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Isolation, Characterization and Antibiotic Resistance Pattern of *Escherichia coli* Isolated from Poultry

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Abstract: A total of 85 samples was collected from moribund birds with different pathological conditions. *E. coli* was isolated to be characterized and to study the antibiotic drug resistance pattern of different serotypes and their pathogenicity. Maximum isolates were recovered from cases with perihepatitis (44.61 %), followed by enteritis (33.85 %). Serotyping of these isolates revealed 32 'O' serotypes, predominantly O11, O79 and O111 accounting for 26.15 %. The percentage of other serotypes varied from 1.54-4.61 %. *In vitro* Pathogenicity testing indicated that 95.38 % of the isolates were positive for Congo red dye binding, 70.77 % were Mannitol Resistant Haemagglutination (MRHA) positive, 20 % isolates were Mannitol Sensitive Haemagglutination (MSHA) positive and 9.23 % were non haemagglutinating isolates. Antibiogram profiles indicated maximum resistance to Nitrofurazone (90.77 %), followed by tetracycline (83.08 %) and cotrimoxazole (76.92 %). High sensitivity to ciprocloxacin and enrofloxacin (83.08 %), chloramphenicol (81.54 %), pefloxacin (76.92 %) and norfloxacin (75.39 %) were noticed. It was concluded that *E. coli* is one of the major pathogen responsible for various types of disease conditions in poultry leading to economic losses to poultry industry and that antibiotics used should be specific and as per the sensitivity testing.

Key words: Poultry · Pathogenicity · *E. coli* · Serotypes · Pathological conditions

INTRODUCTION

Colibacillosis is an economically important disease, which is prevalent throughout the world [1]. *E. coli* has been implicated in variety of disease conditions in poultry such as colispeticaemia, coligranuloma, air sacculitis, peritonitis, peicarditis, omphalitis and oophoritis, accounting for about 5-50 % mortality in poultry flocks [2]. Several reports are available on the involvement of serotypes of *E. coli* in poultry diseases [3] and so on. The pathogenic and non-pathogenic strains in poultry are differentiated based on the virulence which has been attributed to various factors like fimbriae, production of colicin, motility and embryo lethality. Hence, detection of these strains becomes important for effective treatment and control [4].

Antimicrobial therapy helps in reducing both the incidence and mortality associated with avian colibacillosis [5]. Unscrupulous use of antibiotics to prevent infection has resulted in emergence of large number of drug resistant enteropathogenic *E. coli* posing problems to control of these infections. 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 [6]. In view of the significance of *E. coli* infection in poultry, this study has been undertaken to isolate and characterize *E. coli* form different pathological conditions and to determine the *in vitro* pathogenicity and to identify the prevalent serotypes and their antibiotic drug resistance pattern.

MATERIALS AND METHODS

Collection of Samples: Tissues were collected based on clinical findings and pathogonomic lesions observed during detailed post mortem examination of poultry at Department of Pathology, Veterinary College, Bangalore;

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Poultry Disease Diagnostic Laboratory, Bangalore and Central Disease Investigation unit, IAH And VB, Bangalore, India. A total of 85 samples from 55 birds of 1-7 weeks of age were collected in sterile containers following aseptic precautions and transported to laboratory. Tissues were collected form cases exhibiting perihepatitis (31), enteritis (27), airsacculitis (5), yolk sac infection (7), pnemonitis (1) and pericarditis (14).

Isolation and Identification: Tissue samples were plated on Mac Conkey agar (HIMEDIA) and incubated at 37°C for 24 hours. The lactose fermenting colonies were reinoculated to Eosin Methylene Blue (HIMEDIA) agar and colonies producing metallic sheen were transferred to Nutrient agar slants and incubated at 37°C for 24 hours and stored at 4°C for further identification. Identification of isolates were done according to Kreig *et al.* [7] based on staining and Bio-chemical tests (Catalase, Oxidase, Indole, Methyl Red, VP test, Citrate utilization, Nitrate reduction, H_2S production in TSI, Gelatin liquification and Urease).

Serotyping: The isolates were sent to National *Salmonella* and Escherichia centre, Kasauli, Himachal Pradesh, India for further confirmation and Serotyping.

***In Vitro* Pathogenicity Testing:** Various serotypes were tested for pathogenicity based on Congo red dye binding test as per the technique of Berkhoff and Vinal [8]. Trypticase Soy Agar was supplemented with 0.003% Congo red dye (Sigma) and 0.15% bile salts. Each isolate was cultured on a separate plate and incubated at 37°C for 24 hrs. After 24hrs incubation, the cultures were left at room temperature for 48 hrs to facilitate annotation of results. Invasive *E. coli* were identified by their ability to take up Congo red dye. Appearance of red colonies was recorded as positive and colonies that did not bind the dye and remained white or grey were considered negative.

Haemolysis production test - *E. coli* isolates were propagated on blood agar supplemented with 5% washed sheep erythrocytes. Blood agar plates were then incubated at 37°C for 24 hrs and colonies producing clear zones of haemolysis were recorded as hemolysin positive [9]. Haemagglutination study was performed based on the method outlined by Duguid *et al.* [10]. Based on HA activity, the serotypes were classified as MRHA (Mannitol Resistant- Highly virulent), MSHA (Mannitol Sensitive- moderately virulent) and NHA (Non haemagglutinating- avirulant).

Antibiogram: Antibiotic sensitivity test was performed according to the procedure of Bauer *et al.* [11] utilizing Mueller Hinton Agar plates (HIMEDIA) by placing 20 mm antibiotic discs of 14 commonly used antimicrobial agents and measuring the diameter of zone of inhibition.

RESULTS

Tables 1 and 2 represent different pathological conditions and the organs from which the isolates were obtained. *E. coli* were recovered from 65 (76.47 %) samples out of the total 85 samples collected. Highest percent of isolates were recovered from cases of hepatitis (44.61 %) followed by enteritis (33.85 %) and pericarditis (16.92 %).

In the present study, 65 *E. coli* isolates were typed serologically into 32 different 'O' groups and 7 were untypable. The predominant serotypes were O79, O11 and O111 together accounting for 26.15 %. The other serotypes isolated were O1, O5, O35, O51, O78, O102, O117, O120, O152 and O165. However, low prevalence of S erotypes O2 and O78 was recorded in this study as compared to the study conducted earlier. The distribution of different serotypes with respect to pathological conditions from which *E. coli* were isolated are presented in Table 2 and it was observed that no particular serotype could be attributed to a particular disease condition or a particular age group.

***In Vitro* Pathogenicity Testing:** The results of the Congo red (CR) binding assay indicates that majority (95.38 %) were positive and only three isolates recovered from enteritis were negative. None of the isolates recovered tested positive for Haemolysis production. Results of Haemagglutination (HA) studies using chicken erythrocytes indicated that of the 65 isolates, 46 (70.75 %) were MRHA positive, 14 (20 %) were MSHA positive 9.23 % were non haemagglutinating, indicating that majority of the isolates were pathogenic.

Antibiogram: The sensitivity and resistance pattern of these isolates for various antibiotics are presented in Table 3. It was observed that none of the antibiotics used were found to be cent percent effective.

Table 1: Organ wise recovery of E coli from different cases

	Liver	Intestine	Heart	Yolk sac	Lungs
Liver	12	7	10	-	-
Intestine	7	15	-	-	-
Heart	10	-	1	-	-
Yolk sac	-	-	-	2	-
Lungs	-	-	-	-	1

Table 2: Frequency of E coli serotypes from poultry in relation to pathological conditions

Sl. No	Condition	E coli serotypes isolated	Total number	Percentage
1	Perihepatitis	Five O79 , two O78 and O165, One each of O3, O4, O5, O11, O25, O35, O37, O51, O61, O84, O102, O106, O111, O117, O126, O138 & O152 Three untypable	29	44.61
2	Enteritis	Two each of O1, O11, O111 & O152, one each of O8, O10, O22, O35, O48, O51, O79, O95, O102, O115, O149. Three untypable	22	33.85
3	Pericarditis	Two O111, one each of O2, O5, O78, O79, O10, O109, O117, O120 & One untypable	11	16.92
4	Yolk sac infection	One each of O11 & O20	2	3.08
5	Pneumonitis	One O11	1	1.54
	Total		65	100

Note: Figures in bold indicates the serotypes isolated from only the respective conditions, whereas others have been isolated from different conditions also.

Table 3: Antibiogram of E coli isolates from Poultry

				Percentage		
Sl. No	Antimicrobial agent	Code	Concentration per disc in µg	Sensitive	Moderately sensitive	Resistant
1	Ciprofloxacin	Cf	10	83.08	0.00	16.92
2.	Enrofloxacin	Ex	10	83.08	1.54	15.38
3.	Chloramphenicol	C	30	81.54	1.54	16.92
4.	Pefloxacin	Pf	5	76.92	3.08	20.00
5.	Norfloxacin	Nx	10	75.39	7.69	16.92
6.	Ampicillin	A	25	72.31	0.00	27.69
7.	Neomycin	N	30	49.23	29.23	21.54
8.	Gentamycin	G	10	46.15	13.85	40.00
9.	Apramycin	Ap	15	30.77	46.15	33.08
10.	Kanamycin	K	30	20.00	36.61	35.39
11.	Cephalaxin	Cp	30	13.85	44.61	41.54
12.	Tetracycline	T	10	7.69	9.23	83.08
13.	Cotrimoxazole	Co	25	3.08	20.00	76.92
14.	Nitrofurazone	Nr	100	1.54	7.69	90.77
15.	Chlortetracycline	Cte	30	6.00	5.00	89.00
16.	Erythromycin	E	15	2.52	3.86	94.19
17.	Streptomycin	S	10	19.00	0.00	81.00
18.	Colistin	Cl	10	55.00	39.00	6.00
19.	Oxytetracycline	OT	10	15.00	8.00	77.00
20.	Lincomycin	L	5	54.25	6.25	39.50

DISCUSSION

Higher rates of isolation from hepatitis followed by enteritis was indicating the acute nature of the disease [12, 13] and also indicating the predominant role of *E. coli* in causing enteritis [14]. However, some workers have reported much lower incidence of *E. coli* in case of enteritis [15, 16]. Incidence of pericarditis in poultry was always encountered in association with perhepatitis and enteritis indicating that these isolates could be highly virulent [17, 18].

The serotypes isolated in this study were in accordance with Ibrahim *et al.* [19] and Singh and Gupta [20]. The very low per cent Serotypes O2 and O78 may probably be due to variation in serotypes over a period of time in a particular area [21]. The correlation between the isolates and the disease condition could not be established [22].

The results of the present *in vitro* pathogencity testing were in agreement with Berkhoff and Vinal [8], who also reported a strong correlation between expression of CR phenotype and virulence in avian *E. coli* and suggested that it was associated with the presence of β-D-glucan in bacterial cell wall. Previous studies also indicated that isolates of virulent avian *E. coli* can be identified by their ability to bind Congo red [20]. The characteristic of CR binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker. Nevertheless, Yoder [23] has reported that Congo red binding did not correlate well with pathogenicity.

The negativity of all the isolates to haemolysis was in agreement with Osman *et al.* [24], who attributed heavy mortality in chicks due to non-haemolytic strains indicating that avian pathogenic *E. coli* to be independent of haemolytic activity. Several workers have also isolated MRHA positive *E. coli* from pathogenic cases in poultry [24, 25].

The results of Antibiogram in the present study are in variance with the findings of other workers, indicating that antibiotic pattern varies with different isolates, time and development of multiple drug resistance among different *E. coli* isolates related to transmissible R factor/ plasmid [26]. The transmission of resistance plasmids of *E. coli* from poultry to human have also been reported [27].

In conclusion, the present study clearly demonstrates that *E. coli* is one of the major pathogen responsible for various types of disease conditions in poultry leading to economic losses to poultry industry. Almost all serotypes of *E. coli* isolated have been found to be pathogenic, but no particular serotype could be attributed to a particular disease condition or a particular age group. The antibiotic sensitivity pattern revealed that special emphasis need to be given for judicious selection of antibiotics, preferably after antibiotic sensitivity testing and judicious use of such antibiotics at an optimum dose for sufficient duration to ensure effective treatment and control of various diseases caused by *E. coli* in poultry.

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