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Article

Isolation and identification of bacterial pathogens from cloacal swabs of turkeys and their antimicrobial sensitivity patterns

Polash Chandra Roy[1], Md. Khaled Hossain[2], Nazmi Ara Rumi[3*], Md. Shajedur Rahman[4], Md. Shahin Mahmud[5] and Ishaq Hossion[6]

[1]Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh
[2]Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh
[3]Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh
[4]Department of Medicine, Surgery & Obstetrics, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh
[5]Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh
[6]Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh

[*]Corresponding author: Nazmi Ara Rumi, Lecturer, Department of Microbiology, Hajee Mohammad Danesh Science & Technology University, Dinajpur, Bangladesh. Phone: +8801774410088; E-mail: rumi_dvm@yahoo.com

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Abstract: The present study was carried for the isolation, identification of bacterial pathogens from cloacal swabs of turkeys during the period from January-June, 2016. The entire research work was conducted in the Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur. The study was performed with 48 cloacal swab samples. The cloacal swab samples were collected carefully from three different Turkey Farms randomly and transferred aseptically to the laboratory. On the basis of morphology, staining, cultural and biochemical characteristics it was found that among the isolates 25(52.08%) samples were positive *E. coli*, 10(20.83%) samples were positive for *Salmonella* spp., 9(18.76%) samples were positive for both *E. coli* and *Salmonella* spp. and 4(8.33%) samples shown no growth in subculture media. Antibiogram profiles indicate that *E. coli* isolated were 100% sensitive to Azithromycin, Kanamycin and Ciprofloxacin, 80% sensitive to Cefradine, Vancomycin and Levofloxacin, 60% sensitive to Cefotetan and Nitrofurantoin and 40% sensitive to Erythromycin. The isolates were 100% resistant to Cloxacillin and Cefixime. On the other hand, *Salmonella* spp. were 100% sensitive to Azithromycin, Kanamycin, Levofloxacin and Ciprofloxacin, 80% sensitive to Nitrofurantoin and Teicoplanin, 60% sensitive to Vancomycin, Erythromycin and Cefixime and 20% sensitive to Cefotetan. The isolates were 100% resistant to cefradine and cloxacillin. So, for *E. coli* Azithromycin, Kanamycin and Ciprofloxacin were more sensitive and for *Salmonella* spp. Azithromycin, Kanamycin, Ciprofloxacin and Levofloxacin were highly sensitive. Diversified bacterial species were present in cloacal swabs of Turkeys. However, *E. coli*, *Salmonella* spp. infection might make the birds vulnerable for easy access of infection. It could be concluded that *E. coli* and *Salmonella* spp. may pass through the feces to the environment. It causes a potential human health hazards and can cause illness.

Keywords: turkey; cloacal swabs; antibiogram studies

1. Introduction

The turkey is a large bird in the genus *Meleagris*, which is not native to Bangladesh. But now a day it is familiar to Bangladesh. *Meleagris gallopavo*, commonly known as the Wild Turkey, is native to the forests of North America. The domestic turkey is a descendant of the Wild Turkey (Smith, 2006). Turkeys are suitable for commercial egg, meat production and can be raised as pets. They are very beautiful and help to increase the beauty of our home. For business purpose, turkeys are highly meat productive. But not suitable for commercial egg production. They grow faster and become suitable for slaughter purpose earlier like broiler chickens and quails. Poultry feces are waste products can also be defined as the by-product that resulted from the digestion of food intake by poultry birds. There are several billions of bacteria present in poultry feces including pathogenic and non-pathogenic species, the normal flora and the opportunistic ones (Adegunloye, 2006). Fewer studies have sought to understand the turkey microbiome. Some work has focused on comparison of the caecal microbiomes of wild and domestic birds (Scupham *et al.*, 2008) or examination of the turkey microbiome in relation to pathogen colonization, such as *E. coli* (Scupham, 2009). There is a difference in the bacterial genera present in the cloacal swab of different turkeys, as well as bacterial populations in the turkey intestinal tract. A number of possible contributing factors have been speculated, including management practices, the presence of known or unknown bacterial pathogens, disruptions of the gastrointestinal microbial communities, problems with nutrient absorption, or dwarfed immune development in turkey (Calvert, 2012). Thus, the purpose of this study was to examine bacterial community succession in turkeys raised under different conditions and to compare the bacterial communities of turkey's cloacal swabs. Bacterial disease that causes concern in the turkey industry. It results in production losses via decreased feed efficiency, slower growth rate, and increased morbidity and mortality rates, and may predispose the poult to other diseases. Research has focused on the aerobic bacteriological (Schmidt *et al.*, 1988) etiologies of turkey enteritis. Many bacteria (Goodwin *et al.*, 1989), have been seen in the intestinal wall of the turkey in the crop, ileum, and cecum (Fuller and Turvey, 1971). The bacteria have been observed to be attached to the enterocytes and have been associated with diarrhea in turkeys (Goodwin *et al.*, 1989). However, others may be primary or opportunistic pathogens capable of causing a variety of turkey diseases. Avian pathogenic *E. coli* strains are the etiologic agents of colibacillosis in birds and are an important problem for the turkey industry (Soon *et al*., 2008). *E. coli* strains cause a number of diseases in domestic turkey, ultimately leading to disease and death, or to a decrease in egg and meat production or condemning of carcasses (Sackey *et al.*, 2001). In turkey, consequences of *E. coli* infections include egg peritonitis, omphalitis, coligranuloma, swollen head syndrome, cellulitis, and colisepticaemia, and death of the birds (Hofstad *et al.*, 1992). *Salmonella enterica* can cause a wide range of illnesses, ranging from gastroenteritis to acute, life-threatening enteric fever. Salmonellosis is one of the most prevalent infectious foodborne diseases in the world (McCarthy *et al*., 2009). Examining the antibiotic susceptibility patterns of pathogens is important toward tailoring treatment to the ever-changing resistance patterns and distribution of pathogenic bacteria (NCCLS, 2001). The high nutrient content of bird excrement provides an excellent sanctuary for potentially harmful organisms. Bird droppings do pose a public health risk and cause illness. Humans become infected by inhaling dust containing dried feces, urine, or respiratory secretions of infected birds. Considering all the above mentioned points, the present work was designed to isolate and identify the bacterial pathogens from cloacal swabs of turkeys and to determine antibiotics sensitivity patterns of the isolated bacteria for rational use of antibiotics in Turkey farms.

2. Materials and Methods

2.1. Collection of samples

This study was carried out throughout the period of January-June, 2016 at bacteriological laboratory in the Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200. A total number of 48 cloacal swab samples were included in this study. The sample were collected from three different places like Turkey research farm, HSTU, Dinajpur, Mokhlesur Turkey Farm, Thakurgaon Sadar, Thakurgaon and Arman Turkey Farm, Saidpur, Nilphamari. The samples were carried to the laboratory in an ice box contained ice and processed for the isolation and characterization of bacteria subsequently and kept in incubator at 37^0C for 24 hours for the isolation and identification.

2.2. Isolation of associated bacteria

Bacteriological examination was carried out using standard method for aerobic bacteria (Brown, 2005). For the isolation of bacteria, all samples were serially diluted and plated on Nutrient agar and subsequently incubated at 37 ^{o}C for 24 hours. Primary culture was performed in Nutrient agar and Nutrient broth media. For sub-culturing, suspected bacteria were inoculated separately onto different bacteriological agar media under aseptic condition

and incubated at 37 ^{o}C for 24 hours. Pure cultures were achieved as per the procedures described by OIE (2000), Merchant and Packer (1967) and Cowan (1985).

2.3. Identification of associated bacteria

Cultural, morphological and biochemical characteristics were studied in order to identify the bacterial flora. Gram's staining was performed to study the morphology and staining characteristics of bacteria according to the technique described by Merchant and Packer (1967). Biochemical tests, such as sugar fermentation, coagulase, catalase, MR, VP, and indole tests, were performed as per the standard methods (Cheesbrough, 1985).

2.4. Antibiogram study

Antimicrobial drug sensitivity test was performed on freshly prepared, dried up Mueller Hinton agar (Oxoid) against 8 commonly used antibiotics by disc diffusion method or Kirby-Bauer method (Bauer *et al.*, 1966) according to the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2015).

3. Results

3.1. Cultural characteristics

Cultural characteristics of each type of bacteria isolated from cloacal swab of turkey were studied for the examination of size, shape, colony characteristics, and pigment production in various solid media. The pure cultures of the organism from each mixed culture were obtained by repeated streak plate method by using different simple, enriched and selective solid media for study. The individual culture characteristics of bacterial isolates are presented in Table 1.

Table 1. Cultural characteristics of the organisms isolated from cloacal swabs of turkey.

Isolated organism	NA	EMB Agar	MacConkey agar	SS agar	SCA
E.coli	Smooth, circular, White to grayish white colony	Smooth, Large, circular, blueblack colonies with slightly green metallic sheen	Smooth pinkish colony	Slight growth and pink to rose-red colonies	No change of green color
***Salmonella* spp.**	Circular, smooth, opaque and translucent	Pink color, circular and smooth colony	Smooth and circular white/transparent colony	Black centered, smooth, small round colony	Green color converted into bluish color

Legends: NA = Nutrient Agar, MC = MacConkey, EMB = Eosin Methylene Blue, SS = Salmonella- Shigella, SDA= Sebouraued dextrose agar, SCA= Simmons citrate agar, *E. coli=Escherichia coli.*

3.2. Staining characteristics

The staining characteristics of the isolated organisms were determined according to Gram's staining technique and the results are presented in Table 2.

Table 2. Morphology, staining and motility characteristics of bacterial isolates.

Bacterial isolates	Shape	Arrangement	Gram's staining reactions	Motility characteristic
E. coli	Rod or Coccobacilli	Single or paired	Gram negative	Motile
***Salmonella* spp.**	Small rod	Single	Gram negative	Non motile

3.3. Biochemical tests

Bacteria isolated from the cloacal swabs were subjected to various types of biochemical tests such as Triple sugar iron agar, methyl red test, *Voges–Proskauer* test, MIU test and buffer peptone water test (Indole test) in order to determine their biochemical characters and degree of variation in their reactivity pattern. The result was presented in Table 3.

Table 3. Biochemical tests of the isolated *E. coli and Salmonella* spp. from cloacal swabs of turkey.

Isolated bacteria	MR	Indole	VP	TSI			MIU
				Butt	Slant	H2S	
E.Coli	+	+	+	Y	Y	-	+
Salmonella spp.	+	+	-	Y	R	+	+

Legends
MR= Methyl Red, VP= *Voges–Proskauer*, TSI= Triple Super Iron, " +"= Positive, "-"= Negative, Y= Yellow, R= Red, Indole= Buffer Peptone water, MIU= Motility Indole and Urease test

3.4. Bacterial flora isolated from turkeys

E. coli and *Salmonella* spp. were isolated from the 48 turkey samples. A total of 48 turkey samples were collected from different turkey farms. Out of 48 turkey samples, 44 were positive and 4 were no growth. Among 44 positive samples, 25 *E. coli,* 10 *Salmonella and* 9 both *E. coli* and *Salmonella* were isolated. However, 4 samples were not grown in sub culture media. The summary of isolation of bacteria from turkeys is shown in Table 4.

Table 4. Isolated bacteria with percentage from turkeys.

Bacteria isolated	No. of isolated
E. coli	25
Salmonella spp.	10
E. coli + Salmonella spp.	9
No Growth	4

3.5. Results of antibiotic sensitivity assay of isolated bacteria

The isolated bacterial pathogens were selected randomly for the antibiotic sensitivity and resistance patterns against commonly used antibiotics. The results of sensitivity against antibiotic discs (zone of inhibition) were categorized as resistant (-), intermediate (++) and sensitive (+++).

3.5.1. Antibiotic sensitivity pattern of *E. coli*

The antibiotic sensitivity pattern of *E coli* under the study revealed that all of the isolates (5) were 100% sensitive to Azithromycin (AZM), Kanamycin (k) and Ciprofloxacin (CIP) , 80 % sensitive to Cefradine (CH), Vancomycin (VA) and Levofloxacin (LE), 60% sensitive to Cefotetan (CN) and Nitrofurantoin (N) and 40% sensitive to Erythromycin (E). The isolates were 100 % resistant to Cloxacillin (COX) and Cefixime (CFM) (Table 5).

Table 5. Antibiotic sensitivity pattern of *E. coli* (n = 5).

Antibacterial agents	No. of isolates			Percentages (%)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Azithromycin (AZM)	5	0	0	100	00	00
Cefotetan (CN)	0	3	2	00	60	40
Cefradine (CH)	4	1	0	80	20	00
Cloxacillin (COX)	0	0	5	0	00	100
Vancomycin (VA)	4	1	0	80	20	00
Nitrofurantoin (N)	0	3	2	00	60	40
Erythromycin (E)	0	2	3	00	40	60
Levofloxacin (LE)	4	1	0	80	20	00
Kanamycin (k)	5	0	0	100	00	00
Cefixime (CFM)	0	0	5	0	00	100
Ciprofloxacin (CIP)	5	0	0	100	00	00
Teicoplanin (TE)	4	1	0	80	20	00

3.5.2. Antibiotic sensitivity pattern of *Salmonella* spp. (n = 5)

The antibiotic study revealed that all of the isolates (5) were 100% sensitive to Azithromycin (AZM), Kanamycin (k), Levofloxacin (LE) and Ciprofloxacin (CIP), 80 % sensitive to Nitrofurantoin (N) and Teicoplanin (TE), 60% sensitive to Vancomycin (VA), Erythromycin (E) and Cefixime (CFM) and 20% sensitive to Cefotetan (CN). The isolates were 100 % resistant to Cefradine (CH) and Cloxacillin (COX) (Table 6).

Table 6. Antibiotic sensitivity pattern of *Salmonella* spp. (n = 5).

Antibacterial agents	No. of isolates			Percentages (%)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Azithromycin (AZM)	5	0	0	100	00	00
Cefotetan (CN)	1	0	4	20	00	80
Cefradine (CH)	0	0	5	0	00	100
Cloxacillin (COX)	0	0	5	0	00	100
Vancomycin (VA)	3	2	0	60	40	00
Nitrofurantoin (N)	4	1	0	80	20	00
Erythromycin (E)	3	2	0	60	40	00
Levofloxacin (LE)	5	0	0	100	00	00
Kanamycin (k)	5	0	0	100	00	00
Cefixime (CFM)	3	2	0	60	40	00
Ciprofloxacin (CIP)	5	0	0	100	00	00
Teicoplanin (TE)	4	1	0	80	20	00

4. Discussion

In this study, there were two types of bacteria were isolated from 48 cloacal swabs samples. The isolated organisms were bacteria as *E. coli and Salmonella* spp. Then out of 48 samples *E. coli* 25(52.08%), *Salmonella* spp. 10(20.83%), both *E. coli* and *Salmonella* spp. 9 (18.76%) and no growth 4(8.33%). The distribution of *E. coli* and *Salmonella* spp. of bacterial isolates in different cloacal swab samples were found in variable condition. So, results of the present study indicated that two types of bacteria were present in the cloacal swab samples which were collected from different turkey farms, especially in turkeys from HSTU turkey research farm and other different turkey farms. The incidence of *E. coli* and *Salmonella* spp. isolated from cloacal swab samples collected from turkey compared with the findings of Tiffany (2014), Jessica *et al.* (2013), Bielke *et al*. (2003) and Boyer *et al*. (1962) with slight variation. The different isolates of *E. coli* and *Salmonella* spp. showed identical results in different biochemical tests including Methyl-Red, Voges-Proskauer, and Indole test and similar type of biochemical reaction as reported by Menconi *et al.* (2010) and Bryan (I965).

In this study, colony characteristics of *E. coli* (Table 2) observed in NA, EMB and SS agar were similar to the findings of Nayak *et al.* (2004) and Buxton and Fraser (1977). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative short rod arranged in single or paired and motile which was supported by several authors (Buxton and Fraser, 1977; Merchant and Packer, 1967). The colony characteristics of *Salmonella* spp. observed in NA, SS agar, were similar to the findings of Potturi *et al*. (2005). In Gram's staining, the morphology of the isolated bacteria exhibited Gram negative small rod arranged in single or paired and motile which was supported by several authors (Kumar *et al.*, 1971; Tempe *et al.*, 2003).

The *E. coli* isolates revealed a complete fermentation of 5 basic sugars by producing both acid and gas which was supported by Bielke *et al.* (2003). The isolates also revealed positive reaction in MR test and Indole test but negative reaction in VP test (Buxton and Fraser, 1977).

The antibiotic study revealed that all of the isolates (5) of *E. coli* were 100% sensitive to Azithromycin, Kanamycin and Ciprofloxacin, 80% sensitive to Cefradine, Vancomycin and Levofloxacin, 60% sensitive to Cefotetan and Nitrofurantoin and 40% sensitive to Erythromycin. The isolates were 100% resistant to Cloxacillin and Cefixime. Akond *et al.* (2009) reported *E. coli* strain from poultry sources were resistant to penicillin, ciprofloxacin, erythromycin, ampicillin. Sensitivity were recorded to Gentamycin, Chloramphenicol and Neomycin. The antibiotic study revealed that all of the isolates (5) of *Salmonella* were 100% sensitive to Azithromycin, Kanamycin, Levofloxacin and Ciprofloxacin, 80% sensitive to Nitrofurantoin and Teicoplanin, 60% sensitive to Vancomycin, Erythromycin and Cefixime and 20% sensitive to Cefotetan. The isolates were 100% resistant to Cefradine and Cloxacillin. So, for *E. coli* Azithromycin, Kanamycin and Ciprofloxacin were

more sensitive and for *Salmonella* spp. Azithromycin, Kanamycin, Ciprofloxacin and Levofloxacin were highly sensitive.

5. Conclusions

In the context of this study, it may be concluded that the cloacal swabs collected from turkeys contain both *E. coli and Salmonella* spp. That might make the birds vulnerable for easy access of infection and also the bacterial pathogens may pass through the faeces to the environment and cause a potential human health hazards and can cause illness. By the antibiogram test, it may be concluded that Azithromycin, Kanamycin and Ciprofloxacin were more sensitive drug for *E. coli* and Azithromycin, Kanamycin, Ciprofloxacin and Levofloxacin were highly sensitive for *Salmonella* spp.

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Conflict of interest

None to declare.

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