isolates causing UTI, and other extra-intestinal infections should be screened for ST131 as this strain can give rise to treatment failure, recurrent UTI, prolonged stay in the hospital and fatal septicemic complications. Detection of MDR *E. coli* ST131 strain will be beneficial for the clinicians to be aware of this pathogen which is responsible for the severe nosocomial infections.

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