Original Article

Assocation of Antimicrobial Resistance in *Campylobacter* Isolated from Food-Producing Animals with Antimicrobial Use on Farms

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SUMMARY: On 1,374 food-producing animal farms in 2001-2004, we investigated the influence of antimicrobial usage within 6 months before a survey conducted on antimicrobial resistance in *Campylobacter*. Tetracyclines and fluoroquinolones were used for therapeutic purposes on 107 and 21 farms, respectively. Oxytetracycline-resistant *C. jejuni* and *C. coli* were found in 57.1% (8/14) and 92.5% (37/40) of the farms, respectively, when the animals were treated with tetracyclines. However, they were found in 43.2% (112/259) and 74.3% (122/164) of the farms, respectively, when the animals were not treated with tetracyclines. Enrofloxacin-resistant *C. jejuni* and *C. coli* were found in 66.7% (2/3) and 16.7% (1/6) of the farms, respectively, when the animals were treated with fluoroquinolones. However, they were found in 15.5% (42/270) and 28.8% (57/198) of the farms, respectively, when the animals were not treated with fluoroquinolones. The percentage of oxytetracycline resistance in *C. coli* was significantly elevated when the animals were treated with tetracyclines (*P* < 0.05). The farms on which antimicrobials included by their resistance pattern were used accounted for 9.1% (4/44) and 24.1% (14/58) of the farms where enrofloxacin-resistant *C. jejuni* and *C. coli* were isolated, respectively. It is likely that several factors account for the presence of fluoroquinolone-resistant *Campylobacter*.

INTRODUCTION

*Campylobacter*, mainly *Campylobacter jejuni*, is a causative agent of foodborne disease throughout the world and its infection in humans is mainly associated with the consumption of undercooked poultry meats and the handling of raw poultry products (1). Antimicrobial agents are commonly used for persons with severe infections to decrease the length and severity of the illness. Fluoroquinolones are prescribed for humans with bacterial infections and *Campylobacter* infections. Ever since fluoroquinolones were approved for use in veterinary medicine, human infections with fluoroquinolone-resistant *Campylobacter* have increased (2-6). Several experiments have shown that fluoroquinolone-resistant *Campylobacter* emerged due to fluoroquinolone use for chickens infected with *C. jejuni* (7-10). Thus, the appearance of fluoroquinolone-resistant *C. jejuni* is a consequence of fluoroquinolone use for chickens, although veterinary antimicrobial products, including fluoroquinolones, are never used for the treatment of *Campylobacter* infection in poultry because of the absence of clinical signs.

Fluoroquinolone drugs have been used for the treatment of bacterial pneumonia and diarrhea in food-producing animals in Japan since their approval by the Japanese Ministry of Agriculture, Forestry and Fisheries (JMAFF) in 1991 as medicine for veterinary use. JMAFF reported that fluoroquinolones accounted for 0.6% (approximately 6.3 tons) of the total sales amount of antimicrobial agents for animal health purposes (1,059 tons) in 2001 (11). Between 2000 and 2003, all *Salmonella* isolates from apparently healthy food-producing animals were susceptible to enrofloxacin (ERFX) (12), and the frequencies of ERFX resistance in *Escherichia coli* were low in apparently healthy food-producing animals (13). As for *Campylobacter* in Japan, fluoroquinolone resistance has been found in *C. jejuni* isolates from cattle, layer chickens and broiler chickens and in *C. coli* isolates from pigs, but the level of fluoroquinolone resistance in *C. jejuni* and *C. coli* remained constant between 2000 and 2004 (11.9 - 20.0% and 22.7 - 35.2%, respectively) (13,14). The objective of the present study was to determine the present usage situation of antimicrobials, including fluoroquinolones in food-producing animals, and the association of antimicrobials with the prevalence of fluoroquinolone-resistant *Campylobacter*.

MATERIALS AND METHODS

Antimicrobial usage within 6 months before the survey: Information on the use of veterinary antimicrobial products within 6 months before the survey was collected by veterinarians from the prefectural livestock hygiene service centers on 1,374 farms (378 cattle farms, 359 pig farms, 284 broiler chicken farms and 353 layer chicken farms) throughout Japan during the period from 2001 to 2004.

Prevalence of antimicrobial-resistant *Campylobacter*.

The results of the antimicrobial susceptibility test for isolates of *Campylobacter* examined in another study (13,14) were used. In brief, *Campylobacter* was isolated from fecal samples of apparently healthy food-producing animals between 2001 and 2004 using modified CCDA (Oxoid, Hampshire, UK). The isolates were identified biochemically and using polymerase chain reaction (15). The *Campylobacter* isolates included 273 isolates of *C. jejuni* and 204 isolates of *C. coli* from feces samples of 77 cattle, 151 pigs, 93 broiler chickens...
and 156 layer chickens reared on different farms. Antimicrobial susceptibility testing was carried out by an agar dilution method according to the National Committee for Clinical Laboratory Standards (16) recommendations. C. jejuni ATCC 33560 was used as a quality control strain. The MICs of each antimicrobial were interpreted according to our previous report (17).

Association of antimicrobial resistance with antimicrobial use: The relationship between antimicrobial use and the resistance rates was evaluated by the chi-square test and Fisher’s exact test. Relative risks with 95% confidence interval (CI) of antimicrobial use were estimated utilizing the statistical software Epi Info version 3.3.2 (Centers for Disease Control and Prevention, Atlanta, Ga., USA).

RESULTS

The most widely used antibiotics were tetracycline antibiotics (used on 107 farms [7.8%] of the 1,374 farms surveyed), followed by penicillin antibiotics (89 farms, 6.5%), aminoglycoside antibiotics (63 farms, 4.6%), and macrolides and lincosamide (59 farms, 4.3%). The remaining antimicrobial substances were used on less than 4% of the farms surveyed. Fluoroquinolone drugs were used for therapeutic purposes on 21 farms (1.5%) (Table 1).

Oxytetracycline (OTC)-resistant C. jejuni isolates were found in 57.1% (8/14) of the farms positive for isolation on which the animals were treated with tetracycline antibiotics, and in 43.2% (112/259) of the farms positive for isolation on which the animals were not treated with tetracycline antibiotics (Table 2). OTC-resistant C. coli isolates were found in 92.5% (37/40) of the farms positive for isolation on which the animals were treated with tetracycline antibiotics, and in 74.3% (122/164) of the farms positive for isolation on which the animals were not treated with tetracycline antibiotics. The percentage of OTC-resistant C. coli isolates was significantly higher for animals treated with tetracycline antibiotics than for animals not treated with tetracycline antibiotics (P < 0.05), but was not significantly elevated in C. jejuni. Relative risk was significantly elevated in C. coli (relative risk, 1.24; 95% confidence limits, 1.10-1.41; P = 0.024) but not in C. jejuni (1.32; 0.82-2.12; P = 0.456).

ERFX-resistant C. jejuni isolates were found on 66.7% (2/3) of the farms positive for isolation on which the animals were treated with fluoroquinolones, and on 15.5% (42/270) of the farms positive for isolation on which the animals were not treated with fluoroquinolones (Table 3). ERFX-resistant C. coli isolates were found on 16.7% (1/6) of the farms positive for isolation on which the animals were treated with fluoroquinolones, and on 28.8% (57/198) of the farms positive for isolation on which the animals were not treated with fluoroquinolones. There was no significant difference between the prevalence of ERFX-resistant C. jejuni/coli in animals treated with fluoroquinolone drugs and that in animals not

| Table 1. Antimicrobial use in food-producing animals between 2001 and 2004 |
|-------------------------|-------------------|----------------|----------------|-------------------|------------------|
|                        | Cattle            | Pig             | Broiler chicken | Layer chicken    | Total            |
| No. of farms studied   | 378               | 359             | 284            | 353              | 1,374            |
| No. of antimicrobial use (%) | 53 (14)          | 123 (34)        | 69 (24)        | 23 (7)           | 268 (20)         |
| Substance              | Penicillins       | Cephalosporins  | Aminoglycosides | Tetracyclines    |                  |
|                        | 17                | 4               | 20             | 12               |                  |
|                        | 55                | 41              | 0              | 59               | 107              |
|                        | 14                | 2               | 2              | 22               | 3                |
|                        | 3                 | 0               | 0              | 9                |                  |
|                        | 14                | 0               | 0              | 2                |                  |
|                        | 7                 | 2               | 3              | 8                |                  |
|                        | 21                | 0               | 0              | 1                |                  |
|                        | 11                | 14              | 13             | 1                |                  |

*: One or more antimicrobials were used on some farms.

| Table 2. Prevalence of oxytetracycline (OTC)-resistant Campylobacter in animals treated with or not treated with tetracycline antibiotics within 6 months before the survey |
|-------------------------|----------------|----------------|-----------------|-----------------|------------------|
| Animal species          | Use of tetracyclines | No. of farms positive for isolation | No. of farms with isolates resistant to OTC | No. of farms positive for isolation | No. of farms with isolates resistant to OTC |
| C. jejuni               | C. coli         |                             |                              |                              |
| Cattle                  | +               | 1                           | 1                            | 1                        | 1                 |
|                          | –               | 69                          | 28                           | 6                          | 3                 |
| Pigs                    | +               | 1                           | 1                            | 34                         | 32                |
|                          | –               | 0                           | 0                            | 116                        | 101               |
| Broiler chickens        | +               | 7                           | 3                            | 2                          | 2                 |
|                          | –               | 73                          | 33                           | 11                         | 5                 |
| Layer chickens          | +               | 5                           | 3                            | 3                          | 2                 |
|                          | –               | 117                         | 51                           | 31                         | 13                |
| Total                   | +               | 14                          | 8                            | 40                         | 37                |
|                          | –               | 259                         | 112                          | 164                        | 122               |

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treated with fluoroquinolone drugs. Furthermore, relative risk
was not significantly elevated in C. jejuni (4.29; 1.84 - 10.00;
P = 0.069) or in C. coli (0.58; 0.10 - 3.51; P = 0.677).

ERFX-resistant C. jejuni was isolated on five (33.3%) of
15 farms where antimicrobial drugs were used and on 39
(15.1%) of 258 farms where no antimicrobial was used.
ERFX-resistant C. coli was isolated on 17 (25.4%) of 67 farms
where antimicrobial drugs were used and on 41 (29.9%) of
137 farms where no antimicrobial drugs were used. There
was no significant difference between the prevalence of
ERFX-resistant C. jejuni/coli in animals treated with anti-
microbial drugs and that in animals not treated with anti-
microbial drugs. However, antimicrobial drugs used on four
(80%) of the five farms on which ERFX-resistant C. jejuni
was isolated were fluoroquinolones (two farms), and anti-
microbials included by their resistance pattern (two farms;
tetracycline antibiotics on nine farms, streptomycin antibiotics
on three farms, tylosin on two farms and lincomycin on one
farm). In total, the farms on which antimicrobials included
by their resistance pattern were used accounted for 9.1% (4/44)
and 24.1% (14/58) of the farms where ERFX-resistant C.
ejuni and C. coli were isolated, respectively.

**DISCUSSION**

We demonstrated that ERFX-resistant *Campylobacter* was
prevalent on farms on which fluoroquinolone drugs had not
recently been used for animals. Even though fluoroquinolone
drugs were used on only a small number of farms in the present
study, the percentages of ERFX resistance in *C. jejuni* and
*C. coli* from food-producing animals have been constant
in recent years (13,14). Although fluoroquinolone-resistant
*Campylobacter* has emerged from fluoroquinolone use for
infected animals (7-10), our results indicated that several
factors are present which have enabled the fluoroquinolone
resistance to *Campylobacter* in food-producing animals to
persist.

The emergence of fluoroquinolone resistance in *C. jejuni*
was found after the distribution of fluoroquinolone drugs in
humans and animals in Japan as well as in other countries (2-
6). In Japan, all clinical isolates of *C. jejuni* were susceptible
to nalidixic acid in 1981 -1982 (3), whereas 22% of clinical
isolates of *C. jejuni* were resistant to fluoroquinolone in 1996-
2000 (5). The number of strains resistant to fluoroquinolones
and nalidixic acid has increased significantly since 1992
in Japan (6). Fluoroquinolone-resistant *Campylobacter* has
emerged from fluoroquinolone use for infected chickens (7-10)
and pigs (18). The present study revealed that tetracycline
antibiotics were the most frequently used among veterinary
antimicrobial products and that fluoroquinolone drugs were
used on few farms studied. JMAFF reported that tetracycline
antibiotics were the most widely distributed drugs among
veterinary antimicrobial products and that the sales amount
of tetracycline antibiotics accounted for 43% (456 tons)
of the total sales amount of antimicrobial agents for animal
health purposes (1,059 tons) in 2001 (11). The sales volume
of fluoroquinolones accounted for 0.6% (approximately 6.3
tons) of the total sales amount of antimicrobial agents (11).
Thus, the sales amount of fluoroquinolones is less than that
of tetracycline antibiotics in veterinary medicine. In addition,
there are regulatory guidance documents on the use of spe-
cific antimicrobial substances in food-producing animals.
(e.g., fluoroquinolones and the third (or more) generation cephalosporins) because of the importance for public health. In brief, the application for approval of the drug for use in animals is not accepted until the end of the re-examination period of the corresponding drug for use in humans. Furthermore, the description states that the drug is not considered a first-choice drug. After marketing, monitoring data on the amount sold and the appearance of antimicrobial resistance in target and foodborne pathogens must be submitted to the JMAFF. These regulations on fluoroquinolone drugs used for food-producing animals may affect the situation of fluoroquinolone usage in food-producing animals.

ERFX-resistant isolates could be potentially selected by the use of antimicrobials other than fluoroquinolone drugs. We previously reported that co-selection contributes to the persistence of chloramphenicol-resistant *E. coli* in food-producing animals (19). Since point mutations in the quinolone resistance-determining region of *gyrA* are responsible for fluoroquinolone resistance in *Campylobacter* (1,10,17), a genetic linkage of the resistance to other antimicrobial resistance rarely exists. However, in multidrug-resistant *Campylobacter* with fluoroquinolone resistance, the bacteria can be selected by use of the antimicrobials included by their resistance pattern. Avrain et al. (20) reported an increase in tetracycline-resistant *Campylobacter* from broiler chickens treated with tetracycline antibiotics. The results of the present study also showed a similar relationship. Moreover, since erythromycin (EM)-resistant *C. coli* exhibits cross-resistance to tylosin and lincomycin (14), the use of these antimicrobials can be a selective pressure of EM-resistant *C. coli* with fluoroquinolone resistance. In addition, multidrug resistance was more frequently observed in *C. coli* than in *C. jejuni* (17), implying strong effects of this phenomenon in *C. coli*. Attention should be given to the influence of veterinary antimicrobial products other than fluoroquinolones on the prevalence of fluoroquinolone-resistant *Campylobacter*.

Improvement in biosecurity on farms is important for preventing infection with microbial pathogens in food-producing animals. The cleaning and disinfection of animal facilities before animal movement is essential, because fluoroquinolone-resistant *Campylobacter* continuously circulates in poultry flocks (21). Varieties of *C. jejuni* strains with antibiograms and genotypes were shown to predominate by flock in the absence of antimicrobial selective pressure (21), suggesting the importance of preventing the intrusion from several environmental sources such as waste (22) and wildlife animals (23) to animal herds. Therefore, biosecurity programs on farms should be reassessed to minimize the risk of *Campylobacter* infection in animals.

In conclusion, the regulation of fluoroquinolone drugs used for food-producing animals should be continued in order to prevent an increase in fluoroquinolone-resistant foodborne bacteria of animal origin. Prudent use of antimicrobials is essential, because the overuse and misuse of fluoroquinolones increases the risk of fluoroquinolone-resistant bacteria emerging, and multidrug-resistant *Campylobacter* with fluoroquinolone resistance may be selected by the use of antimicrobials other than fluoroquinolones. Moreover, biosecurity programs on farms will contribute to the control of fluoroquinolone-resistant *Campylobacter* infection in food-producing animals.

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**REFERENCES**


