

1. “Determine the outbreak mechanisms and development of a surveillance model for multi-drug resistant bacteria”
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4. The emergence of super drug-resistant bacteria worldwide has caused intractable infections. The overuse of antibacterial agents in the agro-fish and medical care sectors has contributed to this major public health threat. In addition, these super-resistant bacteria have spread around the globe due to the transborder movement of people and globalization of agro-fishery products, creating a global crisis. In this study, we examine the mechanisms of spread of multidrug-resistant bacteria from a microbiological, pharmacological, and anthropological point of view in Vietnam, where the prevalence of drug-resistant bacteria has increased significantly. We also aim to establish a resistant bacteria monitoring system based on our findings.

Our previous studies have demonstrated that many residual antibiotics are present at relatively high levels in foods. In our studies performed this year, we found that there were regional differences in the detection rates of residual antibiotics (e.g., 0–10%) in seafood; no major regional differences were observed for pork and chicken meat. In our analysis of residual antibiotics in eggs, approximately 10% of eggs contained residual antibiotics. Moreover, we observed differences in the residual-antibiotic positive rates of eggs between farmers. These results indicate that there may be differences in the management of antibiotic administration among farmers. Measurement of the decomposition products of highly utilized antibiotics in the area revealed high frequencies of degradation products in the environment, including pond water. Thus, these findings suggested that monitoring of both antibiotic residues and degradation products is necessary in area with high antibiotic use; such monitoring will facilitate assessment of actual antibiotic use in the area.

Characterization of extended-spectrum beta lactamase (ESBL)-producing *Escherichia coli* strains isolated from fecal specimens of healthy individuals (residents) demonstrated that the same ESBL-producing *E. coli* clone could be isolated for approximately 6 months. Moreover, many ESBL-producing bacteria with different genetic backgrounds were isolated from each examined participant. These analyses also showed that 34.8% of ESBL-producing *E. coli* isolates had the same genetic background, as assessed by pulsed-field gel electrophoresis (PFGE) analysis. The remaining ESBL-producing *E. coli* exhibited heterologous genetic backgrounds. The



results of S1-PFGE analysis for assessment of the genetic homology of plasmids showed that 30.4% of ESBL-producing *E. coli* isolates exhibited similar patterns.

Studies of food contaminated with ESBL-producing *E. coli* showed that 45.4% of food samples were contaminated, with 92.7% of chicken samples, 34.8% of pork samples, 29.3% of seafood samples, and 24.3% of beef samples showing contamination with ESBL-producing *E. coli*. The detection rates were higher in retail stores than in specimens from the slaughterhouse. Thus, improper handling of meat in retail stores may contribute to the emergence of these multidrug-resistant bacteria.

In our analysis of the mechanisms through which super drug-resistant bacteria have emerged, we used a mouse model in which cephalosporin was administered at high concentrations. This antibiotic administration induced a high-level resistance in ESBL-producing *E. coli*, with multidrug resistance to heterologous antimicrobial agents. Genomic analysis of isolates recovered after administration of cephalosporin revealed that the multidrug resistance could be explained by the expression of resistance genes that were originally presented on the plasmid but were not expressed before treatment with the antibiotic. In addition, the high resistance to antibiotics may be due to increased copy numbers of the resistance gene on the plasmid as well as transfer of the resistance gene to the chromosome.

For human resource development, one long-term Vietnamese trainee received a doctorate degree in Japan last year. Four other long-term Vietnamese trainees are currently working toward completion of a PhD program in graduate school.