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Serotyping and Antimicrobial Susceptibility Pattern of Avian Pathogenic *Escherichia coli*

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A B S T R A C T

Poultry industry in India is facing major economic setback due to persistent disease problems like colibacillosis. The incidence and severity extra intestinal infection like complicated chronic respiratory disease have increased rapidly. In this context the role of *E.coli* in causing extra intestinal infection needs to be explored. The study of virulence determinants associated with these bacteria help us in understanding the pathogenic mechanism of disease. The role of various serotypes associated with extra intestinal infection needs further studies. With this view this research was conducted on total 77 *E. coli* which were isolated from 228 tissue samples collected from dead birds suffering with colibacillosis. Out of 228 tissue samples a maximum numbers (41) 53.24 % of *E.coli* isolates were recovered from Navsari Dist. of Gujarat Followed by (36) 23.84% from Anand Dist. of Gujarat. All these 77 *E. coli* isolates were tested for susceptibility to eight suitable antibiotics by disc diffusion method. As per antibiogram pattern the antibiotic drug resistance was highest to Penicillin (P), 77 (100%), and Oxytetracycline (O), 77 (100%), followed by 74 (96.10%) to Ampicillin (AMP), Enrofloxacin (EX), 70 (90.90%), Streptomycin (S), 64 (83.11%), Chloramphenicol (C), 65 (84.41%), Gentamicin (GEN), 59 (76.62%) whereas only 20 (25.97%) isolates were resistant to Cefatrixone (CTR). All these isolates were tested for invasiveness by Congo red binding test, 64 (83.11%) showed red colonies after 48 hrs of incubation at room temperature and were found to be invasive, while 13 (16.88%) showed colorless colonies turned out to be negative (non-invasive) for Congo red binding test. The Serotyping of selected 43 isolates was done at NSEC, CRI, Kasauli (HP). India. The results revealed UT (6), O_{88} (6), O_{83} (4), O_{126} (1), O_{49} (3), O_{145} (2) and O_{84}(5), O_{7}(2), O_{157}(9), O_{8}(4), O_{119}(1), Among these serotypes the most frequent serotypes detected were O_{157}(9) O_{88} (6), UT (6), O_{84}(5), O_{8}(4), O_{49} (3), O_{145} (2),O_{7}(2),.

Introduction

Poultry is one of the fastest growing livestock sector in India with around eight percent growth rate per annum. The application of new technologies resulted in the multifold and multifaceted growth of this sector.

In spite of all the measures, the poultry industry in India suffers a major setback due to the disease outbreaks. The incidence and severity of colibacillosis have increased rapidly and it is likely to be a major problem in the poultry industry., *E. coli* grouped as (a) commensal *E. coli* (b) intestinal pathogenic *E. coli* and (c) extra intestinal pathogenic *E. coli*. The strains that are responsible for extra intestinal infections are termed as extra intestinal pathogenic *E. coli* (ExPEC) (Russo and Johnson 2000). There are six phylogenetic group A, B1, B2, C, D and E, Most of the ExPEC strains phylogenetically belong to B2 and to a lesser extent D groups (Smith *et al.,* 2007.)

The diversity of serotypes associated with colibacillosis was reported by (Landman *et al.,*2014). In Europe, six serogroups (O1, O2, O5, O8, O18 and O78) were accounted for 56.5% of APEC isolates and 22.5% of non-pathogenic isolates (Schouler *et al.,* 2012).

The differentiation of pathogenic and non-pathogenic strains of *E.coli* is made on the basis of the virulence attributes, including those encoding for adhesions (F1, P, and stg fimbriae, curli, and EA/I), anti-host defense factors (ompA, iss, lipopolysaccharide, and K1), iron acquisition systems (aerobactin, iroproteins, yersiniabactin, and the sit iron acquisition locus), auto transporters (tsh,vat, and aatA).

The indiscriminate use of antibiotics for prevention of early chick mortality resulted in rapid spread of antimicrobial resistance among avian *Escherichia coli*. In order to devise a strategy to deal with them, antimicrobial resistance pattern of *E.coli* associated with extra intestinal infection was studied.

Materials and Methods

Collection of samples

In this study a total of 228 tissue swab viz. liver, heart, lung, kidney, spleen were aseptically collected from freshly dead chicken after postmortem examination.

All samples were transferred to the microbiology laboratory under chilled condition. All the work involving handling of *E. coli* organism and infected tissue material was performed by taking appropriate bio-safety precautions and following all aseptic conditions

Isolation and identification

All tissue swabs were inoculated directly on MacConkey agar and on Eosin methylene blue agar (EMB) and incubated at 37°C for 16-18hrs. When evidence of growth was observed in the form of Lactose fermenting pinkish colonies on MAC and Metallic sheen with dark centered colonies on EMB; further identification was carried out on Chromogenic *E.coli* Agar. Colonies showing blue color were considered as *E. coli* and further identification was carried out by conventional and commercial biochemical test kit.

Congo Red (CR) binding assay

The phenotypic characterization of *E. coli* isolates for determination of their invasiveness property was carried out by using Congo Red (CR) binding assay, with the objective of distinguishing between pathogenic and non-pathogenic microorganisms as described by (Berkhoff and Vinal, 1986).

Antibiotic sensitivity test

The antibiotic sensitivity test was carried out for all *E.coli* isolates as per method described by Bauer *et al.,* (1966).

Serotyping of *E. coli* isolates

Total 43 multidrug resistant *E.coli* isolates were sent for Serotyping at NSEC, CRI, Kasauli (HP). India.

Results and Discussion

In this chapter results of different test are presented systematically. Wherever deemed fit data have been tabulated. The results, their rationales, outcome, have been discussed in the light of set doctrines and work of other researchers on the topic Total 228 tissue samples were collected from dead birds affected with colibacillosis. All the samples were processed by following standard bacteriological procedure in the department of Veterinary Microbiology. The recovery rate of *E. coli* from total of 228 tissue samples was 77 (33.77%). (Table 1). Identification of all recovered isolates was done using biochemical test. All *E.coli* showed biochemical profile as Indole positive MR positive, VP negative, Citrate negative, Oxidase negative and Catalase positive reactions. A maximum numbers (41) 53.24 % of *E.coli.* isolates were recovered from tissue specimens collected from dead birds of poultry farms located in Navsari Dist. of Gujarat Followed by (36) 23.84% from Anand Dist. of Gujarat. The findings of this research work are in agreement with findings of Archna mishra *et al.,*(2002), Veere Gowda *et al.,* (1996) and Saha *et al.,*(2007) who reported 20.00%, 23.80% and 28.93% prevalence of avian *E. coli*. Whereas Rajasekaran (2001) and Sharada *et al.,* (2001) reported 58.00% and 76.47% recovery rate for *E. coli* isolated *from* colibacillosis affected birds. All 77 *E. coli* isolates recovered from Colibacillosis were tested for susceptibility to eight suitable antibiotics by disc diffusion method. Out of 77 isolates of *E. coli*, the antibiotic drug resistance was highest to Penicillin (P), 77 (100%), and Oxytetracycline (O), 77 (100%), followed by 74 (96.10%) to Ampicillin (AMP), Enrofloxacin (EX), 70 (90.90%), Streptomycin (S), 64 (83.11%),, Chloramphenicol (C), 65 (84.41%), Gentamicin (GEN), 59 (76.62%) whereas only 20 (25.97%) isolates were resistant to Cefatrixone (CTR) (Table 3). Multiple drug resistance was observed in all isolates of *E. coli.* The highest sensitivity (74.02%) observed to Cefatrixone (CTR) followed by Gentamycin (G) (23.37 %), Streptomycin (16.88%) and Chloraphenicol (C) (23.37 %). Our findings are in agreement with Sahoo *et al.,* (2012). who reported high resistance to commonly used antibiotics like Chlortetracycline (88.58%), Ampicillin (74.29%), Tetracycline (74.29%), Streptomycin (85.72%), Penicillin-G (82.86%), Amikacin (82.86%), Furazolidone (77.14%), Amoxicillin (71.43%) and Cotrimoxazole (71.43%), similar findings of multiple drug resistance were also recorded by(Mohamed *et al.,* and Radwan *et al.,* 2014).The highest resistance to Oxytetracycline was also recorded during this study. This resistance is principally plasmid mediated and an inducible trait. Mechanisms of resistance include decreased accumulation of tetracycline due to either acquisition of an energy-dependant efflux path way or to decreased influx, or to decrease access of tetracycline to the ribosome (site of action) due to acquisition of ribosome protected proteins and enzymic inactivation bacteria that have been resistant to one tetracycline frequently exhibit resistance to the other tetracyclines. In this study,96.10% per cent resistant to, Ampicillin was recorded which may be attributed to the production of β-lactamase group of enzymes, which have been

reported in high percent Tetracycline and Streptomycin have been utilized for several decades, The present study from south Gujarat region of India also indicates highest 100 % resistance to Oxytetracycline and 83.11 % to Streptomycin therefore, preventive and therapeutic effects on APEC strains should no longer be expected from these antibiotics. The antibiogram patterns differ from nation to nation and even within different regions of nation as a result of exposures to different antibiotics. There is strong correlation between expression of CR phenotype and virulence in avian *E. coli* (Berkhoff and Vinal 1986), they suggested that it was associated with the presence of p-D-glucan in bacterial cell wall.

The characteristic of CR binding constitutes a moderately stable, reproducible and easily distinguishable phenotypic marker. Out of 77 *E. coli* isolates tested for invasiveness by Congo red binding test, 64 (83.11%) showed red colonies after 48 hrs of incubation at room temperature and were found to be invasive, while 13 (16.88%) showed colorless colonies turned out to be negative (non-invasive) for Congo red binding test (Table 4) The present results are in agreement with earlier observations of (Berkhoff and Vinal 1986, Deshmukh and Karpe 2006) who opined that the test distinguished between invasive and non-invasive serotypes of *E. coli*. The similar findings for Congo red binding activity were observed by Aziz *et al.,* (1995) and Seifi *et al.,* (2015). They reported 82.6% and 88.75% CR positive *E. coli* strains, respectively. Whereas, Berkhoff and Vinal (1986) Shankar *et al.,* (2010) and Bashar *et al.,* (2011) reported 100%, 98.47% and 69% of *E. coli* isolates with CR binding activity respectively.

The Serotyping of all the 42 isolates was done at NSEC, CRI, Kasauli (HP). India. The results revealed UT (6) 13.95%, O_{88} (6) 13.95%, O_{83} (4) 9.30 %, O_{126} (1) 2.32%, O_{49} (3) 6.97%, O_{145} (2) 4.65% and O_{84} (5) 11.62%, O_7 (2) 4.65%, O_{157}(9)20.60%, O_8 (4) 9.30%, O_{119}(1) 2.32 % (Table 2).

Among these serotypes the most frequent serotypes detected were O_{157}(9) O_{88} (6), UT (6), O_{84}(5), O_8(4), O_{49} (3), O_{145} (2),O_7(2), The occurrence of different *E. coli* serotypes in coli septicaemia was reported from time to time by various researchers *viz*., Ghosh (1989); Krishnamohan Reddy *et al.,* (1994a); They opined that the outbreaks of the disease might be influenced by extent of intensity of the various strains present in the environment.

The findings of present research work are in agreement with those of Ashraf *et al.,* (2015) who also reported O157 and UT (untypable) serotypes from chicken.

Table.1 Recovery rate of *E. coli* from tissue samples

Total no of tissue samples	No .of pure *E.coli* isolates	Percentage %
228	**77**	**33.77%**

Table.2 Serotyping test results

Sr. No.	Serotype	Total no of *E. coli* isolates send for serotyping	Total no of specific serotypes identified	Percentage of specific serotype identified
1	UT (Untypable)	43	6	13.95%
2	**O_{88}**	43	6	13.95%
3	**O_{83}**	43	4	9.30%
4	**O_{126}**	43	1	2.32%
5	**O_{145}**	43	2	4.65%
6	**O_{84}**	43	5	11.62%
7	**O_{7}**	43	2	4.65%
8	**O_{157}**	43	9	18.60%
9	**O_{8}**	43	4	9.30%
10	**O_{119}**	43	1	2.32%
11	**O_{49}**	43	3	6.97%

Table.3 Antibiotic sensitivity test results

Sr. no.	Antimicrobial agents(Concentration)	Total no	Sensitive	%	Resistant	% Resistance
1	Penicillin (P) (10mcg)	77	0	0%	77	100%
2	Oxytetracycline (0) (30mcg)	77	0	0%	77	100%
3	Ampicillin (AMP) (10mcg)	77	3	3.89%	74	96.10%
4	Enrofloxacin (EX) (10mcg)	77	7	9.09%	70	90.90%
5	Chloraphenicol (C) (10mcg)	77	12	15.58%	65	84.41%
6	Sreptomycin (S) (10mcg)	77	13	16.88%	64	83.11%
7	Gentamycin (G) (10mcg)	77	18	23.37%	59	76.62%
8	Cefatrixone (CTR) (10mcg)	77	57	74.02%	20	25.97%

Table.4 Congo Red binding assay results

Total no of *E.coli*	*E.coli* Positive for Congo red	*E.coli* %positive	*E.coli* Negative for Congo red	*E.coli* % negative
77	64	83.11%	13	16.88%

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