学位論文内容の要旨
Abstract of the dissertation

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学位論文題名
The title of the doctoral dissertation

Genetic analysis of extended-spectrum ß-lactamase-producing *Enterobacteriaceae* from humans and poultry in Zambia.

(ザンビアのヒトおよび家禽から分離された基質特異性拡張型 β ラクタマーゼ産生腸内細菌の遺伝学的解析)

Antimicrobial resistance (AMR) among *Enterobacteriaceae* from humans and animals raises considerable concern globally. While AMR has a broad scope encompassing several pathogens, multidrug-resistant (MDR) gram-negative bacteria are most dreaded because of their fatal health outcomes. Most MDR *Enterobacteriaceae* produce hydrolytic enzymes called extended-spectrum ß-lactamases (ESBLs) that degrade ß-lactam antibiotics to render them ineffective. Despite the many ESBL classes described, the CTX-M-type ESBLs dominate, with reports documented on every populated continent. Strains carrying *bla*$_{\text{CTX-M}}$ genes are usually found in clinical samples; however, animal reservoirs have also emerged, likely due to the extensive use of antimicrobial growth promoters. The CTX-M-type enzymes are encoded by *bla*$_{\text{CTX-M}}$ genes, which usually exist on plasmids with other AMR genes, thus explaining the associated MDR. However, chromosomal *bla*$_{\text{CTX-M}}$ genes have also been reported; therefore, investigating the association between chromosomal *bla*$_{\text{CTX-M}}$ and MDR is crucial. Furthermore, there is a need to evaluate the possibility of transmission of *bla*$_{\text{CTX-M}}$-carrying MDR strains between food animals and humans.

Sixty-six MDR *Enterobacteriaceae* isolates collected from poultry (n = 20) and hospital patients (n = 46) in Lusaka, Zambia, were sequenced on MiSeq and MinION and assembled to nearly complete genomes. Three species were identified among the human strains; *Escherichia coli* (n = 36), *Klebsiella pneumoniae* (n = 9), and *Enterobacter cloacae* (n = 1), while all the poultry strains were *E. coli* (n = 20). *In silico* genotyping detected four alleles of the *bla*$_{\text{CTX-M}}$ gene, namely *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-15}}$, *bla*$_{\text{CTX-M-27}}$, and *bla*$_{\text{CTX-M-55}}$ across

65/66 (98.5%) isolates. The *bla*$_{\text{CTX-M}}$ gene existed on plasmids in 58/65 (89.2%) strains and on chromosomes in the remaining 7/65 (10.8%) isolates. In one *E. cloacae* and three *E. coli* strains, the *bla*$_{\text{CTX-M-15}}$ gene was found on large (> 10 kb) chromosomal insertions bordered by the IS*Ecp1* insertion sequence at one end. The nucleotide sequences of these insertions resembled previously reported plasmids and harbored multiple AMR genes that mostly correlated with the observed phenotypic AMR profiles.

Phylogenic analysis and hierarchical clustering of *E. coli* strains revealed the clustering of 4/20 (20%) poultry and 9/36 (25%) clinical isolates belonging to O17:H18-ST69, suggesting clonal transmission. The O17:H18-ST69 strains from poultry and humans shared an IncI-complex plasmid carrying five AMR genes and an IncFI plasmid harboring nine AMR genes. Further comparison showed that the strains also possessed unique AMR plasmids distinct for each niche. Specifically, the four poultry strains had an IncFII(pCoo) plasmid carrying the *bla*$_{\text{CTX-M-55}}$ allele, and the nine human isolates harbored the *bla*$_{\text{CTX-M-14}}$ allele on an IncHI plasmid.

The IS*Ecp1*-mediated transposition of the *bla*$_{\text{CTX-M-15}}$ gene with various AMR genes from plasmids to chromosomes in *E. cloacae* and *E. coli* indicated it as a general mechanism across multiple *Enterobacteriaceae* species. Furthermore, the potential low fitness cost of chromosomal MDR insertions may promote the maintenance of MDR strains in antibiotic-free environments, undermining antibiotic stewardship programs. Finally, the clonal dissemination of MDR *E. coli* O17:H18-ST69 between poultry and humans underscores the need for strategic and concerted efforts from human and animal health sectors to prevent and control foodborne MDR.