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Escherichia coli infection in ducks in the Mekong Delta: Bacterial isolation, serogroup distribution and antibiotic resistance

Ho Thi Viet Thu[1*], Doan Tran Loan Anh[1] and Le Van Dong[2]

[1]*College of Agriculture and Applied Biology, Can Tho University, Vietnam*

[2]*Tra Vinh University, Vietnam*

[*]*Correspondence: Ho Thi Viet Thu (email: htvthu@ctu.edu.vn)*

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ABSTRACT

An investigation on duck Escherichia coli infection was carried out by examination of 241 suspicious colibacillosis outbreaks from 1 city and 4 provinces in the Mekong Delta. The study procedure involves several steps including bacterial isolation and identification, O serogroup typing and antibiotic resistant determination. The results showed that 990 from 994 ducks were confirmed to be infected by E. coli. E. coli bacteria were found from feces in almost diseased ducks (99.0%) and many organ samples; the highest rate of positive isolates was reported from livers (78.3%), followed by lungs (71.8%), spleens (67.4%), and the lowest one was in bone marrows (58.9%). The typing of 300 E. coli isolates with 10 important groups of mono O antisera revealed that 265 isolates were identified and belonged to 10 O serogroups. The most commonly isolated O group was O2 (16.7%), followed by O78 (15.0%), O81 (9.7%), O35 (9.3%), O1 (8.0%), O36 (7.0%), O111 (7.7%), O92 (5.7%), O18 (5.3%), and the lowest one was O93 (4.0%). A total of 659 E. coli isolates were tested for their sensitivity to commonly used antibiotics, these avian pathogenic E. coli isolates demonstrated moderate to high resistances (20.2 % to 67.4 %) to 7/15 antibiotics tested, and very little amikacin and fosfomycin resistances (3.0 and 6.4%). It is imperative that susceptibility tests should be carried out on infecting pathogen prior to treatment of ducks colibacillosis in field in order to avoid treatment failure and reduce selective pressure that could result in spreading avian pathogenic E. coli in the environment.

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1 INTRODUCTION

Avian colibacillosis is a complicated disease with many localized and systemic infections caused by avian pathogenic *Escherichia coli* (APEC) including colisepticemia, salpingitis, anopthalmitis, osteoarthiritis, synovitis, coligranuloma, airsaculitis, and cellulitis. Nowadays, there is general agreement that avian colibacillosis is one of the leading causes of mortality and morbidity associated with economic losses in the poultry production throughout the world (Roshdy *et al.*, 2012; Zhuang *et al.,* 2014). Economic losses can be due to decreased hatching rates, decreased egg production, mortality, lowered production, carcass condemnation at slaughter and costs associated with

treatment, and prophylaxis. More than 1,000 serotypes are known, but only a few are considered as important in avian pathology. Earlier studies by Sojka and Carnaghan (1961) identified the serotypes O1, O2, O35, and O78 as the most dominated. However, recent studies have shown that the serotypes O1, O2, and O78 are widely spread and represent 15-61% of the isolates, yet other types still exist (Dho-Moulin and Fairbrother, 1999). In Vietnam, duck production is well developed in the Mekong Delta, accounting for 48.3% of the poultry population in Vietnam (FAO, 2008). *E. coli* infection in ducks was recognized as a popular and important duck disease in the Mekong Delta with 74.50% of ducks in Long An infected (Nguyen Trong Phuoc, 1997), and mortality of *E. coli* infection ducks could be high as 40 to 50% (Nguyen Xuan Binh *et al*., 2000). In addition, the frequent use of antibiotics in drinking water and duck feed for preventive and treatment purposes which have been responsible for selective pressure of *E. coli* bacteria lead to a lot of *E. coli* strains develop antibiotic resistance to multi-antibiotics (Vo Thi Tra An *et al.,* 2010; Tran Thi Thuy Giang *et al*., 2014), and *E. coli* infections become harder to treat. Another concern is that *E. coli* bacteria are the most popular agents which cause food poisoning, and they are transmitted to human by food chains from animal products including duck eggs and meat. The main purpose of this study was to examine the incidence of O serogroups, antibiotic resistance of *E. coli* in diseased ducks in the Mekong Delta.

The result will be useful information in disease control, and contribution of *E. coli* antibiotic resistant alleviation strategy.

1 MATERIAL AND METHODS

1.1 Bacterial isolation and identification

1.1.1 Sample collection

E. coli infection suspicious ducks from 241 flocks from Can Tho city and 4 provinces (Vinh Long, Hau Giang, Dong Thap and Tra Vinh) were collected and screened for *E. coli* infection. In each flock, 4-6 diseased ducks were sampled, and *E. coli* bacteria were isolated from internal organs (lung, liver, spleen), bone marrow, and feces from diseased ducks.

1.1.2 E. coli isolation and identification

E. coli was cultured on MacConkey and nutrient agar (NA) medium for morphological characterization. After 24 hrs, all *E. coli* colonies were pink, round and convex on MacConkey medium, 3-5 of these colonies were collected for growing on NA. After 24 hrs, *E. coli* appeared creamy white on NA medium. *E. coli* were identified by biochemical tests with Indole, Methyl Red, Voges-Proskauer, Simmons citrate from Merck Co (Germany) according to Bryan *et al*. (2013). Duck was confirmed to be infected with *E. coli* when *E. coli* bacteria were found at least from 1 internal organ or bone marrow.

1.2 O-serogroup typing

Ten *E. coli* O-antisera (O1, O2, O18, O35, O36, O78, O81, O92, O93, O111) antigens (SSI Diagnostica, Denmark) were available for testing. Sixty representatives of APEC isolates in each province or city were chosen for sero-typing. Totally, 300 APEC isolates were typed by screening the potential O-serotype by slide agglutination test, according to the manufacturer's.

1.3 Antibiotic resistant examination

Antibiotic resistant examination was studied by antibiotic susceptibility tests with 15 antibiotics commonly used in poultry farming in the Mekong delta by antibiotics discs of amikacin (30µg), ampicillin (10µg), ceftazidime/clavulanic acid (30µg), cefuroxime (30µg), ciprofloxacin (5µg), colistin (10µg), doxycylin (30µg), florfenicol (30µg), fosfomycin (200µg), gentamycin (10µg), norfloxacin (10µg), ofloxacin (5µg), streptomycin (10µg), tetracycline (30µg), and trimethoprim/sulfamethoxazole (1,25/23,75µg) distributed by Nam Khoa Biotek Co. Ltd (Vietnam). In this study, two to three isolates from each outbreak were chosen for testing. Totally, 569 APEC isolates were used in antibiotic susceptibility tests.

Antimicrobial susceptibility was determined by agar diffusion method according to Bauer *et al*. (1966). Pure cultures of *E. coli* were grown overnight in NA at 37^{o}C in 24hrs, then the bacterial concentration was adjusted based on 0.5 McFarland turbidity, approximately bacterial suspension of $1.5x10^{8}$ bacteria/ml. One hundred µL of the culture suspension was spread onto each Mueller Hinton Agar (Merck, Germany) plate surface, and three or four antimicrobial discs were placed on the surface of the agar plate. These plates were incubated at 37^{o}C for 16 to 20 hrs. The results were interpreted as sensitive, intermediate, or resistant based on aseptic diameter measurement according to the Clinical and Laboratory Standards Institute (CLSI, 2017).

Statistical analysis

The data obtained were analyzed by Minitab software 13.2 (Ryan *et al*., 2000), using Goodness

to fit test and Chi square to assess significant differences in the prevalence of serogroups and antibiotic resistance rates.

2 RESULTS AND DISCUSSIONS

Conventional diagnosis method for the disease is based on *E. coli* isolation and identification from typical lesion of colibacillosis (Barnes *et al*., 2008), and positive isolation and identification of *E. coli* from visceral organ of suspected ducks is an indication of colibacillosis. The results of confirmed colibacillosis by *E. coli* isolation and identification showed that 226 out of 241 (93.8%) suspected colibacillosis duck flocks were confirmed to be colibacillosis flocks, and 990 from 994 of examined ducks (99.6%) in colibacillosis flocks were colibacillosis ducks. Since the definite aim of the study is to assert colibacillosis ducks and flocks by *E. coli* isolation and identification from suspicious clinical cases based on typical symptoms and lesions, the percentages of positive ducks and flocks were nearly 100.0%, and there was no significant difference between positive duck percentages of surveyed areas.

Table 1: Results of confirmed colibacillosis diagnosis by *E. coli* isolation and identification

City/ provinces	No. colibacillosis flocks/ No. examined flocks	No. ducks in colibacillosis flocks	No. colibacillosis ducks	(%)
Can Tho	49/52	200	200	100.0
Hau Giang	52/55	202	202	100.0
Vinh Long	42/48	200	200	100.0
Dong Thap	43/45	214	213	99.5
Tra Vinh	40/41	178	175	98.3
Total	226/241	994	990	99.6

Table 2: Incidence of *E. coli* recovered from feces and internal organs of colibacillosis ducks

Duck sample	No. of tested	No. of positive	(%)
Feces	990	980	99.0[a]
Liver	990	775	78.3[b]
Lung	990	711	71.8[c]
Spleen	990	667	67.4[d]
Bone marrow	990	583	58.9[e]

Values in the same column with different letter are significantly different (P<0.05)

E. coli bacteria were found from feces in almost diseased ducks (99%) and all types of organ samples collected, the highest rate of positive isolates was reported from livers (78.3%), followed by lungs (71.8%), spleens (67.4%), and the lowest one was in bone marrows (58.9%). Avian colibacillosis is a complicated disease with many localized and systemic infections depending on bacterial localization. Primary enteritis is a common manifestation of *E. coli* infection in mammals, but it is considered rare in birds. The very high percentage of *E. coli* recovered from diseased duck feces due to *E. coli* is a common inhabitant of the duck intestine, and it is widely disseminated in fecal materials so that the presence of *E. coli* from duck feces may be from septicemia colibacillosis, *E. coli* primary enteritis, and even healthy ducks. In ducks, coliform septicemia is quite popular, in this case *E. coli* (usually O78) can be recovered from any of internal organ (Leibovitz, 1972). Since, localization of *E. coli* in bone and synovial tissue was a common sequel of septicemia, the frequency of positive isolates was lower than from other internal organs.

Table 3: O-serogroup distribution of APEC isolates of ducks (n=300)

Serotype	No. of positive	Prevalence (%)
O1	24	8.0
O2	50	16.7**
O18	16	5.3
O35	28	9.3
O36	21	7.0
O78	45	15.0*
O81	29	9.7
O92	17	5.7
O93	12	4.0
O111	23	7.7
Others	35	11.7
P=0.047		

*Prevalence with * is significant difference at level (P=<0.05) and with ** (P=<0.001)*

In this study, 10 O-serogroups were identified from 300 APEC isolates. Five serogroups (O1, O2, O35, O78 and O81) accounted for 58.7% of pathogenic strains. Among these, O2 and O78 were predominant serogroups, and the prevalence of O78 group (15.0%) and O2 group (16.7%) showed significant differences with other O-type ones. This result was different from recent reports of colibacillosis in Muscovy ducks (Nguyen Thi Lien Huong, 2017) and from Bau and Dom ducks in the north of Vietnam, in which O2 and O78 were not

detected (Dang Thi Vui and Nguyen Ba Tiep, 2016), but it was quite similar to the study results in chickens in Ho Chi Minh city (To Minh Chau *et al*., 2002), 3 determined serotypes of *E. coli* isolates were O1:K1, O2:K1, O78:K80. Besides, lots of international studies also showed that O1, O2, O8, O18 and O78 were detected more frequently in chickens, turkeys or other birds (Ewers *et al*., 2004, 2007; McPeake *et al*., 2005; Vandekerchove *et al*., 2005; Yaguchi *et al*., 2007; Dziva and Stevens, 2008; Ozawa *et al*., 2008). There has been not much research on serotyping of *E. coli* from ducks, especially in the Mekong Delta. In this study, O2 and O78 APEC were firstly reported from ducks in Vietnam. The results suggested that distribution of APEC O-serogroups from ducks in Vietnam are very complex and different from hosts and geographic regions. These problems cause difficulties in disease prevention by vaccine. Further work is needed to verify distribution of O-serogroup from different origins and different hosts.

Table 4: Results of antibiotic susceptibility tests from *E. coli* isolates (n=659)

Antibiotic(s)	Abb	Resistant		Intermediate		Sensitive	
		No.	(%)	No.	(%)	No.	(%)
Ampicillin	Am	421	63.9**	0	0.0	238	36.1
Trimethoprim + Sulfamethoxazole	Bt	444	67.4**	0	0.0	215	32.6
Norfloxacin[SEP]	No	133	20.2	169	25.6	357	54.2
Streptomycin	Sm	434	65.9**	0	0.0	225	34.1
Amikacin	Ak	20	3.0	153	23.2	486	73.7*
Fosfomycin	Fos	42	6.4	169	25.6	448	68.0
Doxycycline[SEP]	Dx	103	15.6	153	23.2	403	61.2
Cefuroxime[SEP]	Cu	102	15.5	322	48.9	235	35.7
Gentamycin	Ge	143	21.7	101	15.3	415	63.0
Colistin[SEP]	Co	64	9.7	0	0.0	595	90.3**
Florfenicol[SEP]	FFc	134	20.3	401	60.8	124	18.8

*Prevalence in the same column with * is significant difference at level (P=<0.05) and with ** (P=<0.001)*

Abb: Abbreviation

Antibiotics have been used extensively for treatment of poultry diseases since 1950s. Occurring in parallel with use of an antimicrobial has been in progressive development of resistance which was initially identified following introduction of tetracyclines (Sojka, 1965). In recent years, the acceleration of antibiotic resistance in *E. coli* bacteria has been reported in many countries including Viet Nam (Thi Thu Hao Van *et al*., 2007; Price *et al*., 2013; Nguyen Thi Nhung *et al*., 2017). Therefore, it is very important to examine the susceptibility of these microorganisms involved in the disease outbreaks in order to avoid choosing ineffective antibiotics. In this study, APEC isolates demonstrated moderate to high resistance (20.2% to 67.5%) to 7/15 antibiotics (No, FFc, Ge, Te, Am, Sm and Bt), and the strongest resistance were to trimethoprim/ sulfamethoxazole (67.4%) and streptomycin (65.9%). These results are quite similar to many reports on *E. coli* antibiotic resistance in Vietnam and other countries (Truong Ha Thai *et al*., 2017; Miles *et al*., 2006; Vandemaele *et al*., 2002). The long use and misuse of antibiotics have contributed to the emergence and spread of antimicrobial resistant microorganisms (Levy, 1994). Besides, increasing uses of antibiotics as additives in poultry feed for growth promotion and disease preventive purposes lead to selective pressure for antibiotic resistance in bacteria in poultry, and it has been rapidly increased (Van den Bogaard *et al*., 2011). Colistin is a highly affective antibiotic against *E. coli* and has been commonly used for animal colibacillosis prevention and treatment, so there have been many reports about the resistance of *E. coli* to this antibiotic (Nguyen Thi Nhung *et al.*, 2015; Truong Ha Thai *et al.*, 2017). However, this study results revealed that high percentage of APEC (90.3%) was sensitive to colistin. This matter can be explained by high sensitivity of ducks to this antibiotic so that it was rarely used in treatment and prevention duck diseases. Fosfomycin and amikacin are novel antibiotics, they have been introduced and come into commercial uses in the 1970s (Hendlin *et al*., 1969; Gilbert, 1995), and there are not many commercial products of two antibiotics which have been used in veterinary medicine in Vietnam, so the rates of APEC resisting to these antibiotics were still low (6.4% and 3.0%, respectively); especially, all amikacin products are injection forms and only used for mammals. These results are evidence of long antibiotic usage being a contributing factor to antimicrobial resistance. Although ciprofloxacin has been prohibited in using for animals, duck raisers can easily purchase it at pharmacy counters or chemical shops. This explains why there were not many *E.*

coli isolates (40.2%) showed susceptible to ciprofloxacin. This problem suggested a strict law in trading antibiotic must be applied in order to prevent antimicrobial resistance and to preserve antibiotics for human disease treatment. Since antibiotic resistance and sensitivity of bacteria have no relationship with serogroups of bacteria, the susceptibility of *E. coli* in each O serogroup to antibiotics was not analyzed in this study.

3 CONCLUSIONS

Duck colibacillosis occurs frequently in the Mekong Delta with two predominant serogroups O2 and O78. There is emerging of drug resistance in APEC associated duck colibacillosis. APEC showed moderate to high resistance to a lot of antibiotics, but low resistance to amikacin and fosfomycin.

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