

Original Research Article

Study of Multiple Drug Resistance in *Escherichia coli* strain of Poultry Origin

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ABSTRACT

In recent years *Escherichia coli* has emerged as a more problematic organism causing considerable losses worldwide due to increased susceptibility of birds. Resistance of *E. coli* to antibiotics is well known, which is a major obstacle in the control of infection. This problem is more vibrant in developing countries like ours due to uncontrolled use of antibiotics. Antimicrobial drug sensitivity of the isolates was tested for different antibiotics using the modified disc diffusion method. The antibiogram of seventy *Escherichia coli* isolates was studied against 12 antibiotics viz. ofloxacin, pefloxacin, ampicillin, amikacin, gentamicin, piperacillin, chloramphenicol, co-trimoxazole, tetracycline, cefotaxime, ceftizoxime and ciprofloxacin. Out of seventy *Escherichia coli* isolates tested, highest 69 (98.57 percent) isolates exhibited resistance against cefotaxime followed by 60 (85.71 percent) isolates showing resistance against piperacillin, 58 (82.86 percent) isolates showed resistance against tetracycline, 37 (52.86 percent) isolates were resistance to co-trimoxazole, 16 (22.86 percent) isolates were resistant to ceftizoxime, 14 (20 percent) isolates were resistant to pefloxacin, 11 (15.71 percent) isolates against ampicillin, 10 (14.26 percent) isolates showed resistance against ofloxacin and 7 (10 percent) isolates were resistant to ciprofloxacin and chloramphenicol both. Only one isolate (earch 1.43 percent) was found resistant against gentamicin and amikacin. Multiple drug resistance has been a consistence finding in the study of antibiogram of enterobacterial isolates. Out of 70 *Escherichia coli* isolates tested 11 (15.71 percent) isolates, 7 (10 percent) isolates and 47 (67.14 percent) isolates were found resistance to 2 antibiotics, 3 antibiotics and 4 or more antibiotics combinations respectively out of 12 antibiotics tested. The authors attributed the pattern of multiple drug resistant to large scale indiscriminate use of different antimicrobials as feed additives and for therapeutic purpose.

Keywords

Escherichia coli,
antimicrobial,
multiple drug
resistance,
poultry origin

Introduction

In recent years *Escherichia coli* has emerged as a more problematic organism causing considerable losses worldwide due to increased susceptibility of birds. Many serotypes of *Escherichia coli* are normal inhabitant of large intestine, caecum and lower part of small intestine of healthy fowl and majority of them are nonpathogenic (Roy *et al.*, 2004). Resistance of *Escherichia coli* to antibiotics is well known, which is a

major obstacle in the control of infection. The antimicrobial agents are of great value for devising curative measures against bacterial infections.

However, emerging trend of resistance in bacterial strains against antimicrobial agents has now become one of the major public health concerns in both developing and developed countries (Levy, 1982).

Materials and Methods

Faecal samples were collected from local poultry farms of Kumarganj (Faizabad) and surroundings for isolation of from birds showing *Escherichia coli* symptoms of diarrhoea. Samples were collected aseptically in sterile vials using cotton swabs and brought to laboratory on ice packs.

The samples were kept at refrigeration temperature (4°C) until further processing. Sample proressing was done as per method described by Cruikshank *et al.*, (1975) for isolation and isolates identified on the basis of Edward and Ewing (1972).

Antimicrobial drug sensitivity test

Antimicrobial drug sensitivity of the isolates was tested for different antibiotics (table –1) using the modified disc diffusion method of Bauer *et al.*, (1966). The test organism was inoculated in 2.0 ml of brain heart infusion (BHI) broth and incubated at 37°C for 24 hours.

Their after, a uniform lawn of culture was prepared on Muller Hinton agar plates with the help of sterile swab soaked with the culture. Standard discs of the antibiotics were placed on to the surface of agar plate keeping disc distance of about 12 mm and disc were slightly pressed with the help of forceps to make complete contact with agar surface. The plates were then incubated at 37°C for 24 hours. Sensitivity of *Escherichia coli* strains to an antibiotic was determined by appearance of growth inhibition around the disc. Diameter of clear zone of inhibition surrounding the disc was measured for individual antibiotic and was compared with standard inhibition zone as supplied by the manufacturer to interpret the results. The zone diameter was classified as resistant, moderately sensitive or sensitive.

Results and Discussion

The antibiogram of seventy *Escherichia coli* isolates was studied (Table – 2, Graph-1) against 12 antibiotics viz. ofloxacin, pefloxacin, ampicillin, amikacin, gentamicin, piperacillin, chloramphenicol, co-trimoxazole, tetracycline, cefotaxime, ceftizoxime and ciprofloxacin. Out of seventy *Escherichia coli* isolates tested, highest 69 (98.57 percent) isolates exhibited resistance against cefotaxime followed by 60 (85.71 percent) isolates showing resistance against piperacillin, 58 (82.86 percent) isolates showed resistance against tetracycline, 37 (52.86 percent) isolates were resistance to co-trimoxazole, 16 (22.86 percent) isolates were resistant to ceftizoxime, 14 (20 percent) isolates were resistant to pefloxacin, 11 (15.71 percent) isolates against ampicillin, 10 (14.26 percent) isolates showed resistance against ofloxacin and 7 (10 percent) isolates were resistant to ciprofloxacin and chloramphenicol both. Only one isolate (each 1.43 percent) was found resistant against gentamicin and amikacin.

Mukhopadhyay and Mishra (1992), Mishra (1995) also reported higher resistance against cefotaxime. Investigation regarding resistance pattern against some antibiotics were found very similar to the present study.Kumar *et al.*, (2003) reported 78.43 percent of the isolates to be resistant to oxytetracycline, 35.29 percent to pefloxacin and 3.92 percent to chloramphenicol. Begum *et al.*, (2003) found highest resistance for ciprofloxacin (89 percent) followed by gentamicin (89 percent) and lowest to cefotaxime (4 percent), among the *Escherichia coli* isolates from drinking water in Guwahati area.

Antibiotic sensitivity of the *Escherichia coli* isolates from the mutton showed highest

sensitivity to chloramphenicol (95.92 percent), followed by amikacin (69.39 percent), ciprofloxacin (67.37 percent), gentamicin (67.35 percent), tetracycline (59.18 percent), co-trimoxazole (46.94 percent) and ampicillin (8.16 percent), these findings are slightly different from those recorded in the present study. The difference may be attributed to the isolates which is mutton in this case. The results of antibiogram study of *Escherichia coli* from poultry in field condition reported by Goswami *et al.*, 2002, are in confirmately with the present finding as they also found ciprofloxacin very sensitive and resistance percentage for tetracycline, ampicillin, gentamicin and chloramphenicol to be 98.30 percent, 62.74 percent, 5.88 percent for last two drugs respectively. Maximum sensitivity for gentamicin has also been recorded by Mishra *et al.*, 2002. Similar finding have also been reported after antibiogram study conducted by Sinha *et al.*, 2000, on broiler chicken meat as most sensitivity was for gentamicin, ciprofloxacin, chloramphenicol, and co-trimoxazole. Antibiotic sensitivity result after study conducted on *Escherichia coli* serotypes in poultry farms of Arunachal Pradesh showed gentamicin as effective whereas tetracycline was found completely ineffective, it might be due to change in area under study. Result of Butura *et al.*, (1971) were found to be suggestive in favour of present study as 98 percent were sensitive to chloramphenicol and 32 percent to tetracycline. Antibiogram of *E. coli* serotypes from chicken with haemorrhagic enteritis (bloody diarrhoea) conducted by Sharma and Joshi, (1987) is also in accordance to present study with slight variation as they reported high sensitivity for ampicillin and chloramphenicol while 100 percent resistant to tetracycline. Mukhopadhyay and Mishra, (1992) studied the antibiotic sensitivity in chicks affected

with colibacillosis in some poultry products of West Bengal and found effective to cefotaxime, ampicillin and co-trimoxazole and none antimicrobial was found 100% effective against all *Escherichia coli* isolates, these findings are similar to present study with variation of cefotaxim found to be highly resistant with 98.57 percent. Sensitivity percent for pefloxacin (77.60 percent) was found very nearer to present study by Vakani *et al.*, 1997, while wide variation in sensitivity result occurred for chloramphenicol (65-60 percent), ciprofloxacin (53.73 percent) and gentamicin (49.20 percent). This might be due to difference in study pattern and tract (Godawari, Andhra Pradesh) under study.

Isolates of *Escherichia coli* serotypes from chicken in Sikkim give similar antibiotic sensitivity results for chloramphenicol and gentamicin by Mishra (1995), as under present study. David *et al.*, (1991) under antibiogram testing of *E. coli* isolates from poultry found (90 percent) isolates resistant to tetracycline which is very nearer to the result of present study. Resistance against gentamicin and co-trimoxazole found in this study is in accordance with earlier findings of Dwivedi, 2006 (3.49 percent), Yadav and Sharma 2006 (46.94 percent) respectively. The sensitivity to gentamicin of *Escherichia coli* isolates observed (79.56 percent) and (100 percent) to ciprofloxacin by Chowdhury and Das (2003) studied in diarrhoeic claves, which is contrary to present study. This variation may be due to indiscriminate use and irrational treatment in veterinary field without following the actual dose of these drugs. Suggestive findings were observed by Kakati *et al.*, (2003). On antibiogram study of *Escherichia coli* isolates from pork herd Guwahati and Shillong as resistance percentage (100 percent) and sensitivity percentage (9.09 percent) are nearer to the present study for

cefotaxime and tetracycline respectively. Study conducted by Seh *et al.*, (2000) on antibiogram of *Escherichia coli* serotypes in uterine secretion of repeat breeding and normal breeding cows in Kashmir is in accordance to present study as 99.9 percent isolates showed sensitivity to gentamicin, ciprofloxacin and chloramphenicol. Slight variations in result of sensitivity to gentamicin might be due to species variation. The difference recorded in this study may be attributed to change in the preference of antibiotics for treatment as well as control of infections in poultry in recent times and antibiotic may be effective at the time when it was introduced and become resistant once due to its inadvertently use for a long duration. So no single antibiotic is effective at all time. It is appropriate to select an antibiotic based on the antibiogram pattern obtained at that time rather than on reports (Chowdhury and Das, 2003). Least resistance recorded against gentamicin may be due to less frequent use of this antibiotic in poultry production (Dwivedi, 2006). A direct relationship between increased use of antibiotics and increased prevalence of resistant bacteria, has been well documented (Mc Gown, 1987, Linton *et al.*, 1972). The sensitivity and resistance pattern of *Escherichia coli* was not constant fact and could differ with different isolates, drugs and laboratory conditions (Chowdhury and Das, 2003). A total of 121 *E. coli* strains were isolated from broiler chickens (96 extraintestinal pathogenic (ExPEC) strains from diseased broiler chickens and 25 avian fecal *E. coli* (AFEC) from healthy ones). Antimicrobial resistance patterns revealed a complete resistance to gentamicin, pefloxacin, amoxicillin, and enrofloxacin among examined strains followed by varying degrees of resistance for the rest of tested agents. The highest resistance was recorded against norfloxacin, in 24 isolates (96%), in

contrast to the lowest resistance was recorded against colistin sulphate, in 14 strains (56%). These findings suggest the need for the prudent use of antimicrobials with broiler chickens and act as a warrant for the possibility of avian sources to transmit these resistant isolates to humans (Moemen A. Mohamed *et al.*, 2014). A total of 174 *E. coli* isolates collected from healthy poultry, bovine and ovine recovered between December 2009 and June 2013 in different geographic location in Tunisia, were assessed and examined for resistance to antimicrobial agents. Avian isolates showed the highest rates of antibiotic resistance: tetracycline (74.7 %) trimethoprim/sulfamethoxazole and amoxicillin with the same rate of resistance (57 %). Interestingly, avian *E. coli* isolates were more resistant than bovine and ovine ones. These results provide novel insights into the epidemiological characteristics of poultry, bovine and ovine *Escherichia coli* isolates in Tunisia, and suggest the need for the prudent use of antimicrobial agents in husbandry (Abbassi MS *et al.*, 2017).

Overall, 94% of the isolates showed resistance to at least one drug with 83% being resistance to at least three different classes of antimicrobials (Yassin *et al.*, 2017). In present study also the majority of isolates exhibited resistance against more than one antibiotic (Table-3). Out of 70 isolates tested 11 (10.00 percent) isolates for 3 antibiotics, 47 (67.14 percent) isolates have shown resistance for 4 or more antibiotics. the resistance against pepracillin and cefotaxime was shown by highest 7 (10.00 percent) isolates followed by combination of tetracycline and cefotaxime antibiotics. Similarly, among isolates showing resistance for antibiotics in combination of 3, highest 7 (10.00 percent) isolate showed resistance against tetracycline, piperacillin and cefotaxime.

Table.1 Antibiotics used to study drug resistance pattern of bacterial isolates

S. No.	Groups	Name of antibiotic	Code of antibiotic	Concentration per disc	Effect
1.	Quinolones	Oflloxacin	Of	5mcg	Cidal
		Pefloxacin	Pf	10mcg	Cidal
2.	Aminoglycosides	Ampicillin	A	20mcg	Cidal
		Amikacin	Ak	30mcg	Cidal
		Gentamicin	G	10mcg	Cidal
		Piperacillin	Pc	100mcg	Cidal
3.	Chloramphenicol	Chloramphenicol	C	30mcg	Static
4.	Sulphonamide + Trimethoprim	Co-trimoxazole	Co	25mcg	Cidal
5.	Tetracycline	Tetracycline	T	30mcg	Static
6.	Cephalosporine	Cefotaxime	Ce	30mcg	Cidal
		Ceftizoxime	Ck	30 mcg	Cidal
7.	Quinolones and fluoroquinolones	Ciprofloxacin	Cf	5mcg	Cidal

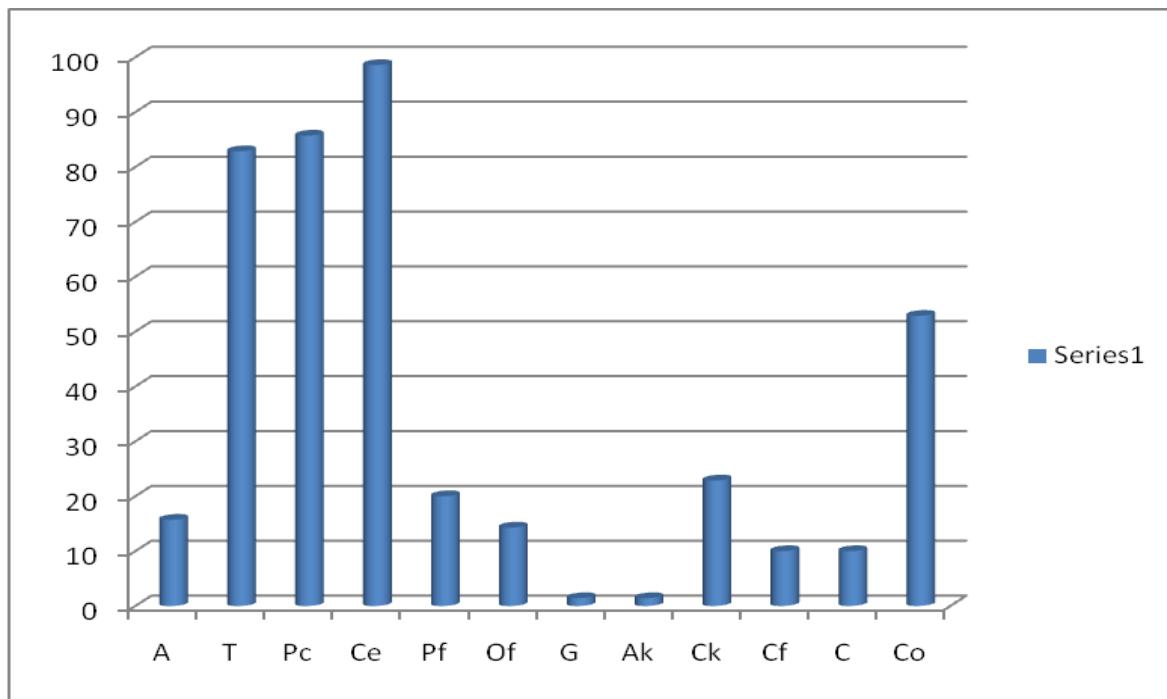
Table.2 Antibiotic resistance pattern of *Escherichia coli* isolates

Antibiotic	Resistance		Sensitive	
	No.	Percent	No.	Percent
Ampicillin	11	15.71	59	84.29
Tetracylin	58	82.86	12	17.14
Piperacillin	60	85.71	10	14.29
Cefotaxime	69	98.57	01	1.43
Pefloxacin	14	20.00	56	80.00
Oflloxacin	10	14.29	60	85.71
Gentamicin	1	1.43	69	98.57
Amikacin	1	1.43	69	98.57
Ceftizoxim	16	22.86	54	77.14
Ciprofloxaain	7	10.00	63	90.00
Chlorampheniol	7	10.00	63	90.00
Co-trimoxazole	37	52.86	33	47.14

Table.3 Multiple drug resistance pattern of enterobacteria isolated from poultry

Organism	No. of isolates tested	Combination of resistant antibiotic	Name of antibiotic (number)
E. coli	70	2	Pc + Ce (7) T + Ce (4)
		3	T+Pc+Ce (7)
		4 or more	T+Pc+Ce+Co (14) T+Pc+Ce+Ck (5) T+Ce+Pf+Co (1) A+T+Pc+Ce (1) T+Pc+Ce+Pf (2) T+Pc+Ce+Of+Co (1) T+Pc+Ce+Pf+C+Co (2) A+T+Pc+Ce+Co (5) T+Pc+Ce+Ck+Co (5) T+Pc+Ce+Pf+C+Co (2) T+Pc+Ce+Pf+Ck+Co (1) A+T+Pc+Ce+Ck+Co (1) T+Pc+Ce+Pf+Of+G+Cf (1) A+T+Pc+Ce+Pf+Of+Ck (1) A+T+Pc+Ce+Pf+Of+Ck (1) T+Pc+Ce+Pf+Of+Ak+Cf (1) T+Pc+Ce+Pf+Of+Cf+Co (1) T+Pc+Ce+Pf+Of+Ck+Cf+C+Co (1) A+T+Pc+Ce+Pf+Of+Ck+Cf+C+Co (1)

Graph.1 Graphical representation of antimicrobial resistance pattern of *E. coli* isolates



Under resistance for 4 and more than 4 antibiotics, highest 14 (20.00 percent) isolates showed resistance against tetracycline, piperacillin, cefotaxime and co-trimoxazole followed by 5 (7.14 percent) isolates for combinations of four antibiotics viz. tetracycline, piperacillin, cefotaxime and ceftizoxime.

Multiple drug resistance has been a consistent finding in the study of antibiogram of enterobacterial isolates. Out of 70 *Escherichia coli* isolates tested 11 (15.71 percent) isolates, 7 (10 percent) isolates and 47 (67.14 percent) isolates were found resistance to 2 antibiotics, 3 antibiotics and 4 or more antibiotics combinations respectively out of 12 antibiotics tested. Similar, findings were reported earlier by various workers. Mishra *et al.*, (2002) reported that 98.00 percent of the *Escherichia coli* isolates from domestic poultry exhibited simultaneous resistance to 2 to 10 antibiotics, maximum number of isolates (11 each) showed resistance to 7 or

9 drugs at a time. Similar, observations have also been reported by Chakraborty and Nag (1998), Jindal *et al.*, (1999), Chatterjee and Kashyap (2006), Ghanbarpour and Derakhshanfar (2004). Dwivedi (2006) also reported multiple drug resistance where 28 (19.58 percent), isolates were resistant to 2 antibiotics whereas 23 (16.08 percent) isolates were resistant to 3 antibiotics and 18 (12.58 percent) isolates were resistant to 4 antibiotics out of 9 antibiotics tested. In present study highest 7 (10 percent) isolates of *Escherichia coli* showed resistance against piperacillin and cefotaxime while 7 (10 percent) were resistant to tetracycline, piperacillin and cefotaxime antibiotic combinations. Maximum of 14 (20 percent) isolates were found resistant for the combination of tetracycline, cefotaxime and co-trimoxazole antibiotics.

The isolates of *Escherichia coli* from poultry were frequently reported to be resistant to one or more drugs especially if they have been widely used over a long period (Calnek

et al., 1997). Chowdhury and Das (2003) found none of the isolates out of 137 *Escherichia coli* isolates tested, resistant to only one antibiotics, only one strain was resistant to 2 antimicrobials where as 4.38 percent, 15.32 percent, 17.52 percent, 24.82 percent and 13.14 percent isolates were found to be resistant to 3, 4, 5, 6 and 7 antimicrobial agents respectively. The authors attributed the pattern of multiple drug resistant to large scale indiscriminate use of different antimicrobials as feed additives and for therapeutic purpose. In this study majority of *Escherichia coli* isolates of diarrhoeic poultry origin revealed the multiple antimicrobial drug resistance. Out of 70 *Escherichia coli* isolates tested 11 (15.71 percent) isolates, 7 (10 percent) isolates and 47 (67.14 percent) isolates were found resistance to 2 antibiotics, 3 antibiotics and 4 or more antibiotics combinations respectively out of 12 antibiotics tested.

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