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pMEX01, a 70 kb plasmid isolated from *Escherichia coli* that confers resistance to multiple β-lactam antibiotics

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ABSTRACT

Multidrug-resistant microorganisms are of great concern to public health. Genetic mobile elements, such as plasmids, are among the most relevant mechanisms by which bacteria achieve this resistance. We obtained an *Escherichia coli* strain CM6, isolated from cattle presenting severe diarrheic symptoms in the State of Querétaro, Mexico. It was found to contain a 70 kb plasmid (pMEX01) with a high similarity to the pHK01-like plasmids that were previously identified and described in Hong Kong. Analysis of the pMEX01 sequence revealed the presence of a *bla*$_{CTX-M-14}$ gene, which is responsible for conferring resistance to multiple β-lactam antibiotics. Several genes putatively involved in the conjugative transfer were also identified on the plasmid. The strain CM6 is of high epidemiological concern because it not only displays resistance to multiple β-lactam antibiotics but also to other kinds of antibiotics.

Introduction

Plasmids play a crucial role in the dissemination of CTX-M type extended-spectrum β-lactamases (ESBLs), and their characterization may provide important insight into understanding multidrug-resistant bacterial strains. β-Lactams are among the most widely used antimicrobial agents for the treatment of bacterial infections. However, the intensive exposure of bacteria to β-lactam antibiotics has induced the emergence of several antibiotic resistance strategies, with

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the development of β-lactamases capable of inactivating β-lactams being one of the most relevant. In gram-negative bacteria, the production of ESBLs is the principal cause of resistance to β-lactam antibiotics.[1,2] Plasmids encoding CTX-M-type cefotaximase were reported in Germany in the late 1980s,[3,4] and they are currently distributed worldwide, including North America,[5] Latin America[6] and Asia.[7] CTX-M has become so widespread that it is now considered to be the most prevalent β-lactamase among clinical isolates of *Escherichia coli* worldwide.[8] Diverse studies in Hong Kong,[9,10] China,[11] and South Korea[12] have reported that CTX-M-14 is the most frequently found β-lactamase enzyme in *E. coli*, *Klebsiella pneumoniae*, and *Shigella* isolates. In Mexico, the presence of GES and CTX-M type β-lactamases have been found in *Enterobacteriaceae* clinical isolates.[13] In a previous study, we sampled multidrug-resistant *E. coli* strains from cattle presenting severe diarrheic symptoms in the State of Querétaro, Mexico, and analyzed their drug resistance spectra, plasmid profiles and conservation using shock waves.[14] In this study, we analyzed the complete nucleotide sequence of the pMEX01 plasmid from *E. coli* strain CM6, which has a high similarity with the previously described pHK01-like plasmids.[15]

Materials and methods

Isolation and identification of bacteria

Fecal samples were obtained from cattle presenting severe diarrheic symptoms in the State of Querétaro, Mexico during 2014. Samples from cattle that did not respond to standard antibiotic treatment were selected for further analysis. A total of 10 isolates were identified as *E. coli* by analyzing 16S ribosomal deoxyribonucleic acid (rDNA) sequences, which were amplified using the primers fD1 (CCGAATTCGTCGACAACAGAGTTTGATCCTGGCTCAG) and rD1 (CCCGGGATCCAAGCTTAAGGAGGTGATCCAGCC)[15] and by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry,[16] using a MicroFlex LT mass spectrometer (Bruker Daltonics, Bremen, Germany).

Antibiotic resistance profile

The plasmid pMEX01 was extracted from *E. coli* strain CM6 and transformed by electroporation (250 V, 20 Ω and 250 μFD) into the *E. coli* strain BL21 STAR (Invitrogen, Carlsbad, CA, U.S.A.). We then determined the antibiotic resistance profiles of the *E. coli* strain CM6 and the *E. coli* strain BL21 STAR containing pMEX01. All antimicrobial testing was performed on agar-solidified LB. The β-lactam antibiotics assayed included carbenicillin, cephalexin, ceftriaxone, cefotaxime and dicloxacillin. Kanamycin, rifampicin, chloramphenicol, polymyxin, spectinomycin and streptomycin were also tested. The minimum inhibitory concentration (MIC) of each *E. coli* strain were evaluated by the criteria of the Clinical and Laboratory Standards Institute.[17] *E. coli* BL21 STAR without the pMEX01 plasmid was used as a control.

Sequencing and bioinformatics

E. coli strain CM6 was selected for plasmid isolation and sequencing. The plasmid was extracted from an overnight culture of the selected strain grown in LB broth (BD DIFCO, Mexico) and purified using a Zyppy Plasmid Miniprep Kit (Zymo Research, CA, U.S.A). The plasmid was sequenced with an Illumina HiSeq 2000 sequencing platform at the Macrogen Korea Institute (Seoul, Republic of Korea) using a whole-genome shotgun library strategy. The plasmidic DNA sequence was reconstructed using multiple bioinformatic tools. The sequence reads were assembled using the genome assembler program SPAdes v3.1.1.[18] The initial contigs were compared with the Plasmids database from NCBI GenBank (accessed April 2016) using Blast+. The best matches indicated a high similarity with pHK plasmid family, then we use pHK17a as a template to reorder the contigs and reconstruct the full plasmid. Open reading frames (ORFs) were deduced and annotated by using NCBI Glimmer v3.02.[19–21] ORF sequences were analyzed against protein family databases to obtain information about their functionality.[22,23]

Nucleotide sequence

The nucleotide sequence for the pMEX01 plasmid has been deposited in GenBank under the accession number KU695535.

Results

Sequence assembly and comparative analysis of the pMEX01 plasmid

The plasmid (pMEX01) from *E. coli* strain CM6 was isolated and sequenced. The sequencing of pMEX01 produced 26,133,370 short-sequences as 2×100 paired-end reads. The initial assembly of the sequence reads produced 1247 contigs, with an average size of 4879 bp, an expected 2119× average coverage and a calculated N50 value of 20,000. The fine assembly using pHK17a sequence as a reference showed the presence of a circular replicon consisting of 70,093 nucleotides, with an average GC content of 52.27% (Fig. 1).

The pMEX01 plasmid is comparable in size and is highly similar to other plasmids isolated and sequenced from *E. coli* in Asia, including pHK17a (99%), pHK01 (99%), pHK08 (99%), and pHK09 (99%); pKF3-70 (98%) from *K. pneumoniae*; pEG0356 (99%) (except for the $bla_{CTX\text{-}M\text{-}14}$ allele) from *S. sonnei*; and pO26-L (98%) a plasmid from a Canadian *E. coli* isolate that carries a tetracycline resistance gene cluster (Fig. 2). Our assembly covers 88.71% of the reference sequence (pHK17a) with an average coverage of 7547×. A sequence analysis of pMEX01 showed that it belongs to the incompatibility group IncFII. Analyses using several algorithms and approaches showed that the pMEX01 plasmid had 98 potential ORFs, with only 8 showing similarities to unknown proteins from the NCBI non-redundant microbial protein database (Supplementary Table S1). pMEX01 contains a number of primary structural features that are highly similar to pHK01-like plasmids. These include a DNA transfer region (*tra* genes), required for efficient

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