Escherichia coli モデル株を用いたbla_<CTX-M-14> のプラスミドから染色体への転位の観察

（様式第３－２号）

Abstract

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Characterization of *bla*$_{CTX-M-14}$ transposition from plasmid to chromosome in *Escherichia coli* experimental strain.

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Abstract

Background: Mostly, CTX-M-type extended spectrum β-lactamase (ESBL) encoding gene is found on transferable plasmids. Among the several insertion sequence elements involved in mobilizing *bla*$_{CTX-M}$, the insertion sequence IS*Ecp1* exists on the upstream region of *bla*$_{CTX-M}$ is known as one of the most important elements. IS*Ecp1* is bracketed by a left terminal inverted repeat sequence (IRL) and right terminal inverted repeat sequence (IRR). IS*Ecp1* transfer *bla*$_{CTX-M}$ using IRL and alternative IRR (IRRalt) which exists on distal position of original IRR. Several recent studies conducted in clinical and community settings have reported the presence of chromosomally located *bla*$_{CTX-M}$ in ESBL-producing bacterial isolates. In this study, we aimed to characterize the frequency and molecular nature of the IS*Ecp1*-mediated transposition of *bla*$_{CTX-M-14}$ from a plasmid to a chromosome.

Method: To observe IS*Ecp1*-mediated transposition event, *E. coli* DH5α transformed with two kinds of plasmids, namely CRISPR/Cas9 plasmid and *bla*$_{CTX-M-14}$ plasmid was used as an *E. coli* experimental strain. Chromosomally-located *bla*$_{CTX-M-14}$ in *E. coli* was confirmed by S1 nuclease pulsed-field gel electrophoresis and Southern blot hybridization analysis. Genetic structure of chromosomally-located *bla*$_{CTX-M}$ transposition units and its surrounding sequences were analyzed by inverse PCR and adapter ligation-mediated PCR followed by sequencing analysis.

Results and Discussions: We determined 102 different chromosomal transposition sites of *bla*$_{CTX-M-14}$ in 126 *E. coli* isolates following five independent screening procedures. The characterization of the 102 different chromosomal transposition sites of *bla*$_{CTX-M-14}$ observed in this study revealed the presence of 5-bp direct repeat (DR) sequences and identical IRL in 80 *E. coli* isolates. However, 5'-flanking sequences of the right terminal DR sequences in the 80 *E. coli* isolates were highly diverse, and consensus sequences of IRRalt were not observed. In case of our *E. coli* experimental strain, the

frequency of the IS*Ecp1*-mediated transposition of $bla_{\text{CTX-M-14}}$ from a plasmid to a chromosome was determined to be 0.51% (SD = 0.37). Collectively, IRRalt unevenly used by IS*Ecp1* could be one of factors providing opportunity for IS*Ecp1*-mediated transposition of $bla_{\text{CTX-M-14}}$ with high frequency. The molecular nature of IS*Ecp1* could plausibly be a factor contributing to the high detection rates of *E. coli* possessing chromosomally located $bla_{\text{CTX-M-14}}$ in both clinical and community settings.