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RESEARCH ARTICLE

Antibiogram profile of Escherichia coli isolated from migratory birds

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Özet

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Amaç: Bangladeş'in Hakaluki Haor (lagoon) bölgesinde kış sezonunda göçmen kuşlardan izole edilen *Escherichia coli*'nin antibiyotik duyarlılığını belirlemektir.

Gereç ve Yöntem: Toplamda 100 adet *Escherichia coli* izole edildi ve in vitro şartlarda antibiyotik duyarlılık testi yapıldı. İzolatlar kolistin sülfat, gentamisin, azitromisin, levofloksasin, siprofloksasin, tetrasiklin, amoksisilin ve metronidazol yönünden duyarlılığı araştırıldı. Duyarlılık testi disk difüzyon yöntemine göre yapıldı.

Bulgular: Escherichia coli'nin kolitsin sülfata %96, levofloksasine %89 ve siprofloksasine %88 oranında yüksek düzeyde duyarlı olduğu belirlendi. Azitromisine %79, gentamisine %77, amoksisiline %63 ve tetrasikline ise %46 düzeyinde duyarlı olduğu belirlendi. Metronidazole ise hiçbir izolatın duyarlılığı bulunamadı.

Öneri: Kolistin sülfat, levofloksasın ve siprofloksasının göçmen kuşlardan izole edilen *Escherichia coli*'ye güçlü etkiye sahip olduğu ve insan ile diğer kanatlılardan elde edilen *Escherichia coli* izolatlarında çapraz direnç varlığı araştırılmalıdır.

Abstract

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Aim: The aim of the study was to conduct the in vitro antibiotic susceptibility test of *Escherichia coli* isolated from the migratory birds available in winter season at Hakaluki Haor (lagoon) of Bangladesh

Materials and Methods: A total of 100 *Escherichia coli* strains were isolated and subjected for in vitro antibiotic susceptibility test. Colistin sulphate, gentamicin, azithromycin, levofloxacin, ciprofloxacin, tetracycline, amoxicillin and metronidazole were used to detect the antibiotic susceptibility pattern of those isolates. The antibiogram status of each antibiotic was determined by measuring the diameter of the zone of inhibition due to the diffusion of the agent into the medium surrounding the disc.

Results: A high of 96%, 89% and 88% *Escherichia coli* isolates showed the sensitivity to colistin sulphate, levofloxacin and ciprofloxacin, respectively, followed by azithromycin (79%), gentamicin (77%), amoxicillin (63%) and tetracycline (46%). No isolate was sensitive to metronidazole.

Conclusion: Colistin sulphate, levofloxacin and ciprofloxacin have the potential effects against *Escherichia coli* in migratory birds and further study should be performed to evaluate the cross linkage of this *Escherichia coli* isolate with that of found in other poultry and human.

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► Introduction

Escherichia coli (E. coli) is a very common and notorious pathogen of commercial poultry, migratory birds (Hofstad et al 1992) and important zoonotic pathogen (Damare et al 1979) as well resulting elevated fecal coliform densities in the water column (Hussong et al 1979) which lead to food and water-borne human diarrhea worldwide in developing countries causing 800000 deaths out of 650 million cases per year primarily in children under the age of five years (Turner et al 2006). Coliform bacteria are often benign, but some strains may adversely affect disease and mortality risks.

In the public health arena, coliform counts in water supplies and food samples are used as a correlative index for human health risk. Various types of antimicrobial agents are being used but the number of multi-drug resistant *E. coli* is continuously increasing (Hussain et al 1982). Though antibiotics are widely used in order to combat the *E. coli* (Houndt and Ochman 2000, Vidaver 2002), they develop resistance to antibiotics (Koshland 1994). Most antibiotic resistance genes reside on horizontally mobile elements (HMEs) of viruses, conjugative plasmids, integrons, transposons etc. and transmitted from one organism to another (Heinemann 1998), and can persist in bacterial genomes in the absence of selective pressure by antibiotics (Jabes et al 1989).

Resistance can be acquired by horizontal gene transfer from other microbes; many bacteria and fungi constitute natural sources of drug resistance genes and may serve as reservoirs in the environment (Maiden 1998). Basically, winter season is the high time for migratory birds to harbor in such Haors, marshlands, and lagoons in Bangladesh. Migratory birds thus spread various diseases in their surroundings (Bartoloni et al 2004). Considering the above facts, the present research work was undertaken to determine the current status of drug sensitivity and resistance pattern of the *E. coli* isolated from migratory whistling swan to take the desirable steps against this pathogen.

Materials and Methods

Freshly voided fecal samples of waterfowls, common gulls, whistling swans etc. were collected aseptically and pure cultures of *E. coli* were obtained by using Eosin Methylene Blue (EMB) agar and identified the isolates by observing gross colony morphology, grams staining and motility test using hanging drop (Merchant and Packer 1967) and subjected to different biochemical tests such as sugar fermentation test, Indole production test, Methyl-Red and Voges-Proskyer (MR-VP) test (Cowan 1985) for final confirmation.

Commercially available antimicrobial discs (BENEX Limited, USA) including colistin sulphate (CS, 15 µg), gentamicin (GNT, 10 µg), azithromycin (AZM, 15 µg),

levofloxacin (LVX, 5 μg), ciprofloxacin (CIP, 5 μg), tetracycline (TE, 30 μg), amoxicillin (AMX, 10 μg) and metronidazole (MET, 80 μg) were used for the test to determine the drug sensitivity and resistance pattern and to interpret their disease potential. Antimicrobial sensitivity pattern of *E. coli* isolates allowed for the rapid determination of the efficacy of a drug by measuring the diameter of the zone of inhibition that results from diffusion of the agent in to the medium surrounding the disc. By sterile syringe 0.2 mL of broth culture of the test organism (prepared in nutrient broth by overnight culture) was poured on EMB agar and Nutrient agar separately. Inoculated plates containing homogenous texture were closed and allowed to dry for approximately 3-5 minutes. Then all the samples were tested aseptically against antibiotic discs and incubated at 37 °C for 24 hours (Wiggins 1996) as inverted pattern. After incubation the plates were examined and the diameter of the zone of complete inhibition was measured by mm scale.

▶ Results

The overall results of antibiotic susceptibility test are summarized in Table 1.

Table 1. Antibiotic susceptibility of E.coli strains isolated from migratory birds (n=100).

Antibiotics	HS %	MS %	LS %	R %
Colistin sulphate	96	-	-	4
Gentamicin	-	77	-	23
Azithromycin	-	79	-	21
Levofloxacin	89	-	-	11
Ciprofloxacin	88	-	-	12
Tetracycline	-	-	46	54
Amoxycilin	-	63	-	37
Metronidazole	-	-	-	100

HS: Highly sensitive, MS: Moderately sensitive, LS: Less sensitive, R: Resistance, -: Not detected

Most of the $E.\ coli$ isolates from these migratory birds found to be highly sensitive to CS (96%), LVF (89%) and CIP (88%), AZM (79%), GNT (77%), AMX (63%), TC (46%) and MET (00%).

▶ Discussion

The sensitivity % of CS was found near 100% i.e, 96% and it remains peak form here out of all tested antibiotics. Due to lack of information availability on CS the result we got could not compared though Jeremy and George (1963) reported the excellent antibiogam activity of CS on cultured *E. coli*.

LVF and CIP showed 89% and 88% sensitivity respectively and the result is partially verified by Fu et al (1992) and they shown that LVX was two- to four fold less active than CIP against most members of the family Enterobacteriaceae, such as *E. coli*. The related articles were also not available on migratory water-

fowls, though available on chicken. But, Middleton and Ambrose (2005) found 100% sensitivity of *E. coli* to CIP isolated from migratory Canadian geese (*Branta canadensis*) that is very similar to the findings of this study (88%). On the contrary, Yang et al (2004) showed that high percentage of isolates recovered from chicken displayed sensitivity to LVX (21%), and CIP (36%) that is very dissimilar and may be due to species variations and location as well.

AZM, GNT and AMX showed moderate type sensitivity against the introduced isolates resulted in 79%, 77% and 63%, respectively. Data to construct comparison are not available on that regards in waterfowls. MET represented no sensitivity (0%) to that isolates but literature of MET on migratory waterfowls is not available.

In this study, MET showed 100% resistance followed by TC (54%), AMX (37%), GNT 23%), AZM (21%), CIP (12%), LVF (11%) and CS (4%). Drug resistant bacteria recovered from wild birds (Niida et al 1983) and migratory birds (Kanai et al 1983) creating the diseases bridges to environmental pollution involving humans and other animals (Terakado et al 1971) and consequently may play the vital role in the spread of drug resistant E. coli isolates. The prevalence of human pathogenic strains of E. coli in Canada goose feces was 55%. More detailed studies of E. coli in Canada goose feces by Kullas et al (2002) showed that the prevalence of human pathogenic serogroups was 25% in Colorado. Masao et al (1995) reported that 80% of drug resistant isolates recovered from whistling swans had resistance to more than one drug that is not detected in this study. They also described that isolates were more resistant to TC in aspect of whistling swans which is also detected in this study (resistant about 54%). This resistance pattern may be related with their feeding system, routes of their migration (Kanai et al 1983) though these were not considered in this study.

Bacteria with antimicrobial drug resistance could be imported into this region either by migratory birds or through human refuse (food, excretions) from fishermen, settlers, and prospectors in the area. The region around the Hakaluki Haor (lagoon) constitutes the harbor of a large number of waterfowl, geese, shorebirds and other migratory birds during winter season. It is strongly decided with the findings of Bartoloni et al (2004) on many of these birds species; are largely confined to one side of the Haor, and act as vector and reservoir of various pathogens like *E. coli* which consequently transmit antibiotic resistant bacteria to other host ranges during migration or occasionally wander across such types of marshlands.

► Conclusions

Antibiogram pattern of *E. coli* strains isolated from migratory waterfowls showed higher sensitivity to

CS, LVX and CIP, and represented no sensitivity to MET. These findings will be helpful for further studies on treatment patterns, detection of mutation linkages, phylogenic and epidemiological studies.

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