

# Characterization of $\beta$ -lactamase-producing *Escherichia coli* isolated from farm animals and meat origin

Midori Hiroi

*Department of Environmental Health Sciences, Graduate School of Nutritional and Environmental Sciences*

In Japan, cefotaxime resistant (CTX-M)-type extended-spectrum- $\beta$ -lactamase (ESBL)-producing *Enterobacteriaceae* are important in nosocomial infections. CTX-M-type ESBLs are the most prevalent type of ESBL in clinical isolates of *Escherichia coli*. In this study,  $\beta$ -lactamase-producing *E. coli* isolates recovered from food producing animals and meat origin were characterized. We first collected fresh fecal samples from healthy farm animals during 2004 to 2006 and commercial meat samples in Shizuoka prefecture. By isolation of *E. coli* from these samples, a total of 305 isolates (45 isolates from 50 beef cow faeces, 41 isolates from 50 milk cow faeces, 45 isolates from 50 pig faeces, 32 isolates from 50 layer faeces, 42 isolates from 50 broiler faeces, 60 isolates from 100 chicken meat, 25 isolates from 100 pork meat and 15 isolates from 100 beef meat) were obtained. All isolates were tested for sensitivity to ampicillin, CTX, tetracycline, chloramphenicol, ciprofloxacin, imipenem, and fosfomycin by microdilution method to determine minimum inhibitory concentration (MIC). By this test, 19 CTX resistant *E. coli* isolates (MIC $\geq$ 2  $\mu$ g/mL) were found. A double-disk synergy test for detection of ESBLs revealed 9 of the 19 isolates with synergy between CTX and clavulanate (an inhibitor of ESBL), suggesting production of ESBLs from these *E. coli* isolates. Characterization of  $\beta$ -lactamase genes including ESBL type of *bla*<sub>SHVs</sub>, and *bla*<sub>CTX-Ms</sub> in the 19 isolates by PCR amplification followed by sequencing, showed two, four, and two isolates with *bla*<sub>SHV-2</sub>, *bla*<sub>CTX-M-2</sub>, and *bla*<sub>CTX-M-14</sub>, respectively. The remaining isolates had non-ESBL-type  $\beta$ -lactamases of *bla*<sub>CMY-2</sub> and *bla*<sub>TEM-1</sub>, or mutations at *ampC* promoter region. By pulsed-field gel electrophoresis, two of ESBL-producing isolates, which were obtained from two different farms, showed the similar PFGE patterns, suggesting that these two ESBL-producing *E. coli* are genetically related and may diffuse in some parts of Shizuoka prefecture. These findings suggest that it is necessary to monitor the spread of ESBL-producing bacteria and further epidemiological study including animals and humans and their environments will be required.