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Complete genome of the multidrug-resistant *Escherichia coli* strain KBN10P04869 isolated from a patient with acute myeloid leukemia

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급성백혈병 환자에서 분리된 다제내성 대장균 KBN10P04869의 유전체 염기서열분석

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Recently, we isolated a multidrug-resistant *Escherichia coli* strain KBN10P04869 from a patient with acute myeloid leukemia. We report the complete genome of this strain which consists of 5,104,264 bp with 4,457 protein-coding genes, 88 tRNAs, and 22 rRNAs, and the co-occurrence of multidrug- resistant genes including ^{bla}CMY-2, ^{bla}TEM-1, ^{bla}CTX-M-15, ^{bla}NDM-5, and ^{bla}OXA-18.

Keywords: *Escherichia coli*, carbapenem, multidrug-resistance

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped bacterium; clinically, it is one of the major causative pathogens of urinary tract infection, intra-abdominal infection, and primary bacteremia (Javaloyas *et al*., 2002). In the last two decades, there have been great concern regarding multidrug-resistant (MDR) *E. coli*, due to its increased incidence and its resistance to a broad range of β-lactams and other groups of antimicrobial agents (Rodríguez-Baño *et al*., 2006).

We isolated an antibiotic-resistant *E. coli* strain KBN10P04689, which caused bacteremia from the bloodstream of an acute myeloid leukemia patient. Antibiotics susceptibility testing with VITEK2 (bioMérieux) showed that the strain was resistant to carbapenem, non-extended spectrum cephalosporin, extended-spectrum cephalosporin, cephamycin, fluoroquinolones, folate pathway inhibitors, monobactams, and penicillins.

To investigate the genomic potential of the drug-resistant *E. coli* strain KBN10P04689 extensively, the strain was cultured at 37°C on blood agar. Then, genomic DNA was extracted using the i-genomic BYF Mini Kit (iNtRON Biotechnology). Genome sequencing was performed using PacBio RS II single-molecule real-time (SMRT) sequencing technology (Pacific Biosciences). A standard PacBio library with an average of 20-kb inserts were prepared and sequenced, yielding an average genome coverage of >179.2X. *De novo* assembly of the 101,776 reads with an average of 15,304 nucleotides (total 1,557,581,797 bp) was conducted using the hierarchical genome-assembly process (HGAP) pipeline of SMRT Analysis v2.3.0 (Chin *et al*., 2013).

The genome consists of one circular chromosome (4,840,855 bp with 50.57% G + C content) and three circular plasmids, pKBN10P04689A (107,229 bp with 51.69% G + C content), pKBN10P04689B (104,701 bp with 46.96% G + C content) and pKBN10P04689C (51,479 bp with 46.37% G + C content)

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Table 1. General features of *E. coli* strain KBN10P04869 complete genome

Attribute	Chromosome	pKBN10P04869A	pKBN10P04869B	pKBN10P04869C
Assembly size (bp)	4,840,855	107,229	104,701	51,479
Contigs	1	1	1	1
GC content (%)	50.57	51.69	46.96	46.37
DNA coding region (%)	84%	68%	82%	79%
Predicted ORFs	4211	91	94	61
rRNA	22			
tRNA	85		3	
Genes assigned to COGs	3,558	60	21	25
Genes with Pfam domains	3,820	74	24	34
Genes with signal peptides	416	4	2	6
Genes with transmembrane helices	1,032	11	8	10

Table 2. Results of antimicrobial test and resistant genes

Antibiotics	Susceptibility (MIC)	Antimicrobial resistant genes	
		Genes (Locus_tag)	Position
Amikacin	S (8)	*acc(6')-Ib-cr* (YKEC1_4527)	pKBN10P04869A
Gentamicin	R (≥32)	*aac(6')-Ib-cr* (YKEC1_4527) *aac(3)-IIb* (YKEC1_4558)	pKBN10P04869A
Piperacillin-tazobactam	R (≥128)	^{bla}TEM-1 (YKEC1_4537, YKEC1_4562) ^{bla}OXA-181 (YKEC1_4795) ^{bla}NDM-5 (YKEC1_4575)	pKBN10P04869A pKBN10P04869C pKBN10P04869A
Ertapenem	R (≥8)	^{bla}NDM-5 (YKEC1_4575)	pKBN10P04869A
Imipenem	R (≥16)	^{bla}NDM-5 (YKEC1_4575)	pKBN10P04869A
Cefazolin	R (≥64)	^{bla}TEM-1 (YKEC1_4537, YKEC1_4562)	pKBN10P04869A
Cefotaxime	R (≥64)	^{bla}CTX-M-15 (YKEC1_4533)	pKBN10P04869A
Ceftazidime	R (≥64)	^{bla}CTX-M-15 (YKEC1_4533) ^{bla}NDM-5 (YKEC1_4575)	pKBN10P04869A
Cefepime	R (≥64)	^{bla}NDM-5 (YKEC1_4575) ^{bla}OXA-181 (YKEC1_4795)	pKBN10P04869A pKBN10P04869C
Cefoxitin	R (≥64)	^{bla}CMY-2 (YKEC1_2769) ^{bla}NDM-5 (YKEC1_4575)	Chromosome pKBN10P04869A
Ciprofloxacin	R (≥4)	*aac(6')-Ib-cr* (YKEC1_4527) *qnrS1* (YKEC1_4788)	pKBN10P04869A pKBN10P04869C
Trimethoprim/ sulfamethoxazole	R (≥320)	*dfrA12* (YKEC1_4578) *sul1* (YKEC1_4570, YKEC1_4581) *sul2* (YKEC1_4546)	pKBN10P04869A
Tigecycline	S (≤0.5)	-	-
Aztreonam	R (≥64)	^{bla}CMY-2 (YKEC1_2769)	Chromosome
Ampicillin	R (≥32)	^{bla}TEM-1 (YKEC1_4537, YKEC1_4562)	pKBN10P04869A
Amoxicillin-clavulanic acid	R (≥32)	^{bla}OXA-181 (YKEC1_4795)	pKBN10P04869C
Chloramphenicol	ND	*catB3* (YKEC1_4533)	pKBN10P04869A
Colistin	ND	*arnA* (YKEC1_1480)	Chromosome
Tetracycline	ND	*tetB* (YKEC1_4586)	pKBN10P04869A

ND: not determined

(Table 1).

Different types of antibiotic-resistant genes encoding enzymes for structure-altering or inactivating antibiotics and modifying target sites, and encoding the enzymes resistant to inhibitors of a metabolic pathway (trimethoprim-sulfamethoxazole) were found in the chromosome, pKBN10P04689A and pKBN10P04689C. The chromosome harbors two antibiotic-resistant genes, ^{bla}CMY-2 (YKEC1_2769) and *arnA* (YKEC1_1480). In the plasmid pKBN10P04689A, the genes related to gentamicin resistance (*aac(3)-IIb*/YKEC1_4558), penicillin and cefazolin resistance (^{bla}TEM-1/YKEC1_4537 and YKEC1_4562), extended-spectrum cephalosporin resistance (^{bla}CTX-M-15/YKEC1_4533) and folate pathway inhibitor resistance (*dfrA12*/ YKEC1_4578, *sul1*/ YKEC1_4570 and YKEC1_4581, *sul2* /YKEC1_4546) were identified. In addition, the plasmid contains ^{bla}NDM-5 (YKEC1_4575), which has been reported to confer resistance against piperacillin-tazobactam and carbapenem, extended-spectrum cephalosporins, and ^{bla}NDM-5 or its variant, which has recently spread to a large extent (Hawkey and Jones, 2009). Besides, the genes involved in erythromycin, chloramphenicol and tetracycline resistance (*mphA*/YKEC1_4553, *catB3*/YKEC1_4533, and *tetB*/ YKEC1_4586) were detected. The plasmid pKBN10P04689C harbors genes that confer resistance to amoxicillin-clavulanic acid (^{bla}OXA-181/YKEC1_4795) and resistance to fluoroquinolones, including ciprofloxacin (*qnrS1*/YKEC1_4788). Although the *aac(6')-Ib-cr* gene (YKEC1_4527), which encodes a bifunctional protein to catalyze the acetylation of fluoroquinolones as well as aminoglycosides such as amikacin and gentamicin, was detected in pKBN10P04869A, the strain shows susceptibility against amikacin, as observed after an antimicrobial test (Table 2).

The complete genome sequence of the *E. coli* strain KBN10P04869 has been deposited at DDBJ/EMBL/GenBank under the accession numbers CP026473 (chromosome), CP026474 (pKBN10P04869A), CP026475 (pKBN10P04869B), and CP026476 (pKBN10P04869C). This strain is available from the Kyungpook National University Hospital Culture Collection for Pathogens.

적 요

저자들은 최근 급성골수성백혈병 환자로부터 다제내성대장균 균주 KBN10P04869를 분리했다. 균주는 4,457개의 단백질 코딩 유전자, 88개의 운반 RNA, 22개의 리보솜 RNA를 포함하는 5,104,264 염기쌍으로 구성되고, ^{bla}CMY-2, ^{bla}TEM-1, ^{bla}CTX-M-15, ^{bla}NDM-5, ^{bla}OXA-18를 포함한 다제내성유전자를 가지고 있다. 저자들은 이 균주의 총유전체를 보고하는 바이다.

Conflict of Interest

No potential conflicts of interest relevant to this article are reported.

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