IN VITRO ANTIBIOTIC SUSCEPTIBILITY PROFILES OF AVIAN *ESCHERICIA COLI* IN AND AROUND FAISALABAD

M. Saleem, G. Muhammad, M. Siddique and T. Zia
Faculty of Veterinary Science,
University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

One hundred *E. coli* isolates were recovered from livers and hearts of chickens of different age groups suffering for colisepticaemia. These isolates wre subjected to *in vitro* antibiotic susceptibility test using eleven antimicrobial agents which included enrofloxacin, ciprofloxacin, norfloxacin, flumequin, oxolanic acid, cefazolin, gentamicin, kanamycin, amoxycillin, neomycin and sulphamethoxazole + trimethoprim by disc diffusion technique. All isolates were sensitive to enrofloxacin and ciprofloxacin whereas 98 per cent of isolates displayed sensitivity to norfloxacin. Ninety four, , 87, 80, 79, 77, 47, 32 and 8 per cent isolates showed sensitivity to cefazoline, gentamicin, flumequine, neomycin, oxolanic acid, amoxycillin, kanamycin and sulphamethoxazole + trimethoprim, respectively. A total of 22 antibiogram patterns were recorded among 100 isolates with 67 per cent displaying resistance to two or more antimicrobials (multiple drug resistance).

INTRODUCTION

Escherichia coli is involved in many health problems in poultry, especially where poultry is reared in intensive system and cause substantial production losses to poultry industry by its association with various disease conditions, where as a primary pathogen or as a secondary pathogen. Feed and drinking water are often contaminated with *E. coli* and may act as a source of infection. Egg transmission of pathogenic *E. coli* is common and can be responsible for high mortality and morbidity of chicks (Haneef and Siddique, 1990).

A wide array of antibiotics/antibacterials are being used for the treatment and prophylaxis of different bacterial diseases including those caused by *Escherichia coli*. The indiscriminate use of antibiotics as therapeutic preventative or as growth promoting agents may lead to the emergence of antibiotic resistant bacterial microflora in poultry (Heller and Smith, 1973; Nazer, 1980; Rosenberger *et al.*, 1985; Tariq, 1989; Allen *et al.*, 1993; Ginns *et al.*, 1996). Pervious studies have shown multiple resistance of *Escherichia coli* strains to different antimicrobial agents. A shift in sensitivity pattern fromm time to time in a given locality in well established (Krishnamohan *et al.*, 1995). Therefore, for the rational use of antibiotics there is a constant need to periodically determine the antibiotic susceptibility profiles of the important pathogens. Present study was undertaken to determine the antibiogram pattern of *E. coli* isolates in and around Faisalabad which is one of the major poultry pockets in Pakistan.

MATERIALS AND METHODS

One hundred livers and hearts of sick and dead birds of different age groups suffering from suspected *E. coli* infection were collected from twenty five poultry farms located in and around Faisalabad. Immediately after arrival in the laboratory, samples were streaked onto MacConkey's agar with the help of sterilized platinum loop. Streak plate method was adopted to obtain discrete colonies. The plates were incubated at 37°C for 24 hours. The isolate were identified on the basis of cultural characters i.e., colony morphology on MacConkey's agar, Gram's staining, motility and various biochemical and sugar fermentation reactions according to the procedures of Cruickshank (1975). *In vitro* antibiotic susceptibility of pathogenic antibiotics was determined using the disc diffusion method (National Committee for Clinical Laboratory Standards, 1990). Mueller Hinton (Difco) agar was used for antibiotic susceptibility testing. After overnight incubation at 37°C, the radius of zone of inhibition of each antibiotic was measured in millimeters, and interpreted according to the zone size of interpretative charts. The reference strain *Staphylococcus aureus* (ATCC 25923 (American Type Culture Collection, Rockville, Maryland, USA) was used as quality control.

RESULTS AND DISCUSSION

In vitro antibiotic sensitivity test showed that the maximum sensitivity to enrofloxacin (100%) and ciprofloxacin (100%), followed by norfloxacin (98%), cefazolin (94%), gentamicin (878%), flumequine (80%), neomycin (79%) and oxolanic acid (77%) and low sensitivity to amoxycillin (47%), kanamycin (32%), sulphamethoxazole + trimethoprim (8%). The very high sensitivity to enrofloxacin, ciprofloxacin and norfloxacin (Table 1 and Table 2) may be due to the faily recent introduction of these quinolones, their broad spectrum of activity and their limited use thus far by the poultry farmers. The results concurred with those of Ibrahim *et al.* (1997) and Shafi *et al.* (1998).

Table 1: Antibiotic susceptibility profiles of avian *Escherichia coli* isolates (n = 100) to eleven antimicrobial drugs.

Sr. No.	Antibiotics	Disc potency (µg)	Antibiotic susceptibility profiles		
			Susceptible No. (%)	Intermediate No. (%)	Resistant No. (%)
1	Enrofloxacin	5	100(100)	-	-
2	Ciprofloxacin	5	100(100)	-	-
3	Norfloxacin	10	98(98)	2(2)	-
4	Cefazolin	30	94(94)	1(1)	5(5)
5	Gentamicin	10	87(87)	-	13(13)
6	Flumequine	30	80(80)	12(12)	8(8)
7	Neomycin	30	79(79)	-	21(21)
8	Oxolanic acid	2	77(77)	-	23(23)
9	Amoxycillin	20	47(47)	6(6)	47(47)
10	Kanamycin	30	32(32)	15(15)	53(53)
11	Sulfamethoxazole + Trimethiprime	23.75 + 1.25	8(8)	-	92(92)

Table 2: Different antibiogram patterns of 100 avian *Escherichia coli* isolates to 11 atimicrobial drugs.

Sr. No.	Name of Antibiotics, the isolates are sensitive to												
1	En	Cip	Nor	Cz	G	F	Neo	Oxo	Am	K	Sxt	11	6
2	En	Cip	Nor	Cz	G	F	Neo	Oxo	Am	K		10	27
3	En	Cip	Nor	Cz	G	F	Neo	Oxo	Am			9	11
4	En	Cip	Nor	Cz	G	F	Neo	Am	K			9	2
5	En	Cip	Nor	Cz	G	F	Oxo	Am	K			9	1
6	En	Cip	Nor	Cz	G	Oxo	Am	K				8	2
7	En	Cip	Nor	Cz	G	F	Oxo	Am				8	10
8	En	Cip	Nor	Cz	G	F	Neo	Oxo				8	8
9	En	Cip	Nor	Cz	G	F	Neo	K				8	4
10	En	Cip	Nor	Cz	F	Neo	Oxo	K				8	5
11	En	Cip	Nor	Cz	G	F	Neo	Sxt				8	2
12	En	Cip	Nor	Cz	G	F	Neo	Oxo				8	2
13	En	Cip	Nor	Cz	G	F	Neo	K				8	2
14	En	Cip	Nor	Cz	F	Neo	Am	K				8	2
15	En	Cip	Nor	Cz	F	Neo	Oxo	Am				8	2
16	En	Cip	Nor	Cz	G	F	Neo					7	4
17	En	Cip	Nor	Cz	G	F	Oxo					7	1
18	En	Cip	Nor	Cz	G	F	Am					6	1
19	En	Cip	Nor	Cz	G	Am						6	2
20	En	Cip	Nor	Cz	Neo	Am						6	3
21	En	Cip	Nor	Cz	Neo	Am						5	2
22	En	Cip	Nor	Cz	Am								1

En = Enrofloxacin Cip = Ciprofloxacin Nor = Norfloxacin Cz = Cefazolin
G = Gentamicin F= Flumequine Neo = Neomycin Oxo = Oxolanic acid
Am = Amoxycillin K = Kanamycin Sxt = Sulfamethoxazole + Trimethorpim

Low sensitivity to amoxycillin, neomycin and sulphamethoxazole + trimethoprim observed in present study was in line with the findings of Kaul *et al.* (1992), Krishnamohan *et al.* (1995), Arshad *et al.* (1995) and Mishra (1995).

This could be due to variation of *E. coli* serotypes and transfer of drug resistance among the *E. coli* isolates as a consequence of indiscriminate use of these antibiotics. Sixty seven of 100 isolates demonstrated a multiple resistance i.e., resistance to two or more antimicrobials. A total of 22 antibiogram patterns were recorded among 100 *E. coli* isolates (Table 2). This high number is reflective of heterogeneity of isolates to different antimicrobials tested.

The results of the present *in vitro* study indicated that at present quinolones (e.g. enrofloxacin, ciprofloxacin, norfloxacin) can be recommended for the treatment of *E. coli* associated disease problems on poultry farms located in and around Faisalabad. The external validity of these findings out side this geographic region needs to be tested.

REFERENCES

Arshad, M., M.I. Ali, M. Anwar and M. Ashfaque, 1995. Studies on isolation and antibiotic sensitivity of bacteria closely associated with omphalitis in broiler chicks. National Seminar on Epidemiology of LIvestock and Poultry Disease. College of Veterinary Sci., Lahore. pp: 19-20.

Cruickshank, R., 1975. Microbiology, 12th Ed. The English Language Book Society and E. Livingstone, London. U.K. pp: 211-215.

Ginas, C.A., G.F. Browining, M.L. Benhan, G.A. Anderson and K.G. Whithear, 1996. Antimicrobial resistance and epidemiology of *E. coli* in broiler breeder chickens. Avian Path., 25: 591-605.

Haneef, W. and M. Siddique, 1990.Prevalence and antibiogram of *Escherichia coli* isolates in and around Faisalabad. Proc. 3rd Intl. Cong. Pakistan Vet. Med. Assoc., pp: 285-292.

Heller, E.D. and H.W. Smith, 1973. The incidence of antibiotic resistance and other characteristics amongst *E. coli* strains causing fatal infection in chickens. The utilization of these characteristics to study the epidemiology of infection. J. Hygiene, 71: 241-245.

Ibrahim, A.I., A.A. Elatter and M.S. El-Shahidy, 1997. Studies on *E. coli* isolates from respiratory affected broilers and protection evaluation of different prepared bacterins. Assiut Vet. Med. J., 37: 152-162.

Kaul, L., P.L. Kaul and N.M. Shah, 1992. An outbreak of colibacillosis in broiler chicks at an organized poultry farm under semi arid zone in North Gujrat. Indian Vet. J., 69: 373-374.

Krishnamohan, R.Y., K. Shobe, N. Dorairajan and N. Punniamurthy, 1995. *In vitro* sensitivity studies of *Escherichia coli* to quinolone antibiotics. Indian Vet. J., 72: 752-753.

Mishra, D.C., 1995. Prevalence and antibiogram of *Escherichia coli* serotypes from chickens in Sikkim. Indian Vet. J., 72: 1219-1221.

National Committee for Clinical Laboratory Standards, 1990. Performance standard for antimicrobial disc susceptibility test. 4th Ed. Approved Standard M2-A3. NCCL Villanova Pa., USA.

Nazer, A.H.K., 1980. Transmissible drug resistance in *E. coli* isolated from poultry and their carcasses in Iran. Cornell Vet., 70: 365-372.

Rosenberger, J.K., P.A. Fries, S.S. Could and R.A. Wilson, 1985. *In vitro* and *In vivo* characterizations of avian *Escherichia coli*. II. Factors associated with pathogenicity. Avian Dis., 29: 1094-1107.

Shafi, K., M. Ashfaque, M.S. Khan and M.A. Sheikh, 1998. Drug trials on *E. coli* isolated from broilers. Intl. Seminar on Microbial Diseases of Livestock and Poultry, March 21-24. pp: 23-25.

Tariq, M.A., 1989. Further studies on the status of drug resistance in pathogenic avian *Escherichia coli*. Pakistan Vet. J., 9: 165-167.