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Detection of chromosomal $bla_{CTX\text{-}M\text{-}15}$ in *Escherichia coli* O25b-B2-ST131 isolates from the Kinki region of Japan

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ABSTRACT

Escherichia coli O25b-B2-ST131 isolates harbouring $bla_{CTX\text{-}M\text{-}15}$ are distributed worldwide. The $bla_{CTX\text{-}M\text{-}15}$ transposition unit has often been found on plasmids and the genetic contexts have been examined; however, less is known about the frequency and contexts of the $bla_{CTX\text{-}M\text{-}15}$ transposition unit on the chromosome. This study was performed to determine the chromosomal location of the $bla_{CTX\text{-}M\text{-}15}$ transposition unit and to analyse the molecular structure of the region surrounding the $bla_{CTX\text{-}M\text{-}15}$ transposition unit in *E. coli* O25b-B2-ST131 isolates. Twenty-two *E. coli* O25b-B2-ST131 strains harbouring $bla_{CTX\text{-}M\text{-}15}$ that had been isolated from university hospital patients and nursing home residents in the Kinki region of Japan were examined. Inverse PCR (iPCR) targeting $bla_{CTX\text{-}M\text{-}15}$ was performed to classify the molecular structure of the region surrounding the $bla_{CTX\text{-}M\text{-}15}$ transposition unit. The isolates were classified into nine types (types A–I) considering the iPCR results; type A was the most prevalent type (13/22 isolates). Sequences of the iPCR-amplified DNA fragments showed that the $bla_{CTX\text{-}M\text{-}15}$ transposition unit consisted of IS*Ecp1*, $bla_{CTX\text{-}M\text{-}15}$ and orf477Δ. A homology search of the obtained sequences showed that the $bla_{CTX\text{-}M\text{-}15}$ transposition unit was inserted into different chromosomal regions in eight of the nine classified types. Although 21 of the 22 *E. coli* isolates possessed chromosomally located $bla_{CTX\text{-}M\text{-}15}$ transposition units, clonal spread was not evident on pulsed-field gel electrophoresis (PFGE) analysis. Taken together, these data indicate that certain *E. coli* O25b-B2-ST131 strains harbouring chromosomal $bla_{CTX\text{-}M\text{-}15}$ have emerged and spread in the Kinki region of Japan.

1. Introduction

Escherichia coli O25b-B2-ST131 isolates, which are found throughout the world, produce CTX-M-15-type extended-spectrum β-lactamases (ESBLs) that are known to cause antimicrobial-resistant nosocomial and community-acquired infections [1,2].

CTX-M-type ESBLs are classified according to their amino acid sequences and basically consist of four main groups, i.e. CTX-M-1 group (CTX-M-1, CTX-M-3, CTX-M-15, etc.), CTX-M-2 group (CTX-M-2, CTX-M-4, CTX-M-5, etc.), CTX-M-8 group (CTX-M-8, CTX-M-40 and CTX-M-63) and CTX-M-9 group (CTX-M-9, CTX-M-13, CTX-M-14, etc.), as well as some minor variants [3]. β-Lactamases of *Kluyvera* spp. are regarded as ancestral of the CTX-M-type ESBLs spread in many countries [3]. Genetically, multiple insertion sequence (IS)-mediated incorporation of β-lactamase genes of different *Kluyvera* spp. is considered a possible factor in sequence variations in CTX-M-type ESBLs [4].

Most of the transposition units in the CTX-M-1 group consist of a left inverted repeat (IR-L), IS*Ecp1*, a right inverted repeat (IR-R), a spacer sequence between the IS*Ecp1* and $bla_{CTX\text{-}M}$, $bla_{CTX\text{-}M}$, orf477 and another IR-R of the 3′ end [5–7]. It has been reported that mobilisation of the $bla_{CTX\text{-}M}$ transposition unit is mediated by the IS*Ecp1*, IR-L and IR-R [8,9] and that the presence of the IR-L contributes to higher transposition efficiency [10]. Three types of spacer sequences in CTX-M-1 group transposition units, e.g. 48, 79 and 127 bp, have been reported [5–7] and it is suggested that the expression level of CTX-M-type ESBLs is affected by the kind of spacer sequences [11].

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Genes encoding ESBLs are usually located on transmissible antimicrobial resistance plasmids. However, recent studies have shown that certain bla_{CTX-M} genes are located on the bacterial chromosome [12–14]. Although the chromosomal location of $bla_{CTX-M-15}$ has been described in previous reports, the genetic structure surrounding the chromosomally located $bla_{CTX-M-15}$ transposition unit has not been studied in detail.

Inverse PCR (iPCR) is a powerful technique that can be used to amplify the unknown flanking region of a known target region [15]. Therefore, iPCR with a robust high-fidelity PCR enzyme and specific outward primers against $bla_{CTX-M-15}$ is applicable in order to obtain sequence information of longer flanking regions of the $bla_{CTX-M-15}$ transposition unit. Although there are few reports describing the applications of iPCR for genotyping, it was worth considering iPCR targeting bla_{CTX-M} to characterise the genetic structure surrounding the bla_{CTX-M} transposition unit in antimicrobial resistance genes.

Currently, the meaning of the chromosomal location of the bla_{CTX-M} transposition unit is not clear. The chromosomal location of certain antimicrobial genes could offer more stable retention than location on conventional plasmids and might facilitate the distribution of antimicrobial-resistant bacteria even without antibiotic selection pressure. Therefore, detection of chromosomally located bla_{CTX-M} transposition units and characterisation of their molecular structure are particularly important for understanding the spread of antimicrobial-resistant bacteria.

In this study, iPCR targeting $bla_{CTX-M-15}$ and subsequent sequence analysis were used to detect chromosomally located $bla_{CTX-M-15}$ transposition units and to characterise the molecular structure surrounding $bla_{CTX-M-15}$ in 22 *E. coli* O25b-B2-ST131 strains.

2. Materials and methods

2.1. Escherichia coli isolates

In this study, 22 *E. coli* O25b-B2-ST131-$bla_{CTX-M-15}$ isolates were examined, comprising 10 clinical isolates (indicated by the letter N) and 12 isolates (indicated by JO) from nursing home residents. The clinical isolates isolated from urine, sputum and other specimens obtained from eight inpatients and two outpatients (N447 and N449) were selected from 2898 *E. coli* isolates collected in 2008 at Kansai Medical University Hirakata Hospital in the Kinki region of Japan [16]. The isolates from nursing home residents were selected from 31 *E. coli* strains that produced CTX-M-type ESBLs isolated from 144 stool specimens of elderly residents in one of the three private nursing homes (nursing home A) described in our previous report [17].

2.2. Characterisation of the Escherichia coli isolates

The 22 *E. coli* isolates examined in this study were determined to be *E. coli* O25b-B2-ST131 strains that produce the CTX-M-15-type ESBL using conventional microbiological techniques and the double disk diffusion test to confirm the ESBL phenotype in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines (document M100-S23). The sequence type, phylogenetic group and O types of these *E. coli* strains were determined by using previously described protocols [18–21].

2.3. Bacterial DNA extraction, and detection and genotyping of bla_{CTX-M}

Bacterial DNA was extracted from cultures of the *E. coli* isolates using a PureLink™ Genomic DNA Mini Kit (Life Technologies Japan, Tokyo, Japan). By following the protocols described by Monstein et al., bla_{CTX-M} was detected in these *E. coli* O25b-B2-ST131 strains [22]. Their genotype ($bla_{CTX-M-15}$) was determined by sequencing DNA fragments amplified by PCR with the primers CTXMF and CTXMR1.

2.4. Conventional PCR and inverse PCR

Purified bacterial DNA samples were used as templates for conventional PCR and iPCR. For conventional PCR, 100 pg of purified bacterial DNA was amplified with a PrimeSTAR Max DNA Polymerase (Takara Bio Inc., Shiga, Japan) according to the manufacturer's instructions. For iPCR, 100 ng of purified bacterial DNA was digested with *Pst*I or *Hin*1I and was recircularised by ligation. Then, 0.5 ng of the processed bacterial DNA was subjected to iPCR using the PrimeSTAR Max DNA Polymerase with primers U1R and U2R. After iPCR, a portion of the amplified DNA sample was digested with the restriction enzyme that was used for template processing, i.e. *Pst*I or *Hin*1I. Amplified intact DNA fragments and *Pst*I- or *Hin*1I-digested DNA fragments were then analysed by 1% agarose gel electrophoresis.

2.5. Sequence analysis

In general, iPCR-amplified DNA fragments were directly sequenced using a BigDye® Terminator v.3.1 Cycle Sequencing Kit (Life Technologies Japan) following the manufacturer's instructions. A part of the DNA fragments was sequenced after cloning into the pCR®-Blunt II vector (Life Technologies Japan). Sequences of the amplified DNA fragments were confirmed by sequencing both DNA strands of the template DNA by primer walking, which was performed using the primers listed in Table 1. The GenBank accession nos. of the sequences are AB683462–AB683465 and AB701567–AB701571. The obtained sequences were analysed with the Basic Local Alignment Search Tool (BLAST) [23] and the insertion sites of the $bla_{CTX-M-15}$ transposition unit were identified with reference to the complete genome sequence of the uropathogenic *E. coli* O25b-B2-ST131 strain NA114 (GenBank accession no. NC_017644) [24].

2.6. Pulsed-field gel electrophoresis (PFGE)

*Xba*I- or I-*Ceu* I-digested genomic DNA samples from the *E. coli* isolates were analysed on a CHEF-DR II System (Bio-Rad, Hercules, CA) following the PulseNet protocol [25]. The *Xba*I-digested *Salmonella enterica* serovar Braenderup H9812 sample was kindly provided by the US Centers for Disease Control and Prevention (Atlanta, GA) through the National Institute of Infectious Diseases (Tokyo, Japan). The I-*Ceu* I-digested samples were analysed by PFGE and were transferred onto a Zeta-Probe blotting membrane (Bio-Rad). The DNA fragments containing the $bla_{CTX-M-15}$ transposition unit were visualised by hybridisation with digoxigenin-labelled $bla_{CTX-M-15}$ cDNA following the manufacturer's instructions (Roche Diagnostics Japan, Tokyo, Japan).

3. Results

3.1. Screening of Escherichia coli O25b-B2-ST131 isolates possessing $bla_{CTX-M-15}$

Escherichia coli strains that showed an ESBL phenotype on the double disk diffusion test were subjected to CTX-M grouping and sequence analysis to determine the CTX-M genotype. After confirmation of ESBL phenotype and $bla_{CTX-M-15}$, the phylogenetic group, sequence type and O type of the *E. coli* isolates were determined. Finally, 10 and 12 *E. coli* O25b-B2-ST131 isolates were selected,