Antibiotic resistance profile of *Escherichia coli* isolates from Colibacillosis in and around Pantnagar, India

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Abstract

Aim: The present study was designed to study antibiotic resistance profile of *E. coli* isolates from colibacillosis in layers in and around Pantnagar.

Materials and Methods: A total of 20 isolates of *E. coli* were recovered from 35 cases of colibacillosis in layers during necropsy. Antibiogram was studied via disc diffusion method against 12 antibiotics.

Results: Results showed multiple drug resistance in 52.63% *E. coli* isolates. Serotyping of these isolates revealed 10 'O' group serotypes, predominantly O80 and O84 accounting for 31.57%. O80, O110, O119 and O132 have previously been isolated from human suggesting its zoonotic importance. A high degree of resistance was seen against cephalexin (73.68%) whereas chloramphenicol was found to be maximally (100%) effective. Emergence of enhanced mechanism of resistance to a variety of frequently used antibiotics is an increasing public health problem.

Conclusion: It can be concluded that animals and human are at potential risk of acquiring infection with multi drug resistant strain of *E. coli*.

Key words: Antibiotic sensitivity, *E. coli*, Layers, Serotypes

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Introduction

E. coli has become a great concern in both human and veterinary practices. Although ubiquitous in nature, it plays a vital role in maintaining homeostasis of intestinal physiology of poultry [1]. It is not detrimental as long as it is kept in check by other intestinal microflora [2] but whenever there is imbalance, it results in colibacillosis, a disease of severe economic significance to all poultry producers, worldwide, characterized by a diverse array of lesions [3, 4]. This disease is of immense zoonotic importance since poultry meat is the commonest source of animal protein consumed by human population in most parts of the world [5]. There is increase in both incidence and severity of colibacillosis and current trends indicate that it is likely to continue and become an even greater problem in the poultry industry [6]. Now, there is considerable increase in prevalence of this disease in layers indicative of an alarming situation [7].

Antibiotics are extensively used in poultry industry either as a growth promoter or to control infectious diseases [8]. Concern about antibiotic resistance and its transmission to human pathogens is important because these resistant bacteria may colonize the human intestinal tract and may contribute resistance genes to human endogenous microflora through R-factor, conjugative plasmid, or chromosomal elements as reviewed by Kabir [9]. Therefore, disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem.

Due to the significance of *E. coli* infection in poultry industry, the present study was envisaged with the objectives of isolation of *E. coli* from various poultry samples and to study their antibiotic resistance pattern against wider range of antibiotics.

Materials and Methods

Collection of samples: A total of 35 faecal and carcass samples were collected using sterile cotton swab (Himedia, India), under strict aseptic condition, from morbid white leg horn birds of varied age groups (0 to 6 weeks of age) that were brought to Department of Veterinary Microbiology from areas in and around Pantnagar. Sampling were made as per the guidelines

Table-1. Antibiotic susceptibility pattern of *E. coli* isolate from poultry samples

Sr. No.	Antibiotics	Concentration per disc (µg)	Percentage		
			Sensitive	Intermediate	Resistant
1	Amikacin	30	78.95	15.79	5.26
2	Cephotaxime	30	10.53	89.47	-
3	Cephalexin	30	-	26.32	73.68
4	Norfloxacin	10	73.68	10.53	15.79
5	Chloramphenicol	30	100.00	-	-
6	Sulphamethizole	300	84.21	-	15.79
7	Pefloxacin	5	26.31	47.38	26.31
8	Furazolidone	50	52.63	31.58	15.79
9	Enrofloxacin	10	68.42	-	31.58
10	Nitrofurantoin	300	73.68	26.32	-
11	Co-trimoxazole	25	84.21	-	15.79
12	Neomycin	30	-	68.42	31.58

of Institutional Animal Ethics Committee. Samples were collected based on clinical findings and pathognomonic lesions observed during necropsy.

Transportation of sample: After collection, all the samples were being transported to the laboratory and processed immediately.

Isolation and identification: The samples were inoculated into peptone water (Himedia, India) and incubated at 37ºC for 18 h. Subsequently the cultures were streaked on Mc Conkey agar (Himedia, India) and incubated overnight at 37ºC. The lactose fermenter colonies were reinoculated on Eosin Methylene Blue (EMB) agar (Himedia, India) and incubated overnight at 37 ºC. Indole, methyl red, Voges-Proskauer, Simon's citrate test (IMViC), catalase, oxidase, urease, H_2S production in TSI and sugar fermentation test were performed with the colonies that showed growth characteristics of *E. coli* on EMB agar.

Serotyping: The isolates were sent to National Salmonella and Escherichia centre, Kasauli, Himanchal Pradesh, India for further confirmation and 'O' group serotyping.

Antibiotic susceptibility testing: Antibiogram of various serotypes was prepared using disc diffusion method, as described by Cruickshank *et al.* [10], against 12 commonly used antibiotics. The results were interpreted according to the criteria recommended by National Committee for Clinical Laboratory Standards (NCCLS) [11]. The antibiotic discs used in this study were amikacin (30 µg/disc), cephalexin (30 µg/disc), cephotaxime (30 µg/disc), chloramphenicol (30 µg/disc), sulphamethizole (300 µg/disc), nitrofurantoin (300 µg/disc), norfloxacin (10 µg/disc), pefloxacin (5 µg/disc), neomycin (30 µg/disc), furazolidone (50 µg/disc), enrofloxacin (10 µg/disc) and co-trimoxazole (25 µg/disc).

Results and Discussion

In the present study, *E. coli* were recovered from 20 (57.14%) samples out of 35 samples collected. 20 *E. coli* isolates were typed serologically into 10 different 'O' groups including O60, O80, O84, O95, O102, O110, O114, O119, O120 and O132. Two rough and one untypable isolate were also recovered. The predominant serotypes were O80 and O84, accounting for 31.57%. Many other workers also noted the *E. coli* serotypes obtained in the present investigation. O80, O110, O119 and O132 have already been isolated from human suggesting its zoonotic impotance (WHO report, 1998) [12]. O119 was also reported from diseased bird by Srinivasan *et al* (2003) [13]. O84, O95, O102 and O120 were isolated from colibacillosis in poultry by Sharada *et al* (2010) [14].

Antibiotic susceptibility pattern of *E. coli* isolate from poultry samples has been outlined in Table-1. It was observed that chloramphenicol was 100 % sensitive followed by sulphamethizole, co-trimoxazole (84.21% each) and amikacin (78.95%). This finding is in agreement to earlier studies done by other workers. Akond *et al* [8] and Sharada et al [14] showed that chloramphenicol is 80% effective against *E. coli* isolated from poultry and poultry environment. Alam *et al* [16] found that *E. coli* isolated from layers were sensitive to chloramphenicol. Omer *et al* [7] reported that *E. coli* isolated from colibacillosis are highly sensitive to co-trimoxazole which is in support to our findings. Mitra *et al* [9] showed that amikacin can be an effective drug in controlling poultry colibacillosis. However, in contrast, Rahman *et al* [17] reported resistance against chloramphenicol whereas Sharada *et al* [14] showed a high level resistance to co-

Table-2. Resistance spectrum of *E. coli* tested for 12 commonly used antibiotics

Sr. No.	Serotypes	Antibiotics Used	Resistant Antibiotics	Resitance %
1	O60	12	1	8.33
2	O80	12	0	0
3	O84	12	6	50
4	O95	12	1	8.33
5	O102	12	3	25
6	O110	12	2	16.67
7	O114	12	3	25
8	O119	12	6	50
9	O120	12	2	16.67
10	O132	12	3	25
11	Rough	12	0	0
12	Untypable	12	1	8.33

trimoxazole (76.92%). A high degree of resistance was found to be against cephalexin (73.68%) followed by neomycin and enrofloxacin (31.58% each) which is in full agreement with Sharada *et al* [14]. Nath *et al* [18] also showed resistance against cephixime in significant portion of the *E. coli* isolates. Wasyl *et al* [19] showed poultry as a reservoir of third-generation cephalosporin-resistant *E. coli.* Enrofloxacin was the most effective antibiotic against *E. coli* infection in earlier days but its indiscriminate usage in food animals (poultry) leads to fluoroquinolone resistance in zoonotic Gram negative bacilli as reported by Oteo *et al* [20] where all *E. coli* tested were resistant to ciprofloxacin, an another fluoroquinolone. Pathogen acquires antibiotic resistance through episomal transfer of resistance factor [21]. Percentage resistance spectrum of *E. coli* isolates tested for 12 antibiotics is shown in Table-2. 52.63% of the *E. coli* isolates of present study exhibited multiple resistances to more than 2 antibiotics. However, O84 and O119 showed resistance against 50% antibiotics.

Conclusion

From the present findings, it can be clearly demonstrated that multiple antibiotic resistant zoonotic *E. coli* are alarmingly high in poultry birds in and around Pantnagar. Therefore, to keep an eye on antibiotic resistance, introduction of surveillance programme is strongly recommended since transmission of resistant plasmids from food animals (poultry) to humans can occur. Synergistic antimicrobial combinations must be practiced only after sensitivity testing, at an optimal dose for sufficient time duration, in order to avoid antibiotic resistance.

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Competing interests

Authors declare that they have no competing interests.

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