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DRUG RESISTANCE AND CONJUGATIVE R PLASMIDS IN *ESCHERICHIA COLI* STRAINS ISOLATED FROM MIGRATORY WATERFOWL

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ABSTRACT: We evaluated drug resistance and R plasmids of 554 strains of *Escherichia coli* isolated from feces of migratory waterfowl, including whistling swans (*Cygnus columbianus*), pintails (*Anas acuta*) and black-tailed gulls (*Larus crassirostris*) collected from the San-in District, Japan, between each November and March, 1983 to 1984, 1984 to 1985, and 1985 to 1986. Seven antimicrobial agents were tested: dihydrostreptomycin (DSM), kanamycin, spectinomycin, ampicillin (ABPC), oxytetracycline (OTC), chloramphenicol, and sulfadimethoxine (SDMX). Many strains were resistant to several drugs; in particular, all strains were resistant to SDMX. Both multiple drug resistant strains and drug resistance patterns occurred most frequently in strains isolated from whistling swans, followed by black-tailed gulls, and pintails, respectively. Of 233 strains, 128 (55%) carried transmissible R plasmids. The drugs with the largest number of resistance patterns observed were, in descending order, OTC, DSM, ABPC, and SDXM.

Key words: Drug resistance, R plasmids, *Escherichia coli*, migratory waterfowl, whistling swan, pintail, black-tailed gull, *Cygnus columbianus, Anas acuta, Larus crassirostris*.

INTRODUCTION

Strains of bacteria resistant to antimicrobial drugs have spread widely in humans as well as in domestic animals, following the widespread use of various drugs for control and prevention of diseases, and with their use in animal feed for growth promotion (Kinjo, 1979). Drug-resistant strains also have been detected from wild animals found in close contact with human environments (Roland et al., 1985; Ruotman et al., 1985).

In Japan, drug resistant strains and R plasmids of *Escherichia coli* occur in a variety of wild birds (Nakamura et al., 1982). However, there has been only one report on the antibiogram of Enterobacteriaceae isolated from migratory waterfowl (Sato et al., 1979).

Since migratory birds fly all over the world, and could contribute to the spread of drug resistant strains, it is important to identify any drug resistant strains among these birds.

Our objective was to determine the prevalence of drug resistance and R plasmids among *E. coli* isolated from migratory waterfowl coming to San-in District in Japan during the winter season.

MATERIALS AND METHODS

We collected 1,819 fecal samples: 984 from whistling swans (*Cygnus columbianus*), 606 from pintails (*Anas acuta*), and 229 from black-tailed gulls (*Larus crassirostris*). Samples were collected from the Japan Sea Coast in Tottori Prefecture and from Lake Nakanoumi in Shimane Prefecture, Japan (35°5′N, 133°12′ to 134°14′E), over three time periods: November 1983 to March 1984, November 1984 to March 1985, and November 1985 to March 1986. Feces were distinguished by size and shape. Whistling swan feces are about 10 mm in diameter and 50 to 80 mm in length with a cigarette shape. Pintail feces are small with a poorly defined shape. Black-tailed gull feces are small and watery, with no well-defined shape. One complete sample each of fresh feces for whistling swans and pintails was collected into a screw-capped tube, and four or five pieces of feces of black-tailed gulls were collected in the same way. These were stored at −20 C until examined.

As control samples, feces of 54-day-old Hyline chicks (Keizai-ren poultry farm, Tottori, Japan) were obtained. Eight days before sampling, the chicks were given 0.005% ouleomycin (Takeda Seiyaku, Osaka, Japan), 0.01% tylosin (Takeda Seiyaku) and 0.01% furasolidone (Ueno Seiyaku, Osaka, Japan) in their feed for 5 days.

TABLE 1. Frequencies of drug resistance in *Escherichia coli* strains isolated from migratory waterfowl and chickens, San-in District, Japan, 1983 to 1986.

Birds	Number of strains tested	Number of resistant strains						
		DSM[a]	KM	SPCM	ABPC	OTC	CP	SDMX
Whistling swan	279	51	37	201	34	71	5	279
Pintail	196	33	2	12	5	29	0	196
Black-tailed gull	79	26	1	18	8	17	1	79
Chicken	468	376	401	367	183	397	14	426

[a] DSM, dihydrostreptomycin; KM, kanamycin; SPCM, spectinomycin; ABPC, ampicillin; OTC, oxytertacyclin; CP, chloramphenicol; SDMX, sulfadimethoxine.

Four or five colonies resembling those of *E. coli* were taken from each fecal sample and grown on desoxycholate hydrogen sulfide lactose (DHL) agar plates (Nissui Seiyaku, Tokyo, Japan). They then were identified as *E. coli* according to Cowan (1974).

Seven antimicrobial agents were used: dihydrostreptomycin (DSM) (Kyouwa Yakuhin, Tokyo), kanamycin (KM) (Meiji Seiyaku, Tokyo), spectinomycin (SPCM) (Sumitomo Seiyaku, Osaka, Japan), ampicillin (ABPC) (Fujisawa Seiyaku, Tokyo), oxytetracycline (OTC) (Takeda Seiyaku), chloramphenicol (CP) (Sankyou Seiyaku, Tokyo, Japan) and sulfadimethoxine (SDMX) (Daiichi Seiyaku, Tokyo). All drugs first were dissolved in sterile distilled water to a concentration of 1,000 μg/ml. They then were diluted serially by two-fold dilutions in distilled water.

Drug sensitivity tests were conducted in heart infusion agar plates (Difco, Detroit, Michigan, USA) or Mueller-Hinton agar plates (Baltimore Biological Laboratories, Fair Lawn, England) (for SDMX) by a serial two-fold dilution method of drugs recommended by the Japanese Society of Chemistry (Japanese Society of Chemotherapy, 1981). Minimum inhibitory concentrations (MIC) were read as the lowest concentration resulting in complete inhibition of growth after 40 hr incubation at 37 C. The drug concentration used in selective plates were as follows: DSM was 25 μg/ml; KM, SPCM, ABPC, OTC, and CP were at 50 μg/ml each; and SDMX was at 400 μg/ml.

Nalidixic acid (NA)-resistant *E. coli* were used as the recipient of conjugative R plasmid (Omae, 1982). Donor and recipient strains were cultured in heart infusion broth for 6 to 8 hr at 37 C; 1 ml of culture from the donor and 9 ml culture from the recipient were mixed, and incubated for 1 hr at 37 C with shaking. Later, 100 μl of the mixture was spread on each of the recipient selective medium (DHL agar containing 25 μg/ml of NA), donor selective medium (DHL agar containing 12.5 μg/ml of DSM, 25 μg each of KM, SPCM, ABPC, OTC, CP, and 400 μg/ml of SDMX), and transconjugate selective medium (DHL agar containing 25 μg/ml of NA and one of the donor drugs), After incubation for 18 to 24 hr at 37 C the colonies grown on each plate were selected and resistance pattern markers of the conjugants were confirmed after being cultured three times with transconjugate selective medium.

RESULTS

We collected 554 *E. coli* ioslates from 121 of the 1,819 fecal samples tested. We collected 279 *E. coli* from 54 of 984 whistling swans, 196 *E. coli* from 51 of 606 pintails and 79 *E. coli* isolates from 16 of 229 black-tailed gulls.

The frequency of resistance to each antimicrobial agent in *E. coli* varied by avian source of the bacteria and by drug type (Table 1). Collectively, *E. coli* from waterfowl were most resistant to SDMX, followed in decreasing order by SPCM, DSM, OTC, ABPC, KM, and CP. Zero or low resistance was found to CP and KM except for an *E. coli* from a whistling swan.

As a control, drug resistant *E. coli* isolated from feces of 54-day-old Hy-line chicks were tested. Strains of bacteria resistant to each drug were isolated more frequently from chicks than from waterfowl (Table 1). However, only 91% of the chick *E. coli* strains were resistant to SDMX compared to 100% in waterfowl (Table 1).

Resistance to more than one to six drugs, and various combinations of drug resistance patterns were observed in some waterfowl strains of *E. coli*.

In whistling swans, 224 (80%) of the drug

TABLE 2. Resistance patterns and conjugative R plasmids of *Escherichia coli* strains isolated from 279 whistling swans fecal samples, Japan, 1983 to 1986.

Original strains (before transmission)		R plasmids (after transmission)	
Resistance patterns[a]	Number of strains	Resistance patterns[a]	Number of strains
DSM, KM, SPCM, ABPC, OTC, SDMX	18	DSM, SPCM, ABPC, OTC	1
		DSM, KM, OTC	1
		SPCM, OTC,	1
		ABPC, OTC	4
		OTC, SDMX	1
		OTC	5
DSM, KM, ABPC, OTC, CP, SDMX	1	DSM, OTC	1
DSM, KM, SPCM, OTC, SDMX	13	DSM, OTC	1
		OTC, SDMX	1
		OTC	7
DSM, KM, OTC, SDMX	5	DSM, OTC	1
		OTC, SDMX	1
		OTC	3
DSM, SPCM, OTC, SDMX	1	OTC	1
DSM, SPCM, CP, SDMX	4	DSM, SPCM, CP	1
		SPCM, CP, SDMX	1
		SDMX	1
DSM, ABPC, OTC, SDMX	2	OTC, SDMX	1
		OTC	1
SPCM, ABPC, OTC, SDMX	8	ABPC, OTC	6
		OTC	2
DSM, SPCM, SDMX	3	not tested	
DSM, OTC, SDMX	2	OTC, SDMX	1
		OTC	1
SPCM, ABPC, SDMX	4	ABPC	2
SPCM, OTC, SDMX	11	OTC	11
DSM, SDMX	2	not tested	
SPCM, SDMX	13	not tested	
APBC, SDMX	1	ABPC	1
OTC, SDMX	10	OTC	8
SDMX	23	SDMX	3
Total	121	Total	69

[a] DSM, dihydrostreptomycin; KM, kanamycin; SPCM, spectinomycin; ABPC, ampicillin; OTC, oxytertacyclin; CP, chloramphenicol; SDMX, sulfadimethoxine.

resistant isolates had resistance to more than one drug. Resistance to two drugs was most prevalent ($n = 152$), followed by resistance to four ($n = 20$), three ($n = 20$), six ($n = 19$) and five ($n = 13$) drugs, respectively.

In pintail, 43 (22%) of 196 *E. coli* isolates had some drug resistance. Resistance to three drugs was most prevalent ($n = 25$), followed by resistance to two ($n = 10$) and four ($n = 8$) drugs, respectively.

In black-tailed gulls, 36 (46%) of 79 *E. coli* isolates had resistance to more than one drug. Resistance to two drugs was most prevalent ($n = 14$), followed by resistance to four ($n = 11$) and three ($n = 10$) drugs, respectively.

In total, 17, 12, and 13 resistance patterns were detected in drug resistant *E. coli* isolated from whistling swans, pintails and black-tailed gulls, respectively. Isolates resistant to only one drug all were resistant to SDMX.

Of 233 drug resistant strains, 128 (56%) of the isolates carried R plasmids (Table 2, 3, and 4). After conjugation, resistance of isolates to one to four drug patterns was observed with drug resistant *E. coli* isolated from whistling swans, while resis-

TABLE 3. Resistance patterns and conjugative R plasmids of *Escherichia coli* strains isolated from 196 pintail fecal samples, Japan, 1983 to 1986.

Original strains (before transmission)		R plasmids (after transmission)	
Resistance patterns[a]	Number of strains	Resistance patterns[a]	Number of strains
DSM, KM, SPCM, SDMX	1	not tested	
DSM, KM, OTC, SDMX	1	OTC	1
DSM, SPCM, ABPC, SDMX	2	DSM, SDMX	1
		SDMX	1
DSM, SPCM, OTC, SDMX	2	OTC	2
DSM, ABPC, OTC, SDMX	2	DSM, OTC	2
DSM, SPCM, SDMX	1	DSM	1
DSM, OTC, SDMX	23	DSM, OTC	4
		OTC, SDMX	2
		OTC	17
SPCM, ABPC, SDMX	1	not tested	
DSM, SDMX	1	not tested	
SPCM, SDMX	5	SDMX	2
OTC, SDMX	4	OTC, SDMX	1
		OTC	3
SDMX	17	not tested	
Total	60	Total	37

[a] DSM, dihydrostreptomycin; KM, kanamycin; SPCM, spectinomycin; ABPC, ampicillin; OTC, oxytertacyclin; CP, chloramphenicol; SDMX, sulfadimethoxine.

tance to only one or two drugs was observed with isolates from pintails and black-tailed gulls. Thus we isolated 11 resistance patterns in *E. coli* from whistling swans, six resistance patterns in pintails, and four resistance patterns in black-tailed gulls. Most resistance patterns were observed against OTC, followed by DSM, ABPC,

TABLE 4. Resistance patterns and conjugative R plasmids of *Escherichia coli* strains isolated from 79 black-tailed gull fecal samples, Japan, 1983 to 1986.

Original strains (before transmission)		R plasmids (after transmission)	
Resistance patterns[a]	Number of strains	Resistance patterns[a]	Number of strains
DSM, ABPC, OTC, CP, SDMX	1	OTC	1
DSM, KM, SPCM, SDMX	1	not tested	
DSM, SPCM, OTC, SDMX	4	OTC	4
DSM, APBC, OTC, SDMX	5	DSM, OTC	1
		OTC	4
SPCM, ABPC, OTC, SDMX	1	OTC	1
DSM, SPCM, SDMX	4	not tested	
DSM, OTC, SDMX	4	DSM, OTC	1
		OTC	3
SPCM, ABPC, SDMX	1	SPCM	1
SPCM, OTC, SDMX	1	OTC	1
DSM, SDMX	7	DSM	4
SPCM, SDMX	6	not tested	
OTC, SDMX	1	OTC	1
SDMX	16	not tested	
Total	52	Total	22

[a] DSM, dihydrostreptomycin; KM, kanamycin; SPCM, spectinomycin; ABPC, ampicillin; OTC, oxytertacyclin; CP, chloramphenicol; SDMX, sulfadimethoxine.

and SDMX. The frequency for SPCM was low (Tables 2, 3, and 4).

DISCUSSION

Wild animals with drug resistant bacteria (Niida et al., 1983) may be partly responsible for diseases linked to environmental pollution involving humans and other animals (Terakado et al., 1971). However, wild birds also may play a very important role in the spread of drug resistant bacteria (Kanai et al., 1981).

In Japan, drug resistant strains in wild birds usually have occurred among domestic birds (Sato et al., 1978, 1979; Kinjo, 1979; Kanai et al., 1981; Nakamura et al., 1982) but do occur among wild birds (Kanai et al., 1981).

Among the three species of birds we studied, drug resistant strains most frequently were detected in whistling swans. If infection by drug resistant strains was related to their feeding (Kanai et al., 1981), then rice paddies may be a source. However, their sources of food, and migratory routes still are unknown. After whistling swans, drug resistant strains in black-tailed gulls were next most frequent. These birds probably became contaminated because they stayed along the coast near the river in association with humans and domestic animals.

All strains were SDMX-resistant; this may have been caused by the feeding characteristics of these birds. The frequency of SDMX-resistant strains for chickens also was high.

We isolated KM- and CP-resistant strains of *E. coli* less frequently from migratory waterfowl than from domestic animals such as healthy or diseased calves, pigs and chickens (Hosoda et al., 1990). This difference may be because domestic animals are living in an environment contaminated with antimicrobial agents.

In the past, when *E. coli* were tested against either TC, SM, SA, or a combination of these drugs, *E. coli* from wild birds usually were resistant only to one of those drugs (Nakamura et al., 1982).

However, in the present study, many multiple drug resistant strains were observed in all three species of birds. Our findings resembled the drug resistance patterns observed among humans and domestic animals (Hosoda et al., 1990). Sato et al. (1978) reported that the *E. coli* from carrion crows (*Corvus corone*) and Japanese jungle crows (*Corvus macrorhynchos*) had R plasmids more frequently (77%) than Kanai et al. (1981) reported in other wild birds (9 to 15%).

Escherichia coli strains isolated from migratory waterfowl carried transmissible R plasmids at a similar high degree of frequency as in domestic mammals (Kanai et al., 1983b) and fowl (Kanai et al., 1983a). Isolates resistant to more than one drug also occurred at a prevalence similar to that reported from domestic animals and fowls. Because there was no difference observed in the detection of drug resistant strains in birds from 1983 to 1985, it may have been common for migratory waterfowl to have drug resistant strains and R plasmids at that time. Thus, birds may be responsible for the spread of R plasmids over a wide area.

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