

## Isolation of Glycopeptide-Resistant Enterococci from Chickens in Japan

Avoparcin is a glycopeptide antibiotic that has been used as a growth-promoting agent for food animals in many countries, except the United States and Canada. In human medicine, glycopeptide-resistant enterococci (GRE) have become an increasingly serious problem in the treatment of nosocomial infections (6, 8, 10). In Europe, GRE have been isolated from animals, meat, the environment, and healthy humans outside hospitals (1, 2, 4, 7, 9), suggesting the possibility of transmission of GRE from food animals to humans via the food chain (2–4). In Japan, two glycopeptides have been approved as feed additives for chickens—avoparcin in 1985 and orienticin in 1994—but no papers have been published on the isolation of GRE from chickens in Japan. Then, a nationwide survey was conducted to isolate GRE from the fecal droppings of chickens on farms in Japan.

Prior to isolation of enterococci from fecal droppings, the distribution of avoparcin from the manufacturers to chicken farms was traced for 1 year across the country. Avoparcin was confirmed to have been brought into 24 prefectures in the period from December 1995 to November 1996. Fecal droppings were sampled from 39 broiler farms in these 24 prefectures. Fecal droppings of chickens not exposed to avoparcin were sampled from 11 broiler farms and 35 layer farms in 23 prefectures where no avoparcin had been distributed in the same period. Fecal samples were taken during a 1-week period from 11 December to 18 December 1996. Enterococci were isolated on bile esculin azide agar plates. MICs were determined for avoparcin, orienticin, and vancomycin by the agar dilution method with Mueller-Hinton agar.

Two hundred and sixty-three enterococci were isolated from the 35 broiler farms confirmed to have used avoparcin. Eight (3.0%) of them were GRE, originating from three (8.6%) farms. Of these eight strains, seven were identified as *Enterococcus faecalis* and the remaining one was identified as *E. faecium*. Five of the seven *E. faecalis* strains originated from the same farm, and the remaining two strains originated from an-

other farm. All of the GRE were proved by PCR to harbor the *vanA* gene. No GRE were found among 295 enterococci isolated from broiler and layer farms not exposed to avoparcin (Table 1).

In Japan, the amounts of avoparcin recently marketed were 8,902 kg in 1993, 5,214 kg in 1994, 1,840 kg in 1995, and 986 kg in 1996, accounting for 2.78, 1.87, 0.68, and 0.40% of all growth-promoting antibiotics, respectively (5). Even though the consumption of avoparcin was smaller than that in Denmark (1) and has decreased each year, avoparcin use was associated with the presence of GRE in the feces of chickens after only a decade of use. The presence of GRE in the fecal droppings of chickens in this survey led to the ban of avoparcin and orienticin in food animals on 18 March 1997 in Japan.

We thank Y. Ike, Gunma University School of Medicine, for helpful advice. We are also grateful to colleagues at Livestock Health Service Centers across the country for fecal sampling and for providing us with the enterococcal isolates.

### REFERENCES

1. Aarestrup, F. M. 1995. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. *Microb. Drug Resist.* **1**:255–257.
2. Bates, J., J. Z. Jordens, and D. T. Griffiths. 1994. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. *J. Antimicrob. Chemother.* **34**:507–516.
3. Chadwick, P. R., N. Woodford, E. B. Kaczmarek, S. Gray, R. A. Barrell, and B. A. Oppenheim. 1996. Glycopeptide-resistant enterococci isolated from uncooked meat. *J. Antimicrob. Chemother.* **38**:908–909.
4. Klare, I., H. Heier, H. Claus, G. Böhme, S. Martin, G. Seltmann, R. Hanenbeck, V. Antanassova, and W. Witte. 1995. *Enterococcus faecium* strains with vanA-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. *Microb. Drug Resist.* **1**:265–272.
5. Ministry of Agriculture, Forestry and Fisheries. 1993–1996. Bulletin of the Tokyo Fertilizer and Feed Inspection Station. Tokyo Fertilizer and Feed Inspection Station, Tokyo, Japan (In Japanese.)
6. Pegues, D. A., C. F. Pegues, P. L. Hibberd, D. S. Ford, and D. C. Hooper. 1997. Emergence and dissemination of a highly vancomycin-resistant vanA strain of *Enterococcus faecium* at a large teaching hospital. *J. Clin. Microbiol.* **35**:1565–1570.
7. Schouten, M. A., A. Voss, and J. A. A. Hoogkamp-Korstanje. 1997. VRE and meat. *Lancet* **349**:1258.
8. Vandamme, P., E. Vercauteren, C. Lammens, N. Pensart, M. Ieven, B. Pot, R. Leclercq, and H. Goossens. 1996. Survey of enterococcal susceptibility patterns in Belgium. *J. Clin. Microbiol.* **34**:2572–2576.
9. van den Bogaard, A. E., L. B. Jensen, and E. E. Stobberingh. 1997. Vancomycin-resistant enterococci in turkeys and farmers. *N. Engl. J. Med.* **337**:1558–1559.
10. Well, C. L., B. A. Juni, S. B. Cameron, K. R. Mason, D. L. Dunn, P. Ferrieri, and F. S. Rhame. 1995. Stool carriage, clinical isolation, and mortality during an outbreak of vancomycin-resistant enterococci in hospitalized medical and/or surgical patients. *Clin. Infect. Dis.* **21**:45–50.

H. Yoshimura  
 M. Ishimaru  
 Y. S. Endoh

National Veterinary Assay Laboratory  
 Ministry of Agriculture, Forestry and Fisheries  
 1-15-1 Tokura, Kokubunji, Tokyo 185-8511  
 Japan

M. Suginaka  
 S. Yamatani

Tokyo Fertilizer and Feed Inspection Station  
 Ministry of Agriculture, Forestry and Fisheries  
 1-3-3 Ohtemachi, Chiyodaku, Tokyo 100-0004  
 Japan

TABLE 1. In vitro susceptibilities to avoparcin, orienticin, and vancomycin of enterococci isolated from fecal droppings of chickens

| Source <sup>a</sup> (no. of isolates and antibiotic) | MIC (µg/ml) |      |      | No. of resistant isolates (%) |
|--|-------------|------|------|-------------------------------|
|  | Range       | 50%  | 90%  |                               |
| <b>Broiler farms</b>                                 |             |      |      |                               |
| Using avoparcin (263)                                |             |      |      |                               |
| Avoparcin  | 0.78–>100   | 1.56 | 3.12 | 8 (3.0)                       |
| Orienticin   | 0.05–≥50    | 0.2  | 0.78 | 8 (3.0)                       |
| Vancomycin   | 0.39–>100   | 0.39 | 3.12 | 8 (3.0)                       |
| Not using avoparcin (81)                             |             |      |      |                               |
| Avoparcin  | 0.78–3.12   | 1.56 | 1.56 |                               |
| Orienticin   | 0.05–1.56   | 0.2  | 0.78 |                               |
| Vancomycin   | 0.39–3.12   | 0.78 | 3.12 |                               |
| <b>Layer farms not using avoparcin (214)</b>         |             |      |      |                               |
| Avoparcin  | 0.78–3.12   | 1.56 | 1.56 |                               |
| Orienticin   | 0.05–1.56   | 0.2  | 0.78 |                               |
| Vancomycin   | 0.39–3.12   | 0.78 | 1.56 |                               |

<sup>a</sup> Use or lack of use of avoparcin is indicated for the period of December 1995 to November 1996.