沖縄の家畜由来細菌の薬剤耐性：I.
豚および鶏の糞便由来大腸菌について(畜産学科)

Drug Resistant Strains of Bacteria Isolated from Domestic Animals in Okinawa

I. Fecal *Escherichia coli* from pigs and chickens

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I INTRODUCTION

Various antimicrobials have received widespread popularity as additives of animal feeds for growth promotion purposes and for diesease prevention. It is obvious that rapid increased yield of animal products is much indebted to these antimicrobials.

However, the continuous use of antimicrobial drugs in animals for various purposes results in a significant increase in the number of drug resistant bacterial strains (4,5).

Furthermore, it is noticeable that a majority of the drug resistant strains carry transferable R factors (1,3,5,7~10).

Such drug resistant bacterial strains carring R factors in animals may be able to transmit to human beings through contaminated animal products such as milk, meat, egg and others, and also be able to transfer their resistance to the resident bacterial strains in a human alimentary tract (6). The antimicrobials, in consequence, are loosed of their therapeutic value in man.

From above point of view, disorderly use of antimicrobials for various purposes in animals have a tendency to take precaution (2). Now, these antimicrobials have also been used commonly for livestock in Okinawa. However, no reports have been made concerning the distribution of drug resistant bacteria and also R factors among strains of animal origins.

In the present study, the authors, firstly, tried to determine how these drug resistant bacteria are widespread in the fecal *Escherichia coli* isolated from healthy pigs and chickens in Okinawa.

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II MATERIALS AND METHODS

1. Isolation of fecal *E. coli* from pigs and chickens

The strains of *E. coli* used were collected from apparently healthy pigs and chickens who were fed with commercial feeds, in all over the island of Okinawa.

To isolate *E. coli*, rectal swabs were inoculated onto BTB-lactose agar plates. Two or three typical lactose-fermenting colonies per each sample were picked up and identified by the IMViC system.

The strains confirmed *E. coli* which were used for following studies were 175 strains from pigs and 179 from chickens, respectively.

2. Antibiotics used

The following 5 antibiotics were used in the testing system ; aminobenzyl penicillin (PC, Meiji) , dihydrostreptomycin sulfate (SM, Meiji), oxytetracycline hydrochloride (TC, Pfeizer), chloramphenicol (CM, Sankyo) and kanamycin sulfate (KM, Yamanouchi) .

3. Antibiotics sensitivity test

The sensitivity of bacterial strains to antibiotics was tested by agar plate dilution method.

Each antibiotic was made 2 fold dilution with physiological saline and incorporated into melted agar medium in 1 / 10 volume at concentrations indicated in the text. The melted agar thus prepared was poured into petri dishes and made plates. Thereafter, the agar plate was inoculated by streaking on it a loopful of the nutrient broth culture of each *E. coli* strain to be tested.

The inoculated plates were incubated at 37°C for 18 hours, and the presence or absence of growth was scored. When no growth was observed at concentration of 25 micrograms per 1 *ml*, the strain was recorded as resistant.

III RESULTS

1. Drug resistant fecal *E. coli* from pigs

A total of 175 strains isolated from pigs were tested for drug resistance and the results obtained were summarized in table 1.

Table 1. Drug resistance patterns of fecal *E. coli* strains isolated from pigs in Okinawa

Resistance patterns		Number of strains	Per cent	Number of strains in each group	Per cent
5	PC TC SM CM KM	3	1.7	3	1.7
4	PC TC SM CM	2	1.1	68	38.8
	PC TC SM KM	63	36.0		
	PC TC CM KM	0	0		
	PC SM CM KM	0	0		
	SM CM TC KM	3	1.7		
3	PC TC SM	53	30.3	75	42.9
	PC TC CM	0	0		
	PC CM SM	0	0		
	PC TC KM	7	4.0		
	PC SM KM	4	2.3		
	PC CM KM	0	0		
	TC SM CM	3	1.7		
	TC SM KM	8	4.6		
	TC CM KM	0	0		
	SM CM KM	0	0		
2	PC TC	5	2.9	26	14.9
	PC SM	7	4.0		
	PC CM	0	0		
	PC KM	2	1.1		
	TC SM	10	5.8		
	TC CM	0	0		
	TC KM	2	1.1		
	SM CM	0	0		
	SM KM	0	0		
	CM KM	0	0		
1	PC	2	1.1	3	1.7
	TC	1	0.6		
	SM	0	0		
	CM	0	0		
	KM	0	0		
0	Sensitive	0	0	0	0
	Total	175	100	175	100

No strains were found to be sensitive for all 5 antibiotics tested, namely, all strains were drug resistant to one or more antibiotics.

A noteworthy fact was that among 175 strains, 172 (98.3%) were found to be multiple drug resistance. Of these drug resistant strains, 75 (42.9%) were triple-, 68 (38.8%) were quadruple-reistant and other 3 (1.7%) were resistant to all 5 antibiotics used, and such strains, resistant to 3 or more antibiotics, were account for 83.4% of all strains tested.

The drug resistance patterns and isolation frequencies of these strains were also shown in table 1.

The most common resistance pattern was PC-TC-SM-KM which was found in 63 strains (36.0%), the 2nd common was PC-TC-SM in 53 strains (30.3%) and followed by TC-SM in 10, TC-SM-KM in 8, in decreasing orders.

2. Drug resistant fecal *E. coli* from chickens

The isolation frequencies and resistance patterns among 179 strains tested were shown in table 2.

Table 2. Drug resistance patterns of fecal *E. coli* strains isolated from chickens in Okinawa

Resistance patterns		Number of strains	per cent	No. of strain in each group	Per cent
5	PC TC SM CM KM	2	1.1	2	1.1
4	PC TC SM CM	1	0.6	1	0.6
	PC TC SM KM	0	0		
	PC TC CM KM	0	0		
	PC SM CM KM	0	0		
	SM CM TC KM	0	0		
3	PC TC SM	98	54.7	102	56.9
	PC TC CM	0	0		
	PC CM SM	0	0		
	PC TC KM	4	2.2		
	PC SM KM	0	0		
	PC CM KM	0	0		
	TC SM CM	0	0		
	TC SM KM	0	0		
	TC CM KM	0	0		
	SM CM KM	0	0		
2	PC TC	63	35.2	64	35.8
	PC SM	0	0		
	PC CM	0	0		
	PC KM	0	0		
	TC SM	1	0.6		
	TC CM	0	0		
	TC KM	0	0		
	SM CM	0	0		
	SM KM	0	0		
	CM KM	0	0		
1	PC	8	4.5	10	5.6
	TC	2	1.1		
	SM	0	0		
	CM	0	0		
	KM	0	0		
0	Sensitive	0	0	0	0
	Total	179	100	179	100

Of these, all strains showed resistance to one or more antibiotics used. Multiple resistant strains accounted for 94.4% (169 strains) of all strains tested. Furthermore, 102 of 169 were triple resistance which corresponded to 56.9% of total strains.

In these cases, the triple resitance pattern of PC-SM-TC was found most frequently in 98 strains (54.7%). Secondly, the double reistance pattern of PC-TC was found in 63 strains (35.2%).

It was a noticeable fact that the strains belonged to above 2 types of resistance pattern took 89.9% of total strains. However, quadruple or more resistant strains were found in only 3 (1.7%) which was different from those of pigs.

IV DISCUSSION

The purpose of present survey was to determine how widespread drug resistant *E. coli* strains were in animal population in Okinawa. In spite of use antimicrobials for feed additives for long years in Okinawa, no investigations from above point of view have been performed. This was a reason why the authors have to start present survey.

Though a number of animal species, individuals, *E. coli* strains and antibiotics used were not sufficient to solve the question, a state of distribution of drug resistant bacteria in animal population in Okinawa could roughly be grasped.

All fecal *E. coli* strains tested which were isolated from apparently healthy pigs and chickens were resitant to one or more antibiotics used, that was, PC, TC, SM, CM and KM.

The strains of multiple resistance to 3 or more antibiotics were isolated in 83.4% from pigs and 58.6% from chickns, respectively.

Mitsuhashi and his co-workers (7,9) reported that all *E. coli* strains isolated from 151 pigs and 38% from 108 chickens were resistant to TC, CM, SM and sulfanilamide, or certain combinations thereof. They also reported that among 278 resistant strains isolated from pigs, 87% were of multiple resistance and among 54 resistant strains of chickens, 76% were also of multiple resistance. Furthermore, they confirmed that among these resistant strains, 40% of pigs and 22% of chickens carried R factors.

A recent surveys by Terakado et al (1972) , Kashiwazaki et al (1972) also indicated that a high insidence of drug resistance, mostly of infective type, was found among strains of *E. coli* isolated from pigs.

From the above findings, it was suggested that a high incidence of drug resistant *E. coli* strains did exist in animal being exposed to continuous levels of antimicrobial drugs and also that strains carried R factors were usually prevalent among pigs and chickens in Japan. Similar results were obtained and reported in other countries (4,6).

The results obtained in this study confirmed that drug resistant *E. coli* strains were similarly widespread in these animals in Okinawa. The authors, however, could not show in the present report that the distribution of R factors among *E. coli* strains tested. This problem is now under investigation.

V SUMMARY

A survey for drug resistance was conducted among fecal *Escherichia coli* strains isolated from apparently healthy pigs and chickens in Okinawa.

The results obtained were as follows :

1. All strains tested, 175 from pigs and 179 from chickens, were reistant to one or more antibiotics used ; penicillin (PC) , streptomycin (SM) , chloramphenicol (CM) , tetracycline (TC) or kanamycin (KM) .

2. Of 175 strains of pigs, 172 (98.3%) were of multiple resistance, in which triple resistant strains were obtained most frequently in 42.9%, and follwed by quadruple resistant (38.8%) and double (14.9%) .

3. The most common resistance configuration observed was the quadruple pattern of PC - TC - SM - KM (36.0%) and followed by triple of PC-TC-SM (30.3%) , double of TC-SM (5.8%) and triple of TC-SM-KM (4.6%) in a decreasing orders.

4. Among 179 strains isolated from chickens, 169 were found to be multiple resistant. Of these multiple resistant strains, 102 (56.9%) were triple resistant and 64 (35.8%) were double.

5. In the case of chicken strains, the most common resistance configuration observed was triple pattern of PC-TC-SM which corresponded to 54.7% of all strains tested. The second most frequently occurring resistance pattern was double resistance of PC-TC (35.2%).

6. It was suggested from above findings that the incidence of multiple resistant strains was widespread among pigs and chickens in Okinawa.

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沖縄の家畜由来細菌の薬剤耐性

I. 豚および鶏の糞便由来大腸菌について

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要　　約

沖縄各地に飼育されている健康な豚および鶏の糞便より大腸菌を分離し，ペニシリン（PC），ストレプトマイシン（SM），クロラムフェニコール（CM），テトラサイクリン（TC），エリスロマイシン（EM），カナマイシン（KM）など6種の抗生物質に対する感受性試験を寒天平板希釈法で実施した。得られた成績は以下の如くである。

1. 供試した菌株，豚由来の175株および鶏由来の179株，何れも使用した抗生物質の1つあるいはそれ以上に耐性を示した。

2. 豚由来大腸菌175株中172株(98.3%)が多剤耐性株で，うち3剤耐性株が全体の42.9%と優位を占め，次いで4剤耐性の38.8%，2剤耐性14.9%の順であった。

3. これら耐性菌の耐性型は，PC-TC-SM-KMの4剤およびPC-TC-SMの3剤耐性型が多く，それぞれ36.0%および30.3%を示した。その他，TC-SM型が5.8%，TC-SM-KM型が4.6%得らたた。

4. 鶏由来179株については，169株が多剤耐性株で，これらのうち102株，全体の56.9%が3剤，64株，35.8%が2剤耐性株であった。

5. 鶏由来の耐性株中，最も高頻度にみられる耐性型はPC-TC-SMの3剤耐性型で全株の54.7%を占めた。次いで，PC-TCの2剤型が35.2%であった。

6. 以上の成績より，沖縄の豚，鶏の間にも多剤耐性株が高率に分布しているといえる。

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