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Epidemiological Survey of Drug-resistant *Escherichia coli* Isolated from the River Water in Kagoshima Prefecture

Karoku OKAMOTO, Eiji ISHITOBI and Junzo AMEMIYA
(*Laboratory of Veterinary Public Health*)
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Introduction

Kagoshima Prefecture is an area conspicuous for livestock-industry where about 300,000 cattle, 1,200,000 swine and 36,000,000 fowl have been raised per annum. The raw sewage excreted by those reaches the totals of approximately 17,100 tons, which are to be restored to agricultural farmland after being fertilized as compost, or to be burned up for the purpose of utilizing as a heat-source. After being disposed of in variety grades, most of the waste-water from the livestock-industry was allowed to be flowing into the river whose water was further to be utilized as drinking-water as well as for industrial or agricultural water.

Recent results of our surveillance study[12] revealed that the incidence of drug-resistant *E. coli* in the domestic animals had not decreased since the enforcement of the legal reajustment of drug usage for animal husbandry in 1976. In the increasing of resistant bacteria, the role of anti-microbial drugs for food additive is still a moot point[7,9,13,17]. Spread of drug resistant bacteria to the environment including river water has been put surveys in many countries[1-4,11,14-16] but less frequently in Japan[8], which makes this a matter of our primary concern.

The incidence of drug-resistant *E. coli* isolated from the river water in Kagoshima Prefecture and the frequency of R plasmid carriers among them are dealt with in this paper.

Materials and methods

Water samples collected from 34 major rivers in Kagoshima were kindly provided by Kagoshima Prefectural Institute for Environmental Science, and most of the samples from the two rivers, the Kotsuki and the Manose were collected by the authors from March to October in 1986. Five ml of samples was added to the same amount of Enterococcus Confirmary Broth (Eiken) in 2-fold concentration, and incubated for 48 hours at 44.5℃ in waterbath. An aliquot of those was subcultured on Eosin Methylen-blue Agar (Nissui) at 37℃ over night. The isolated typical *E. coli* was tested for drug susceptibility and for R plasmid by the methods previously reported[12]. The threshold concentrations for discriminating the resistance were as follows; 25 μg/ml of aminobenzyl penicillin (ABPC), kanamycin (KM), tetracycline (TC) or chroramphenicol (CP), 12.5 μg/ml of streptomycin (SM) and 800 μg/ml of sulfadimethoxine (SDM).

Results

E. coli was isolated from 198 (69%) out of 286 samples from the river waters, and drug-resistance was found in 215 (47%) out of 459 isolants. Frequency in isolation of *E. coli* from the

Manose was 81% and significantly more than those in the Kostuki (Table 1). Seventy eight percentage of isolants from the former and 30% of those from the latter were resistant to one or more drugs (Fig. 1). Similar tendency was noted in plasmid carrier; 53% and 32% of the resistant isolants carried R plasmid for some drug, respectively. On the other hand, the general water analyses showed that the Kotsuki was more contaminated than the Manose in the points of SS and coliforms. Samples from other rivers represented the results more or less similar to those of the Manose.

As to the resistance to individual drug, the incidences of resistance to SM, TC and SDM were significantly higher than other drugs, especially for the isolants from the Manose in which these incidences were over 60% (Table 2). Percentages of plasmid-carriers in the resistant isolants from the Kotsuki were significantly less than those of the Monose or others concerning to KM and TC (Table 3).

Out of 215 resistant isolants, 137 isolants (63%) were resistant to plural drugs (Table 4). Multiple-drug resistance was found in 18%, 63% and 32% of the isolants which held 60%, 81% and 44% of the resistant isolants from the Kotsuki, the Manose and others, respectively (Fig 1). Remarkably, 38 isolants from the Manose showed the resistant pattern of SM+TC+SDM which held over 50% of all the resistant isolants. Plasmid carriers for multiple drugs reached 37 (39%) out of

Table 1. Antimicrobial resistance of *E. coli* isolated from the river in Kagoshima and its relation to the general water quality

		Kotsuki River	Manose River	Others	Total
Total sample		210	36	40	286
Positive for *E. coli* (%)		141 (67.0)	29 (81.0)#	28 (70.0)	198 (69.0)
Total isolants		282	95	82	459
Resistant strains (%)		85 (30.1)#	74 (77.9)	56 (68.3)	215 (46.8)
R–plasmid carriers (%)		27 (31.8)#	39 (52.7)	28 (50.0)	94 (43.7)
Water analysis*	pH	7.5	7.1	7.5	
	BOD	1.8	1.5	3.7	
	COD	2.3	2.2	4.0	
	SS	14.8	4.3	14.0	
	Coliform	5.5×10^4	6.8×10^3	2.7×10^4	

* : These results were cited from the environmental white paper in Kagoshima 1983–84.

\# : Statistically different from other groups ($p<0.05$).

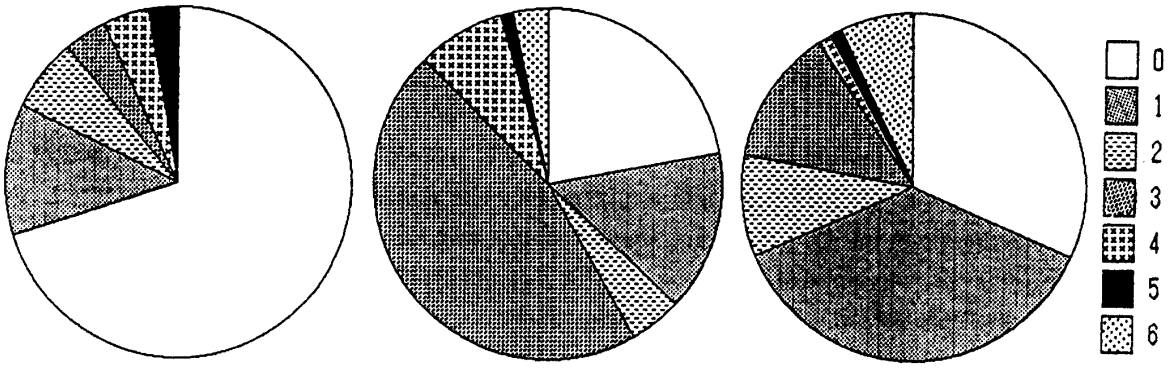

Fig. 1. Incidence of multiresistant *E. coli* isolants in water samples from the Kostuki (left), the Manose (middle) and other rivers (right) in Kagoshima. Figures indicate the number of drugs that the isolants are resistant to.

Table 2. Incidence of resistant isolants to individual drug

	Kotsuki River	Manose River	Others	Total
KM	11 (3.9)#	12 (12.6)	8 (9.8)	31 (6.8)
SM	43 (15.2)	57 (60.0)#	20 (24.4)	120 (26.1)
ABPC	24 (8.5)	14 (14.7)	13 (15.9)	51 (11.1)
CP	9 (3.2)#	5 (5.3)#*	7 (8.5)*	21 (4.6)
TC	62 (22.0)#	59 (62.1)	31 (37.8)*	152 (33.1)
SDM	47 (16.7)#	61 (64.2)	42 (51.2)	150 (32.7)

Difference of the shoulder abbrevation means statistical difference ($p<0.05$).

Table 3. Percentages of R plasmid carriers in resistant isolants

	Kotsuki River	Manose River	Others	Total
KM	0 (0)#	6 (50.0)	6 (75.0)	12 (38.7)
SM	9 (20.9)#	19 (33.3)#*	9 (33.8)*	37 (30.8)
ABPC	9 (37.5)	5 (35.7)	5 (38.5)	19 (37.3)
CP	2 (22.2)	3 (60.0)	2 (28.6)	7 (33.3)
TC	4 (6.5)#	19 (32.2)	11 (35.5)	34 (22.4)
SDM	13 (27.7)	19 (31.2)	14 (33.3)	46 (30.7)

Difference of the shoulder abbrevation means statistical difference($p<0.05$).

94 resistant isolants and the carriers for 3 or more drugs were significantly more frequently in the Manose or others than in the Kotsuki (Table 5).

No seasonal variation was found statistically in the incidence of *E. coli* or the frequency of drug resistance throughout the experimental period in the Kotsuki. The incidences of drug resistance among the points of the river in which the samples were collected, were differed each other (Fig. 2). The incidence was increased downstream in the Manose whereas relatively higher incidences were observed at some upper streams of the Kotsuki.

Discussion

Some cases of infection with the drug-resistant *Salmonella* were suspected to be caused by their transmission from animal to man[7], and the increasing outbreaks due to these organisms have been found in horses that were often more extensively treated with antibiotic therapy because of the economic value of individual animals[5]. Transmission of the resistance of these pathogens from enteric bacteria was assumed to have been carried out by R plasmid, and the contribution of water as a medium of spreading bacteria containing plasmid was discussed elsewhere[1-4,8,11,14-16]. In this report, examinations were executed on drug-resistance and R plasmid of *E. coli* strains isolated from the main rivers in Kagoshima including the Kotsuki and the Manose which are the sources of water supply for Kagoshima city (530,000 population) and Kaseda city (26,000 population).

Table 4. Resistance pattern and percentage in the resistant isolants from river water

No. of drugs	Resistance pattern						Kotsuki River		Manose River		Others		Total	
	KM	SM	ABPC	CP	TC	SDM	Number	Sum(%)	Number	Sum(%)	Number	Sum(%)	Number	Sum(%)
6	○	○	○	○	○	○		0 (0)	3	3 (4.1)	6	6 (10.7)	9	9 (4.2)
5		○	○	○	○	○	4						4	
	○	○	○	○		○	2	9 (10.6)		1 (1.4)	1	1 (1.8)	3	11 (5.1)
	○	○	○		○	○	2		1				3	
	○	○		○	○	○	1						1	
4		○	○		○	○	4		3				7	
	○	○	○		○		4	11 (12.9)	2	8 (10.8)		1 (1.8)	6	20 (9.3)
	○	○			○	○	2		1		1		4	
		○		○	○	○	1		2				3	
3		○			○	○	8		38		10		56	
	○	○			○				4				4	
			○		○	○	2	11 (12.9)		43 (58.1)		10 (17.9)	2	64 (29.8)
		○	○			○	1						1	
	○				○	○			1				1	
2					○	○	9		2		7		18	
		○				○	6		1				7	
		○			○		4	20 (23.5)	2	5 (6.8)		8 (14.3)	6	33 (15.3)
		○		○			1						1	
			○		○						1		1	
1						○	5		9		17		31	
					○		21				6		27	
			○				5	34 (40.0)		14 (18.9)	7	30 (53.6)	12	78 (36.3)
				○					5				5	
		○					3						3	
Sum (%)	31	120	51	21	152	150		85 (100.0)		74 (100.0)		56 (100.0)		215 (100.0)

Table 5. Pattern and percentage of R plasmid carrier

No. of drugs	R plasmid pattern						Kotsuki River		Manose River		Others		Total	
	KM	SM	ABPC	CP	TC	SDM	Number	Sum(%)	Number	Sum(%)	Number	Sum(%)	Number	Sum(%)
5	○	○	○	○		○		0 (0)		1 (2.7)		0 (0)	1	1 (1.1)
4		○	○		○	○			2				2	
	○	○	○	○				0 (0)		2 (5.1)	2	4 (14.3)	2	6 (6.4)
	○	○	○			○					1		1	
	○	○			○	○					1		1	
3	○	○			○				4				4	
		○			○	○	1	1 (3.7)	1	7 (17.9)	1	1 (3.6)	3	9 (9.8)
	○	○	○						1				1	
				○	○	○			1				1	
2		○				○	3		4		4		11	
		○			○		2		3				5	
			○			○	2	8 (29.6)		8 (20.5)		5 (17.9)	2	21 (22.3)
		○		○			1						1	
				○	○				1				1	
	○		○								1		1	
1						○	7		10		7		24	
					○		1		7		9		17	
			○				7	18 (66.7)	1	21 (53.8)	1	18 (64.2)	9	57 (60.6)
		○					2		3				5	
				○			1						1	
	○										1		1	
Sum(%)	12	37	19	7	34	46		27 (100.0)		39 (100.0)		28 (100.0)		94 (100.0)

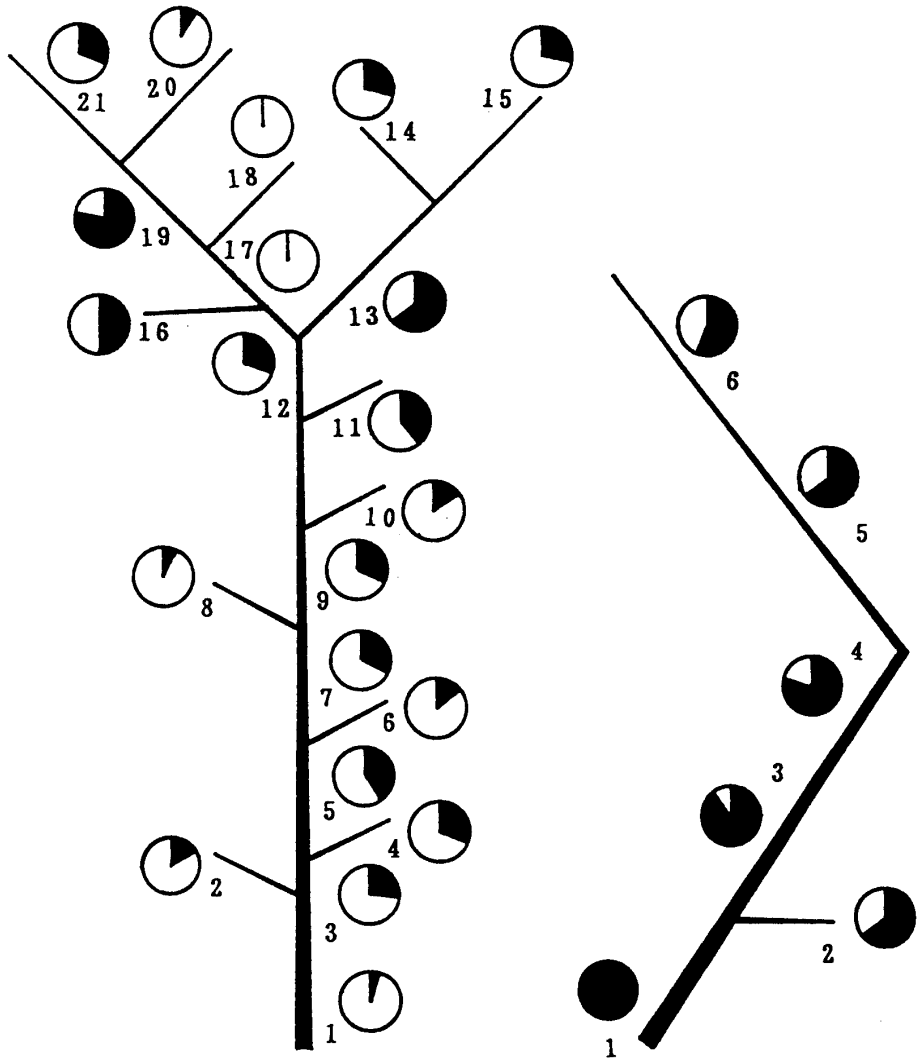

Fig. 2. Incidence of resistant *E. coli* (dark zone) in water samples at various points of the Kotsuki (left) and the Manose (right).

Forty-seven percentages of total isolants were resistant to some of 6 drugs examined and 64% of them were multiply resistant. The incidences of resistance were much higher in the Manose than in the Kotsuki, although the general water analyses showed better results in the former than in the latter. Moreover, some of the upper streams of the latter were contaminated more heavily than the lower stream of the same river flowing through the urbanized area. The higher incidence might be due to the pollution by waste-water from livestock industries because they are located at the basin of the stream showing high incidence. In our previous study about the isolants from cattle and swine[12], the incidence of resistance was found in 54% and and 100% of the isolants, and the percentage of multiple resistance of them was 52% and 94%, respectively. The resistant pattern of SM+TC+SDM was also characteristic in the isolants either from the river water or from feces of livestock.

In the reports of the survey on the river water polluted with effluent from cow stables or domestic wastes, about 40% of *E. coli* were resistant and half of them were resistant to three or more drugs[2]. A lower frequency of multiple resistance was reported in coliforms isolated from polluted river (less than 50%) compared with *E. coli* isolated from raw sewage (78%)[1]. It was reported that populations of multi-resistant coliforms in river water were differed depending on the urbanization of

their basins (7～33%) and those in the final effluent from sewage-treatment-plants were influenced by the types of plants (13～58%)[4]. The survival of *E. coli* in water has been revealed to be influenced by temperature, nutrients, sediments, UV light, or natural microbial flora[6,10]. Survival period of drug-r4sistant bacteria in water has been reported relatively shorter than those of sensitive organisms[15]. These reports are helpful to speculate the source of serious contamination with resistant bacteria and to speculate on the epidemiological significance.

The carrier of R plasmid held 43% of total resistant isolants and was more frequent in the isolants from the Manose than those from the Kotsuki. These frequencies were not less than those in our survey for livestock where 38 and 48 percentages of resistant isolants from cattle and swine possessed plasmid, respectively[12]. These high proportions of plasmid carrier recalled us the proposal that bacteria with R plasmid should be reckoned in future water quality deliberations and in fixing of discharge regulation[3]. On the other hand, Shaw and Cabelli[15] disagreed the proposal because the majority of the strains donated their plasmids at frequencies of less than 10^{-4} per donor cells, while 60% of the multiresistant *E. coli* holding 8.3% of the strains isolated from river water were capable of transferring all or part of their resistance. It should be noted that the incidence of resistance in our study was much higher than their report, although we did not examine the transfer-frequencies of R plasmid, yet.

It was reported that UV light irradiation effectively reduced the number of coliforms in waste-water effluents but, at the same time, the percentages of population resistant to TC or CP was increased after irradiation by the mechanism attributable to R plasmid[11]. In this aspect, further studies on sewage treatment should be carried out to reduce the drug resistant population in river water. Attentions should be paid on the possible spread of drug-resistant enteric bacteria over the environment as well as on to the other dangerous pathogens which have been controlled strictly according to the laws from the viewpoint of human and animal hygiene.

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Summary

Surveys were made on the distribution of resistance to antimicrobial drugs among the *E. coli* isolants from the river water in Kagoshima Prefecture. Out of 286 samples collected from 34 major rivers, 69% were positive for *E. coli* and 47% of 415 isolants were resistant to one or more drugs. The resistant pattern of SM+TC+SDM was most frequent among the multiple resistant strains holding 64% of resistant isolants. Forty-four percentage of resistant isolants carried R plasmid and 39% of them were for plural drugs. Isolation frequency of *E. coli*, incidence of resistance and percentage of plasmid carrier were significantly higher in the Manose than in the Kotsuki. This might be attributable to livestock-industries, because the number of livestock was more numerous in the former basin than in the latter.

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