High Prevalence of Chromosomal bla\textsubscript{CTX-M-14} in Escherichia coli Isolates Possessing bla\textsubscript{CTX-M-14}

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In several bacterial species, chromosomal bla\textsubscript{CTX-M-14} or bla\textsubscript{CTX-M-2} has been identified (1–5). The prevalence of chromosomal bla\textsubscript{CTX-M-14} among Escherichia coli strains producing CTX-M-14-type extended-spectrum β-lactamase (ESBL) remains unclear. We preliminarily analyzed the location of bla\textsubscript{CTX-M-14} in E. coli isolates possessing this gene by inverse PCR and conventional sequencing analysis, as described previously (5) and detected at least five chromosomal locations of bla\textsubscript{CTX-M-14} (AB780367, AB780368, AB914799, AB915399, and AB915400). Multiple copies of the insertion sequence have become an obstacle to evaluation of bla\textsubscript{CTX-M-14} location by whole-genome sequencing using the next-generation sequencer. Therefore, we evaluated the location of bla\textsubscript{CTX-M-14} in 74 E. coli isolates possessing bla\textsubscript{CTX-M-14}, including 16 isolates (JO isolates) obtained from nursing home residents in the Kinki region of Japan and 58 isolates (KC isolates) obtained from asymptomatic healthy individuals living in a Thai rural community by pulsed-field gel electrophoresis (PFGE) using S1 nuclease (S1-PFGE) and Southern blot hybridization (6–8).

Locations of bla\textsubscript{CTX-M-14} of the E. coli isolates examined were summarized in Fig. 1 along with genetic information of the E. coli isolates. Chromosomal bla\textsubscript{CTX-M-14} was detected in 46 (62.2%) of the 74 E. coli isolates examined. Among the 58 KC isolates and the 16 JO isolates, 33 (56.9%) and 13 (81.3%) possessed chromosomal bla\textsubscript{CTX-M-14}, respectively. Nine (15.5%) and three (18.8%) of the 33 KC and 13 JO isolates, respectively, possessed both chromosomal and plasmid-mediated bla\textsubscript{CTX-M-14}. As negative control, we used a Klebsiella pneumoniae clinical strain which possesses bla\textsubscript{TEM} and bla\textsubscript{SHV} but not bla\textsubscript{CTX-M-14} (9). One to three bands were detected in the examined E. coli isolates by Southern blotting; however, no band was detected in the negative control (data not shown). Our preliminary observation using KC002 (AB914799), KC012 (AB915399), KC140 (AB915400), and JO72 (AB780367 and AB780368) was consistent with results obtained by S1-PFGE and Southern blotting hybridization. These results strongly suggested that our evaluation of bla\textsubscript{CTX-M-14} location in the examined E. coli isolates was appropriately performed. No statistical significance was observed by the \( \chi^2 \) test among isolate group, phylogenetic group, location of bla\textsubscript{CTX-M-14}, and prevalence of chromosomal bla\textsubscript{CTX-M-14}.

Susceptibility of the E. coli isolates examined to amikacin (AMK), gentamicin (GEN), ciprofloxacin (CIP), norfloxacin (NOR), tetracycline (TET), imipenem (IPM), and meropenem (MEM) was also examined. Antibiotic resistance profiles of the JO and the KC isolates were not noticeably different among each of the isolate groups, regardless of bla\textsubscript{CTX-M-14} location.

Our results showed that 46 of the 74 examined isolates (62.2%) possessed at least one copy of chromosomal bla\textsubscript{CTX-M-14}. In this study, we could not determine all sequences surrounding chromosomal bla\textsubscript{CTX-M-14} in the E. coli isolates possessing chromosomal bla\textsubscript{CTX-M-14}. In addition, the copy number and stability of chromosomal bla\textsubscript{CTX-M-14} in the E. coli isolates remain unclear. Nevertheless, taking into account that the chromosomal location of bla\textsubscript{CTX-M-14} transposition units was detected even in the KC isolates from asymptomatic healthy individuals living in a rural area in Thailand, the chromosomal location of the bla\textsubscript{CTX-M-14} transposition unit might be one factor contributing to the worldwide spread of E. coli strains producing CTX-M-14-type ESBL.

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**FIG 1** Summary of \( \text{bla}_{\text{CTX-M-14}} \) location in the E. coli isolates producing CTX-M-14-type ESBL. Information on isolates possessing \( \text{bla}_{\text{CTX-M-14}} \) only on the chromosome (A) and on plasmids, with or without the gene on the chromosome (B), is summarized. Black squares indicate resistance to antibiotic, an identified location of \( \text{bla}_{\text{CTX-M-14}} \), and classification of the detected plasmids harboring \( \text{bla}_{\text{CTX-M-14}} \). Gray squares indicate intermediate sensitivity to antibiotic. The location of \( \text{bla}_{\text{CTX-M-14}} \) is indicated as C (chromosomal) or P (plasmid). Plasmids were classified by size: group 1, 104.5 kb or shorter; group 2, from 104.5 to 150 kb; group 3, from 150 to 250 kb; group 4, from 250 to 310 kb; group 5, 310 kb or longer. Phy. grp, phylogenetic group; ST, sequence type.


