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Antibiotic Resistance Pattern Of Escherichia Coli Isolated From Poultry In Bangalore

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Citation

Abstract

A total of 85 samples were collected from moribund birds with different pathological conditions like perihepatitis, enteritis, air sacculitis and pneumonia. In this investigation thirty two avian pathogenic Escherichia coli (APEC) strains isolated from broiler chickens with colisepticemia and examined for susceptibility to antimicrobials of veterinary and human significance. 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 antibiotic activities of 20 antibiotic substances against the isolates were determined by disc diffusion test (Kirby Bauer method). Multiple resistances to antibiotics were observed in all the isolates. Antibiogram profiles indicated maximum resistance to Nitrofurazone (90.77 %), followed by tetracycline (83.08 %) & cotrimoxazole(76.92 %). High sensitivity to ciprocloxacin and enrofloxacin (83.08 %), chloramphenicol (81.54 %), pefloxacin (76.92 %) and norfloxacin (75.39 %) were noticed.

INTRODUCTION

Escherichia coli is one of the most important agent causing secondary bacterial infection in poultry and may also be a primary pathogen (Gross, 1994). Colibacillosis is the most frequently reported disease in surveys of poultry diseases or condemnations at processing (Saif, 2003). In the past few years, both the incidence and severity of colibacillosis have increased rapidly, and current trends indicate that it is likely to continue and become an even greater problem in the poultry industry (Altekruse et al., 2002). Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed et al., 1993). E. coli may be sensitive to many antibiotics. However, isolates of E. coli from poultry are frequently resistant to one or more antibiotics, especially if they have been widely used in poultry industry over a long period (e.g., tetracyclines) (Allan et al., 1993; Blanco et al., 1997). Antibiotics once effective at controlling E. coli infections are now ineffective due to the bacterium's acquired resistance to these compounds. Resistance to two or more classes of antibiotics is now commonplace in both veterinary (Gonzalez and Blanco, 1989) and human (Dennesen et al., 1998) medicine.

Concern has been expressed about possible harmful effects on humans through the use of drugs in agriculture, as follows: 1) increased microbial drug resistance, 2) drug residues in food, 3) allergic reactions and sensitisation to antimicrobials, and 4) drug toxicity (Bazile-Pham-Khac et al., 1996). Concern about antibiotic resistance and its transmission to human pathogens is important because 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 (Tabatabaei and Nasirian, 2003). In view of the significance of E. coli infection in poultry, this study has been undertaken to isolate and to study their antibiotic drug resistance pattern

MATERIALS AND METHODS

Collection of Samples: The 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; Poultry Disease Diagnostic Laboratory, Bangalore and Central Disease Investigation unit, IAH & 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: The tissue samples were plated on Mac Conkey agar (HIMEDIA) and incubated at 37o 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 37o C for 24 hours and stored at 4o C for further identification. Identification of isolates were done according to Kreig et al (1984) 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.

Antibiogram: Antibiotic sensitivity test was performed according to the procedure of Bauer et al (1966) utilizing Mueller Hinton Agar plates (HIMEDIA) by placing 20 mm antibiotic discs. The following antibiotic discs were applied: Ciprofloxacin (Cf/ 10μg), Chloramphenicol (C/ 10 μg), Enrofloxacin (Ex/ 30 μg), Pefloxacin (Pf/ 5 μg), Norfloxacin (Nx/ 10 μg), Ampicillin (A/ 25 μg), Neomycin (N/ 30 μg), Gentamycin (G/ 10 μg), Apramycin (Ap/ 15 μg), Kanamycin (K/ 30 μg), Cephalaxin (Cp/ 30 μg), Tetracycline (T/ 10 μg), Cotrimoxazole (Co/ 25 μg), Nitrofurazone (Nr/ 100 μg), Chlortetracycline (Cte/ 30 μg), Erythromycin (E/ 15 μg), Streptomycin (S/ 10 μg), Colistin (Cl/ 10 μg), Oxytetracycline (OT/ 10 μg) and Lincomycin (L/ 5 μg)

RESULTS AND DISCUSSION

In the present investigation, 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 %) indicating the acute nature of the disease (Krishnamohan Reddy et al., 1994 and Blanco et al., 1996) followed by enteritis (33.85 %) and pericarditis (16.92 %) indicating the predominant role of E. coli in causing enteritis (GROSS, 1994). However some workers have reported much lower incidence of E. coli in case of enteritis (Panneerselvam et al., 1988 and Yadav and Malik, 1971). Incidence of pericarditis in poultry was always encountered in association with perihepatitis and enteritis indicating that these isolates could be highly virulent (Ghosh, 1998; Krishanmohan Reddy and Koteeswaran, 1994).

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 %, which were in

accordance with Ibrahim et al (1998) and Singh and Gupta (1996). The other serotypes isolated were O1, O5, O35, O51, O78, O102, O117, O120, O152 and O165.

The sensitivity and resistance pattern of these isolates for various antibiotics are presented in Table. It was observed that none of the antibiotics used were found to be cent percent effective. In this study, multiple antibiotic sensitivity and resistance pattern was observed in all of the examined strains similar to the findings of previous studies (Guerra et al., 2003 ; Saenz et al., 2003). E. coli isolates showed variable percentages of sensitivity and resistance to the different antibiotics. High levels of resistance were against Erythromucin (94.19 %), Nitrofurazone (90.77), Chlortetracycline (89.00 %), Tetracycline (83.08 %), Oxytetracycline (77.00 %) and Cotrimoxazole (76.92 %). E coli isolates are resistant to these drugs because of regular usage in poultry industry for control of pathogenic avian colibacillosis. E. coli isolates of this study were highly sensitive to ciprofloxacin (83.08 %), Enrofloxacin (83.08 %), Chloramphenicol (81.54 %), Pefloxacin (76.92), Norfloxacin (75.39 %), Ampicillin (72.31 %), Colistin (55.00 %) and Lincomycin (54.25 %). Moderate sensitivity to antibiotics such as Neomycin, apramycin, Gentamycin, Cephalaxin was exhibited by the isolates. However the results of this 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 (Holmberg et al, 1984). The transmission of resistance plasmids of E. coli from poultry to human have also been reported (Maansouri and Shareifi, 2002).

Figure 1

Antibiogram of E coli isolates from Poultry

Sl. No	Antimicrobial agent	Code	Concentration per disc in µg	Sensitive	Moderately sensitive	Resistant
1	Ciprofloxacin	Cf	10	83.08	0	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	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.	Oxytetracycline	OT	10	15.00	8.00	77.00
14.	Cotrimoxazole	Co	25	3.08	20.00	76.92
15.	Nitrofurazone	Nr	100	1.54	7.69	90.77
16.	Chlortetracycline	Cte	30	6.00	5.00	89.00
17.	Erythromycin	E	15	2.52	3.86	94.19
18.	Streptomycin	S	10	19	0	81.00
19.	Colistin	Cl	10	55.00	39.00	6.00
20.	Lincomycin	L	5	54.25	6.25	39.50

CONCLUSION

In conclusion, the findings clearly demonstrates multiple antimicrobial resistant E. coli isolates, are commonly present among diseased broiler chickens in India. Resistance to existing antimicrobials is widespread and of concern to poultry veterinarians. The significant increase in the incidence of resistance against antibiotics in the E. coli strains isolated from broiler chickens is probably due to increased use of antibiotics as feed additives for growth promotion and prevention of diseases, use of inappropriate antibiotics for treatment of diseases, resistance transfer among different bacteria and possible cross resistance between antibiotics used in poultry. Thus, introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is strongly needed in developing countries because in addition to animal health problems, transmission of resistant clones and resistance plasmids of E. coli from food animals (especially poultry) to humans can occur. Hence, 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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