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A national surveillance of antimicrobial resistance in *Escherichia coli* isolated from food-producing animals in Japan

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A nationwide investigation of antimicrobial susceptibility in *Escherichia coli* isolated from food-producing animals was performed in Japan. MICs of 18 antimicrobial agents were determined for a total of 1018 isolates. Higher resistance rates were observed against sulfadimethoxine, oxytetracycline and dihydrostreptomycin, followed by ampicillin and kanamycin. Resistance was more frequently observed among broiler isolates, followed by isolates from pigs. Almost 10% of broiler isolates were resistant to fluoroquinolones and extremely high MICs (100 mg/L) were observed. In general, antimicrobial resistance rates in *E. coli* have declined in recent years, with the exception of resistance to fluoroquinolones among broiler isolates, which has increased.

Keywords: *Escherichia coli*, antimicrobial resistance, cattle, pig, broiler

Introduction

The WHO has recently published its 'global principles for the containment of antimicrobial resistance in animals intended for food'.[1] These principles aim to reduce the overuse of antimicrobials in food animals, to protect human as well as animal health. In order to achieve this, the WHO and other organizations require programmes to monitor antimicrobial resistance in zoonotic bacteria, animal pathogens and 'indicator bacteria'. In accordance with these requirements, we conducted a national surveillance of antimicrobial resistance in the veterinary field in Japan.

Many studies on bacterial resistance have been published to date, but most have focused on human and animal pathogens, and have been based on data generated to support therapy.[2] However, the selective pressure of antimicrobial use for therapy should be taken into account when interpreting these studies. *Escherichia coli* is the most common enterobacterium, and can serve as an indicator bacterium that easily acquires antimicrobial resistances commonly found in different animal species.[2,3] The use of *E. coli* as an indicator bacterium allows a representative stratified random sampling strategy from healthy animals, enabling direct comparison of resistance between different animals and analysis of trends over time.[3] The use of *E. coli* as an indicator bacterium is also important because changes in the resistance of this species may serve as an 'early warning system' for resistance in potentially pathogenic bacteria.[2]

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. This paper deals with the antimicrobial susceptibility of *E. coli* isolates from groups of the major food-producing animals throughout Japan, determined under the JVARM.

Materials and methods

Sampling of faeces

Sampling was carried out by the Livestock Hygiene Service Centers of all 47 prefectures across Japan from June to December 1999. Fresh faecal samples were collected from healthy beef cattle, pigs and broilers at the farm. In general,

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four samples per animal species were collected from different farms in each prefecture. Faecal samples were placed in sterile plastic specimen tubes on ice and transported to our laboratory for bacterial isolation within 3 days.

Culture procedures for isolation of E. coli

Faeces were plated on to desoxycholate–hydrogen sulphate–lactose agar (Eiken Co. Ltd, Japan) directly or, occasionally, after enrichment in trypticase soy broth (BBL; Becton-Dickinson, Cockeysville, MD, USA) containing vancomycin (Sigma Chemical Co.). Candidate colonies were then plated on to nutrient agar (Eiken), identified biochemically and kept at 4°C in Dorset egg medium (Nissui Pharmaceuticals Co. Ltd, Japan) until use. For individual samples, two *E. coli* isolates were selected randomly for the purpose of determining susceptibility.

Antimicrobial susceptibility testing

Eighteen widely used antimicrobial agents approved in Japan as veterinary medicines were tested. The MIC for each isolate was determined by a standardized agar dilution method, as described by the Japanese Society of Chemotherapy,[4] using Mueller–Hinton agar (Difco). *E. coli* ATCC 25922, *E. coli* ATCC 35218, *E. coli* NIHJ, *Staphylococcus aureus* ATCC 25923, *S. aureus* 209P and *Pseudomonas aeruginosa* ATCC 27853 were used as quality controls. Breakpoint was defined from a population of the isolates tested in this study.

Results and discussion

A total of 1018 *E. coli* isolates were obtained from 515 faecal samples taken from all 47 prefectures across Japan, of which 356 isolates were derived from 178 cattle, 358 isolates from 179 pigs and 304 isolates from 158 broilers. The MICs for these isolates are shown in Table 1. Higher resistance rates were observed against sulfadimethoxine (from 69.7% for broilers to 44.7% for cattle), oxytetracycline (69.4% to 25.3%) and dihydrostreptomycin (50.3% to 20.8%), followed by ampicillin (40.1% to 8.4%) and kanamycin (32.6% to 3.4%). All of these antimicrobials are commonly used and classically approved in Japan. Resistances were more commonly observed among broiler isolates, followed by pig isolates. Comparison of these data with previous nationwide surveillances of 1976–1977[5,6] and 1992–1994[7] revealed that present resistance levels are generally lower, especially among strains originating from cattle, even if the breakpoints of the data are adjusted. Consumption of classical antimicrobials is estimated to have decreased during the past decade,[8] and our resistance data may reflect this.

Trimethoprim is only used in combination with sulphonamides, and comparable data are limited. The MIC distribution of trimethoprim showed clearly distinguished peaks in our study, and the resistance rates were 28.6%, 13.1% and 2.2% in isolates from broilers, pigs and cattle, respectively. These levels were higher than those of indicator isolates in Denmark,[3] but lower than those of clinical isolates in Denmark[3] and indicator pig isolates in The Netherlands and Sweden.[2]

The use of chloramphenicol was banned in all animals except pets in Japan in 1998, so that the emergence of resistance due to its use would no longer take place. However, our study has shown that chloramphenicol resistance levels are still comparable to those of previous Japanese studies,[5–7] indicating that continuous monitoring is clearly necessary.

Resistance rates were found to be <10% for the other antimicrobials, with the exception of quinolones. Gentamicin and apramycin resistance levels were found to be comparable to previous levels in Japan[7] and other countries.[2,3] Neither antimicrobial is approved for use in cattle or broilers in Japan, accordingly, apramycin resistance was only observed in seven pig isolates. Although gentamicin resistance was detected in broiler isolates, it may have been caused by off-label use or the clonal spread of resistant isolates, as suggested by Aarestrup *et al.*[3] Simultaneous resistance to gentamicin and apramycin, which has been reported in other countries,[3] was observed in our study for all apramycin-resistant isolates. Colistin showed substantial activity against *E. coli* isolates from all animal origins, with an MIC_{50} of 0.39 mg/L and an MIC_{90} of 0.78 mg/L, after three decades of use of this antimicrobial. Resistance to bicozamycin was low for all animal isolates, although the breakpoint was high, at 100 mg/L. Regardless of the animal origin, only a limited number of isolates were resistant to cephalosporins, including the third-generation cephalosporin, ceftiofur, which is an important human medicine. The use of cephalosporins is restricted in Japan and oral formulations for mass administration of these antimicrobials have not been available since their introduction to the veterinary market around 1986. Such restricted use may have resulted in lower levels of resistance against cephalosporins.

Resistance levels against quinolones were markedly higher in broiler isolates. Table 2 shows the distribution of MICs of the fluoroquinolones and a comparison of the resistance rates observed in this study with those of a previous survey. In the survey of 1992–1993,[7] only a limited number of isolates were resistant to fluoroquinolones, regardless of the animal origin. However, the resistance rates increased among the broiler isolates from 0% in 1992 to 9.9% for enrofloxacin and 1.3% in 1993 to 10.5% for ofloxacin, whereas resistance rates were comparable or decreased in isolates from other animals. Among the broiler isolates, extremely high MICs of fluoroquinolones (100 mg/L) were also determined, although the MIC_{50}s of enrofloxacin and ofloxacin were ≤0.05 and 0.1 mg/L, respectively, demonstrating that *E. coli* are still extremely sensitive to fluoroquinolones. Regionally, fluoroquinolone-resistant *E. coli* were isolated on 24 farms, located in 16 pre-

Table 1. MIC for *E. coli* isolated from food-producing animals in Japan

Antimicrobial agent	Breakpoint (mg/L)	Cattle isolates ($n = 356$)			Pig isolates ($n = 358$)			Broiler isolates ($n = 304$)		
		MIC_{50} (mg/L)	MIC_{90} (mg/L)	number of resistant isolates (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	number of resistant isolates (%)	MIC_{50} (mg/L)	MIC_{90} (mg/L)	number of resistant isolates (%)
Ampicillin	12.5	3.13	6.25	30 (8.4)	3.13	>50	81 (22.6)	3.13	>50	122 (40.1)
Cefazolin	25	1.56	1.56	0 (0.0)	1.56	1.56	0 (0.0)	1.56	3.13	2 (0.7)
Cefuroxime	50	3.13	6.25	0 (0.0)	3.13	6.25	0 (0.0)	3.13	6.25	0 (0.0)
Ceftiofur	3.13	0.39	0.39	0 (0.0)	0.39	0.39	0 (0.0)	0.39	0.78	2 (0.7)
Dihydrostreptomycin	50	3.13	>100	74 (20.8)	6.25	>100	154 (43.0)	50	>100	153 (50.3)
Kanamycin	12.5	1.56	3.13	12 (3.4)	1.56	>100	68 (19.0)	3.13	>100	99 (32.6)
Gentamicin	3.13	0.78	0.78	0 (0.0)	0.78	0.78	10 (2.8)	0.78	0.78	15 (4.9)
Apramycin	12.5	3.13	3.13	0 (0.0)	3.13	3.13	7 (2.0)	3.13	3.13	0 (0.0)
Colistin	1.56	0.39	0.78	11 (3.1)	0.39	0.78	3 (0.8)	0.78	0.78	7 (2.3)
Chloramphenicol	50	6.25	12.5	11 (3.1)	6.25	>100	80 (22.3)	6.25	>100	52 (17.1)
Oxytetracycline	12.5	1.56	>100	90 (25.3)	>100	>100	239 (66.8)	>100	>100	211 (69.4)
Bicozamycin	100	25	50	2 (0.6)	25	50	8 (2.2)	25	50	6 (2.0)
Nalidixic acid	50	3.13	3.13	7 (2.0)	3.13	12.5	3 (0.8)	3.13	>100	112 (36.8)
Oxolinic acid	12.5	0.39	0.39	3 (0.8)	0.39	1.56	0 (0.0)	0.39	100	41 (13.5)
Enrofloxacin	3.13	≤0.05	≤0.05	1 (0.3)	≤0.05	0.1	0 (0.0)	≤0.05	1.56	30 (9.9)
Ofloxacin	3.13	0.1	0.1	1 (0.3)	0.1	0.2	0 (0.0)	0.1	3.13	32 (10.5)
Trimethoprim	12.5	0.39	0.78	8 (2.2)	0.39	>50	47 (13.1)	0.39	>50	87 (28.6)
Sulfadimethoxine	400	200	>400	159 (44.7)	400	>400	232 (64.8)	>400	>400	212 (69.7)

Table 2. MIC distribution of fluoroquinolones for *E. coli*

Antimicrobial agent	Origin	Number of isolates with MIC (mg/L) of[a] ≤0.05	0.1	0.2	0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100	% Resistance[b]	% Resistance reported by Ishimaru *et al.*[7c]
Enrofloxacin	cattle	331	18	3		1	2				1				0.3	2.2
	pigs	267	66	24	1										0.0	0.4
	broilers	175	17	16	51	9	6	3	1	15	8		3		9.9	0.0
Ofloxacin	cattle	63	276	10	3	1	2			1					0.3	1.8
	pigs	58	230	51	18	1									0.0	2.1
	broilers	48	138	6	18	50	12	5	7	12	7		1		10.5	1.3

[a]The numbers of isolates from broilers with decreased susceptibility (MIC 0.39–1.56 mg/L) were 66 (21.7%) for enrofloxacin and 80 (26.3%) for ofloxacin.
[b]The breakpoints of enrofloxacin and ofloxacin were 3.13 mg/L.
[c]Data from 1992 for enrofloxacin and from 1993 for ofloxacin.

fectures across Japan, indicating that fluoroquinolone resistance is widely dispersed in Japan.

E. coli with decreased susceptibility to fluoroquinolones were also observed (Table 2). Among broiler isolates, the number of *E. coli* isolates with MICs ranging from 0.39 to 1.56 mg/L for enrofloxacin and ofloxacin was 66 (21.7%) and 80 (26.3%), respectively. Ozeki *et al.*[9] reported that in a study of *E. coli* isolates from patients with urinary tract infections, ofloxacin MICs for the majority of the strains with single and double mutations in the *gyrA* gene (at Ser-83 and Asp-87) were 0.39–3.13 and 6.25–100 mg/L, respectively. They suggest that an increase in the incidence of strains with moderately decreased susceptibility portends a further increase in the incidence of strains with clinically significant resistance to fluoroquinolones. Notably, *E. coli* with decreased susceptibility to fluoroquinolones was more frequently observed in our study among isolates from broilers than previously reported.[7] Fluoroquinolones are one of the most valuable antimicrobial classes available to treat human infection, and have been restricted for veterinary use in Japan since their introduction to the veterinary market around 1992. Despite this, consumption of fluoroquinolones is estimated to be increasing, with about two-thirds being used for broilers, according to Yoshimura's estimate for 1997.[10] Thus, appropriate and prudent use of fluoroquinolones and continuous monitoring should be considered in the future.

In conclusion, antimicrobial resistance in *E. coli* as an indicator bacterium has generally decreased in Japan, with the exception of fluoroquinolone resistance among broiler isolates, which has increased.

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