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Detection of an *Escherichia coli* Sequence Type 167 Strain with Two Tandem Copies of bla_{NDM-1} in the Chromosome

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ABSTRACT New Delhi metallo-β-lactamase-1 (NDM-1)-producing *Enterobacteriaceae* has disseminated rapidly throughout the world and poses an urgent threat to public health. Previous studies confirmed that the bla_{NDM-1} gene is typically carried in plasmids but rarely in chromosome. We discovered a multidrug-resistant *Escherichia coli* strain Y5, originating from a urine sample and containing the bla_{NDM-1} gene, which did not transfer by either conjugation or electrotransformation. We confirmed the possibility of its chromosome location by S1-pulsed-field gel electrophoresis (PFGE) and XbaI-PFGE, followed by Southern blotting. To determine the genomic background of bla_{NDM-1}, the genome of Y5 was completely sequenced and compared to other reference genomes. The results of our study revealed that this isolate consists of a 4.8-Mbp chromosome and three plasmids, it is an epidemic clone of sequence type (ST) 167, and it shows 99% identity with *Escherichia coli* 6409 (GenBank accession no. CP010371), which lacks the same bla_{NDM-1} gene-surrounding structure as Y5. The bla_{NDM-1} gene is embedded in the chromosome along with two tandem copies of an insertion sequence common region 1 (IS*CR1*) element (*sul1-ARR-3-cat-*bla_{NDM-1}*-bleo-*IS*CR1*), which appears intact in the plasmid from *Proteus mirabilis* (GenBank accession no. KP662515). The genomic context indicates that the IS*CR1* element mediated the bla_{NDM-1} transposition from a single source plasmid to the chromosome. Our study is the first report of an *Enterobacteriaceae* strain harboring a chromosomally integrated bla_{NDM-1}, which directly reveals the vertical spreading pattern of the gene. Close surveillance is urgently needed to monitor the emergence and potential spread of ST167 strains that harbor bla_{NDM-1}.

KEYWORDS New Delhi metallo-β-lactamase-1, chromosome, IS*CR1*, *Enterobacteriaceae*

The bacteria containing the New Delhi metallo-β-lactamase gene (bla_{NDM-1}) have swiftly spread worldwide and are considered a serious public health concern (1, 2). Studies confirmed that mobile-resistance elements, such as plasmids, transposons (Tn) and insertion sequences (IS), are responsible for the distribution of this superresistant gene, and they are regarded as major mechanisms driving the dramatically increased prevalence of carbapenem-resistant *Enterobacteriaceae* isolates (3–5).

Plasmids constitute the main vehicles carrying the bla_{NDM-1} gene and are the primary media for its horizontal dissemination. Additional bla_{NDM-1} plasmids ranging

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TABLE 1 Antimicrobial susceptibility testing of *E. coli* Y5 isolate

Isolate	MIC (μg/ml)[a]													
	FEP	CTX	SAM	TZP	CPS2/1	IPM	MEM	IPM/IPM+EDTA	CST	AMK	TGC	MIN	CIP	ATM
Y5	>256	>256	>32	>256	>256	>256	>32	>32/<1	0.5	32	1	24	>32	>256

[a]FEP, cefepime; CTX, cefotaxime; SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; CPS2/1, cefoperazone/sulbactam 2:1; IPM, imipenem; MEM, meropenem; CST, colistin; AMK, amikacin; TGC, tigecycline; MIN, minocycline; CIP, ciprofloxacin; ATM, aztreonam.

from 40 kb to 400 kb in size have been fully sequenced and documented for their effective transferability within species (3–5). Recent surveys demonstrated the large similarities of the genetic structures surrounding the bla_{NDM-1} gene among different members of the *Enterobacteriaceae* family, which further highlights the potential for the extensive spread of this determinant of resistance. Such transposable elements, like IS*26* and IS*5*, which are frequently disseminated among diverse species or strains, most likely constitute the other horizontal-transfer mechanisms and are directly involved in the spread of the bla_{NDM-1} gene cross-species (6).

Reports have cited the emergence of the bla_{NDM-1} gene in the chromosomes of nonfermenter strains but not in *Enterobacteriaceae* isolates(7). Notably, few reports have revealed vertical-spreading patterns associated with this gene. Therefore, neither evidence nor research on the vertical-spreading pattern of the bla_{NDM-1} gene has been discussed recently. In this study, we report the discovery of the first clinical strain of *Escherichia coli* sequence type 167 (ST167) with two tandem chromosomal copies of bla_{NDM-1} originating from China. We completely sequenced the genome to analyze the genomic background of antimicrobial-resistance determinants and reveal their dispersal mechanism.

RESULTS

Antimicrobial susceptibility analysis. Antimicrobial susceptibility results showed that the *E. coli* strain Y5 was resistant to multiple antimicrobial agents, including cephalosporins, carbapenems, fluoroquinolones, and aminoglycosides, but it was susceptible to colistin and tigecycline. Additionally, the stain was positive for the metallo-β-lactamase (MBL) phenotype (Table 1).

Location of the bla_{NDM-1} gene. Southern blot results presented a positive signal band of ~54 kb according to the XbaI-PFGE assay but not the S1-PFGE assay (see Fig. S1 in the supplemental material). Further plasmid transfer experiments were unsuccessful, which strongly suggested that the gene was most likely carried chromosomally.

Genomic sequence analysis. The assembly of whole-genome sequencing data generated a circular chromosome and three plasmids, with mean coverage and quality values of >82 and >43, respectively (Table 2). The size of the genome was 4,839,266 bp, with 50.81% GC content, 7 rRNA operons, 83 tRNAs, and 3,890 predicted protein-coding sequences (Table 2 and Fig. 1). Three plasmids measuring ~61 kb to ~136 kb

TABLE 2 Genome and plasmids of *E. coli* Y5

Genomic structure	Mean sequencing and alignment indices		Size (kb)	GC content (%)	CDS no.	Accession no.	Resistance genes present	*rep* group
	Coverage	Quality						
Y5 chromosome	169.36	48.77	4,839	50.81	3,890	CP013483	*mph(A)*, *sul1*, *ARR-3*, bla_{NDM-1}, *sul1*, *aadA16*, *aac(6′)Ib-cr*, *aac(3)-IId*, *bleo*	—
Y5-3 plasmid	123.79	47.49	136	51.95	90	KT997783	*acc(3)-IId*, *aph(3¢)-Ia*, *strB*, *strA*, *sul2*, *tet(A)*	*rep*A
Y5-5 plasmid	136.91	47.89	124	52.76	154	KU043115	*aac(6′)Ib-cr*, *aadA5*, bla_{OXA-1}, $bla_{CTX-M-15}$, *sul1*, *tet(A)*	*rep*E
Y5-6 plasmid	82.69	43.80	61	50.29	78	KU043116	$bla_{CTX-M-14}$, bla_{CMY-42}	*rep*E

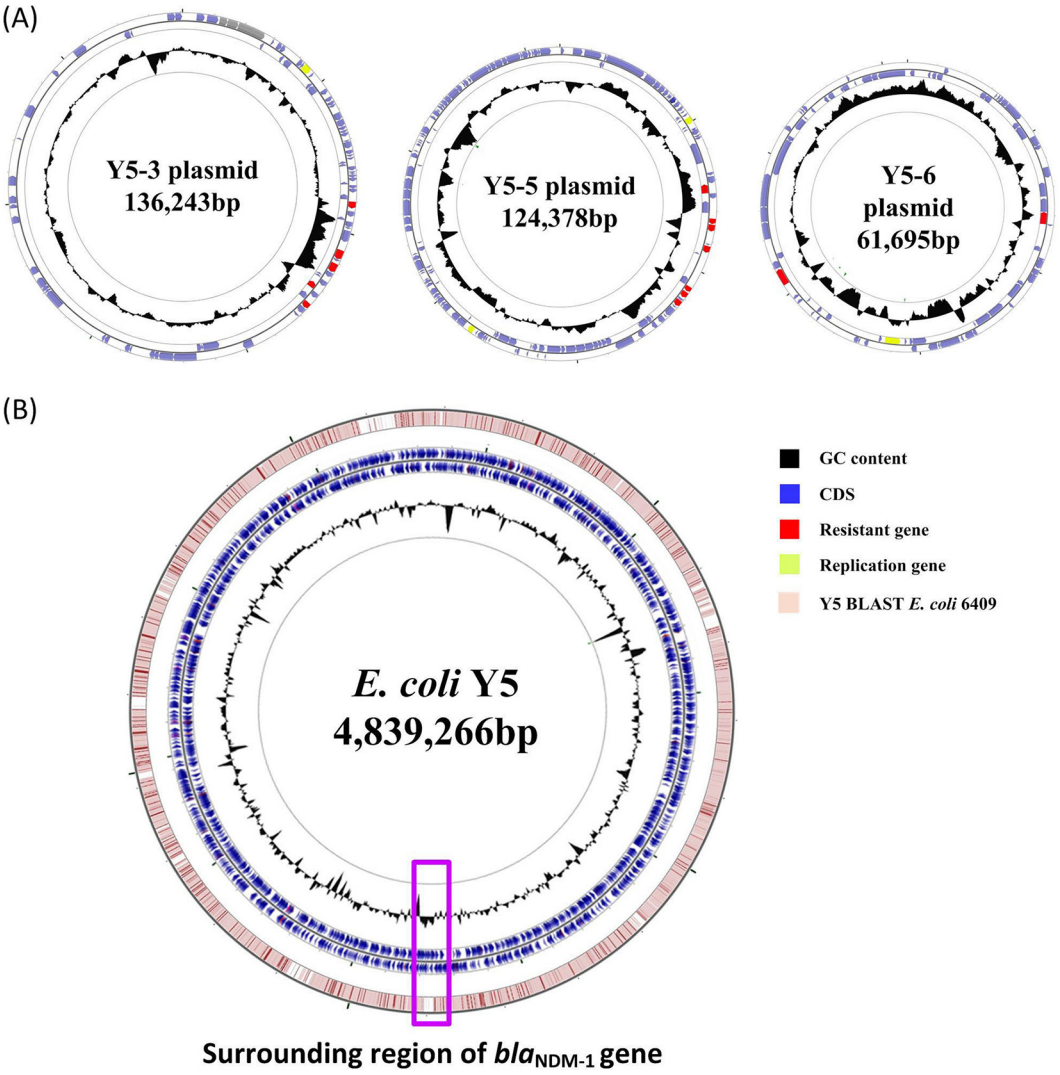

FIG 1 Circular maps of the *E. coli* Y5 genome and its plasmids. (A) Circular graphs of three plasmids. (B) Circular graph of the Y5 genome sequence and genome alignment. Blue arrows denote coding sequences, red arrows denote resistance genes, and replication genes are denoted by green arrows. Genome alignment between Y5 and *E. coli* 6409 is the outer circle in pink, and GC content is the inner circle in black. The region surrounding the bla_{NDM-1} gene is highlighted with a purple frame.

in size and having between ~50.29% and ~52.76% GC content were grouped into identifiable replicon types (Table 2).

The isolate belonged to an epidemic-resistant clone of ST167. Multiple resistance genes responsible for resistance to β-lactams, aminoglycosides, fluoroquinolones, macrolides, rifampin, sulfonamide, and tetracycline, were identified in the strain (Table 2).

Genomic background of the bla_{NDM-1} gene. The bla_{NDM-1} gene was located upstream of an *intI1* gene in the chromosome and was embedded within two tandem copies of a long-repeat fragment (Fig. 2). The fragment, which is an insertion sequence common region 1 (IS*CR1*) element, was flanked by two copies of *sul1* and comprised a cluster of genes (*sul1-ARR-3-cat-*bla_{NDM-1}*-bleo-*IS*CR1*).

DISCUSSION

Enterobacteriaceae isolates positive for the bla_{NDM-1} gene have swept across the globe since its first report in 2009 (8). Many studies identified the gene location as resident within a plasmid and with horizontal transfer as the main spreading mechanism (6, 9–11). Our findings constitute the first report of its emergence in a chromosomal location in a clinical *E. coli* isolate revealing the vertical-spreading pattern for this gene.

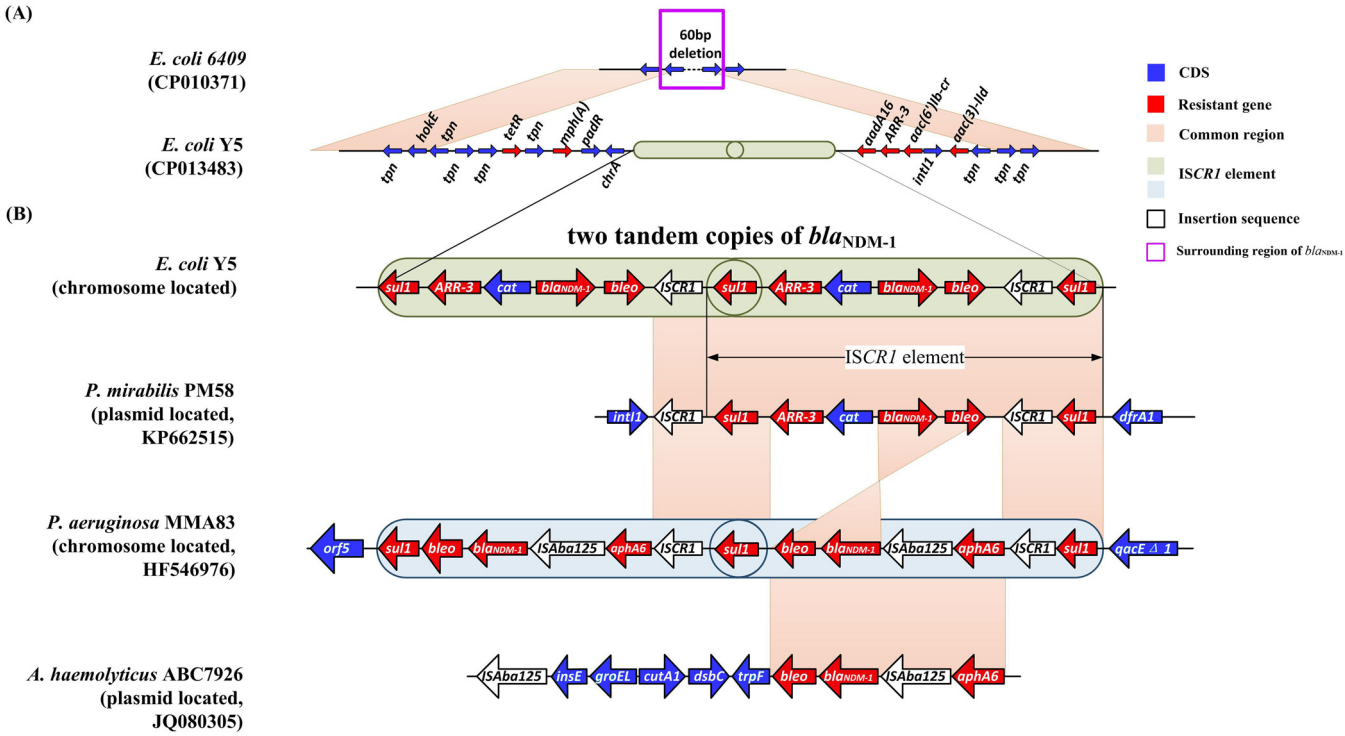

FIG 2 Alignment of sequences surrounding the region of bla_{NDM-1}. Blue arrows are for the coding sequences and red arrows are for resistance genes. The pink boxes represent the common regions among different strains. IS*CR1* elements are indicated in light green and gray. White arrows indicate insertion sequences. The region surrounding the bla_{NDM-1} gene is highlighted with a purple frame. *mph*(*A*), macrolide-2′-phosphotransferase I; *padR*, transcriptional regulator, PadR family; *chrA*, chromate transport protein A; *sul1*, sulfonamide resistance gene; *ARR-3*, rifampin resistance gene; *catB3*, chloramphenicol resistance gene; bla_{NDM-1}, New Delhi metallo-β-lactamase gene; *bleo*, bleomycin resistance gene; IS, insertion sequence; *aadA16*, aminoglycoside-(3′) (9)-adenylyltransferase gene; *arr3*, rifampin ADP-ribosylation transferase; *aac*(*6′*)*Ib-cr*, aminoglycoside N(6′)-acetyltransferase gene; *intI1*, integron integrase gene; *aac*(*3*)*-IId*, aminoglycoside-(3)-N-acetyltransferase type II; *dfrA1*, dihydrofolate reductase; *aphA6*, aminoglycoside 3′-phosphotransferase type 6; *qacEΔ1*, QacEdelta1; *tpn*, transposer; *hokE*, HokE protein; *tetR*, transcriptional regulator, TetR family.

Reports of carbapenem-resistant *Enterobacteriaceae* have dramatically increased recently and shown a geographical distribution not only in America and Europe but also in Asia (12). In China, many provinces, including Zhejiang, Hunan, and Henan, reported the emergence of *Enterobacteriaceae* positive for the bla_{NDM-1} gene (1, 3, 10, 13). The patient in this study was from Yuhuangding Hospital, located in Yantai, Shandong province, a coastal city in the Huadong region of China. The patient had never traveled abroad. Although there was no evidence to rule out a nosocomial infection, we preferred to consider that this Y5 strain might have originated from the patient herself, given that no further bla_{NDM-1}-positive isolates were detected 1 month prior to or following the event in our hospital.

Enterobacteriaceae isolates producing NDM-1-type carbapenemase are often associated with lethal infectious diseases, especially when separated from blood or secretions (13). Bloodstream infections caused by such "super bugs" are always life-threatening due to their extreme drug resistance (13). However, our and previous studies proved that symptoms are mild and localized when urinary tract infections (UTIs) occur. Obstruction of the urinary tract is one of the most important direct risk factors involved in such infections; therefore, relief of the obstruction is as important as is the use of antibiotics (10). Approximately 80% of UTIs are caused by *E. coli*, a bacterium that is often found in the human gut, which escapes and translocates up the urinary tract to cause infection. Therefore, it is reasonable to postulate that this *E. coli* Y5 strain might have originated from and colonized in the gut of the patient, with subsequent infection occurring in an ascending manner.

The *E. coli* Y5 isolate reported here was a strain of ST167, an epidemic clone of significant public health concern that typically carries β-lactamase-resistant plasmids, including bla_{CMY}, bla_{CTX-M}, and bla_{SHV}. bla_{NDM} variants (bla_{NDM-5} and bla_{NDM-7}) were

recently detected in plasmids of *E. coli* ST167, further highlighting its potential as a threat to public health (9, 11, 14).

Sequence alignment of the genomes revealed high degrees of similarity (99% identity and 91% query coverage) between the Y5 strain and another ST167 strain (*E. coli* 6409 [GenBank accession no. CP010371]), except for an additional insertion around the bla_{NDM-1} gene. The insertion ranged 20 kb upstream and downstream of the site, exhibited >60% GC content, contained 15 drug-resistance genes, and substituted a 60-bp sequence of *E. coli* 6409 (Fig. 1 and 2). This genomic data provided direct evidence that bla_{NDM-1} was carried chromosomally and also proved that it was exogenous in origin. Further analysis of the insertion sequence surrounding bla_{NDM-1} revealed two tandem copies of an IS*CR1* element. IS*CR1*, which was first reported by Wang et al., lacks terminal inverted repeats and is transposed by a mechanism called rolling-circle transposition. IS*CR1* is often neighbored by a wide array of antibiotic-resistance genes and shows a potential for mobilizing adjacent antibiotic-resistance genes (15, 16).

Considering the important role of the IS*CR1* element in bla_{NDM-1} transposition, sequence alignment was performed in our study. In 2013, Jovcic et al. reported that bla_{NDM-1} was carried in a similar IS*CR1* element in *Pseudomonas aeruginosa* according to restriction enzyme-based cloning (7). Their IS*CR1* element was embedded within a chromosome and also presented two tandem copies; however, the sequence within the element was significantly different (Fig. 2). They found a more composite Tn structure consisting of two insertion sequences (IS*CR1* and IS*Aba125*), a portion of which was highly homologous with the Tn*125* in an *Acinetobacter haemolyticus* ABC7926 plasmid (GenBank accession no. JQ080305) (Fig. 2). Given that both IS*CR1* and IS*Aba125* were capable of transfer, the determination of which is responsible for mobilizing the bla_{NDM-1} gene from the plasmid to chromosome is unlikely. Here, we revealed that the sequence of the IS*CR1* element in the Y5 isolate showed 99% similarity to a plasmid, PM58, from *P. mirabilis* (GenBank accession no. KP662515.1) (Fig. 2) (13). Additionally, we observed an integrin-1 gene (*intI1*) upstream of two tandem copies of the IS*CR1* element at a point where cassette integration usually occurs. This alignment information indicated that the IS*CR1* element played an important role in the genetic transmission of bla_{NDM-1} and likely mediated its transposition from a single-source plasmid to the chromosome.

In summary, this is the first report of an *E. coli* isolate with chromosomally carried bla_{NDM-1}, although plasmid-borne bla_{NDM-1} is already widespread. By aligning the genomic sequences surrounding this bla_{NDM-1} isolate, we discovered that the IS*CR1* element was critical to bla_{NDM-1} mobility between chromosome and plasmid. Therefore, bla_{NDM-1} appears to be transmissible via multiple mechanisms and should be monitored with greater vigilance.

MATERIALS AND METHODS

Strain information and case history. A female patient, aged 32 years-old, came to Yantai Yuhuangding Hospital reporting serious pain in the right portion of her back accompanied by fever (the highest point reaching 38.6°C) for 10 days in September, 2013. She was diagnosed with ureteral and kidney stones, hydronephrosis, and a urinary tract infection (UTI) according to computed-tomography scan results and was admitted to our hospital for a ureterolithotomy after her menstrual period. Her temperature and systemic inflammatory index were normal and the white blood cell (WBC) count was 8.11×10^9 cells/liter, with 78.2% neutrophils and 14.3% lymphocytes in the blood. However, the WBC count in a urine sample was 67 cells/μl and the red blood cell count was 65 cells/μl, indicating a local UTI. She received nonstandard antibiotic treatment, including cefepime (2 g, every 8 h [q8h]) for 1 day and piperacillin-tazobactam (3.375 g, q8h) for 3 days. *E. coli* strain Y5, which was extremely resistant to multiple antimicrobial agents, including piperacillin-tazobactam, cephalosporin, and carbapenem, was isolated from urine cultures (at $>10^5$ CFU/ml) using the Vitek 2 system (bioMérieux, France). Soon, the patient was transferred to Shandong Provincial Hospital (a higher-level hospital) for further treatment. A follow-up survey showed that she recovered shortly after surgery for removal of renal and ureteral calculi, and bacteria were no longer isolated.

Antimicrobial-susceptibility testing and bla_{NDM-1} location. Antimicrobial susceptibility was measured with Etest strips (bioMérieux, Sweden) according to standard operating procedures. Briefly, direct colony suspension was adjusted to that of a 0.5 McFarland standard in saline (corresponds to $\sim 1.5 \times 10^8$ CFU/ml), and then the suspension was swabbed to a Müller-Hinton agar (MHA) plate. The strip was

applied to the MHA surface and the plate was inoculated in ambient air at 35°C for 16 to 18 h (http://www.biomerieux-diagnostics.com/Etest). The MBL phenotype and MICs for 13 antibiotics were determined and interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (17). The presence of the bla_{NDM-1} gene was established by PCR and confirmed by sequencing.

Because previous reports identified the bla_{NDM-1} gene as being located in plasmids, we performed S1-pulsed-field gel electrophoresis (PFGE) and XbaI-PFGE, followed by Southern blotting with a bla_{NDM-1} probe (6). In accordance with published methods, conjugation mating was tested between *E. coli* Y5 and *E. coli* J53, and electrotransformation was tested using competent *E. coli* DH5α cells (6).

Whole-genome sequencing and annotation. *E. coli* strain Y5 was cultured to mid logarithmic phase in 50 ml of LB medium at 37°C. DNA for sequencing was extracted via a QIAamp DNA minikit (Qiagen, Valencia, CA) and further purified using a PowerClean DNA cleanup kit (Mo Bio Laboratories, Carlsbad, CA) according to the protocols of the manufacturers. The quality of DNA was determined by gel electrophoresis and by a NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE). Single-molecule real-time (SMRT) sequencing reads were generated using a PacBio RS II platform (Pacific Biosciences, Menlo Park, CA). Sequencing reads were *de novo* assembled with the PacBio Hierarchical Genome Assembly Process 3 (HGAP3.0)/Quiver software package. The resulting assembly was confirmed with optical maps generated with 30-fold coverage on the Argus mapping station according to the OpGen protocol (OpGen, Inc., Gaithersburg, MD).

The assembled genome was annotated using GeneMark and Rapid Annotation by using Subsystems Technology (http://rast.nmpdr.org/rast.cgi) (18, 19). Coding DNA sequences (CDS) and protein similarities were determined by BLASTX (http://blast.ncbi.nlm.nih.gov/Blast.cgi), whereas protein domains were identified by InterProScan (20). ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) was used to detect antimicrobial resistance genes at 98% identity (21). The BacWGSTdb (http://bacdb.org/BacWGSTdb/) server was used for *in silico* multilocus sequence typing (MLST) analysis (22). Whole genomes were compared using Artemis Comparison Tool release 11.1.1, and the CGView server (http://stothard.afns.ualberta.ca/cgview_server/) was used to generate graphical maps, sequence features, base composition plots, analysis results, and sequence similarity plots (23).

Accession number(s). The chromosome and three plasmids of *E. coli* strain Y5 were deposited in GenBank under accession numbers CP013483, KT997783, KU043115, and KU043116 (Table 2).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01581-16.

TEXT S1, PDF file, 0.1 MB.

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The authors declare no conflicts of interest.

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