BRIEF NOTE

**Low Incidence of *Enterobacteriaceae* in Wild Ducks (*Aythya* spp.)
and Antibigrams of the Isolates**

Gihei SATO and Masayoshi ASAGI

*Department of Veterinary Public Health, Obihiro University of Agriculture
and Veterinary Medicine, Obihiro-shi, Hokkaido 080*

Hiroshi YAMAMOTO

*Department of Veterinary Microbiology, Faculty of Agriculture,
Tottori University, Tottori-shi, Tottori 680*

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Antibiograms of *Enterobacteriaceae* isolated from wild birds and animals have been examined to clarify the ecology of the R plasmid. We [6] reported that pigeons and crows had antibiotic-resistant *Escherichia coli* with high frequency and that many of the resistant isolates had conjugative R plasmids. However, to our knowledge, there has been no report on the antibiogram of *Enterobacteriaceae* isolated from wild ducks (*Aythya* spp.). This paper deals with the low incidence of *Enterobacteriaceae* with or without drug resistance in the ducks.

Forty-four ducks used for this study were accidentally caught in fishing nets and died in Shinjiko lake in Shimane Prefecture of Honshu island from Dec. 1977 to Feb. 1978. They had been kept at -20°C until the present examination. In Japan, the wild ducks are classified into 6 species [4]. In this study, however, species of the birds were not identified.

All the birds were examined for *Enterobacteriaceae* including *E. coli*, *Salmonella* and others. A loopful of the contents of the lower part of the intestine of each carcass was spread on deoxycholate-hydrogen sul-

fide-lactose agar (DHL, Eiken) plates with or without the addition of chloramphenicol (CP; $25\text{ }\mu\text{g/ml}$), tetracycline (TC; $25\text{ }\mu\text{g/ml}$) or streptomycin (SM; $12.5\text{ }\mu\text{g/ml}$), and incubated overnight at 37°C . The antibiotics were added to the agar media to select drug-resistant bacteria. A loopful of the intestinal contents was also placed into 4 ml of heart infusion broth (Eiken) for enrichment culture and incubated overnight at 37°C . A loopful of the heart infusion broth cultures was subcultured onto DHL agar plates with or without the addition of CP, TC or SM and incubated overnight at 37°C . Approximately 1 cm of the lower part of the intestine was then cut lengthwise, placed into about 15 ml of selenite brilliant green broth (SBG, Nissui) for isolation of *Salmonella*, and incubated overnight at 43°C . A loopful of the SBG broth cultures was spread on brilliant green agar plates (Eiken) and incubated overnight at 37°C . Two to ten colonies grown on each DHL agar plate were examined for *Enterobacteriaceae*. At least ten colonies per bird were used for this purpose. The identification of the isolates was made according to the procedure of Cowan [2] and

Table 1. Isolation of *Enterobacteriaceae* from wild ducks (*Aythya* spp.)

No. of bird harboring <i>Enterobacteriaceae</i>	Cultivation*	Species of isolates	Drug resistance pattern** of isolates	Detection of conjugative R plasmid
K3	Direct and enrichment	<i>Enterobacter cloacae</i>	CER. APC. CL	—
K39	Enrichment	<i>Citrobacter intermedius</i> biovar b	Sensitive	•
K42	Direct and enrichment	<i>Enterobacter cloacae</i>	CER. APC. CL	—

* Cultivation procedure by which *Enterobacteriaceae* could be isolated.

** CER, cephaloridine ; APC, aminobenzyl penicillin ; CL, colistin.

Buchanan and Gibbons [1]. The drug-sensitivity tests were conducted by the agar dilution method [5]. Thirteen kinds of drugs were used for the tests as follows: TC and CP (25 $\mu\text{g}/\text{ml}$); SM and spectinomycin (12.5 $\mu\text{g}/\text{ml}$); sulfadimethoxine (800 $\mu\text{g}/\text{ml}$); kanamycin, aminobenzyl penicillin, gentamicin, cephaloridine (CER), nalidixic acid and rifampin (25 $\mu\text{g}/\text{ml}$); colistin (CL; 12.5 U/ml); furatrizine (6.3 $\mu\text{g}/\text{ml}$). An isolate was recorded as resistant when its growth has not been inhibited by the given concentrations of the drugs. The detection of conjugative R plasmids including thermosensitive ones was made according to a previous description [5].

As shown in Table 1, only three (6.8%) of the 44 birds examined harbored *Enterobacteriaceae*. No growth of *Salmonella* was recorded on brilliant green agar plates. *Enterobacter cloacae* was isolated from 2 of the 3 birds and *Citrobacter intermedius* biovar b was from the remaining one. *E. cloacae* isolates showed resistance to CER, APC and CL. But conjugative R plasmid was not detected from the resistant isolates. It is of interest that wild ducks of the *Aythya* spp. had *Enterobacteriaceae* with very low frequency and no *E. coli* was isolated even by the enrichment culture. Our previous report [6] indicates that 91.1% of feral pigeons and all crows which were examined

in the similar manner after having been kept at -20°C yielded *E. coli*. Since wild ducks of the *Aythya* spp. usually inhabit the sea or center of lakes at a distance from human beings and have specific food habits [3], their intestinal bacterial flora appears to be different from that of other wild birds such as pigeons and crows living close to a human environment. From these data, it appears that wild ducks of the *Aythya* spp. play little role in the spread of the R plasmid, though *E. cloacae* isolated showed resistance to the three drugs described above.

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要 約

ハジロガモにおける腸内細菌の低分離率と分離菌の薬剤耐性型（短報）：佐藤儀平・浅木正義（帯広畜産大学獣医公衆衛生学教室），山本 博（鳥取大学農学部家畜微生物学教室）——腸内細菌を分離し，その薬剤耐性を調べるため，1977～78年に宍道湖で得られたハジロガモ（*Aythya* spp.）44羽の死体の腸管下部を直接および増菌培養した．腸内細菌の検出率は極めて低く，2羽から *Enterobacter cloacae* が，1羽から *Citrobacter intermedius* biovar b が分離されたにとどまった．分離された *E. cloacae* は，セファロリジン，アンピシリンおよびコリスチンに耐性を示したが，接合性 Rプラスミドは検出されなかった．また，*C. intermedius* は検査した 13 種の薬剤にすべて感受性であった．