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Characterization of Escherichia coli isolated from migratory water fowls in Hakaluki Haor, Bangladesh

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ABSTRACT

A total of 135 fecal samples were collected to characterize the Escherichia coli isolates from the migratory waterfowls (whistling Swan) harbored in the Hakaluki Haor of Bangladesh in the year of 2008 and 2009. Out of 135 fecal samples, 100 samples were distinguished as positive for isolates of Escherichia coli following cultural, biochemical and motility test. Amongst the recovered isolates only 38% were found upbeat to enterotoxin production propensity on mice inoculation test. Finally, out of 38 % enterotoxigenic E. coli (ETEC) positive isolates no any isolates found to be positive for the aptitude of heat stable (ST) toxin yield. So, the presence of ETEC in migratory waterfowls indicating the possibilities of them to act as vector and reservoir of E. coli to spread further infection to animals and humans. This work indicates the first time ETEC characterization from the migratory birds of Bangladesh.

Key words: Migratory birds, Escherichia coli and toxin profile

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Introduction

Within the enterobacteriaceae family, E. coli is the leading species responsible for various types of diseases encountering in the lower portion of the intestine (Thompson, 2007) of human, warm blooded animals and birds (Pelczar et al., 1986). Different strains of E. coli are often host-specific, making it possible to determine the source of fecal contamination in environmental samples (Feng et al., 2002). The potential for transport and dissemination of E. coli by migratory birds is of concern regarding drug resistance and public health (Damare et al., 1979). Migratory birds might be involved in dispersal of E. coli (Middleton and Ambrose, 2005) as their biological carriers, (Zdenek, 2004) may lead to gastroenteritis, urinary tract infections, and neonatal meningitis and in rare cases it is also responsible for haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia and Gram-negative pneumonia to human and other related hosts (Todar, 2007). Migratory birds play a vital role for the transmission of diseases from one place to another and may cross the host-species barrier (Hussong et al., 1979) leading to a notorious impacts on the ecosystem.

Enterotoxigenic E. coli (ETEC) strains are non-invasive, and they do not leave the intestinal lumen. ETEC is the leading bacterial cause of diarrhea in animals and commonly to children in the developing world, as well as the most common cause of traveler's diarrhea (Nataro and Kaper, 1998). Each year, ETEC

causes more than 200 million cases of diarrhea and 380,000 deaths, mostly in children in developing countries (Qadri et al., 2005). ETEC produces two district enterotoxins: a high-molecular weight, immunogenic, heat labile toxin (LT) and/or a low-molecular weight, non-immunogenic, heat stable toxin (ST) (Greenberg and Guerrant, 1981). Historically, zoonotic disease research has emphasized occupational or animal-origin food borne exposures. Although fecal contamination of raw food products in fields is an important source of zoonotic infection (Tauxe, 1997). Consequently, environmental reservoirs of microbes of public health importance and their epidemiological linkages on zoonoses need to be investigated.

In Bangladesh, there are many Haors, bills, lagoons, lakes, marshland etc. wherein various types of migratory birds harbor mainly during winter season. This research was conducted with a view to isolate and identify *E. coli* from the migratory whistling swan and to determine the toxin profile of that isolates to orient a future research module on their antibiogram profile, genetic linkages, transmission modes, mutational status etc.

Material and Methods

Isolation and identification of E. coli isolates : Fecal samples of whistling swans were collected from different scattered regions of Hakaluki Haor using sterile cotton buds and transferred the buds immediately into the sterile nutrient broth. The samples were kept in box, wrapped with ice and transferred immediately to the laboratory. Pure culture of E. coli was isolated using Eosin Methylene Blue (EMB) agar according to the methods described by Tsubokura et al., (1995). To identify the E. coli isolates the gross colony morphology produced on EMB agar, McConkey agar, SS agar etc. were observed.

Grams staining and motility test using hanging drop method were also performed to observe the properties and traits of asking isolates according to the methods described by (Cowan, 1985). Then the isolates were subjected to different biochemical tests according to Merchant and Packer (1967) such as sugar fermentation test, Indole production test, Methyl-Red and Voges-Proskyer (MR-VP) test.

Determination of Toxin Profile : With the view to maintain the stock culture for each isolate Nutrient agar slants were used. Inoculation induced in the slant as streaking pattern and incubated at 370C for 24 hours. Culture was kept at room temperature after overlaying with glycerol. Overnight cultures were centrifuged at 1200 rpm for 30 minutes and supernatants were poured into new vials and stored in room temperature after adding 5µg gentamicin (Gentipra R) in each vial. To detect the purity of the toxins, streaking of supernatant was applied on EMB agar and incubated at 370C for 24 hours. Forty numbers of 3 days old Swiss albino suckling mice were divided into two groups namely A and B consisting of twenty mice in each group. The mice of group A were administered orally with 3 □l of crude culture supernatant containing enterotoxin using separate micropipette and were kept in incubation at 370C for 24 hours to observe toxic effects. On the contrary, the mice of group B were kept as control in the same environment. Finally, the supernatant was used for detection of heat-stable (ST) toxin by Infant Mouse Assay (IMA) in case of those petridishes devoid of any colony formation

Detection of heat stable toxin by IMA : Nine number of day old Swiss albino suckling mice arranged in three groups consisting of three mice in individual group were also inoculated with crude culture supernatant at a volume of 0.2 ml and kept at room temperature for 4 hours. The abdomen was opened and the entire intestine was removed after sacrificing by cervical dislocation. The weight of the gut and the remaining carcass were taken separately for the interpretation of ST by inducing IMA according to Giannella, (1976) described the method of STs remarks as either positive (0.070~0.085) or negative (< 0.070) after calculating the ratio of gut weight against remaining carcass weight for individual mouse.

Embryo lethality assay : A total of ten randomly selected isolates subjected to chicken embryo lethality (ELA) test following the method disclosed by Gibbs et al., (2007) with a view to identify the presence of putative virulence factors and pathogenic potentials. The test isolates kept overnight in broth cultures and then washed in phosphate buffered saline (PBS) and inoculated into the allantoic cavity of ten (one embryo for one sample) 12 day-old embryonated chicken eggs (ECE) at a volume of 0.1 ml containing >200 colony forming unit (CFU). The ECE candled twice daily to count the death embryos up to 6 days of post inoculation. No any control test was performed regarding ELA due to lack of availability of repository *E. coli* isolates yet from migratory birds in Bangladesh. The ELA result for test isolates was concluded on the basis of embryo mortality <10%, 10-29% and >29% as avirulent, moderately virulent and virulent strain respectively (Wooley et al., 200).

Results

Isolation and Identification of fecal E. coli isolates

The gross colony morphology on EMB agar, McConkey agar and S-S agar represented by 100 samples out of 135 as yellow green metallic sheen, bright pink or red and pinkish colonies respectively. Similarly as before, 100 samples found as gram negative rod shaped isolates on Gram staining during microscopic examination. Motile traits observed in all positively found gram negative rod on Hanging drop motility test.

Biochemical characterization of the isolates

A series of biochemical tests selective for *E. coli* were performed with the suspected Gram-negative rod shaped isolates (100 culturally positive samples) and all the suspected isolates produced acid and gas due to the fermentation process in sugar fermentation test. All the isolates also showed Indole test positive (red ring), Methyl Red positive (bright red), Voges Proskauer test negative (colorless/ no change) and Citrate negative (no change/ green color).

Enterotoxigenicity test

Enterotoxigenic *E. coli* (ETEC) isolates were detected based on mortality and survivability of 3 days old mice (n=40) during 24 hours of post inoculation. Out of 100 positive isolates, 38 (38%) *E. coli* was found to be positive for ETEC.

Determination of STs toxin by IMA

All the positive isolates showed IMA value with a range of 0.092 – 0.103 (Table 1) indicating that all isolates were found negative for ST in this study.

Table 1. Determination of STs toxin

Isolates	Crude culture supernatant (toxin)			Gut weight : Carcass weight		Remarks
	Quantity	Route	Incubation	Ranges of standard value	*Ranges of obtained value*	
W. swan	0.1 ml	Oral	4 hours	0.070 to 0.085	0.092 to 0.103	ST (- ve)

Embryo lethality assay

Out of ten embryos only two embryos died on 2nd day, three embryos died on 4th day and the remaining five embryos still alive on 6th day of post inoculation. A marked hemorrhage on skin and edema found on postmortem of died embryos on 2nd day. Some sort of petechial hemorrhage on skin also found on death embryos after 4 days post inoculation. Remaining twelve live embryos did not develop any remarkable lesions. So, in context of ELA out of 100 positive isolates; 20 % were virulent, 30% were moderately virulent and remaining 50% were avirulent isolates.

Discussion:

The findings of *E. coli* isolation and identification of this study is strongly similar with Dana et al., (2007); where they used swabs collected from Canada goose to inoculate in brain heart infusion broth (BHIB) and incubated overnight at 37°C. BHIB cultures were subsequently streaked for isolated colonies on MacConkey agar plates; 1 lactose-fermenting colony was selected from each goose sample that exhibited growth on agar. The microscopic traits belonged to the gram negative (- ve) short rod, single, pair or short chain which are the characteristic features of *E. coli*. Damare et al., (1979) also isolated the *E. coli* from the gut of Canada geese (*Branta canadensis*) and whistling swans (*Cygnus columbianus columbianus*). Middleton and Ambrose (2005) also isolated the *E. coli* from fecal samples of Migratory Canada Geese (*Branta Canadensis*) showing the similar morphology. **Tsubokura** et al., (1995) also isolated 554 stains of *Escherichia coli* from feces of migratory watefowl, including whistling swans (Cygnus columbianus). On hanging drop method all isolates revealed motile traits as postulated by Buxton and Fraser, 1977; Jones, 1987).

Out of 135 fecal samples 74.07% found positive as *E. coli* isolates in this research that is very identical with the result found by Aguirre et al., (1992). They recovered 54% *E. coli* isolates from adult black-bellied whistling ducks trapped at Laguna La Nacha, Tamaulipas, Mexico. Dawn et al., (2004) got 65.33% prevalence of *E. coli* (294 isolates out of 450 fecal samples) recovered from free living waterfowls through the zoological settings and their result is also similar to this study. On the other hand, Geoffrey et al., (2006) found one *Escherichia coli* O157 positive out of 231 composite samples recovered from wild bird feces in southwest Scotland and noted the isolate as phage type 21/28 and possessed vtx_2, *eaeA*, and enterohemorrhagic *E. coli hlyA* genes. The prevalence of *E. coli* isolate in their study was very low (0.433%) in contrast to this study that might be due to using composite fecal samples in their study. Data on prevalence of *E. coli* from migratory birds are not so available.

Out of 100 positive *E. coli* isolates the ETEC found as 38% on enterotoxigenecity test in this study indicating the strong possibilities of occurrence of enterotoxocity

as traveler's diarrhea to surrounding animals and humans especially to children. This finding is strongly agreed with Nataro and Kaper (1998) and Qadri et al., (2005) where they disclosed the common causal agent of infantile and visitors' diarrhea of third world developing coutries as ETEC due to poor sanitation and no hygienic water. The sanitation and water condition around the Hakaluki Haor was absolutely awful. In this connection, some case studies of visitors' diarrheal occurence demonstrated around the Hakaluki Haor during the sample collection of this study. This case occurrence extremely supported with the findings of ETEC of this study.

In aspect of ST, this result discloses similar finding with the Giannella, (1976), but he described that the ST production was optimal in Casamino Acids-yeast extract media, but both Trypticase soy and brain heart infusion broths resulted in several false negative reactions. Gülhan et al., (2009) recovered 10 ETEC positive strains from gulls in Turkey, but they didn't get any positive isolates for ST, indicating similar result with this work, though lethal toxin (LT) was not performed here.

On ELA, the virulent isolates demonstrated hemorrhages and edema on death embryos on 2 days post incubation, moderately virulent represented petechial hemorrhages, and avirulent isolates with no lesions; highly analogous with that described by Gibbs et al., (2003) in aspect of *E. coli* from the feces of Yellow-Headed Blackbirds (*Xanthocephalus xanthocephalus*) in North Dakota. Gibbs and Wooley (2003) also compared the ELA with intravenous chicken challenge (ICC) method in case of chicken colibacillosis, that is not studied here due to devoid of any such occurrences in migratory birds examined. Montgomery et al., (2005), Wooley et al., (2000) and Nolan et al., (1992) also reported the virulent *E. coli* recovered from poultry tend to embryonic death and hemorrhagic skin lesions with edematous body of ECE of 24 hours post inoculation; that notify the similarities with this study. But, in case of migratory birds the data are not so available to compare the result of ELA of this study. So, it can be concluded that the Hakaluki Haor bears higher predisposition to spreading virulent *E. coli* infection to other animals and humans as well.

There are many such findings available in aspect of chicken rather than migratory waterfowls and particularly in Bangladesh there is no any past evidence of characterization and toxin profile of *E. coli* recovered from migratory whistling swan. The results of this study will be act as a milestone in designing further study plan on molecular epidemiology related with that migratory waterfowls. So, further studies would be conducted highlighting on the molecular linkages and mutation level of *E. coli* of migratory birds with that recovered from other species of poultry.

References:

1. Aguirre AA, Quan TJ, Cook RS, GMcLean RG, (1992). Cloacal flora isolated from wild black-bellied whistling ducks (*Dendrocygna autumnalis*) in Laguna La Nacha, Mexico. *Avian Dis*, 36, 459-462.
2. Buxton A, Fraser G, (1977). *Animal Microbiology. Vol. 1. Escherichia coli*, Blackwell Scientific Publications, Oxford, London, Edinburg, Melbourne. pp: 92-102.
3. Cowan ST, (1985). Cowan and Steel's manual for identification of medical bacteria. (2nd Edn.), Cambridge University Press, Cambridge, London. PP: 138-139.
4. Damaré JM, Hussong D, Weiner RM, Colwell RR, (1979). Aerobic and facultatively anaerobic bacteria associated with the gut of Canada geese (*Branta canadensis*) and whistling swans (*Cygnus columbianus columbianus*). Appl Enviro Microbiol, 38, 258-266.
5. Dana C, David JVD, David ES, David GW, Margie DL, Sherry A, Mark S, John JM, (2007). Antimicrobial Georgia Division of Public Health, Atlanta, Georgia, USA.
6. Dawn MF, Clifton MM, Teresa YM, Catherine AB, Raymond FW, (2004). Survey of Parasites and Bacterial Pathogens from Free-Living Waterfowl in Zoological Settings. Avian Dis, 48, 759-767.
7. Feng P, Weagant S, Grant M, (2002). *Enumeration of Escherichia coli and the Coliform Bacteria*. Bacteriological Analytical Manual (8th Edn.), FDA/Center for Food Safety & Applied Nutrition. *(Retrieved on 2007-01-25).*
8. Geoffrey F, Judith E, Hazel IK, Alastair WS, George JG, Lesley JA, Barti AS, Tom WP, (2006). Analysis of Feces Samples Collected from a Wild-Bird Garden Feeding Station in Scotland for the Presence of Verocytotoxin-Producing *Escherichia coli* O157. Appl Enviro Microbiol, 72, 2265-2267.
9. Giannella RA, (1976). Suckling mouse model for detection of heat stable *Escherichia coli* enterotoxin: characteristics of the model. Infect Immun, 14, 95-99.
10. Gibbs PS, Wooley RE, (2003). Comparison of the intravenous chicken challenge method with the embryo lethality assay for studies in avian colibacillosis. Avian Dis, 47, 672-680.
11. Gibbs PS, Nolan LK, Maurer J, Wooley RE, (2003). Prediction of chicken-embryo lethality with avian *Escherichia coli* traits complement resistance, colicin V production, and presence of the increased

serum survival gene cluster (*iss*). Avian Dis, 47, 370-379.
12. Greenberg RN, Guerrant RL, (1981). *Escherichia coli* heat-stable enterotoxin. Pharmacol Ther, 11:507-531.
13. Gülhan T, İlhan Z, Aksakal A, Solmaz H, Ekın İH, (2009) The determination of enterotoxin types (LT, ST) of *Escherichia coli* strains of animal origin. Yüzüncü yıl Üniversitesi Veteriner Fakültesi Dergisi, 20, 27-31.
14. Holmes RK, Twiddy EM, Pickett CL, (1996). Purification and characterization of type 11 heat-labile enterotoxin of *E. coli*. Infect Immun, 53, 464-473.
15. Hussong D, Damaré JM, Limpert RJ, Sladen WJ, Weiner RM, Colwell RR, (1979). Microbial impact of Canada geese (*Branta canadensis*) and whistling swans (*Cygnus columbianus columbianus*) on aquatic ecosystems. Appl Enviro Microbiol, 37, 14-20.
16. Jones TO, (1987). Intramammary antibiotic preparations and cephalosporin resistance in *Salmonella typhimurium* 204c. *Vet Record*, 120, 399-400.
17. Merchant IA, Packer RA, (1967). Veterinary Bacteriology and Virology. (7th Edn.), The Iowa State University Press, Ames, Iowa, USA, PP:211-305.
18. Middleton JH, Ambrose A, (2005). Enumeration and Antibiotic Resistance Patterns Of Fecal Indicator Organisms Isolated From Migratory Canada Geese (*Branta Canadensis*). *J Wild Dis*, 41, 334-341.
19. Montgomery RD, Jones LS, Boyle CR, Luo Y, Boyle JA, (2005). The embryo lethality of *Escherichia coli* isolates and its relationship to various in vitro attributes. Avian Dis, 49, 63-69.
20. Nataro JP, Kaper JB, (1998). "Diarrheagenic *Escherichia coli*". *Clin Microbiol Rev*, 11, 142-201.
21. Nolan LK, Wooley RE, Brown J, Spears KR, Dickerson HW, Dekich M, (1992). Comparison of a complement resistance test, a chicken embryo lethality test, and the chicken lethality test for determining virulence of avian *Escherichia coli*. Avian Dis, 36, 395-397.
22. Pelczar JR, Michel J, Krieg NR, Chan ECS, (1986). Microbiology. 5th edn, PP: 272.
23. Qadri F, Svennerholm AM, Faruque AS, Sack RB, (2005). Enterotoxigenic *Escherichia coli* in developing countries: epidemiology, microbiology, clinical features, treatment, and prevention. Clin Microbiol Rev, 18, 465-483.
24. Tauxe RV, (1997). Emerging foodborne diseases: an evolving public health challenge. Emerg Infect Dis, 3, 425-434.
25. *Thompson A, (2007). E. coli* Thrives in Beach Sands. *Live Science*. Retrieved on 2007-12-03.
26. *Todar K, (2007).* Pathogenic *E. coli*. *Online Textbook of Bacteriology. University of Wisconsin-Madison Department of Bacteriology*. Retrieved on 2007-11-30.
27. Tsubokura M, Matsumoto A, Otsuki K, Animas SB, Sanekata T, (1995). Drug resistance and conjugative R plasmids in Escherichia coli strains isolated from migratory waterfowl. *J Wild Dis*, 31, 352-357.
28. Wooley RE, Gibbs PS, Brown TP, Maurer JJ, (2000). Chicken embryo lethality assay for determining the virulence of avian *Escherichia coli* isolates. Avian Dis, 44, 318-324.
29. Yamamoto T, Yokota T, (1983). Plastids enterotoxigenic *Escherichia coli* HI0407: evidence of two heat stable enterotoxin genes and a conjugal transfer system. J Bacteriol, 153, 1352-1360.
30. Zdenek H, (2004). An Annotated checklist of pathogenic microorganisms associated with migratory birds. *J Wild Dis*, 40, 639-659.