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**Original Article**

# Antimicrobial Resistance in Shiga Toxin-Producing *Escherichia coli* O157 and O26 Isolates from Beef Cattle

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**SUMMARY**: This study was conducted to determine the prevalence of antimicrobial resistance in Shiga toxin-producing *Escherichia coli* (STEC) O157 ($n$ = 241) and O26 ($n$ = 11) isolated from beef cattle and to characterize their antimicrobial resistance profiles. Resistance to dihydrostreptomycin was detected most frequently (STEC O157, 9.5%; STEC O26, 54.5%), followed by resistance to oxytetracycline (7.9%; 45.5%) and ampicillin (5.4%; 36.4%). Resistance to one or more antimicrobial agents was detected in 13.3% (32/241) of the STEC O157 isolates and 54.5% (6/11) of the STEC O26 isolates. The antimicrobial resistance rate in the STEC O26 isolates was significantly higher than that in the STEC O157 isolates ($P$ = 0.002, Fisher's exact test). The antimicrobial resistance rate in the STEC O157 isolates possessing both $stx_1$ and $stx_2$ genes was 26.3% (15/57), while that in the isolates possessing $stx_{2c}$ gene alone was 3.9% (3/77). These findings suggest that the antimicrobial resistance in STEC O157 is associated with serogroups and the Shiga toxin genotype.

## INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a major human food-borne pathogen worldwide and causes various illnesses, ranging from mild intestinal disease to a life-threatening complication known as hemolytic-uremic syndrome (1). Although many STEC O serogroups cause human STEC infections, STEC O157 has been the cause of most outbreaks of such infections in Japan (2). In general, beef cattle are considered the main reservoir of STEC. We previously reported that STEC O157 and STEC O26 were, respectively, isolated from 8.9% (218/2,436) and 0.4% (10/2,436) of beef cattle in Japan (3).

There is evidence that humans are infected with antimicrobial-resistant pathogenic *E. coli* isolates from cattle (4). Kobayashi et al. (5) reported that antimicrobial resistance was observed in 23.7% (28/118) of STEC isolates obtained from healthy dairy cows between 2006 and 2007. Dairy beef is commonly consumed in Japan, but accounted for only 24% of the country's total beef production from 2006 to 2009 (6). Therefore, the prevalence and characteristics of antimicrobial-resistant STEC isolates from beef cattle, as well as dairy cattle, should be investigated.

Antimicrobial chemotherapy for STEC infections remains controversial (7,8), although many patients with diarrhea receive empirical antimicrobial chemotherapy in hospitals. This study was conducted to determine the prevalence of antimicrobial resistance in STEC O157 and O26 isolates obtained from beef cattle.

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## MATERIALS AND METHODS

**Isolates:** We used 252 STEC isolates obtained from 234 heads of apparently healthy beef cattle in 119 beef farms in Japan between November 2007 and March 2008 in this study. All the isolates possessed both *eaeA* and enterohemorrhagic *E. coli* (EHEC)-*hlyA* genes. Of the 252 STEC isolates, 241 were obtained from 224 heads of beef cattle in 113 beef farms in a previous study (3). The remaining 11 isolates were obtained from 10 heads of beef cattle from 6 beef farms in Japan during the same period. The Shiga toxin genotypes identified in all the isolates are listed in Table 1.

**Antimicrobial agent susceptibility testing:** The minimum inhibitory concentration (MIC) values of 16 antimicrobial agents were determined using the agar dilution method as per the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (9). *Enterococcus faecalis* ATCC29212, *E. coli* ATCC25922, and *Staphylococcus aureus* ATCC29213 were used as the quality control strains. We tested the following antimicrobial agents: ampicillin (ABPC), cefazolin (CEZ), ceftiofur (CFT), dihydrostreptomycin (DSM), gentamicin (GM), kanamycin (KM), apramycin (APM), oxytetracycline (OTC), bicozamycin (BCM), chloramphenicol (CP), colistin (CL), nalidixic acid (NA), enrofloxacin (ERFX), sulfadimethoxine (SDMX), trimethoprim (TMP), and fosfomycin (FOM). The resistance breakpoints were adopted from those defined by the CLSI (10). We obtained the breakpoints not defined by the CLSI from a report on the Japanese Veteri-

Table 1. Shiga toxin genotypes of STEC O157 and STEC O26 isolates

| Shiga toxin genotype | No. of genotypes (%) among tested isolates | | No. of genotypes (%) among antimicrobial resistant isolates | |
|---|---|---|---|---|
| | STEC O157 | STEC O26 | STEC O157 | STEC O26 |
| $stx_1$ | 6 (2.5) | 11 (100.0) | 1 (3.0) | 6 (100.0) |
| $stx_1$ + $stx_2$ | 53 (22.0) | | 14 (43.8) | |
| $stx_1$ + $stx_{2c}$ | 16 (6.6) | | 6 (18.8) | |
| $stx_1$ + $stx_2$ + $stx_{2c}$ | 4 (1.7) | | 1 (3.0) | |
| $stx_2$ | 20 (8.3) | | 3 (9.4) | |
| $stx_{2c}$ | 77 (32.0) | | 3 (9.4) | |
| $stx_2$ + $stx_{2c}$ | 61 (25.3) | | 4 (12.5) | |
| $stx_{2\text{-NV206}}$ | 4 (1.7) | | | |
| Total | 241 | 11 | 32 | 6 |

Table 2. Primers used for additional PCR

| Target | Nucleotide sequence (5′→3′) | Accession no. | Position |
|---|---|---|---|
| $bla_{\text{ACC-1}}$ | ATGCAGAACACATTGAAGCTG<br>CTACTTATTCCCTTCCAATGA | AJ133121 | 650–670<br>1810–1790 |
| $bla_{\text{ACT-1}}$ | ATGATGATGACTAAATCCCT<br>CTACAGCGCGCTCAAAATACG | U58495 | 28–47<br>1173–1153 |
| $bla_{\text{MOX-3}}$ | ATGCAACAACGACAATCCATCCTG<br>TTACCTGGCCAGTTGCGTCAGGAT | EU515248 | 1–24<br>1149–1126 |
| $bla_{\text{FOX-1}}$ | ATGCAACAACGACGTGCGTTCGCG<br>TCACTCGGCCAACTGACTCAGGATG | X77455 | 701–724<br>1839–1815 |
| $bla_{\text{MIR-1}}$ | ATGATGACAAAATCCCTAAGC<br>TTACTGCAGCGCGTCGAGGAT | M37839 | 928–948<br>2073–2053 |

nary Antimicrobial Resistance Monitoring System (JVARM) (11). In this study, we defined the breakpoint for APM as 32 mg/L by taking into consideration the the midpoint between the peaks of each MIC distribution.

In addition, susceptibilities of 3 CFT-resistant isolates to 8 β-lactam agents (ABPC, piperacillin, cefotaxime [CTX], ceftazidime [CTZ], cefpodoxime [CPD], flomoxef, aztreonam [AZT], and imipenem) were tested according to the CLSI guidelines (9,10) using the commercially available broth microdilution test (Eiken Co., Tokyo, Japan). *E. coli* ATCC25922 and Pseudomonas aeruginosa ATCC27853 were used as the quality control strains.

**β-Lactamase study:** CFT-resistant isolates were tested by a double-disk clavulanate synergy test, as described previously (12), to detect CFT-resistant isolates producing extended-spectrum β-lactamases (ESBLs). The synergy between clavulanate and CTX, CTZ, CPD, or AZT disks (Nissui, Tokyo, Japan) was investigated. The detection of β-lactamase genes and amplification of the promoter region of the *ampC* gene were carried out by PCR. First, we used 9 primer sets for the detection of β-lactamase genes ($bla_{\text{CTX-M}}$, $bla_{\text{TEM}}$, $bla_{\text{SHV}}$, $bla_{\text{PSE-1}}$, $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-9}}$, $bla_{\text{CMY-1}}$, $bla_{\text{CMY-2}}$, and $bla_{\text{FOX}}$) (13,14). Five additional primer sets were used to detect the β-lactamase genes in 2 CFT-resistant STEC O157 isolates (Table 2). The cycle conditions for PCR using these 5 primer sets were 30 amplification cycles at 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, followed by 72°C for 7 min. A multiplex PCR method reported by Dallenne et al. (15) was used to detect the genes of AmpC β-lactamase groups (ACC, FOX, MOX, DHA, CIT, and EBC). Amplification of the promoter region of the *ampC* gene in the 2 CFT-resistant STEC O157 isolates was conducted using a primer set, as reported by Kojima et al. (13). The nucleotide sequences of β-lactamase and the promoter region of the *ampC* genes were detected by direct DNA sequencing.

## RESULTS AND DISCUSSION

The MIC distributions of the 16 antimicrobial agents against the STEC isolates are listed in Table 3. Resistance to DSM was detected most frequently (STEC O157, 9.5%; STEC O26, 54.5%), followed by resistance to OTC (7.9%; 45.5%) and ABPC (5.4%; 36.4%). The rates of resistance to ABPC, DSM, and OTC in *E. coli* isolates obtained from healthy beef cattle in Japan in 2007 were 9.2%, 19.2%, and 26.2%, respectively, and these were the highest resistance rates from among those for the 15 tested antimicrobial agents (ABPC, CEZ, CFT, DSM, GM, KM, APM, OTC, BCM, CP, CL, NA, ERFX, SDMX, and TMP) (11). Penicillins, tetracyclines, and streptomycins are classically approved in Japan and have been commonly used in animal husbandry for long periods (16,17). In the present study, resistance to one or more antimicrobial agent was detected in 13.3% (32/241) of the STEC O157 isolates and 54.5% (6/11) of the STEC O26 isolates. The rate of antimicrobial resistance in STEC O26 isolates was significantly higher ($P = 0.002$, Fisher's exact test) than that in STEC O157 isolates, although the number of STEC O26 isolates tested was small. Mora et al. (18) have reported that STEC isolates are obtained from humans, cattle, sheep, and food in Spain, and the

Table 3. The MIC distiribution of antimicrobials for STEC O157 ($n$ = 241) and STEC O26 ($n$ = 11) from beef cattle

| Antimicrobial | | MIC (mg/L) | | | | | | | | | | | | | Break point (mg/L) | Resistant isolate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0.063 | 0.125 | 0.25 | 0.5 | 1 | 2 | 4 | 8 | 16 | 32 | 64 | 128 | >128 | | No. (%) |
| ABPC | STEC O157 | | | | | | 188 | 40 | | | 1 | | | 12 | 32[1] | 13 (5.4) |
| | STEC O26 | | | | | | | 7 | | | | | | 4 | | 4 (36.4) |
| CEZ | STEC O157 | | | | | 62 | 165 | 12 | | | | 1 | | 1 | 32[1] | 2 (0.8) |
| | STEC O26 | | | | | 1 | 8 | 1 | | | | | | 1 | | 1 (9.1) |
| CFT | STEC O157 | 17 | 6 | 150 | 66 | | | | | | 2 | | | | 8[2] | 2 (0.8) |
| | STEC O26 | | | 9 | 1 | | | | | | | | | 1 | | 1 (9.1) |
| DSM | STEC O157 | | | | | | | 192 | 23 | 3 | 3 | 12 | 6 | 2 | 32[2] | 23 (9.5) |
| | STEC O26 | | | | | | | 5 | | | | 2 | | 4 | | 6 (54.5) |
| GM | STEC O157 | | | | 129 | 110 | | | 1 | 1 | | | | | 16[1] | 1 (0.4) |
| | STEC O26 | | | | 4 | 7 | | | | | | | | | | 0 (0.0) |
| KM | STEC O157 | | | | | 6 | 130 | 103 | | | | 1 | | 1 | 64[1] | 2 (0.8) |
| | STEC O26 | | | | | | 4 | 5 | | | | | | 2 | | 2 (18.2) |
| APM | STEC O157 | | | | | | | 58 | 182 | | | 1 | | | 32[3] | 1 (0.4) |
| | STEC O26 | | | | | | | | 11 | | | | | | | 0 (0.0) |
| OTC | STEC O157 | | | | | | 6 | 208 | 8 | | | | | 19 | 16[2] | 19 (7.9) |
| | STEC O26 | | | | | | | 5 | 1 | | | 2 | 2 | 1 | | 5 (45.5) |
| BCM | STEC O157 | | | | | | | | | 2 | 231 | 4 | | 4 | 128[2] | 4 (1.7) |
| | STEC O26 | | | | | | | | | 5 | 6 | | | | | 0 (0.0) |
| CP | STEC O157 | | | | | | | 49 | 185 | 2 | 1 | 3 | 1 | | 32[1] | 5 (2.1) |
| | STEC O26 | | | | | | | 2 | 7 | | | | | 2 | | 2 (18.2) |
| CL | STEC O157 | | | | 111 | 127 | | | | | 1 | | | 2 | 16[2] | 3 (1.2) |
| | STEC O26 | | | | | 10 | | | 1 | | | | | | | 1 (9.1) |
| NA | STEC O157 | | | | | | 1 | 234 | 4 | 1 | | | | 1 | 32[2] | 1 (0.4) |
| | STEC O26 | | | | | | | 11 | | | | | | | | 0 (0.0) |
| ERFX | STEC O157 | 240 | | | 1 | | | | | | | | | | 2[1] | 0 (0.0) |
| | STEC O26 | 11 | | | | | | | | | | | | | | 0 (0.0) |
| SDMX | STEC O157 | | | | | | | | | | | | 39 | 202 | | |
| | STEC O26 | | | | | | | | | | | 1 | 1 | 9 | | |
| TMP | STEC O157 | 1 | 11 | 79 | 137 | 7 | 1 | 1 | | | | 1 | | 3 | 16[2] | 4 (1.7) |
| | STEC O26 | | | | 2 | 7 | | | | | | | | 2 | | 2 (18.2) |
| FOM | STEC O157 | | | | | | 3 | 74 | 129 | 28 | 5 | | 2 | | 256[1] | 0 (0.0) |
| | STEC O26 | | | | | | 2 | 2 | | | 6 | | | 1 | | 1 (9.1) |

[1]: The value was a CLSI breakpoint.
[2]: The value was a NVAL breakpoint.
[3]: The value was set as the midpoint between the peaks of each MIC distribution.
ABPC, ampicillin; CEZ, cefazolin; CFT, ceftiofur; DSM, dihydrostreptomycin; GM, gentamicin; KM, kanamycin; APM, apramycin; OTC, oxytetracycline; BCM, bicozamycin; CP, chloramphenicol; CL, colistin; NA, nalidixic acid; ERFX, enrofloxacin; SDMX, sulfadimethoxine; TMP, trimethoprim; FOM, fosfomycin.

rate of antimicrobial resistance in STEC O26 (60.0%, 24/40) was higher than in STEC O157 (41.1%, 58/141). To our knowledge, this is the first report to show that the antimicrobial resistance rate in STEC O26 is higher than that in STEC O157. In addition, 24 (75.0%) of the 32 STEC O157 isolates and all 6 STEC O26 isolates exhibited resistance to multiple antimicrobials (Table 4). Thus, STEC O26 isolates may acquire antimicrobial resistance more easily than STEC O157 isolates do.

In Japan, FOM is the most commonly used agent in antimicrobial chemotherapy for STEC infections because of its minimal side effect profile; fluoroquinolones and cephalosporins are also commonly used (19–21). In the present study, although ERFX demonstrated strong activity (MIC $\leq$ 0.5 mg/L) against all the isolates, we detected resistance to CFT and FOM in STEC isolates from beef cattle. One STEC O26 isolate (9.1%, 1/11) and 2 STEC O157 isolates (0.8%, 2/241) exhibited resistance to CFT in this study. The CFT-resistant STEC O26 isolate (53-1A) was obtained from Holstein-Friesian (HF) cattle raised in Hokkaido. The 2 CFT-resistant STEC O157 isolates (325-6F and 27-4C) were obtained from Japanese Black (JB) cattle raised in Saga Prefecture and first-generation hybrid (HF $\times$ JB) cattle raised in Niigata Prefecture, respectively. Susceptibilities of these 3 CFT-resistant isolates to 8 $\beta$-lactam agents were investigated, and the antimicrobial resistance profiles of $\beta$-lactam agents in these CFT-resistant isolates were found to differ from each other (Table 5). The double-disk clavulanate synergy test yielded positive and negative results for the CFT-resistant STEC O26 and STEC O157 isolates, respectively. Moreover, we detected the $bla_{\text{CTX-M-14}}$ gene in the STEC O26 isolate, while no $\beta$-lactamase genes were detected in either STEC O157 isolate. These results suggest that the STEC O26 isolate produced CTX-M-14 ESBL, and the STEC O157 isolates produced cephalosporinases. It has been reported that $bla_{\text{CTX-M-14}}$ (identical to $bla_{\text{CTX-M-18}}$) has been detected in *E. coli* from broilers (13,22), and in STEC O26 isolated from a girl in Japan (23). As for the CFT-resistant STEC O157 isolates, we conducted an additional PCR assay for 5 $\beta$-lactamase genes ($bla_{\text{ACT-1}}$, $bla_{\text{FOX-1}}$, $bla_{\text{ACC-1}}$, $bla_{\text{DHA}}$, and $bla_{\text{MOX}}$) and a multiplex PCR reported by Dallenne et al. (15), but the 2 STEC O157 isolates yielded no PCR products. Furthermore, there were no mutations in the *ampC* promoter regions linked to the hyperexpression of AmpC chromosomal cephalosporinase in the 2 STEC O157 isolates (24–26). Therefore, the determinants for CFT resistance in the 2 STEC O157 isolates could not be

Table 4. Antimicrobial resistance profiles of STEC O157 ($n$ = 32) and STEC O26 ($n$ = 6) isolates

| No.[1)] | Antimicrobial resistance profile | Genotype | STEC O157 | STEC O26 |
|---|---|---|---|---|
| 1 | ABPC | $stx_{2c}$ | 1 | |
| | DSM | $stx_{2c}$ | 1 | |
| | | $stx_2$ + $stx_{2c}$ | 2 | |
| | OTC | $stx_1$ + $stx_2$ | 1 | |
| | BCM | $stx_1$ + $stx_{2c}$ | 1 | |
| | CP | $stx_1$ + $stx_2$ | 1 | |
| | CL | $stx_{2c}$ | 1 | |
| 2 | ABPC + DSM | $stx_1$ | | 1 |
| | | $stx_1$ + $stx_2$ | 1 | |
| | DSM + OTC | $stx_1$ | | 2 |
| | | $stx_1$ + $stx_{2c}$ | 5 | |
| | | $stx_2$ | 1 | |
| | | $stx_1$ + $stx_2$ + $stx_{2c}$ | 1 | |
| | OTC + CP | $stx_1$ + $stx_2$ | 2 | |
| 3 | ABPC + DSM + OTC | $stx_1$ | | 1 |
| | | $stx_1$ + $stx_2$ | 7 | |
| | ABPC + DSM + TMP | $stx_2$ | 1 | |
| | | $stx_2$ + $stx_{2c}$ | 1 | |
| | DSM + OTC + CP | $stx_1$ + $stx_2$ | 1 | |
| 4 | GM + KM + BCM + CL | $stx_1$ | 1 | |
| | ABPC + DSM + OTC + TMP | $stx_2$ | 1 | |
| 6 | ABPC + CEZ + CFT + BCM + CP + TMP | $stx_1$ + $stx_2$ | 1 (27-4C) | |
| 7 | ABPC + DSM + KM + OTC + CP + TMP + FOM | $stx_1$ | | 1 |
| 8 | ABPC + CEZ + CFT + DSM + KM + OTC + CP + TMP | $stx_1$ | | 1 (53-1A) |
| | CEZ + CFT + DSM + KM + APM + BCM + CL + NA | $stx_2$ + $stx_{2c}$ | 1 (325-6F) | |
| Total | | | 32 | 6 |

[1)]: Number of antimicrobial agents to resistance.

Table 5. MICs of $\beta$-lactam agents for 3 CFT-resistant isolates

| Isolate | MICs (mg/L) of | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ampicillin (32)[1)] | Piperacillin (128) | Cefotaxime (64) | Ceftazidime (32) | Cefpodoxime (8) | Flomoxef | Aztreonam (32) | Imipenem (16) |
| 53-1A (STEC O26) | >32 | >64 | >32 | <1 | >32 | <8 | >16 | <1 |
| 325-6F (STEC O157) | 8 | 16 | >32 | >32 | >32 | 16 | >16 | 2 |
| 27-4C (STEC O157) | 32 | 16 | 8 | 4 | 16 | 32 | 8 | <1 |

[1)]: Resistance breakpoint (mg/L) defined by the CLSI. The breakpoint of flomoxef has not yet been defined

identified in the present study.

FOM resistance was detected in a STEC O26 isolate in this study. The FOM-resistant STEC O26 isolate was obtained from HF cattle raised in Hokkaido. In Japan, Horii et al. (27) reported that FOM resistance was detected in 2 clinical isolates of STEC O26 in 1996 and 1997. The appearance of FOM resistance in STEC isolates is a significant concern since FOM is commonly used as first-line treatment for human STEC infections. At present, the prevalence of antimicrobial resistance against CFT, ERFX, and FOM in STEC O157 and STEC O26 isolates remains low, suggesting that the use of these agents in antimicrobial chemotherapy for STEC infections remains effective.

The relationship between antimicrobial resistance and the Shiga toxin genotype is a significant concern in human medicine, but this may not be the case in veterinary medicine. Several studies have demonstrated that clinical severities of STEC infection in humans were associated with Shiga toxin genotype (28–30). The resistance rate was 26.3% (15/57) in STEC O157 isolates possessing both $stx_1$ and $stx_2$, while it was 3.9% (3/77) in STEC O157 isolates possessing $stx_{2c}$ alone (Table 1). Consequently, STEC O157 isolates possessing both $stx_1$ and $stx_2$ accounted for 46.9% (15/32) of antimicrobial-resistant STEC O157 isolates, although they accounted for 23.7% (57/241) of the STEC O157 isolates tested. STEC O157 isolates possessing both $stx_1$ and $stx_2$ had higher virulence than STEC O157 possessing $stx_{2c}$ alone (28). Although cattle infected with STEC O157 do not always present with clinical signs, antimicrobial agents have been used in food-producing animals for some therapeutic purposes and growth promotion (17). The selective pressure imposed by the use of antimicrobial

agents may be associated with not only the occurrence of antimicrobial resistance in STEC O157, but also an increase in the population of STEC O157 possessing both $stx_1$ and $stx_2$ in beef cattle.

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**Conflict of interest** None to declare.

## REFERENCES

1. Mainil, J.G. and Daube, G. (2005): Verotoxigenic *Escherichia coli* from animals, humans and foods: who's who? J. Appl. Microbiol., 98, 1332–1344.
2. National Institute of Infectious Diseases and Tuberculosis and Infectious Diseases Control Division, Ministry of Health, Labour and Welfare (2009): Enterohemorrhagic *Escherichia coli* infection in Japan as of April 2009. Infect. Agents Surveillance Rep., 30, 119'–120'.
3. Sasaki, Y., Tsujiyama, Y., Kusukawa, M., et al. (2011): Prevalence and characterization of Shiga toxin-producing *Escherichia coli* O157 and O26 in beef farms. Vet. Microbiol., 150, 140–145.
4. Ramchandani, M., Manges, A.R., DebRoy, C., et al. (2005): Possible animal origin of human-associated multidrug-resistant uropathogenic *Escherichia coli*. Clin. Infect. Dis., 40, 251–257.
5. Kobayashi, H., Kanazaki, M., Ogata, T., et al. (2009): Changing prevalence of O-serogroups and antimicrobial agent susceptibility among STEC strains isolated from healthy dairy cows over a decade in Japan between 1998 and 2007. J. Vet. Med. Sci., 71, 363–366.
6. Ministry of Agriculture, Forestry, and Fisheries in Japan (2009): Production of various kinds of meat. Monthly Stat. Agric. Forest. Fish., 682, 43.
7. Paton, J.C. and Paton, A.W. (1998): Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. Clin. Microbiol. Rev., 11, 450–479.
8. Wong, C.S., Jelacic, S., Habeeb, R.L., et al. (2000): The risk of the haemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. N. Engl. J. Med., 342, 1930–1936.
9. Clinical and Laboratory Standards Institute (2008): Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; 3rd edition: Approved standard M31-A3. Clinical and Laboratory Standards Institute, Wayne, Pa.
10. Clinical and Laboratory Standards Institute (2008): Performance standards for antimicrobial susceptibility testing; 18th Informational Supplement M100-S18. Clinical and Laboratory Standards Institute, Wayne, Pa.
11. National Veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries (2009): A Report on the Japanese Veterinary Antimicrobial Resistance Monitoring System—2000 to 2007—. Available at 〈http://www.maff.go.jp/nval/tyosa_kenkyu/taiseiki/pdf/jvarm2000_2007_final_201005.pdf〉.
12. Luzzaro, F., Mantengoli, E., Perilli, M., et al. (2001) Dynamics of a nosocomial outbreak of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum β-lactamase. J. Clin. Microbiol., 39, 1865–1870.
13. Kojima, A., Ishii, Y., Ishihara, K., et al. (2005): Extended-spectrum-β-lactamase-producing *Escherichia coli* strains isolated from farm animals from 1999 to 2002: report from the Japanese Veterinary Antimicrobial Resistance Monitoring Program. Antimicrob. Agents Chemother., 49, 3533–3537.
14. Xu, L., Ensor, V., Gossain, S., et al. (2005): Rapid and simple detection of $bla_{CTX-M}$ genes by multiplex PCR assay. J. Med. Microbiol., 54, 1183–1187.
15. Dallenne, C., Da Costa, A., Decré, D., et al. (2010): Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. J. Antimicrob. Chemother., 65, 490–495.
16. Asai, T., Kojima, A., Harada, K., et al. (2005): Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in *Escherichia coli* isolated from the feces of food-producing animals in Japan. Jpn. J. Infect. Dis., 58, 369–372.
17. Harada, K. and Asai, T. (2010): Role of antimicrobial agent selective pressure and secondary factors on antimicrobial resistance prevalence in *Escherichia coli* from food-producing animals in Japan. J. Biomed. Biotechnol. Doi: 10.1155/2010/180682.
18. Mora, A., Blanco, J.E., Blanco, M., et al. (2005): Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol., 156, 793–806.
19. Higami, S., Nishimoto, K., Kawamura, T., et al. (1997): Retrospective analysis of the relationship between HUS incidence and antibiotics among patients with *Escherichia coli* O157 enterocolitis in the Sakai outbreak. J. Jpn. Assoc. Infect. Dis., 72, 266–272 (in Japanese).
20. Ikeda, K., Ida, O., Kimoto, K., et al. (1999): Effect of early fosfomycin treatment on prevention of haemolytic uremic syndrome accompanying *Escherichia coli* O157:H7 infection. Clin. Nephrol., 52, 357–362.
21. Kasahara, H., Ueda, H., Kurogami, Y., et al. (2011): Outbreak of EHEC O26 infection through playing with water in a park spreading in a nursery school, July–August 2010—Nagano. Infect. Agents Surveillance Rep., 32, 132–133 (in Japanese).
22. Hiroi, M., Harada, T., Kawamori, F., et al. (2011): A survey of β-lactamase-producing *Escherichia coli* in farm animals and raw retail meat in Shizuoka Prefecture, Japan. Jpn. J. Infect. Dis., 64, 153–155.
23. Ishii, Y., Kimura, S., Alba, J., et al. (2005): Extended-spectrum β-lactamase-producing Shiga toxin gene ($stx_1$)-positive *Escherichia coli* O26:H11: a new concern. J. Clin. Microbiol., 43, 1072–1075.
24. Caroff, N., Espaze, E., Gautreau, D., et al. (2000): Analysis of the effects of −42 and −32 *ampC* promoter mutations in clinical isolates of *Escherichia coli* hyperproducing AmpC. J. Antimicrob. Chemother., 45, 783–788.
25. Siu, L.K., Lu, P.L., Chen, J.Y., et al. (2003): High-level expression of ampC β-lactamase due to insertion of nucleotides between −10 and −35 promoter sequences in *Escherichia coli* clinical isolates: cases not responsive to extended-spectrum-cephalosporin treatment. Antimicrob. Agents Chemother., 47, 2138–2144.
26. Tracz, D.M., Boyd, D.A., Hizon, R., et al. (2007): *ampC* gene expression in promoter mutations of cefoxitin-resistant *Escherichia coli* clinical isolates. FEMS Microbiol. Lett., 270, 265–271.
27. Horii, T., Kimura, T., Sato, K., et al. (1999): Emergence of fosfomycin-resistant isolates of Shiga-like toxin-producing *Escherichia coli* O26. Antimicrob. Agents Chemother., 43, 789–793.
28. Nishikawa, Y., Zhou, Z., Hase, A., et al. (2000): Relationship of genetic type of Shiga toxin to manifestation of bloody diarrhea due to enterohemorrhagic *Escherichia coli* serogroup O157 isolates in Osaka city, Japan. J. Clin. Microbiol., 38, 2440–2442.
29. Kawano, K., Okada, M., Haga, T., et al. (2008): Relationship between pathogenicity for humans and *stx* genotype in Shiga toxin-producing *Escherichia coli* serotype O157. Eur. J. Clin. Microbiol. Infect. Dis., 27, 227–232.
30. Nakamura, H., Ogasawara, J., Kita, T., et al. (2008): Typing of Stx2 genes of *Escherichia coli* O157 isolates from cattle. Jpn. J. Infect. Dis., 61, 251–252.