O GROUPS AND ANTIBIOTIC SENSITIVITY OF *ESCHERICHIA COLI* ISOLATED FROM DISEASED CHICKENS*

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Reports on the serotypes of *Escherichia coli* associated with the diseases of animals were reviewed by SOJKA. In Japan, few reports have been published on this problem in poultry. MIKAMI stated that the majority of the 672 strains of *E. coli* isolated from normal day-old chicks taken from 3 farms in Hokkaido belonged to the antigenic groups O60, 8-18, Y813, O53 and O1, while strains of O2 were hardly seen. INOUE et al. isolated the following strains of hemolytic *E. coli* from 3- and 7-month-old chickens showing hemorrhagic symptoms; O15, O21 and O88. Each strain was seen only once. SATO et al. indicated that the most dominant O groups of *E. coli* which were isolated from 45 chickens infected with *Mycoplasma gallisepticum* were O2 and O78. SAKAZAKI & YOSHIMURA found that more than one third of the strains of *E. coli* isolated from diseased chickens belonged to the strain O2a : K1.

This paper deals with O groups and antibotic sensitivity of *E. coli* strains isolated from chickens on Hokkaido.

MATERIALS AND METHODS

Disease status of T farm The chickens involved in this study were submitted by the T farm in Hokkaido to this department during the 12 months period from April 1964 to March 1965. They were all from a closed flock of primarily New Hampshire (NH) breeding and they were raised on a commercial feed product. Coccidiosis, infectious coryza, CRD, fowl pox and so-called lymphomatosis were prevalent on this farm, but salmonellosis, infectious bronchitis, infectious laryngotracheitis and Newcastle disease were not recognized at the time of our study. One-half of the 242 chickens examined developed respiratory symptoms and 116 (74.4%) of the 156 serum samples taken showed positive agglutination to *Mycoplasma gallisepticum*.

E. coli cultures tested Cultures were taken from the heart, liver, spleen, kidneys

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and lungs of 242 birds, 220 of which were sacrificed and 22 which were dead. The trachea, air-sacs and infraorbital sinuses were examined for inflammatory changes and cultures were taken when any abnormality was found. Thus, 251 strains of *E. coli* were obtained from 89 of diseased chickens necropsied. These 89 birds included both broiler and stock chickens. Twenty-seven strains of *E. coli* were cultivated from 6 diseased chickens ageing from 6 to 30 days, 86 from 25 birds of 31~70 days, 81 from 38 birds of 71~150 days and 57 from 20 adult birds. The biochemical characteristics of these cultures were tested by the routine methods (tab. 1).

TABLE 1 *Biochemical characteristics of the 251 isolates*

SUBSTRATE OR TEST	POSITIVE	%
Cytochrome oxidase	0	0
Nitrate reductase	251	100
Glucose	251	100
Gas from glucose	245	97.6
Lactose	234	93.2
Indol	243	96.8
Methyl red	251	100
Voges-Proskauer	0	0
Citrate, Simmons'	0	0
" , Christensen's	173	68.9
Hydrogen sulfide	0	0
KCN broth	0	0
Motility	139	55.4
Hemolysis, sheep red cell	1	0.4

TABLE 2 *Composition of the pooled antisera*

POOLED ANTISERA	O GROUPS INCLUDED
A	113, 137, 139
B	15, 69, 71, 73, 125, 126
C	78, 131, 140, 141
D	8, 21, 22, 28 $^{a}_{c}$, 44
E	55, 75, 86, 144, 8-18*
F	1, 6, 11, 83, 111, Y813*
G	2, 5, 25, 53, 60
H	88, 109
I	16, 26, 138
J	3, 18, 54, 112$^{a}_{c}$
K	4, 115

* Undetermined O groups: Isolated from diseased chickens by MIKAMI (1964)

Antisera for typing The standard strains of *E. coli* employed for antisera production were kindly supplied by Dr. SAKAZAKI. Two strains of undetermined O groups, 8–18 and Y 813, isolated by MIKAMI from chickens showing systemic infection were also used. These O groups are listed in table 2. The O antiserum was prepared by immunizing rabbits with chrome-alum treated cells of *E. coli* according to SAKAZAKI & NAMIOKA. However, in this study, suspensions of *E. coli* cultures were heated at 120°C for 2.5 hours and used for preparing some of the antisera. A total of 11 lots of pooled antisera were prepared by mixing antisera of 2 to 6 different O groups so that antisera of individual lots have the same agglutination titers (tab. 2.).

Antigen for O grouping Saline suspensions of the isolates were heated at 100°C for 1 hour and then washed twice with saline. Then a 0.5% phenol saline was used to adjust the density equivalent to a #3 McFarland's tube. When the antigens were not agglutinated by any lot of the pooled antisera, the suspensions were heated to 120°C for 2.5 hours to destroy any possible type A K antigen which might interfere with the O agglutination.

O grouping by the single tube agglutination test O grouping of the strains was conducted in two steps. At first, each strain was tested with the pooled (A～K) antisera to estimate the O group to which it belonged. Secondly, the O group of each strain was determined by the O serum composing the pooled serum which agglutinated the antigen. The following procedure was used for the agglutination test: 0.5 ml of the antigen to be tested was added to 0.5 ml of O antiserum which diluted to the titer of 1 tube lower than the end titer of complete agglutination. For example, if the final agglutination titer of a certain antiserum was 1 : 12,800, it was diluted 1 : 6,400 for the test. After 18 to 20 hours incubation, in a water bath at 50°C, the results were recorded.

Determination of K antigen The determination of K antigens of strains isolated from coli-granuloma like lesions (1 pullet) was made according to SAKAZAKI & NAMIOKA, EDWARDS & EWING[5] and SOJKA. Anti-OK serum was obtained from 2 male NH chickens injected with whole cells of an isolate treated with chrome-alum. The K antigens were demonstrated in cultures by an O inagglutibility test with formalized chrome-alum treated cells. The OK antiserum absorption test was done with both phenolized whole cells and O antigens, heated to 100°C for 1 hour.

Sensitivity test for antibiotics The antibiotics used in the sensitivity test were tetracycline (TC), streptomycin (SM) and chloramphenicol (CP). Each nutrient broth culture was incubated for 18 hours at 37°C, then one drop of each culture was used to inoculate on Sodium laurylsulfate lactose agar plates containing 25 mg of TC (25 γ/ml), 50 mg of SM (50 γ/ml) and 50 mg of CP (50 γ/ml) per 1,000 ml, respectively and on control medium without any antibiotics. The cultures which did not show any colony growth on agar containing antibiotics were judged as sensitive. The results were read after 18 hours' incubation at 37°C.

RESULTS

O grouping of the chicken strains Of the 251 strains isolated, 147 (58.6%) were classified into 17 O groups. Of this strains, 120 (81.6%) were classified into one of the

TABLE 3 *O grouping of the E. coli isolated from diseased chickens*

O GROUPS	NO. OF STRAINS ISOLATED		%	
O2	54	(19)*	21.5	
O78	26	(8)	10.4	
O8	21	(13)	8.4	
O1	19	(4)	7.6	
8-18	9	(4)	3.6	
O109	3	(3)	1.2	
O140	3	(3)	1.2	
O53	2	(2)	0.8	
O88	2	(2)	0.8	
O21, O25, O26, O54, O73, O131, O139, Y813	1	respectively	0.4	respectively
Total of typable, 17 O groups	147		58.6	
Untypable	104		41.4	

* ( ): No. of chickens of positive cultures

following four O groups; O1, O2, O8, and O78 (tab. 3). All 19 strains of O1 originated from chickens of broiler flocks. It was interesting to note that the strains taken from kidney and lung tissue belonged to the O groups 10 and 8 respectively. On the other hand, the strains found in other organs belonged to 6 or less different O groups. These findings suggest that both the kidneys and lungs are exposed to environmental contamination by *E. coli* more frequently than the other organs. Of the 17 chickens yielding *E. coli* from almost all tissues examined, 9 showed systemic infections with single O groups of O1, O2 and O78. In general, strains of the same O group isolated from the same bird had uniform biochemical characteristics and antibiotic sensitivities.

Serotypes from coli-granuloma like lesions Two of the four strains isolated from one chicken showing coli-granuloma like lesions were of the serotype O8:K? (L):H- similar to ULBLICH's description, and the remaining strains belonged to O2. Seven of the nineteen non-motile strains classified to O8 in this study had the same K antigen as the L type, which were isolated from 6 chickens not having coli-granuloma like lesions.

Cross reaction in O grouping One-side reaction was observed with the antigens and antisera of the standard and test strains. This is shown in table 4. The following isolates were agglutinated simultaneously with their respective antisera and used as test strains; 21 strains of O8 with O60 antiserum, 9 strains of 8-18 with O4 and O115 antisera, and one strain of Y813 with O4 antiserum. Unlike the O140 standard strain, two of the three O140 isolates were agglutinated with O53 antiserum, but by the absorption test they

TABLE 4 *Antigenic relationships among the standard or test "O" groups of E. coli used in this study*

O ANTISERUM	RELATIONSHIPS WITH OTHER "O" GROUPS	
	Reciprocal	Unilateral
3	4	—
4	3	—
8	—	60
18	—	139
25	—	109
53	—	4
71	—	5
73	—	3
83	—	21 & 22
131	—	83
8–18	—	4 & 115
Y813	—	4

were found to be of O140 O grouping. Of the 104 unclassified strains, 5 (from 2 chickens) were agglutinated simultaneously with O2, O109 and O137 sera, and 5 others (also 2 birds) with O18, O139 and Y813 sera. These 10 isolates could not be grouped into specific O groups by the sera absorbed with these isolates and the standard or test strains (O2, O109, O137; O18, O139, Y813) for the antiserum preparations.

Antibiotic sensitivity of the chicken strains Table 5 shows that TC resistant strains were the most common resistant strains found. On the other hand, no CP resistant strains were found. In correlating O groups to antibiotic resistance, 89.5% (17 of 19) of the O1 strains were resistant to TC, 57.1% (12 of 21) of the O8 strains were resistant to TC and to both TC and SM. However, only 7.4% (4 of 54) of the O2 strains were resistant to TC and SM, and 15.4% (4 of 26) of the O78 strains to both TC and SM.

TABLE 5 *Antibiotic sensitivity*

RESISTANT TO	NO. OF STRAINS
Tetracycline (25γ/ml)	64 (25.5%)
Streptomycin (50γ/ml)	1 (0.4%)
Chloramphenicol (50γ/ml)	0 (0 %)
Tetracycline (25γ/ml) & Streptomycin (50γ/ml)	12 (4.8%)
Total	77 (30.7%)

DISCUSSION

Our isolates of *E. coli* had biochemical characteristics similar to those described by EDWARDS & EWING[5] and SOJKA. We did however find some differences between the definition of *E. coli* given by the Enterobacteriaceae Subcommittee and the properties of our isolates.

SOJKA & CARNAGHAN reported that 401 of 797 strains of *E. coli* that they isolated from chickens, turkeys and other birds, suffering from various diseases, were typed into the following groups O2 (199 strains), O8 (56), O1 (54), O78 (45), O71 (17), O11 (12), O22 (4), O73 & O138 (each 3) and O103 & O140 (each 0); and the remaining 396 (49.8%) strains were not typable with any of the above-mentioned 11 antisera. However, 40.7% (99 of 243) of the strains isolated from chickens with coli-septicaemia, belonged to O2, 26 (10.7%) to O78, 24 (9.9%) to O1, 8 (3.3%) to O71 with remainders belonged to 21 of the 141 O groups, and only 31 (12.8%) of which were not typable. Our findings concur with those of EDWARDS & EWING[4], GROSS, GLANTZ et al. and some others[1,3,8,10,11] who reported that the dominant O groups of the *E. coli* isolated from diseased birds belonged to the groups O1, O2, O8 and O78.

MIKAMI examined O groups of the 672 strains of *E. coli* isolated from internal organs, unabsorbed yolks and droppings of normal day-old chicks submitted by farms in Hokkaido including the T farm. One half of the strains that he isolated originated from the T farm. He stated that the dominant O groups of *E. coli* found were O60 (139 strains, 20.7%), 8-18 (69, 10.3%), Y813 (62, 9.2%), O53 (45, 6.7%) and O1 (34, 5.1%). According to MIKAMI only 18 strains (2.7%) belonged to the group O2 and the group O78 was not isolated. In the present study, the prevalent O groups found were O2 (21.5%), O78 (10.4%), O8 (8.4%) and O1 (7.6%), but the groups 8-18, Y813 and O53 were negligible. Group O60 was not isolated, even though our cultures were taken from the same source as MIKAMI's. This discrepancy between MIKAMI's results and ours did not seem to be due to either the difference of sampling methods used (MIKAMI collected 2 strains both from one of the visceral organs and the unabsorbed yolk, and 2 from droppings per chick) or the age difference of the chickens examined. We did conclude that the discrepancy may due to the difference in the condition of the chickens involved, normal or diseased, and/or the unilateral reactions found in some of the antisera used. The low frequency of group O2 isolates, in MIKAMI's study, appears to have been caused by the fact that his strains originated from normal chicks. Furthermore, some of O60 groups from MIKAMI's stock cultures were agglutinated by both O8 and O60 antisera at their end titiers, but they were not agglutinated with O60 antiserum which was absorbed

with O8 antigen. MIKAMI used only unsaturated O60 antiserum.

Although there have been reports[2,9] of coli-granuloma lesions in birds since HJÄRRE & WRAMBY isolated regular mucoid cultures of *E. coli* from tuberculoid granulomatous lesions in the intestines and liver of birds. There has been no report of *E. coli* isolation from coli-granuloma lesions in Japan. However in the present study, two of the isolates from coli-granuloma like lesions in a chicken belonged to one of the three O groups of coli-granuloma strains described by ULBRICH and these isolates had an undetermined L type of K antigen.

In this study, all of the 251 cultures were sensitive to chloramphenicol (CP), while 64 strains (25.5%) were resistant to tetracycline (TC), 1 (0.4%) to streptomycin (SM) and 12 (4.8%) to both TC and SM.. MIKAMI and SAKAZAKI et al. also studied the antibiotic sensitivity of *E. coli* in chickens using the same drug concentrations as we did in this study. MIKAMI stated that 181 (26.9%) of the 672 strains that he isolated were resistant to TC, 3 (0.4%) to SM, 40 (6.0%) to both TC and SM, and all were sensitive to CP, the greater part of which was isolated from the same farm as in this study. SAKAZAKI et al. cultured the droppings from a total 640 chickens in the districts of Hokkaido, Nagano, Osaka and Okayama, respectively, which yielded *E. coli* strains sensitive to CP, but 24～53% of the specimens yielded strains resistant to TC, 3～5% to SM and approximately 10% to both TC and SM. We concluded then from our studies that the strains of *E. coli* isolated from the T farm showed the same antibiotic sensitivity as the strains of *E. coli* isolated from a general survey of Japan.

SUMMARY

By using *Escherichia coli* antisera from 45 different O groups, 157 (58.6%) of the 251 strains isolated, from diseased chickens, were classified into 17 O groups. The prevalent O groups found were O2 (54 strains, 21.5%), O78 (26, 10.9%), O8 (21, 8.4%) and O1 (19, 7.6%); these strains of 4 O groups comprised 81.6% of the typable strains and 47.9% of the total 251 strains. The serotype O8 : K ? (L) : H- was isolated from a chicken showing coli-granuloma like lesions. All of the 251 strains were sensitive to chloramphenicol, but 64 strains (25.5%) were resistant to tetracycline, 1 (0.4%) to streptomycin and 12 (4.8%) to both tetracycline and streptomycin.

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