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Short Communication

Correlation between the Usage Volume of Veterinary Therapeutic Antimicrobials and Resistance in *Escherichia coli* Isolated from the Feces of Food-Producing Animals in Japan

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SUMMARY: We compared the overall usage of veterinary therapeutic antimicrobials in Japan to the proportion of antimicrobial-resistant. *Escherichia coli* isolated from the feces of apparently healthy food-producing animals in 2001. The annual sales volume of veterinary antimicrobials, which is published and accumulated information, was subdivided according to the target animal species (cattle, pigs, broiler chickens, and layer chickens). The antimicrobial susceptibility of the isolates was examined against 7 classes of 11 antimicrobials. The rates of antimicrobial resistance among the isolates were found to correlate significantly with the usage of antimicrobial agents in cattle, pigs, and broiler and layer chickens. Therefore, the overall usage of veterinary antimicrobials appears to contribute to the appearance of antimicrobial resistance in *E. coli* isolates from apparently healthy food-producing animals.

Veterinary antimicrobial agents are essential for the treatment of sick animals, but excessive use may lead to antimicrobial resistance (1-3). Whereas resistance to such agents reduces the general efficacy of antimicrobial therapy in veterinary medicine, the development of resistance among animal-derived isolates also represents a potential hazard to humans via food-borne infections caused by resistant bacteria (3). The increasing incidence of the antimicrobial resistance of *Enterobacteriaceae* isolates such as *Escherichia coli* from food-producing animals is of great concern in the field of public health; in response to these increases, the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) was formed in 1999 (4). The two main objectives of the JVARM are to monitor the populations of antimicrobial-resistant isolates obtained from food-producing animals and to monitor the veterinary usage of antimicrobials in food-producing animals.

The monitoring of the veterinary usage of antimicrobials in food-producing animals is considered as a risk-management option for responding to antimicrobial resistance in food-producing animals (5). Several studies have already investigated the relationship between antimicrobial usage in humans and the emergence of resistant bacterial populations. The use of a particular antimicrobial agent is known to increase resistance to that antimicrobial, both in hospitals (6) and outside of hospitals (7); this has also been demonstrated in a nationwide study (8). However, few studies have been reported to date that provide an overview of the correlation between the therapeutic veterinary use of antimicrobials and the occurrence of resistance among bacteria isolated from food-producing animals. In the current study, we conducted an initial trial to investigate the relationship between the volume of use of therapeutic antimicrobials in the veterinary setting in Japan and the isolation of antimicrobial-resistant populations of *E. coli* isolated from apparently healthy food-producing animals.

The system implemented in the present study for monitoring antimicrobial usage has been described elsewhere (4). In brief, pharmaceutical companies that produce and/or import antimicrobials for animals are required to submit data annually in accordance with the governing pharmaceutical affairs law. The annual weight of the approved active antimicrobials (i.e., including only therapeutic antimicrobials) is subdivided according to animal species. The sales volume of each of the antimicrobials supplied in the year 2001 (9) was defined as the antimicrobial usage in each species of animal (cattle, pigs, broiler chickens, and layer chickens). As regards the β-lactam antibiotics, the volume of usage of the penicillins (i.e., the sum of aspoxicilin, amoxicillin, ampicillin (AMP), cloxacillin, benzylpenicillin, and mecillinum) and cephalosporins (the sum of cefazolin, ceftiofur and cefquinome) were calculated separately. The sum of the volumes of tetracycline, oxytetracycline (OTC), doxycycline, and chlortetracycline usage was deemed as tetracycline usage, since OTC-resistant bacteria exhibit cross-resistance among these antimicrobials (10). As regards the aminoglycoside antibiotics, the volumes of use of kanamycin (KM), gentamicin (GM), and streptomycin (including dihydrostreptomycin [DSM]) were divided, as isolates resistant to one of these agents are not consistently cross-resistant to the other agents (10). The usage of quinolones (sum of nalidixic acid [NA] and oxolinic acid) and fluoroquinolones (the sum of enrofloxacin [ERFX], ofloxacin, orbifloxacin, norfloxacin, danofloxacin and difloxacin) were considered separately, since the development of fluoroquinolone resistance in *E. coli* requires more mutation of the gene-coding DNA gyrase than does quinolone resistance (10). Methoprim

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Table 1. Usage volumes of selected antimicrobial agents for veterinary use in food-producing animals

Class or Active substance	Correspondence to resistance	usage volume (Kg) in: Cattle	Pig	Broiler	Layer	Contents
Penicillin	Ampicillin	7,180.4	32,747.5	6,449.7	4,650.7	The sum of aspoxicilin, amoxicillin, ampicillin, cloxacillin, benzylpenicillin, and mecillinum usage
Cephalosporins	Cefazolin	184.1	188.3	0.0	0.0	The sum of cefazolin, ceftiofur and cefquinome usage
Streptomycin	Dihydrostreptomycin	3,195.0	30,051.0	7,221.3	4,089.4	Streptomycin including dihydrostreptomycin usage
Kanamycin	Kanamycin	805.0	7,115.8	1,580.4	2,062.2	Kanamycin usage
Gentamicin	Gentamicin	7.3	7.8	0.0	0.2	Gentamicin usage
Colistin	Colistin	1.3	773.6	192.2	187.7	Colistin usage
Tetracycline	Oxytetracycline	19,031.9	291,579.5	65,345.0	25,611.9	The sum of tetracycline, oxytetracycline, doxycycline, and chlortetracycline usage
Bicozamycin	Bicozamycin	217.0	313.0	0.0	0.0	Bicozamycin usage
Quinolone	Nalidixic acid	1.5	12.7	224.4	9.0	The sum of nalidixic acid and oxolinic acid usage
Fluoroquinolones	Enrofloxacin	332.7	1,292.9	3,580.8	256.2	The sum of enrofloxacin, ofloxacin, orbifloxacin, norfloxacin, danofloxacin and difloxacin usage
Methoprim	Trimethoprim	295.4	16,805.5	2,322.1	377.4	The sum of ormethoprim and trimethoprim usage

Table 2. Antimicrobial susceptibility of *E. coli* isolates from apparently healthy food-producing animals during 2001 in Japan

Antimicrobial agent	Break point (mg/L)	No. of resistance (%) Cattle *n* = 88	Pig *n* = 82	Broiler *n* = 63	Layer *n* = 73
Ampicillin	32	12 (13.6)	27 (32.9)	28 (44.4)	9 (12.3)
Cefazolin	32	0 (0)	0 (0)	4 (6.3)	0 (0)
Dihydrostreptomycin	32	16 (18.2)	44 (53.7)	39 (61.9)	19 (26.0)
Kanamycin	64	6 (6.8)	18 (22.0)	25 (39.7)	6 (8.2)
Gentamicin	4	0 (0)	3 (3.7)	4 (6.3)	0 (0)
Colistin	16	0 (0)	0 (0)	0 (0)	0 (0)
Oxytetracycline	16	29 (33.0)	55 (67.0)	47 (74.6)	29 (39.7)
Bicozamycin	128	0 (0)	2 (2.4)	2 (3.1)	1 (1.4)
Nalidixic acid	32	1 (1.1)	2 (2.4)	15 (23.8)	5 (6.8)
Enrofloxacin	2	0 (0)	0 (0)	2 (3.1)	4 (5.5)
Trimethoprim	32	1 (1.1)	17 (20.7)	17 (27.0)	6 (8.2)

usage was considered as the sum of both ormethoprim and trimethoprim (TMP) usage.

The usage volumes of antimicrobial agents calculated for each animal species are shown in Table 1. Among the antimicrobials examined here, more tetracycline was used, in all of the animal species studied, than any of the other agents; following tetracycline in this regard were penicillin and streptomycin.

Representing the *Enterobacteriaceae*, *E. coli* was used for the present investigation, as it is generally present in the intestinal tracts of different animal specie. This choice of *E. coli* enabled the use of a representative stratified random sampling strategy (11). A total of 306 *E. coli* samples isolated in 2001 from 306 feces samples of apparently healthy food-producing animals were supplied by Livestock Hygiene Service Centers randomly selected from 12 of the 47 prefectures in Japan. All of the isolates were stored in Trypticase soy broth (TSB) containing 15% glycerol at –80°C until use. The isolates were tested for their susceptibility to selected antimicrobials by an agar dilution method with Mueller-Hinton agar according to the recommendations of the National Committee for Clinical Laboratory Standards (NCCLS) (12). The following quality-control strains were also tested: *E. coli* American Type Culture Collection (ATCC) 25922, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Enterococcus faecalis* ATCC 29212. All of the results, except for those for DSM, OTC, and ERFX were interpreted by using the NCCLS criteria (13). The break points of DSM, OTC, and ERFX were determined on the basis of the MIC population of the isolates, as well as on the results of our previous report (11).

The highest resistance rates of *E. coli* isolates from the four animal species examined here were observed for OTC ranging from 33.0 to 74.6% (Table 2). Higher resistance rates were observed for DSM (18.2 to 61.9%), followed by AMP (13.6%) in cattle; AMP (32.9%), KM (22.0%), and TMP (20.7%) in pigs; AMP (44.4%), KM (39.7%), TMP (27.0%), and NA (23.8%) in broiler chickens; and AMP (12.3%) in layer chickens. The resistance rates for the other drugs were less than 10%.

We cross-sectionally compared the usage volume of each antimicrobial with the resistance rate of the *E. coli* isolated from apparently healthy food-producing animals. The statistical analysis was conducted using Pearson's correlation coefficient and Student's *t* test in order to clarify the overall correlation between the two factors.

The antimicrobial resistance rates of the isolates were found to correlate significantly with the usage volume of antimicrobial agents in cattle (r = 0.958, $P < 0.01$), pigs (r = 0.787, $P < 0.01$), broiler chickens (r = 0.699, $P < 0.05$), and layer chickens (r = 0.945, $P < 0.01$) (Figure 1). The rates of resistance to antimicrobials frequently used in food-producing animals were elevated in *E. coli* isolated from apparently healthy food-producing animals. In 2001, official statistics database was created regarding the veterinary sales volume of therapeutic antimicrobials for each of the animal species examined here. Thereafter, the regulatory authority annually published data regarding the subsequent yearly sales volumes. Preliminary analyses using the data from the DANMAP reports in 2001 and 2002 (14,15) represented the correlation between the usage volume of antimicrobials and the isolation of resistant population in *E. coli* from cattle and pigs (data not shown). Therefore, the overall usage of veterinary antimicrobials is thought to contribute to the appearance of antimicrobial resistance among *E. coli* isolates from apparently healthy food-producing animals.

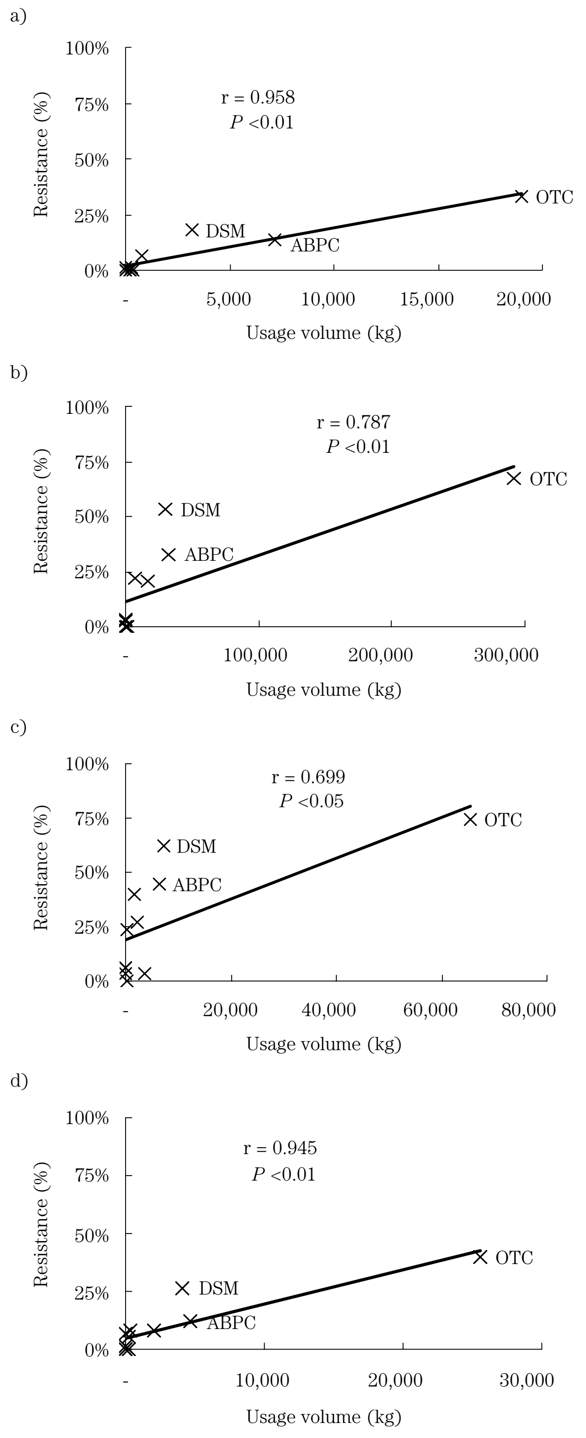

Fig. 1. Relationship between antimicrobial usage and antimicrobial resistance population in animal species. (a), cattle; (b), pigs; (c), broiler chickens; (d), layer chickens.

A lower correlation between antimicrobial usage and resistant bacterial populations was observed in broiler chickens than in the cases of the cattle, pigs, and layer chickens. In layer chickens, the actual administration of antimicrobial usage was considerably lower than in the other animals, because the administration of antimicrobials to a flock that lays eggs is regulated (3). The regimen of antimicrobial administration to animal species for meat production was shown to differ from that used in non-meat-producing species (16). Namely, in cattle, a veterinarian administers antimicrobials, primarily by injection, to individual cattle diagnosed with an infection. In the remaining animal species, metaphylaxis of antimicrobials such as administration by the addition of the drugs to the feed or the drinking water is frequently performed for certain therapeutic purposes. Furthermore, there are remarkable differences in the expected lifetimes of each species (cattle>pigs>broiler chickens). Broiler chickens are slaughtered at about 2 to 3 months of age, and therefore the usage of antimicrobials is limited to an early stage of life. Accordingly, a number of different factors in each species may affect the correlation between antimicrobial usage and the emergence of resistant bacterial populations in each of these species.

Moreover, complex mechanisms such as cross-resistance and co-resistance may participate in the emergence and maintenance of antimicrobial resistance among *E. coli* populations. In the present study, although cross-resistance was taken into consideration in the process of calculating the usage volume of each antimicrobial agent, co-resistance may have contributed to the selective pressure of antimicrobial resistance. Kumai et al. (17) previously reported that most OTC-resistant isolates from pigs exhibited multiple drug resistance and harbored a streptomycin-resistance gene within the class 1 integron. Harada et al. (18) indicated that the therapeutic usage of streptomycin and/or methoprim in the cattle and pig industries was involved in the persistence of *E. coli* resistant to chloramphenicol, the use of which in food-producing animals was banned in 1998. Further studies will still be needed in order to clarify the accurate relationship between antimicrobial usage and the emergence of resistance.

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