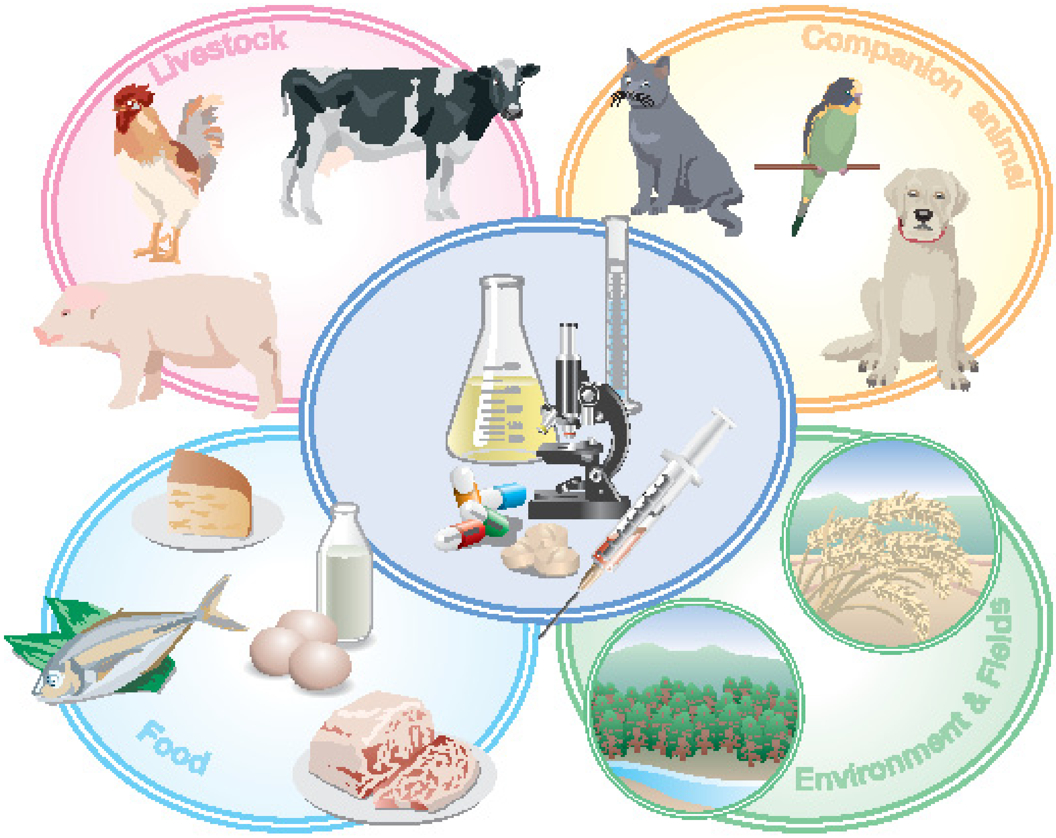

A Report on the Japanese Veterinary Antimicrobial Resistance Monitoring System

-2000 to 2007-

National Veterinary Assay Laboratory

Ministry of Agriculture, Forestry and Fisheries

2009

Contents

Introduction

Antimicrobial agents are still used for growth promotion in animal husbandry in Japan. The effect on human health has been a concern since Swann et al. reported that antimicrobial-resistant bacteria arising from the use of veterinary antimicrobial agents were transmitted to humans through livestock products, and consequently reduced the efficacy of antimicrobial drugs in humans. In addition, the development of antimicrobial resistance in bacteria of animal origin reduces the efficacy of veterinary antimicrobial drugs.

In Japan, the Japanese Veterinary Antimicrobial Resistance Monitoring System (JVARM) was formed in 1999 in response to international concern about the impact of antimicrobial resistance on public health. The JVARM program conducted preliminary monitoring for antimicrobial-resistant bacteria in 1999, followed by the first and second stages of the program carried out in 2000-2003 and 2004-2007, respectively.

Veterinary antimicrobial use is a selective force for the appearance and prevalence of antimicrobial-resistant bacteria in food-producing animals. However, antimicrobial-resistant bacteria are found in the absence of an antimicrobial selective pressure. The trends in antimicrobial resistance in foodborne and indicator bacteria from apparently healthy food-producing animals, and the relationship between antimicrobial usage and prevalence of resistant bacteria under the JVARM program from 1999 to 2007 are outlined in this report.

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Tamura, Y. 2003. The Japanese veterinary antimicrobial resistance monitoring system, In: OIE International Standards on Antimicrobial resistance 2003, OIE headquarters, Paris, France. pp 206-210.

I The Japanese Veterinary Antimicrobial Resistance Monitoring System

1. Objectives

The objectives of JVARM are to monitor the occurrence of antimicrobial resistance in bacteria in food-producing animals and the consumption of antimicrobials for animal use. These objectives allow the efficacy of antimicrobials in food-producing animals to be determined, promotion of prudent use of such antimicrobials to be encouraged, and the effect on public health to be ascertained.

2. Outline of JVARM

JVARM (summarized in Figure 1) comprises three components: monitoring the quantities of antimicrobials used in animals; resistance monitoring in zoonotic and indicator bacteria isolated from healthy animals; and resistance monitoring in animal pathogens isolated from diseased animals. In Japan, the Ministry of Agriculture, Forestry and Fisheries (MAFF) is responsible for animal husbandry, but not food hygiene. Thus, all bacteria are isolated from food-producing animals on farms, but not from food products.

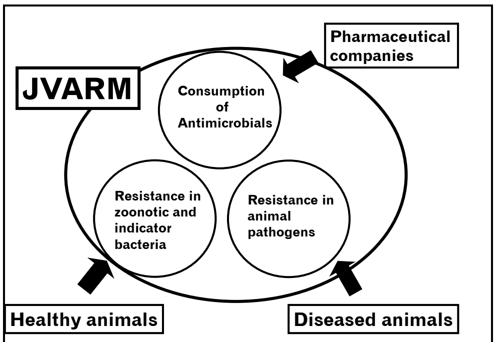

Fig.1. Outline of JVARM

(1) Monitoring of Antimicrobial Consumption

The monitoring implementation system of antimicrobial consumption is shown in Figure 2. Pharmaceutical companies that produce and import antimicrobials for animals are required to submit data to the National Veterinary Assay Laboratory (NVAL) annually in accordance with the Pharmaceutical Affairs law. NVAL subsequently collates analyses and evaluates the data. MAFF headquarters then publishes this data in a yearly report entitled “Amount of medicines and quasi-drugs for animal use”.

The annual weight in kilograms of the active ingredients in approved antimicrobials used in animals is collected. This includes only antimicrobials for therapeutic animal use. Data are then subdivided into animal species. This method of analysis only provides an estimate of the consumption

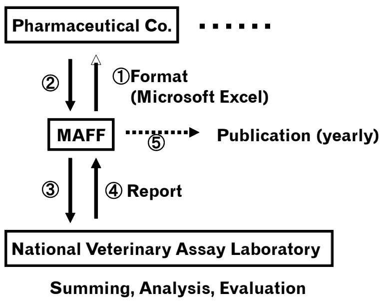

Fig.2. Monitoring of Antimicrobial Consumption

(2) Monitoring of Antimicrobial Resistant Bacteria

Bacteria used in antimicrobial susceptibility testing are continuously collected and include: zoonotic and indicator bacteria isolated from apparently healthy animals, and pathogenic bacteria isolated from diseased animals. Zoonotic bacteria include: *Salmonella* species, and *Campylobacter jejuni* or *C. coli;* indicator bacteria include *Escherichia coli* and *Enterococcus faecium* or *E. fecalis*. Animal pathogens include species of *Salmonella*, *Staphylococcus*, *Actinobacillus pleuropneumoniae*, *Pasteurella multocida*, *Streptococcus* and *Mannheimia haemolytica*. The zoonotic and indicator bacteria are isolated from faecal samples collected from cattle, pigs, and broiler and layer chickens. Six samples per animal are collected annually in each prefecture. One sample is limited from one farm. Two strains per sample are collected for antimicrobial susceptibility testing. Animal pathogens are isolated from samples submitted for diagnosis. Minimum Inhibitory Concentrations (MICs) of antimicrobial agents for target bacteria are determined using the agar dilution method as described by the Clinical Laboratory Standard Institute (CLSI; formerly National Committee for Clinical Laboratory Standards).

4. JVARM Implementation System

The JVARM implementation system is shown in Figure 3. Livestock Hygiene Service Centers (LHSCs), which belong to prefecture offices, participate in JVARM. The LHSCs function as participating laboratories of JVARM, and are responsible for the isolation and identification of target bacteria, as well as MIC measurement. They send results and tested bacteria to NVAL, which functions as the reference laboratory of JVARM and is responsible for preservation of the bacteria, collating and analyzing all data and reporting to MAFF headquarters. In addition, NVAL conducts research into the molecular epidemiology and resistance mechanisms of the bacteria.

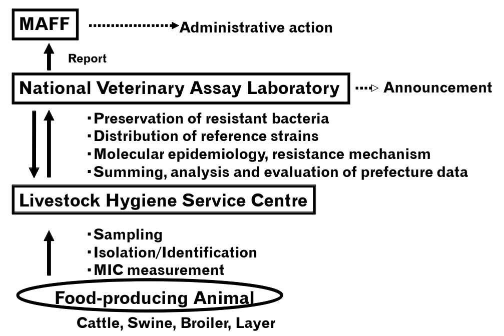

Fig.3. Monitoring of Resistant bacteria

5. QA/QC Systems

Quality control procedures are implemented in participating laboratories that perform antimicrobial susceptibility testing to help monitor the precision and accuracy of the test procedure, the performance of the appropriate reagents, and the personnel involved. Strict adherence to standardized techniques is necessary for the collection of reliable and reproducible data from participating laboratories. Quality control reference bacteria are also tested in each participating laboratory to ensure standardization. Moreover, NVAL holds a national training course on antimicrobial resistance every year to provide training in standardized laboratory methods for the isolation, identification and antimicrobial susceptibility testing of target bacteria.

6. Publication of Data

Because a problem with antimicrobial resistance directly influences animal and human health, it is of paramount importance to distribute information on antimicrobial resistance as soon as possible. We have officially taken three steps to publicize such information; initially through the MAFF weekly newspaper called “Animal Hygiene News”, then by publication in scientific journals and via the NVAL website (URL http://www.maff.go.jp/nval/tyosa_kenkyu/taiseiki/index.html).

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II. An overview on the availability of veterinary antimicrobial products in Japan used for therapy or growth promotion

The number of animals slaughtered for meat in slaughterhouses and poultry slaughtering plants are shown in Table 1. In the last decade, there has been no remarkable change in the number of meat animals produced, except in cattle. The number of slaughtered beef cattle decreased by approximately 20%: from 1.5 million in 1995 to 1.2 million in 2003. The scale of pig and poultry farms increased each year. However, the number of farmers around Japan has decreased because of the absence of successors.

The sales volume of veterinary medical products from 2000 to 2005 is shown in Figure 4. The total antimicrobial consumption for animals increased temporarily from 2000 (970 tons) to 2001 (1060 tons) and has since decreased to 870 tons in 2005. In 2001, the total sales amount of veterinary antimicrobial products used for animal health purposes in Japan was 1059 tons including tetracyclines (456 tons, 43%), sulfonamides (175 tons, 17%), macrolides (142 tons, 13%) and penicillins (103 tons, 10%; Table 2). The sales volume of fluoroquinolones and cephalosporins accounted for 0.6% (approx. 6.3 tons) and 0.2% (approx. 1.7 tons), respectively, of the total sales amount of antimicrobial agents for animal health purposes. These veterinary antimicrobial products were mainly used for pigs (54%), fish (20%), broiler chickens (11%), cattle (8%) and layer chickens (4%). Of the total sales amount of veterinary antimicrobial products for pigs, tetracyclines accounted for 51.1% (292 tons).

The use of antimicrobial feed additives commenced in the 1950s. In Japan, all antimicrobial feed additives must be subjected to a national assay before distribution. The current trends in assay acceptable amounts of feed additives (converted to bulk products) are shown in Figure 5. From 2000 to 2005, the total volume was fairly constant, averaging 171 tons. Polyethers and polypeptides comprised a large percentage of feed additives (average of 97 and 44 tons, respectively), whereas those of other compounds, including tetracyclines, aminoglycosides, and macrolides, comprised less than 5% of the total volume (average of 6.8, 5.0 and 1.1 tons, respectively).

Presently, the total usage volume of antimicrobial drugs is much greater than that of antimicrobial feed additives in Japan. Thus, veterinary antimicrobial drugs are given priority as risk factors associated with bacterial antimicrobial resistance.

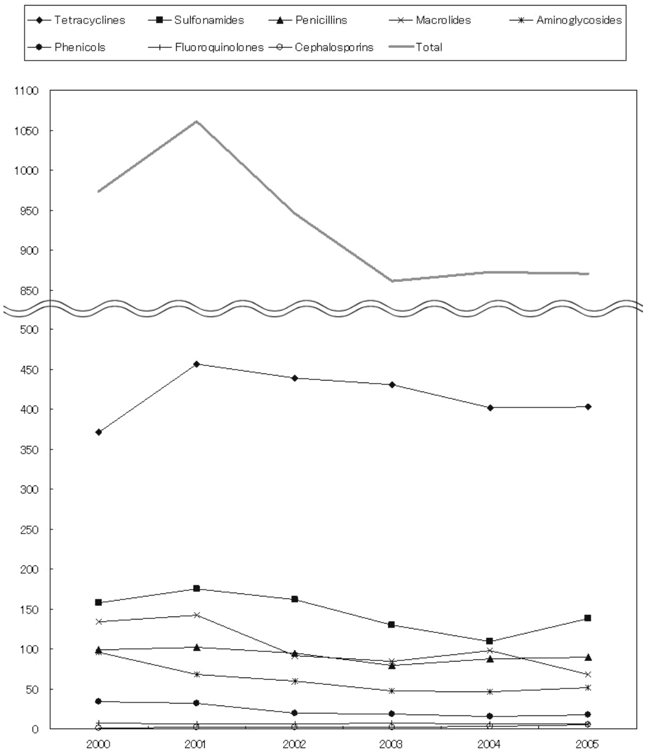

Figure 4. Trends in veterinary antimicrobials sold from pharmacies in Japan (in tons of active compound).

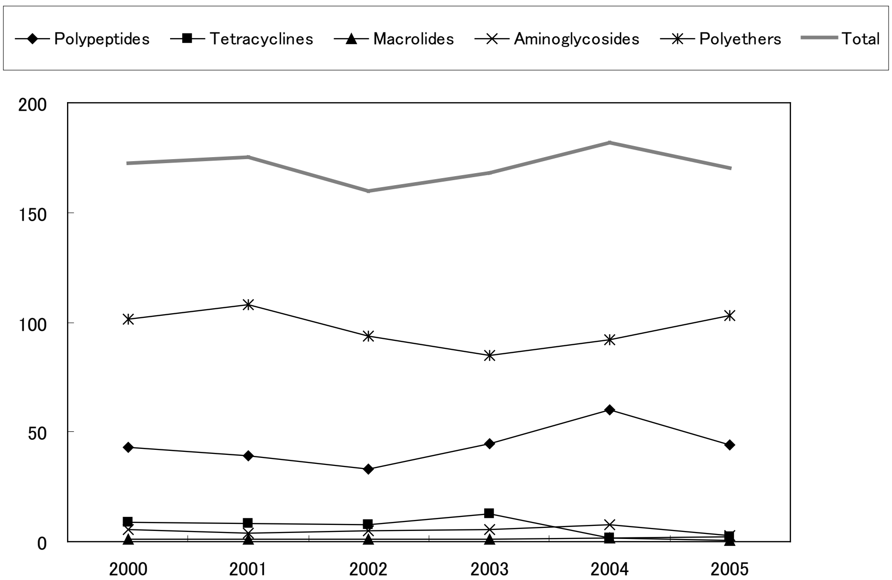

Figure 5. Trends in assay acceptable amounts of antimicrobial feed additives in Japan (in tons of active compound).

III. Monitoring of antimicrobial resistance

1. 1st stage of 1. JVARM (2000-2003)

1) *Escherichia coli*

In total, 2,205 isolates of *E. coli* (646 from cattle, 558 from pigs, 471 from layer chickens and 530 from broiler chickens) collected from 2000 to 2003 were available for antimicrobial susceptibility testing (Table 3). Antimicrobial resistance was found for 14 of 16 antimicrobials tested (Table 4).

Resistance rates against almost all antimicrobials studied were stable in *E. coli* isolates during the period of 2000 to 2003. Resistance was frequently found against dihydrostreptomycin and oxytetracycline in food-producing animals.

Resistance in pig and broiler chicken isolates were most common against dihydrostreptomycin (resistance rates in pigs/broilers, 49.0-55.4%/44.4-58.1%), oxytetracycline (64.0-71.8%/60.6-74.3%), ampicillin (25.0-33.6%/40.9-55.9%), kanamycin (12.4-26.8%/19.2-38.5%), chloramphenicol (16.9-25.6%/10.9-17.9%) and trimethoprim (19.1-31.4%/16.2-43.4%). Incidence of nalidixic acid resistance was high in the isolates of *E. coli* from broiler chickens (27.4-32.0%), intermediate in those from pigs (2.0-6.6%) and layer chickens (4.3-7.5%) and low in those from cattle (0-1.7%). Low frequency of enrofloxacin resistance was observed among isolates of *E. coli* from cattle (0-1.2%) pigs (0-4.1%), broiler (2.6-6.9%) and layer chickens (0-5.8%). Resistance to cefazolin and ceftiofur were found in *E. coli* isolates only of broiler origin (2.7-7.1% and 2.7-5.1%, respectively) between 2000 and 2002. In 2003, resistance to cefazolin was found in nine *E. coli* isolates from all animal species.

2) *Enterococci*

A total of 1,181 isolates of *E. faecalis* (n = 610) and *faecium* (n = 571) from four food-producing animals were collected from 2000 to 2003 (Table 3). Antimicrobial resistance in isolates of *E. faecalis* and *faecium* was found for 9 and 8 of the 12 antimicrobials tested, respectively (Tables 5 and 6). Resistance rates against all of the antimicrobials studied were stable in *E. faecalis* and *faecium* isolates between 2000 and 2003. Antimicrobial resistance was more frequently found in *E. faecalis* isolates than *E. faecium* isolates.

Resistance was more frequently found in *E. faecalis* and *faecium* isolates from pigs and broiler chickens than cattle and layer chickens (Tables 3 and 4). Resistance in *E. faecalis* isolates was frequently found against dihydrostreptomycin, kanamycin, oxytetracycline, erythromycin and lincomycin in pigs, broiler and layer chickens. Resistance against chloramphenicol was most common in pig isolates of *E. faecalis* (Table 5). Resistance in *E. faecium* isolates was most common against oxytetracycline in the four food-producing animals, and against dihydrostreptomycin, erythromycin and lincomycin in pig and broiler chicken isolates (Table 6).

3) *Campylobacter*

A total of 647 *C. jejuni* and 426 *C. coli* isolates were obtained over 5 years (Table 3). *C. jejuni* was isolated mainly from feces of cattle, layers and broilers, whereas *C. coli* was isolated mainly from feces of pigs (Table 7).

Fluoroquinolone (FQ) resistance in *Campylobacter* isolates, *C. jejuni* isolated from cattle and *C. coli* isolated from pigs tended to increase (Table 8). In Japan, enrofloxacin was approved for veterinary medicine in 1991. Nowadays, seven different FQs have been approved for veterinary use. Recently, even though the use of FQ has decreased as knowledge concerning the risk of FQ resistance has increased, lowered FQ resistance in *Campylobacter* has never been observed in Japan.

All of the *C. jejuni* isolates were susceptible to erythromycin, but around half of the *C. coli* isolates were resistant to erythromycin independent of animal origin.

C. jejuni and *C. coli* isolates were resistant to oxytetracycline (51.9% to 61.6%, respectively). Oxytetracycline resistance was observed in both *C. jejuni* and *C. coli* in all animals. The amounts of tetracyclines used for treatment of animals were the greatest among the antimicrobials. Moreover, tetracyclines have been approved as feed-additive antimicrobial agents for growth promotion in Japan.

Resistance frequencies in *C. coli* to dihydrostreptomycin, erythromycin, oxytetracycline and quinolones were higher than those in *C. jejuni*. Only ampicillin resistance was found at higher frequencies in *C. jejuni* than *C. coli* isolates. The frequencies of ampicillin resistance were much higher in *C. jejuni* isolates from layers (26.9 to 39.6%) and broilers (20 to 20.7%) than those from cattle.

4) *Salmonella*

Salmonella was isolated from 16 of 650 cattle (2.5%, 25 isolates), 20 of 527 pigs (3.8%, 39 isolates), 57 of 283 broiler chickens (20.1%, 91 isolates) and 15 of 444 layer chickens (3.4%, 25 isolates). A total of 183 isolates were obtained between 2000 and 2003. Twenty-nine serovars were identified as shown in Table 1. The major serovars of isolates were *S.* Typhimurium in cattle (76.0%, 19/25) and pigs (43.6%, 17/39) and *S.* Infantis in broiler chickens (71.4%, 65/91). A wide variety of serovars were found in isolates from layer chickens (Table 9).

Resistance was observed for 9 of 20 antimicrobials tested, 77.6% for dihydrostreptomycin and 67.8% for oxytetracycline (Table 10). *Salmonella* resistant to dihydrostreptomycin was approximately 10% more prevalent than that resistant to oxytetracycline, though the national level of veterinary use of tetracycline antibiotics is much greater than that of streptomycin.

Except for ampicillin, there were no significant differences in resistance rates of the antimicrobials tested in the 4 years of this study. The resistance rate to ampicillin decreased from 29.7% in 2000 to 0% in 2003 (Table 10). However, no isolates originating from cattle, in which resistance to ampicillin was frequently found, were obtained in 2003. A small number of isolates were used for antimicrobial susceptibility tests in 2001 and 2003.

The present study showed that 131 (71.6%) isolates were resistant to two or more of the antimicrobials tested (Table 3). Resistance rates of layer chicken isolates to two or more antimicrobials (10.7%, 3/28) were the lowest among those of the four animal species. The majority of multiantimicrobial-resistant (MAR) isolates were derived from cattle, pigs, and broiler chickens. *S.* Typhimurium accounted for 70.0% of the MAR isolates from cattle (18/22) and pigs (17/28), and *S.* Infantis accounted for 78.2% (61/78) of the MAR isolates from broiler chickens. Oxytetracycline and dihydrostreptomycin resistance in *Salmonella* have been shown to be strongly associated with the dissemination of *S.* Typhimurium in cattle/pigs and *S.* Infantis in poultry.

2. 2nd stage of JVARM (2004-2007)

1) *Escherichia coli*

In total, 1,979 isolates of *E. coli* (541 from cattle, 520 from pigs, 466 from layer chickens and 452 from broiler chickens) collected from 2004 to 2007 were subjected to antimicrobial susceptibility testing (Table 3). Resistance rates against almost all antimicrobials studied were stable in *E. coli* isolates during the periods of 2004 to 2007 (Table 11).

Resistance in pig and broiler chicken isolates were most common against dihydrostreptomycin (resistance rates in pigs/broilers, 43.4-47.1%/33.1-45.8%) and oxytetracycline (57.5-69.1%/53.9-66.4%), ampicillin (22.1-27.0%/42.1-53.3%), kanamycin (7.5-17.8%/13.7-24.3% 12.4-26.8%/19.2-38.5%), chloramphenicol (13.5-24.3%/5.7-14.0%) and trimethoprim (27.0-30.2%/20.6-30.4%), as seen in the first stage of this study. Incidence of nalidixic acid resistance was high in the *E. coli* isolates from broiler chickens (27.1-30.5%), intermediate in those from pigs (4.6-8.8%) and layer chickens (8.9-22.3%) and low in those from cattle (0-5.4%). Frequency of enrofloxacin resistance remained low, but slightly increased in isolates of *E. coli* from broiler (5.3-8.6%) and layer chickens (0.8-7.7%). Resistance to cefazolin and ceftiofur were found in *E. coli* isolates from all animal species. In addition, resistance frequencies to cefazolin and ceftiofur increased in *E. coli* isolates only of broiler origin between 2004 and 2007 (10.5-18.6% and 9.5-16.7%, respectively).

2) *Enterococci* (see Table 12 & 13).

A total of 951 isolates of *E. faecalis* (n = 575) and *faecium* (n = 376) from the four food-producing animals were isolated from 2004 to 2007 (Table 3). Antimicrobial resistance in isolates of *E. faecalis* and *faecium* is shown in Table 12 and 13. Antimicrobial resistance in isolates were found for 10 of the 11 tested antimicrobials in *E. faecalis* and for all tested antimicrobials in *E. faecium* (Tables 12 and 13). Resistance rates against all of the antimicrobials studied were stable in *E. faecalis* and *faecium* isolates between 2004 and 2007. Antimicrobial resistance was more frequently found in *E. faecalis* isolates than *E. faecium* isolates. Oxytetracycline resistance in *E. faecium* isolates originating from all food-producing animals was detected at a high frequency. Dihydrostreptomycin, EM and LCM resistance were found in isolates originating from pig and broiler chickens.

Resistance in *E. faecalis* and *E. faecium* isolates was frequently found against dihydrostreptomycin, kanamycin, oxytetracycline, erythromycin and lincomycin in all food-producing animals. Resistance rates of isolates originating from pig and broiler chickens tended to be higher than those originating from cattle and layer chickens. kanamycin, chloramphenicol and enrofloxacin resistance in *E. faecalis* and *E. faecium* increased in the second stage of JVARM compared with the first stage. Increased incidence of kanamycin resistance was found in *E. faecium* isolates originating from broiler chickens (45.5%), layer chickens (34%) and pig (42.2%). Enrofloxacin resistance rate in *E.faecium* isolates was higher than those of *E. faecalis*. Incidence of enrofloxacin resistance was high in the *E. faecium* isolates originating from broiler chickens (44.4%), intermediate in those from layer chickens (21%) and pig (13.7%) and low in those from cattle (8.0%). Enrofloxacin resistance rates of *E. faecalis* isolates originating from broiler chickens (7.9%) and layer chickens (2.4%) were low, and those originating from cattle and pigs were not detected. Enrofloxacin resistance in *E. faecium* isolates increased in the second stage of JVARM compared with the first stage, with the increase in the rate of enrofloxacin resistance in broiler chickens being quite remarkable.

Vancomycin resistance was found in *E. faecium* isolates originating from cattle (1.3%) and layer chickens (2.4%), but the incidence was low, and resistance was not recognized in the other isolates.

3) *Campylobacter*

A total of 394 *C. jejuni* and 285 *C. coli* isolates were obtained between 2004 and 2007 (Table 3). *C. jejuni* was isolated mainly from feces of cattle, layers and broilers, whereas *C. coli* was isolated mainly from pig feces.

Resistance to oxytetracycline was more frequently found in *C. coli* isolates (62.8 to 83.1%) than *C. jejuni* isolates (40.9 to 57.5%; Table 14). Oxytetracycline resistance was observed in both *C. jejuni* and *C. coli* in isolates from all origins.

Dihydrostreptomycin resistance was observed in 44.1-60.5 % of *C. coli* isolates and in 3.8-10.0% of *C. jejuni* isolates.

FQ resistance in *C. jejuni* and *C. coli* significantly increased in the second stage of JVARM compared with the first stage. Increased incidence of FQ resistance was found in *C. jejuni* isolated from broiler chickens and *C. coli* isolated from pigs.

Erythromycin resistance was not found in any of the *C. jejuni* isolates, and frequently found in *C. coli* isolates in the second stage of JVARM. Thus, the trend in resistance was stable between the first and second stages.

4) *Salmonella*

A total of 179 isolates, including 30 from pigs, 27 from layer chickens and 122 from broiler chickens, were obtained between 2004 and 2007. Seventeen serovars were identified (shown in Table 15). The predominant serovar were *S.*

Infantis (88 isolates, 49.2%), followed by *S.* Schwarzengrund (29 isolates, 16.2%) and *S.* Typhimurium (13 isolates, 7.2%). *S.* Infantis was the predominant serovar isolated from broiler chickens (85.3%, 81/95). However, *S.* Schwarzengrund was isolated from broiler chickens after 2005.

Resistance to dihydrostreptomycin (average, variation during 2004-2007: 70.4%, 58.5 to 82.8%) and oxytetracycline (65.4%, 54.3 to 78.1%) were most frequently found in *Salmonella* isolates between 2004 and 2007 (Table 16). Resistance frequencies to kanamycin (35.2%, 20.5 to 51.2%) and trimethoprim (43.0%, 30.8 and 63.4%) were relatively high.

3. Association between antimicrobial usage with antimicrobial resistant bacteria in food-producing animals

In Japan, the prevalence of resistant strains of *Escherichia coli* against each category of antimicrobials is proportionate to the total amount of the respective antimicrobials used in animals (Asai T., et al., Jpn. J. Infect. Dis. 58: 369-372, 2005). Therefore, the national overall usage volume of therapeutic antimicrobials is likely to be related to the occurrence of antimicrobial resistance among commensal *E. coli* isolates from food-producing animals. At the farm level, the use of these antimicrobials in animals may not contribute directly to the development of resistant microorganisms.

1) Prevalence of antimicrobial-resistant bacteria in the absence of antimicrobial selective pressure

Antimicrobial-resistant bacteria have been isolated from animals in which no antimicrobials had been used. In Japan, cefazolin-resistant *E. coli* and *Salmonella* strains have been isolated from broiler chickens in spite of no cephalosporins being approved for use in poultry. The strains were either CTX-M-type or CMY-2 enzyme-producing strains, or had mutations in the ampC promoter region (Kojima A, et al., Antimicrob. Agents Chemother. 49: 3533-3537, 2005, Ishihara et al., Acta Vet. Scand. 51: 35).

The resistance type and PFGE profile of predominant *C. jejuni* strains obtained from two different broiler farms changed in the absence of antimicrobial selective pressure. The broiler flocks on one farm were repeatedly infected with two or three *C. jejuni* clones. The flocks on the other farm were infected with at least seven clones and the predominant clone was different in each flock (Ishihara K, et al., J. Vet. Med. Sci. 68: 515-518, 2006).

Since the late 1990's, the predominant salmonella from broiler chickens has been *Salmonella* Infantis, resistant to both streptomycin and tetracycline. *S.* Infantis strains isolated between 1993 and 1998, and those obtained between 2001 and 2003 showed similar antimicrobial resistance and the same PFGE profile. No antibiotics from the streptomycin or tetracycline group had been used between 2001 and 2003 on the farm (Asai T, et al., Microbiol. Immunol. 51: 111-115, 2007).

The proportion of DT104 in *S.* Typhimurium isolates from cattle and pigs significantly ($P < 0.01$) decreased from 71.9% and 31.4% in 1999-2001 to 30.8% and 4.1% in 2002-2005, respectively. Results showed that the predominant bovine resistance in *S.* Typhimurium was to ampicillin, dihydrostreptomycin, kanamycin, and oxytetracycline. By contrast, resistance to dihydrostreptomycin and oxytetracycline was observed in porcine isolates. Veterinary use of chloramphenicol for food-producing animals was prohibited in

Japan in 1998, but thiamphenicol and florfenicol have been approved for treatment of bacterial diseases in both animal species. Moreover, penicillin antibiotics have been used frequently in swine practice. Thus, the changing proportion of antimicrobial resistance pattern in *S.* Typhimurium isolates may not be due to veterinary use of antimicrobial drugs (Kawagoe et al., J Vet Med Sci. 69: 1211-1213, 2007).

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2) Characteristic prevalence of antimicrobial resistance

Multiple-antimicrobial resistance has frequently been found in *S.* Typhimurium and *S.* Infantis in Japan. Fifty-three percent of *S.* Typhimurium strains isolated from food-producing animals between 1999 and 2001 were DT104 strains, most of which were resistant to ampicillin, chloramphenicol, dihydrostreptomycin, oxytetracycline and sulfadimethoxine (Esaki H, et al., Microbiol. Immunol. 48: 553-556, 2004). The majority of *S.* Infantis isolates from broiler chickens were resistant to dihydrostreptomycin and oxytetracycline (Asai T, et al., J. Food Prot. 2006. 9: 214-216.). These isolates, therefore, exhibited higher resistant rates for streptomycin than tetracycline (Asai T, et al., J. Vet. Med. Sci. 68: 881-884, 2006).

3) Contribution of multiple antimicrobial resistance to prevalence of resistant bacteria

Harada et al. demonstrated the higher resistance rate in *E. coli* strains from diseased animals compared with healthy animals (Harada K, et al., J. Vet. Med. Sci. 67: 999-1003, 2005). They speculated the contribution of co-resistance to development of antimicrobial-resistance, since chloramphenicol-resistant strains can still be isolated from diseased cattle and pigs although the use of chloramphenicol in food-producing animals was banned in 1998 (Harada K, et al., Am. J. Vet. Res. 67: 230-235, 2006).

Harada et al. further found the increase in rates of kanamycin- and trimethoprim-resistant *E. coli* strains in apparently healthy pigs in association with the use of tetracycline (Harada K, et al., Microbiol. Immunol. 51: 493-499, 2007).

IV. Current risk management of antimicrobial resistance linked to antimicrobial products

Veterinary medical products (VMPs), which include antimicrobial products used for prophylaxis and therapy, are regulated by the Pharmaceutical Affairs Law (Law No.145 of 1960). The purpose of the law is to regulate matters pertaining to drugs, quasi-drugs and medical devices so as to ensure their quality, efficacy and safety at each stage of development, manufacturing (importing), marketing, retailing and usage. In addition to therapeutic or prophylactic use, growth promotion is another important use of antimicrobials and has significant economical consequences in the livestock industry. Feed additives (FAs), which include antimicrobial products used for growth promotion, are regulated by the Law Concerning Safety Assurance and Quality Improvement of Feed (Law No.35 of 1953). Compared with the antimicrobial VMPs, FAs are used at lower concentrations and longer periods. Antimicrobial growth promoters cannot be used for milking cows, laying hens, and in pigs and chickens 7 days preceding slaughter for human consumption.

There are specific requirements for marketing approval of antimicrobial VMPs in Japan. For the approval of antimicrobial VMPs, data concerning the antimicrobial spectrum, antimicrobial susceptibility tests of recent field isolates of targeted bacteria, indicator bacteria and foodborne bacteria; and the resistance acquisition test, are attached to the application for consideration of public and animal health issues. For the approval of VMPs for food-producing animals, data concerning the stability of the antimicrobial substances under natural circumstances is also attached. The antimicrobial substance in the VMP is thoroughly described in the dossier and the period of administration is limited to 1 week where possible.

General and specific data are evaluated at an expert meeting conducted by MAFF. The data of VMPs used in food-producing animals are also evaluated by the Food Safety Commission. The Pharmaceutical Affairs and Food Sanitation Council, which is an advisory organization to the Minister, evaluate the quality, efficacy, and safety of the VMP (e.g. residue levels for VMPs to be used in food-producing animals). If the VMP satisfies all requirements, the Minister of MAFF approves the VMP. There are two stages at which post-marketing surveillance of VMPs occurs in Japan: during re-examination of new VMPs, and during re-evaluation of all VMPs. After the re-examination period has ended for the new VMP, the field investigation data about efficacy, safety, and public and livestock health, is attached to the application. For new VMPs, results of monitoring for antimicrobial resistance should be submitted according to the requirements of the re-examination system. For all approved drugs, MAFF conducts literature reviews about efficacy, safety, residues and resistant bacteria as per the requirements of the re-evaluation system.

Because most of the antimicrobial VMPs have been approved as drugs requiring directions or prescriptions by a veterinarian, these VMPs cannot be used without diagnosis and instruction by a veterinarian. The distribution and use of VMPs, including veterinary antimicrobial products, is routinely inspected by the regulatory authority (MAFF).

For marketing and use of VMPs, veterinarians prescribe the drug, and place restrictions on its use so that the drug does not remain beyond MRLs in livestock products. As for the label, there are restrictions relating to the description on the "direct container" and on the "package insert". The description on the label must include (1) the prescribed drug, (2) disease and bacterial species indicated, (3) the route, dose and period of administration, (4) prohibition/withdrawal periods, (5) precautions for use, such as side effects, and handling, and (6) in the case of specific antimicrobial drugs (fluoroquinolone and the third generation cephalosporins), the description includes an explanation that the drug is not considered as the first-choice drug. For the specific antimicrobial drugs fluoroquinolone and third generation cephalosporins, which are particularly important for public health, the application for approval of the drug for use in animals is not accepted until the end of the period of re-examination of the corresponding drug for use in humans. After marketing, monitoring data on the amount sold and the appearance of antimicrobial resistance in target pathogens and foodborne pathogens must be submitted to MAFF.

In Japan, the basic Law on Food, Agriculture and Rural Areas was established in 1999 to stabilize and improve people's lifestyle and to develop the national economy. This law aimed to improve the management of food hygiene and quality to ensure food safety, and improve food quality. The risk assessment for antimicrobial resistance in bacteria arising from the use of antimicrobials in animals, especially those that are common to human medicine, is provided to MAFF by the Food Safety Commission (FSC). FSC is an organization for risk assessment, and is independent from risk management organizations such as MAFF and MHLW. The risk assessment for antimicrobial resistance in bacteria from the use of antimicrobials in animals is undertaken on the basis of their new guidelines that are based on the OIE guidelines of antimicrobial resistance, Codex and FDA guidelines.

Antimicrobial VMPs are essential in animal husbandry in Japan. Growth promotion is another important use of antimicrobials in the livestock industry. In the present conditions, with the increased risk of outbreak due to emerging bacterial diseases as well as viral diseases such as foot-and-mouth disease and avian influenza, clinical veterinarians need various classes of antimicrobials to treat endemic and unexpected disease in domestic animals. The risk assessments of antimicrobial resistance in food-producing animals have not yet been completed by FSC. To perform appropriate risk-management on antimicrobial resistance, the benefits/risks of antimicrobial VMPs should be scientifically evaluated.

V. Future Veterinary Antimicrobial Resistance Monitoring

Antimicrobial agents are essential for the prevention, control and treatment of bacterial infections in veterinary medicine and are still available for growth promotion in animal husbandry in Japan. The use of antimicrobial agents, however, can cause the emergence, prevalence, and dissemination of bacteria resistant to antimicrobial agents. Multiple factors appear to be involved in the occurrence and prevalence of antimicrobial-resistant bacteria under the selective pressure from antimicrobial usage. In other words, the prevalence of a specific resistance may not be controlled by the regulation of the corresponding class of antimicrobial drug. Without knowledge of the actual magnitude of cross and co-resistance, antimicrobial resistance created by veterinary usage of antimicrobials cannot be fairly controlled.

At present, national level monitoring of antimicrobial use to elucidate all factors contributing to the prevalence of antimicrobial resistance is limited. As described in this review, contradictory results between the prevalence of antimicrobial resistance and antimicrobial use are obtained from national veterinary monitoring in Japan. Resistance to critically important antimicrobials such as fluoroquinolones and cephalosporins may need to be especially monitored; not only at a national level but also at a farm or individual level before measured risk management decisions can be made. Hereafter, further detailed monitoring may be implemented in the veterinary field. The following steps are recommended: 1) national (or district) level monitoring, 2) farm level monitoring, and 3) individual (or herd) level monitoring. These efforts will develop conservative risk management strategies for antimicrobial resistance.

JVARM began in 1999, conforming to the OIE report on antimicrobial resistance, studying the prevalence of antimicrobial-resistant bacteria in food-producing animals. For risk analysis of antimicrobial resistance, further steps could be taken to ensure animal and public health in Japan. In several countries, national antimicrobial resistance monitoring systems have been established including both animal and public health. At present, however, there is no global monitoring system in Japan or coordination between these areas. Joint efforts are now needed to establish a national antimicrobial monitoring system that includes both animal and public health to solve the food-safety problem of antimicrobial resistance.

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This JVARM report was written by JVARM members of the National Veterinary Assay Laboratory and Food and Agricultural Materials Inspection Center.

This report included data gathered between 2000 and 2007, in addition in part to data from 1999 (Preliminary trial of the JVARM program).

Director of the Veterinary Assay Laboratory

Kenichi Omae (1997.9-2001.3)
Norio Hirayama (2001.4-2003.5)
Hirotaka Makie (2003.6-)

Appendix I (Materials and Methods)

Sampling

Sampling was carried out by the Livestock Hygiene Service Center in all forty-seven prefectures across Japan. Fresh fecal samples were collected from healthy cattle, pigs, and layer and broiler chickens on each farm.

In brief, the 47 prefectures were divided into four groups, selected evenly on the basis of geographical difference from northern to southern areas (11 or 12 prefectures per year). Sampling and bacterial isolation were carried out at Livestock Hygiene Service Centers. Freshly voided fecal samples were taken from healthy beef cattle, pigs and broiler and layer chickens at the farm. In most cases, six samples per animal species were collected from different farms in each prefecture.

Isolation and identification

Escherichia coli

E. coli isolates from each sample were kept using desoxycholate-hydrogen sulfate-lactose agar (DHL agar, Eiken, Japan). These isolates were then stored at -80°C until further use in tests.

Enterococcus

Fecal samples were incubated in the following two ways; direct culturing using the Bile-Esculin Azide agar (BEA, Difco Laboratories, Detroit, MI, USA), or using the enrichment procedure with Buffered Peptone Water (Oxoid, Basingstoke, Hampshire, England). The former plates were incubated at 37°C for 48-72h; latter tubes were incubated at 37°C for 18-24h and subsequently passaged onto plates used for the direct culturing method. Isolates were presumptively identified as enterococci by colony morphology. These isolates were subcultured onto heart infusion agar (Difco) supplemented with 5% (v/v) sheep blood whereupon hemolysis was observed and Gram-staining was tested. Isolates were tested for catalase production, growth in heart infusion broth supplemented with 6.5% NaCl, and at 45°C. Hydrolysis of L-pyrolydonyl-β-naphtylamide, pigmentation, motility, and API 20 STREP (bioMérieux, March l'Etoile, France) was also performed. Further identification was achieved using D-Xylose and sucrose fermentation tests if necessary (Facklam and Sahm, 1995). All isolates were stored at –80°C until testing.

Campylobacter

During 1999 to 2001, *Campylobacter* isolation was performed by one of the following two methods: (1) direct inoculation onto Campylobacter blood-free selective agar (mCCDA: Oxoid, UK) and/or (2) inoculation for enrichment in CEM broth (Ono et al., 1995). After 2002, isolation was performed only by the direct inoculation method. Isolates were identified biochemically and using PCR (Linton et al., 1997). In principle, two isolates per sample were selected for antimicrobial susceptibility testing. These isolates were suspended in 15% glycerin to which Buffered Peptone Water (Oxoid) had been added. They were then stored at –80°C until further use in tests.

Salmonella

One gram of fecal sample was inoculated into 10 ml of Hajna tetrathionate broth, followed by incubation at 42°C for 18h, or an additional 5–7 days at room temperature as a delayed secondary enrichment culture. After incubation, each culture was streaked onto DHL and brilliant green agar plates, each containing 20 ml/L of novobiocin. Candidate colonies were identified biochemically. Identification of isolates for serovar was then performed by slide and tube agglutination according to the latest versions of the Kauffmann-White scheme. All isolates were stored at –80°C until testing.

Antimicrobial susceptibility test

The minimum inhibitory concentration (MIC) of isolates obtained during the period from 1999 to 2000 was determined by a standardized agar dilution method as described by the Japanese Society of Chemotherapy (Mitsuhashi et al., 1981). MHA (Difco) was used for all isolates except for *Campylobacter* where MHA (Oxoid) supplemented with 5% defibrinated horse blood was used for determination of MIC of *Campylobacter*.

The MICs of antimicrobial agents between 2001 and 2003 were determined using the agar dilution method according to the guidelines of Clinical Laboratory Standards Institutes (CLSI: formally, NCCLS). *Staphylococcus aureus* ATCC 29213, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. *C. jejuni* ATCC33560 and *C. coli* ATCC33559 were used as quality control for MIC determination of *Campylobacter*.

Resistant breakpoints

Resistant breakpoints were defined microbiologically in serial studies. The intermediate MIC of two peak distributions was defined as the breakpoints when the MICs for the isolates were bimodally distributed (Working Party of the British Society for Antimicrobial Chemotherapy, 1996).

The MICs of each antimicrobial established by the CLSI were interpreted using the CLSI criteria. The breakpoints of the other antimicrobial agents were microbiologically determined.

Table 1 Number of animals slaughtered in slaughterhouses and poultry slaughtering plants (1,000 heads)

	Cattle	Calves	Horse	Pig	Broiler	Fowl*
1995	1499.8	12.2	22.1	17560.9	606019.1	68917.5
1996	1391.2	8.9	19.7	16832.6	584089.6	67800.4
1997	1335.3	9.5	21	17106.1	578745.7	69868.5
1998	1326.9	14.5	22.2	17232.3	572087	73445.1
1999	1344.9	11.7	19	16899.7	574509.3	71309.6
2000	1292.7	7.2	18.2	16543	572362.3	72401.4
2001	1114.7	6.3	17.8	16215.9	555907	66331.7
2002	1249.2	4.8	18.6	16295.5	596106.3	75028.2
2003	1243.6	9.2	19.3	16574.6	598856.7	81113.3

*Most of these fowls are old layer chickens.

Table 2 Sales Amount of Antimicrobial VMPs in 2001.

	Class or Active substance	Amount (tons)									
		Beef cattle	Dairy cow	Horse	Pig	Broiler	Layer	Dog/cat	Fish	Others	Total
Antibiotics	Aminoglycosides	4	6.4	0.5	39.6	10.5	6.5	0.9	0	0	68.3
	Cephalosporins	0.2	1.3	0	0.2	0	0	0	0	0	1.7
	Tetracyclines	19	15.1	0	291.6	65.4	25.6	0	38.2	0.6	455.5
	Penicillins	7.2	8.9	0.7	32.7	6.4	4.7	0.6	41.4	0	102.5
	Peptides	0	0	0	0.8	0.2	0.2	0	0	0	1.2
	Macrolides	0.4	0.2	0	25.5	8.3	9.8	0	97.2	0.6	142
	Lincosamides	0	0	0	13.6	2.9	0	0.2	2.2	0	19
	Antifungal antibiotics	0	0	0	0	0	0	0.1	0	0	0.1
	MIscellaenous antibiotics	0.7	0.4	0	8.7	0	0	0.5	1.7	0	11.9
Synthetic	Quinolone	0	0	0	0	0.2	0	0	2.9	0	3.2
	Sulfonamineds	9.1	11.3	0.7	114.1	14.7	2.5	0.4	21.4	0.6	174.6
	Thiamphenicol and derivatives	0.8	0.1	0	11.2	2.3	0.1	0	17.3	0	31.9
	Nitrofuran and derivatives	0	0	0	0	0	0	0	6.4	0	6.4
	Fluoroquinolones	0.3	0.2	0	1.3	3.6	0.3	0.6	0	0	6.3
	MIscellaenous antibacterials	0.3	0.1	0	31	2.3	0.4	0.1	0.1	0.1	34.3
	Total	42.1	43.9	1.8	570.3	116.8	50	3.3	228.8	1.9	1058.9

Table 3 Number of bacteria used in this study by animal and isolation year.

Species	Animal	2000	2001	2002	2003	2000-2003	2004	2005	2006	2007	2004-2007
E. coli	Cattle	162	172	179	133	646	124	138	149	130	541
	Pig	149	152	136	121	558	136	152	126	106	520
	Broiler Chicken	145	117	110	99	471	138	107	105	102	452
	Layer Chicken	162	139	107	122	530	113	121	120	112	466
	Total	618	580	532	475	2205	511	518	500	450	1979
E. faecalis	Cattle	10	17	6	4	37	7	7	12	6	32
	Pig	40	37	38	39	154	36	11	27	17	91
	Broiler Chicken	33	54	39	42	168	50	54	40	59	203
	Layer Chicken	55	79	48	69	251	54	79	64	52	249
	Total	138	187	131	154	610	147	151	143	134	575
E. faecium	Cattle	42	26	21	17	106	11	28	23	13	75
	Pig	59	31	21	17	128	21	41	21	19	102
	Broiler Chicken	55	26	38	59	178	20	34	26	19	99
	Layer Chicken	53	32	35	39	159	19	32	19	30	100
	Total	209	115	115	132	571	71	135	89	81	376
C. jejuni	Cattle	43	28	26	34	131	37	12	4	22	75
	Pig	1	0	2	0	3	0	2	0	0	2
	Broiler Chicken	53	43	32	40	168	37	25	24	57	143
	Layer Chicken	77	59	52	48	236	58	51	12	53	174
	Total	174	130	112	122	538	132	90	40	132	394
C. coli	Cattle	3	5	2	2	12	0	0	0	5	5
	Pig	98	68	37	86	289	72	49	28	64	213
	Broiler Chicken	1	4	5	15	25	0	4	3	7	14
	Layer Chicken	5	11	12	22	50	11	15	12	15	53
	Total	107	88	56	125	376	83	68	43	91	285
Salmonella	Cattle	19	4	2	0	25	0	0	0	0	0
	Pig	29	4	2	4	39	8	6	9	7	30
	Broiler Chicken	29	13	37	12	91	17	31	47	27	122
	Layer Chicken	14	1	9	4	28	10	4	8	5	27
	Total	91	22	50	20	183	35	41	64	39	179

Table 4 Antimicrobial susceptibility of *E. coli* isolates from animals (2000-2003)

Antimicrobials (Breakpoint)[a]	Animal species	MIC range (mg/L)		Resistance (%)				
		2000[b]	2001-2003	2000[b]	2001	2002	2003	2000-2003
Ampicillin (25/32)	Cattle	0.78-≥50	0.50-≥512	15.2	12.2	10.2	9.0	11.7 [a]*
	Pig	0.78-≥50	1-≥512	32.9	33.6	25.0	29.8	30.6 [b]
	Broiler	0.78-≥50	1-≥512	55.9	45.3	40.9	44.4	47.3 [c]
	Layer	0.78-≥50	0.50-≥512	17.9	10.8	25.2	9.0	15.7
Cefazolin (50/32)	Cattle	0.78-6.25	0.50-256	0	0	0	0.8	0.2 [a]
	Pig	0.39-12.5	0.50-≥512	0	0	0	0.8	0.2 [a]
	Broiler	0.39-≥100	0.50-≥512	4.1	6.0	2.7	7.1	4.9 [b]
	Layer	0.78-25	0.50-≥512	0	0	0	0.8	0.2 [a]
Ceftiofur (6.25/8)	Cattle	0.1-1.56	≤0.125-2	0	0	0	0	0 [a]
	Pig	0.1-0.78	≤0.125-≥512	0	0	0	0.8	0.2 [a]
	Broiler	0.1-12.5	≤0.125-≥512	4.1	3.4	2.7	5.1	4.0 [b]
	Layer	0.1-0.78	≤0.125-8	0	0	0	0.8	0.2 [a]
Dihydrostreptomycin (50/32)	Cattle	0.78-≥100	1-≥512	29.1	20.9	33.9	28.6	28.0 [a]
	Pig	1.56-≥100	1-≥512	49.0	54.6	50.0	55.4	52.2 [b]
	Broiler	1.56-≥100	1-≥512	50.3	58.1	51.8	44.4	51.4 [b]
	Layer	1.56-≥100	2-≥512	30.2	23.7	31.8	17.2	25.8 [a]
Gentamicin (3.13/16)	Cattle	0.2-≥100	0.25-4	1.8	0	0	0	0.5 [a]
	Pig	0.2-≥100	≤0.125-64	2.0	3.3	2.9	4.1	3.0 [b]
	Broiler	0.2-≥100	≤0.125-128	4.1	7.7	1.8	1.0	3.8 [b]
	Layer	0.39-≥100	0.25-64	1.9	0	6.5	0	1.9
Kanamycin (12.5/64)	Cattle	0.78-≥100	0.50-≥512	6.1	6.4	7.9	3.8	6.2 [a]
	Pig	0.78-≥100	1-≥512	26.8	22.4	15.4	12.4	19.7 [b]
	Broiler	0.78-≥100	1-≥512	28.3	38.5	26.4	19.2	28.5 [c]
	Layer	0.78-≥100	1-≥512	17.9	8.6	12.1	6.6	11.7 [a]
Apramycin (12.5/64)	Cattle	1.56-6.25	1-32	0	0	0	0	0 [a]
	Pig	1.56-≥100	1-≥512	1.3	3.3	2.9	0	2.0 [b]
	Broiler	1.56-6.25	1-32	0	0	0	0	0 [a]
	Layer	1.56-6.25	1-32	0	0	0	0	0 [a]
Oxytetracycline (12.5/16)	Cattle	0.39-≥100	0.5-≥512	37.6	34.3	39.5	36.1	36.8 [a]
	Pig	0.78-≥100	0.25-≥512	71.8	68.4	64.0	70.2	70.2 [b]
	Broiler	0.39-≥100	0.5-≥512	69.7	74.3	68.2	60.6	68.6 [b]
	Layer	1.56-≥100	0.5-≥512	45.1	38.1	46.7	32.8	40.8 [a]
Bicozamycin (100/128)	Cattle	12.5-≥100	8-≥512	0	0	0.6	0.8	0.3 [a]
	Pig	12.5-≥100	16-≥512	3.4	2.0	0	4.1	2.3 [b]
	Broiler	6.25-≥100	8-≥512	4.1	3.4	0	3.0	2.8 [b]
	Layer	12.5-50	16-≥512	0.0	1.4	2.8	0.8	1.1
Chloramphenicol (50/32)	Cattle	3.13-≥100	2-≥512	3.0	0.6	2.8	2.3	2.2 [a]
	Pig	3.13-≥100	2-≥512	24.0	18.4	16.9	25.6	21.1 [b]
	Broiler	3.13-≥100	2-≥512	17.9	17.9	10.9	12.1	15.1 [c]
	Layer	1.56-≥100	2-≥512	6.2	7.9	7.5	2.5	6.0 [a]
Colistin (1.56/16)	Cattle	0.39-12.5	0.25-≥512	0	0.6	0	0.8	0.3
	Pig	0.39-12.5	0.25-64	0	0	0	0.8	0.2
	Broiler	0.39-1.56	0.5-8	0	0	0	0	0
	Layer	0.39-6.25	0.25-4	0	0	0	0	0
Olaquindox (-[c]/-)	Cattle	3.13-50	4-64					
	Pig	6.25-50	8-128					
	Broiler	12.5-50	8-64					
	Layer	6.25-50	4-64					
Nalidixic acid (50/32)	Cattle	0.1-≥100	1-≥512	1.2	1.7	0	0	0.8 [a]
	Pig	0.2-≥100	1-≥512	2.0	2.6	3.7	6.6	3.6 [b]
	Broiler	0.1-≥100	1-≥512	32.0	27.4	30.9	31.3	23.4 [c]
	Layer	≤0.05-≥100	1-≥512	4.3	6.4	7.5	6.6	6.0 [a]
Enrofloxacin (3.13/2)	Cattle	≤0.05-50	≤0.125-0.5	1.2	0	0	0	0.3 [a]
	Pig	≤0.05-50	≤0.125-32	0.7	0	3.7	4.1	2.0 [b]
	Broiler	≤0.05-≥100	≤0.125-32	6.9	2.6	3.6	4.0	4.5 [b]
	Layer	≤0.05-6.25	≤0.125-16	3.1	5.8	0.9	0	2.6 [b]
Trimethoprim (12.5/16)	Cattle	0.1-≥50	≤0.125-≥512	3.0	2.4	7.3	4.5	4.3 [a]
	Pig	0.2-≥50	≤0.125-≥512	28.2	19.1	20.6	31.4	24.6 [b]
	Broiler	0.1-≥50	≤0.125-≥512	43.4	23.9	27.3	16.2	29.1 [b]
	Layer	≤0.05≥50	≤0.125-≥512	11.7	8.6	21.5	11.5	12.8 [c]
Sulfadimethoxine (-/-)	Cattle	≥100	64-≥512					
	Pig	≥100	64-≥512					
	Broiler	≥100	64-≥512					
	Layer	≥100	128-≥512					

a) (mg/L: breakpoint of Japanese Society of Chemotherapy/Clinical Laboratory Standards Institutes)

b) MIC determination according to guidelines of Japanese Society of Chemotherapy in 2000; Clinical Laboratory Standards Institutes in 2001-2003.

c) Not applicable because MICs distribution were showed unimodal in this study.

* Significant diferences between different characters (P<0.01).

Table 5 Antimicrobial susceptibility of *E. faecalis* isolates from animals (2000-2003)

Antimicrobials (Breakpoint)[a]	Animal species	MIC range (mg/L) 2000[b]	MIC range (mg/L) 2001-2003	Resistance (%) 2000[b]	2001	2002	2003	2000-2003
Ampicillin (-[c]/-)	Cattle	0.78-1.56	0.5-1					
	Pig	0.78-1.56	0.25-4					
	Broiler	0.78-1.56	≤0.125-2					
	Layer	0.39-1.56	0.25-4					
Dihydrostreptomycin (200/256)	Cattle	12.5-3200	16->512	30.0	76.5	16.7	0	45.9
	Pig	50->3200	16->512	45.0	54.1	52.6	48.7	50.0 [a*]
	Broiler	25->3200	8->512	51.5	42.6	43.6	45.2	45.2 [a]
	Layer	50->3200	16->512	34.5	25.3	50	29	33.1 [b]
Gentamicin (200/256)	Cattle	1.56->3200	4->512	30.0	11.8	16.7	0	16.2 [a]
	Pig	6.25->3200	2->512	20.0	8.1	13.2	30.8	18.2 [a]
	Broiler	12.5-50	2->512	0.0	3.7	2.6	4.8	3.0 [b]
	Layer	6.25->3200	4->512	1.8	2.5	4.2	5.8	3.6 [b]
Kanamycin (400/512)	Cattle	6.25->3200	16->512	30.0	35.3	16.7	0	27.0
	Pig	25->3200	8->512	42.5	18.9	44.7	41.0	37.0
	Broiler	25->3200	16->512	24.2	25.9	33.3	40.5	31.0
	Layer	25->3200	8->512	21.8	7.6	33.3	37.7	23.9
Oxytetracycline (12.5/16)	Cattle	0.39->100	0.25-256	30.0	47.1	50	100	48.6 [a]
	Pig	0.39->100	0.25-256	75.0	81.1	73.7	69.2	74.7 [b]
	Broiler	3.13->100	≤0.125-256	81.8	68.5	66.7	71.4	71.4 [b]
	Layer	0.78-100	≤0.125-512	61.8	60.8	54.2	59.4	59.0 [a]
Erythromycin (6.25/8)	Cattle	0.2->100	≤0.125->512	30.0	23.5	16.7	25	24.3 [a]
	Pig	≤0.1->100	≤0.125->512	60.0	54.1	34.2	64.1	53.2
	Broiler	0.2->100	≤0.125->512	48.5	74.1	51.3	54.8	58.9 [b]
	Layer	0.2->100	≤0.125-512	34.5	29.1	41.7	43.5	36.7 [a]
Lincomycin (-/128)	Cattle	12.5->100	8-512		35.3	50	25	27.0 [a]
	Pig	12.5->100	1->512		56.8	42.1	64.1	40.3
	Broiler	12.5>100	8->512		72.2	51.3	57.1	49.4 [b]
	Layer	12.5->100	1->512		38.0	41.7	47.8	33.1 [a]
Chloramphenicol (50/64)	Cattle	6.25-50	4-32	30.0	0	0	0	8.1 [a]
	Pig	6.25-100	2-128	12.5	27.0	39.5	25.6	26.0 [b]
	Broiler	6.25-100	2-128	9.1	3.7	2.6	14.3	7.1 [a]
	Layer	6.25-50	1-64	3.6	2.5	6.3	2.9	3.6 [a]
Aviramycin (NT[d]/64)	Cattle	NT	1-2	NT	0	0	0	0.0
	Pig	NT	0.5-256	NT	0	2.6	23.1	8.8 [a]
	Broiler	NT	0.25-128	NT	0	0	2.4	0.7 [b]
	Layer	NT	0.5-128	NT	0	0	1.4	0.5 [b]
Vancomycin (-/32)	Cattle	0.39-0.78	0.5-4		0	0	0	0
	Pig	0.39-3.13	0.5-8		0	0	0	0
	Broiler	0.78-3.13	0.5-8		0	0	0	0
	Layer	0.78-3.13	0.5-8		0	0	0	0
Virginiamycin (NT/-)	Cattle	NT	1-4	NT				
	Pig	NT	0.25-16	NT				
	Broiler	NT	1-32	NT				
	Layer	NT	0.25-16	NT				
Enrofloxacin (25/16)	Cattle	0.78	0.5-4	0.0	0	0	0	0.0
	Pig	0.39-1.56	0.25-32	0.0	2.7	0	0	0.6
	Broiler	0.78-50	0.25-64	6.1	1.9	5.1	7.1	4.8
	Layer	0.39-6.25	0.25-32	0.0	0	0	2.9	0.8

a), b), c), * See the footnotes in Table 2.
*d) Not tested

Table 6 Antimicrobial susceptibility of *E. faecium* isolates from animals (2000-2003)

Antimicrobials (Breakpoint)[a)]	Animal species	MIC range (mg/L)		Resistance (%)					
		2000[b)]	2001-2003	2000[b)]	2001	2002	2003	2000-2003	
Ampicillin (50/-[c)])	Cattle	0.39-3.13	≤0.125-64	0					
	Pig	0.39-12.5	≤0.125-8	0					
	Broiler	0.2-50	≤0.125-32	3.6					
	Layer	≤0.1-6.25	≤0.125-4	0					
Dihydrostreptomycin (200/256)	Cattle	6.25-3200	8->512	7.1	19.2	9.5	5.9	10.4	a*
	Pig	≤0.1->3200	8->512	39.0	29	47.6	35.3	37.5	b
	Broiler	12.5->3200	8->512	36.4	26.9	47.4	23.7	33.1	b
	Layer	12.5-3200	8->512	7.5	25	17.1	15.4	15.1	a
Gentamicin (200/256)	Cattle	1.56-25	2-16	0	0	0	0	0.0	
	Pig	0.78-25	2-8	0	0	0	0	0.0	
	Broiler	1.56-25	2-512	0	0	2.6	0	0.6	
	Layer	0.78->3200	1->512	1.9	0	0	5.1	1.9	
Kanamycin (3200/-)	Cattle	12.5-200	1->512	0					
	Pig	12.5->3200	8->512	16.9					
	Broiler	12.5->3200	8->512	25.5					
	Layer	6.25->3200	16->512	3.8					
Oxytetracycline (12.5/16)	Cattle	0.39->100	≤0.125-128	23.8	53.8	33.3	23.5	33.0	a
	Pig	0.39->100	≤0.125-256	59.3	51.6	66.7	47.1	57.0	b
	Broiler	0.39->100	≤0.125-256	85.5	53.8	84.2	54.2	70.2	b
	Layer	0.39->100	≤0.125-256	35.8	37.5	25.7	23.1	30.8	a
Erythromycin (100/128)	Cattle	≤0.1->100	≤0.125->512	2.4	15.4	4.8	0	5.7	a
	Pig	≤0.1->100	≤0.125->512	23.7	25.8	42.9	29.4	28.1	b
	Broiler	0.2->100	≤0.125->512	34.5	15.4	28.9	16.9	24.7	b
	Layer	0.2->100	≤0.125->512	3.8	18.8	11.4	12.8	10.7	a
Lincomycin (-/128)	Cattle	0.39->100	≤0.125->512		7.7	38.1	5.9	17.2	a
	Pig	0.39->100	≤0.125->512		35.5	38.1	29.4	34.8	b
	Broiler	0.39->100	0.25->512		7.7	44.7	18.6	24.4	
	Layer	0.39->100	≤0.125->512		25	14.3	15.4	17.9	a
Chloramphenicol (-/-)	Cattle	3.13-50	2-32						
	Pig	3.13-50	1-32						
	Broiler	6.25-25	2-32						
	Layer	3.13-25	1-32						
Aviramycin (NT[d)]/64)	Cattle	NT*	1-4	NT	0	0	0	0	a
	Pig	NT	1-256	NT	0	9.5	0	2.9	a
	Broiler	NT	≤0.125-256	NT	3.8	23.7	13.6	14.6	b
	Layer	NT	0.5-128	NT	6.3	0	5.1	3.8	
Vancomycin (-/32)	Cattle	0.39-12.5	0.5-8		0	0	0	0	
	Pig	0.39-6.25	0.5-8		0	0	0	0	
	Broiler	0.39-12.5	0.5-8		0	0	0	0	
	Layer	0.39-12.5	0.25-8		0	0	0	0	
Virginiamycin (NT/-)	Cattle	NT	≤0.125-4	NT					
	Pig	NT	0.25-8	NT					
	Broiler	NT	0.25-32	NT					
	Layer	NT	≤0.125-32	NT					
Enrofloxacin (-/-)	Cattle	0.39-6.25	0.25-16						
	Pig	0.2-6.25	0.25-16						
	Broiler	0.78-12.5	0.5-32						
	Layer	0.39-6.25	0.5-8						

a), b), c), * See the footnotes in Table 2.

d) Not tested

Table 7 Isolation rate of *Campylobacter* from fecal samples

year	Origin											
	Cattle			Pigs			Layers			Broilers		
1999	26	/ 183	(14.2)	43	/ 180	(23.9)				43	/ 156	(27.6)
2000	38	/ 155	(24.5)	65	/ 148	(43.9)	48	/ 160	(30.0)	30	/ 116	(25.9)
2001	25	/ 90	(27.8)	45	/ 91	(49.5)	44	/ 91	(48.4)	25	/ 88	(28.4)
2002	16	/ 96	(16.7)	22	/ 91	(24.2)	36	/ 89	(40.4)	20	/ 66	(30.3)
2003	22	/ 111	(19.8)	48	/ 97	(49.5)	41	/ 88	(46.6)	29	/ 69	(42.0)
total	127	/ 635	(20.0)	223	/ 607	(36.7)	169	/ 428	(39.5)	147	/ 495	(29.7)

Table 8 Trends in antmicrobial resistance among *Campylpbacter* spp.

Antimicrobial agents	origin	species	Year 1999	2000	2001	2002	2003
Ampicillin	Cattle	*C. jejuni*	NT	NT	NT	0	0
		C. coli	NT	NT	NT	0	0
	Pigs	*C. jejuni*	NT	NT	NT	0	-
		C. coli	NT	NT	NT	2.9	0
	Layers	*C. jejuni*	NT	NT	NT	26.9	39.6
		C. coli	NT	NT	NT	25	13.6
	Broilers	*C. jejuni*	NT	NT	NT	20.7	20
		C. coli	NT	NT	NT	0	0
Dihydrostreptomycin	Cattle	*C. jejuni*	5.9	14	7.1	0	0
		C. coli	-	100	20	0	5.9
	Pigs	*C. jejuni*	0	0		50	-
		C. coli	76.6	64.3	58.8	57.1	55.8
	Layers	*C. jejuni*	-	0	0	1.9	10.4
		C. coli	-	0	0	8.3	4.5
	Broilers	*C. jejuni*	2.8	0	9.5	6.9	0
		C. coli	0	0	0	40	0
Erythromycin	Cattle	*C. jejuni*	0	0	0	0	0
		C. coli	-	100	20	0	0
	Pigs	*C. jejuni*	0	0		0	-
		C. coli	55.3	44.9	47.1	57.1	47.7
	Layers	*C. jejuni*	-	0	0	0	0
		C. coli	-	40	0	0	0
	Broilers	*C. jejuni*	0	0	0	0	0
		C. coli	0	100	0	40	13.3
Oxytetracycline	Cattle	*C. jejuni*	41.2	60.5	35.7	42.3	32.4
		C. coli	0	100	20	100	100
	Pigs	*C. jejuni*	0	100		100	-
		C. coli	91.5	88.8	89.7	85.7	86
	Layers	*C. jejuni*	-	29.9	36.7	28.8	50
		C. coli	-	60	0	50	54.5
	Broilers	*C. jejuni*	55.6	43.4	52.4	51.7	37.5
		C. coli	33.3	100	50	80	46.7
Chloramphenicol	Cattle	*C. jejuni*	*	*	7.1	0	5.9
		C. coli	*	*	0	0	0
	Pigs	*C. jejuni*	*	*		0	-
		C. coli	*	*	41.2	37.1	22.1
	Layers	*C. jejuni*	*	*	0	7.7	0
		C. coli	*	*	18.2	0	9.1
	Broilers	*C. jejuni*	*	*	9.5	0	0
		C. coli	*	*	50	0	0
Nalidixic acid	Cattle	*C. jejuni*	8.8	16.3	25	19.2	17.6
		C. coli	-	33.3	80	0	50
	Pigs	*C. jejuni*	33.3	0		100	-
		C. coli	21.3	24.5	23.5	28.6	34.9
	Layers	*C. jejuni*	-	2.6	3.3	3.8	4.2
		C. coli	-	40	0	33.3	22.7
	Broilers	*C. jejuni*	16.7	7.5	40.5	17.2	20
		C. coli	0	100	0	40	53.3
Enrofloxacin	Cattle	*C. jejuni*	8.8	16.3	25	15.4	17.6
		C. coli	-	33.3	80	0	50
	Pigs	*C. jejuni*	33.3	0		100	-
		C. coli	21.3	24.5	23.5	25.7	34.9
	Layers	*C. jejuni*	-	2.6	3.3	3.8	4.2
		C. coli	-	40	0	33.3	22.7
	Broilers	*C. jejuni*	16.7	5.7	40.5	17.2	17.5
		C. coli	0	100	0	40	53.3

NT, non tested; -, no isolate; *, the breakpoints were not difined.

Table 9 *Salmonella* serovars isolated from food-producing animals between 2000 and 2003

Serovars	Cattle	Pigs	Broiler	Layer	Total
Infantis		2	65	2	69
Typhimurium	19	17			36
Agona		4	4	2	10
Thompson			2	4	6
Enteritidis			3	2	5
Virchow			4	1	5
Dublin	4				4
Brandenburg		3			3
Hader			3		3
Anatum		4			2
Bareilly				2	2
Blockley			2		2
Corvallis				2	2
Derby		2			2
Haifa			2		2
Havana				2	2
Istanbul			2		2
Mbandaka	2				2
Minesota		2			2
Mons				2	2
Montevideo				2	2
Newport			2		2
Othmarschen				2	2
Tennessee				2	2
Albany				1	1
Isangi				1	1
Pakistan		1			1
Zanzibar		1			1
Untypable		3	2	1	3
Total	25	39	91	28	183

Table 10 Antimicrobial susceptibility of *Salmonella* isolates (n = 183) from food-producing animals

			No. resistance (%)				No. resistance (%)				
Antimicrobial agents	Break point (mg/ml)	MIC range (mg/ml)	2000 (n = 91)	2001[b)] (n = 22)	2002 (n = 50)	2003 (n = 20)	Cattle (n = 25)	Pig (n = 39)	Broiler (n = 91)	Layer (n = 28)	Total
Ampicillin	32	0.5-512<	27 (29.7)	4 (18.2)	6 (12.0)	0 (0)	18 (72.0)	15 (38.5)	4 (4.4)	0 (0)	37 (20.2)
Cefazolin	32	1-16	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Cefuroxime		2-16									
Ceftiofur		0.5-2									
Apramycin		0.5-8									
Destomycin A		8-64									
Dihydrostreptomycin	32	8-512<	68 (74.7)	17 (77.3)	43 (86.0)	14 (70.0)	22 (88.0)	32 (82.1)	79 (86.8)	9 (32.1)	142 (77.6)
Gentamicin	8	≦0.125-1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Kanamycin	64	0.5-512<	31 (34.1)	6 (27.3)	8 (40.0)	12 (40.0)	6 (24.0)	5 (12.8)	54 (59.3)	0 (0)	65 (35.5)
Oxytetracycline	16	1-512	57 (62.6)	15 (68.2)	38 (76.0)	14 (70.0)	18 (72.0)	26 (66.7)	76 (83.5)	4 (14.3)	124 (67.8)
Bicozamycin	128	16-512<	2 (2.2)	2 (9.1)	3 (6.0)	0 (0)	0 (0)	2 (5.1)	1 (1.1)	4 (14.3)	7 (3.8)
Chloramphenicol	32	1-512<	22 (24.2)	4 (18.2)	6 (12.0)	0 (0)	17 (68.0)	13 (33.3)	2 (2.2)	0 (0)	32 (17.5)
Colistin	16	0.5-64	1 (1.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.6)	1 (0.5)
Nalidixic acid	32	2-512	7 (7.7)	2 (9.1)	4 (8.0)	4 (20.0)	4 (16.0)	0 (0)	13 (14.3)	0 (0)	17 (9.3)
Oxolinic acid	2	≦0.125-16	7 (7.7)	2 (9.1)	4 (8.0)	4 (20.0)	4 (16.0)	0 (0)	13 (14.3)	0 (0)	17 (9.3)
Enrofloxacin	2	≦0.125-1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Ofloxacin	2	≦0.125-2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0(0)
Olaquindox		8-128									
Trimethoprim	16	512<	19 (20.9)	4 (18.2)	17 (34.0)	6 (30.0)	1 (4.0)	4 (10.3)	40 (44.0)	1 (3.6)	46 (25.1)
Sulphadimethoxine		256-512<									

Table 11 Antimicrobial susceptibility of *E. coli* isolates from animals (2004-2007)

Antimicrobials (Breakpoint: mg/L)		Isolation years 2004	2005	2006	2007
Ampicillin	MIC range (mg/L)	0.5->512	1->512	0.5->512	1->512
(32)	Resistant No.	125	141	117	96
	%	24.5	27.2	23.4	21.3
	Cattle (%)	9.7	12.3	8.1	9.2
	Pigs (%)	22.1	27.0	24.6	22.6
	Layer (%)	17.8	21.5	23.3	15.2
	Broiler (%)	42.1	53.3	43.8	42.2
Cefazolin	MIC range (mg/L)	1->512	≤0.125->512	0.25->512	1->512
(32)	Resistant No.	21	26	16	24
	%	3.8	5.0	3.2	5.3
	Cattle (%)	0	2.9	0	2.3
	Pigs (%)	1.5	0.0	0	0
	Layer (%)	2.2	3.3	4.2	1.8
	Broiler (%)	10.5	16.8	10.5	18.6
Ceftiofur	MIC range (mg/L)	≦0.125-512	≤0.125->512	≦0.125->512	≦0.125->512
(8)	Resistant No.	19	21	14	25
	%	3.7	4.1	2.8	5.6
	Cattle (%)	0	0.7	0	1.5
	Pigs (%)	1.5	0.0	0	0.9
	Layer (%)	2.5	4.1	3.3	4.5
	Broiler (%)	10.5	14.0	9.5	16.7
Dihydrostreptomycin	MIC range (mg/L)	0.25->512	1->512	1->512	1->512
(32)	Resistant No.	160	180	158	141
	%	32.3	34.7	31.6	31.3
	Cattle (%)	21	20.3	16.1	19.2
	Pigs (%)	47.1	46.1	46	43.4
	Layer (%)	22	27.3	27.5	23.2
	Broiler (%)	33.1	45.8	41	43.1
Gentamicin	MIC range (mg/L)	≦0.125-128	0.25-128	0.5-128	0.25-64
(16)	Resistant No.	11	9	10	3
	%	2.2	1.7	2	0.7
	Cattle (%)	0	0.7	0.7	0
	Pigs (%)	3.7	2.0	4	1.9
	Layer (%)	0	1.7	0	0
	Broiler (%)	4.5	2.8	3.8	1
Kanamycin	MIC range (mg/L)	0.25->512	0.5->512	1->512	1->512
(64)	Resistant No.	58	65	68	26
	%	11.4	12.5	13.6	5.8
	Cattle (%)	4	2.2	2.7	1.5
	Pigs (%)	14	17.8	16.7	7.5
	Layer (%)	10.2	7.4	15.8	1.8
	Broiler (%)	16.5	24.3	22.9	13.7
Apramycin	MIC range (mg/L)	1-32	1-32	2-64	2-32
(ND)	Resistant No.	-	-	-	-
	%	-	-	-	-
	Cattle (%)	-	-	-	-
	Pigs (%)	-	-	-	-
	Layer (%)	-	-	-	-
	Broiler (%)	-	-	-	-
Oxytetracycline	MIC range (mg/L)	0.25-512	0.5->512	0.5->512	0.5-512
(16)	Resistant No.	232	264	233	190
	%	45.4	51.0	46.6	42.2
	Cattle (%)	26.6	23.2	28.2	26.2
	Pigs (%)	63.2	69.1	60.3	57.5
	Layer (%)	32.2	46.3	45	35.7
	Broiler (%)	54.1	66.4	58.1	53.9
Bicozamycin	MIC range (mg/L)	8->512	8->512	8->512	8->512
(128)	Resistant No.	5	6	9	5
	%	1.0	1.2	1.8	1.1
	Cattle (%)	0	0.7	1.3	1.5
	Pigs (%)	0.7	2.0	4.8	2.8
	Layer (%)	2.5	0.8	0.8	0
	Broiler (%)	0.7	0.9	0	0
Chloramphenicol	MIC range (mg/L)	2->512	2-512	1->512	1-512
(32)	Resistant No.	60	72	33	37
	%	11.7	13.9	6.6	8.2
	Cattle (%)	4	7.2	2	3.8
	Pigs (%)	21.3	24.3	13.5	17
	Layer (%)	11	8.3	5.8	3.6
	Broiler (%)	9.8	14.0	5.7	9.8
Colistin	MIC range (mg/L)	0.25-128	0.25-8	0.25->128	0.25-8
(16)	Resistant No.	1	0	1	0
	%	0.2	0.0	0.2	0
	Cattle (%)	0	0.0	0.7	0
	Pigs (%)	0	0.0	0	0
	Layer (%)	0.8	0.0	0	0
	Broiler (%)	0	0.0	0	0
Nalidixic acid	MIC range (mg/L)	1->512	1->512	1->512	1->512
(32)	Resistant No.	61	69	60	53
	%	11.9	13.3	12	11.8
	Cattle (%)	0	4.3	2	5.4
	Pigs (%)	8.8	4.6	4.8	6.6
	Layer (%)	10.2	22.3	15.8	8.9
	Broiler (%)	27.1	27.1	30.5	28.4
Enrofloxacin	MIC range (mg/L)	≦0.125->64	≤0.125-≥32	≦0.125->64	≦0.125->32
(2)	Resistant No.	12	19	14	11
	%	2.3	3.7	2.8	2.4
	Cattle (%)	0	1.4	0	1.5
	Pigs (%)	2.9	1.3	0.8	0
	Layer (%)	0.8	7.4	3.3	2.7
	Broiler (%)	5.3	5.6	8.6	5.9
Sulfadimethoxine	MIC range (mg/L)	64->512	32->512	64->512	32->512
(ND)	Resistant No.	-	-	-	-
	%	-	-	-	-
	Cattle (%)	-	-	-	-
	Pigs (%)	-	-	-	-
	Layer (%)	-	-	-	-
	Broiler (%)	-	-	-	-
Trimethoprim	MIC range (mg/L)	≦0.125->512	≤0.125->512	≦0.125->512	≦0.125->512
(16)	Resistant No.	90	108	77	71
	%	17.6	20.8	15.4	15.8
	Cattle (%)	1.6	4.3	2.7	3.1
	Pigs (%)	30.1	27.0	27	30.2
	Layer (%)	14.4	18.2	10.8	12.5
	Broiler (%)	22.6	36.4	24.8	20.6

Table 12 Antimicrobial susceptibility of *E. faecalis* isolates from animals (2004-2007)

Antimicrobials (Breakpoint: mg/L)		Isolation years 2004	2005	2006	2007
Ampicillin	MIC range (mg/L)	≦0.125-1	0.25-8	≦0.125-4	0.25-4
(16)	Resistant No.	0	0	0	0
	%	0.0	0.0	0.0	0.0
	Cattle (%)	0.0	0.0	0.0	0.0
	Pigs (%)	0.0	0.0	0.0	0.0
	Layer (%)	0.0	0.0	0.0	0.0
	Broiler (%)	0.0	0.0	0.0	0.0
Dihydrostreptomycin	MIC range (mg/L)	16->512	32->512	4->512	8->512
(128)	Resistant No.	72	56	42	51
	%	49	37.1	29.4	38.1
	Cattle (%)	57.1	57.1	8.3	33.3
	Pigs (%)	55.6	54.5	37.0	47.1
	Layer (%)	42.6	24.1	21.9	44.2
	Broiler (%)	50.0	50.0	42.5	30.5
Gentamicin	MIC range (mg/L)	2->512	4-256	1->512	2->512
(32)	Resistant No.	17	10	36	22
	%	11.6	6.6	25.2	16.4
	Cattle (%)	28.6	14.3	0.0	33.3
	Pigs (%)	13.9	9.1	22.2	29.4
	Layer (%)	9.3	10.1	28.1	23.1
	Broiler (%)	10.0	0.0	30.0	5.1
Kanamycin	MIC range (mg/L)	16->512	8->512	4->512	16->512
(128)	Resistant No.	59	37	50	40
	%	40.1	24.5	35.0	29.9
	Cattle (%)	14.3	57.1	0.0	0.0
	Pigs (%)	38.9	18.2	29.6	23.5
	Layer (%)	38.9	8.9	31.3	30.8
	Broiler (%)	46.0	44.4	55.0	33.9
Oxytetracycline	MIC range (mg/L)	≦0.125->512	0.25-256	0.25->512	≦0.125->512
(16)	Resistant No.	100	95	94	90
	%	68.0	62.9	65.7	67.2
	Cattle (%)	28.6	71.4	25.0	16.7
	Pigs (%)	61.1	81.8	81.5	82.4
	Layer (%)	70.4	50.6	64.1	61.5
	Broiler (%)	76.0	75.9	70.0	72.9
Erythromycin	MIC range (mg/L)	≦0.125->512	≦0.125->512	≦0.125->512	≦0.125->512
(64)	Resistant No.	62	63	43	63
	%	42.2	41.7	30.1	47.0
	Cattle (%)	0.0	14.3	0.0	0.0
	Pigs (%)	47.2	63.6	33.3	82.4
	Layer (%)	35.2	26.6	31.3	40.4
	Broiler (%)	52.0	63.0	35.0	47.5
Lincomycin	MIC range (mg/L)	0.25->512	1->512	0.25->512	0.25->512
(128)	Resistant No.	59	66	42	60
	%	40.1	43.7	29.4	44.8
	Cattle (%)	0.0	14.3	0.0	0.0
	Pigs (%)	50.0	63.6	37.0	76.5
	Layer (%)	27.8	27.8	29.7	38.5
	Broiler (%)	52.0	66.7	32.5	45.8
Chloramphenicol	MIC range (mg/L)	2-128	4-256	2-128	2-128
(32)	Resistant No.	15	17	13	14
	%	10.2	11.3	9.1	10.4
	Cattle (%)	0.0	42.9	0.0	0.0
	Pigs (%)	19.4	36.4	22.2	52.9
	Layer (%)	3.7	3.8	3.1	1.9
	Broiler (%)	12.0	13.0	12.5	6.8
Aviramycin	MIC range (mg/L)	1->128	0.5->128	0.5->128	0.25->128
(16)	Resistant No.	8	7	11	10
	%	5.4	4.6	7.7	7.5
	Cattle (%)	0.0	0.0	0.0	0.0
	Pigs (%)	11.1	36.4	25.9	47.1
	Layer (%)	3.7	1.3	0.0	0.0
	Broiler (%)	4.0	3.7	10.0	3.4
Vancomycin	MIC range (mg/L)	0.25-4	0.5-4	0.5-32	≦0.125-8
(32)	Resistant No.	0	0	2	0
	%	0.0	0.0	1.4	0.0
	Cattle (%)	0.0	0.0	0.0	0.0
	Pigs (%)	0.0	0.0	0.0	0.0
	Layer (%)	0.0	0.0	3.1	0.0
	Broiler (%)	0.0	0.0	0.0	0.0
Virginiamycin	MIC range (mg/L)	≦0.125-32	0.25-8	≦0.125-64	≦0.125-16
(-)	Resistant No.				
	%				
	Cattle (%)				
	Pigs (%)				
	Layer (%)				
	Broiler (%)				
Enrofloxacin	MIC range (mg/L)	0.25-32	0.25-32	≦0.125-4	0.25-8
(4)	Resistant No.	6	6	2	8
	%	4.1	4.0	1.4	6.0
	Cattle (%)	0.0	0.0	0.0	0.0
	Pigs (%)	0.0	0.0	0.0	0.0
	Layer (%)	3.7	3.8	0.0	1.9
	Broiler (%)	8.0	5.6	5.0	11.9

Table 13 Antimicrobial susceptibility of *E. faecium* isolates from animals (2004-2007)

Antimicrobials (Breakpoint: mg/L)		Isolation years 2004	2005	2006	2007
Ampicillin	MIC range (mg/L)	≦0.125-4	≦0.125-64	≦0.125-4	≦0.125-64
(16)	Resistant No.	0	1	0	2
	%	0.0	0.7	0.0	2.5
	Cattle (%)	0.0	0.0	0.0	0.0
	Pigs (%)	0.0	0.0	0.0	0.0
	Layer (%)	0.0	0.0	0.0	0.0
	Broiler (%)	0.0	2.9	0.0	10.5
Dihydrostreptomycin	MIC range (mg/L)	16->512	8->512	8->512	4->512
(128)	Resistant No.	18	27	13	5
	%	25.4	20.0	14.6	6.2
	Cattle (%)	9.1	0.0	4.3	0.0
	Pigs (%)	33.3	22.0	28.6	5.3
	Layer (%)	10.5	18.8	5.3	0.0
	Broiler (%)	40.0	35.3	19.2	21.1
Gentamicin	MIC range (mg/L)	1->512	0.5-32	2-64	1-32
(32)	Resistant No.	5	1	3	1
	%	7.0	0.7	3.4	1.2
	Cattle (%)	0.0	0.0	0.0	7.7
	Pigs (%)	19.0	2.4	4.8	0.0
	Layer (%)	5.3	0.0	10.5	0.0
	Broiler (%)	0.0	0.0	0.0	0.0
Kanamycin	MIC range (mg/L)	8->512	8->512	8->512	8->512
(128)	Resistant No.	30	42	35	19
	%	42.3	31.1	39.3	23.5
	Cattle (%)	0.0	7.1	8.7	0.0
	Pigs (%)	66.7	41.5	38.1	21.1
	Layer (%)	21.1	43.8	63.2	13.3
	Broiler (%)	60.0	26.5	50.0	57.9
Oxytetracycline	MIC range (mg/L)	0.25->512	≦0.125-256	≦0.125->512	≦0.125->512
(16)	Resistant No.	41	68	36	36
	%	57.7	50.4	40.4	44.4
	Cattle (%)	36.4	35.7	21.7	7.7
	Pigs (%)	52.4	58.5	52.4	78.9
	Layer (%)	47.4	37.5	31.6	30.0
	Broiler (%)	85.0	64.7	53.8	57.9
Erythromycin	MIC range (mg/L)	≦0.125->512	≦0.125->512	≦0.125->512	≦0.125->512
(64)	Resistant No.	26	29	11	9
	%	36.6	21.5	12.4	11.1
	Cattle (%)	18.2	7.1	4.3	0.0
	Pigs (%)	38.1	22.0	23.8	15.8
	Layer (%)	36.8	18.8	5.3	10.0
	Broiler (%)	45.0	35.3	15.4	15.8
Lincomycin	MIC range (mg/L)	≦0.125->512	0.25->512	0.25->512	≦0.125->512
(128)	Resistant No.	23	32	14	6
	%	32.4	23.7	15.7	7.4
	Cattle (%)	18.2	10.7	4.3	0.0
	Pigs (%)	38.1	24.4	33.3	15.8
	Layer (%)	21.1	18.8	5.3	3.3
	Broiler (%)	45.0	38.2	19.2	10.5
Chloramphenicol	MIC range (mg/L)	4-128	2-128	1-128	2-32
(32)	Resistant No.	5	5	5	3
	%	7.0	3.7	5.6	3.7
	Cattle (%)	0.0	0.0	0.0	0.0
	Pigs (%)	0.0	4.9	14.3	15.8
	Layer (%)	10.5	6.3	10.5	0.0
	Broiler (%)	15.0	2.9	0.0	0.0
Aviramycin	MIC range (mg/L)	0.5->128	0.25->128	0.5->128	≦0.125->128
(16)	Resistant No.	4	15	5	8
	%	5.6	11.1	5.6	9.9
	Cattle (%)	0.0	0.0	0.0	0.0
	Pigs (%)	4.8	9.8	9.5	31.6
	Layer (%)	5.3	9.4	0.0	0.0
	Broiler (%)	10.0	23.5	11.5	10.5
Vancomycin	MIC range (mg/L)	0.25-4	0.5->128	0.25-16	≦0.125-8
(32)	Resistant No.	0	1	0	0
	%	0.0	0.7	0.0	0.0
	Cattle (%)	0.0	3.6	0.0	0.0
	Pigs (%)	0.0	0.0	0.0	0.0
	Layer (%)	0.0	0.0	0.0	0.0
	Broiler (%)	0.0	0.0	0.0	0.0
Virginiamycin	MIC range (mg/L)	≦0.125-16	0.25-32	0.25-64	≦0.125-16
(-)	Resistant No.				
	%				
	Cattle (%)				
	Pigs (%)				
	Layer (%)				
	Broiler (%)				
Enrofloxacin	MIC range (mg/L)	0.25-16	0.5-16	≦0.125-32	0.25-8
(4)	Resistant No.	16	32	18	19
	%	22.5	23.7	20.2	23.5
	Cattle (%)	18.2	7.1	4.3	7.7
	Pigs (%)	23.8	9.8	9.5	15.8
	Layer (%)	10.5	18.8	31.6	23.3
	Broiler (%)	35.0	58.8	34.6	42.1

Table 14 Antimicrobial susceptibility of *Campylobacter* isolates from food-producing animals between 2004 and 2007 (2nd stage)

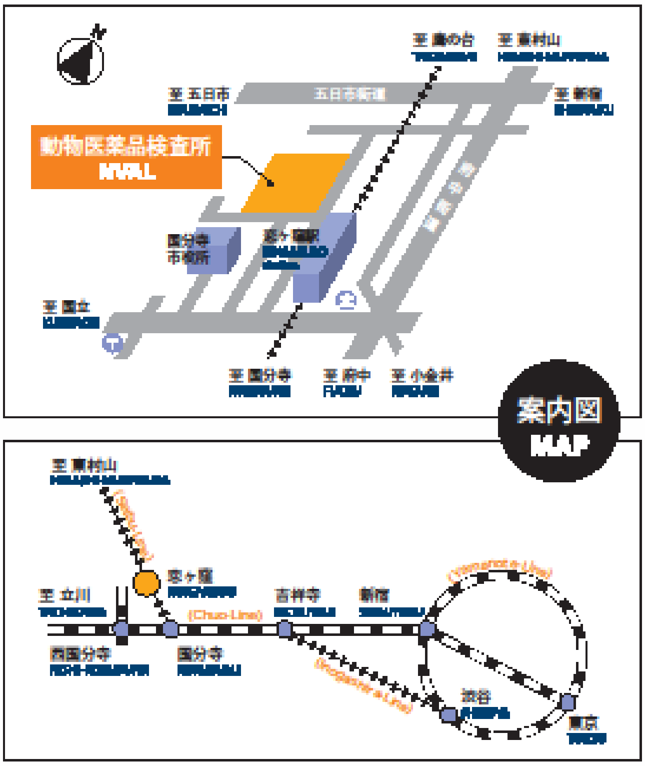
		2004				2005				2006				2007			
agent	species	MIC 50 (mg/L)	MIC 90 (mg/L)	No. resistant	rate (%)	MIC 50 (mg/L)	MIC 90 (mg/L)	No. resistant	rate (%)	MIC 50 (mg/L)	MIC 90 (mg/L)	No. resistant	rate (%)	MIC 50 (mg/L)	MIC 90 (mg/L)	No. resistant	rate (%)
Ampicillin	*C. jejuni*	4	32	16	12.1	4	32	15	16.7	4	32	6	15.0	8	32	21	15.9
	C. coli	4	8	7	8.4	4	8	4	5.9	4	16	0	0.0	4	8	1	1.1
Dihydrostreptomycin	*C. jejuni*	0.5	1	5	3.8	0.5	1	4	4.4	1	2	4	10.0	0.5	1	6	4.5
	C. coli	16	>512	39	47	2	256	30	44.1	512	>512	26	60.5	16	>512	43	47.3
Gentamicin	*C. jejuni*	0.25	1			0.5	0.5			0.25	4			0.5	0.5		
	C. coli	0.5	2			0.5	1			0.5	4			0.5	1		
Erythromycin	*C. jejuni*	1	4	0	0	2	2	0	0	1	4	0	0.0	1	4	0	0.0
	C. coli	256	>512	44	53	4	256	24	35.3	4	>512	15	34.9	1	256	30	33.0
Oxytetracycline	*C. jejuni*	4	128	54	40.9	4	128	41	45.6	32	128	23	57.5	2	128	59	44.7
	C. coli	64	256	69	83.1	64	256	52	76.5	32	256	27	62.8	64	256	63	69.2
Chloramphenicol	*C. jejuni*	1	8	7	5.3	2	4	1	1.1	2	8	2	5.0	4	8	4	3.0
	C. coli	4	32	20	24.1	4	16	7	10.3	4	32	12	27.9	4	32	31	34.1
Nalidixic acid	*C. jejuni*	4	128	20	15.2	4	256	19	21.1	4	256	15	37.5	8	256	34	25.8
	C. coli	8	128	22	26.5	8	128	18	26.5	8	256	14	32.6	64	256	51	56.0
Enrofloxacin	*C. jejuni*	<0.125	2	16	12.1	<0.125	4	16	17.8	<0.125	8	15	37.5	<0.125	8	33	25.0
	C. coli	<0.125	4	21	25.3	<0.125	8	17	25	<0.125	4	13	30.2	2	8	49	53.8
Sulufadimetoxin	*C. jejuni*	256	>512			8	512			64	>512			256	>512		
	C. coli	>512	>512			512	>512			256	>512			>512	>512		

Table 15 *Salmonella* serovars isolated from food-producing animals between 2004 and 2007

	Pig					Broiler chicken					Layer chicken					
Serovar	2004	2005	2006	2007	subtotal	2004	2005	2006	2007	subtotal	2004	2005	2006	2007	subtota	Total
S.Infantis						14	19	32	16	81		2		5	7	88
S.Schwarzengrund							8	12	7	27			2		2	29
S.Typhimurium	4	2	2	5	13											13
S.Enteritidis			1		1						2		2		4	5
Hader						1	2			3	2				2	5
S.Mbandaka								1		1	2		2		4	5
S.Cerro											2		2		4	4
S.Montevideo						2		2		4						4
Thompson	2				2						1				1	3
S.Agona			2		2				2	2						4
S.Choleraesuis		2			2											2
S.Kottbus		2			2											2
S.Litchfield			2		2											2
S.Livingstone			2	2	4											4
S.Newport							2			2						2
S.Manhattan									2	2						2
Bareily											1				1	1
Untypable	2				2							2			2	4
Total	8	6	9	7	30	17	31	47	27	95	10	4	8	5	22	179

Table 16 Antimicrobial susceptibility of *Salmonella* isolates (n = 179) from food-producing animals between 2004 and 2007 (2nd stage)

	BP (mg/l)	2004 (n = 35) MIC50 (mg/l)	MIC90 (mg/l)	No. resistant	rate (%)	2005 (n = 41) MIC50 (mg/l)	MIC90 (mg/l)	No. resistant	rate (%)	2006 (n = 64) MIC50 (mg/l)	MIC90 (mg/l)	No. resistant	rate (%)	2007 (n = 39) MIC50 (mg/l)	MIC90 (mg/l)	No. resistant	rate (%)	Total No. resistant	rate (%)
Ampicillin	32	1	4	3	8.6	1	1	2	4.9	1	512	8	12.5	1	1	0	0	13	7.3
Cefazolin	32	1	2	0	0	1	4	0	0	1	2	2	3.1	1	1	0	0	2	1.1
Dihydrostreptomycin	32	64	128	21	60	32	128	24	58.5	64	256	53	82.8	128	>512	28	71.8	126	70.4
Kanamycin	64	2	>512	9	25.7	>512	>512	21	51.2	2	>512	25	39.1	2	>512	8	20.5	63	35.2
Gentamicin	16	0.5	1	0	0	0.5	1	0	0	0.5	1	0	0	0.5	1	0	0	0	0.0
Oxytetracycline	16	64	256	19	54.3	128	256	23	56.1	128	256	50	78.1	128	512	25	64.1	117	65.4
Colistin	16	1	2	0	0	1	4	0	0	1	4	0	0	0.5	4	0	0	0	0.0
Chloramphenicol	32	4	16	2	5.7	4	8	2	4.9	8	8	0	0	4	8	1	2.6	5	2.8
Nalidixic acid	32	4	8	3	8.6	4	8	4	9.8	4	8	5	7.8	4	16	3	7.7	15	8.4
Enrofloxacin	2	≦0.125	≦0.125	0	0	≦0.125	≦0.125	0	0	≦0.125	≦0.125	0	0	≦0.125	≦0.125	0	0	0	0.0
Trimethoprim	16	1	512	14	40	64	>512	26	63.4	0.25	>512	25	39.1	0.5	512	12	30.8	77	43.0



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