HOKKAIDO UNIVERSITY

Title	Elucidation of the dissemination mechanisms of fluoroquinolone-resistant Escherichia coli ST131 in the community
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Description	この博士論文の全文は利用可能日以降に公開されます。利用可能日以前の閲覧方法は https://www.lib.hokudai.ac.jp/dissertations/copy-guides/ をご参照ください。
Degree Grantor	北海道大学
Degree Name	博士(獣医学)
Dissertation Number	甲第17140号
Issue Date	2026-09-25
DOI	https://doi.org/10.14943/doctoral.k17140
Doc URL	https://hdl.handle.net/2115/100759
Type	doctoral thesis
File Information	Maeda_Aiko_summary.pdf, 全文の要約



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SUMMARY

In the 21st century, antimicrobial resistance (AMR) bacterial infections have emerged as a major global public health threat. Among them, the worldwide dissemination of fluoroquinolone-resistant (FQ-R) *E. coli*—particularly the international high-risk clone ST131—remains a critical and unresolved challenge. Although existing AMR countermeasures have largely focused on healthcare settings, resistance rates of FQ-R *E. coli*, most of which belong to ST131, have not shown a clear decline. This suggests that hospital-centered strategies alone may be insufficient for controlling the spread of these clones. Recent evidence further indicates that ST131 is not confined to clinical environments but is also present in healthy community members and in companion animals sharing close living spaces with humans, raising the possibility that these animals may act as community-level reservoirs. However, the transmission dynamics of ST131 in households and the community, as well as its mechanisms for surviving and persisting across different hosts, remain insufficiently understood.

This doctoral dissertation addressed this gap by focusing on the underexplored role of companion animals within the One Health framework. The overarching aim was to characterize the distribution of FQ-R *E. coli* in companion animals, elucidate routes of community and household transmission involving humans and animals, and identify molecular determinants that enable ST131 to survive and colonize the intestinal environment. By integrating molecular epidemiology with functional genomics, this work sought to provide scientific evidence that companion animals can harbor human-adapted pandemic clones and may contribute to community-associated AMR through household transmission.

In CHAPTER I, FQ-R *E. coli* isolates from companion animals in Sapporo were characterized by identifying antimicrobial susceptibility, clonality, antimicrobial resistance genes, and virulence factors (VFs). The study revealed that 33.0% of 106 companion animals that visited veterinary clinics in Sapporo carried FQ-R *E. coli*, and 55.8% of these isolates were

identified as the international high-risk clone ST131. Whole-genome sequence (WGS) revealed that the ST131 isolates were distributed across five ST131 clades/sub-clades, indicating substantial diversity in the dissemination of ST131 in companion animals. Another high-risk clone, ST1193, was also isolated. Genetically, all isolates harbored mutations within the quinolone resistance-determining regions, and 51.4% carried *bla*$_{\text{CTX-M}}$ genes. Furthermore, numerous VFs were identified, including eight (*csgA*, *fdeC*, *fimH*, *yehB*, *yehC*, *yehD*, *nlpI*, and *terC*) shared by all isolates. These findings indicate that companion animals share certain *E. coli* lineages, including ST131 and ST1193, with humans, serving as a potential reservoir.

In CHAPTER II, to elucidate community-level dissemination of FQ-R *E. coli* including ST131, a One Health genomic analysis of 2,358 *E. coli* isolates collected contemporaneously between 2021 and 2022 from humans, companion animals, livestock, and environmental sources (wastewater and rivers) within a single regional community (Sapporo and surrounding areas) was conducted. WGS data demonstrated that ST131 and ST1193 predominated in humans, companion animals, and wastewater, whereas livestock and river isolates displayed clearly distinct population structures. Core-genome SNP analysis further revealed the sharing of genetically similar ST131 strains between humans and companion animals and provided evidence of direct transmission within households. These results suggest that within this community, transmission involving human–companion animal contact may play a more substantial role than pathways involving livestock or environmental waters. In addition, long-read sequencing of representative isolates demonstrated that *bla*$_{\text{CTX-M-27}}$-harboring plasmids shared highly conserved backbone structures and near-identical nucleotide sequences across isolates from humans, companion animals, and sewage. These findings suggest that regional dissemination of extended-spectrum β-lactamase (ESBL)-producing ST131 is driven not only by clonal expansion but also by the independent spread of ESBL-encoding mobile genetic elements across these reservoirs.

In CHAPTER III, a high-throughput functional genomics approach called Transposon-Directed Insertion-site Sequencing (TraDIS) was employed to comprehensively screen for bacterial factors essential for the survival and adherence of ST131 within simulated intestinal environments of companion animals and humans. Using a canine fecal suspension model, critical genes essential for survival in the canine intestine were successfully identified and validated. These included highly conserved core genes, *oxyR* (oxidative stress regulator) and *lpxL* (Lipid A biosynthesis enzyme), which defend against host immune responses, as well as two ST131-lineage-specific genes (*SME173_02031* and *SME173_01788*) whose specific functions contribute uniquely to survival. Conversely, although candidate genes for adherence to human intestinal epithelial cells were screened, their essentiality could not be reproduced in single-gene deletion mutants, highlighting the multifactorial complexity of bacterial adherence. These findings indicate that ST131 utilizes a dual strategy, combining fundamental bacterial defense systems with unique lineage-specific adaptations, to withstand the hostile intestinal environment of companion animals. This robust survival capability facilitates the establishment of ST131 in companion animals and may also enable its adaptation in humans as a stable reservoir, thereby driving the community dissemination of ST131.

Together, this dissertation provides an integrated One Health perspective on the community dissemination and intestinal persistence of the high-risk clone ST131. Comprehensive local sampling and genomic analysis demonstrated that ST131 primarily spreads through human-associated routes, with spillover to companion animals, which in turn can sustain intestinal colonization through a combination of universal and lineage-specific mechanisms. Evidence of within-household sharing between humans and companion animals—encompassing both clonal transmission and the co-circulation of closely related ESBL plasmids—further highlights the potential contribution of the human–animal interface to community-level AMR dissemination. In addition, TraDIS-based functional genomics

identified oxidative-stress response systems, lipid A biosynthesis pathways, and ST131-specific genes as contributors to gut survival, advancing molecular understanding of the colonization basis of ST131.

These findings collectively support the need to extend AMR countermeasures beyond hospital-centered approaches, emphasizing surveillance in community populations, including companion animals; interventions targeting the human–animal interface; and the incorporation of molecular microbiology to better understand regional dissemination factors. Such strategies may facilitate the development of more effective and comprehensive AMR control frameworks in the future.