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Brief report

IS*Ecp1* element in association with $bla_{CTX\text{-}M}$ genes of *E. coli* that produce extended-spectrum β-lactamase among the elderly in community settings

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ABSTRACT

Introduction: This study analyzed the relationship between the IS*Ecp1* element and $bla_{CTX\text{-}M}$ genes of *Escherichia coli* isolates that produce extended-spectrum β-lactamase (ESBL) in community settings.
Methods: Nineteen *E. coli* isolates that produced CTX-M-type β-lactamase were collected from four communities of elderly people in Shenyang, China. Polymerase chain reaction (PCR) amplification and direct sequencing were used to detect the insertion of the IS*Ecp1* element into the genetic environment of the $bla_{CTX\text{-}M}$ genes.
Results: The IS*Ecp1* element was associated with several $bla_{CTX\text{-}M}$ gene types, including CTX-M-14, CTX-M-24, CTX-M-22, and CTX-M-79. Sequence analysis revealed that all of the IS*Ecp1*-like DNA sequences contained the putative promoter region that is involved in CTX-M genes transcription. IS*Ecp1* insertion sequences were observed 42–127 bp upstream of the open reading frames (ORFs) that encode the CTX-M enzymes in all 15 strains. The CTX-M-79 β-lactamase-encoding gene was observed with a different IS*Ecp1* insertion site and variable sequences between the IS*Ecp1* and $bla_{CTX\text{-}M\text{-}79}$ gene. For one strain (T298), the IS*Ecp1* element was disrupted by IS*10*.
Conclusion: This work confirmed that the IS*Ecp1* elements were closely linked to $bla_{CTX\text{-}M}$ genes in community isolates from Shenyang, China.

El elemento IS*Ecp1* asociado con los genes $bla_{CTX\text{-}M}$ de *E. coli* que producen β-lactamasa de amplio espectro en las personas ancianas en marcos comunitarios

Palabras clave:
ESBL
β-lactamasa CTX-M
Secuencias de inserción

RESUMEN

Introducción: Este estudio analizó la asociación entre el elemento IS*Ecp1* y los genes $bla_{CTX\text{-}M}$ de los aislados de *Escherichia coli* que producen β-lactamasa de amplio espectro (ESBL) en marcos comunitarios.
Métodos: Se tomaron 19 aislados de *E. coli* que producían β-lactamasa de tipo CTX-M en 4 comunidades de personas ancianas de Shenyang, China. Se utilizó la amplificación mediante la reacción en cadena de la polimerasa (PCR) y la secuenciación directa para detectar la inserción del elemento IS*Ecp1* en el trasfondo genético de los genes $bla_{CTX\text{-}M}$.
Resultados: El elemento IS*Ecp1* estuvo asociado con varios tipos de gen $bla_{CTX\text{-}M}$, entre ellos CTX-M-14, CTX-M-24, CTX-M-22 y CTX-M-79. El análisis de secuencia reveló que todas las secuencias de ADN similares a IS*Ecp1* (IS*Ecp1*-like) contenían la región promotora putativa implicada en la transcripción de los genes CTX-M. Las secuencias de inserción de IS*Ecp1* se encontraron a 42–127 bp (pares de bases) aguas arriba de los marcos abiertos de lectura (ORF) que codifican las enzimas CTX-M en las 15 cepas. El gen CTX-M-79 que codifica β-lactamasa tuvo un punto distinto de inserción de IS*Ecp1* y secuencias variables entre IS*Ecp1* y el gen $bla_{CTX\text{-}M\text{-}79}$. En una cepa (T298), el elemento IS*Ecp1* fue degradado por IS*10*.
Conclusión: Este trabajo confirma que los elementos IS*Ecp1* estuvieron estrechamente ligados a los genes $bla_{CTX\text{-}M}$ en aislados comunitarios de Shenyang, China.

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Introduction

More than 80 CTX-M enzyme variants (www.lahey.org/studies/) are currently recognized as rapidly emerging members of the clavulanic acid-inhibited Ambler class A extended-spectrum β-lactamase (ESBL) family. These enzymes are a specific concern in many areas of the world.[1,2] In some countries, CTX-M-type enzymes are the most frequently isolated ESBLs from *Escherichia coli*.[3] They have been involved in several outbreaks in long-term care facilities and are also becoming a problem in the community.[4]

Insertion sequences (IS), especially IS*Ecp1*, have repeatedly been found adjacent to genes that encode CTX-M-type β-lactamase and appear to play an important role in the mobilization and expression of genes that encode these enzymes.[5–8] Because the novel ESBL CTX-M-79 (GenBank accession no. EF426798) and other CTX-M types from community isolates in China are 99–100% identical to the above CTX-M lactamases, the IS*Ecp1* elements may be in the vicinity of CTX-M-79 and other CTX-M genes. Thus, we investigated the presence of IS*Ecp1* elements in association with the novel ESBL CTX-M-79 and other CTX-M types from community isolates in China.

Methods

The 19 strains of *E. coli* used in this study have been reported previously.[9] They were isolated from rectal flora sampled from elderly people in four different communities in Shenyang, China. The strains were identified using the VITEK 2 system (bioMérieux S.A., Marcy l'Etoile, France). These strains included *E. coli* that produced the enzymes CTX-M-14 (11 strains), CTX-M-22 (three strains), CTX-M-79 (three strains), CTX-M-24 (one strain), and CTX-M-24 and CTX-M-79 together (one strain; Table 1). Pulsed field gel electrophoresis (PFGE) patterns from the ESBL-producers revealed a high degree of diversity.[9]

The genetic organization of the insertion sequence element IS*Ecp1* was investigated by polymerase chain reaction (PCR) amplification. The plasmid DNA for PCR amplification was prepared using commercial isolation kits (TIANGEN, Beijing, China) as recommended by the manufacturer. The primers used for amplification of the IS*Ecp1*-CTX-M-1 group were the following: forward, 5′-GGA AAA CTA TCC GTA CAA GGG AGT G-3′; reverse, 5′-CCG TTT CCG CTA TTA CAA ACC-3′. The primer pairs for amplification of the IS*Ecp1*-CTX-M-9 group were the following: forward, 5′-GGA AAA CTA TCC GTA CAA GGG AGT G-3′; reverse, 5′-GAT GAT TCT CGC CGC TGA AG-3′. Primers for the partial transposase gene of IS*Ecp1* were the following: forward, 5′-AAT ACT ACC TTG CTT TCT GA-3′; reverse, 5′-CAA CCA CCT TTC AAT CAT TTT T-3′. The 25 μl reaction mixture consisted of 40–50 ng of plasmid DNA, 2.0 mM $MgCl_2$, 200 μM of each dNTP, 1 × PCR buffer, 1 unit of TaKaRa TAQ DNA polymerase (Takara Shuzo Co., Ltd, Shiga, Japan) and 0.5 μM of each primer (Sangon, Shanghai, China). PCR amplification was performed with a PCR System 9700 (Applied Biosystems), and the cycling conditions included a 5 min initial denaturation step at 95 °C, followed by 35 cycles of 1 min at 95 °C, 1 min at 56 °C and 1 min at 72 °C, and a final extension step at 72 °C for 5 min. The PCR products were purified with UNIQ-10 Column (Sangon, Shanghai, China) and sequenced on an ABI PRISM 3730 sequencer (Applied Biosystems) in both directions using the dideoxy chain termination method. The nucleotide sequences and deduced protein sequences were analyzed using BLAST and BioEdit software.

The nucleotide sequence data reported in this paper are available in the GenBank nucleotide database under accession no. FJ169498.

Results

The insertion sequence IS*Ecp1* was identified upstream of the $bla_{CTX\text{-}M}$ gene in 15 strains using PCR, but was not observed in four isolates: T236, T332, T435 and S28 (Table 1). The length of the PCR fragment amplified for the partial transposase gene of IS*Ecp1* was 0.5 kb. The PCR products amplified for the IS*Ecp1*-CTX-M group were 1.2 kb, with the exception of one strain T298. For T298, the PCR fragment was approximately 2.5 kb, suggesting the insertion of additional DNA. Direct sequencing of the PCR products identified the insertion of an IS*10* sequence within IS*Ecp1*. The length of the disrupted IS*Ecp1* gene was 1209 bp in strain T298.

The putative promoter region involved in the transcription of the $bla_{CTX\text{-}M}$ genes was contained within all of the IS*Ecp1*-like DNA sequences. Sequence analysis showed that the −35 (TTGAAA) sequences near the right inverted repeat (IRR) sequences (ACGTG-GAATTTAGG 14 bp) at the end of the IS*Ecp1*-like element were located 148 bp upstream of the ATG site of $bla_{CTX\text{-}M\text{-}14}$, and of its point mutant derivative, $bla_{CTX\text{-}M\text{-}24}$, similar to what has been found in other studies (Fig. 1).[5–8] In these cases, the ATG sites of $bla_{CTX\text{-}M\text{-}22}$ were located 208 bp downstream of the −10 (TACAAT) promoter element of the IS*Ecp1*-like sequences, whereas the −10 (TACAAT) promoter element of the IS*Ecp1*-like sequences was located 82 bp upstream of the initiation codon in $bla_{CTX\text{-}M\text{-}79}$.

IS*Ecp1* insertion sequences have been observed 42–127 bp upstream of the ORFs that encode the CTX-M enzymes. In all 13 strains that belong to the CTX-M-9 group (CTX-M-14 or CTX-M-24), a 42 bp region with an identical sequence (named Y sequence) was found upstream of the start codon of the β-lactamase gene (Fig. 1). For three strains (T20, T359, T435), the CTX-M-22 group was characterized by a 48 bp region (W sequence) and the additional 79 bp fragments (V sequence), which was identical to the CTX-M-3 group as described by another study.[5] The CTX-M-79 cluster (assigned as the novel gene FJ169498) was characterized by a 45 bp region upstream of the $bla_{CTX\text{-}M\text{-}79}$ gene, which was shorter than the W sequence with the GAC nucleotide deletion according to Eckert's study (Fig. 1).[5]

Discussion

In the present study, IS*Ecp1* was found in the 15 strains examined (15/19, 79%; Table 1), and the IS*Ecp1* element was associated with several $bla_{CTX\text{-}M}$ genes, such as CTX-M-14, CTX-M-24, CTX-M-22, and CTX-M-79 (which was first reported in Shenyang, China). A detailed analysis of the promoter regions in bacteria containing the CTX-M genes revealed that this IS*Ecp1* element contained the typical −35 (TTGAAA) and −10 (TACAAT) putative promoter regions, suggesting that this IS element may enhance the expression of β-lactamase genes. Previous studies have demonstrated that the IS*Ecp1* elements may act as key factors in the dissemination of such ESBLs.[6–8] Interestingly, in one strain, T298, this IS*Ecp1* element was disrupted by IS*10*. Notably, IS*10* was identified upstream of $bla_{CTX\text{-}M\text{-}8}$ (AF189721) and may play a role in the mobilization of this $bla_{CTX\text{-}M\text{-}8}$ gene.

Interestingly, the insertion site of this IS*Ecp1* element is different from the different $bla_{CTX\text{-}M}$ types. The distances that separate the $bla_{CTX\text{-}M}$ gene from IS*Ecp1* vary within a given cluster of these enzyme-encoding genes, suggesting that different genetic events occur.[7] In the present study, a CTX-M-79 β-lactamase-encoding gene was observed with the specific insertion of IS*Ecp1* and different variable sequences between IS*Ecp1* and $bla_{CTX\text{-}M\text{-}79}$ gene. Our findings suggest that the $bla_{CTX\text{-}M\text{-}79}$ gene may derive from a unique origin. However, a comparison of the same $bla_{CTX\text{-}M}$-type genes from different countries (France and China) revealed that the variable sequences that separate the IS*Ecp1* elements from the $bla_{CTX\text{-}M}$

Table 1
CTX-M types and IS*Ecp1* element in community isolates from Shenyang, China.

Isolate no.	CTX-M group	CTX-M types	IS*Ecp1* element		
			Have or not	The upstream sequence of CTX-M enzyme	Length of sequence
T2	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
T20	CTX-M-1	CTX-M-22	Yes	W sequence	48 bp
				V sequence	79 bp
T24-1	CTX-M-9	CTX-M-24	Yes	Y sequence	42 bp
T24-2	CTX-M-1	CTX-M-79	Yes	W sequence	45 bp
T36	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
T104	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
T105	CTX-M-1	CTX-M-79	Yes	W sequence	45 bp
T181	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
T185	CTX-M-1	CTX-M-79	Yes	W sequence	45 bp
T229	CTX-M-1	CTX-M-79	Yes	W sequence	45 bp
T236	CTX-M-9	CTX-M-14	No	–	–
T249	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
T286	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
T298	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
T332	CTX-M-1	CTX-M-22	No	–	–
T359	CTX-M-1	CTX-M-22	Yes	W sequence	48 bp
				V sequence	79 bp
T435	CTX-M-9	CTX-M-24	No	–	–
T536	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
S24	CTX-M-9	CTX-M-14	Yes	Y sequence	42 bp
S28	CTX-M-9	CTX-M-14	No	–	–

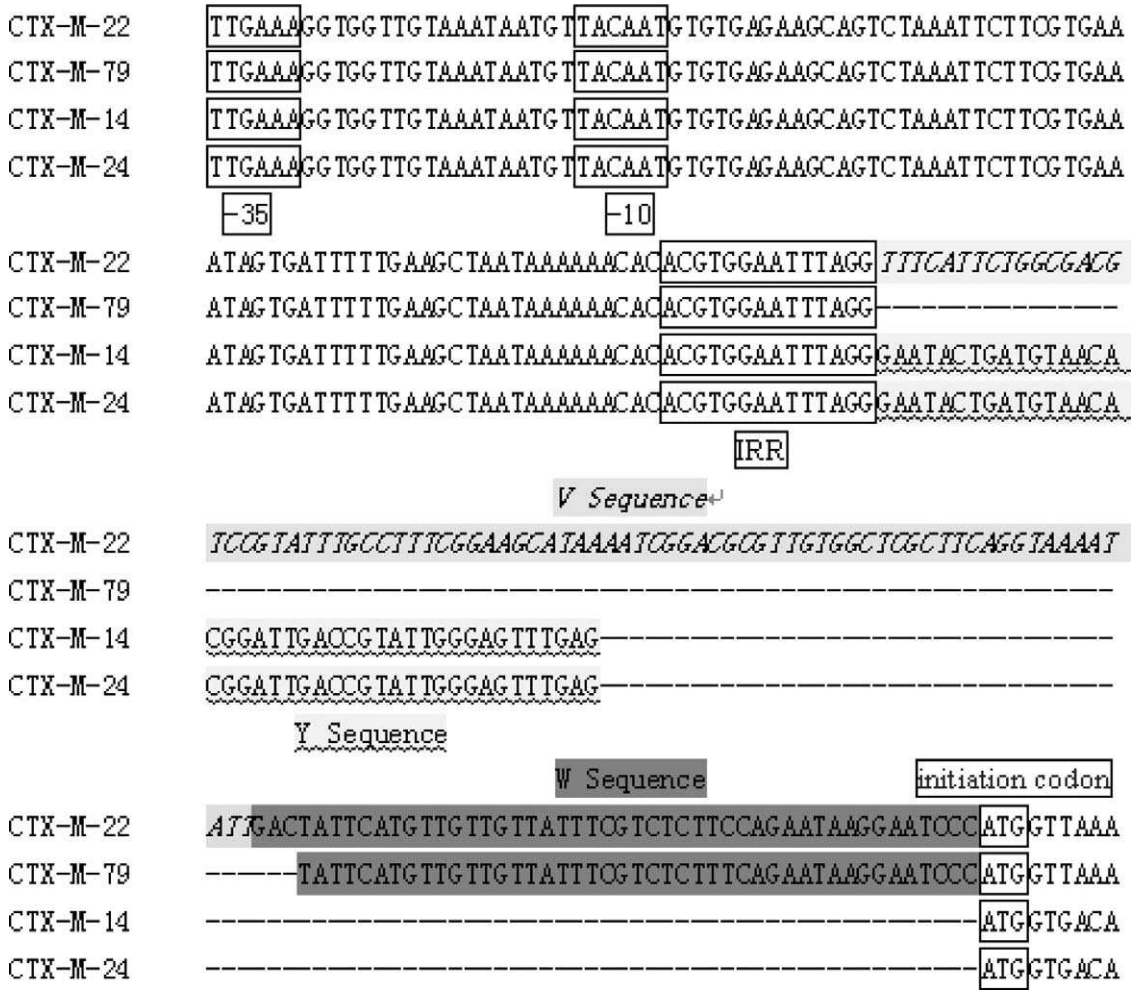

Fig. 1. Alignment of nucleotide sequences including variable sequences between the IS*Ecp1* and different $bla_{CTX\text{-}M}$-type genes for comparison.

genes had highly similar patterns of nucleotide sequence variation between geographic regions,[5] indicating that the insertion events seem to have emerged only once for a given bla_{CTX-M} gene and then subsequently spread throughout the world. This assumption is yet to be confirmed.

Several *enterobacteria* species of the *Kluyvera genus* are known to be natural reservoirs of CTX-M-like genes,[10] but the reservoir of IS*Ecp1*-like sequences among various human intestinal flora has not been identified.[7] Future studies should look for bacterial species in animal intestinal flora, as bla_{CTX-M} genes have also been identified in animal isolates.

Among the ESBLs, the cefotaximases (CTX-M) constitute a rapidly growing cluster of enzymes. IS*Ecp1* may be an efficient tool for the mobilization and expression of bla_{CTX-M} genes.[5–8] The present study clearly showed that the IS*Ecp1* elements were closely associated with bla_{CTX-M} genes from community isolates in Shenyang, China. These data may shed light on the mechanism of the rapid spread of CTX-M producing strains, but our analysis has limitations. Our study investigated only 19 isolates from four communities, which may provide only limited information. Furthermore, an analysis of the downstream region of bla_{CTX-M} genes and plasmid assays were not performed, which deserve further study. Finally, our study was conducted in our region, and the results cannot be generalized to other settings.

Conflict of interests

The authors declare that they have no conflict of interests related to this study.

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