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Prevalence, Isolation, Characterisation and Antibiogram Study of Pathogenic *Escherichia coli* from Different Poultry Farms of Odisha

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Abstract

From 182 birds of different farms of odisha suspected for colibacillosis 317 swab samples containing 51 air sacs, 39 lungs, 53 livers, 36 heart bloods, 45 pericardial fluids, 19 yolk sacs and 74 intestine samples were processed for isolation and identification of pathogenic *E. coli*. and subjected to detail bacteriological and biochemical examination in the laboratory and 105 *E. coli* isolates were isolated following standard procedures. The percentage of isolation of *E. coli* isolates in decreasing order was yolk sac (52.6%) and heart blood (38.4%) in 0-4 week birds. In older birds (4-7week) the highest percentage of isolation was from pericardial fluid (35.8%) followed by heart blood (33.4%). The present study showed that the frequency of occurrence of O9 strain is highest (16.7%) followed by O1, O33 & O51 (13.3%), O23 & O119 (10%), O103 & 79 (6.7%) and serotype O90 (3.3%). The antibiogram study reveals that, *E. coli* isolates found were highly resistant to some of the classical drugs like chlortetracycline (88.58%), streptomycin (85.72%), penicillin-G (82.86%), amikacin (82.86%), furazolidone (77.14%), ampicillin (74.29%), Tetracycline(74.29%), Amoxicillin (71.43%) and cotrimoxazole (71.43%), while isolates were highly sensitive to ceftriaxone and tazobactum (94.29%), ceftriaxone (91.43%), gentamicin (85.72%), chloromphenicol (82.67%), cephotaxime (77.14%), cefixime (74.29%) and ciprofloxacin (74.29%).

Keywords: Antibiogram; *Escherichia coli*; Farms; Poultry

Introduction

India ranked sixth position in the world in poultry production and the industry is a multicrore investment and increasingly more competitive with establishment of large number of farms with intensification. This intensification has lead to increased susceptibility to various disease conditions in the birds. Among these, losses due to colibacillosis appears to be one of the bottle necks as it has been recorded regularly in majority of the farms throughout the year. The organism affects multiple body system of birds causing various symptoms. The high consumption of chicken meat also warrants great care in safeguarding the industry against threatening factors (Kabir, 2010). Again, concern about antibiotic resistance and its transmission to human pathogens is important because these resistant bacteria may colonize the human intestinal tract and may contribute resistance genes to human endogenous flora. The episomal transfer of resistance factor between the intestinal pathogens may lead to evolution of drug resistant bacterial strains in human being which is of public health importance. In view of the significance of *E. coli* infection in poultry, this study has been undertaken to isolate, characterization of different serotypes and to study their antibiotic drug resistance pattern.

Materials and methods

The tissues and swab samples from 317 samples of 182 poultry were collected in sterile containers based on clinical findings and pathogonomonic lesions observed during detailed post mortem examination of poultry of different poultry farms and dead birds submitted to pathology department of Orissa Veterinary College, Bhubaneswar, India were processed. Samples like heart blood (36), pericardial fluid swab (45), liver (53), airsac (51), lungs (39), yolk sac (19) and intestine (74) were

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collected aseptically from fresh carcasses exhibiting perihepatitis, enteritis, airsaculitis, yolk sac infection, pnuemonitis and pericarditis and processed for bacteriological isolation and identification. Briefly, the collected tissues and swabs samples were inoculated into the Brain heart infusion (BHI) broth tubes and incubated at 37° C for 24 hours. After 24 hr of inoculation, the inoculum were plated on Mac Conkeys lactose agar (HIMEDIA) and incubated at 37°C for 24 hours. The lactose fermenting colonies were reinoculated to Eosin Methylene Blue agar (HIMEDIA) and colonies producing metallic sheen were transferred to EMB slants and incubated at 37°C for 24 hours and stored at 4°C for further identification. The *E. coli* organisms were identified based on their morphology, cultural, biochemical and sugar fermenrtation characters as per the method described by Edwards and Ewing (1972) and Cruickshank *et al*. (1975). Various biochemical tests which were performed include catalase test, oxidase test, indole test, Methyl Red (MR) test, Voges Proskauer (VP) test, H_2S production test, and citrate utilization test. Other test like haemolysis on blood agar and motility test were also carried out. Sugars used in the present study were arabinose, glucose, sucrose. maltose, lactose, ramnose, raffinose, mannose, xylose, mannitol, dulcitol and salicin. The pure cultures of *E. coli* isolates in EMB agar slants were submitted to National Salmonella and Escherichia Centre, Central research Institute, Kasauli, Himachal Pradesh, India for confirmation and serotyping by agglutination test. The antibiotic sensitivity tests of the isolates were performed by employing the Bauer- Kirby diffusion method using antibiotic discs (Himedia) as per the method of Bauer *et al*. (1966).

Results

From 182 suspected birds 35 birds were found to be positive for *E. coli* infection as they show characteristic lactose fermenting colonies on Mac Conkey Lactose agar and greenish black metallic sheen colonies on EMB agar and their biochemical and sugar fermenrtation characters were in accordance Edwards and Ewing (1972).

In the present study *E. coli* has been isolated from 10 different farms and also from dead birds submitted to pathology department covering 7 different districts of Odisha having varying environmental condition (Table 1). Out of 35 *E. coli* isolates referred to National Institute of Salmonella and Escherichia, Kasauli, 30 *E. coli* cultures could be serotyped as they were in smooth condition, whereas 2 were in rough conditions, 2 samples were non *E. coli* and one isolate was found to be nonviable. Different serotypes obtained in the present study are O1, O9, O33, O23, O51, O79, O90, O103 and O119 (Table 2). Among the strains isolated, O9 serotype was most frequent (16.7%) followed by O1, O33 and O51 (13.3% each).

Table 1. Frequency of E. coli serotypes from different sources

Sl. No	Source	No. of isolates	Serotypes
1	Poultry farm, OVC, BBSR	4	O23(2), O79(1), UT-1 (1)
2	Central Poultry Farm. BBSR	2	O1(1), O33(1)
3	O.P.L.O.FED. Laxmisagar	3	O23(1), O9(1), UT-2 (1)
4	Pathology Dept, OVC, BBSR	4	O33(1), O51(2), O9(1)
5	Pasupati Farm, Tangi. Cuttack.	2	O51(1), O119(1)
6	Ranjan Poultry Farm, Dhenkanal	2	O1(1), O9(1)
7	Biswal Poultry Farm, Talcher, Angul	2	O1(1), O33(1)
8	State Govt. Farm, Angul	3	O9(2), O33(1)
9	Santosh Poultry Farm, Deogarh.	3	O1(1), O119(2)
10	Rajesh Purohit Poultry Farm, Bhawanipatna	3	O103(2), O90(1)
11	Bhabani Poultry Farm, Sambalpur	2	O51(1), O79 (1)

From the total isolates the highest percentage of *E. coli* isolates was recovered from intestine (22%) followed by liver (17%), air sac (15%), pericardial fluid (14%), lungs & heart (11%) and yolk sac (10%) (Table 3). Again, Age wise prevalence shows that the percentage of isolation of *E. coli* isolates was highest from samples of yolk sac (52.6%) followed by heart blood (38.4%) in 0-4 week birds and in older birds (4-7week) the highest percentage of isolation was from pericardial fluid (35.8%) followed by heart blood (33.4%).

Table 2. Frequency of occurrence of different *E.coli* serotypes (total number of isolates having smooth colony: 30)

Serotypes	No	Percentage
O1	4	13.3
O9	5	16.7
O23	3	10.0
O33	4	13.3
O51	4	13.3
O79	2	6.7
O90	1	3.3
O103	2	6.7
O119	3	10.0
UT	2	6.7

The results of antibiotic sensitivity test of the 35 isolates of *E. coli* showed that the isolates were highly sensitive to ceftriaxone and tazobactum (94.29%), ceftriaxone (91.43%), gentamicin (85.72%), chloromphenicol (82.67%), cephotaxime (77.14%), cefixime (74.29%) and ciprofloxacin (74.29%) and resistance was observed against chlortetracycline (88.58%), streptomycin (85.72%), penicillin-G (82.86%), amikacin (82.86%), furazolidone (77.14%), ampicillin (74.29%), tetracycline (74.29%), amoxicillin (71.43%) and cotrimoxazole (71.43%) (Table 4).

Table 3. Organ wise Prevalence of *E. coli*

Type of sample	Age group (Weeks)	No. of. samples	%
Air sac	0-4	19	31.57
	4-7	32	31.25
Lungs	0-4	13	30.76
	4-7	26	30.76
Liver	0-4	17	35.29
	4-7	36	33.33
Heart Blood	0-4	13	38.46
	4-7	23	30.40
Pericardial Fluid	0-4	17	29.41
	4-7	28	35.71
Yolk Sac	0-4	19	52.63
	4-7	0	00
Intestine	0-4	35	28.57
	4-7	39	30.76
Total		317	33.12

Table 4. Antibiotic sensitivity pattern of various antibiotics on *E. coli* isolates

Name of the Antibiotic	Percents of samples		
	High sensitivity	Moderate sensitivity	Resistant
Ampicillin (A)	8.57	17.14	74.29
Amoxycillin (Ac)	14.28	14.28	71.43
Amikacin (Ak)	5.71	11.43	82.86
Ceftriaxone (Ci)	91.43	8.57	0
Ceftriaxone and Tazobactum (Cit)	94.29	5.71	0
Cefixime (Cfx)	74.29	20.00	5.71
Cephotaxime (Ce)	77.14	11.43	11.43
Cotrimoxazole (Co)	5.71	22.86	71.43
Ciprofloxacin (Cf)	74.29	14.28	11.42
Chloromphenicol (C)	82.67	11.42	5.71
Gentamicin (G)	85.72	11.43	2.85
Penicillin-G (P)	8.57	8.57	82..86
Streptomycin (S)	5.71	8.57	85.72
Furazolidone (Fr)	11.43	11.43	77.14
Tetracycline (T)	8.57	17.14	74.29
Chlortetracycline (Ct)	2.85	8.57	88.58

Discussion

Perusal of literature indicates that different serotypes are prevalent in different regions of India. The *E. coli* O2 and O78 serotype was reported to be most predominant by Sharada *et al*. (2001) and Mc Peake *et al*. (2005) whereas Savita *et al*. (2007) reported O25 in Madya Pradesh, Saha *et al*. (2007) reported O8, O5 and O29 in West Bengal, Srinivasan *et al*. (2003) reported O1, O2, O27 in Namakkal. However, in this present study none of the isolates (except O1) belong to these serotypes. In an independent study conducted by Panda *et al*. (2010) in ducks of Odisha found O9 to be most prevalent which correlates with our finding. So, O9 is currently the most prevalent *E. coli* poultry serotype in Odisha. As both O9 and O51 serotypes has been isolated in both duck and poultry of Bhubaneswar, so they may have come from a common source. The present study indicated 2 (6.7%) serotypes were nontypable which could be due to antibiotic treatment, presence of mixed bacterial and viral infection (Humski *et al*. 1984; Srinivasan *et al*., 2003). Srinivasan *et al*. (2003) reported maximum percentage of positive cases from heart blood swab followed by liver, spleen and bursa. This difference in the recording of isolates from yolk sac might be due to individual susceptibility of birds, unhygienic rearing practices existing in the farm as well as environmental conditions. Similarly, in another study, Smith and Berrange (2006) reported high prevalence of *E. coli* in crop samples.

The result showed that no single antibiotic was 100% effective against *E. coli* isolates. Similarly, multiple drug resistance was observed in almost all the isolates exhibiting simultaneous resistance to more than two antibiotics and again the antibiogram of same serotypes also varies amongst different isolates of *E. coli*. These finding correlates well with the observation of Saha *et al*. 2007; Mishra *et al*. (2002); Mary Jones *et al*. (2000). The resistance pattern shows that the *E. coli* isolates are sensitive to the modern antibiotics which were less used in the poultry farms and there is development of higher resistance to those antibiotics which are traditionally used as feed additives or chemotherapeutic agents.

There is concern about antibiotic resistance and its transmission to human pathogens as these resistant bacteria may colonize the human intestinal tract and may also contribute resistance genes to human endogenous flora. The episomal transfer of resistance factor between the intestinal pathogens may lead to evolution of drug resistant bacterial strains in human being which is of public health importance.

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