Antibiotic-resistant Bacteria from Feces of Livestock, Farmyard Manure, and Farmland in Japan—case report—

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We examined the distribution of antibiotic-resistant bacteria by analyzing 19 samples of feces of livestock, solid farmyard manure (FYM), soil from arable fields and orchards treated with FYM or not, and forest soils. Bacteria resistant to ampicillin, vancomycin, kanamycin, chloramphenicol, rifampicin, and tetracycline were enumerated by the dilution plate method at a rate of 50 mg l−1. The total numbers of antibiotic-resistant bacteria in swine or poultry feces were the same as or up to eight orders of magnitude lower than the total numbers of cultivable bacteria. The occurrence of antibiotic-resistant bacteria in the poultry feces increased with the use of antibiotics as feed additives. The occurrence of antibiotic-resistant bacteria was much lower in FYM samples than fecal samples with some exceptions. Of the soil samples, only that to which solid swine FYM at 40 t ha−1 had been applied yearly for more than 10 years had significantly higher numbers of total and antibiotic-resistant bacteria than the upland soil that had not been treated with FYM. Resistant isolates from fecal samples showed a broad range of multi-drug resistance (MDR), but those from forest soil had a narrow-range MDR, suggesting that the use of antibiotics as feed additives influences the occurrence of broad-range MDR.

Key words: antibiotic-resistant bacteria, soil, feces of livestock, composting, multi-drug resistance

Many antibiotics have been used as human and veterinary medicines and feed additives. In recent years the prevalence of antibiotic-resistant bacteria, not only in clinics but in animal farms and fish farms, has become a serious problem2–8,10,14,15,17,19. Recent studies have suggested that there may be a link between the use of in-feed antibiotics and the prevalence of antibiotic-resistant bacteria in human infections17–20. The fecal waste from thousands of animals reared under intensive conditions often is spread as fertilizer on pastures, sometimes after composting11. Alternatively, swine farms typically construct lagoons to hold such wastes, and they are implicated in the contamination of the environment with resistant bacteria15.

International organizations have strong concerns about the prevalence of antibiotic-resistant bacteria and the use of antibiotics in the production of food animals. The Office International des Epizooties (OIE) published International Standards on Antimicrobial Resistance in 200112. Further, the European Union (EU) has issued a strategy to reduce the prevalence of antibiotic-resistant bacteria by surveillance of the evolution of resistant bacteria and antibiotic consumption and by conducting research into the development of alternative preventive methods. The EU has proposed a complete ban by 2006 on the use of antibiotics as feed additives for the promotion of animal growth (http://ue.eu.int/ueDocs/cms_Data/docs/pressdata/en/agricult/70350.pdf). In Japan, about 2200 t of antibiotics is used each year, and more than half is used for animal production: in 2001, 1060 t was used as veterinary medicines and 230 t as feed addi-

To clarify the effect of soil application of animal waste (or “farmyard manure”; FYM) on the spread of antibiotic resistance to soil-inhabiting bacteria, we surveyed the occurrence of antibiotic resistance among aerobic heterotrophic bacteria of feces of livestock, FYM, and soils obtained from various locations.

Materials and Methods

Samples used

Samples were taken from feces from swine and poultry, FYM, and soils in Ibaraki Prefecture and Aichi Prefecture, Japan between April 2002 and September 2003. We obtained 19 samples in total (six fecal samples, six FYM samples and seven soil samples). All samples were transported on ice and processed within 3 to 5 h. Because of our agreements with the various owners, the exact locations and names of the entities from which we obtained the samples are withheld.

Six fecal samples were used. We collected three swine fecal samples. One was from brood sows (LWD strain, a three-way cross of Landrace×Large White×Duroc) at a pigery of a swine farm, and the other two were from LWD and Meishan piglets at the National Institute of Livestock and Grassland Science (NILGS) in Ibaraki Prefecture. Antibiotics (efrotomycin and colistine) were fed to the piglets at both the swine farm and NILGS as feed additives. We also collected three poultry fecal samples; the first was from broiler chicks at NILGS where antibiotics (salinomycin and colistin) were fed as feed additives, the second was from laying hens obtained from a large farm managed by a company (Laying hens A), and the third was from a small, family-run farm (Laying hens B). Both laying hens A and B were fed with no antibiotics.

Six FYM samples (two swine manures, three poultry manures and one cattle manure) were used. We collected two samples of solid swine FYM. One was made from the swine farm where we obtained the fecal sample and was a compost of feces and sawdust. The other swine FYM sample was a commercially available product (Tazaki farm, Ibaraki, Japan), and was purchased at a shopping center in Ibaraki Prefecture. We collected three samples of solid poultry FYM, including one each from the two poultry farms where we obtained the fecal samples. The corporate farm made a good-quality solid FYM from a well-equipped indoor composting facility. Neither of these poultry farms used materials other than feces. The third sample of commercial poultry FYM (Uchida Farm, Ibaraki, Japan), and one sample of commercial cattle FYM (Wayo farm, Gunma, Japan.) were also purchased at a shopping center in Ibaraki Prefecture.

Seven soil samples were taken from five sources. The first source was an upland field at the National Institute for Agro-Environmental Sciences (NIAES) in Ibaraki Prefecture to which no FYM had been applied for more than 10 years. We obtained three soil samples in April, July, and November 2002. The second source was an upland field on a vegetable farm in which Chinese chive (Allium tuberosum Rotter ex Spreng.) was growing and to which solid poultry FYM had been applied at 10 t ha⁻¹ annually for 10 years. The third source was an upland field in which cabbage (Brassica oleracea L. var. capitata L.) and onion (Allium cepa L.) were planted and to which solid swine FYM had been applied at 40 t ha⁻¹ annually for more than 10 years. The fourth source was an orchard in which Japanese pear (Pyrus pyrifolia Nakai) was planted and to which solid poultry FYM had been applied at 15 t ha⁻¹ yearly for 8 years. All the upland and orchard soils were collected from a depth of 0 to 15 cm. As a reference, the fifth source was a forest soil beneath Japanese beech (Fagus crenata Blume) stands on Mt. Tsukuba where no animal farm facilities are located. We collected one soil sample from the A-horizon (0–15 cm).

Measurement of antibiotic resistance

Total and antibiotic-resistant bacteria were determined by plate counts on agar media with and without ampicillin (Ap), vancomycin (Vm), kanamycin (Km), chloramphenicol (Cp), rifampicin (Rf), or tetracycline (Tc) at a rate of 50 mg l⁻¹. One of reason why these six antibiotics were tested instead of feed additive antibiotics (colistin, salinomycin and efrotomycin) is that they belong to important classes of antibiotics that show different modes of action: inhibition of cell wall synthesis (Ap and Vm), inhibition of protein synthesis (Cp, Km, and Tc), and inhibition of RNA polymerase (Rf). Another reason is that the Vm analogue avoparcin was being used in Japan as a feed additive until recently and Vm-resistant enterococci pose a serious problem in medical clinics and Tc is used for disease treatment and prophylaxis in Japan. A third reason is that all of these six antibiotics are frequently used for the research of antibiotic resistant bacteria.

To culture heterotrophic bacteria, all samples were plated
on PTYG agar, a non-selective medium (per liter: 0.25 g of peptone, 0.25 g of tryptone, 0.5 g of yeast extract, 0.5 g of glucose, 0.03 g of MgSO$_4$·7H$_2$O, 0.0035 g of CaCl$_2$·2H$_2$O, and 15 g of Bacto agar (Difco)) to which cycloheximide (100 mg l$^{-1}$) was added as a fungicide. We used the dilution plate method to determine the population densities of antibiotic-resistant bacteria by making 10-fold dilutions with phosphate-buffered saline (pH 7.0). Aliquots of 100 μl of each of three consecutive dilutions were spread on the surfaces of two agar plates. Three replicates were performed and incubated at 30°C for 7 days. After the incubation, the colonies were counted. The detection limit was 100 colony-forming units per gram of dry matter (CFU g$^{-1}$ DM). Data are reported as the mean and standard deviation of three replicates.

**Examination of multi-drug resistance of isolates**

We investigated the occurrence of multiple-resistance phenotypes in antibiotic-resistant bacteria isolated from four representative samples, which presumably differed in their exposure to antibiotics: high exposure (feces of LWD piglets from NILGS and solid swine FYM bought from a shopping center) and low exposure (upland soil from NIAES not treated with FYM and the A-horizon soil of a Japanese beech forest on Mt. Tsukuba).

For each sample, ten colonies were randomly picked and purified from each PTYG agar plate containing one of the six antibiotics. Because Rf-resistant bacteria were not detected in the commercial solid swine FYM, we obtained a total of 230 isolates. Each purified isolate was streaked onto PTYG agar plates containing 50 mg l$^{-1}$ of each of the five antibiotics other than the one originally used for isolation, and the plates were incubated for 24 h at 30°C for determining the viability of the isolates.

**Results and Discussion**

**Enumeration of antibiotic-resistant bacteria in animal feces**

We determined the numbers of antibiotic-resistant bacteria grown on PTYG agar from six fecal samples (three swine and three poultry). Data are shown in Table 1.

In the three samples of the swine feces, the total numbers of culturable bacteria differed greatly from $8.14\times10^5$ to $3.83\times10^6$ CFU/g dry matter. Depending on the antibiotic and sample source, the total numbers of antibiotic-resistant bacteria in the fecal samples from swine were the same as or up to seven orders of magnitude lower than the total numbers of culturable bacteria. Among the six antibiotics, resistance to Ap and Km occurred most frequently (0.30%-83.3% for Ap and 2.38%-33.9% for Km), whereas resistance to Rf occurred least often (not detected to 7.63×10$^{-1}$%). Among the three swine fecal samples, the proportions of antibiotic-resistant bacteria among total culturable bacteria were much higher for the LWD piglets and the Meishan piglets than the LWD brood sows. For example, in the

<table>
<thead>
<tr>
<th>Origin</th>
<th>Total culturable bacteria</th>
<th>Ap-r</th>
<th>Vm-r</th>
<th>Km-r</th>
<th>Cp-r</th>
<th>Rf-r</th>
<th>Tc-r</th>
</tr>
</thead>
<tbody>
<tr>
<td>LWD brood sows</td>
<td>3.83×10$^{11}$</td>
<td>1.15×10$^9$</td>
<td>5.36×10$^4$</td>
<td>9.10×10$^6$</td>
<td>3.21×10$^6$</td>
<td>N.D.$^b$</td>
<td>3.83×10$^6$</td>
</tr>
<tr>
<td></td>
<td>(3.00×10$^{-10}$%)</td>
<td>(1.40×10$^{-3}$%)</td>
<td>(2.38%)</td>
<td>(8.38×10$^{-4}$%)</td>
<td>(7.63×10$^{-3}$%)</td>
<td>(6.22%)</td>
<td></td>
</tr>
<tr>
<td>LWD piglets</td>
<td>8.14×10$^9$</td>
<td>1.10×10$^8$</td>
<td>1.01×10$^7$</td>
<td>2.76×10$^8$</td>
<td>1.31×10$^7$</td>
<td>6.21×10$^5$</td>
<td>5.06×10$^7$</td>
</tr>
<tr>
<td></td>
<td>(1.35×10$^{-10}$%)</td>
<td>(1.24%)</td>
<td>(3.39×10$^{-10}$%)</td>
<td>(1.61%)</td>
<td>(7.63×10$^{-3}$%)</td>
<td>(6.22%)</td>
<td></td>
</tr>
<tr>
<td>Meishan piglets</td>
<td>2.63×10$^9$</td>
<td>2.19×10$^8$</td>
<td>1.28×10$^7$</td>
<td>5.25×10$^8$</td>
<td>2.28×10$^7$</td>
<td>1.02×10$^6$</td>
<td>3.01×10$^8$</td>
</tr>
<tr>
<td></td>
<td>(8.33×10$^{-10}$%)</td>
<td>(4.87×10$^{-3}$%)</td>
<td>(2.00×10$^8$)</td>
<td>(8.67×10$^{-1}$%)</td>
<td>(3.88×10$^{-2}$%)</td>
<td>(1.14×10$^{-3}$%)</td>
<td></td>
</tr>
<tr>
<td>Broiler chicks</td>
<td>1.14×10$^{13}$</td>
<td>7.50×10$^{11}$</td>
<td>3.51×10$^8$</td>
<td>3.23×10$^{11}$</td>
<td>1.80×10$^8$</td>
<td>1.34×10$^7$</td>
<td>3.66×10$^9$</td>
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<tr>
<td></td>
<td>(6.58%)</td>
<td>(3.08×10$^{-3}$%)</td>
<td>(2.83%)</td>
<td>(1.58×10$^{-3}$%)</td>
<td>(1.18×10$^{-4}$%)</td>
<td>(3.21×10$^{-2}$%)</td>
<td></td>
</tr>
<tr>
<td>Laying hens A</td>
<td>3.19×10$^{12}$</td>
<td>5.82×10$^9$</td>
<td>2.61×10$^7$</td>
<td>1.70×10$^8$</td>
<td>3.12×10$^6$</td>
<td>5.58×10$^5$</td>
<td>6.03×10$^8$</td>
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<td></td>
<td>(1.82×10$^{-3}$%)</td>
<td>(8.18×10$^{-4}$%)</td>
<td>(5.33×10$^{-3}$%)</td>
<td>(9.78×10$^{-5}$%)</td>
<td>(1.75×10$^{-5}$%)</td>
<td>(1.88×10$^{-2}$%)</td>
<td></td>
</tr>
<tr>
<td>Laying hens B</td>
<td>2.64×10$^{11}$</td>
<td>3.84×10$^6$</td>
<td>4.20×10$^7$</td>
<td>1.56×10$^6$</td>
<td>8.40×10$^5$</td>
<td>1.20×10$^5$</td>
<td>1.33×10$^6$</td>
</tr>
<tr>
<td></td>
<td>(1.45×10$^{-3}$%)</td>
<td>(1.59×10$^{-2}$%)</td>
<td>(5.91×10$^{-6}$%)</td>
<td>(3.18×10$^{-6}$%)</td>
<td>(4.55×10$^{-5}$%)</td>
<td>(5.04×10$^{-5}$%)</td>
<td></td>
</tr>
</tbody>
</table>


The values in parentheses are the percentages of antibiotic-resistant bacteria among total culturable bacteria.

*r: bacteria resistant to antibiotic, $^b$ N.D.: not detected.
LWD piglets, 13.5% of bacteria exhibited Ap-resistance, 33.9% Km-resistance and 6.22% Tc-resistance. In contrast, the Meishan piglets, the respective values were 83.3%, 20.0% and 114%, indicating that all culturable bacteria of the feces of the Meishan piglets carried a Tc-resistant phenotype. In contrast, the ratio for the LWD brood sows was 3.00×10⁻³% of bacteria with Ap-resistance, 2.83% with Km-resistance and 1.00×10⁻³% with Tc-resistance. Both the swine farm and NILGS where we obtained the fecal samples used the same antibiotics (erfomycin and colistin) as feed additives, in contrast, the poultry farms where we obtained the fecal samples of laying hens A and B used no antibiotics as feed additives. This is probably the reason for the high occurrence of antibiotic resistance in the broiler chicks.

**Enumeration of antibiotic-resistant bacteria in solid FYM**

The numbers of total culturable bacteria of the three FYM samples originating from fecal samples were two to five orders of magnitude lower than those for the corresponding fecal samples (Table 2). Furthermore, the ratio of antibiotic-resistant to total bacteria was much lower in the FYM samples than the fecal samples with some exceptions (Cp-resistant bacteria of LWD brood sows and Ap-, Km-, Tc-resistant bacteria of Laying hens B). In particular, RF-, Cp- and Vm-resistant bacteria could not be detected in the FYM samples. For example, solid FYM originating from the LWD brood sows feces had 0.21% Ap-resistant bacteria and 1.00×10⁻⁴% Tc-resistant bacteria. The counts of Vm-, Km-, and RF-resistant bacteria were below the detection limits. In contrast, of the bacteria in the LWD brood sows feces, 0.30% were Ap-resistant, 2.38% Km-resistant, 1.00×10⁻³% Tc-resistant, and 1.40×10⁻³% Vm-resistant. Furthermore, the solid poultry FYM originating from the feces of laying hens A had only Ap-resistant bacteria (9.33×10⁻⁶%), and the counts of bacteria resistant to the other five antibiotics were below the detection limits. This situation probably reflects the fact that this FYM was produced

### Table 2. Numbers of antibiotic-resistant bacteria from solid farmyard manure samples

<table>
<thead>
<tr>
<th>Origin</th>
<th>Total culturable bacteria</th>
<th>Ap-r¹</th>
<th>Vm-r</th>
<th>Km-r</th>
<th>Cp-r</th>
<th>RF-r</th>
<th>Tc-r</th>
</tr>
</thead>
<tbody>
<tr>
<td>LWD brood sows</td>
<td>1.07×10⁸</td>
<td>2.27×10⁶</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.24×10⁵</td>
<td>N.D.</td>
<td>1.07×10³</td>
</tr>
<tr>
<td></td>
<td>(2.12×10⁻³%)</td>
<td></td>
<td></td>
<td></td>
<td>(1.17×10⁻³%)</td>
<td></td>
<td>(1.00×10⁻³%)</td>
</tr>
<tr>
<td>Laying hens A</td>
<td>1.34×10⁷</td>
<td>1.25×10³</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
<td>N.D.</td>
</tr>
<tr>
<td></td>
<td>(9.33×10⁻³%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Laying hens B</td>
<td>1.16×10⁸</td>
<td>7.38×10⁵</td>
<td>6.77×10³</td>
<td>2.52×10⁴</td>
<td>N.D.</td>
<td>N.D.</td>
<td>1.85×10³</td>
</tr>
<tr>
<td></td>
<td>(6.36×10⁻³%)</td>
<td>(5.84×10⁻³%)</td>
<td>(2.19×10⁻³%)</td>
<td></td>
<td></td>
<td>(1.59×10⁻³%)</td>
<td></td>
</tr>
<tr>
<td>Swine (commercial)</td>
<td>7.42×10¹³</td>
<td>7.42×10⁹</td>
<td>1.05×10⁹</td>
<td>1.20×10⁴</td>
<td>6.11×10⁶</td>
<td>N.D.</td>
<td>4.67×10⁴</td>
</tr>
<tr>
<td></td>
<td>(1.00%)</td>
<td>(1.42×10⁻³%)</td>
<td>(1.62×10⁻³%)</td>
<td>(8.23×10⁻⁶%)</td>
<td></td>
<td>(6.29×10⁻⁵%)</td>
<td></td>
</tr>
<tr>
<td>Poultry (commercial)</td>
<td>8.84×10⁷</td>
<td>2.17×10³</td>
<td>N.D.</td>
<td>5.42×10³</td>
<td>3.38×10⁶</td>
<td>N.D.</td>
<td>2.99×10⁵</td>
</tr>
<tr>
<td></td>
<td>(2.45×10⁻³%)</td>
<td></td>
<td></td>
<td>(6.13×10⁻³%)</td>
<td>(3.82×10⁻³%)</td>
<td></td>
<td>(3.38×10⁻³%)</td>
</tr>
<tr>
<td>Cattle (commercial)</td>
<td>3.57×10⁸</td>
<td>3.15×10⁸</td>
<td>2.34×10⁸</td>
<td>2.49×10⁸</td>
<td>2.81×10⁸</td>
<td>4.11×10⁷</td>
<td>8.50×10⁴</td>
</tr>
<tr>
<td></td>
<td>(8.22%)</td>
<td>(6.55%)</td>
<td>(6.97%)</td>
<td>(7.87×10⁻⁵%)</td>
<td>(1.15%)</td>
<td>(2.38×10⁻⁵%)</td>
<td></td>
</tr>
</tbody>
</table>

See notes to Table 1.
solely indoors and that a high temperature (about 80°C) was maintained during composting, thereby strongly curtailing the survival of antibiotic-resistant bacteria originating in feces.

We also analyzed three samples of commercially available solid FYM (swine, poultry, and cattle) for home use (Table 2). The numbers of total and resistant bacteria of the poultry FYM were similar to those of the FYM originating from feces of laying hens B, but the total number of cultivable bacteria of the commercial swine FYM was two orders of magnitude higher than that of the FYM originating from the feces of LWD brood sows. Furthermore, the percentages of antibiotic-resistant bacteria among total cultivable bacteria (1.00% Ap-resistant bacteria, 0.14% Vm-resistant bacteria, and 0.16% Km-resistant bacteria) were higher in the purchased swine FYM than in the FYM originating from the feces of LWD brood sows. In the cattle FYM, the percentages of antibiotic-resistant bacteria were the highest among the six FYM samples: 8.22% Ap-resistant bacteria, 6.55% Vm-resistant bacteria, 6.97% Km-resistant bacteria, and 1.15% Rf-resistant bacteria, respectively. Although we cannot demonstrate here whether antibiotic-resistant bacteria originating from feces of livestock can survive or not during the composting process, there is a possibility that this occurrence of antibiotic-resistant bacteria is due to insufficient composting. More studies are needed to elucidate the fate of these organisms during this process, because little is known about the dynamics of antibiotic-resistant bacteria during composting.

Enumeration of antibiotic-resistant bacteria in soils

When we investigated the occurrence of antibiotic-resistant bacteria in soil from the upland fields and forest, we found that even the upland field that had been managed for more than 10 years without the application of FYM contained $10^4$ to $10^6$ CFU g$^{-1}$ dry soil of bacteria resistant to at least one of the six antibiotics (Fig. 1). Previous observations support an indigenous presence of antibiotic-resistant bacteria in arable soils. For instance, Smalla et al. detected Km-resistant bacteria in all of the various soil, river water, sewage, and pig manure slurry samples they examined. The number of Km-resistant bacteria in German upland soil was $10^5$ CFU g$^{-1}$ dry soil using a 1/10 TSA agar containing Km (100 mg l$^{-1}$), and the resistance quotient was 0.56% (Holben, W.E. et al. 1989. Abstracts of 5th Int. Symp. Microbiol. Ecol. p. 146.) showed that the number of Ap-resistant bacteria was one log less than the total number of bacteria, and numbers of Km-, Cp-, Rf-, and Tc-resistant bacteria were three or four logs less. The A horizon of natural forest soil beneath Japanese beech stands, where one would expect the anthropogenic influence to be very low, had almost the same numbers of antibiotic-resistant bacteria as did the upland soil that had not been treated with FYM (data not shown). This ubiquitous distribution of antibiotic-resistant bacteria in nature is well accepted, because antibiotics are naturally

![Graph](image_url)

Fig. 1. Numbers of antibiotic-resistant bacteria from upland field soil either not treated with FYM or treated with 40 t ha$^{-1}$ yearly. Error bars represent standard deviations.

- Total culturable bacteria
- Ampicillin-resistant bacteria
- Vancomycin-resistant bacteria
- Kanamycin-resistant bacteria
- Chloramphenicol-resistant bacteria
- Rifampicin-resistant bacteria
- Tetracycline-resistant bacteria
present in soils to some extent and exposure of soil microorganisms to antibiotic-producing *Streptomyces* might provide the selective pressure needed for selection of resistance genes\(^{16,18}\). However, higher numbers of resistant bacteria occur in polluted habitats than in unpolluted habitats, indicating that human activities contribute substantially to the increased proportion of resistant bacteria occurring in the environment\(^{13}\).

Heavy application of organic manure usually increases the number of total culturable bacteria in soils, and may increase the number of antibiotic-resistant bacteria in soils. It is also likely that further substantial numbers of intestinal bacteria of animal origin are spread on farmlands by the disposal of manure, and the spread of antibiotic-resistance genes from bacteria in the manure to microbes indigenous to soil might create an undesirable reservoir of antibiotic resistance\(^{15}\). We evaluated this hypothesis by sampling field soils to which FYM had, or had not, been applied. Of the three farm soil samples that had been treated with solid FYM (swine or poultry), only that to which solid swine FYM at 40 t ha\(^{-1}\) had been applied yearly for more than 10 years had significantly higher (Student’s *t*-test; *P*<0.05) numbers of total and antibiotic (Ap, Vm, Km, Cp, and Tc)-resistant bacteria than the upland soil that had not been treated with FYM (Fig. 1). However, the two other soils, which had received solid poultry FYM at 10 t ha\(^{-1}\) or 15 t ha\(^{-1}\) yearly, did not demonstrate significantly increased numbers of total and resistant bacteria (data not shown). These results suggest that this hypothesis might be especially true for limited cases.

### Occurrence of multi-drug-resistant bacteria

The antibiotic-resistant isolates from the feces of LWD piglets and from the forest soil yielded contrasting results (Fig. 2). All isolates from the fecal sample (except the Ap-resistant isolate) had a broad-range MDR to the other five antibiotics, whereas most of the forest soil isolates had a narrow-range MDR to only one to four other antibiotics, and only a few were resistant to all the other five antibiotics. The Tc-resistant and Vm-resistant isolates of the commercial solid swine FYM also showed broad-range MDR, as did those obtained from the upland soil not treated with

\[\text{Fig. 2. Multi-drug resistance of antibiotic-resistant bacterial isolates. The horizontal axis indicates the number of antibiotics to which the isolate showed resistance. The vertical axis gives the number of resistant isolates. Data on Rf-resistant isolates from commercial solid swine FYM are not presented because no isolates were obtained.}\]
FYM, but the frequency of MDR was low in these Vm-resistant isolates.

Although we have not yet identified the bacterial species of the isolates that showed MDR, antibiotic use in animal husbandry may be one of the reasons for the broad-range MDR in the samples. Indeed, instead of the six antibiotics, antibiotics such as efrotomycin, colistin, and salinomycin had been used as feed additives in the swine and poultry production facilities. It is well known that plasmids that harbor the MDR phenotype can be easily acquired under exposure to antibiotics. This capability is probably the reason why broad-range MDR was present in the fecal samples despite the apparent lack of exposure to the six antibiotics. Actually, in our preliminary studies, we found that the swine fecal isolates carrying the broad-range MDR were also resistant to colistin (data not shown).

In Denmark, reduction of the use of antibiotics as growth promoters has been reported to decrease the occurrence of antibiotic-resistant bacteria in the feces of poultry, swine, and cattle. In the present study, in the fecal samples of livestock fed with antibiotics as feed additives, a high occurrence of antibiotic-resistant bacteria was observed. In contrast, the two poultry farms (Laying hens A and B) did not use antibiotics as feed additives, and a relatively low occurrence of antibiotic-resistant bacteria was observed. Moreover, composting with feces from hens (Laying hens A) that had little history of exposure to antibiotics reduced the number of antibiotic-resistant bacteria in the final FYM. These results suggest that reduction of the use of antibiotics as feed additives and careful composting can prevent the dissemination of antibiotic-resistant bacteria in the agricultural environment. Furthermore, our study also showed that the occurrence of broad-range MDR in the feces of swine fed with antibiotics was much higher than that of the natural forest soil, suggesting that the occurrence of broad-range MDR in the feces was enhanced by the use of antibiotics as feed additives. It is also suggested that reduction of the use of antibiotics as feed additives can reduce the population of bacteria with broad-range MDR in feces of livestock, and prevent their dissemination to FYM and the agricultural environment.

Acknowledgments

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References

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