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Isolation and Detection of Antibiotic Sensitivity Pattern of *Escherichia coli* from Ducks in Bangladesh and Nepal

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A B S T R A C T

A total of 120 rectal swab samples from ducks (sixty samples from Nepalgunj area of Nepal and 60 from Boyera area under Mymensingh district of Bangladesh) were collected for the isolation of *Escherichia coli* and their antibiogram study. After cultural and biochemical examination, a total of 40 samples from Nepalgunj and 45 samples from Boyera were found positive for *E. coli*. Pathogenicity study of 10 positive isolates from Nepalgunj and 12 positive isolates from Boyera were done to detect the presence of enterotoxin. All inoculated mice died showing typical lesion of extensive hemorrhage and massive edema. The isolates from two different origins showed major difference in their antibiogram study. The isolates of Nepal were highly sensitive to ciprofloxacin, co-trimoxazole, chloramphenicol and amoxicillin; moderately sensitive to nalidixic acid and; less sensitive to kanamycin and resistant to cephalexin. However, the isolates of Bangladesh were highly sensitive to ciprofloxacin, chloramphenicol and amoxicillin; moderately sensitive to nalidixic acid, cephalexin, and co-trimoxazole; less sensitive to kanamycin. This variation of antibiotic sensitivity and resistance patterns among the *E. coli* isolates of Nepal and Bangladesh might be due to strain variations and indiscriminate use of antibiotics in these two different countries.

Keywords: *Escherichia coli*, Duck, Nepal, Bangladesh, Antibiogram

Introduction

Escherichia coli is a Gram negative, enteric bacillus, and best known as a noninvasive commensal that grows in mass culture in human and in animal gut lumen, perhaps keeping other harmful bacteria away from proliferating (Buxton and Fraser, 1987). *E. coli* is found habitually in the large intestine of vertebrates usually as a part of the normal flora (Buxton and Fraser, 1987). *E. coli* remains as commensal and is mainly encountered in the lower portion of the intestine and acts as opportunistic pathogens of human and animal (Levine, 1884). It produces septicemia and diarrhea in ducks and other birds and animals, such as lamb, goatlings, foals and calves (Hofstad *et al.*, 1984). Avian colibacillosis has been noticed to be a major infectious disease in birds of all ages. In Bangladesh, antibiotics are randomly used for the treatment of disease caused by *E. coli*. Due to abuse of antibiotics in those country emergences of multi-drug resistant *E. coli* are continuously increasing (Hussain *et al*., 1982) and similar is the case in Nepal (Baral *et al*., 2012). This study was carried out to characterize the *E. coli* isolates from duck of Bangladesh and Nepal and to find out the effective antibiotic(s) that could be applied to control avian colibacillosis in duck in these areas.

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Materials and Methods

The study was conducted during July 2010 to Nov. 2010 at the Department of Microbiology and Hygiene, Bangladesh Agricultural University (BAU), Mymensingh-2202, Bangladesh. A total of 60 field samples (rectal swabs) were collected from 35 diarrheic and 25 apparently healthy ducks by sterile cotton bud and carried to the laboratory of Nepalgunj Medical College and Hospital, Nepalgunj, Nepal. All these 60 samples were inoculated on Eosine Methylene Blue (EMB) agar plates, incubated for overnight at 37^0C. The suspected colonies were brought to the Laboratory of Bacteriology, Department of Microbiology and Hygiene, at BAU, Mymensingh maintaining cool chain. Similarly, 60 field samples comprising rectal swab were collected by sterile cotton bud from 35 diarrheic and 25 apparently healthy ducks of Boyera area, located around BAU, Mymensingh, Bangladesh and brought to laboratory for isolation and characterization of *E. coli.*

Isolation and characterization of isolated *E. coli*

Pure culture was obtained from the primary culture using EMB agar, MacConkey (MC) agar, Brilliant Green (BG) agar, Salmonella-Shigella (SS) agar and Blood agar. The colony characteristics were observed and Gram staining was performed. Motility test and biochemical tests (Sugar Fermentation test, Indole test, Methyl-Red (MR) test, Voges-Proskauer (VP) test and Catalase test) were performed for further characterization of the isolates of both origins.

Pathogenicity study of the isolates

A total of 10 positive isolates from Nepalgunj and 12 positive isolates from Boyera were subjected for pathogenicity study. A total of 30 new born Swiss Albino suckling mice (2-4 days old) collected from the Laboratory Animal Center of the Department of Microbiology and Hygiene, BAU were divided into two groups (n=15/ group): group A for isolates from Nepalgunj and group B for isolates from Boyera on random basis. Ten EMB colonies of isolates from Nepalgunj and 12 colonies of isolates from Boyra were inoculated into nutrient broth and incubated overnight at 37^0C. The cells were then removed from the broth culture by centrifugation at 25,000 rpm for 20 min. The supernatants were filtered and collected. Each of the 12 mice in group A received 0.1 ml of supernatant by oral route and remaining 3 mice were kept as control. Similarly, 10 mice from group B were administered 0.1 ml of supernatant and 5 mice from this group were kept as control without enterotoxin treatment. Both groups were kept at room temperature for 14 hours. After 14 hours all mice (live and dead) including the control mice, were necropsied. The ratio of gut weight and the remaining carcass weight was calculated for each mouse and the average ratio was calculated (Gyles, 1979).

Antibiogram of isolates

Eight different antibacterial discs (HiMedia, Mumbai, India) were selected randomly for antibiotic sensitivity study against isolated *E. coli* from Bangladesh and Nepal (Table 1). Six isolates from each of the country were subjected for antibiogram study. A disk diffusion test of the Kirby-Bauer (Bauer *et al*., 1966) method was used to determine the susceptibility of *E. coli* isolates to different antibiotic agents. The procedure involved measuring the diameter of the zone of inhibition that results from diffusion of the agent into the medium surrounding the disc. Tentative inhibition zone diameter interpretive criteria were developed using the error-rate-bounded methods recommended by the CLSI (Clinical and Laboratory Standards Institute, 2011).

Results

Isolation and identification of *E. coli* isolates

All the positive *E. coli* isolates from both Nepal and Bangladesh showed characteristics colony morphology in EMB agar, MC agar, Blood agar, SS agar and BG agar media. Characteristic pink, rod, short, Gram negative bacilli were found with Gram staining. All the isolates were found to be motile with hanging drop slide. The five basic sugars (dextrose, sucrose, lactose, maltose and mannitol) were fermented by all the isolates producing acid and gas. In addition, all the isolates were found positive for MR test, negative for VP test and positive for indole.

Table 1. Antimicrobial discs and their concentrations

Antimicrobial agents	Disc concentration (µg/disk)
Chloramphenicol (CL)	30
Cephalexin (CK)	30
Amoxycillin (AX)	30
Erythromycin (ER)	15
Cotrimoxazole (CT)	25
Ciprofloxacin (CP)	5
Nalidixic acid (NA)	30
Kanamycin (KA)	30

µg = Microgram

Table 2. Rectal swab samples collected from duck found positive for *E. coli*

Origin of samples (n)	Conditions of birds (n)	Positive samples for *E. coli*	Prevalence in %
Bangladesh (60)	Healthy (25)	10	40
	Diarrheic (35)	35	100
Nepal (60)	Healthy (25)	5	20
	Diarrheic (35)	35	100

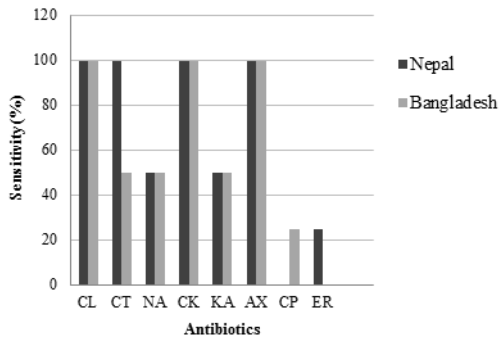

Fig. 1: Antibiogram of *E. coli* isolates of duck collected from Bangladesh and Nepal. Considerable differences in sensitivity shown by *E. coli* against three antibiotics (Cotrimoxazole, Ciprofloxacin and Erythromycin) was revealed in between the isolates of Bangladesh and Nepal.

Pathogenicity test of isolated duck *E. coli*

After 14 hours, all inoculated mice died showing typical lesion of extensive hemorrhage and massive edema of the gut. The ratio of gut weight and the remaining carcass weight was measured and the average ratio of less than 0.070 was considered negative while 0.070-0.085 was considered positive for enterotoxin (Gyles, 1979). Eight isolates from Nepal and ten isolates from Bangladesh were found positive for enterotoxin.

Antibiogram

Antibiogram study of the isolated *E. coli* against 8 commonly used antibacterial agents of different groups revealed that *E. coli* isolated from Bangladesh were highly sensitive to ciprofloxacin, chloramphenicol and amoxicillin and moderately sensitive to nalidixic acid, cephalexin and co-trimoxazole and less sensitive to kanamycin. On the other hand, *E. coli* isolated from Nepal were highly sensitive to ciprofloxacin, co-trimoxazole, chloramphenicol and amoxicillin, and moderately sensitive to nalidixic acid and less sensitive to kanamycin and resistant to cephalexin.

Discussion

The isolated *E. coli* on BA and BG agar produces round, colorless colony with hemolysis and green color colonies respectively. Similar types of cultural characteristics were earlier reported by Buxton and Fraser (1977) and Hasina (2006). In Gram staining, the morphology of the isolated bacteria exhibited Gram negative short rod arranged in single or paired which was supported by several authors (Buxton and Fraser, 1987; Jones, 1987). All the isolated revealed a complete fermentation of five basic sugars as stated by Beutin *et al.* (1997) and Mckee *et al.* (1995).

Antibiogram results showed that this isolated *E. coli* from both Bangladesh and Nepal were highly sensitive against ciprofloxacin, chloramphenicol and amoxicillin. Similar results were obtained in the *E. coli* isolates of duck in South Korea (Unno *et al*., 2010). In addition, the isolates from Nepal showed high sensitivity against co-trimoxazole (Fig). Recently, some *E. coli* isolates of duck in China showed high resistance against ciprofloxacin and chloramphenicol (Ma *et al*., 2012). Again, *E. coli* isolated from Bangladesh showed moderate sensitivity to nalidixic acid, kanamycin and co-trimoxazole; less sensitivity to cephalexin and resistance to erythromycin (Fig. 1). On the other hand, *E. coli* isolates from Nepal showed moderate sensitivity against nalidixic acid and kanamycin; less sensitivity against erythromycin and resistance against cephalexin (Fig. 1).

Conclusion

Based on this study, it may be concluded that chloramphenicol, ciprofloxacin and amoxicillin should be the first choice of treatment against avian colibacillosis in duck in Bangladesh. On the other hand amoxicillin, co-trimoxazole, ciprofloxacin and chloramphenicol should be the first choice of drug for the treatment of avian colibacillosis in duck in Nepal. The resistance pattern observed among the isolated *E. coli* in Bangladesh and Nepal might be related to the extensive and indiscriminate use of antibiotic in duck in these areas.

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